

The copyright of this thesis vests in the author. No quotation from it or information derived from it is to be published without full acknowledgement of the source. The thesis is to be used for private study or non-commercial research purposes only.

Published by the University of Cape Town (UCT) in terms of the non-exclusive license granted to UCT by the author.

UNIVERSITY OF CAPE TOWN



**RESPIRATORY ALLERGY AND ASTHMA ASSOCIATED
WITH PESTICIDE EXPOSURE AMONGST WOMEN IN
RURAL WESTERN CAPE**

VUYELWA NDLOVU

PART 0: PREAMBLE

University of Cape Town

**RESPIRATORY ALLERGY AND ASTHMA ASSOCIATED WITH PESTICIDE EXPOSURE
AMONGST WOMEN IN RURAL WESTERN CAPE**

VUYELWA NDLOVU

STUDENT NUMBER: NDLVUY006

**Thesis submitted to the Faculty of Health Sciences, University of Cape Town in
fulfilment of the requirements of the degree Master of Public Health (Epidemiology
and Biostatistics)**

August, 2012

Supervisor:

**Associate Professor Mohamed Aqiel Dalvie
Centre for Occupational & Environmental Health Research (COEHR)
School of Public Health & Family Medicine
Health Sciences Faculty, University of Cape Town**

Co-Supervisor:

**Professor Mohamed F. Jeebhay
Centre for Occupational & Environmental Health Research (COEHR)
School of Public Health & Family Medicine
Health Sciences Faculty, University of Cape Town**

Declaration

I, Vuyelwa Ndlovu (NDLVUY006), hereby declare that the work in this mini dissertation is based on my original work (except where acknowledgements indicate otherwise) and has not, in whole or in part, been submitted towards another degree at this or any other university.

I empower the University of Cape Town to reproduce for the purpose of research, either the whole or any portion of the contents.

Signature:

Signed by candidate

Date: 10 August 2012

Dedication

To my loving husband and children, Bright, Tiboke and Lethubuhle.

Through thick and thin.

To my parents.

To God Almighty, who makes everything possible.

University of Cape Town

Acknowledgements

I wish to express my appreciation to my supervisor, Associate Professor Mohamed Aqiel Dalvie and co-supervisor Professor Mohamed F. Jeebhay from the Centre for Occupational and Environmental Health Research, School of Public Health and Family Medicine, University of Cape Town for their excellent guidance, advice, commitment, insight and patience while working on this research project.

I would also like to thank the Centre for International Health of the Ludwig-Maximilian-University Munich, Germany (CIHLMU) for funding my studies at the University of Cape Town.

A special thank you goes to my family here in Cape Town and in Zimbabwe for their unwavering support and encouragement during the course of my studies.

The following organisations and individuals are thanked for their roles in the project:

Financial support:

The Women on Farms Project and the University of Cape Town (Prof Jeebhay Allergy Research Fund)

Sampling and provision of fieldwork venue:

Dr Colette Solomon and the rest of the WFP management and staff

Research assistance:

Algernon Africa, School of Public Health and Family Medicine, UCT

Dawn Venter, School of Public Health and Family Medicine, UCT

Mohammed Sharief Abrahams, School of Public Health and Family Medicine, UCT

Deon September, School of Public Health and Family Medicine, UCT

Michael Luma Ekema-Agbaw, Department of Psychology, Virginia Polytechnic Institute and State University

WFP staff

Statistical advice:

Prof F. Little

Laboratories:

National Institute for Occupational Health, Johannesburg

Pathcare

University of Cape Town

Thesis abstract

Background: Pesticide exposure has been increasingly associated with adverse respiratory health effects including asthma and allergy. However, few studies have been conducted among women and workers in developing countries. South Africa is one of the largest users of pesticides on the continent and the Western Cape is one of the main agricultural areas in the country, with uncontrolled use posing an increased health risk to farm workers.

Objectives: This study focused on determining the association between pesticide exposure (predominantly organophosphates and carbamates) and the presence of allergy and asthma among women farm workers and residents.

Methods: A cross-sectional study was conducted on 211 women including those working and living on farms (farm dwellers, n=121) and those residing in neighbouring farm areas (town dwellers, n=90). Outcome measurements included the abbreviated European Community Respiratory Health questionnaire, fractional exhaled nitric oxide (FeNO) levels and immunological tests (a positive Phadiatop test indicating atopy and quantification of specific IgE to house dust mite, storage mite and spider mite). The outcome variables included doctor diagnosed asthma, adult onset asthma, current asthma, allergic sensitisation, allergic airway inflammation and the asthma symptom score. The asthma symptom score was a continuous outcome generated as the sum of positive responses to four questions on asthma symptoms in the last 12 months including wheeze with breathlessness, woken up with chest tightness, attack of shortness of breath at rest and woken by attack of coughing. The asthma symptom score values ranged from 0 for no symptoms to 4 for all symptoms.

Exposure information was obtained from the questionnaire, which had items on household and occupational pesticide use and concentration of whole blood cholinesterase (ChE) obtained from blood samples of workers. The questionnaire also had items on potential host-related confounding factors. Univariate and bivariate exploration of the data were performed. Fischer's Exact method was used for expected values less than 5. Multiple Logistic Regression Analyses were used to test for associations between dichotomous outcomes and exposure variables while controlling for confounding. For the continuous outcome, asthma symptom score, a Negative Binomial Regression Analysis was used. This models the ratio of the mean score among exposed and nonexposed

Results: The median age was 37 years (interquartile range: 28-45 years). There were 9% of participants with low ChE (below laboratory reference standard) among whom 78% were farm dwellers. The prevalence of doctor diagnosed asthma was 11%, current asthma was 6% and 24% ocular nasal symptoms. Being woken by cough was the most common symptom with a prevalence of 37%. After adjusting for confounding variables (age, smoking, years of schooling, atopy), ocular nasal symptoms were associated (OR= 2.97; 95% CI: 0.93-9.50) with immediate re-entry in the pesticide sprayed field. Furthermore, current asthma symptom score was significantly associated with pesticide drift in the home (Ratio of mean asthma symptom score=2.03; 95% CI: 1.38-2.98) compared to the unexposed, being a farm dweller (Ratio of mean asthma symptom score= 2.25; 95% CI: 1.45-3.48) and having a low ChE (Ratio of mean asthma symptom score=1.93; 95% CI: 1.09-3.44). While the prevalence of doctor diagnosed asthma was not significantly different between farm

and town dwellers, those with low ChE had a five-fold increased odds of having FeNO > 50ppm (highly probable allergic asthma) (95% CI: 0.80-28.00; p=0.08). Allergic sensitisation to the various mites was not associated with pesticide exposure.

Conclusion: Exposure to pesticides among women farm workers is associated with increased risk of ocular nasal symptoms and asthma. This study was limited by the small sample size, lack of information on specific pesticides and the cross sectional design. The findings in this study therefore need further exploration in a larger longitudinal study.

University of Cape Town

Table of contents

COVER PAGE	i
PART 0: PREAMBLE	ii
Title page.....	iii
Declaration.....	iv
Dedication.....	v
Acknowledgements.....	vi
Thesis abstract.....	viii
Table of contents.....	x
List of tables.....	xiii
PART A: PROTOCOL	1
1. Introduction.....	2
1.1 Literature review.....	2
1.2 Problem statement.....	6
1.3 Research question.....	8
1.4 Hypothesis	9
1.5 Justification.....	9
1.6 Aim.....	10
1.7 Objectives.....	10
2. Methods	11
2.1 Study design, population and sampling.....	11
2.2 The sampling strategy.....	11
2.3 Sample size calculations.....	12
2.3.1 Inclusion and exclusion criteria.....	13
2.4 Measuring instruments.....	13
2.4.1 Questionnaire.....	13
2.4.2 whole blood cholinesterase (ChE) testing.....	14
2.4.3 Allergy tests: Phadiatop and allergen specific serum IgE levels	14
2.4.4 Airway inflammation as a marker for asthma: Fractional exhaled nitric oxide (FeNO).....	15
2.5 List and definition of variables	16
2.6 Validity and reliability of instruments.....	19
2.7 Pilot study.....	19
2.8 Data analysis strategy.....	19
2.8.1 Data exploration.....	20
2.8.2 Bivariate associations.....	20
2.8.3 Regression analysis	21
3. Ethical considerations.....	21

3.1	Autonomy.....	22
3.2	Benefit.....	22
3.3	Harm.....	23
3.4	Justice.....	23
4.	Logistics.....	23
5.	References.....	24
PART B (STRUCTURED LITERATURE REVIEW).....		28
1.	Introduction.....	29
1.1.	Background	29
1.2.	Objectives of the literature review.....	29
1.3.	Search strategy.....	30
2.	High risk populations and target groups exposed to pesticides.....	30
3.	Epidemiological studies on respiratory health effects associated with pesticides.....	33
3.1	Respiratory health measurement.....	34
3.2	Pesticide exposure assessment.....	35
3.3	Specific pesticides associated with respiratory health problems.....	36
3.4	Studies on women.....	38
3.5	Allergic and non-allergic asthma.....	40
3.6	Studies on rhinitis.....	41
3.7	Studies on children.....	42
3.8	Studies in Africa.....	42
4.	Pathophysiological mechanisms.....	43
5.	Risk factors for rhinitis and asthma associated with pesticides.....	45
6.	Future research.....	47
7.	References.....	48
PART C: ENVIRONMENT INTERNATIONAL JOURNAL MANUSCRIPT		54
Abstract		56
1.	Introduction	58
2.	Materials and methods.....	61
2.1	Study Design, Population and sampling.....	61
2.2	Questionnaire.....	62
2.3	Erythrocyte acetylcholinesterase (AChE) testing.....	63
2.4	Allergy tests: Phadiotop and allergen specific serum IgE levels.....	63
2.5	Airway inflammation as a marker for asthma: Fractional exhaled nitric oxide (FeNO).....	64
2.6	Statistical analysis.....	65
3.	Results	67
3.1	Participation.....	67
3.2	Demographic and socioeconomic characteristics.....	68
3.3	Environmental and occupational exposure to agricultural pesticide spraying among farm dwellers.....	69
3.4	Self-reported household and agricultural pesticide exposure indicators and measured cholinesterase enzyme levels.....	70
3.5	Prevalence of asthma and allergic outcomes.....	72

3.6 Host factors associated with asthma and allergic outcomes.....	74
3.7 Asthma symptom score and pesticide exposure.....	76
3.8 Associations between pesticide exposure and asthma and allergic outcomes.....	77
4. Discussion	81
5. Conclusions and recommendations.....	89
6. Acknowledgements	90
7. References	90
SUPPLEMENTARY TABLES TO JOURNAL MANUSCRIPT	95
PART D: APPENDICES.....	103
Appendix 1a. Questionnaire in English	104
Appendix 1b. Questionnaire in Afrikaans.....	115
Appendix 2. Exhaled Nitric Oxide Pre-test Data Collection Sheet.....	127
Appendix 3. Exhaled Nitric Oxide Data Collection Sheet	131
Appendix 4. Consent Form.....	133
Appendix 5a. Letter of Approval from Research Ethics Committee.....	136
Appendix 5b. Health Research Committee Annual progress Report.....	139
Appendix 6. Table of Epidemiology studies for the structured Literature review	141
Appendix 7. Environment International: Author Instructions.....	148
Appendix 8. Pesticides and the Airways-A Review of the literature (Ndlovu et al., 2011).....	161

List of tables

Table no.	Title	Page
Part A Protocol		
Table 1	Distribution of study participants by area and type of residence	12
Table 2	List of variables	18
Part B Structured Literature Review		
Table 1	Specific Pesticides associated with upper and lower airway respiratory outcomes	38
Table 2	Summary of epidemiological studies of women or including women in the investigation of the effect of pesticides on respiratory health	39
Part C Journal Ready Manuscript		
Table 1	Distribution of study participants by area and type of residence	68
Table 2	Demographic and socioeconomic characteristics of women farm workers in the Boland of the Western Cape (n=211)	69
Table 3	Self-reported household and agricultural pesticide exposure and measured cholinesterase enzyme levels of women farm workers in the Boland of the Western Cape (n=211)	71
Table 4	Prevalence of asthma and allergic outcomes among women farm workers in the Boland of the Western Cape (n=211)	72
Table 5	Unadjusted logistic regression models of host and other potential confounders associated with asthma outcomes among women farm workers in the Boland of the Western Cape	75
Table 6	Adjusted negative binomial regression models of pesticide exposure indicators associated with continuous asthma symptom score among women farm workers in the Boland of the Western Cape	77
Table 7	Adjusted multiple logistic regression models of pesticide exposure indicators associated with asthma outcomes among women farm workers in the Boland of the Western Cape	79
Supplementary tables to journal manuscript		
Table 1	Adjusted multiple logistic regression models of pesticide exposure indicators associated with asthma phenotypes among women farm workers in the Boland of the Western Cape	96
Table 2	Unadjusted negative binomial regression models of host factors and other potential confounders associated with asthma symptom score among women farm workers in the Boland of the Western Cape	97
Table 3	Adjusted multiple logistic regression models of pesticide exposure indicators associated with asthma outcomes among women farm workers in the Boland of the Western Cape	98
Table 4	Unadjusted and adjusted linear regression models of pesticide exposure indicators associated with asthma outcomes among women farm workers in the Boland of the Western Cape	99
Table 5	Unadjusted logistic regression models of host and other potential confounders associated with allergic sensitisation outcomes among women farm workers in the Boland of the Western Cape	100
Table 6	Adjusted multiple logistic regression models of pesticide exposure indicators associated with allergic sensitisation outcomes among women farm workers in the Boland of the Western Cape	101
Table 7	Adjusted multiple logistic regression models of pesticide exposure indicators associated with asthma and ocular nasal symptoms phenotypes among women farm workers in the Boland of the Western Cape	102
Part D Appendices		
Appendix 6	Table of Epidemiology studies for the structured Literature review	142

PART A: PROTOCOL

University of Cape Town

1. Introduction

Women working in farms are exposed to a wide range of allergens and pesticides that may be affecting their respiratory health (Bowers et al., 2009; Hoppin et al., 2008). Few studies have investigated the respiratory health of women farm workers in relation to pesticide exposure especially in developing countries. This study aims to address this issue by determining the risk factors associated with allergy and asthma so as to develop strategies to protect and promote the health of this vulnerable group of workers.

1.1 Literature review

South Africa is cited as the largest consumer of pesticides in Sub Saharan Africa and this is in large part due to the large volumes used in agriculture (Naidoo and Buckley, 2003). Within South Africa, the Western Cape where crop farming is important, pesticide use has been increasing (London, 2003; Dalvie, 2009). In the Western Cape, agricultural pesticides have been detected in the rural environment and among farm workers. Pesticides such as endosulfan and chlopyrifos have been detected in drinking and recreational water sources and sediments in the rural Western Cape (Dalvie et al., 2003; Schulz ,2001a, 2001b). In a study conducted in the Western Cape, chlorpyrifos and endosulfan residues in farm workers were found to be higher than in other countries (Dalvie et al., 2009, Dalvie et al., 2011). Additionally, pesticide residues are also found in South African crops as indicated by a study conducted by Dalvie and London (2009) that found pesticide residues in both local and imported wheat samples.

The effect of pesticide exposure on asthma, allergy and other respiratory problems amongst farm workers has been investigated in many studies (Vanden Driessche et al., 2010 ; Alarcon et al., 2005; Zhang et al., 2002; Faria et al., 2005; Hoppin et al., 2008; Fieten et al., 2009; Slager et al., 2009; Hoppin et al., 2002). However, most of these studies have been done in developed countries and have mostly focused on adult males but it has become increasingly apparent that women are also a high risk group (London et al., 2002; Zhang et al., 2002; Garcia, 2003; Faria et al., 2005; Hoppin et al., 2008; Fieten et al., 2009). Work related pesticide poisoning in women was found to be more common than in men in a Chinese study (Zhang et al., 2011). However, it is uncertain whether this was related to the gendered distribution of work or due to other as yet unexplored biological factors. Two other studies reported that respiratory symptoms or doctor diagnosed asthma were more prevalent and strongly associated with pesticide exposure in women than in men (Zhang et al., 2002; Faria et al., 2005).

In the study by Faria et al. (2005), the higher prevalence of asthma symptoms in women was ascribed to the fact that they were found to have less protection during pesticide exposure than men and were more likely to have been exposed in domestic settings as well.

Few studies have investigated the specific pesticides associated with respiratory problems. Organophosphates and carbamates feature prominently in the available literature on specific types of pesticides associated with asthma, allergy and other respiratory problems. In Costa Rica, Fieten et al. (2009) looked at specific pesticides

and found that the organophosphates chlorpyrifos and terbufos were strongly associated with wheeze (OR= 6.7, 95% CI: 1.6, 28.0 and OR = 5.9, 95% CI: 1.4, 25.6, respectively) when comparing women farm workers exposed to pesticides with those who were unexposed . In other studies of farmers and their spouses wheeze was associated with the herbicides atrazine and alachlor (Hoppin et al., 2002) and chlorimuron-ethyl, a herbicide, and phorate an organophosphate insecticide (Hoppin et al., 2006). Pesticides also appear to have an effect on the upper respiratory tract. Rhinitis was associated with the herbicides paraquat, glyphosate, petroleum oil, carbaryl a carbamate and organophosphates namely diazinon and malathion (Slager et al., 2009; Chatzi et al., 2007; Slager et al., 2010). Among male farmers, the organochlorine DDT (dichlorodiphenyltrichloroethane) was associated with adult onset non-allergic asthma whereas heptachlor (another organochlorine) was associated with adult onset allergic asthma along with halogenated organic fumigants and organophosphate pesticides (Hoppin et al., 2009)

Few studies on the effect of pesticide exposure on respiratory health have been conducted in South Africa. Innes et al. (1990) reported that 17.5% of male rural workers engaged in crop spraying reported chronic organophosphate poisoning, asthma and chronic bronchitis in Cape Town. No association between long-term exposure to paraquat and reported symptoms and lung function was found but a decrease in exercise oximetry was found in a study done in the rural Western Cape (Dalvie et al., 1999). A more recent study in South Africa demonstrated that the spider mite, *T. urticae*, is an important outdoor allergen among table-grape farm workers

and the increased risk of spider mite allergy appeared to be related to high pesticide exposure among crop sprayers (Jeebhay et al., 2007).

Allergic asthma was thought to be uncommon in farmers compared to other occupational sectors, but recent studies have shown that pesticide exposure is associated with allergic asthma. In a study of 19 704 male farmers conducted in the USA, it was found that pesticide exposure was associated with both adult onset atopic and non-atopic asthma (Hoppin et al., 2009). In another study, not only were pesticides associated with atopic asthma but having grown up on a farm was found to have a protective effect on asthma in farm women (Hoppin et al., 2008). Most pesticides used, especially parathion and malathion, were associated with atopic asthma and only permethrin was associated with both allergic and non-allergic asthma. The study by Jeebhay et al. (2007) that was conducted in the Western Cape, found that Spider mite, *Tetranychus urticae*, is an important outdoor allergen responsible for allergic symptoms such as asthma among table grape farm workers. Increased risk of exposure and sensitization to spider mite may be related to pesticide crop spraying and atopy in table grape farm orchards (Jeebhay et al., 2007).

In a number of studies investigating the effect of pesticide exposure on asthma, the outcomes studied were self-reported, doctor-diagnosed asthma or asthmatic symptoms. More objective measures for airways disease including lung function indices were reported in relatively fewer studies. Another limitation of the studies is that in many of them, exposure assessment was based on self-reported exposures obtained from questionnaire data with categorical outcomes. Future studies should focus on developing measures of exposure such as personal-exposure monitoring

using biomarkers especially for short term outcomes such as wheeze in the past month. Specific pesticides should be identified for more detailed study so as to determine possible threshold levels for health effects. Future studies should also focus on understanding the pathophysiological mechanisms that underlie asthma and rhinitis so as to characterise the specific asthma phenotypes using more objective measures for asthma outcomes.

1.2 Problem statement

Pesticide exposure in the agricultural sector is important since about 1.8 billion people in the world are involved in agriculture and most are exposed to pesticides (Alavanja, 2009). Agricultural workers, especially applicators and handlers have therefore been the study population of choice in most studies investigating health effects of pesticides. Pesticide exposure has been associated with asthma, allergy and other respiratory problems (Hoppin et al., 2002; Alarcon et al., 2005; Zhang et al., 2002; Faria et al., 2005; Hoppin et al., 2008; Fieten et al., 2009; Slager et al., 2009;). But many of these studies have been conducted in adult males working in the agriculture industry and there is little data on women agricultural workers.

Much of the evidence also comes from developed countries. It is possible that populations in African and other developing countries may be at higher risk of pesticide exposure since the use of pesticides in these countries, including South Africa, is increasing and is still largely unregulated. (Nweke and Sanders, 2009; Jeebhay and Quirce, 2007). Over 90% of deaths from pesticide poisoning emanate from developing countries even though these countries use only 20% of the world's

total consumption (Kesavachandran, 2009). Large proportions of people employed in agriculture are from poorer communities and generally have limited literacy levels. The living and working conditions on many farms in South Africa, for example, are very poor and the workers are often underpaid. Women are worse off than men because they are often precariously employed. They receive fewer benefits because they are assigned menial jobs around the farm like pruning, sorting, grading and packing while men get the higher paying jobs such as pesticide applicators, tractor drivers and supervisors. As a result women have been regarded as less at risk of developing adverse health effects due to pesticide exposure and relatively less research has been done on this group.

In addition to workplace tasks, women and their children may be exposed to pesticides indirectly, including spray drift at work and into homes on the farm, re-entry into sprayed fields, washing of contaminated overalls at home, contamination of rural water sources and reuse of pesticide containers (London, 1994; London, 1998; Dalvie et al., 2003, Dalvie et al., 2004). A recent study conducted in the Western Cape found pesticides residues in the blood of female workers to be very high (Dalvie et al., 2009).

Because women are always given less challenging tasks in farms, the assumption has always been that they are less at risk of exposure to harmful substances. The limited studies available have shown that they are in fact at risk (Hoppin et al., 2008; Forastieri, 1999; McCoy et al., 2002). The problem is that little is known about the health effects of chronic low dose exposure to pesticides in women particularly in

Africa. There are no previous studies that have been done on women farm workers in South Africa to assess pesticide exposure and the related respiratory health outcomes in this vulnerable group of workers who are also exposed to domestic and sexual violence due to alcohol and drug dependence (London, 2003). Respiratory health problems have been identified among the women farm workers in the Western Cape (Bowers et al., 2009). These reports are consistent with the literature on health hazards in agriculture (Stellman, 1998; Forastieri, 1999, Dalvie et al., 1999). Considering the increasing use of pesticides in the Western Cape and the reports of increasing respiratory problems among the women in the area, it is reasonable to hypothesise that pesticide exposure may play a role in the aetiology of asthma, allergy and other respiratory symptoms. There are studies in other countries that have cited a relationship between pesticide exposure and respiratory symptoms (Senthilselvan et al., 1992; Hoppin et al., 2002; Hoppin et al., 2009; Vanden Driessche et al., 2010 ;Chatzi et al., 2007; Zhang et al., 2002; Faria et al., 2005; Hoppin et al., 2008;Fieten et al., 2009;Slager et al., 2009), however further investigation of such an association is required, particularly in South Africa, where the association between pesticide exposure and respiratory outcomes in women has not been explored. More research is required to quantify the burden of disease, to identify the important risk factors in order to inform policy and implement interventions to prevent and control asthma and allergies in women in farms in the Western Cape.

1.3 Research question

Is pesticide exposure associated with increased risk of allergy, respiratory symptoms and asthma among women farm workers in the Western Cape?

1.4 Hypothesis

The hypothesis of the study is that the pesticides, to which women on farms in the Western Cape are exposed to, are associated with respiratory allergy and asthma.

1.5 Justification

Many studies have been conducted in adult males that have shown that pesticide exposure is associated with respiratory allergy and asthma. Relatively less research has been done on women and children and most of the available evidence comes from developed countries. In most African countries pesticide use is still largely unregulated and widespread due to weather conditions favourable to weeds and pests and there is also lack of training on how to use pesticides properly (Nweke and Sanders, 2009). Despite the increased use and exposure to pesticides, very few studies in Africa have been conducted to assess the association with respiratory symptoms, despite pesticides also being documented as a common problem in developing countries. Respiratory health problems have been identified among the women farm workers in the Western Cape (Bowers et al., 2009) but no previous study has assessed whether pesticide exposure is associated with these health outcomes in the area. In this particular study, this hypothesis is explored.

The analysis of the data will yield information that will be used by environmental advocacy groups, farm employers, policy makers and other stake holders to implement interventions that will reduce the risk of exposure to pesticides and subsequently the associated respiratory problems. This research will produce data that can provide an indication of the burden of respiratory problems associated with

pesticides amongst farm women. Quantifying the burden of disease will assist decision makers in the allocation of resources required for control and prevention strategies. There are few studies in South Africa that have used biomarkers in the measurement of pesticides. The development and use of pesticide biomarkers is important in South Africa as they strengthen the validity of measuring exposure in studies. In this study, cholinesterase enzyme inhibition will be used as a marker of pesticide exposure. The results of this study will contribute to the growing body of knowledge on methodological issues relevant to the direct measure of exposure in the South African context.

1.6 Aim

To investigate the effect of occupational and environmental pesticide exposures on respiratory health amongst women on farms in the Western Cape.

1.7 Objectives

- To describe demographic and socio-economic profile of the population
- To determine pesticide exposure among the women firstly by classifying them into exposure groups based on current farm residence and occupation by measurement of whole blood cholinesterase levels as a marker for exposure to organophosphate and carbamate pesticides.
- To determine the respiratory health problems amongst the women including respiratory allergy and asthma

- To determine host factors associated with pesticide exposure and respiratory allergy and asthma
- To investigate the relationship between pesticide exposure and respiratory allergy and asthma, controlling for relevant confounders

2. Methods

2.1 Study Design, Population and sampling

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. The study areas included Stellenbosch, Ceres, Paarl, Grabouw and Worcester. The Non-Governmental Organisation, Women on farms (WFP), assisted in the study by recruiting women for the study, providing study site and transporting women to the study site.¹

2.2 The sampling strategy

The study sample was recruited by WFP who selected a total of 211 women into the study including 113 women currently living on a farm and 98 in towns. The research

¹ This thesis is based on an existing dataset and the researcher's involvement is mainly the development of exposure and outcome variables and the subsequent analyses and write-up. Some parts of the methods section in the protocol are therefore written in the past tense.

team requested WFP to recruit 100 women from farms affiliated to them located in the 5 most accessible Western Cape agricultural areas which included Stellenbosch, Ceres, Paarl, Grabouw and Worcester and 100 women not living on farms from neighbouring towns in each area. About 40 women, 20 each from farms and towns, from each of the 5 areas were targeted. The sample was not selected through random sampling due to time constraints and logistical difficulties. 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. There were 8 women who lived in town but worked on a farm and 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”. Table 1 summarises the distribution of study participants.

Table 1. Distribution of study participants by area and type of residence

Area	N (%)			
	Town dwellers,(n=90)	Farm dwellers, (n=121)		Total
		Farm residents (n=24)	Farm workers (n=97)	
Ceres	19(45)	9(21)	14(33)	42(100)
Grabouw	3(8)	0(0)	35(92)	38(100)
Paarl	23(59)	3(8)	13(33)	39(100)
Stellenbosch	22(47)	6(13)	19(40)	47(100)
Worcester	23(51)	6(13)	16(36)	45(100)
Total	90(43)	24(11)	97(46)	211(100)

2.3 Sample size calculations

Sample size calculations using a two sample comparison of proportions [Stata Corporation, 2003] (exposed/control ratio = 1, power = 90%, confidence level = 95%) and using the results from a recent study (Hoppin, et al, 2009) shows that for a 225% increase in asthma prevalence (20% to 45%), 160 participants would be required.

2.3.1 Inclusion and exclusion criteria

Only women from the Boland region of Western Cape were included in the study. Men and children were excluded.

2.4 Measuring Instruments

2.4.1 Questionnaire

Since this is part of a larger study, only the relevant sections in the main questionnaire (Appendix 1a, 1b) will be used for analysis in line with the objectives of this study. 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) 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. Smoking history included questions on frequency and duration of smoking as well as passive smoking. The respiratory health section incorporated the abbreviated form of the standardised and validated

European Community Respiratory Health Survey questionnaire (Burney et al., 1994). The questionnaires were translated into Afrikaans and Xhosa. They were then back translated to ensure the accuracy of the translation. Trained interviewers administered questionnaires to participants in the language of their choice. Fieldwork was conducted at the WFP premises during the period 24 October -3 December 2009. WFP arranged for the transport of the participants on a daily basis.

2.4.2 Whole blood cholinesterase (ChE) testing

A whole blood sample (9 ml) was drawn from each participant using a tube containing sodium heparin or EDTA as anticoagulant, by a qualified nurse. The tubes were kept on ice and transported to the South African National Accreditation System (SANAS) accredited Pathcare laboratory at N1 City Hospital, Cape Town for cholinesterase (ChE) analyses.. ChE activity was quantified using the Roche Diagnostics Cholinesterase method (Roche/Hitachi analyser, Cobas^R, Roche Diagnostics GmbH, D-68298 Mannheim), a photometric test based on the principles of a study by Ellman et al. (1961).

2.4.3 Allergy tests: Phadiatop and allergen specific serum IgE levels

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 nurse. The blood was allowed to clot for 1-2 hours at room temperature (20-24 degrees Celsius) and then centrifuged at 1350rpm 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 National Institute for

Occupational Health, UCT where it was stored at -80 degrees Celcius until assayed for further measurement. The samples were sent to the SANAS accredited (ISO 15189:2007), NIOH Immunology laboratory for testing. Presence of sensitisation to common aeroallergens (house dust mites, grass pollens, cat, dog, cockroach etc.) was determined by the Phadiatop[®] test (Phadia AB, Uppsala, Sweden) which is a measure of atopy. The quantification of specific IgE antibodies to house dust mite and specific occupational allergens (spider mite: *Tetranychus urticae* and storage mite, *Lepidoglyphus destructor*) was performed using the UniCAP[®] system (Phadia AB, Uppsala, Sweden).

2.4.4 Airway inflammation as a marker for allergic asthma: Fractional exhaled nitric oxide (FeNO)

Fractional exhaled nitric oxide measurement is a recognised non-invasive method for assessing allergic airway inflammation (Quirce et al., 2010). The nurse determined fractional exhaled NO (FeNO) from single-breath exhalations (Appendix 2 and 3). The technique for adult patients involved inspiration of 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 [ATS/ERS 2005]. Exhaled nitric oxide (NO) 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.

2.5 List and definition of variables

Table 2 lists the variables to be used in the study. The list of covariates includes *a priori* confounding variables (age, BMI, smoking and atopy) and other variables of interest. Exposure variables encompassing domestic, occupational and environmental exposure to pesticides to be generated from the exposure questionnaire will be used as a proxy for pesticide exposure. These include the categorical variables; pesticide use at home, history of living or working on farms, currently living or working on farms, pesticide drift into the home, employment status at the farm, re-entry into the farm after spraying and the continuous variables; number of years in current farm job and number of pesticide spraying days per year. ChE measurements will also be used as a marker for exposure to cholinesterase inhibiting pesticides like the carbamates and organophosphates. ChE will be dichotomised. A ChE level below 6021 IU (low end of the laboratory range) (Data on file at Roche Diagnostics®) is considered low and indicates that the participant has been exposed reasonably highly to pesticides. Outcome variables include the following:

- **Doctor diagnosed asthma**
- **Allergic sensitisation** defined as a measure of allergen-specific circulating IgE antibodies to house dust mite, storage mite and spidermite. IgE levels greater than 0.35ku/l indicate allergic sensitisation

- **Allergic airway inflammation** defined as a measure of elevated fractional concentration of exhaled nitric oxide (FeNO) suggestive of highly probable asthma (FeNO levels >50ppb) and FeNO levels 25-50ppm indicating possible asthma (Dweik et al., 2011). FeNO levels will also be analysed as a continuous variable.

Other outcomes generated from the respiratory symptoms variables in Table 2 include:

- **Current asthma** defined as 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?
- **Adult-onset asthma** defined as Ever having had asthma **AND** having had the first asthma attack at the age of 16 years or later.
- **Asthma symptom score:** An asthma symptom score calculated as the sum of the answers (Y=1/N=0) to four questions on asthma symptoms in the last 12 months: 1) wheeze with breathlessness 2) Woken up with chest tightness 3) Attack of shortness of breath at rest 4) Woken by attack of coughing. The asthma symptom score used in this study was a simplified version of an asthma score proposed and developed by Pekkanen et al. (2005) and used by Sunyer et al. (2007) and Vizcaya et al. (2011).

Table 2. list of variables

Variable	Type	Units/categories
Potential confounders		
Age	Continuous	Years
BMI	Continuous	Kg/m ²
Current smoker	Dichotomous	Yes or no
Atopy (phadiatop positive)	dichotomous	Yes or no
Education	Continuous	years
Currently Employed	Dichotomous	Yes or no
Household Income	Continuous	\$US
Length of stay in residence	continuous	years
Born on a farm	Dichotomous	Yes or no
Alcohol	Dichotomous	Yes or no
Pesticide exposure variables		
Use pesticides at home	Dichotomous	Yes or no
Living with a pesticide applicator	Dichotomous	Yes or no
Domestic use of pesticide applicators	Dichotomous	Yes or no
History of living and working on a farm	Dichotomous	Yes or no
Currently Living and/or working on a farm	Dichotomous	Yes or no
Pesticide drift into home	Dichotomous	Yes or no
Employment status on farms	Categorical	Permanent ,Seasonal and Non-farm worker
Re-entry into sprayed fields	Categorical	Non-farm residents, Delayed re-entry and Immediate re-entry
Farm job duration	Continuous	Years
Number of days of pesticide spraying/year	Continuous	Days
Low cholinesterase (<6021 IU)	Dichotomous	Yes or no
Distance of home from vineyard	Continuous	Metres
When last pesticides sprayed	Continuous	Days
Unprotected source of water for drinking and household use	Dichotomous	Yes or no
Method of applying pesticides	Categorical	Tractor with boom-spray, Tractor without boom-spray, Backpack spray and Quad bike
Come into contact with pesticides when outside the house	Dichotomous	Yes or no
PPE use on farm	Dichotomous	Yes or no
Outcome variables		
Ocular nasal symptoms	Dichotomous	Yes or no
Wheeze	dichotomous	Yes or No
Shortness of breath with wheeze	Dichotomous	Yes or No
Wheeze without cold	Dichotomous	Yes or no
Woken up by chest tightness	Dichotomous	Yes or no
Attack of shortness of breath at rest	Dichotomous	Yes or no
Woken by cough	Dichotomous	Yes or no
Asthma ever	Dichotomous	Yes or no
Doctor diagnosed asthma	Dichotomous	Yes or no
Current asthma	Dichotomous	Yes or no
Adult onset asthma	Dichotomous	Yes or no
Asthma score	Continuous	Score of 0-4
Age at asthma diagnosis	Continuous	Years
Asthma attack in the last 12 months	Dichotomous	Yes or no
Asthma medication	Dichotomous	Yes or No
FeNO	continuous	Parts per billion (ppb)
FeNO>25ppb - possible asthma	Dichotomous	Yes or no
FeNO>50ppb - highly probable asthma	Dichotomous	Yes or no
House dust mite sensitisation (IgE>0.35ku/l)	Dichotomous	Yes or no
Spider mite sensitisation (IgE>0.35ku/l)	Dichotomous	Yes or no
Storage mite sensitisation(IgE>0.35ku/l)	Dichotomous	Yes or no

2.6 Validity and Reliability of instruments

The non-respiratory questions in the questionnaire were based on those used in previous surveys (Dalvie et al., 1999; Dalvie et al., 2004). The questionnaires were translated into Afrikaans and Xhosa. They were then back translated to ensure the accuracy of the translation. Trained interviewers administered questionnaires to participants in the language of their choice. An internationally standardised and validated European Community Respiratory Health Survey questionnaire for identifying asthma and respiratory symptoms (Burney et al., 1994) was used. Objective and calibrated instruments were used to measure ChE levels, airway inflammation and allergic sensitisation in accredited laboratories. To test the reliability of the questionnaire, a pilot study was conducted.

2.7 Pilot study

The pilot study was conducted to test the questionnaire and to work out the logistics for the main study. Fieldwork was conducted during the period 24 October -3 December 2009.

2.8 Data Analysis Strategy

The selected software for analysis is StataCorp. 2009. Stata: Release 11. Statistical Software. College Station, TX: StataCorp LP

2.8.1 Data exploration

First all variables will be described and explored. For numerical variables, histograms and the Shapiro-Wilk test will be used to check for normality as this will determine whether parametric or non-parametric tests are used for further analysis. Frequency distributions for numerical variables will be constructed. Means and standard deviation will be used to describe normally distributed numerical variables and median and range will be used to describe non normal variables. Box and Whisker plots will be used to look for outliers as such observations are important in model building. Categorical variables will be described using frequency distributions and pie charts.

2.8.2 Bivariate Associations

Bivariate analysis will be used to look for associations between variables. Scatter plots will be used for numerical variables. Pearson or Spearman rank correlation will be used to measure degree of correlation and the statistical significance. This will also be an opportunity to see if multi-collinearity is a problem. Wilcoxon rank sum or t test will be used to assess significance of the association between a numerical and dichotomous variable. Contingency tables and the chi-square statistic will be used for categorical data to determine if there is a statistically significant association. Odds ratios at 95% confidence interval will be calculated to determine the degree of association between variables. The Fischer's Exact test will be used for expected values less than 5. The purpose of the bivariate analysis is to determine which of the variables are strongly enough associated with asthma to justify their inclusion in the multivariate analysis. Atopy and smoking will be included in the models on an *a priori*

basis and others will be selected from bivariate testing if $p < 0.1$ or showing at least a 10% change in the β coefficient of the crude OR between exposure and outcome.

2.8.3 Regression analysis

Logistic, linear and negative binomial regression will be used for the multivariate analysis. Multivariate Analyses will be used to test for associations between all the individual outcomes and exposure while controlling for confounding. Appropriate techniques will be used for model building and diagnostic tests will be used to ensure the underlying mathematical assumptions of the tests are met and to remove any influential points from the model to ensure that the predictor coefficients are as valid as possible. For the continuous outcome, asthma symptom score, a Negative Binomial Regression Analysis will be used. This models the ratio of the mean score among exposed and nonexposed

3. Ethical considerations

The study was done in accordance with the Declaration of Helsinki of the 25th world Medical Assembly (WHO, 2000). The study proposal for data collection was sent to the University of Cape Town's Research Ethics Committee for approval (Reference 393/2009) (Appendices 5a and 5b). Informed consent (Appendix 4) was obtained from participants. The questionnaires were conducted in English and Afrikaans (Appendices 1a and 1b) and communication was in the language a participant felt most comfortable in. Confidentiality was preserved in that only the research team had access to the data and only group results were reported on.

3.1 Autonomy

The nature and aims of the study were explained to the participants and they were free to decide whether or not to participate in the study with no coercion. A written informed consent was obtained before participation. Participants were assured that they could withdraw from the study at any time without any consequences. Participants were also given contact details for more information about the study. Personal information was safeguarded and could only be disclosed, if necessary, with permission from the participants. They were assigned study numbers instead of using their names. In the analysis phase, confidentiality of data will continue to be maintained. Only the principal author and the supervisors will have access and only group results will be reported on.

3.2 Benefit

There were no financial incentives awarded to the participants at the start of the study, however, all stakeholders will benefit from the findings of this study. Advocacy groups will use the information for their advocacy work.. Civil society will be made aware of the effect of pesticide exposure on the development of asthma and allergies. The study participants will be informed of the study findings and to address potential intervention and strategies to reduce exposure to pesticides and allergens and subsequently reducing the risk of developing asthma and other allergies.

3.3 Harm

Apart from the minor discomfort of the needle prick to obtain a blood sample, no physical harm came to the participants. However participants consented and were free to withdraw from the study if they were uncomfortable with any of the procedures. Current analyses will not require participant involvement therefore there will be no additional harm to the participants.

3.4 Justice

The particular study site was selected amidst reports of respiratory problems among the women in the area. The findings will be distributed to all the stakeholders and will be made available online. A hard copy of a detailed report will be available at the University of Cape Town Medical School Library. This research will also be published in a journal for peer review.

4. Logistics

Time schedule

	2011												2012			
	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	
Data cleaning and organisation	■	■	■	■	■	■										
Protocol Development and departmental approval						■	■	■	■	■	■					
Data analysis										■	■	■	■	■	■	
Search for relevant literature						■	■	■	■	■	■	■	■	■	■	
Write article for selected journal											■	■	■	■	■	
Complete thesis write up and prepare to submit												■	■	■	■	

5. References

- Alarcon, W.A., Calvert, G.M., Blondell, J.M. et al. 2005. Acute illnesses associated with pesticide exposure at schools. *JAMA*. 294(4): 455-465.
- Alavanja, M.C.R. 2009. Pesticides use and exposure extensive worldwide. *Rev Environ Health*. 24(4): 303-309.
- Bowers, Y., London, L., Holtman, Z., Messina, S., Arendse, B., Lindoor, E., Rhodes, G. 2009. Obstacles to the rights of access to health care for farm worker women in the Western Cape. *Critical Health Perspectives*;
- Burney, P.G.J., Luczynska, P., Chinn, S. et al. 1994. The European Community Respiratory Health Survey. *Eur Respir J*. 7: 954-960.
- Chatzi, L., Alegakis, A., Tzanakis, N., Siafakas, N., Kogevinas, M., Lionis, C. 2007. Association of allergic rhinitis with pesticide use among grape farmers in Crete, Greece. *Occup Environ Med*. 64(6): 417-421.
- Dalvie, M.A., White, N., Raine, R., Myers, J.E., London, L., Thompson, M., Christiani, D.C. 1999. Long-term respiratory health effects of the herbicide, paraquat, among workers in the Western Cape. *Occup. Environ. Med*. 56(6):391-396.
- Dalvie, M.A., Myers, J.E., Thompson, M.L., Robins, T.G., Dyer, S. 2004a. The hormonal effects of long term DDT exposure on malaria vector control workers in Limpopo Province, South Africa. *Environmental Research*. 96(1):9-19.
- Dalvie, M.A., Africa, A., Solomons, A., London, L., Brouwer, D., Kromhout, H. 2009. Pesticide exposure and blood endosulfan levels after first season spray amongst farm workers in the Western Cape, South Africa. *J Environ Sci Health B*. 44(3):271-277.
- Dalvie, M.A., London, L. 2006. The impact of aerial application of organophosphates on the cholinesterase levels of rural residents in the Vaalharts district, Northern Cape Province, South Africa. *Environ Res*. 102(3):326-332.
- Dalvie, M.A., London, L. 2009. Risk assessment of pesticide residues in South African raw wheat. *Crop protection*. 28(10):864-869
- Dalvie, M.A., Cairncross, E., Solomon, A., London, L. 2003. Contamination of rural surface and ground water by endo-sulfan in farming areas of the Western Cape, South Africa. *Environ Health*. 2:1.
- Del Prado-Lu, J.L. 2007. Pesticide exposure, risk factors and health problems among cutflower farmers: a cross sectional study. *Journal of occupational medicine and toxicology*. 2(9).

- Dweik, R.A., Boggs, P.B., Erzurum, S.C., Irvin, C.G., Leigh, M.W., Lundberg, J.O., Olin, A.C., Plummer, A.L., Taylor, D.R. 2011. American Thoracic Society Documents: An Official ATS Clinical Practice Guideline: Interpretation of Exhaled Nitric Oxide Levels (FeNO) for Clinical Applications. *Am J Respir Crit Care Med.* 184:602-615.
- Ernst, P. Pesticide exposure and asthma. 2002. *American journal of respiratory and critical care medicine.* 165(5):563.
- Faria, N.M., Facchini, L.A., Fassa, A.G., Tomasi, E. 2005. Pesticides and respiratory symptoms among farmers. *Rev Saude Publica.* 39(6):973-981.
- Fieten, K.B., Kromhout, H., Heederik, D., Van Wendel de Joode B. 2009. Pesticide exposure and respiratory health of indigenous women in Costa Rica. *Am J Epidemiol.* 169(12):1500-1506.
- Forastieri, V. 1999. The ILO Programme on Occupational Safety and Health in Agriculture. SafeWork. International Labour Office, Geneva. URL: <http://www.ilo.org/public/english/protection/safework/agriculture/agrivf01.htm>
- Garcia, A.M. 2003. Pesticide exposure and women's health. *Am J Ind Med.* 44(6): 584-594.
- Hoppin, J.A., Umbach, D.M., London, S.J. et al. 2009. Pesticide use and adult-onset asthma among male farmers in the Agricultural Health Study. *Eur Respir J.* 34(6):1296-1303.
- Hoppin, J.A., Umbach, D.M., London, S.J., et al. 2008. Pesticides and atopic and nonatopic asthma among farm women in the Agricultural Health Study. *Am J Respir Crit Care Med.* 177(1):11-18.
- Hoppin, J.A., Umbach, D.M., London, S.J., Lynch, C.F., Alavanja, M.C., Sandler, D.P. 2006. Pesticides associated with wheeze among commercial pesticide applicators in the Agricultural Health Study. *Am J Epidemiol.* 163(12):1129-1137.
- Hoppin, J.A., Umbach, D.M., London, S.J., Alavanja, M.C., Sandler, D.P. 2002. Chemical predictors of wheeze among farmer pesticide applicators in the Agricultural Health Study. *Am J Respir Crit Care Med.* 165(5):683-689.
- Innes, D.F., Fuller, B.H., Berger, G.M. 1990. Low serum cholinesterase levels in rural workers exposed to organophosphate pesticide sprays. *S Afr Med J.* 78(10):581-583
- Jeebhay, M.F., Quirce, S. 2007. Occupational asthma in the developing and industrialised world: a review. *The international journal of tuberculosis and lung disease.* 11(2):122-133

- Jeebhay, M.F., Baatjies, R., Chang, Y.S., et al. 2007. Risk factors for allergy due to the two-spotted spider mite (*Tetranychus urticae*) among table grape farm workers. *Int Arch Allergy Immunol*. 144(2):143-149.
- Kesavachandran, C.N., Fareed, M., Pathak, M.K., Bihari, V., Mathur, N., Srivastava, A.K. 2009. Adverse health effects of pesticides in agrarian populations of developing countries. *Rev Environ Contam Toxicol*. 200: 33-52.
- London, L. 2003. Human Rights, Environmental Justice and the Health of Farm Workers in South Africa. *Int J Occup Environ Health*. 9:59-68 (Online) Available http://www.15by2015.org/wpcontent/uploads/2008/01/0901_london_south_africa.pdf
- London, L., de Grosbois, S., Wesseling, C., Kisting, S., Rother, H., Mergler, D. 2002. Pesticide usage and health consequences for women in developing countries: out of sight, out of mind? *Int J Occup Environ Health*. 8(1): 46-59.
- London, L. 1998. Occupational epidemiology in agriculture: A case study in the Southern African context. *Int J Environmental and Occupational Health*. 4:245-256
- Lemiere, C., D'Alpaos, V., Chaboillez, S. et al. 2010. Investigation of occupational asthma: sputum cell counts or exhaled nitric oxide? *Chest*. 137:617–622.
- McCoy, C.A., Carruth, A.K., Reed, D.B. 2002. Women in agriculture: risks for occupational injury within the context of gendered role. *J Agric Saf Health*. 8(1):37-50.
- Nweke, O.C., Sanders III, W.H. 2009. Modern environmental health hazards: a public health issue of increasing significance in Africa. *Environ Health Perspect*. 117(6):863-870
- Ntow, W.J. 2001. Organochlorine pesticides in water, sediment, crops, and human fluids in a farming community in Ghana. *Arch Environ Contam Toxicol*. 40(4):557-563
- Pekkanen, J., Sunyer J., Anto J.M., Burney, P. on behalf of the European Community Respiratory Health Study (ECRHS). 2005. Operational definitions of asthma in studies on its aetiology. *Eur Respir J*. 26: 28–35 DOI: 10.1183/09031936.05.00120104
- Quirce, S., Lemiere, C., de Blay F. et al. 2009. Noninvasive methods for assessment of airway inflammation in occupational settings. *Allergy*. 65: 445–458
- Rother, H.A. 2008. South African farm workers' interpretation of risk assessment data expressed as pictograms on pesticide labels. *Environ Res*. 108(3):419-27

- Senthilselvan, A., McDuffie, H.H., Dosman, J.A. 1992. Association of asthma with use of pesticides. Results of a cross-sectional survey of farmers. *Am Rev Respir Dis.* 146(4):884-887.
- Salameh, P., Waked, M., Baldi, I., Brochard, P., Saleh, B.A. 2006. Respiratory diseases and pesticide exposure: a case-control study in Lebanon. *J Epidemiol Community Health.* 60(3):256-261.
- Schulz, R. 2001a Comparison of spray drift-and runoff-related input of azinphos-methyl and endosulfan from fruit orchards into the Lourens River, South Africa. *Chemosphere* . 45(4-5):543-551
- Schulz, R. 2001b.Rainfall-induced sediment and pesticide input from orchards into the Lourens River, Western Cape, South Africa: importance of a single event. *Water Res* .35(8):1869-1876
- Slager, R.E., Simpson, S.L., Levan, T.D., Poole, J.A., Sandler, D.P., Hoppin, J.A. 2010. Rhinitis associated with pesticide use among private pesticide applicators in the agricultural health study. *J Toxicol Environ Health A.* 73(20): 1382-1393.
- Stellman, J.M. (ed). 1998. Encyclopaedia of occupational health and safety. 4th ed. ILO, Geneva.
- Sunyer, J., Pekkanen, J., Garcia-Esteban, R., Svanes, C., Anto J.M., Burney, P. et al. 2007. Asthma score: predictive ability and risk factors. *Allergy.* 62: 142–148
- Van den Driessche, K.S.J. 2010.Anaphylaxis in an airplane after insecticide spraying. *J Travel Med.* 17(6): 427
- Vizcaya, D.,Mirabelli, M.C., Anto´ JM., Orriols, R., Burgos, F., Arjona, L., Zock, JP. 2011. A workforce-based study of occupational exposures and asthma symptoms in cleaning workers. *Occup Environ Med.* 68:914-919. doi:10.1136/oem.2010.063271
- Zhang, L.X., Enarson, D.A., He, G.X., Li, B., Chan-Yeung M. 2002. Occupational and environmental risk factors for respiratory symptoms in rural Beijing, China. *Eur Respir J.* 20(6): 1525-1531.
- Zhang, X., Zhao, W., Jing, R. et al. 2011. Work-related pesticide poisoning among farmers in two villages of Southern China: a cross-sectional survey. *BMC Public Health.* 11: 429.

PART B: STRUCTURED LITERATURE REVIEW

University of Cape Town

1. Introduction

1.1 Background

Pesticides are used to control vector borne diseases and they are important in food production as they improve both quality and quantity of produce, but emergent risks may outweigh their benefits. Pesticides have become a global public health concern due to the plethora of health problems that studies have shown are associated with their use. There has been a rapid development of the agrochemical industry over the past few decades and this has made pesticides inexpensive and readily available. Organochlorines such as DDT (dichlorodiphenyltrichloroethane), though still widely used in Africa, have made way for the less environmentally persistent pesticides such as the cholinesterase inhibiting organophosphates and carbamates.

South Africa is the largest user of pesticides in Sub Saharan Africa and even though pesticide use is increasing every year, it is still largely unregulated. As a result, there is wide spread human exposure to pesticides and increasing concern on the chronic effects of low dose long term exposure (Davies, 1990). Current agrichemical registration procedures in South Africa continue to rely mostly on data on acute toxicity and do not take into account long-term health effects (London, 1995).

1.2 Objectives of the literature review

This review focused on the available evidence on the effect of chronic and in some cases acute exposure to pesticides (and other risk factors) in the development of airway diseases such as asthma and rhinitis. The objective was to identify high risk

groups, and to identify specific pesticides associated with rhinitis and asthma as well as the pathophysiological mechanisms involved. Methodological issues have been considered, particularly exposure assessment methods and more objective clinical endpoints, which will drive recommendations for future research

1.3 Search strategy

The electronic sources of information included PubMed/MEDLINE, EBSCO, Google Scholar and The Cochrane Library. Paper sources included text books, journals and previous theses through the University of Cape Town Medical Library. Key words for the search included pesticides, health effects, asthma, rhinitis, allergies, farm workers, farm residents, rural residents and cholinesterase. The search included all population groups exposed to pesticides, including men, women and children in farms, and the associated respiratory, both allergic and non- allergic health effects. Data from all study designs and countries were considered.

2. High risk populations and target groups exposed to pesticides

The populations at risk of developing adverse health effects associated with pesticide exposure include workers such as farm workers, workers in the pesticide production industry, pest control workers as well as individuals exposed environmentally such as farm residents and those using household pesticides (Jaga et al., 2003).

Pesticide exposure in the agricultural sector is important since about 1.8 billion people in the world are involved in agriculture and most are exposed to pesticides (Alavanja,

2009). Agricultural workers, especially applicators and handlers have therefore been the study population in most studies investigating health effects of pesticides.

Non-occupational or environmental exposure to pesticides potentially affects a large segment of the general population. Residential exposures are associated with pesticide use by exterminators and from dietary and accidental exposures. Other environmental exposures occur in public places and areas close to farms (Jaga et al., 2003). For example, airline passengers are exposed to in-flight pesticide spraying when commuting between malaria endemic areas (Vanden Driessche et al., 2010). Exposure to pesticides can also occur in the school environment especially in rural children and educators, from nearby farms and pesticide manufacturing plants. Acute symptoms have been reported from schools that were associated with exposure to pesticide drift from neighboring farms (Alarcon et al., 2005). Children are a particular high risk group due to their exploratory behaviour resulting in accidental contact with household pesticides (Badakhsh et al., 2010). The risk would be even higher for children living on farms due to drift, playing with empty pesticide containers or exposure to pesticide contaminated clothing from parents or guardians working on farms (Curl et al., 2002). Living in an agricultural region, has also been associated with pesticide exposure in that increased levels of dialkylphosphate (DAP) metabolites in urine were found in children despite them not living close to a farm nor having parents working on a farm (Koch et al., 2002).

Studies have mostly focused on men but it has become increasingly apparent that women are also a high risk group (London et al., 2002; Zhang et al., 2002; Garcia

,2003; Faria et al., 2005; Hoppin et al., 2008; Fietsen et al., 2009). Work related pesticide poisoning in women was found to be more common than in men in a Chinese study of pesticide applicators (Zhang et al., 2011). It has also been reported in many countries, including Italy and USA, that children under the age of 5 have more pesticide exposure related hospitalisations than any other age group (Olson, 1991; Garry, 2004). Children may have higher pesticide exposure than adults in the same exposure environment due to their higher respiratory rate, proportionately larger skin surface area and higher metabolic rate (Garry, 2004). Additionally, pesticide exposure can occur *in utero* since a number of pesticides can pass through the placenta and to infants through breast milk (Berkowitz et al., 2004; Salam et al 2004; Sunyer et al 2005). There is also evidence that pesticide exposure can affect the genes of exposed parents that can, in turn, affect new offspring and subsequent generations (McCauley et al., 2006).

Populations in African and other developing countries are also at high risk of pesticide exposure since the use of pesticides in many African countries including South Africa is still largely unregulated (Nweke and Sanders, 2009).. In South Africa for instance, pesticides have frequently been detected in ground water and surface water including drinking water sources and sediments located near farms in the Western Cape (Dalvie et al., 2003; Schulz, 2001a, 2001b). Additionally high pesticide levels have been measured amongst rural residents in the Western Cape (Dalvie et al., 2009; Dalvie et al., 2011). Pesticide residues have also been detected in wheat samples used in the production of South African wheat products (Dalvie et al., 2009). The illegal street

selling of pesticides, as has been reported in the City of Cape Town for domestic use leading to child poisonings is an added consideration (Rother, 2010)

3. Epidemiological studies on respiratory health effects associated with pesticides

Pesticide exposure has been associated with asthma and rhinitis in various epidemiology studies (Vanden Driessche et al., 2010 ; Alarcon et al., 2005; Zhang et al., 2002; Faria et al., 2005; Hoppin et al., 2008; Fieten et al., 2009; Slager et al., 2009). Prevalence of diagnosed asthma ranged from 2.2% to 4.3%, wheeze from 9% to 36%, other asthma symptoms from 3.4% to 55% and rhinitis ranged from 31% to as high as 74%. Most of the studies were conducted beyond Africa and mainly among male farm workers.

There were 31 epidemiological studies identified (Appendix 6) that looked at the respiratory effects of pesticide exposure and all, except for 2 (Abu Sham'a et al., 2010; Swaen et al., 2008), showed a positive association between pesticide exposure and respiratory and allergy problems. There were 22 cross sectional, 5 case control, 3 cohort, and 1 hybrid design combining cross sectional and follow up design features. Only a few studies were longitudinal therefore there is limited information on disease development.

3.1 Respiratory health measurement

In 20 of the studies, the outcomes studied were self-reported doctor diagnosed asthma or asthmatic symptoms (Appendix 6). Respiratory disease epidemiology has made great strides using standardised questionnaires and for some outcomes such as

wheeze, these are the only option. In most epidemiology studies, standardised questionnaires such as the European Community Respiratory Health Study (ECRHS) questionnaire, the American Thoracic Society questionnaire, British Medical Research Council Questionnaire and the International Union Against Tuberculosis and Lung Disease Questionnaire were used to ask participants a series of asthma related symptom questions. There are, however, large differences in the way the symptom questions have been reported in the studies assessing respiratory effects of pesticides making comparisons across studies a challenge. Many published articles have only reported results based on individual symptoms. Some have only reported symptom combinations, and some have included both. However, these definitions of asthma may not be ideal for studies on risk factors of asthma because the definitions are dichotomous outcomes (Pekkanen et al., 2005). It is unclear whether asthma is a truly dichotomous disease because current knowledge does not completely rule out the possibility that it might exist as a continuum. Pekkanen et al (2005) therefore conclude by proposing the use of a continuous asthma score when analysing symptoms of asthma in epidemiological studies.

.The limitations for self-reported outcomes are that they are subjective and recall bias is always a possibility. For studies investigating asthma, objective measures such as spirometry can be used in conjunction with questionnaires. While there are no objective measures for wheeze and other respiratory symptoms, the key issue is to ensure that participants understand the definitions during self report.

More objective measures for lung disease including spirometry tests, lung volumes, gas diffusion transfer, were reported in only 10 studies (Salameh et.al., 2006; Chakraborty et al., 2009; Fieten et al.,2009; Abu Sham'a et.al.,2010; Beseler et al., 2009; Swaen et al., 2008; Chatzi et al., 2007; Henandez et al., 2008; Mekonnen et al., Mekonnen et.al., 2002).Non-invasive methods for the evaluation of one of the key characteristics of asthma, airway inflammation, have been increasingly proposed for the investigation of work-related asthma and rhinitis. Fractional exhaled NO (FeNO) measurement is one such method. FeNO is a surrogate marker of eosinophilic airway inflammation (Quirce et al., 2009). Assessment of FeNO is an easier and less time-consuming technique and is ideal in study settings. However, elevated FeNO is not specific for asthma and eosinophilic inflammation because it has been found in other diseases and several conditions may influence exhaled NO (ATS/ERS, 2005).In this review, no studies were found that used FeNO measurement in the assessment of respiratory effects of pesticide exposure because this method has only recently been available for use in field studies.

3.2 Pesticide exposure assessment

Another limitation of the studies is that in a large proportion (65%) of studies, exposure assessment was based on self-reported exposures which were not detailed and merely classifying participants in exposed and unexposed groups. Self-reported exposure may not account for many factors that affect pesticide exposure. These factors include weather conditions at the time of application, such as atmospheric temperature and humidity which may affect the volatility of the product, the perspiration rate of the human body and the use of personal protective equipment by

the workers (Dalmatas et al., 2011). Pesticides enter the bloodstream through skin absorption, inhalation or ingestion. A current review of toxicological studies that includes both human and animal data, describes that the degree of skin absorption depends on the contact time with the skin and which region of the skin is affected for example parathion is absorbed faster in scrotal, head and neck skin (Kumar et al., 2010). Other chemicals that are mixed with the active compound, for example solvents and emulsifiers, can facilitate faster absorption in the skin (Kumar et al., 2010). Inhalation of pesticides may be particularly hazardous because pesticide particles can be rapidly absorbed by the lungs into the bloodstream (Schulze et al., 2001). Another factor that could affect self-reported exposure is poor recall of exposure by participants that could lead to inaccurate exposure estimation. Data on self reported pesticide use are generally good for farmers but not farm workers. Poor recall of exposure could lead to either measurement error and or bias. Biological monitoring can provide an accurate measure of the absorbed dose of pesticide although this applies only to exposure in the last three months for virtually all contemporary pesticides as they are non-persistent. Measurements of the biomarker can then be used to validate the self-reports. Biological monitoring in the form of cholinesterase testing, a marker of organophosphate and carbamate exposure, was used in 5 studies (Ohayo-Mitoko, 2000; Chakraborty et al., 2009; Henandez et al., 2008; Abu Mourad et al., 2005, Jeebhay et al., 2007) , and measurement of ethylenethiourea (ETU), a marker of dithiocarbamate exposure, was used in 2 studies (Boers et al., 2008; Swaen et al., 2008) . Interpretation of ChE monitoring results is complicated by variation in enzymatic activity within an individual or between individuals and the presence of other confounding factors such as liver

disease. Exposure to large doses of organophosphate pesticides is required for significant acetylcholinesterase inhibition to occur, and therefore, it is more appropriately used as an indicator of toxicity at high rather than low pesticide exposure levels (McCauley et al 2006). This means that this method could possibly underestimate the number of individuals exposed to pesticides if the exposure levels are not high enough. It is ideal to monitor the change in a person's cholinesterase levels during the non-spraying and spraying season. Bio-monitoring for specific pesticides is a more sensitive method for measuring pesticide exposure, It is evident that more indices of exposure using bio-markers or early biological effect markers are needed in order to minimise exposure misclassification.

3.3 Specific pesticides associated with respiratory health problems

Few studies have investigated specific pesticides associated with respiratory problems. The specific pesticides that have been found to be associated with respiratory effects in humans are listed in Table 1. These include at least 9 different organophosphates , 2 organochlorines,3 carbamates, a pyrethroid, a macrocyclic lactone and a neonicotinoid insectide; a benzimidazole, 2 dithiocarbamates and a phthalimide fungicide; 2 triazines, a bipyridyl, a sulfonyleurea, a bipyridilium, a chlorphenoxy acid, a glysine and a petroleum herbicide; 3 halogenated organic and an inorganic fumigant and an acyldehyde molluscicide. There were 32 specific active ingredients identified that were associated with respiratory effects

Organophosphates and carbamates insecticides feature prominently in the available literature on specific types of pesticides associated with asthma, allergy and other

respiratory problems. For instance in Costa Rica, Fieten et al. (2009) looked at reported exposures to specific pesticides and found that the organophosphates chlorpyrifos and terbufos were strongly associated with wheeze (OR= 6.7, 95% CI: 1.6, 28.0 and OR = 5.9, 95% CI: 1.4, 25.6, respectively) when comparing women farm workers in plantain plantations where pesticides were sprayed (exposed) to those who were working in organic banana plantations (unexposed). In other studies wheeze was associated with the herbicides atrazine and alachlor (Hoppin et al., 2002) and chlorimuron-ethyl, a herbicide, and phorate an organophosphate insecticide (Hoppin et al., 2006). Pesticides also appear to have an effect on the upper respiratory tract. Rhinitis was associated with the herbicides paraquat, glyphosate, petroleum oil, carbaryl a carbamate insecticide and organophosphate insecticides namely diazinon and malathion (Slager et al., 2009; Chatzi et al., 2007; Slager et al., 2010). The organochlorine insecticide DDT was associated with adult onset non-allergic asthma whereas heptachlor (another organochlorine) was associated with adult onset allergic asthma along with halogenated organic fumigants and organophosphate insecticides (Hoppin et al., 2009). Fungicides (phthalimide, dithiocarbamate and benzimidazole) were associated with rhinitis in three studies (Slager et al., 2009; Chatzi et al., 2007; Slager et al., 2010).

Table I: Specific Pesticides associated with upper and lower airway respiratory outcomes

Author, year	Pesticide			Outcome
	Type	Chemical Group	Active ingredients	
Slager et al 2010	Herbicide	Glycine Petroleum	Glyphosate Petroleum oil	Rhinitis
	Insecticide	Organophosphate Carbamates Pyrethroid	Chlorpyrifos, diazinon, dichlorvos, malathion Carbaryl Permethrin	
	Fungicide	Phthalimide	Captan	
Fieten et al 2009	Insecticide	Organophosphate	Chlorpyrifos, terbufos	Wheeze
Hoppin et al 2009	Insecticide	Organophosphates Organochlorines	Coumaphos, parathion Heptachlor	Adult onset allergic (doctor-diagnosed asthma after the age of 19 yrs with history of doctor diagnosed eczema or hay fever)
	Fumigant	halogenated organic inorganic halogenated organic	Carbon tetrachloride carbon disulfide, ethylene dibromide (80/20 mix)	
Hoppin et al 2009	Insecticide	Organochlorine	DDT (dichloro-diphenyltrichloroethane)	Adult onset non-allergic asthma (doctor-diagnosed asthma after the age of 19 yrs without history of doctor diagnosed eczema or hay fever)
Slager et al 2009	Herbicides	Chlorphenoxy acid Glycine Petroleum	2,4 D Glyphosate Petroleum oil	Rhinitis
	Insecticide	Organophosphate Benzimidazole	Diazinon	
	Fungicide		Benomyl	
Hernandez et al 2008	Herbicide	Bipyridilium	NS	FEV ₁ (Forced expiratory volume in the first second)
	Insecticide	Neonicotinoid	NS	
Chatzi et al 2007	Herbicide	Bipyridyl	Paraquat	Rhinitis
	Fungicide	Dithiocarbamate	NS	
	Insecticide	Carbamates	NS	
Del Prado-Lu 2007	Molluscicide	Acetaldehyde polymer	Metaldehyde	Cough
	Insecticide	Carbamate Organophosphate Macrocyclic Lactone	Methiocarb Profenofos Avermectin	
	Fungicide	Dithiocarbamate	Mancozeb	
Hoppin et al 2006	Herbicide	Sulfonylurea	Chlorimuron-ethyl	Wheeze
	Insecticide	Organophosphate	Phorate	
Abu Mourad 2005	Insecticide	Organophosphate	NS	Chest symptoms including (cold symptoms, dyspnoea, chest pain)
Hoppin et al 2002	Herbicide	Triazine	Atrazine, alachlor	Wheeze
Senthilselvan et al 1992	Insecticide	Carbamate	NS	Self reported asthma
Innes et al 1990	Insecticide	Organophosphate	NS	Nursing sister diagnosed asthma and chronic bronchitis

NS: Not specified

3.4 Studies on women

To date, there have been very few epidemiological studies investigating asthma and other respiratory symptoms associated with pesticides among women or those that

include women in the study sample (Zhang et al., 2002; Faria et al., 2005; Salameh et al., 2006; Hoppin et al., 2008; Fietsen et al., 2009; Beseler et al., 2009). The studies that included women are listed in Table 2 below.

Table 2 Summary of epidemiological studies of women or including women in the investigation of the effect of pesticides on respiratory health

Author, year	Population (n), Design, country, City	Exposure Assessment	Outcome measurements	Summary of findings
Fietsen et al., 2009	Cross-sectional study of plantation worker women in Costa Rica, n =127	Questionnaire used to estimate exposure, Exposed women, n=69 Unexposed women, n=58	Questionnaire used to estimate respiratory symptoms. Spirometry tests to measure FVC, FEV	Among the exposed, prevalence of wheeze was 20% and of shortness of breath was 36% versus 9% and 26%, respectively, for the unexposed. Among nonsmokers, reported exposures to chlorpyrifos (n = 25) and terbufos (n =38) were strongly associated with wheeze (odds ratio =6.7, 95% CI: 1.6, 28.0; odds ratio = 5.9, 95% CI: 1.4, 25.6, respectively)
Hoppin et al., 2008	Case control study of 25 814 farm women (> 20years) from Agricultural Health Study (USA) including 282 with atopic asthma and 420 with non-atopic asthma	Personal use of 50 specific pesticides (years of pesticide use, frequency of application.)	Self-reported history of doctor-diagnosed asthma with or without eczema and/or hay fever	Growing up on a farm was protective for atopic asthma (OR, 0.55; 95% CI, 0.43–0.70) and, to a lesser extent, for nonatopic asthma (OR, 0.83; 95% CI, 0.68–1.02; Pesticide use was almost exclusively associated with atopic asthma. (OR, 1.46; 95% CI, 1.14–1.87).
Hoppin et al., 2006	Cross sectional study of Iowa male commercial pesticide applicators enrolled in the Agricultural Health Study, n=2255, aged 17-83	Assessed exposure to 40 different pesticides using questionnaire	Outcome of interest was wheeze in the past year based on the participant's response in questionnaire	Prevalence of wheeze 21%. Association between wheeze and: Herbicide, chlorimuron-ethyl (OR= 1.62, 95% CI): 1.25, 2.09). Phorate, an OP insecticide, (OR=2.87, 95% CI: 1.70, 4.84).
Salameh et al., 2006	Labanese case control study of asthmatic and non-asthmatic patients from several Lebanese hospitals, n=407	Questionnaire	Questionnaire adapted from American Thoracic Society (doctor-diagnosed asthma)	Any exposure to pesticides was associated to asthma (OR = 2.11 (1.47 to 3.02). Occupational use presented the highest association (OR = 4.98 (1.07 to 23.28); followed by regional exposure (OR 3.51 (2.11 to 5.85). Though there were more males in cases than controls there was no direct comparison of pesticide exposure by gender
Faria et al., 2005	A cross-sectional study of 1,379 farmers from two municipalities of Southern Brazil. subjects aged 15 and older	Self-reported individual exposure to pesticides using questionnaire	An adapted questionnaire developed by the American Thoracic Society used to assess respiratory symptoms	The prevalence of asthma symptoms was 12% and chronic respiratory disease symptoms was 22%. Higher odds ratios for both asthma (OR=1.51; 95% CI: 1.07-2.14) and chronic respiratory disease (OR=1.34; 95% CI 1.00-1.81) symptoms were found in women compared to men. Pesticide poisoning associated with higher prevalence of asthma symptoms (OR=1.54; 95% CI: 1.04-2.58) and chronic respiratory disease symptoms (OR=1.57; 95% CI: 1.08-2.28)
Hoppin et al., 2002	Cross sectional study of 20 468 applicators, aged 16 to 88 years from Agricultural Health Study (USA)	Self-administered questionnaires on 40 currently used pesticides and pesticide application practices	Self reported wheeze	19% reported wheezing in the past year. The herbicides, atrazine and alachlor were associated with wheeze
Zhang et al., 2002	Cross-sectional study of 30 villages, 2 rural counties, n = 22,528 male and female residents aged 15 and older, Beijing, China	Questionnaire on occupational and environmental exposures. Exposure measured by answering yes/no to question	International Union Against Tuberculosis and Lung Disease Questionnaire on Bronchial Symptoms	Exposure to insecticides and other chemicals associated with higher prevalence of respiratory symptoms than unexposed. On regular exposure to insecticides females showed significantly higher risks of chronic cough and chronic phlegm than men

Gender based analysis among those studies that do include men and women, was not done in most of them. Women and their children may also be exposed to pesticides indirectly through spray drift into homes on the farm, washing of contaminated overalls at home, contamination of rural water sources and reuse of pesticide containers (London, 1994, 1998; Dalvie et al., 2003, 2004). Women may be at risk of developing respiratory problems on exposure to pesticides even more so than men as illustrated by the two studies that reported respiratory symptoms or doctor diagnosed asthma were more prevalent and strongly associated with pesticide exposure in women than in men (Zhang et al., 2002; Faria et al., 2005). This is, however, still speculative at best as there are currently no longitudinal studies investigating gender differences in populations exposed to pesticides.

3.5 Allergic and non-allergic asthma

The data are strong that farmers are at lower risk of allergic asthma (Portengen et al., 2002). In a study in the USA, however, it was found that pesticide exposure was associated with both adult onset atopic and non-atopic asthma (Hoppin et al., 2009).

Farm workers are also exposed to various allergens of plants or animals or chemicals including pesticides. The spider mite, *Tetranychus urticae*, has been reported as an important outdoor allergen responsible for allergic symptoms such as asthma among table grape farm workers in South Africa. It has been suggested that increased risk of sensitization to spider mite may be related to pesticide crop spraying in table grape farm orchards (Jeebhay et al., 2007). Hoppin et al. (2008) also found that pesticides were associated with atopic asthma but that having grown up in a farm had a

protective effect on asthma in women farm workers. This suggests that pesticides may have a more important role in allergic asthma than originally thought. Most pesticides used especially parathion and malathion were associated with atopic asthma and only permethrin was associated with both allergic and non-allergic asthma. Women who grew up on farms but did not apply pesticides had the lowest overall risk of atopic asthma compared with women who neither grew up on farms nor applied pesticides (Hoppin et al., 2008).

3.6 Studies on rhinitis

Rhinitis in some epidemiological studies has been defined as the presence of stuffy, itchy, or runny nose in the last 12 months (Slager et al., 2009, 2010). Chatzi et al. (2007) defines allergic rhinitis as the occurrence of two or more nasal symptoms (eg, rhinorrhoea, sneezing, nasal obstruction and nasal itching) during the last 12 months, apart from a cold. There are very few studies that have looked at the association between rhinitis and pesticides. Rhinitis precedes or coexists in asthma and is therefore a part of the asthmatic phenotype and as such just another manifestation of the disease induced by an adverse environment (Guerra et al., 2002). Grape farmers exposed to pesticides are reported to have a higher prevalence of allergic rhinitis symptoms compared to controls. The highest risks were observed for paraquat and other bipyridyls and carbamates (Chatzi et al., 2007). Data from the Agricultural Health Study in North America reported that 74% of commercial pesticide applicators reported at least one episode of rhinitis in the past year. The pesticides strongly associated with current rhinitis were 2,4-D, glyphosate, petroleum oil, diazinon and benomyl (Slager et al., 2009). A similar study conducted in another cohort of

applicators reported that 67% of farmers reported current rhinitis and 39% reported three or more rhinitis episodes (Slager et al., 2010). In this study glyphosate, petroleum oil, organophosphates (chlorpyrifos, diazinon, dichlorvos, malathion), carbaryl and permethrin were predictors of current rhinitis (Slager et al., 2010). Rhinitis has also been reported in greenhouse flower growers and is associated with sensitization to workplace allergens and pesticide application using hand pumps (Riu et al., 2008). It is important to note that the definition of rhinitis varies across studies and this could be the reason for the differences in prevalences.

3.7 Studies on children

Children are particularly vulnerable to the effects of pesticides. In a Lebanese study on school children, a prevalence of 12.4% of chronic respiratory disease associated with exposure to pesticides was found (Salameh et al., 2003). In the USA there was a strong association between pesticide exposure and children diagnosed with asthma by age 5, particularly with exposure to herbicides (Salam et al., 2004).

3.8 Studies in Africa

Despite the increased use and exposure to pesticides, very few studies in Africa have been conducted to assess the association with respiratory symptoms, even though pesticides have been documented as a common problem in developing countries (Jeebhay and Quirce, 2007). Innes et al. (1990) reported that 17,5% of male rural workers engaged in crop spraying reported chronic organophosphate poisoning, asthma and chronic bronchitis in Cape Town. No association between long term exposure to paraquat and reported symptoms and lung function was found but a

decrease in exercise oximetry was found in a study done in the rural Western Cape (Dalvie et al., 1999). In Kenya, a high prevalence of respiratory symptoms was found for workers with more than 30% cholinesterase inhibition (Ohayo-Mitoko et al., 2000) and in Ethiopia, the 15-24 year age group of pesticide sprayers had a significantly reduced forced expiratory vital capacity (FVC) and forced expiratory volume in one second (FEV1), compared to age matched controls (Mekonnen et al., 2004) . A more recent study in South Africa, demonstrated that the spider mite, *T. urticae*, is an important outdoor allergen among table grape farm workers and the increased risk of spider mite allergy appeared to be related to high pesticide exposure among crop sprayers (Jeebhay et al., 2007).

4. Pathophysiological mechanisms

Animal experiments have been used to assess whether the association between pesticides and asthma is biologically plausible. In acute poisonings, the clinical effects are well known. In the case of chronic, low level exposure, biological plausibility is confirmed by multiple experimental toxicological studies on animals.(Salameh et al 2006). Over recent years, animal studies have demonstrated some mechanisms that could explain the apparent association between pesticides and asthma and other respiratory symptoms.

A recent review concluded that pesticides can cause asthma either by acting as chemical irritants and thereby causing bronchio-constriction through neurogenic inflammation or as a result of inhibiting nicotinic and muscarinic receptors in the central nervous system (Hernandez et al., 2011). Exposure to organophosphate

pesticides, which inhibit cholinesterase enzymes, can induce effects on the smooth muscles of the respiratory tract that results in bronchoconstriction, increased secretory glands activity and pulmonary oedema. The immediate cause of death in acute organophosphate poisonings is asphyxia due to the muscarinic effects of the pesticide resulting in bronchoconstriction and increased bronchial secretions as well as nicotinic effects leading to paralysis of the respiratory muscles and depression of the respiratory centre (Temple and Smith, 1989). In the case of chronic, low level exposure, biological plausibility that pesticides act via similar mechanisms at a much lower level has also been shown by a number of experimental toxicological studies on animals (Salameh et al., 2006).

In earlier studies, it was postulated that acetylcholinesterase inhibition could directly explain the observed respiratory disease in farmers using organophosphate and carbamates insecticides (Senthilselvan et al., 1992). However, in an animal study in which airway hyper-reactivity was measured in guinea pigs exposed to chlorpyrifos, it was demonstrated that the organophosphate insecticide can cause airway hyper-reactivity by decreasing neuronal M2 muscarinic receptor function at doses below those causing acetylcholinesterase inhibition (Fryer et al., 2004). The same results were found with parathion and diazinon (Lein and Fryer, 2004).

It has also been shown that pre-existing allergic sensitisation in guinea pigs decreases the threshold dose of parathion required to cause airway hyper-reactivity suggesting that even lower levels of pesticide exposure can induce a reaction in those already sensitised to common allergens (Proskocil et al., 2008). In this study, it was shown that

sensitisation changed the muscarinic and nicotinic mechanism of parathion-induced airway bronchial hyperresponsiveness to one that acted via neurogenic inflammation that was dependent on interleukin-5 (IL-5) that resulted in eosinophil recruitment and activation (Proskocil et al., 2008).

It has also been found that pesticides may modify inflammatory responses to other farm exposures, such as allergens. Carbaryl, a commonly used carbamate insecticide, may enhance the allergic response to house dust mites (Dong et al., 1998). In another study, mice treated with malathion showed increased inflammatory mediators, which resulted in increased macrophage function (Rodgers et al., 1997). These results suggest that organophosphate and carbamate insecticides may be interacting with inflammatory pathways involved in asthma. Inhaled pesticides may cause damage to the airways either directly or through the activation of TRPV1 and TRPA1 (Transient Receptor Potential ion channels) in bronchial C-sensory fibers, inflammatory cells and epithelial cells. This triggers neurogenic inflammation which when sustained over time may lead to nonspecific bronchial hyper-reactivity and the subsequent development of asthmatic symptoms (Veronesi et al., 2001; Caceres et al 2009)). This is an important mechanism underlying irritant induced asthma caused by pesticides.

It has increasingly been demonstrated over the last decade that pesticides such as zineb, alachlor, nitrofen carbaryl do not only have direct irritant effects but also act as endocrine disruptors (ED), directly affecting the immune system leading to the development of asthma and allergy (Chalubinski et al., 2006). In the human body, there are two types of T helper cells (Th) called Th1 and Th2 that are the main

producers of cytokines. Th1 cells produce mainly interferon gamma that produces inflammatory responses and Th2 cells produce mainly interleukins 4, 5, 10 and 13 that promote IgE and eosinophilic responses (Berger, 2000). Therefore a normal response to an immune challenge requires an optimum balance between Th1 and Th2 responses. EDs can influence synthesis of cytokines, immunoglobulins, and cell mediators as well as immune cell activation and survival. Modulation by EDs of interleukin-4 and IgE production, tipping the balance between Th1 and Th2 in favour of Th2, enable them to have a potential effect on allergic immune responses (Chalubinski et al., 2006).

5. Risk factors for rhinitis and asthma associated with pesticides

Various studies in this review suggest that pesticide mixers, sprayers or applicators are at increased risk of developing rhinitis and or asthma associated with pesticide exposure (Zhang et al., 2002; Faria et al., 2005; Hoppin et al., 2008; Fieten et al., 2009; Slager et al., 2009; Chatzi et al., 2007; Hernandez et al., 2008; Innes et al., 1990; Abu Mourad et al., 2005; Abu Sham'a et al., 2010; Swaen et al., 2008; Mekonnen and Agonafir, 2004).

Animal studies show findings that may suggest that atopic individuals are more vulnerable to pesticides at lower doses than their non-atopic counterparts (Proskocil et al., 2008). Other important host factors for pesticide associated asthma include age and gender.

Young children are likely to be at higher risk for developing respiratory symptoms than other age groups due to the high level of exposure to pesticides in domestic and environmental settings (Olson, 1991; Garry, 2004; Badakhsh et al., 2010). Herbicide and pesticide exposure was strongly associated with asthma diagnosis before the age of 5 compared to other age groups in the Children's Health Study of California (Salam et al., 2004). This may be due to the fact that the respiratory, immune and nervous systems in children are still in the developmental phase and hence more vulnerable to the effect of pesticides and herbicides (Salam et al., 2004).

Male sex is a known risk factor for asthma in children below the age of 14, though the reasons are unknown (Horwood et al., 1985). Urine levels of dialkylphosphate (DAP) metabolites that are common to the OP pesticides were also found to be higher in male children than in female children (Koch et al., 2002). Furthermore, there was a slightly higher prevalence of asthma in male children than in female children in Iraq where pesticides and herbicides were some of the exposures being investigated (Alsamarai et al., 2009). This suggests a higher risk of developing respiratory symptoms on exposure to pesticides in boys than in girls. These risk factors in children need further investigation. In contrast to studies in children, two studies have reported that women exposed to pesticides appear to have a higher risk of developing asthma than men (Zhang et al., 2002; Faria et al., 2005). However, it is uncertain whether this is related to the gendered distribution of work or due to other as yet unexplored biological factors.

Regarding environmental exposures, the presence of other aeroallergens such as mites is an important risk factor especially for the development of allergic asthma due to sensitisation to outdoor mites (Spieksma, 1991; Jeebhay et al., 2007). Findings from animal studies suggest that sensitisation to allergens also renders individuals more vulnerable to developing respiratory health effects due to pesticides (Proskocil et al., 2008)..

6. Future research

Future studies should focus on developing more measures of exposure such as personal exposures monitoring using biomarkers. Specific pesticides should be identified for more detailed study so as to determine possible threshold levels for health effects. Objective measures for respiratory outcomes should be used more frequently in studies in order to reduce disease misclassification thereby improving the validity of the study. These measures should ideally be non-invasive in order to increase the number of participants who consent to having the tests done. Furthermore, future studies should also focus on understanding the pathophysiological mechanisms that underlie asthma and rhinitis so as to characterise the specific asthma phenotypes using more objective measures for asthma outcomes. Most of the epidemiological studies reported are cross sectional in nature and therefore have their inherent limitations. Other study designs such as cohort studies that have the potential to study outcomes associated with various different pesticide types should be considered. There is need also to expand the population base of study to include more women and children and populations from developing countries including Africa needs further consideration.

7. References

- Abu Mourad, T. 2005. Adverse impact of insecticides on the health of Palestinian farm workers in the Gaza Strip: a hematologic biomarker study. *Int J Occup Environ Health*. 11(2):144-149.
- Abu Sham'a, F., Skogstad, M., Nijem, K., Bjertness, E., Kristensen, P. 2010. Lung function and respiratory symptoms in male Palestinian farmers. *Arch Environ Occup Health*. 65(4):191-200.
- Alarcon, W.A., Calvert, G.M., Blondell, J.M. et al. 2005. Acute illnesses associated with pesticide exposure at schools. *JAMA* . 294(4):455-65
- Alavanja, MCR. 2009. Pesticides Use and Exposure Extensive Worldwide. *Reviews on Environmental Health*. 24(4): 303–309.
- Alsamarai, A.M., Salih, M.A., Alobaidy, A.H., Alwan, A.M., Abdulaziz, Z.H. 2009. Risk factors for asthma in Iraqi children. *Journal of rural and tropical public health*. 8:45-52
- ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005;171:912–930.
- ATS/ERS, 2005. Standardisation of Lung Function Testing. Standardisation of Spirometry. *Eur Respir J*. 26:319–338.
- Badakhsh, R., Lackovic, M., Ratard, R. 2010. Characteristics of Pesticide-Related Hospitalizations, Louisiana, 1998-2007. *Public Health Rep*. 125(3):457-467.
- Berger, A. 2000. Science commentary: Th1 and Th2 responses: what are they? *BMJ*. 321:424
- Berkowitz, G.S., Wetmur, J.G., Birman-Deych, E. 2004. In Utero Pesticide Exposure, Maternal Paraoxonase Activity, and Head Circumference. *Environmental Health Perspectives*. 112(3):388-391
- Boers, D., van Amelsvoort, L., Colosio, C. et al. 2008. Asthmatic symptoms after exposure to ethylenebisdithiocarbamates and other pesticides in the Europit field studies. *Hum Exp Toxicol*. 27(9):721-727
- Caceres AI, Brackmann M, Elia MD, et al. 2009. A sensory neuronal ion channel essential for airway inflammation and hyperreactivity in asthma. *Proc Natl Acad Sci USA*. 106:9099–9104.

- Chakraborty, S., Mukherjee, S., Roychoudhury, S., Siddique, S., Lahiri, T., Ray, M.R. 2009. Chronic exposures to cholinesterase-inhibiting pesticides adversely affect respiratory health of agricultural workers in India. *J Occup Health*. 51(6):488-497.
- Chalubinski, M., Kowalski, M.L. 2006. Endocrine disruptors – potential modulators of the immune system and allergic response. *Allergy*. 61: 1326–1335.
- Chatzi, L., Alegakis, A., Tzanakis, N., Siafakas, N., Kogevinas, M., Lionis, C. 2007. Association of allergic rhinitis with pesticide use among grape farmers in Crete, Greece. *Occup Environ Med*. 64(6):417-421.
- Curl, C.L., Fenske, R.A., Kissel, J.C. et al. 2002. Evaluation of take-home organophosphorus pesticide exposure among agricultural workers and their children. *Environ Health Perspect*. 110(12):787-92.
- Damalas, C.A., Ilias, G., Eleftherohorinos, G.I. 2011. Pesticide Exposure, Safety Issues, and Risk Assessment Indicators. *Int. J. Environ. Res. Public Health*. 8: 1402-1419; doi:10.3390/ijerph8051402
- Dalvie, M.A., Cairncross, E., Solomon, A., London, L. 2003. Contamination of rural surface and ground water by endo-sulfan in farming areas of the Western Cape, South Africa. *Environ Health*. 2:1.
- Dalvie, M.A., Africa, A., Solomons, A., London, L., Brouwer, D., Kromhout, H. 2009. Pesticide exposure and blood endosulfan levels after first season spray amongst farm workers in the Western Cape, South Africa. *J Environ Sci Health*. 44(3):271-277.
- Dalvie, M.A., London, L. 2006. The impact of aerial application of organophosphates on the cholinesterase levels of rural residents in the Vaalharts district, Northern Cape Province, South Africa. *Environ Res*. 102(3):326-332.
- Dalvie, M.A., London, L. 2009. Risk assessment of pesticide residues in South African raw wheat. *Crop protection*. 28(10):864-869
- Dalvie, M.A., White, N., Raine, R. et al. 1999. Long-term respiratory health effects of the herbicide, paraquat, among workers in the Western Cape. *Occup Environ Med*. 56(6):391-396.
- Davies, J.E. 1990. Neurotoxic Concerns of Human Pesticide Exposure. *American Journal of Industrial Medicine*. 18:327-331.
- Del Prado-Lu, J.L. 2007. Pesticide exposure, risk factors and health problems among cutflower farmers: a cross sectional study. *Journal of occupational medicine and toxicology*. 2(9).

- Dong, W., Gilmour, M.I., Lambert, A.L., Selgrade, M.K. 1998. Enhanced allergic responses to house dust mite by oral exposure to carbaryl in rats. *Fundamental and Applied Toxicology* . 44(1):63-69
- Ernst, P. 2002. Pesticide exposure and asthma. *American journal of respiratory and critical care medicine* . 165(5):563
- Faria, N.M., Facchini, L.A., Fassa, A.G., Tomasi, E. 2005. Pesticides and respiratory symptoms among farmers. *Rev Saude Publica*. 39(6):973-981.
- Fieten, K.B., Kromhout, H., Heederik, D., van Wendel de Joode B. 2009. Pesticide exposure and respiratory health of indigenous women in Costa Rica. *Am J Epidemiol*. 169(12):1500-1506.
- Fryer, A.D., Lein, P.J., Howard, A.S., Yost, B.L., Beckles, R.A., Jett, D.A. 2004. Mechanisms of organophosphate insecticide-induced airway hyperreactivity. *Am J Physiol Lung Cell Mol Physiol*. 286(5):L963-9.
- Garcia, A.M. 2003. Pesticide exposure and women's health. *Am J Ind Med* . 44(6):584-594
- Garry, V.F. 2004. Pesticides and children. *Toxicol Appl Pharmacol* . 198(2):152-163
- Guerra, S., Sherrill, D.L., Martinez, F.D., Barbee, R.A. 2002. Rhinitis as an independent risk factor for adult-onset asthma. *J Allergy Clin Immunol*. 109(3):419-25.
- Hernandez, A.F., Parron, T., Alarcon, R. 2011. Pesticides and asthma. *Current opinion in allergy and clinical immunology* . 11(2):90-96
- Hernandez, A.F., Casado, I., Pena, G., Gil, F., Villanueva, E., Pla A. 2008. Low level of exposure to pesticides leads to lung dysfunction in occupationally exposed subjects. *Inhal Toxicol*. 20(9):839-849.
- Hoppin, J.A., Umbach, D.M., London, S.J., Alavanja, M.C., Sandler, D.P. 2002. Chemical predictors of wheeze among farmer pesticide applicators in the Agricultural Health Study. *Am J Respir Crit Care Med*. 165(5):683-689.
- Hoppin, J.A., Umbach, D.M., London, S.J. et al. 2009. Pesticide use and adult-onset asthma among male farmers in the Agricultural Health Study. *Eur Respir J*. 34(6):1296-1303.
- Hoppin, J.A., Umbach, D.M., London, S.J., Lynch, C.F., Alavanja, M.C., Sandler, D.P. 2006. Pesticides associated with wheeze among commercial pesticide applicators in the Agricultural Health Study. *Am J Epidemiol*. 163(12):1129-1137.

- Hoppin, J.A., Umbach, D.M., London, S.J. et al. 2008. Pesticides and atopic and nonatopic asthma among farm women in the Agricultural Health Study. *Am J Respir Crit Care Med.* 177(1):11-18.
- Horwood, L.J., Fergusson, D.M., Shannon, F.T. 1985. Social and familial factors in the development of early childhood asthma. *Pediatrics* . 75(5):859-868
- Innes, D.F., Fuller, B.H., Berger, G.M. 1990. Low serum cholinesterase levels in rural workers exposed to organophosphate pesticide sprays. *S Afr Med J.* 78(10):581-583
- Jaga, K., Dharmani, C. 2003. Sources of exposure to and public health implications of organophosphate pesticides. *Pan Am J Public Health.* 14(3):171-185.
- Jeebhay, M.F., Quirce, S. 2007. Occupational asthma in the developing and industrialised world: a review. *The international journal of tuberculosis and lung disease.* 11(2):122-133
- Jeebhay, M.F., Baatjies, R., Chang, Y.S. et al. 2007. Risk factors for allergy due to the two-spotted spider mite (*Tetranychus urticae*) among table grape farm workers. *Int Arch Allergy Immunol.* 144(2):143-149.
- Kesavachandran, C.N., Fareed, M., Pathak, M.K., Bihari, V., Mathur, N., Srivastava, A.K. 2009. Adverse health effects of pesticides in agrarian populations of developing countries. *Rev Environ Contam Toxicol.* 200:33-52.
- Koch, D., Lu, C., Fisker-Andersen, J., Jolley, L., Fenske, RA. 2002. Temporal association of children's pesticide exposure and agricultural spraying: report of a longitudinal biological monitoring study. *Environ Health Perspect* .110(8):829-833.
- Kumar, S.V., Fareedullah, M.D., Sudhakar, Y. 2010. Current review on organophosphorus poisoning. *Archives of Applied Science Research.* 2 (4): 199-215
- Lein, P.J., Fryer, A.D. 2005. Organophosphorus insecticides induce airway hyperreactivity by decreasing neuronal M2 muscarinic receptor function independent of acetylcholinesterase inhibition. *Fundamental and Applied Toxicology.* 83(1):166-176
- London, L. 1995. The health hazards of organophosphate use in South Africa. *Pesticide News.* 27: 6-7
- London, L., Wesseling, C., Kisting, S., Rother, H., Mergler, D. 2002. Pesticide usage and health consequences for women in developing countries: out of sight, out of mind? *International Journal of Occupational and Environmental Health.* 8(1):46-59

- McCauley, L.A., Anger, W.K., Keifer, M. et al. 2006. Studying Health Outcomes in Farmworker Populations Exposed to Pesticides. *Environ Health Perspect.* 114:953–960. doi:10.1289/ehp.8526
- Mekonnen, Y., Agonafir, T. 2004. Lung function and respiratory symptoms of pesticide sprayers in state farms of Ethiopia. *Ethiop Med J* .42(4):261-266.
- Mekonnen, Y., Agonafir, T. 2002. Effects of pesticide applications on respiratory health of Ethiopian farm workers. *Int J Occup Environ Health.* 8(1):35-40.
- Nweke, O.C., Sanders III, W.H. 2009. Modern environmental health hazards: a public health issue of increasing significance in Africa. *Environ Health Perspect* .117(6):863-870
- Ohayo-Mitoko, G.J., Kromhout, H., Simwa, J.M., Boleij, J.S., Heederik, D. 2000. Self reported symptoms and inhibition of acetylcholinesterase activity among Kenyan agricultural workers. *Occup Environ Med.* 57(3):195-200.
- Olson, D.K., Sax, L., Gunderson, P., Sionis, L. 1991. Pesticide poisoning surveillance through regional poison control centers. *Am J Public Health.* 81(6):750-753
- Pekkanen, J., Sunyer J., Anto J.M., Burney P. on behalf of the European Community Respiratory Health Study (ECRHS). 2005. Operational definitions of asthma in studies on its aetiology. *Eur Respir J.* 26: 28–35 DOI: 10.1183/09031936.05.00120104
- Portengen, L., Sigsgaard, T., Omland, O., Hjort, C., Heederik D., Doekes G. 2002. Low prevalence of atopy in young Danish farmers and farming students born and raised on a farm. *Clin Exp Allergy.* 32(2): 247-53
- Proskocil, B.J., Bruun, D.A., Thompson, C.M., Fryer, A.D., Lein, P.J. 2010. Organophosphorus pesticides decrease M2 muscarinic receptor function in guinea pig airway nerves via indirect mechanisms. *PLoS One* . 5(5):e10562.
- Riu, E., Monso, E., Marin, A., Magarolas, R., Radon, K., Morera, J. et al. 2008. Occupational risk factors for rhinitis in greenhouse flower and ornamental plant growers. *Am J Rhinol.* (4):361-364.
- Rodgers, K., Xiong, S. 1997. Contribution of inflammatory mast cell mediators to alterations in macrophage function after malathion administration. *Int J Immunopharmacol* . 19(3):149-156
- Rother, H. 2010. Falling through the regulatory cracks: Street selling of pesticides and poisoning among urban youth in South Africa. *International Journal of Occupational and Environmental Health.* 16(2):202-213.

- Salam, M.T., Li, Y.F., Langholz, B., Gilliland, F.D. 2004. Children's Health Study. Early-life environmental risk factors for asthma: findings from the Children's Health Study. *Environ Health Perspect.* 112(6):760-765.
- Salameh, P.R., Baldi, I., Brochard, P., Raheison, C., Abi Saleh, B., Salamon, R. 2003. Respiratory symptoms in children and exposure to pesticides. *Eur Respir J.* (3):507-512.
- Salameh, P., Waked, M., Baldi, I., Brochard, P., Saleh, B.A. 2006. Respiratory diseases and pesticide exposure: a case-control study in Lebanon. *J Epidemiol Community Health.* 60(3):256-261.
- Schulz, R. 2001. Comparison of spray drift-and runoff-related input of azinphos-methyl and endosulfan from fruit orchards into the Lourens River, South Africa. *Chemosphere* . 45(4-5):543-551
- Schulz, R. 2001. Rainfall-induced sediment and pesticide input from orchards into the Lourens River, Western Cape, South Africa: importance of a single event. *Water Res.* 35(8):1869-1876
- Senthilselvan, A., McDuffie, H.H., Dosman, J.A. 1992. Association of asthma with use of pesticides. Results of a cross-sectional survey of farmers. *Am Rev Respir Dis.* 146(4):884-887.
- Slager, R.E., Poole, J.A., LeVan, T.D., Sandler, D.P., Alavanja, M.C., Hoppin, J.A. 2009. Rhinitis associated with pesticide exposure among commercial pesticide applicators in the Agricultural Health Study. *Occup Environ Med.* 66(11):718-724.
- Slager, R.E., Simpson, S.L., Levan, T.D., Poole, J.A., Sandler, D.P., Hoppin, J.A. 2010. Rhinitis associated with pesticide use among private pesticide applicators in the agricultural health study. *J Toxicol Environ Health.* 73(20):1382-1393.
- Spieksma, F. 1991. Domestic mites: their role in respiratory allergy. *Clinical and experimental allergy.* 21(6):655-660
- Sunyer, J., Torrent, M., Garcia-Esteban, R. et al. 2006. Early exposure to dichlorodiphenyldichloroethylene, breastfeeding and asthma at age six. *Clin Exp Allergy.* 36(10):1236-41.
- Swaen, G., van Amelsvoort, L., Boers, D. et al. 2008. Occupational exposure to ethylenebisdithiocarbamates in agriculture and allergy: results from the EUROPIT field study. *Hum Exp Toxicol* . 27(9):715-720.
- Temple, W.A., Smith, N.A. 1989. Organophosphorus Pesticides. International Programme on Chemical Safety Poisons Information Monograph (Group Monograph) G001.

- Vanden Driessche, K.S.J. 2010. Anaphylaxis in an Airplane After Insecticide Spraying. *Journal of travel medicine*. 17(6):427.
- Veronesi B, Oortgiesen M. 2001. Neurogenic inflammation and particulate matter (PM) air pollutants. *Neurotoxicology*. 22:795–810.
- Zhang, L.X., Enarson, D.A., He, G.X., Li, B., Chan-Yeung, M. 2002. Occupational and environmental risk factors for respiratory symptoms in rural Beijing, China. *Eur Respir J*. 20(6):1525-1531.
- Zhang, X., Zhao, W., Jing, R. et al. 2011. Work-related pesticide poisoning among farmers in two villages of Southern China: a cross-sectional survey. *BMC Public Health*. 11:429.

University of Cape Town

PART C: JOURNAL READY MANUSCRIPT

This article has been prepared for the purposes of submission to the Journal, *Environment International*. The *Instructions to Authors* document has been attached (Appendix 7). The author adhered to all the instructions set out by the Journal, however, for the purpose of this thesis, some tables are included in the text and the article refers to supplementary material and the Appendices section of the thesis.

University of Cape Town

TITLE: Respiratory allergy and asthma associated with pesticide exposure amongst women in rural Western Cape

Authors:

Vuyelwa Ndlovu ^{a*}, Mohamed Aqiel Dalvie ^a, Mohamed F. Jeebhay^a

^aCentre for Occupational and Environmental Health Research, School of Public Health and Family Medicine, University of Cape Town, South Africa

***Correspondence directed to:**

Vuyelwa Ndlovu, email: vuyelwa.ndlovu@uct.ac.za or vuyiendlovu@yahoo.com, Tel: 021 4066300, Fax; 021 4066459

2

² **Abbreviations:** FeNO, Fractional exhaled nitric oxide; IgE, immunoglobulin E, ChE, Whole blood cholinesterase; DDT, dichlorodiphenyltrichloroethane; WFP, Women on Farms Project; SANAS, South African National Accreditation System; NIOH, National Institute for Occupational Health; BMI, Body mass index

Abstract

Background: Pesticide exposure has been increasingly associated with adverse respiratory health effects including asthma and allergy. However, few studies have been conducted among women and workers in developing countries. South Africa is one of the largest users of pesticides on the continent and the Western Cape is one of the main agricultural areas in the country, with uncontrolled use posing an increased health risk to farm workers.

Objectives: This study focused on determining the association between pesticide exposure (predominantly organophosphates and carbamates) and the presence of allergy and asthma among women farm workers and residents.

Methods: A cross-sectional study was conducted on 211 women including those working and living on farms (farm dwellers, n=121) and those residing in neighbouring farm areas (town dwellers, n=90). Outcome measurements included the abbreviated European Community Respiratory Health questionnaire, fractional exhaled nitric oxide (FeNO) levels and immunological tests (a positive Phadiatop test indicating atopy and quantification of specific IgE to house dust mite, storage mite and spider mite). The outcome variables included doctor diagnosed asthma, adult onset asthma, current asthma, allergic sensitisation, allergic airway inflammation and the asthma symptom score. The asthma symptom score was a continuous outcome generated as the sum of positive responses to four questions on asthma symptoms in the last 12 months including wheeze with breathlessness, woken up with chest tightness, attack of shortness of breath at rest and woken by attack of coughing. The asthma symptom score values ranged from 0 for no symptoms to 4 for all symptoms.

Exposure information was obtained from the questionnaire, which had items on household and occupational pesticide use and concentration of whole blood cholinesterase (ChE) obtained from blood samples of workers. The questionnaire also had items on potential host-related confounding factors. Univariate and bivariate exploration of the data were performed. Fischer's Exact method was used for expected values less than 5. Multiple Logistic Regression Analyses were used to test for associations between dichotomous outcomes and exposure variables while controlling for confounding. For the continuous outcome, asthma symptom score, a Negative Binomial Regression Analysis was used. This models the ratio of the mean score among exposed and nonexposed

Results: The median age was 37 years (interquartile range: 28-45 years). There were 9% of participants with low ChE (below laboratory reference standard) among whom 78% were farm dwellers. The prevalence of doctor diagnosed asthma was 11%; current asthma was 6% and 24% ocular nasal symptoms. Being woken by cough was the most common symptom with a prevalence of 37%. After adjusting for confounding variables (age, smoking, years of schooling, atopy), ocular nasal symptoms were associated (OR= 2.97; 95% CI: 0.93-9.50) with immediate re-entry in the pesticide sprayed field. Furthermore, current asthma symptom score was significantly associated with pesticide drift in the home (Ratio of mean asthma symptom score=2.03; 95% CI: 1.38-2.98), being a farm dweller (Ratio of mean asthma symptom score= 2.25; 95% CI: 1.45-3.48) and having a low ChE (Ratio of mean asthma symptom score=1.93; 95% CI: 1.09-3.44). While the prevalence of doctor diagnosed asthma was not significantly different between farm and town dwellers, those with low ChE had a five-fold increased odds of having FeNO > 50ppm (highly probable

allergic asthma) (95% CI: 0.80-28.00; p=0.08) though this result was of borderline statistical significance. Allergic sensitisation to the various mites was not associated with pesticide exposure.

Conclusion: Exposure to pesticides among women farm workers is associated with increased risk of ocular nasal symptoms and asthma. This study was limited by the small sample size, lack of information on specific pesticides and the cross sectional design. The findings in this study therefore need further exploration in a larger longitudinal study.

Keywords: Pesticides, Farm women, Asthma, Allergy, Cholinesterase,

Highlights

- Pesticide exposure of farm women is high as evidenced by their low blood cholinesterase
- Pesticides might be associated with upper and lower airway symptoms including asthma
- Low blood cholinesterase might be associated with allergic airway inflammation indicating highly probable asthma

1. Introduction

South Africa is the largest consumer of pesticides in Sub Saharan Africa due to the large volumes used in agriculture (Naidoo and Buckley, 2003). Within South Africa, in the Western Cape Province where crop farming is important, pesticide use has been increasing and agricultural pesticides have been detected in the rural environment and among farm workers (London, 2003; Dalvie et al., 2009a, 2009c, 2011). Pesticides such as endosulfan and chlorpyrifos have been detected in drinking and recreational water sources and sediments in the rural Western Cape (Dalvie et al., 2003; Schulz, 2001a, 2001b). In a study conducted in the Western Cape, chlorpyrifos and endosulfan residues in farm workers was found to be higher than in other countries (Dalvie et al., 2009a; , 2011). Additionally, pesticide residues are also found in South African crops as indicated by a study conducted by Dalvie and London (2009b) that found pesticide residues in both local and imported wheat samples.

A large number of epidemiological studies have reported that farm workers are exposed to pesticides that may affect their respiratory health (Ndlovu et al., 2011) (Appendix 8). For instance wheeze has been associated with the organophosphate insecticides chlorpyrifos and terbufos and the triazine herbicide atrazine and alachlor from the chloroacetanilide family (Fieten et al, 2009; Hoppin et al., 2002, 2006). Pesticides also appear to have an effect on the upper respiratory tract. Rhinitis was associated with herbicides such as paraquat a bipyridyl; glyphosate a glycine and the chlorphenoxy pesticide 2.4D and with insecticides such as carbaryl, a carbamate pesticide and organophosphate pesticides including diazinon and malathion (Slager

et al., 2009; Chatzi et al., 2007; Slager et al., 2010). Recent studies have shown that the organochlorine insecticide DDT (dichlorodiphenyltrichloroethane) was associated with adult onset non-allergic asthma whereas another organochlorine insecticide heptachlor was associated with adult onset allergic asthma along with halogenated organic fumigants and organophosphate insecticides (Hoppin et al., 2009). In the latter study, having grown up on a farm was found to have a protective effect on asthma in women farm workers.

Most studies have been done in developed countries and have mainly focused on men but it has become increasingly apparent that women are also a high risk group (London et al., 2002; Zhang et al., 2002; Garcia, 2003; Faria et al., 2005; Hoppin et al., 2008; Fieten et al., 2009). Work related pesticide poisoning in Chinese women was found to be more common than in men (Zhang et al., 2011). Two other studies reported that respiratory symptoms or doctor diagnosed asthma were more prevalent and strongly associated with pesticide exposure in women than in men (Zhang et al., 2002, Faria et al., 2005). In the study by Faria et al. (2005), the higher prevalence of asthma symptoms in women was ascribed to the fact that they were found to have less protection during pesticide exposure than men and were more likely to have been exposed in domestic settings as well.

Respiratory health problems have also been identified among women farm workers in the Western Cape Province of South Africa (Bowers et al., 2009). However, few studies on the effect of pesticide exposure on respiratory health have been conducted in South Africa. Innes et al. (1990) reported that 17.5% of male rural workers engaged in

crop spraying reported chronic organophosphate poisoning, asthma and chronic bronchitis in Cape Town. No association between long-term exposure to paraquat and reported symptoms and lung function was found but a decrease in exercise oximetry was found in a study done in the rural Western Cape (Dalvie et al., 1999). A more recent study in South Africa demonstrated that the spider mite, *Tetranychus urticae*, is an important outdoor allergen among table-grape farm workers and the increased risk of spider mite allergy appeared to be related to high pesticide exposure among crop sprayers (Jeebhay et al., 2007).

Most epidemiological studies that have investigated the relationship between pesticides and respiratory health have relied on self-reported asthma as an outcome. Future studies should focus on identifying undiagnosed asthma outcomes using objective markers where possible as well as personal-exposure monitoring using biomarkers. Specific pesticides should also be identified.

A study investigating asthma amongst rural women in the Western Cape using sensitive outcome and exposure measures was therefore conducted. The data presented in this paper is part of a bigger study investigating various health effects of pesticide exposure among women on farms in the Western Cape in South Africa.

2. Materials and methods

2.1 Study Design, Population and sampling

An analytical cross-sectional study of women farm workers and residents and women living in towns neighbouring the farms, in the Western Cape region of South Africa was conducted during the period 24 October to 3 December 2009. Sample size calculations using a two sample comparison of proportions [Stata Corporation, 2003] (exposed/control ratio = 1, power = 90%, confidence level = 95%) and using the results from a recent study (Hoppin et al., 2009) showed that for a 2.25 fold increase in asthma prevalence (20% to 45%), 160 participants would be required. The budget for this study only allowed for the recruitment of approximately 200 participants. Recruitment of participants was achieved with the assistance of the Women on Farms Project (WFP), a rural women's rights Non Governmental Organisation, who were requested by the research team, to recruit 100 women from farms affiliated to them located in the 5 most accessible agricultural areas and 100 women from neighbouring towns that were about 5 to 10km away from agricultural areas. The areas included Stellenbosch, Ceres, Paarl, Grabouw and Worcester. About 40 women, 20 each from farms and towns, from each of the 5 areas were targeted. The study sample was not selected through random sampling due to time constraints and logistical difficulties. Farm workers and residents were selected from the 5-10 most accessible farms in each area and town dwellers 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. There were 8 women who lived in a town but worked on a farm and 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” in the article. There were 90 women who neither lived nor worked on a farm and they are referred to as “town dwellers” in the article. A pilot study was conducted to test the questionnaire and to work out the logistics for the main study. The study was done in accordance with the Declaration of Helsinki of the 25th world Medical Assembly (WHO, 2000). The study proposal was approved by the University of Cape Town’s (UCT) Research Ethics Committee (Reference 393/2009). Informed consent was obtained from participants prior to the study.

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 (Burney et al., 1994). The non-respiratory questions in the questionnaire were based on those used in previous surveys (Dalvie et al., 1999; Dalvie et al., 2004). The questionnaire was translated into Afrikaans and Xhosa. They

were then back translated to ensure the accuracy of the translation. Trained interviewers administered questionnaires to participants in the language of their choice. Fieldwork was conducted at the WFP premises. WFP arranged for the transport of the participants on a daily basis.

2.3 Whole blood cholinesterase (ChE) testing

A whole blood sample (9 ml) was drawn from each participant using a tube containing sodium heparin or EDTA as anticoagulant, by a qualified nurse. EDTA does not inhibit cholinesterase activity (Roche Diagnostics). The tubes were kept on ice and transported to the South African National Accreditation System (SANAS) accredited Pathcare laboratory at N1 City Hospital, Cape Town for ChE analyses (the ChE level is the sum of serum butyl cholinesterase and erythrocyte acetylcholinesterase). ChE activity was quantified using the Roche Diagnostics Cholinesterase method (Roche/Hitachi analyser, Cobas^R, Roche Diagnostics GmbH, D-68298 Mannheim), a photometric test which is based on the principles of a study conducted by Ellman et al. (1961).

2.4 Allergy tests: Phadiotop and allergen specific serum IgE levels

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 nurse. The blood was allowed to clot for 1-2 hours at room temperature (20-24 degrees Celsius) and then centrifuged at 1350 rpm 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 Lung Institute, UCT where it was stored at -80 degrees celcius. The samples were then couriered to the SANAS accredited (ISO 15189:2007), National Institute for Occupational Health (NIOH) Immunology laboratory for testing. Presence of sensitisation to common aeroallergens (house dust mites, grass pollens, cat, dog, cockroach etc.) was determined by the Phadiatop[®] test (Phadia AB, Uppsala, Sweden). The quantification of specific IgE antibodies to house dust mite and specific occupational allergens (spider mite: *Tetranychus urticae* and storage mite, *Lepidoglyphus destructor*) was performed using the UniCAP[®] system (Phadia AB, Uppsala, Sweden).

2.5 Airway inflammation as a marker for allergic asthma: Fractional exhaled nitric oxide (FeNO)

Fractional exhaled nitric oxide measurement is a recognised non-invasive method for assessing allergic airway inflammation (Quirce et al., 2010). The nurse determined fractional exhaled NO (FeNO) from single-breath exhalations. The technique for adult patients involved inspiration of 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 (ATS/ERS 2005). The average value of the three measurements was used in the analysis. Exhaled nitric oxide (NO) 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 was also recorded.

2.6 Exposure ascertainment

There were several self-reported exposure variables encompassing domestic, occupational and environmental exposure to pesticides that were developed from the questionnaire. These included household pesticide use, history of living or working on farms, currently living or working on farms, pesticide drift, re-entry into sprayed fields (immediate (same day), delayed) and permanency of farm work (permanent, seasonal) were dichotomous variables and job duration and number of pesticide spraying days per year were continuous variables. ChE measurements were used as an objective marker for exposure to cholinesterase inhibiting carbamates and organophosphates and were dichotomised using a cut-off value of 6021 IU which is the low end of the expected laboratory range (data on file at Roche Diagnostics®).

Outcome characterisation

Outcome variables were predominately dichotomous and included ocular nasal symptoms defined as yes to "Have you had any nasal allergies including hay fever or itchy and watery eyes/nose in the last 12 months?"; doctor diagnosed asthma; allergic sensitisation (specific IgE levels to house dust mite, storage mite and spidermite greater than 0.35ku/l) (UniCAP® system Phadia AB, Uppsala, Sweden); FeNO >50ppb suggestive of highly probable allergic asthma and FeNO 25-50ppm indicating possible asthma. FeNO levels were also analysed as a continuous variable. Current asthma

defined as 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?” and adult-onset asthma defined as doctor diagnosed asthma and having had the first asthma attack at the age of 16 years or later, were other composite dichotomous outcomes generated from the respiratory symptoms questionnaire. Additionally asthma symptom score was a continuous outcome generated as the sum of positive responses to four questions on asthma symptoms in the last 12 months including wheeze with breathlessness, woken up with chest tightness, attack of shortness of breath at rest and woken by attack of coughing. The asthma symptom score was a simplified version of an asthma score proposed by Pekkanen et al. (2005) and used by Sunyer et al. (2007) and Vizcaya et al. (2011).

Statistical analysis

The selected software for analysis was StataCorp. 2009. Stata: Release 11. Statistical Software. College Station, TX: StataCorp LP. Univariate and bivariate exploration of the data were performed. As all continuous variables were not normally distributed, median and interquartile ranges were used to summarise these variables. Box and Whisker plots were used to look for outliers. Categorical variables were described using frequency distributions. Bivariate analysis included simple Logistic Regression Analysis, the Student T-Test or Wilcoxon rank sum Test, and the Chi-square Test, Fischer’s Exact test for expected values less than 5 and Pearson or Spearman rank correlation to measure degree of correlation between continuous explanatory variables.

Multiple Logistic Regression Analyses were used to test for associations between dichotomous outcomes and exposure variables while controlling for confounding. Confounders were selected on an *a priori* basis, according to biological plausibility, or using bivariate testing if $p < 0.1$ or showing at least a 10% change in the β coefficient of the crude OR between exposure and outcome. Atopy (based on a positive Phadiatop test) and current smoking (a dichotomous variable) were selected *a priori* for all outcomes (Jeebhay and Adewole, 2011). Other potential confounders included in bivariate testing were age (continuous variable in years), BMI (a continuous variable calculated from measured weight and height), years of schooling (continuous variable), employment (a dichotomous variable), household income (a continuous variable in US dollars), born on a farm (a dichotomous variable) and drinking alcohol. As a result, the covariates selected for inclusion in the final models were years of schooling, born on a farm, smoking and atopy. These covariates were further confirmed using stepwise selection in model building. Exposure variables were then added separately to all the different outcomes adjusting for these covariates. There were no interactions between variables. Regression diagnostics was applied to determine collinearity, the goodness of fit of the model and to assess for outliers or influential observations.

For the continuous outcome, asthma symptom score, the underlying distribution was not normal thereby violating the assumption for Linear Regression Analyses. The asthma score was a count, which suggested a Poisson regression model. However, because the mean score (0.9) was lower than the standard deviation (1.5), a Negative

Binomial Regression Analysis was used. Model building and checking procedures were similar to that used for Logistic Regression.

3. Results

3.1 Participation

Two hundred and eleven women from the Boland were recruited into the study with 20% (n= 42) coming from Ceres, 18% (n = 38) from Grabouw, 19% (n = 39) from Paarl, 22% (n = 47) from Stellenbosch and 21% (n = 45) from Worcester.

Table 1 summarises the distribution of farm dwellers and town dwellers (as already been defined in section 2.1) that participated in the study.

Table 1.

Distribution of study participants by area and type of residence

Area	N (%)			
	Town dwellers,(n=90)	Farm dwellers, (n=121)		Total
		Farm residents (n=24)	Farm workers (n=97)	
Ceres	19(45)	9(21)	14(33)	42(100)
Grabouw	3(8)	0(0)	35(92)	38(100)
Paarl	23(59)	3(8)	13(33)	39(100)
Stellenbosch	22(47)	6(13)	19(40)	47(100)
Worcester	23(51)	6(13)	16(36)	45(100)
Total	90(43)	24(11)	97(46)	211(100)

The 24 farm residents were included in the farm dweller group because sub-analysis treating them as a different exposure group showed that they had similar outcomes as farm workers (data not shown).

3.2 Demographic and socioeconomic characteristics

The farm dwellers were significantly younger than the town dwellers with median ages of 33 years and 40.5 years respectively (Table 2). BMI was also significantly lower amongst farm dwellers. Household income was slightly but significantly less in the farm dwellers group despite a significantly higher proportion (83%) of farm dwellers being currently employed compared to town dwellers (28%). Educational status was similarly low in both groups with more than half not having matriculated and none with tertiary education.

Table 2.

Demographic and socioeconomic characteristics of women farm workers in the Boland of the Western Cape (n=211)

Characteristics	Farm dwellers (n = 121)	Town dwellers (n = 90)	Total (n = 211)
Demographics (median, IQR^a)			
Age (years)	33(27-40)***	40.5(31-49)	37(28-45)
BMI (kg/m ²) (n=207)	25.2(21.6-30.8)**	28.6(23.8-35.7)	26.44(22.52-32.87)
Education (years of schooling)	9(7-10)	9 (7-11)	9(7-10)
Household income/month (\$US) ^b (n=189)	270(188- 500)**	379(221- 744)	324(199-600)
Length of stay in current residence (years)	15(8-24)*	21.5(12-41)	17(9-29)
Other socio-economic indicators, n (%)			
Currently employed	101(83)***	25(28)	126(60)
Born on a farm	83(69)***	13(14)	96(46)
Drinks alcohol	79(65)**	39(43)	118(56)
Current smoker	69(57)*	36(40)	105(50)

*p < 0.05; **p < 0.01; ***p < 0.001.

^aInter quartile range

^bHousehold income converted to \$US from Rands at a rate of 1\$US to 8 Rands

There was a small proportion of town dwellers that were born on a farm (14%) but substantially less than in the farm dweller group (69%) (p<0.001). Smoking and drinking alcohol was also significantly more common among the farm dwellers.

3.3 Environmental and occupational exposure to agricultural pesticide spraying among farm dwellers (n=121)

The crops grown on farms on which farm dwellers lived and worked were predominantly popular fruit such as grapes, apples, berries, pears, prunes and oranges. Amongst the participants currently living on a farm (n= 113), the average distance from the nearest vineyard was 10 metres (IQR= 5m-20m) and 73% of the women said that they come into contact with pesticide contaminated surfaces or are exposed to pesticide drift when doing chores outside the house like hanging the washing. The use of unprotected sources of water such as rivers or farm dams for drinking and household use was reported by 83% of the farm dwellers.

Participants reported that there were women who were employed as pesticide applicators in nearly 20% of farms but only 4 (3%) participants were applicators. 28 (23%) farm dwellers reported that personal protective equipment (PPE) are not provided on farms and 69(57%) reported that there are no showers on the farm. Tractor with a mist blower was the most common spraying method used on farms and was reported by virtually all the farm dwellers (97%). Other methods such as tractor without a mist blower, backpack spraying and using a quad bike were reported by 28%, 37% and 20% of farm dwellers respectively. The median time since last spray occurred was 1 one day (IQR=0-4 days).

3.4 Self-reported household and agricultural pesticide exposure indicators and measured ChE levels

Over half of the participants in both exposure groups reported that pesticides are used at home (Table 3). Almost a third of the farm dwellers reported living with a pesticide applicator and some (11%) reported using empty pesticide containers for domestic purposes. Over half of the farm dwellers (57%) reported that pesticide drift reaches their home. There were 26 (29%) town dwellers that had previously lived or worked on a farm. Pesticide spraying was highly prevalent on farms that participants currently lived or worked as they reported a median number of spraying days in a year of 96 days (IQR=72-144 days).

Table 3.

Self-reported household and agricultural pesticide exposure and measured cholinesterase enzyme levels of women farm workers in the Boland of the Western Cape (n=211)

Exposure variables	Farm dwellers n=121	Town dwellers n=90	Total n=211
Household pesticide use indicators, n (%)			
Use pesticides at home	67(55)	56(62)	123(58)
Living with a pesticide applicator	36(30)***	1(1)	37(18)
Domestic use of pesticide containers	13(11)***	0(0)	13(6)
Pesticide exposure indicators, n (%)			
History of living or working on farms	121(100)	26(29)	147(70)
Pesticide drift into home	69(57)	n/a	n/a
Farm worker status (n=208)			
Permanent	53(45)***	0(0)	53(25)
Seasonal	40(34)***	4(4)	44(21)
Non-farm worker	25(21)***	86(96)	111(53)
Re-entry into sprayed fields			
Non-farm residents	7(6)***	89(99)	96(45)
Delayed re-entry ^a	33(27)***	1(1)	34(16)
Immediate re-entry ^b	81(67)***	0(0)	81(38)
Job duration (years)	5(1-13)	n/a	n/a
Pesticide spraying days/year in current farm (n=196) Median, IQR	96(72-144)	n/a	n/a
Cholinesterase < 6021 IU (n=206) ^c	14(12) [‡]	4(5)	18(9)

*p < 0.05; **p < 0.01; ***p < 0.001.

n/a: not applicable

^a re-entry into field 1 to 7 days after pesticide spraying

^b re-entry into field on the same day after pesticide spraying

^c cholinesterase level below 6021 IU (low end of the laboratory range) indicating enzyme inhibition and exposure to carbamate and organophosphate pesticides

[‡]p=0.08

On spraying days, about two thirds (67%) of the farm dwellers reported that they re-entered the field on the same day after pesticide spraying. Workers were employed for an average of five years on the farms and about a third of farm dwellers were seasonal farm workers.

ChE levels below the laboratory reference value of 6021 IU (Data on file at Roche Diagnostics®) were significantly more prevalent amongst farm dwellers. There were 18 (9%) participants with low ChE (below laboratory reference standard) among whom 78% were farm dwellers.

3.5 Prevalence of asthma and allergic outcomes

Upper airway symptoms (ocular nasal symptoms) were reported by 24% of the participants (Table 4). The prevalence of lower airway symptoms was higher and significantly more prevalent amongst the farm dwellers (12-46%) than town dwellers (8-24%) for various individual symptom outcomes.

Table 4.

Prevalence of asthma and allergic outcomes among women farm workers in the Boland of the Western Cape (n=211)

Outcome	Prevalence, n (%)		
	Farm dwellers (n=121)	Town dwellers (n=90)	Total (n=211)
Upper airway symptoms (n=211)			
Ocular nasal symptoms	31(26)	19(21)	50(24)
Lower airway symptoms (n=211)			
Wheeze	45(37)*	20(22)	65(31)
Shortness of breath with wheeze	34(28)*	15(17)	49(23)
Wheeze without cold	21(17)	14(16)	35(17)
Woken up by chest tightness	42(35)***	9(10)	51(24)
Attack of shortness of breath at rest	14(12)	7(8)	21(10)

Woken by cough	56(46)***	22(24)	78(37)
Asthma phenotype characteristics			
Doctor diagnosed asthma	11(9)	12(13)	23(11)
Current asthma	4(3)	8(9)	12(6)
Asthma with ocular nasal symptoms	2(2)	4(4)	6(3)
Asthma without ocular nasal symptoms	2(2)	4(4)	6(3)
Asthma score (Median(IQR))	1(0-2)***	0(0-1)	0(0-2)
Asthma symptom score categories:			
0	48(40)***	63(70)	111(53)
1	28(23) [†]	12(13)	40(19)
2	23(19)*	8(9)	31(15)
3	16(13)*	3(3)	19(9)
4	6(5)	4(4)	10(5)
Fractional exhaled nitric oxide levels (n=207)			
FeNO ppb (median, IQR)	9.2(5.67-14)**	12.3(8.3-22.3)	10.3(6-17.3)
FeNO 25ppb-50ppb	11(9)	12(14)	23(11)
FeNO >50ppb	7(6)	8(9)	15(7)
Immunological profile (n=206)			
Atopy (Positive Phadiatop)	46(38) [†]	44(51)	90(44)
Storage mite sensitisation (>0.35kU/l)	23(19)	21(24)	44(21)
Spider mite sensitisation (>0.35kU/l)	18(15)	17(20)	35(17)
Allergic asthma phenotypes			
Atopic asthma	3(2) [†]	7(8)	10(5)
Non-atopic asthma	1(1)	1(1)	2(1)
House dust mite allergic asthma	3(2)	4(4)	7(3)
Storage mite allergic asthma	3(2)	4(4)	7(3)
Spider mite allergic asthma	2(2)	5(6)	7(3)

*p < 0.05; **p < 0.01; ***p < 0.001. Data presented as n(%) except where indicated otherwise in parenthesis as median(IQR)

[†]p=0.07

Doctor diagnosed asthma, current asthma and adult onset asthma were not significantly different in the two exposure groups although a higher prevalence was observed in the town dwellers (Table 4). Among those who reported ever having asthma a large proportion (79%) had adult onset asthma. The prevalence of adult onset asthma among farm dwellers was 7% which was slightly lower than among town dwellers (11%). The median age at asthma diagnosis was 25 years (IQR=17-35 years). However, only 1% reported having an asthma attack in the past 12 months and 6% were currently on medication. Current asthma with and without ocular nasal symptoms had a prevalence of 3% each with both phenotypes being more prevalent among the town dwellers. The asthma symptom score was significantly higher amongst the farm dwellers (p<0.001). Half of the participants reported no asthma-like symptoms with an asthma score of 0 which was significantly higher among town

dwellers. There were significantly more participants with 1-3 symptoms among the farm dwellers (13-23%) than the town women (3-13%).

The median FeNO level was significantly lower among the farm dwellers (Table 4). There was also a greater proportion of town dwellers with FeNO levels between 25ppb and 50ppb indicating possible allergic asthma and more than 50ppb indicating highly probable allergic asthma, compared to farm dwellers.

The overall prevalence of atopy was 44% and the town dwellers had a higher proportion (51%) compared with farm dwellers (38%) ($p=0.07$). The proportion of women sensitised to the domestic allergen (house dust mite) and the allergens at work (storage mite and spider mite) was higher among the town dwellers compared to the farm dwellers.

The prevalence of allergic asthma phenotypes defined as the presence of current asthma and allergic sensitisation were found in 1-5% of the participants, with a relatively higher prevalence of all allergic asthma phenotypes, especially atopic asthma, in the town dweller group. Further detailed analysis of these phenotypes could not be conducted because of the low overall prevalence (Supplementary Table 1).

3.6 Host factors associated with asthma and allergic outcomes

Unadjusted logistic regression models revealed that age, BMI and house hold income were not predictors of asthma outcomes (Table 5). Atopy, on the other hand, was

strongly and positively associated with all outcomes. Higher education level and employment were generally protective for asthma outcomes. Being born on a farm was associated with increased odds of ocular nasal symptoms (2.41, 95%CI: 1.26-4.63) but decreased odds for current asthma, adult onset asthma, doctor diagnosed asthma and asthma outcomes measured by FeNO. Smoking was negatively associated with FeNO. Similar associations were found for the asthma symptom score and host factors in the unadjusted negative binomial regression models (Supplementary Table 2).

University of Cape Town

Table 5.

Unadjusted logistic regression models of host and other potential confounders associated with asthma outcomes among women farm workers in the Boland of the Western Cape

	Asthma outcomes. Odds Ratio (95% Confidence Interval)					
	Ocular nasal symptoms	Current asthma	Doctor diagnosed asthma	Adult onset asthma	FeNO=25ppb-50ppb	FeNO>50ppb
Prevalence, (%) (n=211)	24%	6%	11%	9%	11%	7%
Age (years)	1.00(0.96-1.02)	1.04(0.99-1.09)	1.01(0.98-1.05)	1.02(0.99-1.06)	1.00(0.95-1.03)	0.99(0.95-1.04)
BMI (kg/m ²)	1.02(0.98-1.07)	1.01(0.93-1.10)	1.02(0.96-1.08)	1.03(0.97-1.10)	1.05(0.98-1.11)	1.02(0.95-1.10)
Atopy (Positive Phadiatop)	3.79(1.93-7.48) ***	7.12(1.52-33.40) *	3.37(1.32-8.58) *	3.10(1.13-8.5) *	2.2(0.9-5.34)	21.28(2.74-165.23) ***
Education (years of schooling)	0.96(0.87-1.06)	0.78(0.66-0.92) **	0.85(0.74-0.96) ***	0.85(0.74-0.97) *	0.95(0.83-1.09)	1.18(0.96-1.46)
Currently Employed	0.91(0.48-1.74)	0.46(0.14-1.5)	0.86(0.36-2.07)	0.73(0.28-1.87)	0.56(0.24-1.34)	0.55(0.19-1.58)
Household income/month (\$US)	1.00(0.99-1.00)	1.00(0.99-1.00)	0.99(0.99-1.00)	1.00(0.99-1.00)	1.00(0.99-1.00)	1.00(0.99-1.00)
Born on a farm	2.41(1.26-4.63) **	0.10(0.01-0.79) *	0.61(0.25-1.50)	0.67(0.25-1.79)	0.29(0.10-0.81) *	0.27(0.07-0.99) *
Drink alcohol	1.39(0.72-2.66)	0.37(0.11-1.28)	0.69(0.29-1.65)	0.54(0.21-1.41)	0.84(0.35-2.00)	0.67(0.23-1.91)
Current smoker	0.82(0.43-1.55)	1.44(0.44-4.70)	1.66(0.68-4.02)	1.43(0.55-3.72)	0.62(0.25-1.49)	0.23(0.06-0.84) *

*p < 0.05; **p < 0.01; ***p < 0.001.

Each OR represents a separate unadjusted logistic regression model

3.7 Asthma symptom score and pesticide exposure

Table 6 summarises the results of the associations between asthma symptom score and pesticide exposure indicators using negative binomial regression models adjusted for atopy, born on a farm, smoking and education. The mean asthma symptom scores were consistently higher among those that reported either household or agricultural pesticide exposure, the latter demonstrating stronger association. Women who were either currently or previously living or working on a farm had a two fold higher mean score than their counterparts. These associations remained unchanged even after adjusting for household pesticide use (Supplementary table 3). Furthermore, a borderline significant association was also observed with the number of days of pesticide spraying per year on a farm reported among those currently residing and/or working on the farm. Exposure to pesticide drift in the home was also strongly associated with the asthma score as the exposed had double the mean score compared to the unexposed (95% CI: 1.38-2.98). A sub analysis of farm dwellers only revealed that there was a 57% higher mean score among those exposed to pesticide drift compared to the unexposed (95% CI:1.06-2.32) (Supplementary table 3).

Also presented in Table 6 are the results of the associations between ChE measurements and the asthma symptom score. Those with ChE levels less than the laboratory reference value (6021 IU, Roche Diagnostics®) had a significant twofold higher mean score (95%CI:1.09-3.44) than the women with ChE levels higher than the laboratory reference value.

Table 6.

Adjusted negative binomial regression models of pesticide exposure indicators associated with continuous asthma symptom score among women farm workers in the Boland of the Western Cape

Pesticide exposure variable	Asthma symptom score
	Ratio of mean score MR (95%CI) ^a
Use pesticides at home	1.17(0.82-1.69)
History of living or working on farms	2.22(1.30-3.80)**
Currently living and working on farms	2.25(1.45-3.48)***
Pesticide drift into the home	2.03(1.38-2.98)***
Permanent versus seasonal farmworker	1.06(0.70-1.70)
Immediate versus delayed re-entry	1.21(0.75-1.90)
Number of years in current farm job	1.02(1.00-1.04)
Number of days of pesticide spraying/year in current farm	1.01(1.00-1.01)**
Cholinesterase levels < laboratory reference standard	1.93(1.09-3.44)*

*p < 0.05; **p < 0.01; ***p < 0.001.

^a Negative binomial regression models MR, the ratio of the mean score among exposed and nonexposed. Each MR represents a separate model adjusted for atopy, smoking, born on a farm and years of schooling

3.8 Associations between pesticide exposure and asthma and allergic outcomes

Table 7 summarises the results of the associations between asthma outcomes and pesticide exposure indicators using multiple logistic regression models adjusted for atopy, born on a farm, smoking and years of schooling. Multiple linear regression results using FeNO measurement as a continuous variable did not show statistically significant associations with the pesticide exposure indicators (Supplementary Table 4). Using pesticides at home was weakly but positively associated with ocular nasal symptoms, adult onset asthma and FeNO. The results for FeNO differ from low to high groups suggesting a chance observation. There were non-significant positive associations between FeNO outcomes and currently living or working on a farm whereas the effects were negative for the remaining asthma outcomes. The measure and direction of the effects between all outcomes and currently having a history of living or working on farms did not change even after adjusting for household pesticide use (Supplementary table 3). Exposure to pesticide drift in the home, though not statistically significant, increased the odds of having ocular nasal symptoms by

30% (95% CI: 0.60-2.80) but the association was negative for the rest of the asthma outcomes. However, a sub analysis of farm dwellers only revealed a positive association between doctor diagnosed asthma(OR=1.04, 95% CI: 0.26-4.15), ocular nasal symptoms (OR=1.66, 95% CI: 0.65-4.24), adult onset asthma(1.11, 95% CI: 0.25-4.94) and those exposed to pesticide drift (supplementary table 3).

There were positive associations, though not statistically significant, for ocular-nasal symptoms and almost all asthma outcomes among women who reported that they re-entered the fields on the same day after pesticide spraying compared to those who re-entered the field 1 to 7 days after spraying.

Also presented in Table 7 are the results of the associations between ChE measurements and the asthma outcomes. Positive associations were found between women with ChE levels below the laboratory reference standard and ocular nasal symptoms or FeNO outcomes. Those with ChE levels below the laboratory reference standard had, with borderline significance, an almost 5 fold increased odds of having highly probable asthma based on the FeNO measurements when compared with the women with ChE levels above this reference value. Including variables for sensitisation to housedust mite, storage mite and spidermite into all the multivariate models in Table 6 and 7 did not change the findings (data not shown).

Multiple logistic regression analysis revealed an increase, though not significant, in the odds of allergic sensitisation outcomes (atopy, house dust mite sensitisation, storage mite sensitisation) among those using pesticides at home after adjusting for confounding

variables (Supplementary Tables 5 and 6). There was generally no consistent or significant association between pesticide exposure indicators and sensitisation outcomes.

University of Cape Town

Table 7.

Adjusted multiple logistic regression models of pesticide exposure indicators associated with asthma outcomes among women farm workers in the Boland of the Western Cape

	Asthma outcomes. Odds Ratio (95% Confidence Interval)					
	Ocular nasal symptoms 24%	Current asthma 6%	Doctor diagnosed asthma 11%	Adult onset asthma 9%	FeNO=25ppb-50ppb 11%	FeNO>50ppb 7%
Prevalence, (%) (n=211)						
Use pesticides at home	1.40(0.70-2.90)	0.40(0.10-1.70)	0.97(0.40-2.50)	1.30(0.50-3.50)	3.10(1.02-9.30)*	1.10(0.30-4.20)
History of living or working on farms	1.65(0.57-4.76)	0.69(0.15-3.16)	0.68(0.20-2.25)	0.53(0.14-2.02)	1.17(0.40-3.41)	1.19(0.30-4.77)
Currently Living and working on farms	0.87(0.40-2.10)	0.70(0.20-3.00)	0.80(0.30-2.30)	0.76(0.25-2.30)	1.30(0.50-3.60)	1.90(0.50-7.50)
Pesticide drift into the home	1.30(0.60-2.80)	-	0.90(0.30-2.70)	0.90(0.30-2.80)	0.60(0.20-2.10)	0.70(0.20-3.30)
Permanent vs seasonal farmworker	2.24(0.80-6.30)	0.30(0.02-4.30)	0.19(0.04-1.05) [†]	0.23(0.04-1.30)	0.36(0.08-1.73)	2.54(0.20-30.00)
Immediate vs Delayed re-entry	2.97(0.93-9.50) [†]	-	4.81(0.55-42.20)	3.49(0.40-31.00)	1.24(0.30-5.30)	0.82(0.10-6.05)
Number of years in current farm job	1.00(0.97-1.06)	1.00(0.90-1.10)	1.01(0.95-1.10)	1.01(0.96-1.08)	0.96(0.90-1.05)	1.02(0.90-1.10)
Number of days of pesticide spraying/year in current farm	1.00(0.99-1.01)	1.00(0.99-1.01)	1.00(0.99-1.00)	1.00(0.99-1.01)	1.00(0.99-1.01)	1.00(0.99-1.01)
Cholinesterase levels < Laboratory reference standard	1.10(0.30-3.80)	-	-	-	1.14(0.20-5.70)	4.80(0.80-28.00) [‡]

* p < 0.05; ** p < 0.01; *** p < 0.001. Each OR represents a separate regression model adjusted for a tophy, smoking, born on a farm and years of schooling.

-Odds Ratios not determinable

[†] p < 0.1

Pesticide drift into the home was associated with a non-significant 7% and 10% increase in the odds of storage mite and spider mite sensitisation respectively. Among the farm dwellers only, pesticide drift was associated with a non-significant 12% increase in spider mite sensitisation (Supplementary Table 6).

Multiple logistic regression models of allergic asthma phenotypes (atopic asthma, non-atopic asthma, house dust mite allergic asthma, storage mite allergic asthma and spider mite allergic asthma) which were generated from a combination of current asthma and allergic sensitisation outcomes did not reveal any meaningful information because of the low prevalence of the outcomes (Supplementary Table 1).

Logistic regression models of asthma and ocular nasal symptoms phenotypes indicated that low ChE levels are associated with both asthma with or without ocular nasal symptoms although the association is stronger amongst those without ocular nasal symptoms. A one hundred unit increase in ChE levels, as a continuous variable, was associated with a decrease in the odds of asthma without ocular nasal symptoms (OR= 0.87; 95% CI: 0.77- 0.99) and asthma with ocular nasal symptoms (OR=0.95; 95% CI: 0.82-1.21). There was also a 13- fold increase in the odds of having asthma without ocular nasal for ChE levels below the 25th percentile (Supplementary table 7).

4. Discussion

The findings of this study demonstrated that pesticide exposure, particularly ChE depressing organophosphate and carbamate pesticides, is associated with upper and lower airway symptoms including asthma.

Consistently positive associations were observed between the pesticide exposure variables; farm job, farm residence, household pesticide use and low ChE levels with asthma symptom scores. That the association between asthma symptoms and farm exposure or ChE persists after controlling for household use, and that the majority (78%) of those with low ChE levels are farm workers or residents, suggests that occupational and environmental exposures on farms were independently associated with these outcomes. These results are consistent with the results of previous studies in other settings that have shown high prevalences of these symptoms among farm workers (Hoppin et al., 2002; Innes et al., 1990; Dalvie et al., 1999; Zuskin et al., 1997; Senthilselvan et al., 1992; Mekonnen et al., 2002; Zhang et al., 2002; Hoppin et al., 2009; Beselar et al., 2009; Chakraborty et al., 2009).

The prevalence of ocular nasal symptoms among farm dwellers in our study was 24%, which is much lower than a rhinitis prevalence of 74% in a study by Slager et al. (2009) and 67% in a later study by Slager et al. (2010) among farm workers. It is important to note that the definition of rhinitis varies across studies and this could be the reason for the differences in prevalences. Rhinitis in some epidemiological

studies has been defined as the presence of stuffy, itchy, or runny nose in the last 12 months (Slager et al., 2009, 2010). Chatzi et al. (2007) defines allergic rhinitis as the occurrence of two or more nasal symptoms (eg, rhinorrhoea, sneezing, nasal obstruction and nasal itching) during the last 12 months, apart from a cold. The participants in the other studies were all pesticide applicators whereas in the current study only four women were pesticide applicators. In these studies, cholinesterase depressing pesticides were most prominent but there were also other pesticides associated with rhinitis. The pesticides that were predictors of current rhinitis in other studies included the organophosphates chlorpyrifos, diazinon, dichlorvos, and malathion); carbaryl, a carbamate; permethrin, a pyrethroid insecticide; 2,4-D, a chlorophenoxy acid herbicide; glyphosate, a glycine herbicide; petroleum oil, used as a herbicide; paraquat, a bipyridyl herbicide; captan, a phthalimide fungicide and benomyl, a benzimidazole fungicide (Chatzi et al., 2007; Slager et al., 2009, 2010).

The positive association between low ChE and FeNO > 50 ppb could suggest that exposure to one or more ChE inhibiting organophosphate and/or carbamate pesticides may be contributing to probable allergic asthma. Since pesticide exposure was not associated with sensitisation to any of the mite allergens measured in the study suggests that the pesticides may themselves be respiratory sensitisers causing allergic airway inflammation. Alternatively, this association may be due to an as yet unidentified allergen associated with pesticide exposure. Cholinesterase inhibiting pesticides such as carbaryl and zineb as well as non-cholinesterase inhibiting pesticides such as alachlor and nitrofen have also been shown to directly affect

the immune system through a Th2 dependent mechanism leading to the development of asthma and allergy (Chalubinski et al., 2006). To our knowledge, this is the first study that has investigated the association between depressed cholinesterase levels and elevated FeNO. This relationship should be explored with a longitudinal study that has a larger sample size.

In the current study the association between pesticide exposure and specific asthma phenotypes could not be explored due to the low prevalence of current asthma and lack of power (supplementary Table 1). While this current study did not identify specific pesticides associated with atopic and non-atopic asthma outcomes, the study by Hoppin et al. (2008) of American farm women, demonstrated that the use of organophosphate pesticides such as parathion and malathion was found to be exclusively associated with atopic asthma (OR=1.46; 95% CI: 1.14–1.87). While in a later study Hoppin et al. (2009), found that pesticide use was associated with both adult onset atopic and non-atopic asthma among male farmers. In the latter study, the pesticides associated with atopic asthma included , the organophosphates coumaphos (OR 2.34; 95% CI 1.49–3.70) and parathion (OR 2.05; 95% CI 1.21–3.46) heptachlor (OR 2.01; 95% CI 1.30–3.11), , 80/20 mix (carbon tetrachloride/carbon disulfide) (OR 2.15; 95% CI 1.23–3.76) and ethylene dibromide (OR 2.07; 95%CI 1.02–4.20) while DDT (dichloro-diphenyltrichloroethane), an organochlorine, was the most strongly associated with non-atopic asthma (OR 1.41; 95% CI 1.09–1.84).

In this study spidermite sensitisation was more common among farm dwellers who reported exposure to pesticide drift in homes. However, the lack of an association between low ChE levels and allergic sensitisation to spidermite in this study is in contrast to findings from a previous study among table grape farm workers in the Western Cape. That study demonstrated an association between pesticide exposure (crop sprayers) and those workers with low acetylcholinesterase levels and allergic respiratory disease outcomes due to spidermite, *Tetranychus urticae* (Jeebhay et al., 2007).

Asthma (using different definitions for doctor diagnosed asthma, adult onset asthma and current asthma) was more prevalent among town dwellers. This could be due to the protective effect of being born on the farm for atopic asthma as is well known and also been reported in other studies (Hoppin et al., 2008). Another possible explanation for the lower prevalence of self-reported asthma among the farm dwellers than the town dwellers could be due to the healthy worker effect in that women that worked on farms in the past who had developed asthma, may have left the farm to work in the town. This is a commonly observed phenomenon in cross sectional studies. Furthermore, the relatively higher prevalence of sensitisation to the various mite allergens in the town dweller group suggests that these common aeroallergens compared to the pesticide exposures in the farm dwellers group were mainly responsible for the asthma. This is supported by the results of analyses excluding non-farm women that showed higher prevalences of doctor diagnosed asthma, adult onset asthma and current asthma among those reporting pesticide

drift into homes and those workers who reported immediate re-entry into spraying fields.

The higher prevalence of asthma like symptoms among those with a current or past history of living and/or working on farms with relatively similar allergen sensitisation rates suggests a local irritant effect of the airways may also be responsible for their symptoms. Inhaled pesticides may cause damage to the airways either directly or through neurogenic inflammation which, when sustained over time may lead to nonspecific bronchial hyper-reactivity and the subsequent development of asthmatic symptoms (Hernandez et al., 2011). The latter is supported by the data indicating that farm job and residence and low ChE are associated with asthma with (as commonly seen with high molecular weight proteins) or without ocular nasal symptoms although the association is stronger among those without a history of ocular nasal symptoms. In animal models it was found that organophosphate pesticides can cause asthma through inhibition of acetylcholinesterase or causing parasympathetic prejunctional muscarinic M2 receptor dysfunction leading to bronchial hyper-responsiveness (Hernandez et al., 2011). These are possible mechanisms which could be applicable to human asthma.

The self-reported exposures and ChE levels in this study and also evidence from other studies conducted previously amongst similar communities show that farm workers and residents are exposed to pesticides in the environment in a number of ways (Dalvie et al, 2009 a, b & c; Dalvie and English, 2010; Dalvie et al, 2004; Dalvie et

al., 2003; Dalvie and London, 2001). Although the number of sprayers were low in this study, the percentage of farm workers (11%) that reported the use of empty containers for drinking purposes is higher than that found in previous studies amongst these communities (Dalvie et al, 2001; Dalvie et al, 2003; Dalvie et al, 2004, Holtman et al 2010). Additionally, 67% of farm dwellers reported that workers re-entered the field on the same day after pesticide spraying. Furthermore, nearly a quarter of farm workers reported that PPE was not provided. There were at least three times as many women with low ChE levels (a marker of organophosphate or carbamate pesticide exposures) in the farm dwellers compared to the town dwellers ($p=0.08$). These results demonstrate that these women from the farms are exposed to high levels of pesticides and were at increased risk of developing adverse health effects associated with pesticide exposure. Since exposure/outcome relationships remain when controlling for household pesticide exposure, it is probable that the pesticides sprayed in the field may be largely responsible for the decrease in cholinesterase levels observed in the farm women.

It is likely that pesticide exposures were estimated in this current study in that while sampling participants for the study was not random and represented mostly export farms, the participants were recruited from five major agricultural areas in the Western Cape producing a variety of crops. Although only four women in the study were applicators, the participants reported that women worked as pesticide applicators in nearly 20% of farms. This suggests that the number of women applicators in our study was not representative of the study population and that the

true extent of exposure may have been underestimated. This study could also have been vulnerable to exposure misclassification. Among the women living in towns, there were eight women who were working on farms so these women had to be grouped with farm dwellers thereby reducing the comparison group. There were also 26 women who were town dwellers but had previously lived or worked on a farm. This was, however, adjusted for in the analysis by generating the variable 'history of living or working on farm' which included the town dwellers that had previously lived or worked on a farm. Since all the known confounders were adjusted for in the analysis, there was a reasonable degree of comparability between exposed and unexposed groups.

Despite the potential exposure misclassification based on sampling or subjective reporting, the study also indicated more objective markers for organophosphate and carbamate pesticide exposure. ChE levels in whole blood, which is the sum of serum butyl cholinesterase and erythrocyte cholinesterase, is a validated method for measuring cholinesterase (Haigh et al., 2008). Measuring blood cholinesterase levels is considered a reliable biomarker for pesticide exposure in the past three months and screening for cholinesterase inhibition as a result of exposure to organophosphate pesticides is recommended or specifically mandated as has been reported for California pesticide applicators and handlers (McCauley et al 2006). However, interpretation of ChE monitoring results is complicated by variation in enzymatic activity within an individual or between individuals and the presence of other confounding factors such as liver disease. Exposure to large doses of organophosphate pesticides is required for significant acetylcholinesterase

inhibition to occur, and therefore, it is more appropriately used as an indicator of toxicity at high rather than low pesticide exposure levels (McCauley et al 2006). This means that this method could possibly underestimate the number of individuals exposed to pesticides if the exposure levels are not high enough. It is ideal to monitor the change in a person's cholinesterase levels during the non-spraying and spraying season but in our current study blood samples were collected only once off during spraying season. Despite these limitations the consistent positive associations between depressed cholinesterase levels and asthma outcomes suggests a strong role in these outcomes. While bio-monitoring for specific pesticides is a more sensitive method for measuring pesticide exposure, resource constraints posed limitations in pursuing this further. However, urinary samples collected during this study are in storage and will be analysed at a future stage.

Another limitation of this study is the lack of multiple objective markers for detecting asthma. In this study, FeNO was the only objective marker for asthma measured. FeNO measures allergic airway inflammation which indicates the presence of allergic asthma. It is possible therefore that the non-allergic asthma phenotypes were not well characterised. Other objective markers for lung function and airway hyperresponsiveness, such as spirometry and methacholine challenge tests respectively were not used due to similar resource constraints. Despite these limitations, the use of the ECRHS questionnaire contributed towards obtaining reasonable estimates of asthma in this population as it is a validated instrument that is widely used in epidemiological studies of adult asthma.

This study was also limited by the small sample size, which could have contributed to the wide confidence intervals in our study. The study design was a cross sectional study which is not useful for investigating disease development

5. Conclusions and Recommendations

This study provided evidence that occupational and environmental exposure to pesticides of women working and/or living on farms in the Western Cape, particularly cholinesterase inhibiting organophosphate and carbamate pesticides, is associated with asthma through possibly more than one pathophysiological mechanism.

Health and safety issues as well as labour issues should be addressed on farms to reduce pesticide exposures of women. For instance, regular screening for cholinesterase inhibition as a result of exposure to organophosphate pesticides, together with respiratory symptom questionnaires can be introduced among high risk workers. Simple strategies such as delaying re-entry into farms after spraying can also reduce the risk of the respiratory effects. The high pesticide exposures among the farm women call for tighter regulation of pesticide spraying and the sale of pesticides as they are currently readily available and subject to abuse. Toxic release and use inventories on farms should be documented regularly. Education of farmers, farm management and farm workers on the use of pesticides and their environmental and health effects is vitally important. There is also a need for the implementation of integrated pest management methods.

Future studies need to consider stronger study designs such as a large scale cohort study of farm workers and residents including a larger number of pesticide applicators. Furthermore studies need to incorporate more sensitive outcome and exposure measures including bio-monitoring for specific pesticides. There is need also to expand the population base of study to include more women, children and populations from developing countries.

7. Acknowledgements

This study was supported by the Women on Farms Project and the University of Cape Town (Prof. Jeebhay Allergy Research Fund). Vuyelwa Ndlovu is sponsored by the Centre for International Health of the Ludwig-Maximilian-University, Munich, Germany (CIHLMU).

8. References

- Alarcon, W.A., Calvert, G.M., Blondell, J.M., et al., 2005. Acute illnesses associated with pesticide exposure at schools. *JAMA* . 294(4): 455-465.
- ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005;171:912–930.
- ATS/ERS, 2005. Standardisation of Lung Function Testing. Standardisation of Spirometry. *Eur Respir J*. 26:319–338.
- Beseler CL, Stallones L. 2009. Pesticide poisoning and respiratory disorders in Colorado farm residents. *J Agric Saf Health*. 15(4):327-334.

- Bowers ,Y., London, L., Holtman, Z., Messina, S., Arendse, B., Lindoor, E., Rhodes, G., 2009. Obstacles to the rights of access to health care for farm worker women in the Western Cape. *Critical Health Perspectives* .
- Burney,P.G.J., Luczynska, P., Chinn, S., et al.,1994. The European Community Respiratory Health Survey. *Eur Respir J*.7: 954–960.
- Chakraborty, S., Mukherjee, S., Roychoudhury, S., Siddique, S., Lahiri, T., Ray, M.R. 2009. Chronic exposures to cholinesterase-inhibiting pesticides adversely affect respiratory health of agricultural workers in India. *J Occup Health*. 51(6):488-497.
- Chalubinski, M., Kowalski, M.L. 2006. Endocrine disruptors – potential modulators of the immune system and allergic response. *Allergy*. 61: 1326–1335.
- Chatzi L., Alegakis, A., Tzanakis, N., Siafakas ,N., Kogevinas, M., Lionis, C., 2007. Association of allergic rhinitis with pesticide use among grape farmers in Crete, Greece. *Occup Environ Med*. 64(6): 417-421.
- Dalvie, M.A., White ,N., Raine, R., Myers, J.E., London ,L., Thompson ,M., Christiani, D.C.,1999. Long-term respiratory health effects of the herbicide, paraquat, among workers in the Western Cape. *Occup. Environ. Med*. 56(6):391-396.
- Dalvie, M.A., Cairncross, E., Solomon, A., London, L.,2003. Contamination of rural surface and ground water by endo-sulfan in farming areas of the Western Cape, South Africa. *Environ Health*.2:1.
- Dalvie, M.A., Myers, J.E., Thompson, Mary Lou, Robins, T.G., Dyer, S., 2004. The hormonal effects of long term DDT exposure on malaria vector control workers in Limpopo Province, South Africa. *Environmental Research*. 96(1):9-19.
- Dalvie, M.A., London, L., 2006. The impact of aerial application of organophosphates on the cholinesterase levels of rural residents in the Vaalharts district, Northern Cape Province, South Africa. *Environ Res*.102(3):326-332.
- Dalvie, M.A., Africa, A., Solomons, A., London, L., Brouwer, D., Kromhout, H.. 2009a. Pesticide exposure and blood endosulfan levels after first season spray amongst farm workers in the Western Cape, South Africa. *J Environ Sci Health B*.44(3):271-277.
- Dalvie ,M.A., London, L., 2009b. Risk assessment of pesticide residues in South African raw wheat. *Crop protection*.28(10):864-869
- Dalvie, M.A., Africa, A., London, L., 2009c. Change in the quantity and acute toxicity of pesticides sold in South African crop sectors. 1994-1999. *Environ Int*. 35 (4).

- Dalvie, M.A., Naik, I., Channa, K., London, L., 2011. Urinary dialkyl phosphate levels before and after first season chlorpyrifos spraying amongst farm workers in the Western Cape, South Africa. *J Environ Sci Health B*. 46 (2): 163-172.
- Ellman GL, et al., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol*.7:88-95
- Faria, N.M., Facchini, L.A., Fassa, A.G., Tomasi, E., 2005. Pesticides and respiratory symptoms among farmers. *Rev Saude Publica*.39(6):973-981.
- Fieten, K.B., Kromhout, H., Heederik, D., van Wendel de Joode B., 2009. Pesticide exposure and respiratory health of indigenous women in Costa Rica. *Am J Epidemiol*.169(12):1500-1506.
- Forastieri, V., 1999. The ILO Programme on Occupational Safety and Health in Agriculture. SafeWork. International Labour Office, Geneva. URL: <http://www.ilo.org/public/english/protection/safework/agriculture/agrivf01.htm>
- Garcia, A.M., 2003. Pesticide exposure and women's health. *Am J Ind Med*.44(6): 584-594.
- Haigh, J.R., Lefkowitz, L.J., Capacioc, B.R., Doctora, B.P., Gordon, R.K. 2008. Advantages of the WRAIR whole blood cholinesterase assay: Comparative analysis to the micro-Ellman, Test-mate ChETM, and Michel (delta pH) assays. *Chemico-Biological Interactions*. 175: 417-420
- Hoppin, J.A., Umbach, D.M., London, S.J., Alavanja, M.C., Sandler, D.P., 2002. Chemical predictors of wheeze among farmer pesticide applicators in the Agricultural Health Study. *Am J Respir Crit Care Med*.165(5):683-689.
- Hoppin, J.A., Umbach, D.M., London, S.J., Lynch, C.F., Alavanja, M.C., Sandler, D.P., 2006. Pesticides associated with wheeze among commercial pesticide applicators in the Agricultural Health Study. *Am J Epidemiol*.163(12):1129-1137.
- Hoppin, J.A., Umbach, D.M., London, S.J., et al., 2008. Pesticides and atopic and nonatopic asthma among farm women in the Agricultural Health Study. *Am J Respir Crit Care Med*.177(1):11-18.
- Innes, D.F., Fuller, B.H., Berger, G.M., 1990. Low serum cholinesterase levels in rural workers exposed to organophosphate pesticide sprays. *S Afr Med J*.78(10):581-583
- Jeebhay, M.F., Baatjies, R., Chang, Y.S., et al. 2007. Risk factors for allergy due to the two-spotted spider mite (*Tetranychus urticae*) among table grape farm workers. *Int Arch Allergy Immunol*.144(2):143-149.

- Jeebhay, M.F., Adewole, O.O. 2011. Work- related asthma. *Asthma in Africa*. Book chapter in press.
- London, L., 2003. Human Rights, Environmental Justice and the Health of Farm Workers in South Africa. *Int J Occup Environ Health*.9:59-68 (Online) Available http://www.15by2015.org/wpcontent/uploads/2008/01/0901_london_south_africa.pdf
- London, L., de Grosbois, S., Wesseling, C., Kisting, S., Rother, H., Mergler, D., 2002. Pesticide usage and health consequences for women in developing countries: out of sight, out of mind? *Int J Occup Environ Health*. 8(1): 46-59.
- London, L., 1998. Occupational epidemiology in agriculture: A case study in the Southern African context. *Int J Environmental and Occupational Health* 4:245-256
- McCauley LA, Anger WK, Keifer M, et al. 2006. Studying Health Outcomes in Farmworker Populations Exposed to Pesticides. *Environ Health Perspect*. 114:953–960. doi:10.1289/ehp.8526
- Mekonnen Y, Agonafir T.2002. Effects of pesticide applications on respiratory health of Ethiopian farm workers. *Int J Occup Environ Health*. 8(1):35-40.
- Naidoo, V., Buckley, C.A., 2003. Survey of pesticide wastes in South Africa and review of treatment options. Water Research Commission (WRC) Report No. 1128/1/03. Pretoria: WRC.
- Ndlovu, V., Dalvie, M.A., Jeebhay, M.F., 2011. Pesticides and the airways- A review of the literature. *Current Allergy and Clinical Immunology*. 23(4)
- Pekkanen, J., Sunyer J., Anto J.M., Burney P. on behalf of the European Community Respiratory Health Study (ECRHS). 2005. Operational definitions of asthma in studies on its aetiology. *Eur Respir J*. 26: 28–35 DOI: 10.1183/09031936.05.00120104
- Quirce, S., Lemiere, C., de Blay, F., et al, 2009: Noninvasive methods for assessment of airway inflammation in occupational settings. *Allergy*
- Rother, H.A., 2008. South African farm workers' interpretation of risk assessment data expressed as pictograms on pesticide labels. *Environ Res*. 108(3):419-27
- Senthilselvan, A., McDuffie, H.H., Dosman, J.A., 1992. Association of asthma with use of pesticides. Results of a cross-sectional survey of farmers. *Am Rev Respir Dis*.146(4):884-887.

- Schulz, R., 2001. Comparison of spray drift-and runoff-related input of azinphos-methyl and endosulfan from fruit orchards into the Lourens River, South Africa. *Chemosphere*. 45(4-5):543-551
- Schulz, R., 2001. Rainfall-induced sediment and pesticide input from orchards into the Lourens River, Western Cape, South Africa: importance of a single event. *Water Res*. 35(8):1869-1876
- Slager, R.E., Simpson, S.L., Levan, T.D., Poole, J.A., Sandler, D.P., Hoppin, J.A., 2010. Rhinitis associated with pesticide use among private pesticide applicators in the agricultural health study. *J Toxicol Environ Health A*. 73(20): 1382-1393.
- Sunyer J., Pekkanen J., Garcia-Esteban, R., Svanes, C., Anto J.M., Burney, P. et al. 2007. Asthma score: predictive ability and risk factors. *Allergy*. 62: 142–148
- Van den Driessche, K.S.J., 2010. Anaphylaxis in an airplane after insecticide spraying. *J Travel Med*. 17(6): 427
- Vizcaya, D., Mirabelli, M.C., Anto´ JM., Orriols, R., Burgos, F., Arjona, L., Zock, JP. 2011. A workforce-based study of occupational exposures and asthma symptoms in cleaning workers. *Occup Environ Med*. 68:914-919. doi:10.1136/oem.2010.063271
- Zhang, L.X., Enarson, D.A., He, G.X., Li, B., Chan-Yeung, M., 2002. Occupational and environmental risk factors for respiratory symptoms in rural Beijing, China. *Eur Respir J*. 20(6): 1525-1531.
- Zhang, X., Zhao, W., Jing, R., et al., 2011. Work-related pesticide poisoning among farmers in two villages of Southern China: a cross-sectional survey. *BMC Public Health*. 11: 429.
- Zuskin E, Mustajbegovic J, Schachter EN, Kern J, Pavicic D: Respiratory function in vineyard and orchard workers. *Am J IndMed* 1997; 31: 250–255.

SUPPLEMENTARY TABLES TO JOURNAL MANUSCRIPT

The supplementary tables have been numbered in the order in which they are cited in the manuscript.

University of Cape Town

Supplementary Table 1

Adjusted multiple logistic regression models of pesticide exposure indicators associated with asthma phenotypes among women farm workers in the Boland of the Western Cape

	Allergic Asthma phenotypes. Odds Ratio (95% Confidence Interval)				
	Atopic asthma	Non-atopic asthma	House dust mite allergic asthma	Storage mite allergic asthma	spider mite allergic asthma
Prevalence (%) (n=211)	5	1	3	3	3
Household pesticide use	0.60(0.16-2.30)	-	0.73(0.15-3.50)	0.73(0.15-3.50)	0.37(0.065-2.06)
History of living or working on farms	0.28(0.06-1.25)	0.34(0.02-6.53)	0.46(0.07-2.89)	0.46(0.07-2.89)	0.17(0.03-1.09)
Currently Living and working on farms	0.26(0.06-1.09)	0.72(0.04-12.13)	0.48(0.10-2.35)	0.48(0.10-2.35)	0.23(0.04-1.35)
Exposure to pesticide drift	-	-	-	-	-
Permanent vs seasonal farmworker	-	-	-	-	-
Immediate vs Delayed re-entry	-	-	-	-	-
Number of years in current farm job	0.79(0.56-1.12)	1.11(0.96-1.28)	0.84(0.62-1.13)	0.84(0.62-1.13)	0.60(0.16-2.15)
Number of days of pesticide spraying/year in current farm	0.99(0.98-1.00)	-	1.00(0.99-1.01)	1.00(0.99-1.01)	1.00(0.98-1.00)
Cholinesterase levels < laboratory reference standard	-	-	-	-	-

* p < 0.05; ** p < 0.01; *** p < 0.001.

Each OR represents a separate regression model adjusted for smoking, and years of schooling.

-Odds Ratios not determinable

Supplementary Table 2.

Unadjusted negative binomial regression models of host factors and other potential confounders associated with asthma symptom score among women farm workers in the Boland of the Western Cape

	Asthma symptom score
	Mean Ratio, MR(95% CI)
Age (years)	1.00 (0.99-1.02)
BMI (kg/m ²)	1.01 (0.99-1.04)
Atopy (Positive Phadiatop)	1.22(0.84-1.78)
Education (years of schooling)	0.89(0.84-0.94)***
Currently employed	1.09(0.74-4.96)
Household income/month(\$US)	0.99(0.99-1.00)
Born on a farm	1.45(1.01-2.10)*
Drinks alcohol	1.30(0.89-1.90)
Current smoker	1.38(0.62-2.00) ^a

*p < 0.05; **p < 0.01; ***p < 0.001

Negative binomial regression models MR, the ratio of the mean score among exposed and nonexposed.

^ap=0.07

University of Cape Town

Supplementary Table 3

Adjusted multiple logistic regression models of pesticide exposure indicators associated with asthma outcomes among women farm workers in the Boland of the Western Cape

	Asthma outcomes. Odds Ratio (95% Confidence Interval)						
	Ocular nasal symptoms	Current asthma	Doctor diagnosed asthma	Adult onset asthma	FENO=25ppb-50ppb	FENO>50ppb	Asthma score ^a
History of living or working on farms	1.67(0.58-4.86)	0.70(0.15-3.23)	0.68(0.20-2.25)	0.53(0.14-2.02)	1.26(0.42-3.76)	1.20(0.30-4.85)	2.24(1.31-3.83)**
Currently Living and working on farms	0.89(0.37-2.14)	0.64(0.14-2.90)	0.81(0.28-2.30)	0.78(0.25-2.42)	1.48(0.53-4.15)	2.02(0.50-8.16)	2.28(1.47-3.53)***
Pesticide drift into the home ^b	1.66(0.65-4.24)	-	1.04(0.26-4.15)	1.11(0.25-4.94)	0.44(0.12-1.67)	0.20(0.02-1.92)	1.57(1.06-2.32)

* p < 0.05; ** p < 0.01; *** p < 0.001. Each OR represents a separate regression model adjusted for atopy, smoking, born on a farm, years of schooling and household pesticide use.

Negative binomial regression used for asthma score. It models MR, the ratio of the mean score among exposed and nonexposed.

^b sub analysis of farm dwellers only with each OR representing a separate regression model adjusted for atopy, smoking, born on a farm and years of schooling.

Supplementary Table 4

Unadjusted and adjusted linear regression models of pesticide exposure indicators associated with asthma outcomes among women farm workers in the Boland of the Western Cape

Pesticide exposure variable	Unadjusted log FENO	Adjusted log FENO ^a
	β coefficient (95% CI)	β coefficient (95% CI)
Use pesticides at home	0.08(-0.01-0.18)	0.03(-0.05-0.12)
History of living or working on farms	-0.18(-0.28- -0.08)**	-0.06(-0.18-0.06)
Currently living and working on farms	-0.12(-0.21- -0.02)*	0.01(-0.09-0.11)
Pesticide drift into the home	-0.12(-0.22- -0.02)*	-0.05(-0.15-0.05)
Permanent vs seasonal farmworker	0.12(-0.02-0.26)	0.05(-0.08-0.17)
Re-entry into sprayed fields	-0.02(-0.16-0.12)	-0.24(-0.16-0.10)
Immediate vs Delayed re-entry		
Number of years in current farm job	-0.01(-0.01- -0.02)**	-0.01(-0.01-0.01)
Number of days of pesticide spraying/year in current farm	-0.01(-0.01- -0.01)	-0.01(-0.01-0.01)
Cholinesterase levels < laboratory reference standard	0.13(-0.04-0.30)	0.09(-0.06-0.25)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

^aEach β coefficient represents a separate linear regression model adjusted for atopy, born on a farm, smoking, and years of schooling.

University of Cape Town

Supplementary Table 5

Unadjusted logistic regression models of host and other potential confounders associated with allergic sensitisation outcomes among women farm workers in the Boland of the Western Cape

	Allergic sensitisation outcomes Odds Ratio (95% Confidence Interval)			
	House dust mite sensitisation	Storage mite sensitisation	Spider mite sensitisation	Atopy (Positive Phadiatop)
Age (years)	0.99(0.97-1.02)	1.01(0.98-1.04)	1.01(0.98-1.04)	1.00(0.98-1.03)
BMI (kg/m ²)	1.02(0.97-1.06)	0.99(0.95-1.04)	0.97(0.91-1.02)	1.02(0.98-1.06)
Atopy (Positive Phadiatop)	81.65(18.97-351.47)***	-	11.00(4.06-29.83)***	-
education (years of schooling)	0.95(0.86-1.05)	0.93(0.84-1.03)	0.92(0.82-1.03)	0.97(0.89-1.06)
Currently Employed	0.71(0.38-1.32)	0.72(0.37-1.42)	0.47(0.23-0.98)*	0.58(0.33-1.02)
Household income /month(\$US)	1.00(1.00-1.01)	1.00(1.00-1.01)	1.00(1.00-1.01)	1.00(1.00-1.01)
Born on a farm	0.42(0.22-0.81)**	0.60(0.30-1.19)	0.73(0.35-1.54)	0.64(0.37-1.12)
Drink alcohol	1.33(0.71-2.49)	0.89(0.46-1.74)	2.12(0.96-4.68)	1.26(0.72-2.20)
Current smoker	1.38(0.74-2.57)	1.98(1.00-3.93)	1.57(0.75-3.30)	1.44(0.83-2.50)

*p < 0.05; **p < 0.01; ***p < 0.001.

Each OR represents a separate unadjusted logistic regression model

-OR not determinable

Supplementary Table 6

Adjusted multiple logistic regression models of pesticide exposure indicators associated with allergic sensitisation outcomes among women farm workers in the Boland of the Western Cape

Prevalence (%) (n=211)	Allergic sensitisation outcomes Odds Ratio (95% Confidence Interval)			
	House dust mite sensitisation 27	Storage mite sensitisation 21	Spider mite sensitisation 17	Atopy (Positive Phadiatop) ^a 44
Use pesticides at home	1.38(0.56-3.38)	1.86(0.72-4.80)	0.76(0.33-1.75)	1.12(0.63-2.00)
History of living or working on farms	1.33(0.43-4.12)	0.72(0.23-2.28)	0.48(0.16-1.47)	0.98(0.45-2.12)
Currently Living and working on farms	2.26(0.80-6.40)	1.20(0.44-3.29)	0.90(0.35-2.28)	0.64(0.33-1.24)
Pesticide drift into the home	0.51(0.19-1.33)	1.07(0.39-2.90)	1.10(0.44-2.78)	0.91(0.47-1.75)
Pesticide drift in the farm dweller group only	0.14(0.03-0.61)	0.87(0.24-3.08)	1.12(0.36-3.51)	
Permanent vs seasonal farmworker	0.48(0.12-1.89)	1.49(0.37-5.92)	0.60(0.17-2.19)	1.77(0.76-4.13)
Immediate vs Delayed re-entry	0.99(0.26-3.69)	0.40(0.10-1.65)	1.06(0.31-3.66)	0.94(0.41-2.16)
Number of years in current farm job	1.02(0.96-1.08)	1.01(0.95-1.07)	0.96(0.90-1.02)	0.99(0.95-1.03)
Cholinesterase levels < laboratory reference standard	1.48(0.29-7.70)	1.38(0.25-7.60)	0.83(0.16-4.35)	0.75(0.26-2.16)

* p < 0.05; ** p < 0.01; *** p < 0.001. Each OR represents a separate regression model adjusted for atopy, smoking, born on a farm and years of schooling.

^aEach OR represents a separate regression model adjusted for smoking, born on a farm and years of schooling.

Supplementary table 7

Adjusted multiple logistic regression models of pesticide exposure indicators associated with asthma and ocular nasal symptoms phenotypes among women farm workers in the Boland of the Western Cape

Prevalence % (n=211)	Asthma phenotypes. Odds Ratio (95% confidence Interval)	
	Asthma with ocular nasal symptoms	Asthma without ocular nasal symptoms
	3	3
Use pesticides at home	0.61(0.09-4.21)	0.32(0.05-2.15)
History of living or working on farms	0.34(0.03-4.18)	0.95(0.16-5.85)
Currently Living and working on farms	0.46(0.06-3.64)	1.00(0.16-6.25)
Pesticide drift into the home	-	-
Permanent vs seasonal farmworker	-	0.61(0.03-11.73)
Immediate vs Delayed re-entry	-	-
Number of years in current farm job	1.04(0.93-1.16)	0.91(0.71-1.18)
Number of days of pesticide spraying/year in current farm	-	1.01(1.00-1.02)
Cholinesterase IU x100	0.95(0.82-1.21)	0.89(0.78-1.00)
Cholinesterase levels < laboratory reference standard	-	-
cholinesterase (<10 th percentile)	-	-
cholinesterase (<25 th percentile)	-	13.47(1.72-105.68)*

* p < 0.05; ** p < 0.01; *** p < 0.001. Each OR represents a separate regression model adjusted for a topy, smoking, born on a farm and years of schooling.

-Odds Ratios not determinable

PART D: APPENDICES

University of Cape Town

Appendix 1a

English Questionnaire

University of Cape Town

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											

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)

5.7.8 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)

Bronchitis _____ (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 1b

Afrikaans Questionnaire

University of Cape Town

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 Landlyn telefoon		
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 Egaenoot/leuensmaat		
C Ouers		
D Broers en/of susters		
E Kinders		
F Kinderonderhoudstoelae		
G Staatsouderdomspensioen		
H Onaeskiktheidstoelae		
I Soraafhanklikheidstoelae		
J Pleeasoratoelae		
K Hulntoelae		
L Veraoedina vir heroensbeserinas		
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 Gedurende watter maande van die jaar, ly u honger? _____

_____ (maande van die jaar)

Afdeling 4. LEWENS GESKIEDENIS

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, waartoe 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/Nee)

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 gedurende 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 ver wag 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 tabel

	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 aangewend 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, waarmatoe 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 Wanneerlaas 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 gedurende 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 Watterklere 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 hoeveeljare _____ (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. MEDIESIE, 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 ge oefen 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)

10.16 Hoeveel keer per week het u seks ? _____

- 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)											
Gedurende die maand van u swangerskap, Het u of u man enige vorm van geboortebeperkings gebruik? (Ja/Nee)											
Metode van geboortebepeking*											
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 gesondheidsdienste 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 probleme 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 gedurende 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 probleme 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 probleme 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

University of Cape Town

Appendix 2

Exhaled Nitric Oxide Pre-test Data Collection Sheet

University of Cape Town

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 hay fever?

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.

University of Cape Town

Appendix 3

Exhaled Nitric Oxide Data Collection Sheet

University of Cape Town

**UCT OCCUPATIONAL ALLERGY AND ASTHMA STUDY AMONG WOMEN ON FARMS PROJECT
IN THE WESTERN CAPE PROVINCE OF SOUTH AFRICA - 2006 2009**

EXHALED NITRIC OXIDE DATA COLLECTION SHEET

Date: _____

Time _____

Ambient NO concentration (ppb) _____

Ambient temperature (degrees celcius) _____

Survey Number _____

1. Subject's blood pressure systolic _____

diastolic _____

2. Subject's age (in years) _____

3.1 Subject's height (in centimetres) _____

3.2 Subject's weight (in kilograms) _____

4. Gender: Male (1)

Female (2)

5. Effort number (start) _____

6.1 FENo measurement (ppb) 1st effort _____

6.2 FENo measurement (ppb) 2nd effort _____

6.3 FENo measurement (ppb) 3rd effort _____

Appendix 4
Consent Form

University of Cape Town

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:** You 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 from 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 5a

Letter of Approval from Research Ethics Committee

University of Cape Town



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: shurecta.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.

Yours sincerely

**PROFESSOR M BLOCKMAN
CHAIRPERSON, HSE HUMAN ETHICS**

Federal Wide Assurance Number: FW/A00001637.
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.

S Thomas

Appendix 5b

Health Research Committee Annual Progress Report

University of Cape Town

Annual Progress Report

Date	31/1/2012
HREC REF Number	293 / 2009
Protocol number (if applicable) & Protocol title	HEALTH EFFECTS DUE TO PESTICIDE EXPOSURE AMONGST RURAL WOMAN RESIDENTS IN THE WESTERN CAPE
Principal Investigator	MOHAMED ABDEL PALVIC
Department / Office Internal Mail Address	

List of documentation

NOT APPLICABLE

RESEARCH ETHICS COMMITTEE

2012 -02- 02

HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

HREC office use only (FWA00001637; IRB00001938)			
<input checked="" type="checkbox"/> Approved	<input checked="" type="checkbox"/>	This serves as notification of annual approval, including all documentation described above.	
<input type="checkbox"/> Not approved		See attached comments.	
Type of review	<input checked="" type="checkbox"/> Expedited	<input type="checkbox"/> Full committee	
Expiry date	15 FEBRUARY 2013		
Signature Chairperson of the HREC		Date	3/2/12

Appendix 6

Table of Epidemiology Studies for the Structured Literature Review

University of Cape Town

Table 1 Summary of epidemiological studies investigating the effect of pesticides on rhinitis, asthma and asthma symptoms

Author, year	Population (n), Design, country, City	Exposure Assessment	Outcome measurements	Summary of findings
Abu Sham'a et al 2010	Cross-sectional study of 250 male farmers aged 22 to 77 years, in Palestine	Standardised exposure questionnaire	Respiratory symptoms and spirometry.	No association between pesticides and lung or respiratory symptoms found
Slager et al 2010	Cross-sectional data on rhinitis in the past year and pesticide use from 21,958 Iowa and North Carolina farmers in the Agricultural Health Study	Questionnaire	Rhinitis questionnaire	67% percent of farmers reported current rhinitis. The herbicides glyphosate (OR) = 1.09, 95% CI = 1.05-1.13] and petroleum oil (OR = 1.12, 95% CI = 1.05-1.19) were associated with current rhinitis and increased rhinitis episodes. Of the insecticides, (chlorpyrifos, diazinon, dichlorvos, and malathion), carbaryl, and use of permethrin on animals were predictors of current rhinitis. The fungicide captan was also a significant predictor of rhinitis
Chakraborty et al 2009	Cross sectional study of 724 non smoking males from rural eastern India (median age 41yrs, 376 exposed to OP and carbamates, 348 age and sex matched unexposed)	Acetylcholinesterase (Ellman method)	BMRC and ATS Questionnaire, spirometry, GOLD criteria measuring COPD	Higher prevalence of lower and upper respiratory symptoms and reduced spirometric measurements in exposed. 10.9% of exposed had COPD vs 3.4% in controls, AChE lowered by 34.2% in exposed
Fieten et al 2009	Cross-sectional study of plantation worker women in Costa Rica, n =127	Questionnaire used to estimate exposure, Exposed women, n=69 Unexposed women, n=58	Questionnaire used to estimate respiratory symptoms. Spirometry tests to measure FVC, FEV	Among the exposed, prevalence of wheeze was 20% and of shortness of breath was 36% versus 9% and 26%, respectively, for the unexposed. Among non-smokers, reported exposures to chlorpyrifos (n = 25) and terbufos (n=38) were strongly associated with wheeze (odd ratio =6.7, 95% c i: 1.6, 28.0; odds ratio = 5.9, 95% c i: 1.4, 25.6, respectively)

Table 1 continued: Summary of epidemiological studies investigating the effect of pesticides on **rhinitis, asthma and asthma symptoms**

Author, year	Population (n), Design, country, City	Exposure Assessment	Outcome measurements	Summary of findings
Hoppin et al 2009	Case/control study of male farmers (n = 19704), in the Agricultural Health Study, USA	Personal use of 50 specific pesticides (years of pesticide use, frequency of application.)	Adult-onset asthma, defined as doctor-diagnosed asthma after the age of 20 yrs, Categorized as allergic (n = 127) or non-allergic (n = 314) based on history of eczema or hay fever	For allergic asthma, coumaphos (OR 2.34; 95% CI 1.49–3.70), heptachlor (OR 2.01; 95% CI 1.30–3.11), parathion (OR 2.05; 95% CI 1.21–3.46), 80/20 mix (carbon tetrachloride/carbon disulfide) (OR 2.15; 95% CI 1.23–3.76) and ethylene dibromide (OR 2.07; 95% CI 1.02–4.20). For non-allergic asthma, DDT (dichlorodiphenyltrichloroethane) showed the strongest association (OR 1.41; 95% CI 1.09–1.84)
Hernandez et al 2008	Cross sectional study of workers from the agriculture area of southern Spain. 89 pesticide sprayers and a control group of 25 non-spraying control farmers from the same area.	Structured exposure questionnaire and serum cholinesterase levels was measured	Pulmonary function tests, including spirometry, lung volumes, and diffusing capacity for carbon monoxide. Clinical symptoms by using a structured questionnaire	Exposure to bipyridilium-class herbicides associated with a fall in the diffusing capacity of the lungs, and neonicotinoid insecticides associated with lower pulmonary volumes
Hoppin et al 2008	Case control study of 25 814 farm women (> 20 years) from Agricultural Health Study (USA) including 282 with atopic asthma and 420 with non-atopic asthma	Exposure defined as lifetime use of 48 pesticides, frequency of use. Exposure measured by answering yes/no to question	Self-reported history of doctor-diagnosed asthma with or without eczema and/or hay fever	Growing up on a farm was protective for a topic asthma (odds ratio [OR], 0.55; 95% CI, 0.43–0.70) and, to a lesser extent, for non-atopic asthma (OR, 0.83; 95% CI, 0.68–1.02; Pesticide use was almost exclusively associated with atopic asthma. (OR, 1.46; 95% CI, 1.14–1.87).
Swaen et al 2008	European multi centre prospective cohort study, n=479 adults working in farms	Exposure to EDBC was measured by questionnaire and measurement of urinary ethylenethiourea (ETU) at baseline and after 30 days of exposure.	Questionnaire on allergy, Phadiatop, testing, and specific IgE parameters. These data were also collected at baseline and after 30 days of exposure	No association was found between exposure status, EDBC levels and allergic contact dermatitis, allergic rhinitis, food allergy or atopy as measured by the Phadiatop
Del Prado-Lu, 2007	Cross-sectional study of cutflower farmers aged 15-68 in La Trinidad, Benguet, n=102	Questionnaire on pesticide usage. Blood cholinesterase. Exposed to meta ldehyde methi ocarb, mancozeb profenofos, avermectin methamidophos diazinon lambda cyhalothrin	Individual physical health assessment done by medical doctors	40.2% of exposed farmers had cough

Table1 continued: Summary of epidemiological studies investigating the effect of pesticides on rhinitis, asthma and asthma symptoms

Author, year	Population (n), Design, country, City	Exposure Assessment	Outcome measurements	Summary of findings
Jeebhay et al 2007	A cross-sectional study was conducted on 207 workers employed in table grape farms in the Hex River valley in the Western Cape of South Africa	AChE levels were determined using a finger prick method, using the Test-mate ChE Cholinesterase Test System (model 460; EQM Research Inc.)	a standard abbreviated questionnaire based on the European Community Respiratory Health Survey questionnaire, skin prick tests and IgE quantification using ImmunoCAP system	Work-related wheeze (26%), ocular-nasal (24%) and urticaria/skin symptoms (14%) were more prevalent in the orchards. Sensitization to <i>T. urticae</i> (22%), house dust mite (16%), and 25% were atopic. The prevalence of allergy to <i>T. urticae</i> was 9.5%. Work-related ocular-nasal (OR = 4.9) and skin (OR = 3.7) symptoms were more commonly reported by pesticide crop sprayers. Workers with <i>T. urticae</i> -allergic rhino-conjunctivitis and probable asthma were more likely to be atopic, spray pesticides and have lower erythrocyte cholinesterase levels
Chatzi et al 2007	Cross-sectional study of 120 grape farmers and 100 controls at the Malevisi region in Northern Crete, Greece	Questionnaire, skin prick tests for 16 common allergens, measurement of specific IgE antibodies against 8 allergens,	Questionnaire for allergic rhinitis symptoms, spirometry before and after broncho-dilatation.	Grape farmers exposed to pesticides had higher prevalence rates of allergic rhinitis symptoms (OR, 3.0; 95% CI, 1.4 to 6.2) compared with controls. The highest risks were observed for paraquat and other bipyridyl herbicides (OR, 2.2; 95% CI, 1.0 to 4.8), dithiocarbamate fungicides (OR, 2.5; 95% CI, 1.1 to 5.3) and carbamate insecticides (OR, 3.0; 95% CI, 1.4 to 6.5).
Hoppin et al 2006	Cross sectional study of Iowa male commercial pesticide applicators enrolled in the Agricultural Health Study, n=2255, aged 17-83	Assessed exposure to 40 different pesticides using questionnaire	Outcome of interest was wheeze in the past year based on the participant's response in questionnaire	Prevalence of wheeze 21%. Association between wheeze and: Herbicide, chlorimuron-ethyl (OR= 1.62, 95% (CI): 1.25, 2.09). Phorate, an OP insecticide, (OR=2.87, 95% CI: 1.70, 4.84).

Table1 continued: Summary of epidemiological studies investigating the effect of pesticides on rhinitis, asthma and asthma symptoms

Author, year	Population (n), Design, country, City	Exposure Assessment	Outcome measurements	Summary of findings
LeVan et al 2006	Population-based cohort of adults aged 45-74 years from the Singapore Chinese Health Study, n = 52325	Occupational exposure information was collected at enrolment	Information on respiratory outcomes was obtained during follow-up interviews over a 5 year period	Vapour exposure from chemical solvents, including pesticides was associated with non-chronic cough or phlegm (OR = 1.14, 95% CI: 1.03, 1.27), chronic dry cough (OR = 1.55, 95% CI: 1.19, 2.01), and a adult-onset asthma (OR = 1.34, 95% CI: 1.15, 1.56).
Salameh et al 2006	Lebanese case control study of asthmatic patients and non-asthmatic patients from several Lebanese hospitals, n=407	Questionnaire	Questionnaire (doctor-diagnosed asthma)	Any exposure to pesticides was associated to asthma (OR = 2.11 (1.47 to 3.02). Occupational use presented the highest association (OR = 4.98(1.07 to 23.28); followed by regional exposure (OR 3.51 (2.11 to 5.85)
Abu Mourad et al 2005	Cross-sectional study of 48 Palestinian farm workers in the Gaza Strip	Serum cholinesterase and complete blood count were determined before and after a spraying day of organophosphorus insecticides	Self report of respiratory symptoms	Burning sensations in eyes/face (62.5%), itching/skin irritation (37.5%), and chest symptoms (29.2%) were reported. Serum butyryl cholinesterase (SBUChE) was significantly decreased at the end of the work day.
Faria et al 2005	A cross-sectional study of 1,379 farmers from two municipalities of Southern Brazil. subjects aged 15 and older	Self-reported individual exposure to pesticides using questionnaire	An adapted questionnaire developed by the American Thoracic Society used to assess respiratory symptoms	The prevalence of asthma symptoms was 12% and chronic respiratory disease symptoms was 22%. Higher odds ratios for both asthma (OR=1.51; 95% CI: 1.07-2.14) and chronic respiratory disease (OR=1.34; 95% CI 1.00-1.81) symptoms were found in women compared to men. Occurrence of pesticide poisoning was associated with higher prevalence of asthma symptoms (OR=1.54; 95% CI: 1.04-2.58) and chronic respiratory disease symptoms (OR=1.57; 95% CI: 1.08-2.28).
Sunyer et al 2005	4 year Longitudinal study. 482 children recruited from birth in Spain	Prenatal exposure of organochlorine compounds was measured in cord serum in 405 (83%) children	Asthma was defined on the basis of wheezing at 4 years of age, persistent wheezing, or doctor-diagnosed asthma	Wheezing at 4 years of age increased with (dichlorodiphenyldichloroethylene) DDE concentration relative risk = 2.63 (95% confidence interval 1.19-4.69). Conclusion was that prenatal exposure to DDE residues may contribute to development of asthma.
Mekonnen et al 2004	cross-sectional study in four state farms of Ethiopia, n=171	Exposure questionnaire. 102 pesticide sprayers and 69 non-sprayers	Measurements of lung function and respiratory symptoms	The 15-24 years age group of pesticide sprayers had significantly reduced forced expiratory vital capacity (FVC) and forced expiratory volume in one second (FEV1), compared to that of similar age group non-sprayers.

Table1 continued: Summary of epidemiological studies investigating the effect of pesticides on rhinitis, asthma and asthma symptoms

Author, year	Population (n), Design, country, City	Exposure Assessment	Outcome measurements	Summary of findings
Salam et al 2004	Prevalence case-control study nested within the Children's Health Study of 12 southern California communities, n = 908, ages 8-18yrs	Cases, n = 338 Controls, n = 570 Telephone interview on exposures. Exposure measured by answering yes/no to question	Outcome defined as physician-diagnosed asthma by age 5	Asthma diagnosed by age 5 associated with herbicides (OR = 4.58; 95% CI, 1.36-15.43), pesticides (OR = 2.39; 95% CI, 1.17-4.89),
Salameh et al 2003	Cross-sectional of children randomly selected from Lebanese public schools, n = 3,291 children.	Exposure to pesticides was evaluated by a standardised questionnaire and a residential exposure score	Respiratory symptoms were assessed by using the American Thoracic Society standardised questionnaire.	Prevalence of chronic respiratory disease was 12.4%. Any exposure to pesticides, including residential, para-occupational and domestic, was associated with respiratory disease and chronic respiratory symptoms (chronic phlegm, chronic wheezing, ever wheezing), except for chronic cough.
Hoppin et al 2002	Cross sectional study of 20 468 applicators, aged 16 to 88 years from Agricultural Health Study (USA)	Self-administered questionnaires on 40 currently used pesticides and pesticide application practices	Self reported wheeze	19% reported wheezing in the past year. The herbicides, atrazine and alachlor were associated with wheeze
Mekonnen et al 2002	agricultural farm workers in Ethiopia	203 farm workers and 131 controls	Pulmonary function tests, report of respiratory symptoms	Pulmonary function lower among farm supervisors, followed by the sprayers compared with controls. Farm technicians had the most respiratory symptoms, with wheezing and breathlessness being the most frequent (35.7%).
Zhang et al, 2002	Cross-sectional study of 30 villages, 2 rural counties, n = 22,528 male and female residents aged 15 and older, Beijing, China	Questionnaire included questions on different occupational and environmental exposures. Exposure measured by answering yes/no to question	International Union Against Tuberculosis and Lung Disease Questionnaire on Bronchial Symptoms	Exposure to insecticides and other chemicals associated with higher prevalence of respiratory symptoms than unexposed
Ohayo-Mitoko et al 2000	a hybrid design combining cross sectional and follow up design features. 408 participants from four regions in Kenya from the East African pesticides project	256 exposed subjects and 152 controls, a acetylcholinesterase activity to measure exposure	A structured questionnaire on symptoms experienced was given to all participants	High prevalence for respiratory, eye, and central nervous system symptoms was found for workers with > 30% cholinesterase inhibition
Sprince et al 2000	The Iowa Farm Family Health and Hazard Surveillance Project, n=385	Questionnaire for pesticide exposure	Questionnaire for respiratory symptoms	Applying pesticides to livestock associated with phlegm (OR = 1.91, 95% CI 1.02-3.57), chest ever wheezy (OR = 3.92, 95% CI 1.76-8.72), and flu-like symptoms (OR = 2.93, 95% CI 1.69-5.12) adjusting for age and smoking

Table1 continued: Summary of epidemiological studies investigating the effect of pesticides on rhinitis, asthma and asthma symptoms

Author, year	Population (n), Design, country, City	Exposure Assessment	Outcome measurements	Summary of findings
Beshwari et al. 1999	matched case control study of the pesticide-exposed and nonexposed subjects in UAE, n = 208	Exposed subjects randomly selected from farmer chambers directory using fertilizer or exposed to pesticide, n=103 Controls, n=105 Questionnaire used to estimate exposure Information	Chronic respiratory symptoms were recorded by using the British Medical Research Council Questionnaire	The exposed group had higher prevalence for cough, phlegm, breathlessness, sinusitis, throat discomfort, chronic bronchitis, asthma diagnosis by doctor, allergic rhinitis, skin pruritus (tinea, contact dermatitis) and eczema compared to the unexposed
Dalvie et al 1999	A cross sectional study of 126 workers in the Western Cape, South Africa	Questionnaire-based job exposure matrix	Questionnaires based respiratory symptoms, spirometry, gas transfer, chest radiographs and oxygen desaturation on exercise was measured	No association between long term exposure to paraquat and reported symptoms, spirometry (forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1), FEV1/FVC) and gas transfer (TLCO and KCO) or chest radiography. Arterial oxygen desaturation during exercise was associated with pesticide exposure
Senthilselvan et al 1992	Cross-sectional survey of 1 939 male farmers.	Self-reported pesticide use	Self-reported asthma. Fleisch No.4 pneumotachograph was used to measure the airflow to calculate forced vital capacity (FVC), forced expiratory volume in 1s (FEV1), forced expiratory flow during the middle half of the forced vital capacity (FEF25-75), and the ratio of FEV1 to FVC (FEV1/FVC x 100).	Prevalence of asthma was significantly associated with the use of carbamate insecticides (prevalence OR = 1.8, 95% CI: 1.1 to 3.1). Self-reported asthmatics, had significantly lower mean values for lung function compared to non asthmatics
Innes et al 1990	Screening of 44 farmworkers engaged in fruit crop spraying in the Somerset West, Cape Town,	Automated assay for plasma cholinesterase (CHE) exposure.	Clinical complaints described by the nursing sister in charge of the staff clinic	17,5% of male rural workers engaged in crop spraying suffer from chronic organophosphate poisoning, asthma and chronic bronchitis were common complaints

Appendix 7

Environment International: Author Instructions

University of Cape Town

ENVIRONMENT INTERNATIONAL

A Journal of Environmental Science, Risk & Health



ISSN: 0160-4120

AUTHOR INFORMATION PACK

TABLE OF CONTENTS

XXX

- Description
- Audience
- Impact Factor
- Abstracting and Indexing
- Editorial Board
- Guide for Author

DESCRIPTION

Environment International covers all disciplines engaged in the field of environmental research. It seeks to quantify the impact of contaminants in the human environment, and to address human impacts on the natural environment itself. We recognize that scientific issues related to environmental health and human welfare are inherently interdisciplinary and, therefore, we welcome articles that cover the entire spectrum of sources, pathways, sinks and interactions between environmental pollutants, whether chemical, biological or physical. The primary criteria for publication are scientific quality and environmental significance.

AUDIENCE

Environmental scientists, ecotoxicologists, environmental chemists, environmental health specialists, environmental regulators, ecologists, biologists, hydrologists, geologists, marine and atmospheric scientists.

IMPACT FACTOR

2010: 4.691 © Thomson Reuters Journal Citation Reports 2011

ABSTRACTING AND INDEXING

ABI/Inform
Current Contents
EMBASE
Elsevier BIOBASE
Energy Data Base

Energy Research Abstracts
Environmental Periodicals Bibliography
MEDLINE®
Research Alert
Science Citation Index
Scopus

EDITORIAL BOARD

Editor-in-Chief:

R.E. Alcock, Ghyll Cottage, Environmental Research Solutions, Mill Side, Witherslack, Grange-Over-Sands, LA11 6SG, UK, **Email:** ruth.alcock@virgin.net

Associate Editor:

J.L. Domingo, Laboratory of Toxicology and Environmental Health, School of Medicine, IISPV, Universitat Rovira i Virgili, Sant Llorens 21, 43201 Reus, Catalonia, Spain, Fax: +34 977 759322, **Email:** joseluis.domingo@urv.cat

I. Hertz-Picciotto, UC Davis School of Medicine, Dept. PHS, University of California at Davis, One Shields Avenue, Davis, CA 95616, USA, **Email:** ihp@phs.ucdavis.edu

Advisory Review Editors:

R.J. Letcher, Canadian Wildlife Service, Environment Canada, National Wildlife Research Center, Carleton University (Raven Road), Ottawa, K1A 0H3, Canada, **Email:** robert.letcher@ec.gc.ca

Editorial Board:

D. Barceló, Consejo Superior de Investigaciones Científicas (CSIC), Barcelona, Spain

N. Basu, University of Michigan, Ann Arbor, MI, USA

P.A. Behnisch, BioDetection Systems BV (BDS), Amsterdam, Netherlands

J.N.B. Bell, Imperial College London, Kensington, London, UK

P. Bhattacharya, KTH Royal Institute of Technology, Stockholm, Sweden

J. Blasco, Campus Univ. Rio San Pedro, Puerto Real (Cadiz), Spain

P. Brimblecombe, University of East Anglia, Norwich, UK

A. Covaci, Universiteit Antwerpen, Wilrijk-Antwerpen, Belgium

A. del Valls, Universidad de Cadiz, Cádiz, Spain

R. Duarte-Davidson, Health Protection Agency, Didcot, Oxon, England, UK

J. Hofman, Masaryk University, Brno, Czech Republic

O. Hutzinger, Universität Bayreuth, Haiden, Germany

O.I. Kalantzi, University of the Aegean, Mytilene, Greece

R. Lal, Ohio State University, Columbus, OH, USA

G. Ludewig, University of Iowa, Iowa City, IA, USA

A.A. Meharg, University of Aberdeen, Aberdeen, UK

L. Naeher, University of Georgia, Athens, GA, USA

D.M. Oliver, University of Stirling, Stirling, UK

P. Ricci, University of San Francisco, San Francisco, CA, USA

B. Roig, Ecole des Hautes Etudes en Santé Publique (EHESP), NIMES, France

H. Stapleton, Duke University, Durham, NC, USA

G. Thomas, Lancaster University, Lancaster, UK

I. Twardowska, Polish Academy of Sciences, Zabrze, Poland

N.M. van Straalen, Vrije Universiteit Amsterdam, Amsterdam, Netherlands

Y-G. Zhu, Chinese Academy of Sciences (CAS), Beijing, China

GUIDE FOR AUTHORS

Types of paper

No single format can accommodate all useful contributions to this journal.

Five formats are offered, two of which (Reviews and New Developments), fall within the *Progress in Environmental Science* reviews section:

1. *Editorial articles* are published by the Editor-in-Chief or other Editors, members of the Editorial Board or invited Guest Editors. These focus attention on contemporary important environmental issues in relation to environmental health and are designed to stimulate debate and discussion.
2. *Research Articles* are up-to-date, original papers that present developments in any scientific field pertinent to environmental contamination and environmental health. Informative abstracts are required and articles must be fully referenced. Criteria for publication are weighted toward scientific quality and environmental significance. The manuscript will be evaluated on the basis of its conciseness, clarity, and presentation. The work will be assessed according to its originality, scientific merit, and experimental design. Poorly written manuscripts will be returned to the authors with a request to improve the quality of the paper prior to peer review.
3. *Correspondence* is encouraged. Opinions, perspectives and insight on articles published in *Environment International* are very welcome.
4. *Reviews* represent articles that trace recent developments and discuss trends in a particular field of research. They may be of a broad nature, providing accounts of specific fields of interest in any relevant area. Authors are encouraged to write in a clear and simple manner so that the article is understandable to readers from a broad cross section of disciplines. On average, a text length (excluding references) of c. 8000 words is required. Prospective authors should contact the Editor in Chief in the first instance to discuss the suitability of proposed topics. Articles should not be case studies, not reporting of personal research.
5. *New Developments* are short articles presenting the latest developments in scientific, technological and policy developments relating to environmental contamination and environmental health. On average, a text length (excluding references) of c.3000 words is required. Abstracts are not included in these short articles. Readers are encouraged to suggest subjects for inclusion in this section. Since the journal will serve a multidisciplinary audience, authors are urged to write for non-specialists. In particular, they are discouraged from using expressions that are comprehensible only to a select audience. Clarity should be the guide when preparing manuscripts. All the contributions will be subjected to peer review.

Special issues

Proposals for special themed issues, or special issues arising from conferences, should be discussed with the Editor-in-Chief Dr Ruth Alcock (ruth.alcock@virgin.net). Guest Editors will coordinate the review and submission process of special issue papers and will prepare an introduction. Special prices for bulk orders of a special issue can be arranged.

Contact details for submission

All manuscripts should be submitted electronically through the Elsevier Editorial System (EES) which can be accessed at <http://ees.elsevier.com/envint/>
If you are not able to submit your paper to ENVINT electronically please contact the Editor-in-Chief, Ruth Alcock, Email: ruth.alcock@virgin.net for further instructions.

BEFORE YOU BEGIN

Ethics in publishing

For information on Ethics in publishing and Ethical guidelines for journal publication see <http://www.elsevier.com/publishingethics> and <http://www.elsevier.com/ethicalguidelines>.

Conflict of interest

All authors are requested to disclose any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work. See also <http://www.elsevier.com/conflictsofinterest>.

Submission declaration

Submission of an article implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere including electronically in the same form, in English or in any other language, without the written consent of the copyright-holder.

Changes to authorship

This policy concerns the addition, deletion, or rearrangement of author names in the authorship of accepted manuscripts:

Before the accepted manuscript is published in an online issue: Requests to add or remove an author, or to rearrange the author names, must be sent to the Journal Manager from the corresponding author of the accepted manuscript and must include: (a) the reason the name should be added or removed, or the author names rearranged and (b) written confirmation (e-mail, fax, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed. Requests that are not sent by the corresponding author will be forwarded by the Journal Manager to the corresponding author, who must follow the procedure as described above. Note that: (1) Journal Managers will inform the Journal Editors of any such requests and (2) publication of the accepted manuscript in an online issue is suspended until authorship has been agreed.

After the accepted manuscript is published in an online issue: Any requests to add, delete, or rearrange author names in an article published in an online issue will follow the same policies as noted above and result in a corrigendum.

Copyright

Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (for more information on this and copyright see <http://www.elsevier.com/copyright>). Acceptance of the agreement will ensure the widest possible dissemination of information. An e-mail will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement. Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations (please consult <http://www.elsevier.com/permissions>). If excerpts from other copyrighted works are included, the author(s) must obtain written

permission from the copyright owners and credit the source(s) in the article. Elsevier has preprinted forms for use by authors in these cases: please consult <http://www.elsevier.com/permissions>.

Retained author rights

As an author you (or your employer or institution) retain certain rights; for details you are referred to: <http://www.elsevier.com/authorsrights>.

Role of the funding source

You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement then this should be stated. Please see <http://www.elsevier.com/funding>.

Funding body agreements and policies

Elsevier has established agreements and developed policies to allow authors whose articles appear in journals published by Elsevier, to comply with potential manuscript archiving requirements as specified as conditions of their grant awards. To learn more about existing agreements and policies please visit <http://www.elsevier.com/fundingbodies>.

Open access

This journal offers you the option of making your article freely available to all via the ScienceDirect platform. To prevent any conflict of interest, you can only make this choice after receiving notification that your article has been accepted for publication. The fee of \$3,000 excludes taxes and other potential author fees such as color charges. In some cases, institutions and funding bodies have entered into agreement with Elsevier to meet these fees on behalf of their authors. Details of these agreements are available at <http://www.elsevier.com/fundingbodies>. Authors of accepted articles, who wish to take advantage of this option, should complete and submit the order form (available at <http://www.elsevier.com/locate/openaccessform.pdf>). Whatever access option you choose, you retain many rights as an author, including the right to post a revised personal version of your article on your own website. More information can be found here: <http://www.elsevier.com/authorsrights>.

Language and language services

Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who require information about language editing and copyediting services pre- and post-submission please visit <http://webshop.elsevier.com/languageservices> or our customer support site at <http://support.elsevier.com> for more information.

Submission

Submission to this journal proceeds totally online and you will be guided stepwise through the creation and uploading of your files. The system automatically converts source files to a single PDF file of the article, which is used in the peer-review process. Please note that even though manuscript source files are converted to PDF files at submission for the review process, these source files are needed for further processing after acceptance. All correspondence, including notification of the Editor's decision and requests for revision, takes place by e-mail removing the need for a paper trail.

PREPARATION

Use of wordprocessing software

It is important that the file be saved in the native format of the wordprocessor used. The text should be in single-column format. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. In particular, do not use the wordprocessor's options to justify text or to hyphenate words. However, do use bold face, italics, subscripts, superscripts etc. When preparing tables, if you are using a table grid, use only one grid for each individual table and not a grid for each row. If no grid is used, use tabs, not spaces, to align columns. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier: <http://www.elsevier.com/guidepublication>). Note that source files of figures, tables and text graphics will be required whether or not you embed your figures in the text. See also the section on Electronic artwork. To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your wordprocessor.

Article structure

Subdivision - numbered sections

Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods

Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

Theory/calculation

A Theory section should extend, not repeat, the background to the article already dealt with in the Introduction and lay the foundation for further work. In contrast, a Calculation section represents a practical development from a theoretical basis.

Results

Results should be clear and concise.

Discussion

This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions

The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Appendices

If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Essential title page information

- **Title.** Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
- **Author names and affiliations.** Where the family name may be ambiguous (e.g., a double name), please indicate this clearly. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
- **Corresponding author.** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. **Ensure that telephone and fax numbers (with country and area code) are provided in addition to the e-mail address and the complete postal address. Contact details must be kept up to date by the corresponding author.**
- **Present/permanent address.** If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Abstract

A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

Graphical abstract

A Graphical abstract is optional and should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership online. Authors must provide images that clearly represent the work described in the article. Graphical abstracts should be submitted as a separate file in the online submission system. Image size: Please provide an image with a minimum of 531 × 1328 pixels (h × w) or proportionally more. The image should be readable at a size of 5 × 13 cm using a regular screen resolution of 96 dpi. Preferred file types: TIFF, EPS, PDF or MS Office files. See <http://www.elsevier.com/graphicalabstracts> for examples. Authors can make use of Elsevier's Illustration and Enhancement service to ensure the best presentation of their images also in accordance with all technical requirements: Illustration Service.

Highlights

Highlights are mandatory for this journal. They consist of a short collection of bullet points that convey the core findings of the article and should be submitted in a separate file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point). See <http://www.elsevier.com/highlights> for examples.

Keywords

Immediately after the abstract, provide a maximum of 6 keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Abbreviations

Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Math formulae

Present simple formulae in the line of normal text where possible and use the solidus (/) instead of a horizontal line for small fractional terms, e.g., X/Y. In principle, variables are to be presented in italics. Powers of e are often more conveniently denoted by exp. Number consecutively any equations that have to be displayed separately from the text (if referred to explicitly in the text).

Footnotes

Footnotes should be used sparingly. Number them consecutively throughout the article, using superscript Arabic numbers. Many wordprocessors build footnotes into the text, and this feature may be used. Should this not be the case, indicate the position of footnotes in the text and present the footnotes themselves separately at the end of the article. Do not include footnotes in the Reference list.

Table footnotes

Indicate each footnote in a table with a superscript lowercase letter.

Artwork

Electronic artwork

General points

- Make sure you use uniform lettering and sizing of your original artwork.
- Save text in illustrations as 'graphics' or enclose the font.
- Only use the following fonts in your illustrations: Arial, Courier, Times, Symbol.
- Number the illustrations according to their sequence in the text.
- Use a logical naming convention for your artwork files.
- Provide captions to illustrations separately.
- Produce images near to the desired size of the printed version.
- Submit each figure as a separate file.

A detailed guide on electronic artwork is available on our website:

<http://www.elsevier.com/artworkinstructions>

You are urged to visit this site; some excerpts from the detailed information are given here.

Formats

Regardless of the application used, when your electronic artwork is finalised, please 'save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):

EPS: Vector drawings. Embed the font or save the text as 'graphics'.

TIFF: Color or grayscale photographs (halftones): always use a minimum of 300 dpi.

TIFF: Bitmapped line drawings: use a minimum of 1000 dpi.

TIFF: Combinations bitmapped line/half-tone (color or grayscale): a minimum of 500 dpi is required.

If your electronic artwork is created in a Microsoft Office application (Word, PowerPoint, Excel) then please supply 'as is'.

Please do not:

- Supply files that are optimised for screen use (e.g., GIF, BMP, PICT, WPG); the resolution is too low;
- Supply files that are too low in resolution;
- Submit graphics that are disproportionately large for the content.

Color artwork

Please make sure that artwork files are in an acceptable format (TIFF, EPS or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color on the Web (e.g., ScienceDirect and other sites) regardless of whether or not these illustrations are reproduced in color in the printed version. **For color reproduction in print, you will receive information regarding the costs from Elsevier after receipt of your accepted article.**

Please indicate your preference for color: in print or on the Web only. For further information on the preparation of electronic artwork, please see <http://www.elsevier.com/artworkinstructions>.

Please note: Because of technical complications which can arise by converting color figures to 'gray scale' (for the printed version should you not opt for color in print) please submit in addition usable black and white versions of all the color illustrations.

Figure captions

Ensure that each illustration has a caption. Supply captions separately, not attached to the figure. A caption should comprise a brief title (**not** on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

Tables

Number tables consecutively in accordance with their appearance in the text. Place footnotes to tables below the table body and indicate them with superscript lowercase letters. Avoid vertical rules. Be sparing in the use of tables and ensure that the data presented in tables do not duplicate results described elsewhere in the article.

References

Citation in text

Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

References in a special issue

Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

Reference management software

This journal has standard templates available in key reference management packages EndNote (<http://www.endnote.com/support/enstyles.asp>) and Reference Manager

(<http://refman.com/support/rmstyles.asp>). Using plug-ins to wordprocessing packages, authors only need to select the appropriate journal template when preparing their article and the list of references and citations to these will be formatted according to the journal style which is described below.

Reference style

Text: All citations in the text should refer to:

1. *Single author:* the author's name (without initials, unless there is ambiguity) and the year of publication;
2. *Two authors:* both authors' names and the year of publication;
3. *Three or more authors:* first author's name followed by 'et al.' and the year of publication. Citations may be made directly (or parenthetically). Groups of references should be listed first alphabetically, then chronologically.

Examples: 'as demonstrated (Allan, 2000a, 2000b, 1999; Allan and Jones, 1999). Kramer et al.(2010) have recently shown'

List: References should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication.

Examples:

Reference to a journal publication:

Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2010. The art of writing a scientific article. *J. Sci.Comm.* 163, 51–59.

Reference to a book:

Strunk Jr., W., White, E.B., 2000. *The Elements of Style*, fourth ed. Longman, New York.

Reference to a chapter in an edited book:
Mettam, G.R., Adams, L.B., 2009. How to prepare an electronic version of your article, in: Jones, B.S., Smith, R.Z. (Eds.), *Introduction to the Electronic Age*. E-Publishing Inc., New York, pp. 281–304.

Journal abbreviations source

Journal names should be abbreviated according to

Index Medicus journal abbreviations: <http://www.nlm.nih.gov/tsd/serials/lji.html>;

List of title word abbreviations: <http://www.issn.org/2-22661-LTWA-online.php>;

CAS (Chemical Abstracts Service): <http://www.cas.org/sent.html>.

Video data

Elsevier accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. In order to ensure that your video or animation material is directly usable, please provide the files in one of our recommended file formats with a preferred maximum size of 50 MB. Video and animation files supplied will be published online in the electronic version of your article in Elsevier Web products, including ScienceDirect:

<http://www.sciencedirect.com>. Please supply 'stills' with your files: you can choose any frame from the video or animation or make a separate image. These will be used instead of standard icons and will personalize the link to your video data. For more detailed instructions please visit our video instruction pages at <http://www.elsevier.com/artworkinstructions>.

Note: since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

Supplementary data

Elsevier accepts electronic supplementary material to support and enhance your scientific research. Supplementary files offer the author additional possibilities to publish supporting applications, highresolution images, background datasets, sound clips and more. Supplementary files supplied will be published online alongside the electronic version of your article in Elsevier Web products, including ScienceDirect: <http://www.sciencedirect.com>. In order to ensure that your submitted material is directly usable, please provide the data in one of our recommended file formats. Authors should submit the material in electronic format together with the article and supply a concise and descriptive caption for each file. For more detailed instructions please visit our artwork instruction pages at <http://www.elsevier.com/artworkinstructions>.

Linking to and depositing data at PANGAEA

Electronic archiving of supplementary data enables readers to replicate, verify and build upon the conclusions published in your paper. We recommend that data should be deposited in the data library PANGAEA (<http://www.pangaea.de>). Data are quality controlled and archived by an editor in standard machine-readable formats and are available via Open Access. After processing, the author receives an identifier (DOI) linking to the supplements for checking. As your data sets will be citable you might want to refer to them in your article. In any case, data supplements and the article will be automatically linked as in the following example: doi:10.1016/0016-7037(95)00105-9. Please use PANGAEA's web interface to submit your data (<http://www.pangaea.de/submit/>).

Submission checklist

The following list will be useful during the final checking of an article prior to sending it to the journal for review. Please consult this Guide for Authors for further details of any item.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:

- E-mail address
- Full postal address
- Telephone and fax numbers

All necessary files have been uploaded, and contain:

- Keywords
- All figure captions
- All tables (including title, description, footnotes)

Further considerations

- Manuscript has been 'spell-checked' and 'grammar-checked'
- References are in the correct format for this journal
- All references mentioned in the Reference list are cited in the text, and vice versa
- Permission has been obtained for use of copyrighted material from other sources (including the Web)
- Color figures are clearly marked as being intended for color reproduction on the Web (free of charge) and in print, or to be reproduced in color on the Web (free of charge) and in black-and-white in print
- If only color on the Web is required, black-and-white versions of the figures are also supplied for printing purposes

For any further information please visit our customer support site at <http://support.elsevier.com>.

AFTER ACCEPTANCE

Use of the Digital Object Identifier

The Digital Object Identifier (DOI) may be used to cite and link to electronic documents. The DOI consists of a unique alpha-numeric character string which is assigned to a document by the publisher upon the initial electronic publication. The assigned DOI never changes. Therefore, it is an ideal medium for citing a document, particularly 'Articles in press' because they have not yet received their full bibliographic information. The correct format for citing a DOI is shown as follows (example taken from a document in the journal *Physics Letters B*): doi:10.1016/j.physletb.2010.09.059 When you use the DOI to create URL hyperlinks to documents on the web, the DOIs are guaranteed never to change. AUTHOR INFORMATION PACK 24 Jan 2012 www.elsevier.com/locate/envint 10

Proofs

One set of page proofs (as PDF files) will be sent by e-mail to the corresponding author (if we do not have an e-mail address then paper proofs will be sent by post) or, a link will be provided in the e-mail so that authors can download the files themselves. Elsevier now provides authors with PDF proofs which can be annotated; for this you will need to download Adobe Reader version 7 (or higher) available free from <http://get.adobe.com/reader>. Instructions on how to annotate PDF files will accompany the proofs (also given online). The exact system requirements are given at the Adobe site: <http://www.adobe.com/products/reader/tech-specs.html>.

If you do not wish to use the PDF annotations function, you may list the corrections (including replies to the Query Form) and return them to Elsevier in an e-mail. Please list your corrections quoting line number. If, for any reason, this is not possible, then mark the corrections and any other comments (including replies to the Query Form) on a printout of your proof and return by fax, or scan the pages and e-mail, or by post. Please use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor. We will do everything possible to get your article published quickly and accurately – please let us have all your corrections within 48 hours. It is important to ensure that all corrections are sent back to us in one communication: please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility. Note that Elsevier may proceed with the publication of your article if no response is received.

Offprints

The corresponding author, at no cost, will be provided with a PDF file of the article via e-mail. For an extra charge, paper offprints can be ordered via the offprint order form which is sent once the article is accepted for publication. The PDF file is a watermarked version of the published article and includes a cover sheet with the journal cover image and a disclaimer outlining the terms and conditions of use.

AUTHOR INQUIRIES

For inquiries relating to the submission of articles (including electronic submission) please visit this journal's homepage. Contact details for questions arising after acceptance of an article, especially those relating to proofs, will be provided by the publisher. You can track accepted articles at <http://www.elsevier.com/trackarticle>. You can also check our Author FAQs (<http://www.elsevier.com/authorFAQ>) and/or contact Customer Support via <http://support.elsevier.com>.

© Copyright 2012 Elsevier | <http://www.elsevier.com>

Appendix 8

Published article

Pesticides and the Airways- A Review of the Literature (Ndlovu et al., 2011)

University of Cape Town

ALLERGIES IN THE WORKPLACE

PESTICIDES AND THE AIRWAYS – A REVIEW OF THE LITERATURE

Vuyelwa Ndlovu, *BSc Hons, MPH(Epi) candidate*

Mohamed Aqiel Dalvie, *BSc, BSc (Med) Hons, MSc, PhD*

Mohamed F Jeebhay, *MB ChB, DOH, MPhil (Epi), MPH (Occ Med), PhD*

Centre for Occupational and Environmental Health Research, University of Cape Town, South Africa

Correspondence: Vuyelwa Ndlovu, email: vuyelwa.ndlovu@uct.ac.za or vuyiendlovu@yahoo.com.

ABSTRACT

A literature review was conducted focusing on the effects of pesticides in the development of respiratory diseases including asthma and rhinitis. The objective was to identify high-risk groups, and to identify specific pesticides associated with rhinitis and asthma, as well as the pathophysiological mechanisms involved. Methodological issues have been considered, particularly exposure assessment methods, which will drive recommendations for future research.

A number of studies have reported rhinitis and asthma associated with pesticides. Most studies have been conducted on male farm workers with few data on women and children. Few studies have been conducted in Africa. Furthermore, there is a lack of data on biomonitoring of specific pesticides and clinical endpoints aside from symptoms.

The mechanisms by which pesticides may cause respiratory effects include neurogenic inflammation and muscarinic effects of some pesticide groups such as organophosphates and carbamates. Pesticides may also modify inflammatory responses to other allergens.

Recommendations for future research include more African studies with additional focus on women and children, using more robust exposure markers such as specific pesticide biomonitoring and more objective markers for asthma outcomes.

INTRODUCTION

This review focuses on the available evidence on the effect of chronic and in some cases acute exposure to pesticides (and other host and environmental risk factors) in the development of airway diseases such as asthma and rhinitis. The objective was to identify high risk groups, and to identify specific pesticides associated with rhinitis and asthma as well as the pathophysiological mechanisms involved. Methodological issues have been considered, particularly exposure assessment methods and more objective clinical endpoints, which will drive recommendations for future research. The electronic sources of information included PubMed/ MEDLINE, EBSCO,

Google Scholar and The Cochrane Library. Paper sources included text books, journals and previous theses through the University of Cape Town Medical Library. Key words for the search included pesticides, health effects, asthma, rhinitis, allergies, farm workers, farm residents, rural residents and cholinesterase. The search initially included all population groups exposed to pesticides, including men, women and children

in farms, and the associated respiratory, both allergic and non-allergic health effects. Data from all study designs and countries were considered.

HIGH-RISK POPULATIONS AND TARGET GROUPS

The populations at risk of developing adverse health effects associated with pesticide exposure include workers such as farm workers, workers in the pesticide production industry, pest control workers and individuals exposed environmentally, such as farm residents and those using household pesticides.¹

Pesticide exposure in the agricultural sector is important since about 1.8 billion people in the world are involved in agriculture and most are exposed to pesticides.² Agricultural workers, especially applicators and handlers have therefore been the study population in most studies investigating health effects of pesticides (Fig. 1a-c).

Non-occupational or environmental exposure potentially affects a large segment of the general population. Residential exposures are associated with pesticide use by exterminators and from dietary and accidental exposures. Other environmental exposures occur in public places and areas close to farms.¹ For example, airline passengers are exposed to in-flight pesticide spraying when commuting between malaria endemic areas.³

Exposure to pesticides can also occur in the school environment, especially among rural children and educators, from nearby farms and pesticide manufacturing plants. Acute symptoms have been reported from schools that were associated with exposure to pesticide drift from neighboring farms.⁴ Children are a particular high-risk group because of their exploratory behaviour often resulting in accidental contact with household pesticides.⁵

The risk is even higher for children living on farms because of drift, playing with empty pesticide containers or exposure to pesticide-contaminated clothing from parents or guardians working on farms.⁶ Living in an agricultural region has also been associated with pesticide exposure; increased levels of dialkylphosphate (DAP) metabolites were found in the urine of children who did not live close to a farm or have parents who worked on a farm.⁷

Studies have mostly focused on adult males but it has become increasingly apparent that women are also a high-risk group.⁸⁻¹³ Work-related pesticide poisoning in women was found to be more common

than in men in a Chinese study.¹⁴ It has also been reported in many countries, including Italy and USA, that children under the age of 5 have more pesticide-exposure-related hospitalisations than any other age group.^{15,16} Children may have higher pesticide exposure than adults in the same exposure environment because of their higher respiratory rate, proportionately larger skin surface area and higher metabolic rate.¹⁶ Additionally, pesticide exposure can occur *in utero* since a number of pesticides can pass through the placenta, and to infants through breast milk. There is also evidence that pesticide exposure can affect the genes of exposed parents that can, in turn, affect offspring and subsequent generations

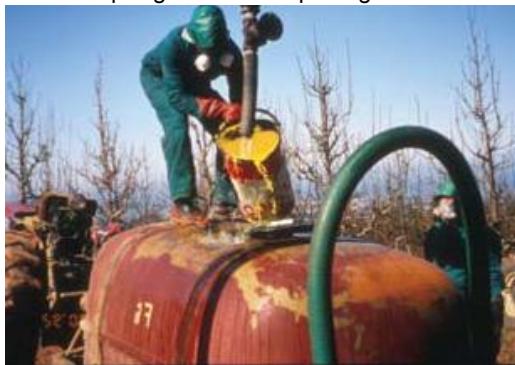


Fig. 1a. High-risk pesticide exposures: mixing.



Fig. 1b. High-risk pesticide exposures: spraying.



Fig. 1c. High-risk pesticide exposures: resultant pesticide drift.

Populations in African and other developing countries are also at high risk of pesticide exposure since the use of pesticides in many African countries including South Africa is still largely unregulated.¹⁷ Over 90% of deaths from pesticide

poisoning emanate from developing countries even though these countries use only 20% of the world's total consumption.¹⁸ In South Africa for instance, pesticides have frequently been detected in ground water and surface water, including drinking water sources and sediments located near farms in the Western Cape.¹⁹⁻²² Additionally high pesticide levels have been measured among rural residents in the Western Cape.^{23,24} Pesticide residues have also been detected in wheat samples used in the production of South African wheat products.²⁵ The street selling of illegal pesticides for domestic use, as has been reported in the City of Cape Town to lead to child poisonings, is an added consideration.²⁶

RESPIRATORY HEALTH EFFECTS ASSOCIATED WITH PESTICIDES

Pesticide exposure has been associated with asthma and rhinitis in various epidemiology studies.^{3,4,10-13,27-44} The pesticides that have been found to be associated with respiratory effects in humans are listed in Table I. Most of the studies were not conducted in Africa and mainly among male farm workers.

In 20 epidemiological studies the outcomes studied were self-reported, doctor-diagnosed asthma or asthmatic symptoms. More objective measures for airways disease including lung function indices were reported in only 9 studies.^{13,31,32,38-43}

Another limitation of the studies is that in a large proportion (65%) of studies, exposure assessment was based on self-reported exposures obtained from questionnaire data with categorical outcomes. Biological monitoring in the form of cholinesterase testing, a marker of organophosphate and carbamate exposure, was used in 5 studies,^{32,34,39,44,45} and measurement of ethylenethiourea (ETU), a marker of dithiocarbamate exposure, was used in 2 studies.^{41,46} It is evident that more robust indices of exposure using biomarkers or early biological effect markers are needed.

There have been very few epidemiological studies investigating asthma and other respiratory symptoms due to pesticides among women or studies that include women in the target population.^{10-13,38,47} Two studies reported that respiratory symptoms or doctor-diagnosed asthma were more prevalent and strongly associated with pesticide exposure in women than in men.^{10,11}

It was previously thought that allergic asthma was less common in farmers than in other occupational sectors and less common in adults than in children. In a study in the USA, however, it was found that pesticide exposure was associated with both adult-onset atopic and non-atopic asthma.²⁸

Farm workers are also exposed to various allergens of plants, animals or chemicals including pesticides. These allergens are associated with IgE-mediated allergic asthma.⁴⁸ The spider mite, *Tetranychus urticae*, has been reported as an important outdoor allergen responsible for allergic symptoms such as

asthma among table-grape farm workers in South Africa. It has been suggested that increased risk of sensitisation to spider mite may be related to atopic asthma but that having grown up on a farm had a protective effect on asthma in women farm workers.¹² This suggests that pesticides may have a more important role in allergic asthma than originally thought. Most pesticides used, like parathion and malathion, were associated with

pesticide crop spraying in table grape farm orchards.⁴⁹ It was also found that pesticides were associated with atopic asthma and only permethrin was associated with both allergic and non-allergic asthma. Women who grew up on farms but did not apply pesticides had the lowest overall risk of atopic asthma compared with women

Table 1: Pesticides associated with upper and lower airway respiratory outcomes			
Pesticide			Outcome
Type	Group	Agents	
Herbicide	Triazine	Atrazine, alachlor	Wheeze ²⁷
Insecticide	- Organophosphates - Organochlorines	Coumaphos, parathion Heptachlor	Adult onset allergic (doctor-diagnosed asthma after the age of 19 yrs with history of doctor diagnosed eczema or hay fever) ²⁸
Fumigant	-halogenated organic -inorganic -halogenated organic	Carbon tetrachloride carbon disulfide ethylene dibromide (80/20 mix)	
Insecticide	Organochlorine	DDT (dichlorodiphenyltrichloroethane)	Adult onset non-allergic asthma (doctor-diagnosed asthma after the age of 19 yrs without history of doctor diagnosed eczema or hay fever) ²⁸
Insecticide	Organophosphate	Chlorpyrifos, terbufos	Wheeze ¹³
Herbicide	Sulfonylurea	Chlorimuron-ethyl	Wheeze ²⁹
Insecticide	Organophosphate	Phorate	
Herbicides	- Chlorphenoxy acid - Glysine - Petroleum	2,4 D Glyphosate Petroleum oil	Rhinitis ³⁰
Insecticide	Organophosphate	Diazinon	
Fungicide	Benzimidazole	Benomyl	
Herbicide	Bipyridyl	Paraquat	Rhinitis ³¹
Fungicide	Dithiocarbamate	NS	
Insecticide	Carbamates	NS	
Herbicide	Bipyridilium	NS	FEV ₁ (Forced expiratory volume in the first second) ³²
Insecticide	Neonicotinoid	NS	
Insecticide	Organosphosphate	NS	Symptoms suggestive of asthma and chronic bronchitis ³³
Insecticide	Organosphosphate	NS	Chest symptoms including (cold symptoms, dyspnoea, chest pain) ³⁴
Insecticide	Carbamate	NS	Self reported asthma ³⁵
Molluscicide	aldehyde	Metaldehyde	Cough ³⁶
Insecticide	- Carbamate - Organophosphate Macrocyclic Lactone	Methiocarb Profenofos Avermectin	
Fungicide	Dithiocarbamate	Mancozeb	
Herbicide	Glysine Petroleum	Glyphosate Petroleum oil	Rhinitis ³⁷
Insecticide	Organophosphate	Chlorpyrifos, diazinon, dichlorvos, malathion	
	- Carbamates - Pyrethroid	Carbaryl Permethrin	
Fungicide	Phthalimide	Captan	

NS: Not specified
who neither grew up on farms nor applied pesticides.¹²

There are very few studies that have looked at the association between rhinitis and pesticides. Rhinitis is also a known risk factor for asthma. Grape

farmers exposed to pesticides are reported to have a higher prevalence allergic rhinitis symptoms compared with controls. The highest risks were observed for paraquat and other bipyridyls and carbamates.³¹ Data from the Agricultural Health Study in North America indicated that 74% of commercial pesticide applicators reported at least one episode of rhinitis in the past year. The pesticides strongly associated with current rhinitis were 2,4-D, glyphosate, petroleum oil, diazinon and benomyl.³⁰ A similar study conducted in another cohort of applicators reported that 67% of farmers reported current rhinitis and 39% reported three or more rhinitis episodes.³⁷ In this study glyphosate, petroleum oil, organophosphates (chlorpyrifos, diazinon, dichlorvos, malathion), carbaryl and permethrin were predictors of current rhinitis. Rhinitis has also been reported in greenhouse flower growers and is associated with sensitisation to workplace allergens and pesticide application using hand pumps.⁵⁰

Children are particularly vulnerable to the effects of pesticides. In a Lebanese study on school children, a prevalence of 12.4% of chronic respiratory disease due to exposure to pesticides was found.⁵¹ In the USA there was a strong association between pesticide exposure and children diagnosed with asthma by age 5, particularly with exposure to herbicides.⁵²

Despite the increased use and exposure to pesticides, very few studies in Africa have been conducted to assess the association with respiratory symptoms, despite pesticides also being documented as a common problem in developing countries.⁴⁵ Innes *et al.*³³ reported that 17.5% of male rural workers engaged in crop spraying reported chronic organophosphate poisoning, asthma and chronic bronchitis in Cape Town. No association between long-term exposure to paraquat and reported symptoms and lung function was found but a decrease in exercise oximetry was found in a study done in the rural Western Cape.⁵³ In Kenya, a high prevalence of respiratory symptoms was found for workers with more than 30% cholinesterase inhibition⁴⁴ and in Ethiopia, the 15-24-year age group of pesticide sprayers had a significantly reduced forced expiratory vital capacity (FVC) and forced expiratory volume in one second (FEV1), compared with age-matched controls.⁴² A more recent study in South Africa demonstrated that the spider mite, *T. urticae*, is an important outdoor allergen among table-grape farm workers and the increased risk of spider mite allergy appeared to be related to high pesticide exposure among crop sprayers.⁴⁹

PATHOPHYSIOLOGICAL MECHANISMS

It is well documented that pesticides cause asthma either through neurogenic inflammation or as a result of the muscarinic effects in the peripheral nervous system. Exposure to organophosphate pesticides can exert effects on the smooth muscles of the respiratory tract resulting in bronchoconstriction, increased activity of the secretory glands and pulmonary oedema. The

immediate cause of death in acute organophosphate poisonings is asphyxia. Contributing factors are the muscarinic actions of bronchoconstriction and increased bronchial secretions, nicotinic action leading to paralysis of the respiratory muscles and depression of the respiratory centre. In the case of chronic, low-level exposure, biological plausibility has also been shown by a number of experimental toxicological studies on animals.³⁸

In earlier studies, it was postulated that acetylcholinesterase inhibition could directly explain the observed respiratory disease in farmers using organophosphate and carbamate insecticides.³⁵ However, in a study in which airway hyperreactivity was measured in guinea pigs exposed to chlorpyrifos, it was demonstrated that the organophosphate insecticide can cause airway hyperreactivity by decreasing neuronal M2 muscarinic receptor function at doses below those causing acetylcholinesterase inhibition.⁵⁴ The same results were found with parathion and diazinon.⁵⁵ It has also been shown that pre-existing allergic sensitisation in guinea pigs decreases the threshold dose of parathion required to cause airway hyperreactivity, suggesting that even lower levels of pesticide exposure can induce a reaction in those already sensitised.⁵⁶ In this study, sensitisation changed the mechanism of parathion-induced airway hyperreactivity to an interleukin-5 (IL-5)-dependent one, resulting in eosinophil recruitment and activation.

It has also been found that pesticides may modify inflammatory responses to other farm exposures, such as allergens. Carbaryl, a commonly used carbamate insecticide, may enhance the allergic response to house dust mites.⁵⁷ In another study, mice treated with malathion showed increased inflammatory mediators, which resulted in increased macrophage function.⁵⁸ These results suggest that organophosphate and carbamate insecticides may be interacting with inflammatory pathways involved in asthma. Inhaled pesticides may cause damage to the airways either directly or through the activation of TRPV1 and TRPA1 ion channels in bronchial C-sensory fibres, inflammatory cells and epithelial cells. This triggers neurogenic inflammation which when sustained over time may lead to nonspecific bronchial hyperreactivity and the subsequent development of asthmatic symptoms.⁵⁹ This is an important mechanism underlying irritant-induced asthma caused by pesticides.

RISK FACTORS FOR RHINITIS AND ASTHMA ASSOCIATED WITH PESTICIDES

Various studies in this review suggest that pesticide mixers, sprayers or applicators are at increased risk of developing rhinitis and/or asthma associated with pesticide exposure.^{3,4,10-13,27-44,49} Some studies suggest that atopic individuals are more vulnerable to pesticides at lower doses than their nonatopic counterparts.⁵⁶ Young children are likely to be at higher risk for developing respiratory symptoms than other age groups because of the high level of exposure to pesticides in domestic and

environmental settings.^{5,15,16} Herbicide and pesticide exposure was strongly associated with asthma diagnosis before the age of 5 compared to other age groups in the Children's Health Study of California.⁵² This may be due to the fact that the respiratory, immune and nervous systems in children are still in the developmental phase and hence more vulnerable to the effect of pesticides and herbicides.

Male sex is a known risk factor for asthma in children below the age of 14, though the reasons are unknown.⁶⁰ Urine levels of dialkylphosphate (DAP) metabolites that are common to the organophosphate pesticides were found to be higher in male children than in female children.⁷ Furthermore, there was a slightly higher prevalence of asthma in male children than in female children in Iraq where pesticides and herbicides were some of the exposures being investigated.⁶¹ This suggests a higher risk of developing respiratory symptoms on exposure to pesticides in boys than in girls. These risk factors in children need further investigation. In contrast to studies in children, two studies have reported that women workers exposed to pesticides appear to have a higher risk of developing asthma than men.^{10,11} However, it is uncertain whether this is related to the gendered distribution of work or due to other as yet unexplored biological factors.

Regarding environmental exposures, the presence of other aeroallergens such as mites is an important risk factor especially for atopic asthma as well as the development of allergic asthma due to sensitisation to outdoor mites.^{49,62} Sensitisation to allergens also renders individuals more vulnerable to developing health effects caused by pesticides.⁵⁶

FUTURE RESEARCH

Future studies should focus on developing more robust measures of exposure such as personal-exposure monitoring using biomarkers. Specific pesticides should be identified for more detailed study so as to determine possible threshold levels for health effects. Future studies should also focus on understanding the pathophysiological mechanisms that underlie asthma and rhinitis so as to characterise the specific asthma phenotypes using more objective measures for asthma outcomes.

Most of the epidemiological studies reported are cross-sectional in nature and therefore have inherent limitations. Other study designs such as cohort studies that have the potential to study outcomes associated with various different pesticide types should be considered. There is a need to expand the population base of study to include more women and children, and populations from developing countries including Africa need further consideration.

Acknowledgements

Vuyelwa Ndlovu is sponsored by the Center for International Health of the Ludwig-Maximilian-University, Munich, Germany (CIHLMU).

Declaration of conflict of interest

Authors declare no conflict of interest.

REFERENCES

1. Jaga K, Dharmani C. Sources of exposure to and public health implications of organophosphate pesticides. *Pan Am J Public Health* 2003; 14(3): 171-185.
2. Alavanja MCR. Pesticides use and exposure extensive worldwide. *Rev Environ Health* 2009; 24(4): 303-309.
3. Van den Driessche KSJ. Anaphylaxis in an airplane after insecticide spraying. *J Travel Med* 2010; 17(6): 427.
4. Alarcon WA, Calvert GM, Blondell JM, et al. Acute illnesses associated with pesticide exposure at schools. *JAMA* 2005; 294(4): 455-465.
5. Badakhsh R, Lackovic M, Ratard R. Characteristics of pesticide related hospitalizations, Louisiana, 1998-2007. *Public Health Rep* 2010; 125(3): 457-467.
6. Curl CL, Fenske RA, Kissel JC, et al. Evaluation of take-home organophosphorus pesticide exposure among agricultural workers and their children. *Environ Health Perspect* 2002; 110(12): 787-792.
7. Koch D, Lu C, Fisker-Andersen J, Jolley L, Fenske RA. Temporal association of children's pesticide exposure and agricultural spraying: report of a longitudinal biological monitoring study. *Environ Health Perspect* 2002; 110(8): 829-833.
8. Garcia AM. Pesticide exposure and women's health. *Am J Ind Med* 2003; 44(6): 584-594.
9. London LdeG, Wesseling C, Kisting S, Rother H, Mergler D. Pesticide usage and health consequences for women in developing countries: out of sight, out of mind? *Int J Occup Environ Health* 2002; 8(1): 46-59.
10. Zhang LX, Enarson DA, He GX, Li B, Chan-Yeung M. Occupational and environmental risk factors for respiratory symptoms in rural Beijing, China. *Eur Respir J* 2002; 20(6): 1525-1531.
11. Faria NM, Facchini LA, Fassa AG, Tomasi E. Pesticides and respiratory symptoms among farmers. *Rev Saude Publica* 2005; 39(6):973-981.
12. Hoppin JA, Umbach DM, London SJ, et al. Pesticides and atopic and nonatopic asthma among farm women in the Agricultural Health Study. *Am J Respir Crit Care Med* 2008; 177(1): 11-18.
13. Fieten KB, Kromhout H, Heederik D, van Wendel de Joode B. Pesticide exposure and respiratory health of indigenous women in Costa Rica. *Am J Epidemiol* 2009; 169(12): 1500-1506.
14. Zhang X, Zhao W, Jing R, et al. Work-related pesticide poisoning among farmers in two villages of Southern China: a cross-sectional survey. *BMC Public Health* 2011; 11: 429.
15. Olson DK, Sax L, Gunderson P, Sionis L. Pesticide poisoning surveillance through regional poison control centers. *Am J Public Health* 1991 ;81(6): 750-753.
16. Garry VF. Pesticides and children. *Toxicol Appl Pharmacol* 2004;198(2): 152-163.
17. Nweke OC, Sanders III WH. Modern environmental health hazards: a public health issue of increasing

- significance in Africa. *Environ Health Perspect* 2009; 117(6):863-870.
18. Kesavachandran CN, Fareed M, Pathak MK, Bihari V, Mathur N, Srivastava AK. Adverse health effects of pesticides in agrarian populations of developing countries. *Rev Environ Contam Toxicol* 2009;200: 33-52.
19. Dalvie MA, Cairncross E, Solomon A, London L. Contamination of rural surface and ground water by endosulfan in farming areas of the Western Cape, South Africa. *Environ Health* 2003; 2: 1.
20. Ntow WJ. Organochlorine pesticides in water, sediment, crops, and human fluids in a farming community in Ghana. *Arch Environ Contam Toxicol* 2001; 40(4): 557-563.
21. Schulz R. Comparison of spray drift-and runoff-related input of azinphos- methyl and endosulfan from fruit orchards into the Lourens River, South Africa. *Chemosphere* 2001; 45(4-5): 543-551.
22. Schulz R. Rainfall-induced sediment and pesticide input from orchards into the Lourens River, Western Cape, South Africa: importance of a single event. *Water Res* 2001; 35(8): 1869-1876.
23. Dalvie MA, Africa A, Solomons A, London L, Brouwer D, Kromhout H. Pesticide exposure and blood endosulfan levels after first season spray amongst farm workers in the Western Cape, South Africa. *J Environ Sci Health B* 2009; 44(3): 271-277.
24. Dalvie MA, London L. The impact of aerial application of organophosphates on the cholinesterase levels of rural residents in the Vaalharts district, Northern Cape Province, South Africa. *Environ Res* 2006; 102(3): 326-332.
25. Dalvie MA, London L. Risk assessment of pesticide residues in South African raw wheat. *Crop Protection* 2009; 28(10): 864-869
26. Rother H. Falling through the regulatory cracks: Street selling of pesticides and poisoning among urban youth in South Africa. *Int J Occup Environ Health* 2010; 16(2): 202-213.
27. Hoppin JA, Umbach DM, London SJ, Alavanja MC, Sandler DP. Chemical predictors of wheeze among farmer pesticide applicators in the Agricultural Health Study. *Am J Respir Crit Care Med* 2002;165(5): 683-689.
28. Hoppin JA, Umbach DM, London SJ, et al. Pesticide use and adult-onset asthma among male farmers in the Agricultural Health Study. *Eur Respir J* 2009; 34(6): 1296-1303.
29. Hoppin JA, Umbach DM, London SJ, Lynch CF, Alavanja MC, Sandler DP. Pesticides associated with wheeze among commercial pesticide applicators in the Agricultural Health Study. *Am J Epidemiol* 2006; 163(12): 1129-1137.
30. Slager RE, Poole JA, LeVan TD, Sandler DP, Alavanja MC, Hoppin JA. Rhinitis associated with pesticide exposure among commercial pesticide applicators in the Agricultural Health Study. *Occup Environ Med* 2009; 66(11): 718-724.
31. Chatzi L, Alegakis A, Tzanakis N, Siafakas N, Kogevinas M, Lionis C. Association of allergic rhinitis with pesticide use among grape farmers in Crete, Greece. *Occup Environ Med* 2007; 64(6): 417-421.
32. Hernandez AF, Casado I, Pena G, Gil F, Villanueva E, Pla A. Low level of exposure to pesticides leads to lung dysfunction in occupationally exposed subjects. *Inhal Toxicol* 2008; 20(9): 839-849.
33. Innes DF, Fuller BH, Berger GM. Low serum cholinesterase levels in rural workers exposed to organophosphate pesticide sprays. *S Afr Med J* 1990; 78(10): 581-583.
34. Abu Mourad T. Adverse impact of insecticides on the health of Palestinian farm workers in the Gaza Strip: a hematologic biomarker study. *Int J Occup Environ Health* 2005; 11(2): 144-149.
35. Senthilselvan A, McDuffie HH, Dosman JA. Association of asthma with use of pesticides. Results of a cross-sectional survey of farmers. *Am Rev Respir Dis* 1992; 146(4): 884-887.
36. Del Prado-Lu JL. Pesticide exposure, risk factors and health problems among cutflower farmers: a cross sectional study. *J Occup Med Toxicol* 2007; 2(9).
37. Slager RE, Simpson SL, Levan TD, Poole JA, Sandler DP, Hoppin JA. Rhinitis associated with pesticide use among private pesticide applicators in the agricultural health study. *J Toxicol Environ Health A* 2010; 73(20): 1382-1393.
38. Salameh P, Waked M, Baldi I, Brochard P, Saleh BA. Respiratory diseases and pesticide exposure: a case-control study in Lebanon. *J Epidemiol Community Health* 2006; 60(3): 256-261.
39. Chakraborty S, Mukherjee S, Roychoudhury S, Siddique S, Lahiri T, Ray MR. Chronic exposures to cholinesterase-inhibiting pesticides adversely affect respiratory health of agricultural workers in India. *J Occup Health* 2009; 51(6): 488-497.
40. Abu Shama F, Skogstad M, Nijem K, Bjertness E, Kristensen P. Lung function and respiratory symptoms in male Palestinian farmers. *Arch Environ Occup Health* 2010; 65(4): 191-200.
41. Swaen G, van Amelsvoort L, Boers D, et al. Occupational exposure to ethylenebisdithiocarbamates in agriculture and allergy: results from the EUROFIT field study. *Hum Exp Toxicol* 2008; 27(9): 715-720.
42. Mekonnen Y, Agonafir T. Lung function and respiratory symptoms of pesticide sprayers in state farms of Ethiopia. *Ethiop Med J* 2004;42(4): 261-266.
43. Mekonnen Y, Agonafir T. Effects of pesticide applications on respiratory health of Ethiopian farm workers. *Int J Occup Environ Health* 2002; 8(1): 35-40.
44. Ohayo-Mitoko GJ, Kromhout H, Simwa JM, Boleij JS, Heederik D. Self reported symptoms and inhibition of acetylcholinesterase activity among Kenyan agricultural workers. *Occup Environ Med* 2000;57(3): 195-200.
45. Jeebhay MF, Quirce S. Occupational asthma in the developing and industrialised world: a review. *Int J Tuberc Lung Dis* 2007; 11(2):122-133.
- and other pesticides in the Europit field studies. *Hum Exp Toxicol* 2008; 27(9): 721-727.

47. Beseler CL, Stallones L. Pesticide poisoning and respiratory disorders in Colorado farm residents. *J Agric Saf Health* 2009; 15(4): 327-334.
48. Ernst P. Pesticide exposure and asthma. *Am J Respir Crit Care Med* 2002; 165(5): 563.
49. Jeebhay MF, Baatjies R, Chang YS, et al. Risk factors for allergy due to the two-spotted spider mite (*Tetranychus urticae*) among table grape farm workers. *Int Arch Allergy Immunol* 2007; 144(2): 143-149.
50. Riu E, Monso E, Marin A, Magarolas R, Radon K, Morera J, et al. Occupational risk factors for rhinitis in greenhouse flower and ornamental plant growers. *Am J Rhinol* 2008; 22(4): 361-364.
51. Salameh PR, Baldi I, Brochard P, Raherison C, Abi Saleh B, Salamon R. Respiratory symptoms in children and exposure to pesticides. *Eur Respir J* 2003; 22(3): 507-512.
52. Salam MT, Li YF, Langholz B, Gilliland FD, Children's Health Study. Early-life environmental risk factors for asthma: findings from the Children's Health Study. *Environ Health Perspect* 2004; 112(6): 760.
53. Dalvie MA, White N, Raine R, et al. Long-term respiratory health effects of the herbicide, paraquat, among workers in the Western Cape. *Occup Environ Med* 1999; 56(6): 391-396.
54. Fryer AD, Lein PJ, Howard AS, Yost BL, Beckles RA, Jett DA. Mechanisms of organophosphate insecticide induced airway hyperreactivity. *Am J Physiol Lung Cell Mol Physiol* 2004; 286(5): L963-9.
55. Lein PJ, Fryer AD. Organophosphorus insecticides induce airway hyperreactivity by decreasing neuronal M2 muscarinic receptor function independent of acetylcholinesterase inhibition. *Fundamental and Applied Toxicology* 2005; 83(1): 166-176.
56. Proskocil BJ, Bruun DA, Thompson CM, Fryer AD, Lein PJ. Organophosphorus pesticides decrease M2 muscarinic receptor function in guinea pig airway nerves via indirect mechanisms. *PLoS One* 2010; 5(5): e10562.
57. Dong W, Gilmour MI, Lambert AL, Selgrade MK. Enhanced allergic responses to house dust mite by oral exposure to carbaryl in rats. *Fundamental and Applied Toxicology* 1998; 44(1): 63-69.
58. Rodgers K, Xiong S. Contribution of inflammatory mast cell mediators to alterations in macrophage function after malathion administration. *Int J Immunopharmacol* 1997; 19(3): 149-156.
59. Hernandez AF, Parron T, Alarcon R. Pesticides and asthma. *Curr Opin Allergy Clin Immunol* 2011; 11(2): 90-96.
60. Horwood LJ, Fergusson DM, Shannon FT. Social and familial factors in the development of early childhood asthma. *Pediatrics* 1985; 75(5): 859-868.
61. Alsamrai AM, Salih MA, Alobaidy AH, Alwan AM, Abdulaziz ZH. Risk factors for asthma in Iraqi children. *Journal of Rural and Tropical Public Health* 2009; 8: 45-52.
62. Spiekma F. Domestic mites: their role in respiratory allergy. *Clin Exp Allergy* 1991; 21(6): 655-660.

