THE RELATIONSHIP BETWEEN ENVIRONMENTAL EXPOSURES TO PESTICIDES MEASURED BY MEANS OF ENVIRONMENTAL EXPOSURE INDICES AND THE ANTHROPOMETRIC OUTCOMES OF BOYS LIVING ON FARMS IN THE RURAL WESTERN CAPE.

ANNE ACHIENG OCHIENG

STUDENT NUMBER: OBLACH001

PART 0: PREAMBLE

THE RELATIONSHIP BETWEEN ENVIRONMENTAL EXPOSURES TO PESTICIDES MEASURED BY MEANS OF ENVIRONMENTAL EXPOSURE INDICES AND THE ANTHROPOMETRIC OUTCOMES OF BOYS LIVING ON FARMS IN THE RURAL WESTERN CAPE.

ANNE ACHIENG OCHIENG
Student Number: OBLACH001

Thesis Submitted in fulfilment of a Masters' Degree in Public Health (Epidemiology & Biostatistics) at the School of Public Health and family Medicine, University of Cape Town, January 2011.

Supervisor:
Mohamed Aqiel Dalvie

Associate Professor & Programme Leader (Chemical exposures and toxicity)

Center for Occupational and Environmental Health Research

School of Public Health & Family Medicine

University of Cape Town

DECLARATION

I, Anne Achieng Ochieng hereby declare that the work on which this dissertation is based is my own except for where it has been acknowledged and referenced. None of this work has been or is being submitted for another degree in another University.

I empower the University of Cape Town to reproduce whole or parts of this work for the purposes of research in whichever way it sees fit.

Signature…….

Full Names……Anne Achieng Ochieng……

Date……01/02/12……..

ACKNOWLEDGEMENTS

Glory and honor to the almighty God for his love, grace and mercy extended to me and my family throughout the years and during my studies.

I would like to thank my supervisor Assoc. Prof Aqiel Dalvie for his invaluable contribution to this work and for holding my hand tirelessly and patiently throughout the process of writing this dissertation. Thanks to Assoc. Prof Francesca Little for her invaluable contribution on the statistical analysis and to the two anonymous reviewers for their thoughtful comments.

Special thanks to the staff at UCT medical school library for their help with referencing and access to articles that I used in the dissertation.

I would also like to thank the team of field workers for the male reproductive health effects in the Western Cape, particularly Mr. Algeron Africa, for co-ordinating the fieldwork and managing the database.

Sincere thanks to Moi Teaching and Referral Hospital for granting me study leave as I did this master's course.

Many thanks to the South African Medical Research Council (MRC) and the University of Cape Town post graduate funding office for financial assistance awarded to me towards my master of Public health programme and The National Research Foundation (NRF) for funding the bigger project.

To my dear friends Linda, Phiona, Alice and Shingai for always being there for me when things got tough. Thanks to my family in Kenya for their prayers and encouragements.

Finally a special thanks to my husband, Ochieng Aoyi, and my two sons, Omondi and Oduor, for unending moral and financial support and the sacrifices they had to make while I was away.

THESIS ABSTRACT

Background

Few epidemiological studies have investigated the effect of pesticides on growth of boys and results are conflicting. Pesticide environmental exposure indices have not previously been developed.

Objective

To investigate the effect of pesticide exposure using environmental exposure indices on pubertal growth of boys.

Methods

A cross-sectional study of 269 boys (176 residing on farms) was conducted in the rural Western Cape in South Africa. Measurements included a questionnaire, height, weight and BMI measurements. A proximity index (PI), spraying intensity index (SI) and combined proximity-spraying index (PSI) was developed, measuring respectively the lifetime average distance of home from spraying, average frequency of farm spraying and PI/SI.

Results

Median age 12.4 years (Inter quartile Range (IQR) = 9.5- 13.3 years). More than 60% boys had height & weight below the < 50^{th} CDC age percentile. After adjusting for confounders, PI and SI was associated with shorter stature **(**β = 1.73cm/10 m; P = 0.02 & β = -1.38 P = 0.05) respectively and PI was also associated with lower weight (β = 1.24 kg/10 m). Associations were

stronger for boys aged < 11 years and were weaker when excluding non-farm boys. There were no other associations between outcome and exposure.

Conclusion

The results provide further evidence that farm boys have lower heights and weights compared to non-farm boys possibly due to hormonally active agricultural pesticides exposure. PI and SI require further development.

DEDICATION

This thesis is dedicated to the memory of my late mum Mrs. Alice Oballa and my father in law Mr. Hesbon Aoyi, they both left when we were not ready to say goodbye.

ABBREVIATIONS

AIC Akaike's Information Criterion

EEI Environmental Exposure Indices

PI Proximity Index

SI Spraying Index

CSP: Combined Spraying and Proximity Index

JEM Job Exposure Matrix

ED Endocrine Disrupting Chemicals

WHO World Health Organization

HPG Hypothalamic-Pituitary-Gonadal axis

DDT Dichlorodiphenyltrichloroethane

CDC Centre for Disease Control

BMI Body Mass Index

GnRH Gonadotrophin-Releasing Hormone

LH Luteinizing Hormone

SMR Sexual Maturity Rating

FSH -Follicle Stimulating Hormone

µg/L -Microgram per Litre

TABLE OF CONTENTS

TABLE OF TABLES

PART A: RESEARCH PROTOCOL

The relationship between environmental exposures to pesticides measured by means of environmental exposure indices and the anthropometric outcomes of boys living on farms in the rural Western Cape.

1 INTRODUCTION.

Contemporary pesticide use has been associated with declining male reproductive health (Anderson 2002; Toppari et al. 1996). Additionally there is emerging evidence that perinatal and childhood exposure to certain organochlorine compounds may affect body size in children, by reducing height and increasing the body mass index, hence increasing the risk of chronic diseases of lifestyle in adulthood (Anderson 2002; Gladen et a1. 2000; Karmaus et al. 2002, Karmaus et al. 2009; Ribas-Fitó et. al. 2006; Verhulst et al. 2009). This has important public health implications in developing countries such as South Africa (SA) where pesticide use is substantial (Dalvie et al. 2009, Naidoo and Buckley 2003) and poorly regulated (Rother et al. 2008).

1.1 PROBLEM STATEMENT.

Previous investigations in the rural Western Cape in South Africa have shown usage and exposure to pesticides, including endocrine disrupting pesticides, to be substantial (Dalvie et al. 2003, Dalvie et al. 2004a, Dalvie et al. 2009; London and Myers 1995). The concern that endocrine disrupting pesticides may affect the body size of exposed children is therefore relevant in this region.

1.2 JUSTIFICATION.

Despite evidence of pesticide exposure among Western Cape farm residents through contaminated food, soil, water and spray drift in addition to occupational exposure in previous studies (Dalvie et al. 2003; Sanusi et al. 2000; Schulz. 2001), the risks of adverse health effects

of long-term pesticide exposure among children living on the farms within Western Cape still remain undetermined. Additionally, characterizing and determining long term environmental pesticide exposure among farm residents is complex and problematic. Although a number of studies have developed job exposure matrices for estimating occupational exposure to pesticides, to date, no published studies could be found that have developed indices for determining environmental exposure to pesticides.

The development of exposure indices to estimate environmental exposure to pesticides would thus greatly improve epidemiological studies investigating the health effects of pesticides in environmentally exposed persons in the absence of bio-monitoring.

1.3 RESEARCH QUESTION.

What is the relationship between environmental exposure to pesticides as quantified by environmental exposure indices and the anthropometric measurements of boys living on farms in the rural Western Cape in South Africa?

1.4 AIM AND OBJECTIVES.

1.4.1 Aim

This is a sub-study of an investigation into the reproductive health effects due to pesticides exposure amongst boys residing in the Western Cape in South Africa. Another sub-study, using a dichotomous exposure variable, found that boys classified as farm boys based on their lifetime living history, had altered reproductive hormone levels, reduced height and weight measurements and reduced testicular size.

This study aims to investigate the relationship between environmental exposures to pesticides measured by means of environmental exposure indices and anthropometric measurements of boys in the rural Western Cape in SA. The environmental exposure indices will be developed from quantitative exposure information collected in the main study.

1.4.2 Objectives

a) To characterize environmental exposures to pesticides of participating boys and to develop environmental exposure indices based on the proximity of homes to the spraying area and the intensity of spraying on the farms.
b) To describe demographic and socio-economic characteristics of the boys.
c) To describe confounders such as phyto-estrogen intake, household pesticide use, smoking history and alcohol consumption.
d) To investigate the relationship between anthropometric development of boys and pesticide exposure using the environmental indices and controlling for relevant confounders.

2 LITERATURE REVIEW

2.1 INTRODUCTION.

Environmental chemicals acting as hormonally active substances have been hypothesized to explain evidence of declining male reproductive health (Skakkebaek and Keiding 1994; Toppari et al. 1996) .Some of these chemicals have been shown to cause male reproductive effects such as abnormal release of reproductive hormones, reproductive organ defects and a decrease in sperm quality and fertility in laboratory animals as well as wild life (Cheek and McLachlan 1998; Toppari et al. 1996). These effects are consistent with the theory that these chemicals disrupt the male endocrine system acting through a number of possible mechanisms including

estrogenic, anti-estrogenic and/or anti-androgenic mechanisms or the aryl hydrocarbon receptor interfering with the male hypothalamus-pituitary-gonadal axis. Additionally, recent reviews indicate that early exposure to certain environmental endocrine disrupting chemicals could increase body weight in humans including DDE which is the main metabolite of DDT (Newbold et al. 2007, Newbold et al. 2008).

There is growing laboratory evidence that anti-androgenic chemical because male reproductive effects (IPCS 2002). Environmental chemicals may also cause male reproductive effects by acting via the thyroid system or non-endocrine mechanisms (Andersen et al. 2000; Andersen et al. 2008; Andrade et al. 2002) .Yet; SA remains the highest pesticide user in Southern Africa posing a potential risk to the exposed populations.

2.2 Pesticide use in South Africa.

An increasing trend in the use of pesticides in SA has been indicated in a few studies (Naidoo & Buckley 2000; Heeren et al. 2003; Dalvie et al. 2009; Maharaj 2005). For example, Dalvie and London (2009) reported that the amount of pesticides sold to 5 major crop sectors in South African agriculture increased from 5400 tons in 1994 to over 6800 tons in 1999. Additionally, significant health effects associated with the domestic use of empty pesticide containers has previously been demonstrated by Heeren et al. (2003) in a case control study among rural women in the Eastern Cape province in SA where children with birth defects were found to be 6.5 times more likely to have been born to women who were using pesticide containers for fetching water.

2.3 Pesticide residues in food in South Africa.

A study conducted by Dalvie and London (2009c) investigating the presence of pesticide residues in wheat produced and imported in South Africa found pesticide residues in both local

and imported wheat samples. Multiple pesticides (> 1 pesticide) were detected in about 30% local samples and 39% imported samples. Eight different agents were detected in total. The most frequently detected pesticides were mercaptothion (99%), permethrin (19%) and chlorpyrifos (17%). Nine (11%) samples exceeded the EU wheat MRL for permethrin (0.05 mg/kg) which included 7 (10%) local samples and 2 (15%) imported samples. The highest fenitrothion level (0.65 mg/kg) corresponded to an intake that was below the estimated short-term safety threshold (Dalvie and London 2009c).

2.4 Pesticide exposure among rural Western Cape residents

Previous studies revealed the use of empty containers for transport and/or storage of water for domestic use (Dalvie et al. 2004c) among the farm residents in the Western Cape. A study investigating pesticide contamination of ground and surface water in the three agricultural areas within the Western Cape indicated widespread low-level contamination of ground water, surface water and drinking water sources by the pesticide endosulfan with about a third of the samples exceeding the European Drinking Water Standard of 0.1 µg/L. Other pesticides detected included, chlorpyrifos, azinphos-methyl, fenarimol, iprodione, deltamethrin, penconazole and prothiofos (Dalvie et al. 2003). A knowledge, attitude and practice cross sectional survey conducted in the same area revealed that farm residents in these areas are potentially exposed to pesticides through various environmental routes including water and are not aware of the harmful effects of pesticides (Dalvie et al. 2004c). London et al. (2002b), further point out the fact that the presence of pesticides in water is not adequately addressed in regulatory controls in SA (London et al. 2002b). The evidence of environmental chemical exposure among the Western Cape farm/rural residents is further supported by other surveys done in this region by (Dalvie and London 2001; Dalvie and London 2006; Dalvie et al. 2006). These surveys revealed the

presence of large quantities of unwanted pesticides including contemporary pesticides such as chlorpyrifos and endosulfan were present on farms (Dalvie and London 2001; Dalvie et al. 2006). In addition, most farms had empty containers on the premises, and most pesticide stores had floors contaminated with chemicals, thus posing a great danger to children who are susceptible to pesticide exposure through several pathways especially in environments where contamination is evident.

2.5 Pesticide exposure pathways among children

Children can be exposed to pesticides through several routes including dietary and drinking water. However, children who live near agricultural farm land could have additional exposures through the proximity of their residences to the spraying areas and possibly method and intensity of spraying in these farms as well as parental work if the clothing are brought and washed at home and use of empty pesticide containers (Lu et al. 2000). Different studies have shown that vineyard treatment can result in drifting beyond the farms into residential areas and schools (Clark et al. 1991; MacNeil and Hikichi 1986; Richter et al. 1992). Most residential areas in agricultural farm land are situated within the farms and children tend to play or carry out chores within these areas. These exposure pathways may contribute substantially to children's exposure and may take place over an extended period of time, hence, methods evaluating long term exposures may be necessary. Studies have shown increased risk of childhood cancer and other diseases among children in agricultural families (Carozza et al. 2009; Savitz and Chen 1990). The possibility of parental occupation and proximity to farm land increasing exposure among children in agricultural families was demonstrated by (Simcox et al. 1995) in a central Washington state study, measurable residues of pesticides in dust samples were detected. Similarly, Lu et al. (2000) found a higher concentration of organophosphorus agricultural

pesticides in house dust of children living in close proximity to orchards as compared to the reference group (1.92 vs 0.27 lg/g; $P < 0.001$), and higher levels of metabolite concentrations in urine. These studies could be indicators that parental occupation and proximity to farmland could increase environmental concentration and exposure among farm residents. An association between increased exposure to pesticides and pesticide spraying among children living within agricultural farms in central Washington State has also been reported by Koch et al. (2002). The latter demonstrated that spraying can increase pesticide exposure even in the absence of parental work or close proximity. The evidence of exposure from the literature discussed thus far warrants a closer look at some of the health effects that have been associated with pesticide exposure, particularly in the male reproductive health which forms an integral part of this study.

2.6 Effects of pesticides on reproductive health

Many contemporary agricultural pesticides are hormonally active (Anderson 2002) with the potential to cause male reproductive health effects in exposed persons. This has important public health implications for South Africa, the highest pesticide user in Southern Africa. Some of the most commonly used pesticides in the Western Cape as identified in previous surveys (London and Myers 1995; London et. al, 2000; London and Rother 2000), have been shown to adversely affect the male reproductive system of laboratory animals and/or wildlife and some have been related to adverse male reproductive outcomes in humans.

Previous studies have linked pesticides with hormonally active properties (Skakkebaek and Keiding 1994) to abnormal male reproductive effects including decline in sperm quality, male reproductive organ defects, and altered fertility in animals and humans. Endocrine disrupting

chemicals (ED) have been demonstrated to affect the hypothalamic pituitary gonadol (HPG) axis (Cheek and McLachlan 1998). The ED chemicals also affect hormone synthesis pathways, thyroid receptors and other hormones including oestrogen and aryl-hydrocarbon (Damstra et al. 2002; Landrigan et al. 2003).

2.7 Pesticides with known endocrine disrupting activity

Several chemicals found in pesticides with endocrine disrupting activities have been known to impair fertility and pubertal development of both lab animals and humans, some of these pesticides are summarized below. Chlorpyryfos a known organophosphate with oestrogenic properties has been shown to have a negative association with reduced sperm quality, androgen index and serum testosterone among American men (Meeker et al. 2004; 2006). The latter has similarly been shown to reduce the serum levels of luteinizing hormone (LH) in ewes (Rawlins et al.1998). In other studies, endosulphan, an organochlorine was associated with decreased serum testosterone, sperm quality and fertility in male rats (Goulet and Hontela 2003). Exposure to endosulphan has also been linked to reduce sexual maturity rating among Indian boys (Saiyed et al. 2003).

Administration of deltamethrin, which is a pyrethroid with weak oestrogenic activity (Andersen et al. 2002), resulted in a change in reproductive behavior and physiology of male rat offspring (Andrade et al. 2002). Similarly, administration of diclorvos was associated with reduced serum testosterone and testicular damage in rats at different dosages (Okamura et al. 2005). Some of the chemicals with known endocrine disrupting activities that are commonly used in the Western Cape are summarized in table 1.

Table 1 summary of pesticides commonly used in the Western Cape with known endocrine disrupting and reproductive health effects				
Pesticide	**Author**	*In vitro* endocrine activity	**Study subjects**	**Reproductive health effect**
Chlorpyrifos (organophosphate) insecticide)	(Andersen et al .2002 ; Meeker et al. 2004, 2006)	Weak estrogenic activity	Humans	Significant negative relationship of urinary metabolite, TCPY (median = 3.2 ug/L) with sperm quality, serum testosterone and androgen index in American men
Cypermethrin (pyrethroid**)**	(Elbetieha et al. 2001)		Humans	Increase in testis weight, reduced fertility, decrease in serum Testosterone, LH, FSH and lower sperm quality
Endosulfan (organochlorine) insecticide	(Saiyed et al. 2003)	Anti-androgenic & estrogenic	Humans	Reduced sexual maturity rating and serum testosterone in environmentally exposed Indian boys
	(Choudhary & Joshi 2003)		Rats	Reduced serum testosterone, testes & accessory glands weight, sperm quality and fertility in male rats administered orally at 5, 10 and 15mg/kg for 30 days.

In the light of the chemicals highlighted in Table 1, it is evident that a number of agricultural pesticides used in the Western Cape, South Africa from different chemical families have the potential to cause male reproductive effects by disrupting the male reproductive system or acting via non-endocrine mechanisms. The end-points of interest in humans include growth and pubertal development, reproductive organ abnormalities, reproductive hormones, semen quality and reduced fertility and the exposure time window is in-utero, child and adult.

2.8 Effects of pesticides on anthropometric outcomes

There is evidence that perinatal exposure to certain organochlorine compounds may affect body size in children, though the human data are scarce and inconsistent. A study conducted by Gladen et al. (2000) in Philadelphia showed that adolescent males with higher prenatal exposure to p,p'-DDE had increases in both height and BMI compared with those with lower exposures; markers of puberty were unaffected (Gladen et al. 2000). Similarly, a study conducted in Germany by Karmaus et al. (2002) showed that reduced height among female but not male children was associated with exposure to higher childhood DDE concentrations. In addition a

prospective cohort study done in the United States (US) revealed significantly reduced height among boys between ages 4-7 in the high exposure group (Ribas-Fitó et al. 2006).While on the contrary, a different study conducted by Gladen at al. (2004) with 304 males born in Philadelphia found no association between anthropometric measurements during their adolescence and prenatal exposure to DDE (Gladen et al. 2004).

2.9 Measurement of pesticide exposures

In addressing some of the challenges in measuring pesticide exposure, few studies have developed job exposure matrices (JEM) to quantitatively estimate the occupational pesticide exposure of farm workers in different agricultural settings including in the Western Cape (London and Myers 1998; Young et al. 2004). The JEM in the Western Cape have subsequently been applied in epidemiological studies investigating the health effects of pesticides in this region (Dalvie et al. 2009a; Dalvie and London 2009b). The JEM used in the Western Cape was designed to estimate occupational pesticides exposure among workers by weighting the time spent on different tasks and the crop sector pesticide usage. Exposure tasks included spraying and mixing as well as indirect exposures such as field contacts, presumed spray drift, and other routes weighted on a scale of 0-10. Crop sector weights were based on market pesticide sales data. However, farm residents, including women and children, are exposed to pesticides through a number of environmental routes. These include homes and schools situated near orchards or vineyards, use of pesticides at home, domestic water sources situated near orchards or vineyards and use of surface water in fields. Other routes of exposure include recreational activities such as swimming in farm dams, children playing in or near orchards and vineyards, use of empty pesticide containers on farms for domestic purposes, eating of crops from orchards and walking through orchards or vineyards immediately after spraying vineyards (Dalvie et al. 2009).

Chemical exposure and contamination among farm residents can also occur during spraying activities especially those living in close proximity to the area. Therefore there is a need for the development of tools that estimate environmental exposure such as environmental exposure indices (EEI) that will capture and characterize long term environmental exposure to pesticides among farm workers and their families.

Careful characterization of chemical exposures is particularly important in the rural agricultural setting, where the environmental measurements or biological monitoring may be lacking, and where the risks of adverse health effects of long-term pesticide exposure are still undetermined. Current characterizations of environmental routes of exposure to agrochemicals are particularly problematic given a lack of knowledge of particular exposure pathways inherent in agricultural settings. For these reasons, accurate quantitative estimates of exposure of farm workers to agrochemicals have proved most deficient in the literature to date and have resulted in biased estimates of the effect because of misclassification of the chemicals responsible hence methods for estimating environmental exposure of farm residents are required.

In the literature, studies investigating environmental exposure generally use single questions on pesticide exposures as indicators of exposure (Brouwer and van Hemmen 1994; London and Myers 1998; Young et al. 2004). No studies could be found in the literature that have developed environmental exposure indices to estimate the environmental exposures experienced by farm residents.

[1]

In conclusion, given the evidence of pesticide effects on anthropometric outcomes and pesticide exposure among Western Cape rural resident, boys living in the area could be at risk of exposure from pesticides with endocrine disrupting properties, and hence a need for a study investigating if their growth are affected. Additionally, there is need to develop a tool quantifying long-term environmental exposure to pesticides.

3 METHODOLOGY

3.1 Study design

This study involves analysis of sub set of data that was collected between April 2007 to March 2008. The main study was a cross-sectional analytic study that investigated the health effects resulting from environmental pesticides exposure on growth, pubertal development and endocrine status of boys and adolescents in the rural Western Cape, South Africa. The choice of such design was influenced by practical consideration given the nature of this study. In addition, a cross sectional design is an easy design to use since it is relatively easy and economical to conduct. In this thesis, the researcher will focus on developing environmental exposure indices from the exposure information collected in the study and the relationship between the exposure indices and growth will be determined.

[1] This thesis is based on secondary data analysis as the data was already collected. The researcher was mainly involved in the development of exposure indices and the relevant data analysis. Some parts of the methodology section in the protocol are therefore written in past tense.

3.2 Study setting

This study was conducted on farm and non-farm school boys from the Hex River Valley where grape farming is practiced, Grabouw where pome fruit farming is predominantly practiced, and Piketberg where wheat and fruit farming is practiced

3.3 Study population

Boys aged 5 to 19 years living on farms were recruited from the above three agriculturally intense areas in the Western Cape where pesticides had previously been detected in water supplies, sediments and farm workers(Dalvie et al. 2003; London et al. 2003; Schults et al. 2001)

3.4 Sampling

The Western Cape department of education provided a list of schools in the selected study areas. The most accessible primary and high schools with pupils from both agricultural and non-agricultural parts in the three areas were selected for the study. Parents of all the boys at the selected schools were asked for provisional consent for their children to be recruited for study through letters, thereafter details of the study was given to parents who agreed to their children's participation. All 94 boys not living on a farm were selected for the study, and 180 boys (60 in each area) out of 398, stratified by age-group, were selected from those living on farms. The age-groups were as follows: 5 to 9 years (before the start of pubertal development), 9.1 to11 years (start of pubertal development), 11.1 to 14 years (advanced and end of pubertal development) and > 14 years (post-puberty). At each school, farm boys were selected by random systematic sampling. The selected participants and their parents/guardians were then asked to avail themselves in school at certain dates for the interviews.

3.5 Recruitment procedures

The fieldwork team relevant to this sub-study consisted of three interviewers and a male nurse. The parents/guardians were encouraged to answer most of the questions. Parents/guardians signed the consent forms in a preferred language on arrival, and oral assent was obtained from the participant after explanation.

3.6 Sample size calculation

Sample size calculations were conducted for weight and height as outcomes in the study as well as other outcomes that are excluded in this analysis. A two sample-test of equality of means (Stata Corporation 2007) was used (exposed/control ratio = 2, i.e. more participants recruited from exposed areas, power = 80%, confidence level = 95%). Results from a previous study (Ribas-Fitó et al. 2006) were used in sample size calculations. The highest sample size for this sub-study was required for weight (n = 174, including 116 exposed and 58 controls) and this indicated sufficient power for this study.

A study sample of 269 boys which consists of a good representation from the age groups 5-9 years (pre-puberty), 9.1-11 years (mid-puberty) and 11.1-14 years (pubertal) as well as those over 14 years (beyond puberty) were recruited (further details of the study sample will be provided in the results section of the manuscript).

Inclusion criteria

Only boys aged 5 -19 years living in rural Western Cape in South Africa in the 3 study areas were included in the study.

Exclusion criteria

There were no exclusion criteria. All boys selected within the age categories who agreed to participate were included in the study.

3.7 DATA COLLECTION TOOLS

3.7.1 Measurements

The measurements conducted in the main study included a questionnaire, weight, height, BMI, physical examination (SMR, testicular volume, testicular and penile abnormality), blood endocrines (LH, FSH, testosterone, inhibin, estradiol) and biological pesticide levels (blood and urine). However, for the purpose of this thesis, only relevant questionnaire items including weight, height and BMI measurements will be used. The biological markers have since been used in another study by (English R, unpublished data).

3.7.2 Questionnaire

The use of mobile technology (Mobile Researcher, Clyral) was implemented in the administration and capture of questionnaires (back translated into English). Trained interviewers administered the questionnaire in the preferred language to the parent or guardian of the participant and captured responses into electronic questionnaires loaded on cellular phones from which they are transferred to a central website via internet. The data could then be downloaded from the website to Excel or a relevant statistical package. A copy of the questionnaire is attached as appendix (B).

The questionnaire included sections on demography, general medical history, genital health history, pubertal development, and mother's personal habits during pregnancy and lifetime environmental exposure to agricultural pesticides, domestic pesticide use, phyto-estrogen intake. The demographics section had items on schooling and age of participant and education, occupation, marital status and household income of parent/guardian. The general medical history section had items on birth weight, lifetime chronic diseases, fetal alcohol syndrome, HIV, back

injuries and pesticide poisonings. The genital health history section had items on mumps and testicular abnormalities, injuries and diseases. While the section on the mother's personal habits during pregnancy included items on alcohol consumption and smoking, diet and the use of soy milk after conception or birth.

The section on lifetime environmental exposure to agricultural pesticides use included distance from the residential home to the farm, the number of times spraying takes place in the farm and the method of spraying used in the farm. This section was based on the participant's living history which extracted information on the different places the participant had stayed during his lifetime. For each home, it was established if the home was located on a farm or not. If the home was located on a farm, further information was asked on spraying intensity on the farm (the number of spraying days per annum on the farm), proximity of the home to pesticides. This section also included information on contact to spraying or contaminated surfaces while playing outside, contaminated water sources, eating of contaminated crops from farms and tasks performed on the farm.

The section on domestic pesticide use had items on pesticide use in the house and garden, fumigation, if persons in the house are sprayers and the use of empty containers. The section on phyto-estrogen intake contained items on dietary content of soy products, other vegetables and nuts.

[2]

[2] **It should be noted that certain questions in section B of the questionnaire pertaining to puberty will not be used as they are not relevant to this sub study. These questions have been used in another study (English 2011)**

Physical Examination

A trained male nurse recorded height, weight (using a calibrated scale) measurements according to standardized methods and calculated BMI (Tanner and Whitehouse 1976).

3.7.3 Development of exposure Indices

Data will be analyzed using STATA version 10.1 (Stata corporation 2007). Three exposure indices including a proximity index, a spraying intensity index and a combined proximity /spraying index will be developed from the exposure information collected in the questionnaire.

a) The proximity index (PI) will be calculated as the average distance of home from the spraying area of all places lived using the following equation:

Proximity Index = $(D_1 Y_1 + D_2 Y_2 \ldots D_x Y_x)/Age$

Where D_i = distance of home from spraying area,

Y_i = number of years lived at the place of residence

D_i for those not living on a farm, distances will be randomly allocated between 500 – 1700 meters using an algorithm in STATA version 10.1 (Stata corporation 2007).

b) The Spraying Index (SI) will be calculated as the lifetime average number of spraying days per year on farms lived using the following using the following equation:

Spraying index = $(B_1Y_1 + B_2Y_2 \ldots + B_xY_x)/age$

Where B_i = total number of days per year sprayed (including boom, tractor and aeroplane spraying) on a farm (days = 0 if not living on a farm)

Y_i = the number of years lived at the place of residence

c) The Combined Spraying Proximity Index (CSP) will be calculated as the ratio of the spraying index to the proximity index using the following equation:

CSP = spraying index/proximity index

The primary exposure variables therefore will be the three exposure indices that will be analyzed as continuous variables.

3.7.4 Outcome measures

The primary outcome variables will be anthropometric measurements (height, weight, body mass index (BMI). These will also all be dichotomized at each quartiles (25^{th}, 50^{th}, 75^{th} percentiles) thus producing 3 dichotomous variables for each outcome. Additionally 2 dichotomous variables will be produced per anthropometric outcome using the 25^{th} and 50^{th} percentile for age (according to CDC growth charts) (Centre for Disease Control and Prevention 2008).

3.8 DATA MANGEMENT AND ANALYSIS

The use of the Clyral's Mobile Researcher software implemented in the administration and capture of questionnaires enabled the PI to have immediate access to the questionnaire data.

All the other data sheets were checked for completeness and consistency by a trained field supervisor before the subject left the examination site. Where indicated, missing or relevant data was obtained from the subject before they left the venue. A checklist was placed outside of the envelope containing each individual subject's data to facilitate completeness of data collection.

Data not captured by Mobile Researcher was entered by a single researcher within one month of the field visit, in the process of which the consistency and coherence of all the responses

including questionnaire data for each subject were checked. In the case of missing or inconsistent questionnaire data, the respondent was re-contacted by the field supervisors.

3.8.1 Data analysis

Analysis will be conducted using STATA version 10.1 (Stata corporation 2007). Univariate, bivariate and multivariate analyses, using both multiple linear and logistic regression analysis, will be performed for relevant variables. Data will be cleaned and explored as necessary. Variables will be assessed for errors e.g. coding, missing data and any other anomalies. Shapiro-wilk test and histograms will be used to test for normality of continuous variable. Scatter plots will be used to illustrate association among continuous variables while box and whisker plots will be used to describe categorical variables. Frequency distribution will be used to describe measures of central tendency in numerical data while contingency tables will be used to describe proportions in categorical data. Regression models will be used to describe the association and effect of exposures on the outcomes.

Analysis will begin with univariate and bivariate explorations of the data. An assessment of relationship between current exposure to pesticides and anthropometric outcomes (height, weight and BMI) will then follow. A further assessment through model building using lifetime exposure indices will then be applied accordingly. The variables to be tested are shown in Table 2.

Linear and Logistic Regression Analysis techniques will be applied during the multivariate analysis using outcomes and exposure variables described before. A standard model building strategy will be used to select the confounders. Potential confounding variables selected on a priori basis and through bivariate data analysis (P-value < 0.1) will be added to the null model

one at a time. The variables that make the most significant contribution to the null model will then be chosen as the baseline model. Several models will then be investigated by successively adding potential confounding variables to the baseline model in a logical stepwise manner. All models will be compared with Aikake's Information Criterion (AIC) statistics and the final model with the lowest AIC for each outcome will be chosen. Finally all the variables will be added to the model and forward and backward stepwise model selection method will be applied to validate the model building strategy. However variables selected on a priori basis will be retained in the models.

Regression diagnostics will be applied to assess the validity of the assumptions underlying linear regression for the linear models and to determine the goodness of fit for the logistic models. Collinearity will also be assessed using Variable inflation factors. Outlying points will be determined using Studentised residuals, Cooks Distance and Dfbeta values.

Further analysis will be conducted:

a) Investigating exposure outcome relationships only amongst those with a history of living on a farm by including those boys who were classified as farm boys in the other sub-study (English 2011, unpublished data). Farm boys will include those who had lived all their lives only on a farm and those who had not lived all their lives on a farm but were born on a farm *and/or* spent the first three years of their life on a farm *and/or* spent more than 3 years of their first 12 years on a farm.

b) Investigating exposure outcome relationships per age-category and

c) Including the dichotomous exposure variable (farm, non-farm) used in the other sub-study in the multivariate model to assess the impact on the strength of association of the exposure indices and also of household income as an indicator of socio-economic status.

Pilot study

Prior to full sample data collection, the protocol and questionnaire were piloted tested on 5 families.

Training

Field workers were trained on the assessment methods, questionnaire administration, collection, storage and transportation of blood samples.

Table 2 Summary of epidemiological models for testing in multivariate analysis

Outcomes	Exposure	Possible confounders/interaction variables
Height/weight/BMI	PI/SI/CSP	Age, household income, mother's smoking and alcohol consumption habits during pregnancy, phyto-estrogen intake, domestic pesticide exposure.

3.9 STUDY LIMITATIONS

The most important limitation to the study is the reliance on exposure history as a marker of long-term exposure to pesticides and the absence of long-term pesticide biomarkers to quantify exposure. However, a good representation of participants in all age-groups in both exposed and unexposed groups were obtained and detailed exposure information and the development of the environmental exposure index also enhances exposure characterization.

Another limitation is the cross-sectional design of the study firstly due to issues about causality and secondly due to the individual variations which characterize anthropometric measurements. Follow-up of participants is planned with this study serving as a baseline. With regard to

variation in measurements, the good representation of participants in all stages of puberty negates this limitation to a certain extent.

3.10 STRENGTHS

The main strengths of the study are the high levels of environmental exposure of the exposed group as shown is previous studies, the recruitment of a sample size which is adequate in terms of power calculations and the age distribution of the boys in terms of the wide range in stages of pubertal development. This enables the study to investigate the entire period of anthropometric development in a highly exposed group of boys.

The use of the environmental exposure indices is another strength as this will enable us to characterize and quantify the lifetime level of exposure in relation to different environmental factors e.g. proximity of residential area.

3.11 ETHICS & COMMUNICATIONS

The study was done in accordance with the Declaration of Helsinki of the 25th world Medical Assembly (WHO 2000). The study proposal has been approved by the University of Cape Town's Research Ethics Committee (REC REF 279/2005). Informed consent was obtained from the parents/guardians and assent was obtained from the children. Children whose parents did not assent for participation were not forced to enroll in the study. Confidentiality was preserved in that only the research team had access to the data and only group results will be reported on. Feedback of individual results will be made to participants. A copy of the written informed consent is attached as Appendix (D).

3.11.1 Autonomy

Participants were given full details of the nature and aims of the study and they were free to decide whether or not to participate in the study without being coerced. A written informed consent was obtained before participation. Participants were assured that they could withdraw from the study whenever they wished to without any consequences. Confidentiality of their personal information was ensured and could only be disclosed, if necessary, after their permission was obtained otherwise subject codes were used for identification instead of names. Confidentiality will further be maintained during analysis as only the researcher will have access to the data and only group results will be reported on.

3.11.2 Benefit

There were no financial incentives awarded to the participants at the start of the study. However, the findings after this analysis would have a population benefit in that it would contribute towards implementation of strategies and possible formulation of health policies to ensure future protection of farm workers/residents and their children from the adverse effects of exposure to hormonally active agricultural pesticides.

3.11.3 Harm/risks

Given the nature of the study, there will be minimal harm to the participants; however the physical examinations could be intimidating to the boys. A male nurse was therefore chosen to do the examinations under private conditions to enhance acceptability and confidentiality. Interviewers were instructed to make every effort to respect the feelings of participants. All participants were free not to answer questions which they were not comfortable with, or withdraw from the study at any time.

3.11.4 Justice

Participants were given contact details, where they could direct their questions either relating to the study or their rights as study participants. After the analysis, the benefits of the research will be made available to the participants and will be widely disseminated through workshops, seminars and journal publications.

3.11.5 Validity and Reliability

The use of Clyral's Mobile Researcher implemented in the capturing of questionnaires enhances the collection of accurate data. All the interviewers (n = 4) as well as the Principal Investigator (PI) attended a half-day training course in the implementation of the mobile technology conducted by Clyral, the contracted technology company. The interviewers also underwent training in the administration of the questionnaire.

The same trained interviewers used mobile technology to interview the mother, father or guardian of participants. The interviews were conducted in the language of preference (Afrikaans); the questionnaire was back-translated into English.

4 BUDGET AND LOGISTICS

4.1 BUDGET

Table 3 Proposed budget for the master's research project

Operational costs	**Total (Rands)**
Consumables – office supplies	1000
Printing and copying	2000
Computer/ laptop	10000
Flash drive/hard drive	2000
Presentation of findings (dissemination activities, conferences , workshops)	15 000
Mini thesis fee	14 000
Grand total	**44 000**

4.2 WORK PLAN FOR 2011

Table 4 Proposed time lines for the research project -2011-2012

Activity/time	February	March	April	May	June	July	August	Sept	oct	Nov	Dec	Jan -2012
Protocol development												
Structured lit review												
Dev Env Exp indices												
Data analysis												
Manuscript												
Final thesis preparation												

5 REFERENCES

Andersen HR, Schmidt IM, Grandjean P, Jensen TK, Budtz-Jørgensen E, et al. 2008. Impaired reproductive development in sons of women occupationally exposed to pesticides during pregnancy. Environ Health Perspect. 116 (4): 566-572.

Andersen HR, Vinggaard AM, Rasmussen TH, Gjermandsen IM, Bonefeld-Jørgensen EC. 2002. Effects of currently used pesticides in assays for estrogenicity, androgenicity, and aromatase activity in vitro. Toxicol Appl Pharmacol. 179 (1): 1-12.

Andrade AJM, Araújo S, Santana GM, Ohi M, Dalsenter PR. 2002. Reproductive effects of deltamethrin on male offspring of rats exposed during pregnancy and lactation.RTP. 36 (3): 310-317.

Brouwer DH, Brouwer EJ. van Hemmen JJ. 1994. Estimation of long-term exposure to pesticides. Am J Ind Med. 25 (4): 573-588.

Carozza SE, Wang Q, Horel S, Cooper S. 2009. Agricultural pesticides and risk of childhood cancers. Int J Hyg Environ Health. 212 (2): 186-195.

Centers for Disease Control and Prevention. Growth Charts. Available :http://www.cdc.gov/growthcharts/ [accessed 08 August 2010].

Cheek AO, McLachlan JA. 1998. Environmental hormones and the male reproductive system. J Androl. 19 (1): 5-10.

Choudhary N Joshi SC 2003. Reproductive toxicity of endosulfan in male albino rats. Bulletin of Bull Environ Contam Toxicol. 70 (2): 285-289.

Clark JM, Marion JR, Tessier DM, Brooks MW, Coli WM. 1991. Airborne drift residues collected near apple orchard environments due to application of insecticide mixtures Bull Environ Contam Toxicol. 46 (6): 829-836.

Colborn T, Clement C. 1992. Chemically-Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection. Adv Mod Environ Toxicol. 21: 403

Collett D. 2002: (Ed) Modelling Binary Data. 2nd Ed. Chapman and Hall/CRC texts in statistical science. Florida: Chapman and Hall/CRC. 408.

Dalvie MA, Cairncross E, Solomon A, London L. 2003. Contamination of rural surface and ground water by endosulfan in farming areas of the Western Cape, South Africa. Environ Health. 2 (1): 1-1.

Dalvie MA, Myers JE, Lou Thompson M, Dyer S, Robins TG, Omar S, et al. 2004a. The hormonal effects of long-term DDT exposure on malaria vector-control workers in Limpopo Province, South Africa. Environ Res. 96 (1): 9-19.

Dalvie MA, Myers JE, Thompson ML, Robins TG, Dyer S, Riebow J,et al. 2004b. The long-term effects of DDT exposure on semen, fertility, and sexual function of malaria vector-control workers in Limpopo Province, South Africa. Environ Res. 96 (1): 1-8.

Dalvie MA, London L, Mbuli S, Cairncross E .2004c. Knowledge and attitudes in the rural Western Cape towards pesticides in water sources. Water SA.30 (1): 43-50.

Dalvie MA, 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):3 26-332.

Dalvie MA, Africa A, London L. 2009a. Change in the quantity and acute toxicity of pesticides sold in South African crop sectors. 1994-1999. Environ Int. 35 (4).

Dalvie MA, Africa A, Solomons A, London L, Brouwer D. Kromhout H. 2009b. 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 MA, London L. 2009c. Risk assessment of pesticide residues in South African raw wheat, Crop Prot. 28 (10): 864-869.

Dalvie MA, Naik I, Channa K, London L. 2011. Urinary dialkyl phosphate levels before and after first season chlorpyifos spraying amongst farm workers in the Western Cape, South Africa. J Environ Sci Health [B]. 46 (2): 163-172.

Ecobichon DJ. 2000. Our changing perspectives on benefits and risks of pesticides: a historical overview. Neurotoxicology. 21 (1-2): 211-218.

Ecobichon DJ. 2001. Pesticide use in developing countries. Toxicology. 160 (1-3): 27-33.

Elbetieha A, Da'as SI, Khamas W, Darmani H. 2001. Evaluation of the toxic potentials of cypermethrin pesticide on some reproductive and fertility parameters in the male rats. Arch Environ Contam Toxicol. 41 (4): 522-528.

Gladen B.C, Ragan N.B, Rogan WJ. 2000. Pubertal growth and development and prenatal and lactational exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene. The J pediatr. 136 (4): 490-496.

Goulet BN, Hontela A. 2003. Toxicity of cadmium, endosulfan, and atrazine in adrenal steroidogenic cells of two amphibian species, Xenopus laevis and Rana catesbeiana. Environ Toxicol Chem. 22 (9): 2106-2113.

Heeren G.A, Tyler J, Mandeya A. 2003. Agricultural chemical exposures and birth defects in the Eastern Cape Province, South Africa: a case-control study. Environ Health. 2 (1): 11-11.

IPCS. 2002. Global Assessment of the State of the Science of Endocrine Disruptors. (Edited by: Terry Damstra, Sue Barlow, Aake Bergman, Robert Kavlock, Glen Van Der Kraak. WHO:Geneva.

Karmaus W, Osuch JR, Eneli I, Mudd LM, Zhang J, Mikucki D, et al. 2009. Maternal levels of dichlorodiphenyl-dichloroethylene (DDE) may increase weight and body mass index in adult female offspring, Occup Environ Med. 66 (3): 143-149.

Karmaus W, Asakevich S, Indurkhya A, Witten J, Kruse H. 2002. Childhood growth and exposure to dichlorodiphenyl dichloroethene and polychlorinated biphenyls. J pediatr. 140 (1): 33-39.

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.

Landrigan P, Garg A, Droller DBJ. 2003. Assessing the effects of endocrine disruptors in the National Children's Study. Environmental Health Perspect. 111 (13): 1678-1682.

London L, Myers, JE. 1995a. Critical issues for agrichemical safety in South Africa. Am J Ind Med. 27 (1): 1-14.

London L, Myers JE. 1995b. Agrichemical use usage patterns and workplace exposure in the major farming sectors in the region of South Africa. SA J Sci. 91: 515-522.

London L, Myers JE. 1995c. General patterns of agrichemical usage in the Southern region of South Africa. S J Sci. 509-514.

London L. Myers J.E. 1998. Use of a crop and job specific exposure matrix for retrospective assessment of long-term exposure in studies of chronic neurotoxic effects of agrichemicals". Occup Environ Med. 55 (3): 194-201.

London L, Dalvie MA, Cairncross E, Solomon A. 2000a. The quality of surface and groundwater in the rural Western Cape with regard to pesticides. WRC Report No: 795/1/00. WRC, Pretoria.

London L, Rother H.A. 2000b. People, pesticides, and the environment: who bears the brunt of backward policy in South Africa?. New Solut. 10 (4): 339-350.

Lu C, Fenske RA, Simcox NJ, Kalman D. 2000. Pesticide exposure of children in an agricultural community: evidence of household proximity to farmland and take home exposure pathways.Environ Res. 84 (3): 290-302.

MacNeil JD, Hikichi M. 1986. Phosmet residues in an orchard and adjacent recreational area. . J Environ Sci Health [B]. 21 (5): 375-385.

Meeker JD, Ryan L, Barr DB, Hauser R. 2006. Exposure to nonpersistent insecticides and male reproductive hormones. Epidemiology. 17 (1): 61-68.

Meeker JD, Ryan L, Barr DB, Herrick RF, Bennett DH, Bravo R, Hauser R. 2004. The relationship of urinary metabolites of carbaryl/naphthalene and chlorpyrifos with human semen quality. Environ Health Perspect. 112 (17): 1665-1670.

Naidoo V, Buckley CA (2003). Survey of pesticide wastes in South Africa and review of treatment options. Water Research Commission (WRC) Report No. 1128/1/03. Pretoria.

Newbold RR, Padilla-Banks E, Snyder RJ, Phillips TM, Jefferson WN. 2007a. Developmental exposure to endocrine disruptors and the obesity epidemic.Reprod Toxicol. 23 (3): 290-296.

Newbold RR, Padilla-Banks E, Snyder RJ, Jefferson WN. 2007b. Perinatal exposure to environmental estrogens and the development of obesity. Mol Nutr Food Res. 51 (9): 12–917.

Newbold RR, Padilla-Banks E, Jefferson WN, Heindel JJ. 2008. Effects of endocrine disruptors on obesity.Int J Androl. 31 (2): 201-208.

Ogden CL, Wei R, Curtin LR. Flegal KM. 2010. The 2000 Centers for Disease Control and Prevention growth charts: several insights after 8 years. Nestlé Nutrition Workshop Series.Paediatric Programme. 65: 181-193.

Okamura A, Kamijima M, Shibata E, Ohtani K, Takagi K, et al. 2005. A comprehensive evaluation of the testicular toxicity of dichlorvos in Wistar rats. Toxicology. 213 (1-2): 129-137.

Ribas-Fitó N, Gladen BC, Brock JW, Klebanoff MA, Longnecker MP. 2006. Prenatal exposure to 1, 1-dichloro-2,2-bis (p-chlorophenyl)ethylene (p,p'-DDE) in relation to child growth.Int J Epidemiol. 35 (4): 853-858.

Richter ED, Chuwers P, Levy Y, Gordon M, Grauer F, et al. 1992. Health effects from exposure to organophosphate pesticides in workers and residents in Israel. Isr J Med Sci. 28 (8-9): 584-598.

Rother H, Hall R, London L .2008. Pesticide use among emerging farmers in South Africa: contributing factors and stakeholder perspectives. Development Southern Africa. 25 (4): 399-424.

Rother HA, London L .1998. Pesticide health and safety policy mechanisms in South Africa: the state of the debate. Occupational and Environmental Health Research Unit Working Paper No.1, Department of Community Health. Cape Town: University of Cape Town.

Rawlings NC, Cook SJ, Waldbillig D. 1998. Effects of the pesticides carbofuran, chlorpyrifos, dimethoate, lindane, triallate, trufluralin, 2,4-D, and pentachloropenol on the metabolic endocrine and reproductive system in ewes. J Toxicol Environ Health. 54 (1): 21-36.

Savitz DA, Chen J. 1990. Parental occupational and childhood cancer: A review of epidemiologic studies. Environ. Health Perspect. .88 (325): 337.

Saiyed H, Dewan A, Bhatnagar V, Shenoy U, Shenoy R, et al. 2003. Effect of endosulfan on male reproductive development. Environ Health Perspect. 111 (16): 1958-1962.

Sanusi A, Millet M, Mirabe P, Wortham H. 2000. Comparison of atmospheric pesticide concentrations measured at three sampling sites: local, regional and long-range transport. Sci Total Environ. 263 (1-3): 263-277.

Skakkebaek NE, Keiding N. 1994. Changes in semen and the testis. BMJ. 309 (69-65): 1316-1317.

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.

Simcox NJ, Fenske RA, Wolz SA, Lee IC Kalman DA. 1995. Pesticides in household dust and soil: exposure pathways for children of agricultural families. Environ Health Perspect. 103 (12): 1126-1134.

Stata Corporation. 2007. 4905 Lakeway Drive, College Station, Texas 77845 USA.

Tanner JM Whitehouse RH. 1976. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child. 51 (3): 170-179.

Toppari J, Larsen JC, Christiansen P, Giwercman A, Grandjean P, et al. 1996. Male reproductive health and environmental xenoestrogens. Environ Health Perspect. 104 (4): 741-803.

Verhulst SL, Nelen V, Hond ED, Koppen G, Beunckens C, Vael C, et al. 2009. Intrauterine exposure to environmental pollutants and body mass index during the first 3 years of life. Environ Health Perspect. 117 (1): 122-126.

World Health Organisation (1990). Public Health Impact of pesticides used in agriculture. WHO. Geneva. Available: http://whqlibdoc.who.int/publications/1990/9241561394.pdf [accessed 20 August 2011].

World Health Organisation. 2000. Declaration of Helsinki – Proposed International Guidelines for Biomedical Research involving Human subjects. Council for International organization of Medical Sciences, revised by 52nd World Medical Assembly, Edinburgh. WHO, Geneva. available: http://www.cioms.ch/publications/guidelines/guidelines_nov_2002_blurb.htm [accessed 12 April 2010].

Young HA, Mills PK, Riordan D, Cress R. 2004. Use of a crop and job specific exposure matrix for estimating cumulative exposure to triazine herbicides among females in a case-control study in the Central Valley of California. Occup Environ Med. 61 (11): 945-951.

PART B: STRUCTURED LITERATURE REVIEW

1 INTRODUCTION

The extensive use of pesticide in the agricultural industry all over the world has been associated with benefits including increased crop yield, improved preservation of farm produce as well as a control of vector- borne diseases caused by rodents and insects e.g. malaria (WHO 1999). However, concerns have been raised over the threats posed by pesticides to human health.

Studies have shown that contemporary agricultural pesticides are hormonally active (Tanner and Whitehouse 1976; Andersen et al. 2002) with the potential to cause male reproductive health effects in exposed persons. This has important public health implications for South Africa, the highest pesticide user in Southern Africa. To date few epidemiological studies have reported on the health effects of long exposure to pesticides on anthropometric outcomes on adolescent boys. There is emerging evidence that perinatal and childhood exposure to certain organochlorine compounds may affect body size in children, by reducing height and increasing the body mass index, hence increasing the risk of chronic diseases of lifestyle in adulthood (Andersen et al. 2002; Gladen et al. 2000; Karmaus et al. 2009; Ribas-Fitó et al. 2006; Verhulst et al. 2009). In the wake of growing concerns about an increasing trend in childhood obesity (Ogden et al. 2002), this could result in serious long term public health implications in South Africa (SA) especially in the Western Cape where previous investigations have shown pesticide use and exposure to be substantial (Dalvie et al. 2004a; Dalvie et al. 2004c; Dalvie et al. 2009a; London 1994; London and Myers 1995b; Karmaus et al. 2002).

1.1 OBJECTIVE

This literature review provides a brief description of pesticides, discusses the use of pesticides within SA, the evidence of pesticide environmental contamination and levels amongst persons

exposed in the rural Western Cape and pesticide exposure pathways among children. The review then focuses firstly on evidence of male reproductive health effects resulting from human exposure to contemporary agricultural pesticides especially reproductive health and growth of boys. Secondly, it focuses on the available approaches to estimating environmental pesticide exposure in exposed persons.

1.2 SEARCH STRATEGY

Literature from online sources were gathered mostly through peer reviewed and print journals. The search was restricted to articles published in English only and conducted through Google scholar as the main search engine. The key data bases used were EBSCO host via academic search premier, Science Direct, Medline and Pub med. The world health organization (WHO) website was visited to get insight into useful information. The search terms used included (Effects of pesticides on health) OR (Pesticide exposure) and (Anthropometric outcomes) OR (Growth) OR (adolescent boys) OR (Endocrine disrupters) OR (weight, height and BMI) OR (pesticide exposure index) OR (Pesticide exposure matrix) OR (Obesity) OR (pesticide exposure indices) OR (exposure assessment measurement methods) OR (male reproductive health).

2 LITERATURE

2.1 Definition of pesticides

The food and Agricultural Organization (FAO) defines a pesticide as:

"Any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest. Pests can be insects, mice and other animals, unwanted plants (weeds), fungi, or microorganisms like bacteria and viruses. Though often misunderstood to refer only to insecticides, the term pesticide also applies to herbicides, fungicides, and various other substances used to control pests. Under United States law, a pesticide is also any substance or

mixture of substances intended for use as a plant regulator, defoliant, or desiccant" (FAO 1986)**.** A similar definition has also been reported by the US Environmental Protection Agency (EPA).

2.2 Types and routes of pesticide exposure

Pesticide exposure can occur through several routes in humans. Figure 1 summarizes the common routes of pesticide exposures as reported by WHO (1990).

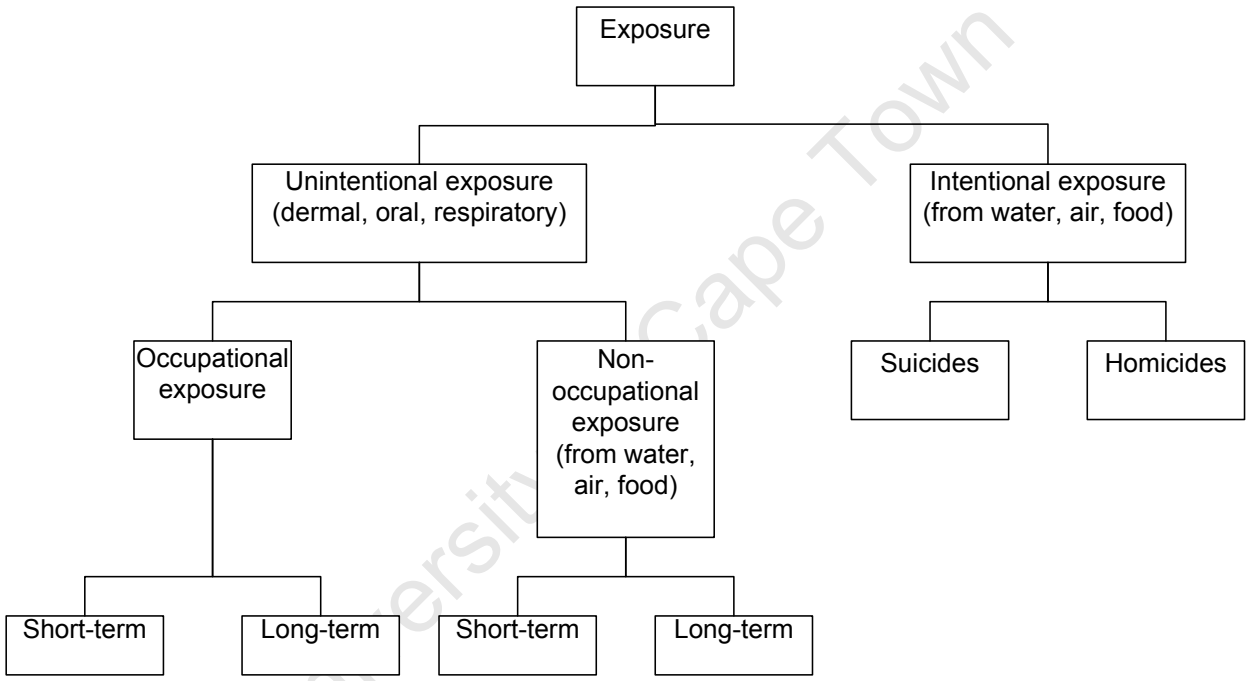

Figure 1 Summary of classification of pesticide exposures (adopted from WHO, 1990)

2.3 Classification of pesticides

Pesticides are generally classified based on their mode of action and the type of pest they control. (Table 1) below summarizes the four major classes of pesticides that are commonly used in the agricultural sector and the examples of each group. (WHO 1990).

Table 1. Summary of classification of commonly used pesticides in agricultural sector		
Pesticide group	**Mode of action and type of pest**	**Example**
Organophosphates	-Insecticides	-Azinfos methyl, diclovors, parathion, malathion, monocrotophos, phosphamidon
	-Affects nervous system, disrupts a neurotransmitter that regulates the enzyme acetylcholine	
Carbamates	-Insectidies and Fungicides	-Carbaryl, methomyl, aldicarb, carbofuran
	-Affects nervous system, disrupts a neurotransmitter that regulates the enzyme acetylcholine	
Organochlorines	-Insecticides	-DDT, chlordane, aldrin, toxaphene, dieldrin, endosulfan
	-alters ion movement in nerve cell membranes	
Pyrethroids	-Insecticides	-Allethrin, permethrin, cyperrmethrin, flumethrin
	-irritants of respiratory system -excitation of neurons	

Pesticides differ in their mode of action, uptake by the body systems, metabolism and toxicity levels to human beings. Toxicity may be acute or chronic and is dependent on the type of chemical, route of exposure and body absorption, the accumulation in the body and one's body immune system (WHO 1990).

2.4 Pesticide use in south Africa

Agriculture is one of the most important income generating activities and the fifth biggest employer in the South Africa (Heeren et al. 2003) Previous data has shown that approximately a tenth of the economically active population is employed in the agricultural sector in South Africa (Heeren et al. 2003).

Previous study (Naidoo and Buckley 2003) indicated that the use of pesticides in South African agriculture is high and continues to increase. For example, insecticide use increased from 2,612 .484 tons in 1997 to 4,363. 371 tons in 2000, and fungicide use from 6,928.639 tons to 8,808.883 tons during the same period. Herbicide use increased slightly from 9,428. 189 tons in 1997 to

9,466.144 tons in 2000 (Maharaj 2005). Although pesticides are used predominantly for agriculture, they are also used residentially and in public places and public buildings to control weeds, vermin, pests and disease vectors (Rother and London 1998).

Recently, (Dalvie et al. 2009a) showed that that the amount of pesticides sold to 5 major crop sectors pesticide sales in South African agriculture increased from 5400 tons in 1994 to over 6800 tons in 1999. Most of the increase in pesticide sales in 1999 was in the grape sector which nearly doubled to 3220 tons. Dalvie et al. (2009a) also report increases in sales in the potato and stone fruit sectors, but a substantial decrease in the pome fruit sector. The grape, pome fruit and potato sectors were the highest. The total kg of pesticides sold per hectare also increased slightly in 1999, but mostly in the stone fruit sector, while sales/hectare decreased in the grape & pome fruit sector. The highest sales/hectare was in the grape, pome and stone fruit sectors (Dalvie et al. 2009a). Additionally, significant health effects associated with the domestic use of empty pesticide containers has previously been demonstrated by (Hereen et al. 2003) in a case control study among rural women in the Eastern Cape province in SA where children with birth defects were found to be 6.5 times more likely to have been born to women who were using pesticide containers for fetching water.

The Western Cape is one of the most agriculturally productive provinces in the country and focuses on agriculture as an important industry and income earner. Crop farming in the form of grapes, pome fruit, stone fruit, potato and wheat farming is especially important.

2.5 Pesticide exposure in SA and among residents of rural Western Cape

Evidence of pesticide exposure among rural farm residents within SA and the Western Cape has been reported (Dalvie et al. 2009a, 2009b, 2009c). Presence of pesticides in food residues in

local and imported wheat samples in this region has also been documented (Heeren et al. 2003; Dalvie and London 2009c; Dalvie et al. 2003). Ground water is considered an important source of exposure since most of the rural residents rely on borehole water for domestic use. A study done within Western Cape showed that endosulfan exceeded the European Drinking Water Standard of 0.1 µg/L in about a third of the samples (Dalvie et al. 2003). Additionally, other pesticides were also detected in soil and water samples including, chlorpyrifos, azinphos-methyl, fenarimol, iprodione, deltamethrin, penconazole and prothiofos (Dalvie et al. 2003; London and Rother 2000; Schulz 2001). Other surveys done in this region have also revealed evidence of environmental exposure to pesticides through the presence of large quantities of unwanted contemporary pesticides such as chlorpyrifos, atrazine and endosulfan as well as presence of empty pesticide containers on the farm premises (Dalvie and London 2001; Dalvie et al. 2003; Dalvie et al. 2006b).

Higher levels of chlorpyrifos and endosulfan than those measured in other settings have been measured amongst farm workers in the Western Cape. Studies have reported lack of knowledge of pesticide exposure among the farm dwellers and pointed out the lack of legislative control measures in the use of pesticides in the SA and the Western Cape region (Dalvie et al. 2004d; London et al. 2005), thus raising serious implications over education and possible protection against exposure in this population . In the latter study, 60% of farm residents reported living within 10 meters away from the spraying area and 48% reported swimming in the farm dams, while 18% reported pesticide spray drifting into their houses during spraying (Dalvie et al. 2004d). The evidence of exposure discussed in the region thus far raises public health concerns

regarding the health of farm workers and their families especially children who live in the farming areas.

2.6 Pesticide exposure pathways among children

Children's exposure to environmental toxicants is a current public health concern (Olden and Guthrie 2000). Children are known to be more susceptible to the effects of these exposures, as they have higher rates of metabolism, less mature immune systems, and different patterns of activity and behavior than adults (Faustman et al. 2000). They have a greater surface to volume ratio than do adults; therefore, they receive a greater dose from the pesticides to which they are exposed. Children metabolize toxicants slower than do adults, therefore, the pesticide dose they receive remains with them longer (Dalvie and London 2006a; Eskenazi et al. 1999; Weiss et al. 2004). Additionally, children can experience chronic low-concentration pesticide exposures that may cause effects not evident in routine clinical examinations (Eskenazi et al. 1999; Landrigan et al. 2001).

Children can be exposed to pesticides through several routes including dietary and drinking water. However children who live near agricultural farm land could have additional exposures to pesticides through the proximity of their residences to the spraying areas, method and intensity of spraying in the farms, as parental work if the clothing are brought and washed at home and use of empty pesticide containers (Landrigan et al. 2003; Lu et al. 2000; Simcox et al. 1995). A number of studies have shown that pesticide spraying of vineyards can result in drift beyond the farms into residential areas and schools. An association between increased pesticide exposure among children living on farms and pesticide spraying have been shown (Clark et al. 1991; Koch et al. 2002; Lu et al. 2000; MacNeil and Hikichi 1986; Richter et al. 1992). Most residential areas in agricultural farm land are situated within the farms and children tend to play or carry out

chores within these areas. These exposures pathways may contribute substantially to children's pesticide exposure and may take place over an extended period of time. Chronic and short term exposure to pesticides have been hypothesised to result in several health problems in both adults and children, including carcinogenic, reproductive endocrine disrupting effects as well as effects on the body's nervous system. The health effects relevant to our study are summarised in the following section.

2.7 Effects of pesticides on male reproductive health

Environmental chemicals acting as hormonally active substances have been hypothesized to explain evidence of declining male reproductive health (Skakkebaek 1994; Richter et al. 1992; Toppari et al. 1996). Some of these chemicals have been shown to cause male reproductive effects such as abnormal release of reproductive hormones, reproductive organ defects and a decrease in sperm quality and fertility in laboratory animals as well as wild life (Cheek and McLachlan 1998; Toppari et al. 1996). These effects are consistent with the theory that these chemicals disrupt the male endocrine system acting through a number of possible mechanisms including estrogenic, anti-estrogenic and/or anti-androgenic mechanisms or the aryl hydrocarbon receptor interfering with the male hypothalamus-pituitary-gonadal axis. There is growing laboratory evidence that anti-androgenic chemicals cause male reproductive effects (IPCS 2002). Environmental chemicals may also cause male reproductive effects by acting via the thyroid system or non-endocrine mechanisms. Some of the most commonly used pesticides in the Western Cape as identified in previous surveys (Andersen et al. 2002; London and Myers 1995b; London et. al. 2000) have been shown to adversely affect the male reproductive system of laboratory animals and/or wildlife and some have been related to adverse male reproductive outcomes in humans.

Table 2 summarizes laboratory and epidemiological studies of some of the chemicals commonly used in Western Cape that have endocrine disrupting activities and effects on the male reproductive health.

Table 2 Endocrine disrupting activity and male reproductive effects of agricultural pesticides commonly used in the Western Cape, South Africa.

Pesticide	**Author**	***In vitro* endocrine activity**	**Study subject**	**Reproductive health effect**
Chlorpyrifos (organophosphate) insecticide)	(Andersen et al.2002; London & Rother 2000; Meeker et al. 2006)	Weak estrogenic activity	Human	Significant negative relationship of urinary metabolite, TCPY (median = 3.2 ug/L) with sperm quality, serum testosterone and androgen index in American men.
Cypermethrin (pyrethroid)	(Elbetieha et al. 2001; Meeker et al. 2006)		Humans	Increase in testis weight, reduced fertility, decrease in serum Testosterone, LH, FSH and lower sperm quality.
	(Elbetieha et al. 2001; Yousef et. Al. 2003)		Rabbits	Reduced plasma testosterone & sperm quality in rabbits administered 24 mg/kg every 2nd day for 12 weeks.
Endosulfan (organochlorine) insecticide	(Saiyed et al. 2003; Yousef et al.2003)	Anti-androgenic & estrogenic	Humans	Reduced sexual maturity rating and serum testosterone in environmentally exposed Indian boys.
	(Choudhary & Joshi 2003; Saiyed et al. 2003)		Rats	Reduced serum testosterone, testes & accessory glands weight, sperm quality and fertility in male rats administered orally at 5, 10 and 15mg/kg for 30 days.
Fenvalerate (pyrethroid)	(Chen et al.2005; Choudhary & Joshi 2003; Xia et al.2004)	-Effects on steroidogenesis signalling cascades and/or steroidogenic enzyme's activity. -Decreased progesterone and estradiol production in a dose-dependent manner at 0-625 µmol/l.	Humans	Significantly reduced sperm quality and raised aneuploidy among Chinese factory workers.
	(Mani et al. 2002)		Rats	Decrease in sperm production, testosterone marker enzymes and serum testosterone in rats for 3 months.
	(Xia et al. 2004; Xu et al. 2004; Young et al. 2004)		Rats	Testicular lesions, reduced sperm motility in rats dozed 3.3 mg/kg.

Pesticide	Author	*In vitro* endocrine activity	Study subject	Reproductive health effect
	(Zhang et al.2010)		Mice	Decreased sperm count, histology, serum and testicular testosterone in adult mice given fenvalerate 60mg/kg/day from postnatal day 35-63.
Iprodione	(Anderson 2002, 2005, 2006; Blystone et al. 2007; Gray et al.1999; Yousef et al.2003).	Anti-androgenic, inhibit aromatase, inhibition of steroidogenesis.	Humans/Rats	Male reproductive developmental abnormalities in rats. delayed puberty (progression of preputial separation), decreased androgen sensitive seminal vesicle and epididymides weights; reduced serum testosterone, 17alpha-hydroxyprogesterone and androstenedione; LH unaffected (200 mg/kg/day.)
Deltamethrin	(Andrade et al. 2002; Blystone et al. 2007)		Rats	In-utero and lactation exposure results in changes in reproductive behaviour and physiology of male rats administered orally at 4 mg/kg from day 1of pregnancy to day 21 of lactation
Dichlorvos	(Andersen et al.2002; Andrade et al. 2002; Okamura et al. 2005)	AR antagonist	Rats	Reduction in sperm quality, serum testosterone and testicular damage in rats dosed 1, 2, 4 mg/kg for 6 weeks.
DNOC**(dinitro-orthocresol) insecticide)**	(Andersen et al. 2002;Takahashi et al. 2004)		Rats	Reduction in sperm quality of rats dosed 4, 7.5 & 15 mg/kg for 5 days
Fenarimol	(Andersen et al. 2002; Takahashi et al.2004;Vinggaard et al. 2005)	Anti-androgeni estrogenic,anti-androgenic, estrogenic, inhibit aromatase activity.	Rats	Sexual differentiation and reduced breeding performance in male rats.
Glyphosate	(Young et al. 2004; Yousef et al.1996)	Aromatase inhibitor (at 210 µM in 24 hours) in human embryonic and placental cells.	Human/Rabbit	*In vitro* reduction in human and rabbit sperm quality.

Evidence that a number of agricultural Pesticides used in the Western Cape, South Africa have have the potential to cause male reproductive effects by disrupting the male reproductive system or acting via non-endocrine mechanisms has been discussed thus far. However, limited studies in literature were found that have investigated the effects of these pesticides on anthropometric outcomes. Some of the studies found are summarized in table 3.

2.8 Epidemiological studies on effects of pesticides on anthropometric outcomes

Exposure to organochlorine pesticides especially DDT has been thought to cause effects on pubertal growth of boys, but the results of epidemiological investigations have been contradictory, (Table 3).

Table 3 Summary of epidemiological studies investigating the relationship between anthropometric measurements and organochlorine pesticides.

Authors (year)	Population& design	Results
Burns et al. 2011	Cohort of Russian boys (n = 499) aged 8-9 years followed for 4 years. The relationship between organochlorine (OC) pesticides (including DDT) and height, weight and BMI was investigated through follow up visits and biomarkers.	Serums OCs were associated with reduced height and BMI.
Gladen et al. 2000	Cohort study of 594 North Carolina boys with prenatal and lactational exposure to background levels of DDT. Height, weight, and stage of pubertal development were assessed through annual mail questionnaires.	Height and weight adjusted for height at puberty increased with prenatal exposure to DDE, as did weight adjusted for height. Boys with high maternal DDE concentration (> 4 ppm fat) were 6.3cm taller than those with lower maternal DDE concentration (0- 1 ppm fat).
Gladen et al.2004	Cohorts follow up study of 394 boys in Philadelphia up to 20years of age. Association between higher exposure to DDE and height, weight, BMI and testosterone levels was investigated through a follow up study.	No associations were found between the outcomes and prenatal exposure to any of the DDT compounds.
Karmaus et al	Cohort follow up study of 343 German children from birth up to 10 years. Association between growth and blood concentration of PCB and DDE at 8years was investigated.	Reduced growth for girls in the high DDE exposure group (1.8cm, P = 0.0275), concentration (>0.44µg/L) as compared to girls in the low DDE exposure quartile (0.08 – 0.2 µg/L). No association was observed in boys.
Smink et al.2008	Cohort study of 482 children in Spain, Menorca followed up from birth up to 6.5 years. The effects of pre-natal exposure to hexachlorobenzene (HCB) on child's weight and body mass index (BMI) at 6 years was investigated, through questionnaire and biomarkers.	Prenatal exposure to HCB was associated with an increase in BMI and weight at age 6.5 years.
(Ribas-Fitó et al. 2006	Prospective cohort study of 1712 boys aged between 4-7 years. Association between maternal exposure to p.p'-DDE and growth during the first seven years of life was investigated.	Decreased height at age 1 year -0.72cm, age 4years -1.14 cm and 7 years -2.19cm was observed among participants in the higher p.p'-DDE exposure group concentration (>60 µg/l),in comparison to the low p.p'-DDE exposure group concentration (<15 µg/l).

A study conducted (Gladen et al. 2000) in Philadelphia showed that adolescent males with higher prenatal exposure *to p,p'*-DDE had increases in both height and BMI compared with those with lower exposures. While a study conducted afterwards on 304 males born in Philadelphia found no association between anthropometric measurements during their post-natal and prenatal

exposure to *p,p'*-DDE (Gladen et al. 2004). On the other hand, a study conducted in Germany by Karmaus et al. (2002) showed that reduced height among female but not male children was associated with exposure to higher childhood DDE concentrations. A prospective cohort study done in the United States (US) found significantly reduced height among boys between ages 4-7 in the high DDT exposure group concentration range (Ribas-Fitó et al. 2006) while a cohort study on Russian boys found reduced peri-pubertal BMI and height associated with serum organochlorine at age 8-9 years. Additionally, a Spanish study (Smink et al. 2008) found hexachlorobenzene exposure to be associated increased weight and BMI.

There is therefore a need for further investigations into the relationship between exposure to endocrine disrupting pesticides and pubertal growth especially in developing countries. There is also a need for epidemiological studies investigating the relationship between exposure to contemporary agricultural endocrine disrupting pesticides and pubertal growth as no published literature could be found on this topic.

2.9 Effect of changes in body size caused by environmental chemicals on chronic diseases of lifestyle

There appears to be an increasing trend in the prevalence of obesity in children (Kelishadi 2007; Olgen et al. 2002) and there is evidence that obese children and adolescents have a higher risk of developing obesity in adulthood (Guo et al. 2002). If perinatal and/or childhood exposure to environmental chemicals affect body size in children and adults, by reducing height and increasing the body mass index, this could increase the risk of chronic diseases of lifestyle in adulthood like type 2 diabetes and hypertension (Guo et al. 2002; Newbold et al. 2007a & 2007b, 2008; Verhulst et al. 2009).

2.10 Estimation of human environmental exposures to pesticides

In the absence of bio-monitoring, quantitative assessment of exposure to pesticides by questionnaire is challenging. Exposure matrices have been used in previous studies in different settings to estimate pesticide exposure of farm workers (Brouwer et al. 1994; Corrao et al. 1989; Karmaus et al. 2002). London and Myers (1998a) developed a job exposure matrix (JEM) to quantitatively estimate the occupational pesticide exposure of farm workers in the Western Cape in SA. This JEM has subsequently been applied in epidemiological studies investigating the health effects of pesticides in this region (London and Myers 1998a; Dalvie et al. 2009a).The JEM developed by London et al. (1998a) determines occupational pesticide exposure by the lifetime number of days worked on a farm weighted for job task and crop sector pesticide usage derived from pesticide sales data. Other exposure variables such as the use of personal protective equipment, past poisoning with pesticides, domestic use of pesticides and pesticide containers, and water sources are used as independent exposure variables but are not incorporated in the JEM.

The job exposure matrices discussed above were developed to estimate occupational exposure to pesticides. However, farm residents including women and children, are exposed to pesticides through a number of non-occupational or environmental routes. These include spray drift into homes and schools situated near orchards or vineyards, use of pesticides at home and use of water containing pesticides for drinking or recreational use (Heeren et al. 2003; Simcox et al. 1995; Fenske at al. 2001). Other routes of environmental pesticide exposure include children playing in or near orchards and vineyards, use of empty pesticide containers on farms for domestic purposes, eating of crops from orchards and walking through orchards or vineyards immediately after spraying vineyards (Young et al. 2004; Dalvie et al. 2009a). Studies have

shown that vineyard treatment can result in pesticide drift beyond the farms into residential areas and schools (MacNeil and Hikichi 1986; Clark et al. 1991; Richter et al. 1992). Pesticide exposure of farm residents can also occur especially those living in close proximity to the area. It has been demonstrated that pesticide residues in homes and urine of residents increases significantly with proximity to pesticide spraying on farms (Burns et al. 2011; Coronado et al. 2011; Lu et al. 2000; McCauley et al. 2000; Quandt et al. 2004). The development of tools such as environmental exposure indices (EEI) that will capture and characterize long term environmental exposure to pesticides among farm residents is therefore important.

Careful characterization of pesticide exposures amongst farm residents is particularly important in settings where biological monitoring may be lacking, and where the risks of adverse health effects of long-term pesticide exposure are still undetermined. Characterizations of environmental routes of pesticide exposure to agrochemicals are, however, complicated. For these reasons the development of methods to quantitatively estimate the environmental exposure of farm residents to agrochemicals have proved most deficient in the literature to date.

In the literature, epidemiological studies investigating health effects due to environmental pesticide exposure generally use single questions on pesticide exposures as indicators of exposure (Brouwer et al. 1994; Dalvie et al. 2009a; London and Myers 1998a; London et al. 1998b; Young et al. 2004). No studies could be found in the literatures that have developed environmental exposure indices. If the environmental exposure of farm residents to pesticides is not characterized appropriately, this can result in biased estimates of health effects in epidemiological studies because of exposure misclassification.

3 CONCLUSION

Endocrine disrupting chemicals have been linked to changes in growth and body size in humans. Many contemporary pesticides used in the South African agricultural sector are endocrine disruptors. The Western Cape is an important agricultural region in South Africa. Western Cape rural residents have shown to be highly exposed to pesticides. Epidemiological studies investigating the effect of endocrine disrupting pesticides on the growth of boys are few, have investigated only organochlorine pesticides and have produced contradictory results. No studies have been conducted in developing countries and on contemporary pesticides. There is therefore a need for more research on this topic. There is also a need to characterize environmental exposure of rural residents to pesticides. No studies were found in the literature that have developed environmental exposure indices (EEI) to quantify human pesticide exposure.

4. REFERENCES

Andersen HR, Schmidt IM, Grandjean P, Jensen TK, Budtz-Jørgensen E, et al. 2008. Impaired reproductive development in sons of women occupationally exposed to pesticides during pregnancy. Environ Health Perspect. 116 (4): 566-572.

Andersen HR, Vinggaard AM, Rasmussen TH, Gjermandsen IM, Bonefeld-Jørgensen EC. 2002. Effects of currently used pesticides in assays for estrogenicity, androgenicity, and aromatase activity in vitro. Toxicol appl pharmacol. 179 (1): 1-12.

Andrade AJM, Araújo S, Santana GM, Ohi M, Dalsenter PR. 2002. Reproductive effects of deltamethrin on male offspring of rats exposed during pregnancy and lactation. RTP. 36 (3): 310-317.

Blystone CR, Lambright CS, Furr J, Wilson VS, Gray LE. 2007. Iprodione delays male rat pubertal development, reduces serum testosterone levels, and decreases ex vivo testicular testosterone production. Toxicol Lett. 174 (1-3): 74-81.

Brouwer DH, Brouwer EJ. van Hemmen JJ. 1994. Estimation of long-term exposure to pesticides. Am J Ind Med. 25 (4): 573-588.

Burns JS, Williams PL, Sergeyev O, Korrick S, Lee MM, Revich B, et al. 2011. Serum Concentrations of Organochlorine Pesticides and Growth among Russian Boys. Environ Health perspect. http://dx.doi.org/10.1289/ehp.1103743 [Online 7 October 2011].

Cheek AO, McLachlan JA. 1998. Environmental hormones and the male reproductive system. J Androl. 19 (1): 5-10.

Chen J, Chen H, Liu R. He J, Song L, et al. 2005. Effects of fenvalerate on steroidogenesis in cultured rat granulosa cells. Biomed Environ Sci. 18 (2): 108-116.

Choudhary N, Joshi SC. 2003. Reproductive toxicity of endosulfan in male albino rats. Bull Environ Contam Toxicol. 70 (2): 285-289.

Clark JM, Marion JR, Tessier DM, Brooks MW, Coli WM. 1991. Airborne drift residues collected near apple orchard environments due to application of insecticide mixtures Bull Environ Contam Toxicol. 46 (6): 829-836.

Colborn T, Clement C. 1992. Chemically-Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection. Adv Mod Environ Toxicol. 21: 403.

Colborn T,vom Saal FS, Soto AM. 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ Health Perspect. 101 (5): 378-384.

Corrao G, Calleri M, Carle F, Russo R, Bosia S, Piccioni P. 1989. Cancer risk in a cohort of licensed pesticide users. Scand J work Environ Health. 15 (3): 203-209.

Coronado GD, Holte S, Vigoren E, Griffith WC, Barr DB, Faustman E, et al. 2011. Organophosphate pesticide exposure and residential proximity to nearby fields: evidence for the drift pathway. J Occup Environ Med. 53 (8): 884-891.

Dalvie MA, London L. 2001. Unwanted pesticides in South Africa: A need for a Public Health intervention? SA J Sci. 97: 309-312.

Dalvie MA, Cairncross E, Solomon A, London L. 2003. Contamination of rural surface and ground water by endosulfan in farming areas of the Western Cape, South Africa. Environ Health. 2 (1): 1-1.

Dalvie MA, Myers JE, Lou Thompson M, Dyer S, Robins TG, Omar S, et al. 2004a. The hormonal effects of long-term DDT exposure on malaria vector-control workers in Limpopo Province, South Africa. Environ Res 96 (1): 9-19.

Dalvie MA, Myers JE, Thompson ML, Robins TG, Dyer S, et al. 2004b. The long-term effects of DDT exposure on semen, fertility, and sexual function of malaria vector-control workers in Limpopo Province, South Africa. Environ Res. 96 (1): 1-8.

Dalvie MA, Myers JE, Thompson ML, Robins TG, Omar S, Riebow J. 2004c. Exploration of different methods for measuring DDT exposure among malaria vector-control workers in Limpopo Province, South Africa. Environ Res. 96 (1): 20-27.

Dalvie MA, London L, Mbuli S, Cairncross E. 2004d. Knowledge and attitudes in the rural Western Cape towards pesticides in water sources. Water SA. 30 (1): 43-50.

Dalvie MA, London L. 2006a. 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 MA, Africa A, London L. 2006b. Disposal of unwanted pesticides in Stellenbosch, South Africa. Sci Total Environ. 15 (361): 1-3.

Dalvie MA, 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 MA, London L. 2009b. Risk assessment of pesticide residues in South African raw wheat. Crop Prot. 28 (10): 864-869.

Dalvie MA, 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).

Elbetieha A, Da'as SI, Khamas W, Darmani H. 2001. Evaluation of the toxic potentials of cypermethrin pesticide on some reproductive and fertility parameters in the male rats. Arch Environ Contam Toxicol. 41 (4): 522-528.

Eskenazi B, Bradman A, Castorina R. 1999. Exposures of children to organophosphate pesticides and their potential adverse health effects. Environ Health Perspect. 107 (3): 409-419.

Faustman EM, Silbernagel SM, Fenske RA, Burbacher TM, Ponce RA. 2000. Mechanisms underlying children's susceptibility to environmental toxicants. Environ. Health Perspect. 108 (3): 13-21.

Food and Agriculture Organisation (FAO). 1986. International code of conduct on the distribution and use of pesticides. available:http://www.fao.org/docrep/005/y4544e/y4544e00.htm. [accessed September 2011].

Fox RD, Reichard DL, Brazee RD, Krause CR, Hall FR. 1993. Downwind residues from spraying a semi-dwarf apple orchard. Trans. ASAE. 36 (2): 333-340.

Gladen B.C, Ragan N.B, Rogan WJ. 2000. Pubertal growth and development and prenatal and lactational exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene. J Pediatr. 136 (4): 490-496.

Gladen BC, Klebanoff MA, Hediger ML, Katz SH, Barr DB. 2004. Prenatal DDT exposure in relation to anthropometric and pubertal measures in adolescent males. Environ Health Perspect. 112 (17): 1761-1767.

Guo SS, Wu, W, Chumlea WC, Roche AF. 2002. Predicting overweight and obesity in adulthood from body mass index values in childhood and adolescence. Am J Clin Nutr. 76 (3): 653-658.

Gray LE Jr, Ostby J, Ferrell J, Sigmon R, Cooper R, Linder R, Rehnberg G, Goldman J, Laskey, J. 1989. Correlation of sperm and endocrine measures with reproductive success in rodents. Prog Clin Biol Res. 302: 193-206.

Heeren G.A, Tyler J, Mandeya A. 2003. Agricultural chemical exposures and birth defects in the Eastern Cape Province, South Africa: a case-control study. Environ Health. 2 (1): 11-11.

International Programme on Chemical Safety (IPCS). 2002. Global Assessment of the State-of-the-Science of Endocrine Disruptors. (Edited by: Damstra T, Barlow S, Kavlock RA, Van Der Kraak G. WHO: Geneva.

Kelishadi R. 2007. Childhood overweight, obesity, and the metabolic syndrome in developing countries. Epidemiol Rev. 29: 62-76.

Karmaus W, Asakevich S, Indurkhya A, Witten J, Kruse H. 2002. Childhood growth and exposure to dichlorodiphenyl dichloroethene and polychlorinated biphenyls. J pediatr. 140 (1): 33-39.

Karmaus W, Osuch JR, Eneli I, Mudd LM, Zhang J, Mikucki D, et al. 2009. Maternal levels of dichlorodiphenyl-dichloroethylene (DDE) may increase weight and body mass index in adult female offspring. Occup Environ Med. 66 (3): 143-149.

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.

Landrigan P, Garg A, Droller DBJ. 2003. Assessing the effects of endocrine disruptors in the National Children's Study. Environ Health Perspect. 111 (13): 1678-1682.

London L. 1994. Agrichemical safety practices on farms in the Western Cape. SAMJ. 84 (5): 273-278.

London L, Myers, JE. 1995a. Critical issues for agrichemical safety in South Africa. Am J Ind Med. 27 (1): 1-14.

London L, Myers JE. 1995b. Agrichemical use usage patterns and workplace exposure in the major farming sectors in the region of South Africa. SA J Sci. 91: 515-522.

London L, Myers JE. 1995c. General patterns of agrichemical usage in the Southern region of South Africa. SA J Sci. 509-514.

London L. Myers J.E. 1998a. Use of a crop and job specific exposure matrix for retrospective assessment of long-term exposure in studies of chronic neurotoxic effects of agrichemicals". Occup Environ Med. 55 (3): 194-201.

London L, Nell V, Thompson ML, Myers JE. 1998b. Effects of long-term organophosphate exposures on neurological symptoms, vibration sense and tremor among South African farm workers. Scand J work Environ Health. 24 (1): 18-29.

London L, Dalvie MA, Cairncross E, Solomon A. 2000a. The quality of surface and groundwater in the rural Western Cape with regard to pesticides. WRC Report No: 795/1/00. WRC, Pretoria.

London L, Rother H.A. 2000b. People, pesticides, and the environment: who bears the brunt of backward policy in South Africa? New Solut. 10 (4): 339-350.

Lu C, Fenske RA, Simcox NJ, Kalman D. 2000. Pesticide exposure of children in an agricultural community: evidence of household proximity to farmland and take home exposure pathways. Environ Res. 84 (3): 290-302.

MacNeil JD, Hikichi M. 1986. Phosmet residues in an orchard and adjacent recreational area. J Environ Sci Health [B]. 21 (5): 375-385.

Maharaj S. 2005. Modelling the behaviour and fate of priority pesticides in South Africa, An MSc Thesis submitted to the University of the Western Cape. 1-160.

Mani U, Islam F, Prasad AK, Kumar P, Suresh KV, et al. 2002. Steroidogenic alterations in testes and sera of rats exposed to formulated Fenvalerate by inhalation. Hum Exp Toxicol. 21 (11): 593-597.

McCauley LA, Lasarev MR, Higgins G, Rothlein J, Muniz J, Ebbert C, et al. 2001. Work characteristics and pesticide exposures among migrant agricultural families: a community-based research approach. Environ Health Perspect. 109 (5): 533-538.

Meeker JD, Ryan L, Barr DB, Hauser R. 2006. Exposure to nonpersistent insecticides and male reproductive hormones. Epidemiology. 17 (1): 61-68.

Naidoo V, Buckley CA. 2003. Survey of pesticide wastes in South Africa and review of treatment options. Water Research Commission (WRC) Report No. 1128/1/03. Pretoria: WRC.

Newbold RR, Padilla-Banks E, Snyder RJ, Phillips TM, Jefferson WN. 2007a. Developmental exposure to endocrine disruptors and the obesity epidemic. Reprod Toxicol. 23 (3): 290-296.

Newbold RR, Padilla-Banks E, Snyder RJ, Jefferson WN. 2007b. Perinatal exposure to environmental estrogens and the development of obesity. Mol Nutr Food Res. 51: 912–917.

Newbold RR, Padilla-Banks E, Jefferson WN, Heindel JJ. 2008. Effects of endocrine disruptors on obesity. Int J of Androl. 31 (2): 201-208.

Olden K, Guthrie J. 2000. Children's health: a mixed review. Environ. Health Perspect. 108 (6): 250-251.

Quandt SA, Arcury TA, Rao P, Snively BM, Camann DE, et al. 2004. Agricultural and residential pesticides in wipe samples from farmworker family residences in North Carolina and Virginia. Environ Health Perspect. 112 (3): 382-387.

Ribas-Fitó N, Gladen BC, Brock JW, Klebanoff MA, Longnecker MP. 2006. Prenatal exposure to 1,1-dichloro-2,2-bis (p-chlorophenyl)ethylene (p,p'-DDE) in relation to child growth. Int J Epidemiol. 35 (4): 853-858.

Richter ED, Chuwers P, Levy Y, Gordon M, Grauer F, et al. 1992. Health effects from exposure to organophosphate pesticides in workers and residents in Israel. Isr J Med Sci. 28 (8-9): 584-598.

Rother HA, London L .1998. Pesticide health and safety policy mechanisms in South Africa: the state of the debate. Occupational and Environmental Health Research Unit Working Paper No.1, Department of Community Health. Cape Town: University of Cape Town.

Saiyed H, Dewan A, Bhatnagar V, Shenoy U, Shenoy R, et al. 2003. Effect of endosulfan on male reproductive development. Environ Health Perspect. 111 (16): 1958-1962.

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. 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.

Simcox NJ, Fenske RA, Wolz SA, Lee IC Kalman DA. 1995. Pesticides in household dust and soil: exposure pathways for children of agricultural families. Environ Health Perspect. 103 (12): 1126-1134.

Skakkebaek NE, Keiding N. 1994. Changes in semen and the testis. BMJ. 309 (69-65): 1316-1317.

Smink A, Ribas-Fito N, Garcia R, Torrent M, Mendez MA, et al. 2008. Exposure to hexachlorobenzene during pregnancy increases the risk of overweight in children aged 6 years. Acta Paediatr. 97 (10): 1465-1469.

Stata Corporation. 2007. 4905 Lakeway Drive, College Station, Texas 77845 USA.

Takahashi KL, Hojo H, Aoyama H, Teramoto S. 2004. Comparative studies on the spermatotoxic effects of dinoseb and its structurally related chemicals. Reprod Toxicol. 18 (4): 581-588.

Tanner JM Whitehouse RH. 1976. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child. 51 (3): 170-179.

Toppari J, Larsen JC, Christiansen P, Giwercman A, Grandjean P, et al. 1996. Male reproductive health and environmental xenoestrogens. Environ Health Perspect. 104 (4): 741-803.

United States Environmental Protection Agency (EPA). Available:http://www.epa.gov/pesticides/about/index.htm. [accessed 20 August 2011].

Verhulst SL, Nelen V, Hond ED, Koppen G, Beunckens C, Vael C, et al. 2009. Intrauterine exposure to environmental pollutants and body mass index during the first 3 years of life. Environ Health Perspect. 117 (1): 122-126.

Vinggaard AM, Hnida C, Breinholt V, Larsen JC. 2000. Screening of selected pesticides for inhibition of CYP19 aromatase activity in vitro. Toxicol In Vitro. 14 (3): 227-234.

Vinggaard AM, Christiansen S, Laier P, Poulsen ME, Breinholt V, et al. 2005. Perinatal exposure to the fungicide prochloraz feminizes the male rat offspring. Toxicol Sci: 85 (2): 886-897.

Weiss B, Amler S, Amler RW. 2004. Pesticides. Pediatric. 113: 1030-1036.

World Health organization(WHO). 1990. Public health impact of pesticides used in agriculture available:http://whqlibdoc.who.int/publications/1990/9241561394.pdf [accessed 20 August 2011].

World Health Organisation (WHO). 1990b. Informal consultation on planning strategy for the prevention of pesticide poisoning. WHO: Geneva.

World Health Organisation (WHO). 1991. Safe use of pesticides. Technical Report No. 813.available http://whqlibdoc.who.int/trs/WHO_TRS_813.pdf [accessed 21 August2011]

Xia Y, Bian Q, Xu L, Cheng S, Song L, et al. 2004. Genotoxic effects on human spermatozoa among pesticide factory workers exposed to fenvalerate. Toxicology. 203 (1-3): 49-60.

Xu L, Zhan N, Liu R, Song L, Wang X. 2004. Joint action of phoxim and fenvalerate on reproduction in male rats. Asian J Androl. 6 (4): 337-341.

Young HA, Mills PK, Riordan D, Cress R. 2004. Use of a crop and job specific exposure matrix for estimating cumulative exposure to triazine herbicides among females in a case-control study in the Central Valley of California. Occup Environ Med. 61 (11): 945-951.

Yousef MI, El-Demerdash F, Al-Salhen K. 2003. Protective role of isoflavones against the toxic effect of cypermethrin on semen quality and testosterone levels of rabbits. J Environ Sci Health [B]. 38 (4): 463-478.

Zhang H, Wang H, Wang Q, Zhao XF, Liu P, et al. 2010. Pubertal and early adult mice exposure to fenvalerate disrupts steroidogenesis and spermatogenesis in mice at adulthood. J Appl Toxicol. 30 (4): 369-377.

PART C: JOURNAL READY MANUSCRIPT

This article has been prepared for the purposes of submission to the *Environmental Health Perspectives* Journal. The *Instructions to Authors* can be found in (Appendix C). 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 lines are not numbered.

Title

The relationship between environmental exposures to pesticides measured by means of environmental exposure indices and the anthropometric outcomes of boys living on farms in the rural Western Cape.

Anne Achieng Ochieng [1, 2] Dalvie Mohammed Aqiel [1, 2].

[1]University of Cape Town. [2]School of Public Health & Family Medicine, Centre for Occupational and Environmental Health Research.

Corresponding Author

Anne Achieng Ochieng,

School of Public Health & Family Medicine

Center for Occupational & Environmental Research

University of Cape Town

Tel: +27 735 386403.

Email: achieng.ochieng@gmail.com

Running Title: Effects of pesticides on anthropometric outcomes of boys

Keywords: Pesticides, Exposure indices, Environment, Anthropometric outcomes, Height, Weight, Body mass Index.

Acknowledgements: Ass proff. Francesca Little (university of Cape Town), South African Medical Research Council (MRC), South African National Research Foundation (NRF), University of Cape Town Research Committee, Algernon Africa (University of Cape Town).

Competing interest statement: None.

Abbreviations:

CI	95% Confidence Interval
CDC:	US Center for Disease Control and Prevention.
EEI:	Environmental Exposure Index
PI:	Proximity index (m)
SI:	Spraying intensity index (D/Y)
CSP:	Combined spraying and proximity index
EDC:	Endocrine disrupting chemical
DDE:	Dichlorodiphenyldichloroethylene
BMI:	Body Mass Index (kg/m^2)
DDT:	Dichlorodiphenyltrichloroethane
OCPs	Organochlorine pesticides

ABSTRACT

Background

Few epidemiological studies have investigated the effect of pesticides on growth of boys and results are conflicting. Pesticide environmental exposure indices have not previously been developed.

Objective

To investigate the effect of pesticide exposure using environmental exposure indices on pubertal growth of boys.

Methods

A cross-sectional study of 269 boys (176 residing on farms) was conducted in the rural Western Cape in South Africa. Measurements included a questionnaire, height, weight and BMI measurements. A proximity index (PI), spraying intensity index (SI) and combined proximity-spraying index (PSI) was developed, measuring respectively the lifetime average distance of home from spraying, average frequency of farm spraying and PI/SI.

Results

Median age 12.4 years (Inter quartile Range (IQR) = 9.5- 13.3 years). More than 60% boys had height & weight below the < 50th CDC age percentile. After adjusting for confounders, PI and SI were significantly associated with shorter stature **(**β **=** 1.73cm/10 fold decrease in distance; P = 0.02 & β = -1.38/ fold increase in spraying frequency P = 0.05) respectively and PI was also associated with lower weight (β = -1.24 kg/10 fold increase in distance). Associations were stronger for boys aged < 11 years and were weaker when excluding non-farm boys. There were no other associations between outcome and exposure.

Conclusion

The results provide further evidence that farm boys have lower heights and weights compared to non-farm boys possibly due to hormonally active agricultural pesticides exposure. PI and SI require further development.

1 INTRODUCTION

Hormonally active substances, including pesticides have been associated with adverse male reproductive health effects (Colborn et al. 1993). Recent laboratory and epidemiological studies have provided evidence that hormonally active substance cause obesity (Heindel and Levin 2005; Newbold et al. 2007, Newbold et al. 2008) and it is hypothesized that they interfere with endocrine signaling pathways during perinatal life (Newbold et al. 2007) thereby influencing growth and development at a later stage. Obese children and adolescents have a higher risk of developing obesity in adulthood (Guo et al. 2002).

An increasing trend in the use of agricultural pesticides in South Africa (SA) has been reported (Dalvie et al. 2009c; Maharaj 2005; Naidoo and Buckley 2003). Many of the most commonly used contemporary pesticides in SA agriculture are hormonally active including prochloraz (Vinngaard et al. 2005), cypermethrin (Yousef et al. 2003) endosulphan, chlorpyrifos, iprodione, fenarimol and fenvalerate and have also been shown to cause male reproductive health effects in laboratory animals and in humans (Andersen et al. 2006; Blystone et al 2007; Mani et al. 2002; Saiyed et al. 2003; Xu et al. 2004; Zhang et al. 2010).

Previous studies in the Western Cape SA, where crop farming is important, have shown that pesticides such as endosulfan, chlopyrifos, iprodium and fenvalerate are present in environmental media including drinking and recreational water sources (Dalvie et al. 2003) Additionally rural residents have been shown to lack knowledge of the potential risks of pesticides. Chlorpyrifos and endosulfan levels measured in farm workers were higher than that measured in other settings (Dalvie et al. 2009a, 2011). Lack of legislative policies to control the

use of pesticides and lack of knowledge of potential dangers of pesticide exposure among the farm residents in this region is also of great concern (Dalvie et al. 2004a; London and Rother 2000).

Exposure to pesticides has been demonstrated to cause effects on pubertal growth and body mass index (BMI) of adolescent boys, however, the results have been contradictory. A study conducted (Gladen et al. 2000) in Philadelphia showed that adolescent males with higher prenatal exposure to *p,p'*-DDE had increased height and BMI compared to those with lower exposures. However, when the study was repeated 4 years later, no associations were found between *p,p'*-DDE and anthropometric measurements (Gladen et al. 2004). On the other hand, a study conducted in Germany (Karmaus et al. 2002) showed that reduced height among female but not male children was associated with exposure to higher childhood DDE concentrations. Also a prospective cohort study done in the United States (US) revealed significantly reduced height among boys between ages 4-7 in the high exposure group (Ribas-Fitó et al. 2006). A recent cohort study of 499 boys aged 8-9 years in the Russian children's study revealed lower mean BMI and height z-scores between the lowest and highest quartiles among DDT exposed boys (Burns et al. 2011).

Careful characterization of chemical exposures is important in the agricultural setting, where biological monitoring may be lacking. London & Myers (1998) developed a job exposure matrix (JEM) to quantitatively estimate the occupational pesticide exposure of farm workers in the Western Cape. Farm residents, including women and children, are exposed to pesticides through a number of routes (Dalvie et al. 2009a, 2009b). We could not find studies in the literature that

have attempted to develop environmental exposure indices for estimating long term pesticide exposure among children who live in close proximity to pesticide spraying areas.

This study aimed to investigate whether pesticide exposure, measured using environmental exposure indices, have discernable effects on growth of boys residing in the rural Western Cape, South Africa. A previous sub-study which used the lifetime living history of boys as an exposure index found that boys who lived on farms had shorter stature and weight compared to boys who did not reside on a farm (English 2011).

2 METHODS AND MATERIALS

Institutional Review Board (IRB) approval for the study was granted by the ethics review board of the University of Cape Town (REC REF: 279/2005).

2.1 Population and study design

An analytical cross-sectional study of 269 boys aged 5 -19 years, from the rural Western Cape Province in South Africa, was conducted. Boys were recruited from the most accessible primary and secondary schools (n = 8) attended by pupils from both farms and towns, in three agriculturally-intense areas (Hex River Valley, Grabouw, Piketberg) where pesticides were previously detected in the environment and in farm workers (Dalvie et al. 2003). Other than age, there were no further exclusion criteria. Provisional parental consent was obtained in advance for boys to be recruited to the study.

A total of 94 boys not currently living on a farm were selected for the study, and 180 boys (60 in each area) were selected out of 398 living on farms. At each school, farm boys were selected by random systematic sampling, stratified by age-group. The age-groups were as follows: 5 to 9 years (pre-pubertal), 9.1 to 11 years (early puberty), 11.1 to 14 years (mid-late puberty) and > 14

years (post-puberty). Selected boys and their parents (preferably the mother) or guardians (n = 274) were invited to participate in the study on specified dates (Karpati et al. 2002). Five of the selected boys were excluded from the study because their parents or guardians did not participate. Prior to full sample data collection, the protocol and questionnaire was pilot tested on 5 families.

2.2 Questionnaire

Trained interviewers administered questionnaires to parents or guardians in Afrikaans, the language of preference. The questionnaire included sections on demography, general medical history, genital health history, the mothers' personal habits during pregnancy, and lifetime environmental exposure to agricultural pesticides, domestic pesticide use, phyto-oestrogen intake and lifestyle factors. Questions were based on previous local studies in similar populations (Dalvie et al. 1999; Dalvie at al. 2004b).

The section on lifetime environmental exposure to agricultural pesticides elicited information on all the places the participant had resided since birth. For each location, it was established whether the home was located on a farm or not. If located on a farm, further information was collected on the frequency of pesticide spraying, the application methods used, and the proximity of the home to the location of the pesticide application. The latter also included information on the boys' participation in spraying activities, contact with the pesticide itself, contact with contaminated surfaces while playing outside, water sources, ingestion of contaminated crops from farms and tasks performed on the farm.

Cellular and internet technology was employed in the capturing and transfer of questionnaire data for analysis.

2.3 Physical examination

A trained male nurse recorded height, weight (using a calibrated scale) according to standardized methods and calculated body mass index (BMI).

2.4 Statistical analysis

Data was analyzed using STATA version 10.1 (Stata Corporation 2007). Three exposure indices including a proximity index, a spraying intensity index and a combined proximity /spraying index were developed from the exposure information collected by questionnaire.

a) The proximity index (PI) was calculated as the average distance of home from the spraying area of all places lived using the following equation:

$$\text{Proximity Index} = (D_1 Y_1 + D_2 Y_2 \ldots D_x Y_x)/\text{Age}$$

Where D_i = distance of home from spraying area,

Y_i = number of years lived at the place of residence

The value of D_i for those not living on a farm distances were randomly allocated between 500 – 1700 meters using an algorithm in Stata 10.1 (Stata Corporation 2007).

b) The Spraying Index (SI) was calculated as the lifetime average number of spraying days per year on farms lived using the following by the equation:

$$\text{Spraying index} = (B_1Y_1 + B_2Y_2 \ldots + B_xY_x)/\text{age}$$

Where B_i = total number of days per year sprayed (including boom, tractor and aeroplane spraying) on a farm (days = 0 if not living on a farm)

Y_i = the number of years lived at the place of residence

c) The Combined Spraying Proximity Index (CSP) was calculated as the ratio of the spraying index to the proximity index using the following equation:

CSP = spraying index/proximity index.

The primary exposure variables therefore were the three exposure indices that were analyzed as continuous variables.

The primary outcome variables were anthropometric measurements (height, weight, body mass index (BMI) which w also continuous. These were also all dichotomized at each quartile (25^{th}, 50^{th}, 75^{th} percentiles) thus producing three dichotomous variables for each outcome. Additionally two dichotomous variables were produced per anthropometric outcome using the CDC 25^{th} and 50^{th} age percentiles (CDC 2008).

Univariate and bivariate exploration of the data were performed. Bivariate analysis included simple Linear Regression Analysis, the Student T-Test or Wilcoxon rank sum Test, and the Chi-square Test. Multiple Linear and Logistic Regression Analyses were used to test for associations between the individual outcomes and exposure while controlling for confounding. Confounders were selected on an *a priori* basis, according to biological plausibility, or using bivariate testing, if $p < 0.1$. Age and household income (marker of socio-economic status) were selected *a priori* for all outcomes (Marmot et al. 2004). Potential confounders included in bivariate testing included all variables measuring demography, medical history, socio-economic status (other than household income), household pesticide exposure, phyto-oestrogen intake and mother's exposure during pregnancy. Age and household income and confounders from bivariate testing were

included in the regression model during the model building process. First, a baseline model was determined including the outcome and the potential confounder with the lowest Aikake's Information Criterion (AIC) statistic. Other confounders were then added to the baseline model and the combination of variables resulting in the lowest AIC was selected as the best model. There was no need to force *a priori* variables into the model as these were selected by the statistical procedures described above. Exposure variables were then added to test for exposure outcome relationships. Regression diagnostics were applied to determine the goodness of fit of the model and to assess for outliers or influential observations.

Further analysis was conducted:

a) Investigating exposure outcome relationships only amongst those with a history of living on a farm by including those boys who were classified as farm boys in the other sub-study (English 2011). Farm boys included those who had lived all their lives only on a farm and those who had not lived all their lives on a farm but were born on a farm *and/or* spent the first three years of their life on a farm *and/or* spent more than 3 years of their first 12 years on a farm.

b) Investigating exposure outcome relationships per age-category and

c) Including the dichotomous exposure variable (farm, non-farm) used in the other sub-study in the multivariate model to assess the impact on the strength of association of the exposure indices and also of household income as an indicator of socio-economic status. All three exposure indices were log transformed as they were not normally distributed. All analyses were performed using Stata 10 statistical software (Stata corporation 2007).

3 RESULTS

3.1 Participation

Two-hundred and sixty-nine participants were recruited (overall response rate of selected boys was 98.2 %) including 37% (100) from Grabouw, 34% (91) from Piketberg and 29% (78) from the Hex River Valley.

3.2 Demographic, socioeconomic status and medical history

There was a good representation of boys in the different age categories (Table 1). The median overall age of the participants was 12.4 years (IQR = 9.5- 13.3 years).

The prevalence of lifetime chronic medical problems for diabetes, epilepsy, and heart problems were below 2%, while 9.3% of participants (n = 25) had asthma and 5.6% (n = 15) had tuberculosis. One boy had HIV and two farm boys had foetal alcohol syndrome. Two farm boys previously experienced pesticide poisoning. Four boys (1.5%) had hypospadias (Table 1).

Table 1 Demographics, household pesticide exposure, phyto-estrogen intake, mother's exposures during pregnancy and exposure to agricultural spraying on farms

Variable	N (%)
Demographics	
Age Groups (years)	41 (15.2)
5-9	77 (28.6)
9.1-11	119 (44.2)
11.1-14	32 (11.9)
> 14	
Sometimes or often going hungry	8 (3)
Household pesticide exposure	
Use household pesticides	152 (56.5)
Fumigate house	159 (5.6)
Household member work with pesticides	22 (8.2)
Pesticide contaminated clothing washed at home	6 (2.2)
Use pesticide containers at home	6 (2.2
Lifetime phyto-estrogen intake	
Vegetables	257 (95)
Nuts	161 (59.9)
Soya	194 (72.1)
Mother's exposures during pregnancy	
Sprayed pesticides	6 (2.2)
Worked in vineyard during pesticide spraying	78 (29.)
Smoked	114 (42.3)
Consumed alcohol	45 (16.7)
Agricultural pesticide exposure in current home amongst boys living on farm (n = 175)	
Pesticides sprayed on farm during current year	171 (97.7)
Farm spraying drifts into house	76 (44.4)
Water source:	
River/dam	87 (34.4)
Municipal	166 (66.5)
Swimming in nearby dams	77 (28.62)
Walking in vineyards after spraying	50 (28.6)
Helping on the farm	32 (11.5)
Eating crops from vineyards	85 (31.6)
Use of empty pesticide containers	12 (04.4)
Mixing of pesticides	2 (0.7)
Lifetime residency	
Lived in current location throughout life	223 (82.9)
Ever lived on a farm	177 (65.8)
Lived only on farm	94 (34.2)
Variable	**Median (IQR)**
Household income (US$)	250 (163-340)
Distance of current home from spraying on farm (m)	12.5 (0.5-325)
Exposure indices	
[a]Proximity index (m)	100.0 (17.5-974.6)
[b]Spraying frequency index (days per year)	36.8 (0-82.5)
[c]Combined spraying /proximity index	36.9 (0-1.20)

[a]The spraying intensity index indicates lifetime the average number of days per year sprayed on a farm on which participants lived.

[b]The proximity index indicates the lifetime average distance from the nearest spraying area on which participants lived.

[c]The combined spraying/proximity index was determined as a ratio of the spraying index to the proximity index

3.3 Household pesticide exposure, phyto-estrogen intake and exposures during pregnancy

More than half of households used pesticides (Table 1). Household fumigation was reported in 5.6% of the households at median 5 times per year (IQR = 4 -5 times per year). Other household pesicide exposures included household members working with pesticides, bringing contaminated clothing home, and the use of empty pesticide containers at home for domestic use.

Phyto-estrogen intake in the form of lifetime vegetable intake was prevalent amongst the vast majority of boys (95%), while intake of nuts and soya was prevalent amongst about two-thirds of boys. Less than 3% of boys smoked, consumed alcohol and/or used drugs.

Few mothers (2.2%) reported that they sprayed pesticides during pregnancy but nearly a third (29.4%) worked in the vineyard while spraying activities took place (Table 1). Nearly half of the mothers smoked and about a fifth consumed alocohol during pregnancy.

3.4 Exposure of participants to agricultural spraying on farms

Boys living on farms are exposed to agricultural pesticides through a number of routes including living near to spraying and exposed to pesticide drifting into homes, coming into contact with pesticides outside the house while spraying occurs, drinking water from unprotected sources, walking in vineyards after spraying, helping in the fields on farms, swimming in farm dams and nearby rivers that contains pesticide residues, eating crops from vineyards and orchards, and using empty pesticide containers (Table 1). The majority of boys (83%) lived in one location throughout their life with the rest living in 2-5 different locations. About two thirds of boys had lived on a farm in their lifetime (Table 1).

3.5 Anthropometric measurements.

The median height of the boys was 137.9 cm (IQR 129.0-148.1), weight was 33 kg (IQR 27.0-43.0) and BMI was 17.5 (IQR 16.0-19.1). The proportion of boys below the CDC 50th height, weight and BMI percentile for age was 71.6% (n = 192), 66.8% (n = 179) and 39.6% (n = 106), respectively and those below the CDC 25th height, weight and BMI percentile for age was 57.1% (n = 153), 41.8% (n = 112) and BMI 19.4% (n = 52), respectively (see supplemental material Table 4).

3.6 Associations between exposure indices and anthropometric measurements adjusting for confounding.

Table 2 summarises the results of Multiple Linear Regression Analyis investigating the associations between the exposure indices and anthropometric measurements. Due to the fact that residuals were not normally distributed the expsoure indices were log transformed to base 10

The results show positive associations between the proximity index and height as well as weight when adjusting for confounding thereby showing that boys who had lived near farms where spraying took place were of shorter stature and lower weight (Table 2). There were negative associations between the spraying index and height and weight when adjusting for confounding thereby showing that boys exposed to more spraying on farms were of shorter stature and lower weight. The regression coeffcients (Table 2) predict that for every 10 fold increase in distance lived away from the farm in a boy's lifetime height and weight increased by 1.73 cm (p = 0.02) and 1.24 kg (p = 0.04), respectively. The model also predicted a 1.38 cm decrease in height for every 10 fold increase in days of spraying done on the farm per year (p = 0.05) (Table 3.2). No statistically significant associations was noted between any of the exposure indices and BMI. There was also no associations between the CPS index and the different outcomes (Table 2).

These results were consistent with:

a) Linear regression analysis using a proximity index whereby the distances of boys not living on farms were assigned an arbitrary distance of 1000 meters (Table A-1, appendix A)

b) Linear regression where the proximity and the spraying indices were categorized into percentiles (See supplemental material, Table 6)

c) Logistic regression using dichotomized outcomes based on quartiles 25^{th} and 50^{th} (Table A-2, appendix A).

Table 2. Summary of associations between the different exposure indexes and outcomes using Multiple Linear Regression Analysis (N= 269) adjuted for age and household income.

Outcome Variables	**Linear models**	
Proximity index (log m)	**Regression Coefficient (95% CI)**	**P-value**
Height (cm)	1.73 (0.23- 3.23)	0.02
Weight (kg)	1.24 (0 .04 -2.45)	0.04
BMI (kg/m^2)	0.21 (-0.18-0.60)	0.28
Spraying Index (log days/year)		
Height (cm)	-1.38 (-2.78-0.03)	0.05
Weight (cm)	-1.09 (-2.22-0.02)	0.06
BMI (kg/m^2)	-0.16 (-0.53-0.19)	0.37
Combined proximity/spraying index		
Height (cm)	-1.01 (-4.37-2.35)	0.55
Weight (kg)	-1.62 (-4.3-1.06)	0.24
BMI (kg/m^2)	-0.62 (-1.49-0.23)	0.16

There was no statistically significant association between the different exposure indices and outcomes when excluding boys who were classified as non -farm boys based on their residential history (See suplemental material Table 5).

3.7 Associations between exposure indices and anthropometric measurements in different age groups.

Table 3 summarizes the associations between the exposure indices and outcomes among boys in different age categories. The proximity index was a significant predictor of height and weight amongst the youngest participants, age group 5 - 9 years (Table 3) with the regression coefficients predicting a 3.66 cm increase in height and 1.84 kg increase in weight for every 10 fold increase in distance lived away from the farm respectively (Table 3). The proximity index was also a significant predictor for height among the age group 9.1 -12 years. The spraying index was also negatively associated with height among boys aged 11 – 14 years.

There were no significant associations between the proximity index and BMI as well as between the CPS index and all the outcomes in all the ages groups. These findings are supported by logistic regression analysis whereby the outcomes were dichotomised at the 25^{th} &50^{th} percentiles. (Tables A-3 – A-5; Appendix A).

Table 3: Relationship between exposure indices and outcomes in the different age groups using Multiple Linear Regression Analysis (adjusted for age and household income)

Exposure variables	Height (cm)		Weight (kg)		BMI (kg/m^2)	
	Regression coefficient (95% CI)	**P-value**	**Regression coefficient (95% CI)**	**P-value**	**Regression coefficient (95% CI)**	**P-value**
Age group 1 (Ages 5-9 years, n = 41)						
Proximity index (log m)	3.66 (0.47-6.8)	0.03	1.84 (0.42-3.25)	0.01	0.33 (-0.49-1.14)	0.42
Spraying index (log days/year)	-0.87 (-3.89-2.14)	0.56	-0.41 (-1.77-0.95)	0.55	-0.05 (-0.78-0.68)	0.88
Combined spraying/proximity index	-1.59 (-7.55-4.36)	0.59	-1.93 (-4.55-0.69)	0.15	-0.93 (-2.34-0.48)	0.19
Age group 2 (9 - 11 years, n = 77)						
Proximity index (log m)	2.44 (0.44-4.44)	0.02	1.76 (0.05-3.47)	0.04	0.44 (-0.29-1.19)	0.24
Spraying index (log days/year)	-0.16 (-1.87-1.56)	0.86	-0.47 (-1.92-0.97)	0.52	-0.17 (-0.79-0.45)	0.59
Combined spraying/proximity index	-0.12 (-4.07-3.82)	0.95	0.84 (-4.17-2.48)	0.62	-0.30 (-1.73-1.12)	0.67
Age group 3 (11-14 years, n = 119)						
Proximity index (log m)	0.54 (-1.89-2.97)	0.66	0.47 (-1.66-2.61)	0.66	-0.04 (-0.71-0.62)	0.89
Spraying index (log days/year)	-2.37 (-4.72 - -0.03)	0.05	-1.35 (-3.43 -0.73)	0.20	0.04 (-0.62-0.69)	0.92
Combined spraying/proximity index	-0.55 (-6.87 -5.77)	0.86	-0.64 (-6.19 - 4.93)	0.82	-0.12 (-1.85-1.61)	0.89
Age group 4 (>14 years, n = 32)						
Proximity index (log m)	1.70 (-3.59-6.99)	0.52	0.95 (-3.66 -5.55)	0.68	0.11 (-1.13-1.35)	0.86
Spraying index (log days/year)	1.20 (-3.99- 6.39)	0.64	-0.38 (-4.89 - 4.13)	0.86	0.55 (-1.74-0.65)	0.36
Combined spraying/proximity index	4.74 (-7.12 -16.59)	0.42	0.14 (-10.25-10.53)	0.98	-1.22 (-3.98 - 1.54)	0.37

Table 4 presents the full statistical model for the association between height and PI and SI when additionally including lifetime residence of the boy on a farm as a predictor. The results show the proximity and the spraying indices weakening as a predictors for height when compared to the results in Table 2 with lifetime residence on a farm a substantially stronger predictor. Age remains a strong socio-economic predictor for height.

Table 4. Linear regression model for the association between the proximity & spraying indices and height adjusting for age, household income and lifetime residence on a farm[a] (N= 269)

Predictors	Regression coefficient (95% CI)	P-value
Proximity index (log m)	-0.08 (-2.56-2.39)	0.95
[b]Farm boy (Yes, No)	-4.19 (-8.76-0.38)	0.07
Age (years)	4.26 (3.69-4.82)	<0.001
Household income (Rands)	2.13 (-0.23-4.49)	0.08
Spraying index (log days/year)	-0.13 (-1.93 - 1.68)	0.89
[b]Farm boy (Yes, No)	-3.91 (-7.47 - -0.35)	0.03
Age (years)	4.26 (3.69 - 4.82)	<0.001
Household income (Rands)	2.14 (-.23 - 4.51)	0.08

[a]Boys were classified as farm boys or non-farm boys based on their lifetime residential history
[b]variable indicating whether boys lived on a farm throughout their lives or not

4 DISCUSSION

The results in this sub-study shows that boys who have resided in closer proximity to agricultural pesticide spraying and/or exposed to higher agricultural pesticide spraying throughout their life are shorter and lighter than boys who have not. However, when boys who do not have a history of living on farms are excluded from the analysis the association disappears suggesting that "farm residence" is the determining factor and that proximity to and intensity of spraying amongst farm boys is not a determining factor for pesticide exposure, although the number of participants is reduced by about a third. When lifetime residence on a farm is included in the statistical models (Table 4) it is a strong predictor of height and weight and the exposure indices are weak predictors, thus providing further indication that farm residence is the determining factor for exposure. It should be noted that lifetime residence on a farm is highly correlated with proximity index, this is why the beta coefficient for PI changes so dramatically in the model which includes both terms (Table 4).Household income remains a strong socio-economic predictor in these models. It is possible that lifetime residence on a farm could to some extent act as a second socio-economic variable controlling for differences between "farm boys" and "non-

farm" boys not accounted by household income. Thus, although PI and SI merely reflected farm residence, they did provide more clarity on the association with height and weight. Additionally, there were not sufficient power to detect associations between farm residence and health outcomes amongst age-groups in the previous sub-study (English 2011) whereas in this study, the use of PI and SI did provide insight of the association at age-group level.

A difference in nutritional status between farm and non-farm boys could have accounted for lower anthropometric measurements found in the former group. However, the two groups were recruited from neighboring areas and household income, an indicator of socio-economic status and a strong determinant of nutritional status, were low in both groups and were controlled for in the analysis (Marmot et al. 2004).

The use of PI and SI particularly amongst farm boys for determining the association between pesticide exposure and height and weight, require further development. Previous studies conducted in the US have provided evidence that organophosphate levels in urine and house dust increase with proximity to the nearest spraying area on farms, with one study showing this association when comparing households within 200 feet of the spraying area to those further away (Coronado et al. 2011) and other studies providing evidence for households within 305 m of farm land. (Lu et al. 2000; McCauley et al. 2001). In our study, reliance on the respondent's estimation of proximity for those living on farms especially for past homes might not have been most accurate. Direct measurement through farm visits could have improved the estimation of proximity to agricultural spraying for homes located on farms. Furthermore, the estimation of proximity to farms for homes not located on farms could be improved through the use of maps or

GPS data instead of assigning arbitrary distances. It should be noted, however, that the amount of pesticide drift in homes is influenced by the application methods, meteorological conditions, topography, characteristics of the crop and decisions made by applicators (Coronado et al. 2011).

The spraying index was probably also affected by reliance on the respondent's estimation of the amount of spraying days on farms and can be improved by contacting the farm management and studying spraying records.

The age-group analysis revealed that the association between PI and height and weight were the strongest for boys aged < 11years (Table 3). This could simply be due to the fact that the effect manifests the strongest at age < 11 years or due to more pesticides absorbed as a result of the larger body surface area to volume ratio of younger boys as well as their slower metabolization of toxicants (Eskenazi. 1999; Weiss et al. 2004). It has also been hypothesized that perinatal and childhood exposure to pesticides could affect the regulation of endocrine set points thereby influencing growth and development at a later stage (Gladen et al. 2000; Ribas-Fito et al. 2006)

The lower height and weight measurements associated with agricultural pesticide spraying is consistent with our hypothesis, as an alteration of GnRH release by the hypothalamus due to exposure to hormonally-active pesticides could have impacted on pubertal growth (Aksglaede et al. 2006; Rice et al. 2003). Altered levels of reproductive hormones found amongst farm boys compared to non-farm boys found in the previous sub-study is further support of our hypothesis (English 2011).

No studies investigating the effect of contemporary pesticides on pubertal growth were found in the literature, but there is laboratory and epidemiological evidence of reduced height measurements amongst DDT exposed boys although results are contradictory. Gladen et al. (2004), did not find an association between DDT exposure and anthropometric measurements of boys in Philadelphia (n = 304) while Gladen et al. (2000) reported an increase in height and BMI for the exposed group in a study of 594 boys in North Carolina. Additionally, reduced height was reported in 342 German children, in 1712 highly DDT exposed children in United States (US) and 349 pre-pubertal Russian boys exposed to organochlorine pesticides (Burns et. al. 2011; Karmaus et al. 2002, Ribas-Fitó et al. 2006).

A limitation in this sub-study is the absence of biomarker data for exposure which would have confirmed recent pesticide levels amongst participants. However, although there are limitations in using PI and SI as discussed earlier, they could be improved for future studies. Further analysis using pesticide bio-monitoring data is currently underway. A follow-up study is intended as the longitudinal design would likely strengthen the measurement of the exposure-outcome relationships due to large individual variations associated with the study outcomes.

Exposure misclassification due to non-farm boys' exposure to contaminated water and food or pesticide drift is possible. However these exposures are likely far less prevalent in non farm groups. An important source of selection bias is the absence of boys not attending school. Farm boys not in school may have been more exposed than those who attended school and their exclusion probably weakened the exposure response associations. Recall bias due the respondents' memory of boys' childhoods and of mother's pregnancies is a factor, especially

when the parent was not present (23%). Furthermore, measurement bias may have been introduced during the physical examination of the boy. However, training of research staff and other quality control measures were introduced to reduce these biases.

5 CONCLUSION.

Our study provides further evidence that farm residence reduces height and weight measurements of pubertal boys that may be due to environmental exposure to hormonally active contemporary agricultural pesticides. A follow-up of the boys as well as a larger study with more boys in the different age categories is required to support this finding. The environmental exposure indices used in this study requires further development. We recommend initiatives to change knowledge, attitudes and practices through the education of farmers, farm workers and other rural residents about the harmful effects of pesticides and the need to reduce exposures.

6 REFERENCES

Aksglaede L, Juul A, Leffers H, Skakkebaek NE, Andersson A. 2006. The sensitivity of the child to sex steroids: possible impact of exogenous estrogens. Human Reprod Update. 12 (4): 341-349.

Andersen HR, Bonefeld-Jørgensen EC, Nielsen F, Jarfeldt K, Jayatissa MN, et al. 2006. Estrogenic effects in vitro and in vivo of the fungicide fenarimol. Toxicol Lett. 16 (2): 142-152.

Blystone CR, Lambright CS, Furr J, Wilson VS, Gray LE. 2007. Iprodione delays male rat pubertal development, reduces serum testosterone levels, and decreases ex vivo testicular testosterone production. Toxicol Lett. 174 (1-3): 74-81.

Burns JS, Williams PL, Sergeyev O, Korrick S, Lee MM, Revich B, et al. 2011. Serum Concentrations of Organochlorine Pesticides and Growth among Russian Boys. Environ Health Perspect .http://dx.doi.org/10.1289/ehp.1103743 [Online 7 October 2011].

Coronado GD, Holte S, Vigoren E, Griffith WC, Barr DB, et al. 2011. Organophosphate pesticide exposure and residential proximity to nearby fields: evidence for the drift pathway. J Occup Environ Med. 53 (8): 884-891.

CDC. (Centres for Disease Control and Prevention). 2009. Clinical Growth Charts. Available: http://www.cdc.gov/growthcharts/clinical_charts.htm. [accessed 08 August 2010].

Colborn T, vom Saal FS, Soto AM. 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ Health Perspect. 101 (5): 378-384.

Dalvie MA, White N, Raine R, Myers JE, London L, Thompson M, 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.

Dalvie MA, Cairncross E, Solomon A, London L. 2003. Contamination of rural surface and ground water by endosulfan in farming areas of the Western Cape, South Africa. Environ Health 2 (1): 1-1.

Dalvie MA, London L, Mbuli S, Cairncross E. 2004a. Knowledge and attitudes in the rural Western Cape towards pesticides in water sources. Water SA. 30 (1): 43-50.

Dalvie MA, Myers JE, Thompson ML, Robins TG, Omar S, Riebow J. 2004b. Exploration of different methods for measuring DDT exposure among malaria vector-control workers in Limpopo Province, South Africa. Environ Res. 96 (1): 20-27.

Dalvie MA, Africa A, Solomons A, London L, Brouwer D, et al. 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 MA, London L. 2009b. Risk assessment of pesticide residues in South African raw wheat. Crop Prot. 28 (10): 864-869.

Dalvie MA, 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): 683-687.

Dalvie MA, Naik I, Channa K, London L. 2011. Urinary dialkyl phosphate levels before and after first season chlorpyifos spraying amongst farm workers in the Western Cape, South Africa. J Environ Sci Health B. 46 (2): 163-172.

English R. 2011. Reproductive health effects due to pesticide exposure amongst boys in the rural Western Cape, South Africa. [MPH Thesis]. University of Cape Town. Cape Town, South Africa

Eskenazi B, Bradman A, Castorina R. 1999. Exposures of children to organophosphate pesticides and their potential adverse health effects. Environ Health Perspect. 107 (3): 409-419.

Gladen BC, Ragan NB, Rogan WJ. 2000. Pubertal growth and development and prenatal and lactational exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene. The J pediatr. 136 (94): 490-496.

Gladen B.C, Klebanoff MA, Hediger M.L, Katz SH, Barr, DB, Davis MD, et al. 2004. Prenatal DDT exposure in relation to anthropometric and pubertal measures in adolescent males. Environ Health Perspect. 112 (17): 1761-1767.

Guo SS, Wu, W, Chumlea WC, Roche AF. 2002. Predicting overweight and obesity in adulthood from body mass index values in childhood and adolescence. Am J Clin Nutr. 76 (3): 653-658.

Heindel JJ, Lewin E. 2005. Developmental Origins and Environmental Influences Introduction. Birth Defects Res A Clin Mol Teratol. 73 (7): 469.

Karmaus W, Asakevich S, Indurkhya A, Witten J, Kruse H. 2002. Childhood growth and exposure to dichlorodiphenyl dichloroethene and polychlorinated biphenyls. J pediatr. 140 (1): 33-39.

Karpati AM, Rubin CH, Kieszak SM, Marcus M, Troiano RP. 2002. Stature and Pubertal Stage Assessment in American Boys: The 1988–1994 Third National Health and Nutrition Examination Survey. J Adolesc Health. 30 (3): 205-212.

London L, Rother HA. 2000. People, pesticides, and the environment: who bears the brunt of backward policy in South Africa? New Solut: 10 (4): 339-350.

Lu C, Fenske RA, Simcox NJ, Kalman D. 2000. Pesticide exposure of children in an agricultural community: evidence of household proximity to farmland and take home exposure pathways. Environ Res. 84 (3): 290-302.

Marmot M (Ed). 2004. The Status Syndrome: How Social Standing Affects Our Health and Longevity. New York: Owl Books.

Maharaj S. 2005. Modelling the behaviour and fate of priority pesticides in South Africa, [MSc Thesis]. University of the Western Cape, Cape Town, South Africa.

Mani U, Islam F, Prasad AK, Kumar P, Suresh Kumar V, et al. 2002. Steroidogenic alterations in testes and sera of rats exposed to formulated Fenvalerate by inhalation. Hum Exp Toxicol. 21 (11): 593-597.

McCauley LA, Lasarev MR, Higgins G, Rothlein J, Muniz J, Ebbert C, et al. 2001. Work characteristics and pesticide exposures among migrant agricultural families: a community-based research approach. Environ Health Perspect. 109 (5): 533-538.

Naidoo V, Buckley CA (2003). Survey of pesticide wastes in South Africa and review of treatment options. Water Research Commission (WRC) Report No. 1128/1/03. Pretoria.

Newbold RR, Padilla-Banks E, Snyder RJ, Phillips TM, Jefferson WN. 2007. Developmental exposure to endocrine disruptors and the obesity epidemic. Reprod Toxicol. 23 (3): 290-296.

Newbold RR, Padilla-Banks E, Jefferson WN, Heindel JJ. 2008. Effects of endocrine disruptors on obesity. Int J Androl. 31 (2): 201-208.

Ribas-Fitó N, Gladen BC, Brock JW, Klebanoff MA, Longnecker MP. 2006. Prenatal exposure to 1,1-dichloro-2,2-bis (p-chlorophenyl)ethylene (p,p'-DDE) in relation to child growth. Int J Epidemiol. 35 (4): 853-858.

Rice C, Birnbaum LS, Cogliano J, Mahaffey K, Needham L, Rogan WJ, et al. 2003. Exposure assessment for endocrine disruptors: some considerations in the design of studies. Environ Health Perspect. 111 (13): 1683-1690.

Saiyed H, Dewan A, Bhatnagar V, Shenoy U, Shenoy R, Rajmohan H, et al.2003. Effect of endosulfan on male reproductive development. Environ Health Perspect. 111 (16): 1958-1962.

Stata Corporation. 2007. 4905 Lakeway Drive, College Station, Texas 77845 USA.

Vinggaard AM, Christiansen S, Laier P, Poulsen ME, Breinholt V, Jarfelt K, et al. 2005. Perinatal exposure to the fungicide prochloraz feminizes the male rat offspring. Toxicol Sci. 85 (2): 886-897.

Weiss B, Amler S, Amler RW. 2004. Pesticides. Pediatrics. 113: 1030-1036.

Xu L, Zhan N, Liu R, Song L, Wang X. 2004. Joint action of phoxim and fenvalerate on reproduction in male rats. Asian J Androl. 6 (4): 337-341.

Yousef MI, El-Demerdash F, Al-Salhen K. 2003. Protective role of isoflavones against the toxic effect of cypermethrin on semen quality and testosterone levels of rabbits. J Environ Sci Health [B]. 38 (4): 463-478.

Zhang H, Wang H, Wang Q, Zhao XF, Liu P, Ji YL, et al. 2010. Pubertal and early adult mice exposure to fenvalerate disrupts steroidogenesis and spermatogenesis in mice at adulthood. J Appl Toxicol. 30 (4): 369-377.

PART D

SUPPLEMENTAL MATERIALS FOR THE JOURNAL ARTICLE

TITLE: The relationship between environmental exposures to pesticides measured by means of environmental exposure indices and the anthropometric outcomes of boys living on farms in the rural Western Cape.

Supplemental material Table 1: Demographic information (N = 296).		
Variable	**N**	**Median (IQR)**
Age range of boys (years)	269	12.4 (9.5 - 13.3)
5-9	41	8.7 (8.3 - 8.8)
0 9.1-11	77	9.8 (9.25 - 10.5)
11.1-14	119	12.7 (12.1 - 13.25)
>14	32	15.0 (14.5 - 16.1)
Birth weight (Kg),	205	2.8 (2.5 - 3.3)
		N %
Parent/guardian married or staying with partner	154	57.3
Parent/guardian employed	176	65.4
Educational status parent/guardian		
No schooling	47	17.7
Matriculated	120	44.6
Household income (dolars)		
0-250	156	57.78
251-499	75	27.78
>500	39	14.44
Household go hungry	8	3

Supplemental material Table 2: Health Problems (N = 269)	
Health problems	**N (%)**
Lifetime chronic disease	
Diabetes	2 (0.7)
Tuberculosis	15 (5.5)
Epilepsy	3 (1.1)
Asthma	25 (9.2)
Heart Problem	2 (0.7)
HIV	1 (0.4)
Foetal Alcohol Syndrome	2 (0.7)
Back problem	1 (0.4)
Other health problems	
Mumps	7 (29.3)
Pesticide poisoning	2 (0.7)

Supplemental material Table 3: Anthropometric measurements		
Anthropometric measurements (units)	**N**	**Median (IQR)**
Height (cm)		
All participants	268	137.9 (129.0 - 148.1)
Age group: 5-9 years	41	124.9 (116.5 - 131.0)
Age group: 9.1-11 years	76	131.0 (127.1 - 137.9)
Age group: 11.1-14 years	119	145.1 (136.8 - 156.1)
Age group: >14 years	32	155.6 (146.6 - 164.8)
Weight (kg)		
All Participants	268	33.0 (27.0 - 43.0)
Age group: 5-9 years	41	24.0 (22.0 - 26.0)
Age group: 9.1-11 years	76	29.0 (26.0 - 32.0)
Age group: 11.1-14 years	119	39.00 (33.0 - 46.0)
Body Mass Index (kg/m^2)		
All Participants	268	17.5 (16.0 - 19.1)
Age group: 5-9 years	41	15.6 (14.2 - 16.8)
Age group: 9.1-11 years	77	16.6 (15.4 - 18.2)
Age group: 11.1-14 years	119	18.0 (17.1 - 19.8)
Age group: >14 years	32	18.7 (17.8 - 20.9)
Age group: >14 years	32	45.50 (42.0 - 55.0)

Supplemental material Table 4: Anthropometric Measurements < CDC 25th & 50th Age Percentile	
Anthropometric measurements	**N (%)**
Body Mass Index (kg/m^2)	
< 50th percentile for age	106 (39.5)
≤ 25th percentile for age	52 (19.4)
Height (cm)	
<50th percentile for age	192 (71.6)
≤25th percentile for age	153 (57.1)
Weight (kg)	
<50th percentile for age	179 (66.8)
≤25th percentile for age	112 (41.8)

Supplemental material Table 5 Summary of associations between the different exposure indexes and outcomes excluding boys who were classified as farm boys using using Linear Regrssion Analysis (N = 177)[a,b].		
Exposure variables		
Proximity index (log m)	**Regression Coefficient (95% CI)**	**P- Value**
Height (cm)	-0.06 (-2.44-2.31)	0.96
Weight (kg)	0.16 (-1.68-1.99)	0.87
BMI (kg/m^2)	0.19 (-0.42-0.79)	0.54
Spraying intensity index (log days/year)		
Height (cm)	0.05 (-1.68-1.79)	0.95
Weight (kg)	-0.19 (-1.54-1.16)	0.78
BMI (kg/m^2)	-0.06 (-0.51-0.38)	0.78
Combined spraying/proximity Index		
Height (cm)	1.38 (-2.09-4.85)	0.43
Weight (kg)	-0.14 (-2.84-2.56)	0.92
BMI (kg/m^2)	-0.49 (-1.39-0.38)	0.27
[a]Adjusted for age and household income		
[b]Results were Based on the boy's residential history		

Supplemental material Table 6: Summary of associations between the proximity and spraying indices categorized into 3 categories (based on 25th, 50th & 75th percentiles) and outcomes using Linear Regression Analysis. (n = 269)[a]

Exposure variables	Height (cm) Regression coefficient (95% CI)	P-value	Weight (kg) Regression coefficient (95% CI)	P-value	BMI (kg/m^2) Regression coefficient (95% CI)	P-value
Proximity index (log m)						
Proximity index category 1 (0 -25th percentile)	-4.34 (-8.03 - -0.65	0.02	-2.27 (-5.19 - 0.65)	0.12	-0.07 (-1.02 - 0.88)	0.88
Proximity index category 2 (>25th -50th percentile)	-3.59 (-7.50 - 0.32)	0.07	-3.90 (-6.99 - -0.81)	0.01	-0.85 (-1.85 - 0.16)	0.09
Proximity index category 3 (>50th -75th percentile)	-4.99 (-8.66 - -1.34)	0.08	-2.77 (-5.66 - 0.13)	0.06	0.01 (-0.93 - 0.95)	0.98
Spraying index (log days/year)						
Spraying index category 1 (0 -25th percentile)	-1.24 (-13.58 - 11.11)	0.84	3.06 (-6.67 - 12.79)	0.54	1.89 (-1.25 - 5.03)	0.24
Spraying index category 2 (>25th -50th percentile)	-1.99 (-14.69 - 10.69)	0.75	1.89 (-8.11 - 11.89)	0.71	1.51 (-1.71 - 4.74)	0.36
Spraying index category 3 (>50th -75th percentile)	0.05 (-13.05 - 13.16)	0.99	2.61 (-7.72 - 12.93)	0.62	1.13 (-2.19 - 4.47)	0.50

[a]Adjusted for age and household income

APPENDIX A. ADDITIONAL RESULTS FOR THE THESIS

Table A-1 Summary of associations between an alternative proximity index (whereby non-farm residents were assigned an arbitary distance of 1000 m) and outcomes using Linear Regression Analysis (N = 269)[a]

Proximity index (m)	Regression Coefficient (95% CI)	P-value
Height (cm)	0.003 (0.000 -0.006)	0.02
Weight (kg)	0.002 (0.000-0.005)	0.05
BMI (kg/m^2)	0.0003 (-0.001-0.001)	0.50

[a]Adjusted for age and household income

Table A-2: Summary of associations between the different exposure indexes and outcomes (dichotomised at the 25th and 50th percentile) using multiple logistic regression[a]. (N =)

	25th percentile		50th percentile	
Exposure variables	**Odds Ratio (95%CI)**	**P- Value**	**Odds Ratio (95% CI)**	**P-Value**
Proximity index (log m)				
Height (cm)	0.76 (0.57- 1.02)	0.06	0.78 (0.55 - 1.09)	0.14
Weight (kg)	0.73 (0.55 - 0.98)	0.04	0.74 (0.54 - 0.99)	0.05
BMI (kg/m^2)	0.95 (0.71 - 1.43)	0.99	1.07 (0.80 - 1.42)	0.65
Spraying index (log days/year)				
Height (cm)	1.33 (1.02 - 1.74)	0.04	1.24 (0.92 - 1.68)	0.16
Weight (kg)	1.18 (0.90 - 1.53)	0.23	1.43 1.071 - 1.90)	0.02
BMI (kg/m^2)	1.09 (0.78 - 1.51)	0.62	0.94 (0.72 - 1.22)	0.63
Combined spraying/proximity Index				
Height (cm)	1.23 (.66 - 2.37)	0.5	1.16 (0.55 - 2.41)	0.7
Weight (kg)	1.48 (0.79 - 2.78)	0.39	1.62 (0.79 - 3.33)	0.19
BMI (kg/m^2)	1.61 (0.79 - 3.28)	0.19	1.32 (0.70 - 2.47)	0.39

[a]Adjusted for age and household income

Table A-2 depicts the logistic regression models when the outcomes were dichotomized into quartiles. There was 27% reduction in the odds of having a weight below the 25th percentile among boys who lived further from the farms (OR = 0.73; CI = 0.55 - 0.98, P –Value = 0.04) (table A-2) showing that those who lived close by were at a risk of falling below the 25th percentile. Similarly those who were exposed to more number of spraying on the farms had a 33% increased risk of having a smaller weight (OR =1.33; CI = 1.02 - 1.74; P-value = 0.04)

Table A-3:Summary of associations between the different exposure indexes and outcomes using multiple logistic regression analysis: Age group1 (Ages 5-9 years; n=41)[a]

	25th percentile		50th percentile	
Exposure variables	**OR (CI)**	**P- Value**	**OR (CI)**	**P- Value**
Proximity index (log m)				
Height (cm)	0.54 (0.24 - 1.23)	0.15	0.39 (0.14 - 1.04)	0.06
Weight (kg)	0.35 (0.14 - 0.86)	0.02	0.49 (0.18 - 1.32)	0.16
BMI (kg/m^2)	0.89 (0.39 - 2.01)	0.79	0.96 (0.43 - 2.14)	0.92
Spraying index (log days/year)				
Height (cm)	1.27 (0.63 - 2.59)	0.50	0.91 (0.42 - 2.01)	0.83
Weight (kg)	1.45 (0.72 - 2.92)	0.29	1.50 (0.64 - 3.52)	0.35
BMI (kg/m^2)	1.53 (0.74 - .17)	0.26	1.23 (0.61 - 2.49)	0.55
Combined spraying/proximity Index				
Height (cm)	1.37 (0.34 - 5.55)	0.66	0.89 (0.20 - 3.96)	0.89
Weight (kg)	3.02 (0.71 -12.92)	0.14	3.55 (0.71 - 12.93)	0.27
BMI (kg/m^2)	5.63 (1.18 - 26.98)	0.03	3.00 (0.67 - 13.55)	0.15
[a]Adjusted for age and household income				

Table A-3 shows results from the logistic regression models when outcomes were dichotomized into quartiles among boys in age group 5- 9 years. There was a 65% reduced risk of having weight below the 25th percentile (OR = 0.35; CI = 0.14 - 0.86; P –value = 0.03) for the boys who lived away from the farms. The results also reveal a 63% increased risk of having a higher BMI for the boys who had a higher combined exposure index (OR = 5.63; CI = 1.18 - 26.98; P – Value = 0.03) (Table A-3).

Table A-4:Summary of associations between the different exposure indexes and outcomes using multiple ogistic Regression Analysis: Age group 2 (ages 9.1 -11 years n=77)[a].

	25th percentile		50th percentile	
Exposure variables	**OR (CI)**	**P- Value**	**OR (CI)**	**P- Value**
Proximity index (log m)				
Height (cm)	0.49 (0.26 - 0.94)	0.03)	0.56 (0.27 - 1.14	0.11
Weight (kg)	0.59 (0.32 - 1.12)	0.11	0.63 (0.33 - 1.18	0.15
BMI (kg/m^2)	0.84 (0.37-1.89)	0.67	0.94 (0.51 - 1.73	0.85
Spraying index (log days/year)				
Height (cm)	1.34 (0.82 - 2.18	0.24	1.21 (0.69 - 2.16	0.49
Weight (kg)	0.94 (0.57 - 1.55	0.80	1.20 (0.72 - 2.02	0.48
BMI (kg/m^2)	0.81 (0.41 - 1.59	0.55	0.80 (0.48-1.32	0.39
Combined spraying/proximity Index				
Height (kg)	1.35 (0.44 - 4.17	0.59	1.55 (0.39 - 6.15	0.53
Weight (kg)	0.72 (0.22 - 2.33	0.59	0.96 (0.28 - 3.27	0.95

BMI (kg/m^2)	0.57 (0.09 - 3.27	0.53	1.17(0.38 - 3.62	0.79
[a]Adjusted for age and household income				

Table A-4 depicts logistic regression results when outcomes were divided into quartiles among boys aged between 9.1 – 11 years. The results reveal a 49 % reduced height among the boys who lived in close proximity to the farms (OR = 0.49; CI = 0.26 - 0.94; P=0.03) (Table A-4).

Table A-5:Summary of associations between the different exposure indexes and outcomes using multiple logistic regression Analysis: Age group 3 (Ages 11.1 -14 years; n=119) - confounders age and income[a].

Logistic models				
	25th percentile		**50th percentile**	
Exposure variables	**OR (CI)**	**P- Value**	**OR (CI)**	**P- Value**
Proximity index (m)				
Height (cm)	1.36 (0.85 - 2.15)	0.19	1.15 (0.69 - 1.87)	0.59
Weight (kg)	1.09 (0.70 - 1.69)	0.69	0.99 (0.63 - 1.55)	0.97
BMI (kg/m^2)	1.20 (0.66 - 2.18)	0.56	1.34 (0.85 - 2.11)	0.2
Spraying index				
Height (cm)	1.36 (0.87 - 2.12)	0.17	1.64 (0.99 - 2.69)	0.05
Weight (kg)	1.22 (0.79 - 1.87)	0.37	1.54 (0.98 - 2.42)	0.06
BMI (kg/m^2)	1.00 (0.56 - 1.78)	0.99	0.80 (0.51 - 1.23)	0.31
Combined spraying/proximity Index				
Height (cm)	0.72 (0.27 - 2.29)	0.58	1.13 (0.30 - 4.20)	0.85
Weight (kg)	1.29 (0.43 - 3.93)	0.65	1.26 (0.38 - 4.15)	0.71
BMI (kg/m^2)	1.01 (0.23 - 4.48)	0.99	0.49 (0.14 - 1.72)	0.27
[a]Adjusted for age and household income				

Table A-5 summarizes the logistic regression results when outcomes were divided into quartiles among boys aged between 11.1-14 years of age. The results reveal a 64% increased risk of falling below the 50th percentile of the Z- scores for height among boys who were exposed to higher number of spraying in this age group (OR = 1.64 CI = 0.99 - 2.69; P – Value = 0.05) (Table A-5).

APPENDIX B- QUESTIONAIRE

CHILD QUESTIONNAIRE

Male reproductive health effects due to pesticides amongst farm residents in the Western Cape

Date _____Room Temperature _____

Survey Number _____

Name of the Interviewer _____

Study Area _____

School _____

Source of drinking water _____

Specify the source of drinking water _____

Details of parent:

Relationship to participants: mother, father, other (circle which one is applicable)

If other, specify _____

Highest Standard/Grade passed at school: _____

Diplomas/Tertiary Education: _____ (Y/N)

Employment status _____ (yes, no, student, retired, other)

If employed, Job Title: _____

If farm worker, Exposure group: _____

(Supervisor, Sprayer/Mixer, Non- Sprayer Farmworker, Non Farmworker)

Marital Status _____

(Married, living with someone as married, widowed, divorced, separated, single with girl friend, single with no girl friend)

What is your monthly household income (in Rands)? _____

How often does your family go hungry or have no food to eat:

Never _____
Seldom____
Sometimes_____
Often_____

Details of son:

Date of birth _____Age (_____)

Gender: ____ (Male/Female)

Birth weight:_____(kg)

Current standard/grade at school: _____

Address _____

A. GENERAL MEDICAL HISTORY

A1. How do you judge your son's health in general? _____(Excellent, Very good, Good, Bad)

A2. Did he have/does he have:

Disease	**Yes, No, Don't Know**	**Year Diagnosed**
Diabetes		
TB		
Fits		
High Blood Pressure		
Asthma		
Heart Problems		

Back Problems		
HIV		
Foetal Alcohol Syndrome		
Other Specify:		

A3.a) Did he have /does he have any other chronic illnesses (longer than three months) apart from those listed above? __ (1 = Yes, 2 = No)
b) If yes, specify _____

A4. Has he taken any daily medication during the last 3 months? ___
(Yes, No)

A5. Has he ever been poisoned by pesticides? _____ (Yes, No, Don't know)
If yes, give details (date, name of doctor, name of hospital)
_____

B. GENITAL HEALTH HISTORY AND PUBERTY

B1. Did your son ever had mumps? ___ (Yes, No, DN)

B2. If yes, how old was he when he had mumps?_____years old

B3. Do you think your child has already entered puberty? _____ (Yes No)
If : Yes
a. At what age do you think your child entered puberty? ____ years, _____ months

b. What was the first sign of puberty you saw in your child?
_____

If : NO (not yet entered puberty)

c. At what age do you expect your child to enter puberty?____ years, _____ months

d. What is the first sign of puberty you expect to see?
_____

B4. Would you say that your son's growth spurt (in height) has started yet? (A growth spurt is defined as growth in height that is faster than usual.)

_____

(No, Yes, barely, Yes, definitely, Development completed, Don't know)

If yes, at what age _____(years)

B5. Would you say that growth of his underarm and pubic hair has started yet?

_____

(No, Yes, barely, Yes, definitely, Development completed, Don't know)

If Yes, at what age? _____(years)

B6. Have you noticed any changes in his skin, especially pimples?

_____

(No, Yes, barely, Yes, definitely, Development completed, Don't know)

B7. Have you noticed a deepening of his voice?

_____(No, Yes, barely, Yes, definitely, Development completed, Don't know)

If yes, at what age _____(years)

B8. Has he started to grow hair on his face_____

(No, Yes, barely, Yes, definitely, Development completed, Don't know)

B9.Compared with other boys his age, would you say your son's physical development is:

_____

(much earlier than the other boys, somewhat earlier than the other boys, about the same as the other boys, somewhat later than the other boys, much later than the other boys)

B10. Was your son born with abnormally developed testicles? ___ (yes, no, DN)

if Yes, did he go for an operation or received medication? _____ . What was the date he went for an operation or received medication? _____

B11. Has your son ever had an injury, resulting in swelling/discolouring in the testicular area? ____ (yes, no, DN)

B12. Has he ever had an operation in the testicular area? If YES, which date

B13. Has he been sterilized? _____(Yes, No)

B14.Has your son ever had any other diseases in the testicular area? ____ (Yes, No, Don't Know)

If "Yes", specify and give the date

_____

B15. Did your son already had his first wet dreams? _____

If yes, at what age? _____

B16. From the diagram, what stage of development do you consider your child?

Pubic hair and genital development :_____ (a, b, c, d or e)

C. LIVING HISTORY

Please answer the following questions regarding the places where your son has lived in his lifetime (C1-C16 is for current residence, Sections CA-CD is only applicable for residences before current residence starting from the most recent one)

C1Where does he live currently? _____ (Name of town or city)

C2 For how long has he been living there? _____(years, months)

C3 Is his home located on a farm, town or city**?** _____

C4 If his home is located on a farm, how far from the house is the nearest vineyard/field? _____ (meters)

C5 Are pesticides sprayed on the vineyard/field during the year? ____ (yes, no, DN)

IF No (go to C7)
IF YES, complete the following:

How many times a year are pesticides applied by means of

a) a tractor with a boom sprayer _____ (number of times a year)

b) a tractor with persons using hand or backpacks? _____ (number of times a year)

c) aeroplane _____ (number of times a year)

C6 Does the pesticides spraying come into the house? ____ (yes, no, DN)

C7 Does your son come into contact with pesticides outside the house while spraying occurs (for e.g. playing near spraying area) ? _____ (yes, no)

C8 Does your son go into in the field/vineyards soon after spraying or come into contact with sprayed surfaces? ____ (yes, no)

C9 What are the sources of drinking water at his house? _____ (municipal water, storage dam on mountain, borehole/spring, river water, farm dam, rain water tank, etc)

C10 What are the sources of water for recreational use (bathing, washing of clothes) at his house? _____ (municipal water, storage dam on mountain, borehole/spring, river water, farm dam, rain water tank, etc)

C11 Does your son play swim or play in dams/rivers? _____(yes, no)
If yes, where is the dam/river located _____
(on farm, just outside farm, more than 100m away, out of town

C12 Does your son perform help on the farm? _____ (yes, no)
If Yes,
What does he do _____ and
How often?_____ (every day, twice a week, once a week, once a month, school holidays)

C13 Is he involved in spraying or mixing pesticides? _____ (yes, no)

C14 Does he work in the pesticide store? _____ (yes, no)

C15 Does your son come into contact with empty pesticide containers? ___(yes, no)
If yes, how _____ (for eg play, drinking water, burning)

C16 Does your son eat from the crops in the vineyard/field soon after spraying?
_____ (yes, no)

The following questions are about the place your son lived before his current home

CA1 Where did you son live before? _____ (Name of town or city)

CA2 For how long did he live there? _____(years, months)

CA3 Was that home located on a farm, town or city**? _____**

C3 If the place was on a farm, what kind of farm

CA4 If his home was located on a farm, how far from the house was the nearest

vineyard/field? _____ (meters)

CA5 Was pesticides sprayed on the vineyard/field during the year?
____ (yes, no, DN)

IF No (go to C7)
IF YES, complete the following:

CA6 Did the pesticides spraying come into the house? ____ (yes, no)

CA7 Did your son come into contact with pesticides outside the house while spraying occurs (for e.g. playing near spraying area) ? _____ (yes, no)

CA8 Did your son go into in the field/vineyards soon after spraying or come into contact with sprayed surfaces? ____ (yes, no)

CA9 What were the sources of drinking water at his house? _____ (municipal water, storage dam on mountain, borehole/spring, river water, farm dam, rain water tank, etc)

CA10 What were the sources of water for recreational use (bathing, washing of clothes) at his house? _____ (municipal water, storage dam on mountain, borehole/spring, river water, farm dam, rain water tank, etc)

CA11 Does your son play swim or play in dams/rivers? _____(yes, no)

If yes, where is the dam/river located _____
(on farm, just outside farm, more than 100m away, out of town

C12 Did your son help on the farm? _____ (yes, no)

If Yes, What did he do? _____ and How often?_____ (every day, twice a week, once a week, once a month, school holidays)

CA13Was he involved in spraying or mixing pesticides? _____ (yes, no)

CA14 Did he work in the pesticide store? _____ (yes, no)

CA15 Did your son come into contact with empty pesticide containers? ___(yes, no)

If yes, how _____ (for eg play, drinking water, burning)

CA16 Did your son eat from the crops in the vineyard/field soon after spraying?
____ (yes, no)

The following questions are about the place your son lived before his previous home

CA1 Where did you son live before ? _____ (Name of town or city)

CA2 For how long did he live there? _____(years, months)

CA3 Was that home located on a farm, town or city**? _____**

C3 If the place was on a farm, what kind of farm?

CA4 If his home was located on a farm, how far from the house was the nearest vineyard/field? _____ (meters)

CA5 Was pesticides sprayed on the vineyard/field during the year?____ (yes, no, DN)

IF No (go to C7)
IF YES, complete the following:

CA6 Did the pesticides spraying come into the house? ____ (yes, no)

CA7 Did your son come into contact with pesticides outside the house while spraying occurs (for e.g. playing near spraying area) ? _____ (yes, no)

CA8 Did your son go into in the field/vineyards soon after spraying or come into contact with sprayed surfaces? ____ (yes, no)

CA9 What were the sources of drinking water at his house? _____ (municipal water, storage dam on mountain, borehole/spring, river water, farm dam, rain water tank, etc)

CA10 What were the sources of water for recreational use (bathing, washing of clothes) at his house? _____ (municipal water, storage dam on mountain, borehole/spring, river water, farm dam, rain water tank, etc)

CA11 Does your son play swim or play in dams/rivers? _____(yes, no)

If yes, where is the dam/river located _____
on farm, just outside farm, more than 100m away, out of town

C12 Did your son help on the farm? _____ (yes, no)

If Yes, What did he do? _____ and How often?
_____(every day, twice a week, once a week, once a month, school holidays)

CA13 Was he involved in spraying or mixing pesticides? _____ (yes, no)

CA14 Did he work in the pesticide store? _____ (yes, no)

CA15 Did your son come into contact with empty pesticide containers? ___(yes, no)

If yes, how _____ (for eg play, drinking water, burning)

CA16 Did your son eat from the crops in the vineyard/field soon after spraying?
_____ (yes, no)

The following questions are about the place your son lived before his previous home

CA1 Where did you son live before? _____ (Name of town or city)

CA2 For how long did he live there? _____(years, months)

CA3 Was that home located on a farm, town or city**? _____**

CA4 If his home was located on a farm, how far from the house was the nearest vineyard/field? _____ (meters)

CA5 Was pesticides sprayed on the vineyard/field during the year?____ (yes, no, DN)

IF No (go to C7)
IF YES, complete the following:

CA6 Did the pesticides spraying come into the house? ____ (yes, no)

CA7 Did your son come into contact with pesticides outside the house while spraying occurs (for e.g. playing near spraying area) ? _____ (yes, no)

CA8 Did your son go into in the field/vineyards soon after spraying or come into contact with sprayed surfaces? ____ (yes, no)

CA9 What were the sources of drinking water at his house? _____ (municipal water, storage dam on mountain, borehole/spring, river water, farm dam, rain water tank, etc)

CA10 What were the sources of water for recreational use (bathing, washing of clothes) at his house? _____ (municipal water, storage dam on mountain, borehole/spring, river water, farm dam, rain water tank, etc)

CA11 Does your son play swim or play in dams/rivers? _____(yes, no)
If yes, where is the dam/river located _____
(on farm, just outside farm, more than 100m away, out of town

C12 Did your son help on the farm? _____ (yes, no)

If Yes,What did he do? _____ and

How often?_____ (every day, twice a week, once a week, once a month, school holidays)

CA13 Was he involved in spraying or mixing pesticides? _____ (yes, no)

CA14 Did he work in the pesticide store? _____ (yes, no)

CA15 Did your son come into contact with empty pesticide containers? ___(yes, no)

If yes, how _____ (for eg play, drinking water, burning)

CA16 Did your son eat from the crops in the vineyard/field soon after spraying? _____ (yes, no)

The following questions are about the place your son lived before his previous home

CA1 Where did you son live before? _____ (Name of town or city)

CA2 For how long did he live there? _____(years, months)

CA3 Was that home located on a farm, town or city**? _____**

CA4 If his home was located on a farm, how far from the house was the nearest vineyard/field? _____ (meters)

CA5 Was pesticides sprayed on the vineyard/field during the year?____ (yes, no, DN)

IF No (go to C7)
IF YES, complete the following:

CA6 Did the pesticides spraying come into the house? ____ (yes, no)

CA7 Did your son come into contact with pesticides outside the house while spraying occurs (for e.g. playing near spraying area) ? _____ (yes, no)

CA8 Did your son go into in the field/vineyards soon after spraying or come into contact with sprayed surfaces? ____ (yes, no)

CA9 What were the sources of drinking water at his house? _____ (municipal water, storage dam on mountain, borehole/spring, river water, farm dam, rain water tank, etc)

CA10 What were the sources of water for recreational use (bathing, washing of clothes) at his house? _____ (municipal water, storage dam on mountain, borehole/spring, river water, farm dam, rain water tank, etc)

CA11 Does your son play swim or play in dams/rivers? _____(yes, no)

If yes, where is the dam/river located _____
(on farm, just outside farm, more than 100m away, out of town

C12 Did your son help on the farm? _____ (yes, no)

If Yes, What did he do? _____ and
How often? _____ (Every day, twice a week, once a week, once a month, school holidays)

CA13 Was he involved in spraying or mixing pesticides? _____ (yes, no)

CA14 Did he work in the pesticide store? _____ (yes, no)

CA15 Did your son come into contact with empty pesticide containers? ___(yes, no)

If yes, how _____ (for eg play, drinking water, burning)

CA16 Did your son eat from the crops in the vineyard/field soon after spraying? **_____ (yes, no)**

D. HOUSEHOLD PESTICIDE EXPOSURE

D1 Do you use any pesticides in your garden or in your home (eg doom, rat poison, fleas)?
____ (yes, no)

D2 If yes, for how long have you been using pesticides at home?_____ (number of years)

D3 How frequently do you use pesticides at home _____ (every day, 3 times a week, once a week, once a month, less than once a month)

D4 Do you have your house fumigated?
If yes, for how long? _____(number of years)
How frequently?
_____
(every day, 3 times a week, once a week, once a month, less than once a month)

D5 Does any person in the house work with pesticides? (Yes, No)

If yes, how many? _____

Since when has there been a person that work with pesticides? _____ (year)

Does any pesticide contaminated clothes get washed at home _____(yes,no)

If yes, does it get washed with the rest of the washing? _____ (yes, no)

D6 Does your son eat fruit or vegetables from your garden _____ (yes, no)

D7 Do you use empty pesticide containers at home for domestic purposes? (Yes, No)
If yes, what do you use them for? _____

Since when have you been using empty containers at home _____ (year)

E. DIET

E1 Does your son eat meat/fish? _____ (Yes, No)

E2 How many times a week does he eat meat/fish _____

E3 In his lifetime, how many times a week did he eat meat/fish _____

E4 Does he eat vegetables? ___ (Yes, No)

E5 How many times a week does he eat vegetables _____

E6 How many times a week does he eat soy products ____

E7 In his lifetime, how many times a week did he eat vegetables _____

E8 In his lifetime, how many times a week did he eat soy products ____

E9 Does your son like to eat nuts? __
How many times a week does he eat nuts? ____

E10 In his lifetime, how many times a week did he eat nuts? ___

E11 Was he on soya milk after birth? ___

For how long? _____

E12 Does your son eat meals provided by the school? (Yes, No)

If yes, what do they provide? _____

Please specify the meals _____

F. MOTHERS HABITS DURING PREGNANCY

F1 When you were pregnant with this son, did you spray or mix pesticides ___? (Yes, No)

If yes, for how many weeks____?

F2 During the pregnancy, did you work in the vineyard/orchard while pesticides were

sprayed? ____ (Yes, No)

F3 Did you work in the vineyard/orchard while pesticides were not sprayed?___
(Yes, No)

F4 During the pregnancy, did you smoke?___ (Yes, No)
If yes, how many cigarettes per day? ____

F5 During the pregnancy, did you drink alcohol?__ (Yes, No)
If yes, how many bottles per week? ____ (if papsak, estimate number of bottles)

F6 During the pregnancy, how many times a week did you eat meat/fish _____

F7 During the pregnancy, how many times a week did you eat vegetables _____

F8 During the pregnancy, how many times did you eat soya beans or soy products ____

F9 During the pregnancy, how many times a week did you eat nuts ___

G. SMOKING AND ALCOHOL

G1 Does your son smoke currently or did he smoke before? _____ (yes, no)

If yes, for how long? _____ (number of years)

G2 Does your son drink alcohol currently or did he drink alcohol before? _____ (yes, no)

If yes, for how long? _____ (number of years) and how many bottles per week_____ (estimate if papsak)

G3 Does your take drugs or smoke dagga currently or before? _____ (yes, no)

If yes, for how long? _____ (number of years)

APPENDIX C ENVIRONMENTAL HEALTH PERSPECTIVES INSTRUCTIONS TO AUTHORS 2011

Who We Are

Environmental Health Perspectives (*EHP*) is a monthly open-access journal that publishes peer-reviewed research and news concerning human health and the environment. One of the overarching principles of the journal is to provide a forum for the objective and balanced presentation of scientifically credible information. Although *EHP* is sponsored by the National Institute of Environmental Health Sciences (NIEHS), its editorial policies are independent of the institute.

All papers submitted to *EHP* are evaluated by a group of consulting editors to determine whether the topic is within the scope of the journal and to evaluate adherence to word limits and journal format. Papers also are assessed for originality, scientific quality, environmental health significance, clarity of presentation, and conciseness. Before papers are sent for peer review, they are screened for possible plagiarism (see "Scientific Integrity" below), and authors must submit a Competing Financial Interests Declaration form on behalf of all authors (see "Competing Financial Interests" below). Papers selected for review are assigned to an Associate Editor, who identifies reviewers and makes recommendations to the Editor-in-Chief. Members of the Editorial Review Board serve as a pool of potential reviewers of papers. Both the Board of Associate Editors and the Editorial Review Board are composed of leading scientists from all segments of the environmental health sciences. The overall acceptance rate of papers submitted to the journal in 2010 was 15%.

In 2004 *EHP* became an open-access journal. All News and Research Articles published since the beginning of the journal in 1972 are available free online (http://www.ehponline.org/). *EHP* is committed to promoting the discussion and exchange of information internationally, as described in detail at http://www.ehponline.org/international/. *EHP* also is committed to promoting the use and understanding of scientific literature through its Science Education Program (http://www.ehponline.org/education).

What We Publish

The environmental health sciences include many fields of study and increasingly comprise multidisciplinary research areas. *EHP* publishes articles from a wide range of scientific disciplines encompassing mechanistic research, experimental and observational human studies, and *in vitro* and *in vivo* animal research with a clear relationship to human health effects. Studies involving exposure science, climate change, ecologic issues, or effects on wildlife populations are welcome, but the relevance of the findings to human health should be made clear. Physicians and others working in environmental medicine may submit Grand Rounds articles or Case Reports for consideration. *EHP* also addresses ethical, legal, social, and policy issues related to environmental public health. Because children are uniquely sensitive to their environments, *EHP* devotes a research section specifically to issues surrounding children's environmental health.

EHP provides additional information on environmental health issues through its News and Book Review sections and its Editorials. Although *EHP* welcomes ideas for News, Book Reviews, and Editorials, the journal does not accept unsolicited manuscripts of these types. Please contact the Editor-in-Chief for further information.

About your Manuscript

Types of Manuscripts

Manuscripts in the categories below are considered for publication. All manuscripts are peer reviewed except Correspondence. See "Article Length" below for details concerning word limits.

Correspondence (≤ 750 words) should address specific scientific issues or questions raised by Research or News Articles published in the print version of the journal within the previous 6 months. Authors of papers cited in Correspondence will be given the opportunity to respond. Letters addressing issues raised in previously published letters are discouraged. Correspondence may include a brief table or small figure if it is critical to the discussion. New data must not be included. Authors may include data from or redrawing of previously published materials as long as the work is cited and written permission from the original authors and/or publishers has been granted for republication in both printed and electronic form. Each figure is considered equivalent to 250 words toward the total word count. Correspondence that cites abstracts or unpublished observations is not acceptable and will not be published. Letters that are highly polemic or personal in nature will not be published. Correspondence is not peer reviewed and is

published at the discretion of the *EHP* editors. Conclusions and opinions expressed by the authors do not necessarily reflect the policies of *EHP*.

Commentaries (≤ 5,000 words) present information and personal insight on a particular topic. Commentaries should not be extended critiques of single articles appearing in *EHP* or elsewhere. Factual data should be included to substantiate arguments. *EHP* reserves the right to reject Commentaries without review if they are perceived as being too polemic or personal in nature. *EHP* also reserves the right to propose that Commentaries be reviewed as one side of a point/counterpoint debate. Assuming the original author agrees, *EHP* will ask another author to address the opposite side of an argument. If both papers are accepted, *EHP* will publish them together. Manuscripts on ethical, legal, social, or policy issues may also be accepted in this category.

Research Articles (≤ 7,000 words) report original scientific research and discovery in the broad field of environmental health sciences. Research Articles may come from any field of scientific research relevant to the study of human health and the environment.

Emerging Issue Reviews (≤ 5,000 words) identify emerging ideas, concepts, or trends in the area of environmental health sciences. These papers have a highly focused narrative (about two to three print pages) and a limited set of references. Because the intent of the Emerging Issue Review is to get new and novel ideas into the literature in a timely fashion, the review of these manuscripts will be expedited.

Substantive Reviews (≤ 10,000 words) provide an overview, integration of information, and critical analysis of a particular field of research or theme related to environmental health sciences. Previous research should be comprehensively reviewed regardless of whether the findings are consistent with expectations or the review authors' hypotheses. However, it is appropriate for authors to discuss the strengths and weaknesses of individual studies; focus on high-quality studies that add to the weight of the evidence on the topic under review; identify information gaps; and make recommendations for future research. Lengthy historical perspectives generally are not appropriate.

Quantitative Reviews and Meta-Analyses (≤ 10,000 words) present, contrast, and (when appropriate) combine data across studies to address a specific study question related to environmental health. Inclusion criteria and strategies used to search the literature should be

explicitly described, along with analytic methods used to evaluate or combine data. The potential for publication bias and heterogeneity among studies should be investigated, and graphical displays of data contributed by individual studies are encouraged. The strengths and weaknesses of individual studies and potential causes of discordant findings among studies also should be discussed. As with Substantive Reviews, authors should integrate and critically analyze information from previous research, identify information gaps, and make recommendations for future research.

Meeting Reports (≤ 5,000 words) provide an overview of outcomes of conferences, symposia, or workshops. Authors should submit reports that review the state of the science for a particular area, identify research gaps and needs, and explain how the outcome of the conference addresses those gaps and needs. Meeting Reports may review existing information, summarize research findings on specific topics, and recommend methods, courses of action, or research needs for the scientific community. *De novo* data, participant lists, dialogue of workgroups or committees, and discussion of the internal organization of the meeting are not allowed. Meeting Reports must be submitted to *EHP* no later than 9 months after the events they describe. Prospective authors should consult with the Editor-in-Chief before submitting a Meeting Report.

Grand Rounds (≤ 6,000 words) present discussions of case presentations of patients or community health issues with a clearly established link of relevance to environmental exposures and environmental health, including children's health. The format requires that a case scenario be presented to illustrate the environmental issues under consideration, followed by a discussion of the clinical and public health implications of these issues. Visual images (e.g., X rays, microscopic pathology) or other graphics are encouraged.

Case Reports (≤ 6,000 words) differ from Grand Rounds articles in that the diagnosis pertaining to the clinical presentation is not necessarily conclusive. Instead, evidence for an environmental etiology may be indirect—for example, a case report of hepatitis suspected to be related to a chemical that has not been previously linked with hepatitis. Visual images (e.g., X rays, microscopic pathology) or other graphics are encouraged.

Originality of Submission

Contributions submitted to *EHP* must be original works of the author(s) and must not have been previously published in print or online or simultaneously submitted to another publication.

Previously published material (e.g., figures, tables) may be included in Commentaries and Reviews, assuming the original authors have given permission to reproduce the material and all copyright issues have been resolved. For original Research Articles, previously published schemata or illustrative figures are acceptable with the proper attribution. Text or narrative from guidance documents, technical reports, and position papers by various government and nongovernmental organizations may be considered if they include new information. *EHP* will consider papers from dissertations that have been published in their entirety by a university in partial fulfillment of a degree. Manuscripts presented at a scientific meeting but not published in full or under review for publication in a proceedings or similar format also will be considered. Previously published material may be included in the Supplemental Material of the paper. As indicated in *Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication* [International Committee of Medical Journal Editors (http://www.icmje.org/urm_full.pdf)], it is the responsibility of the author to make a full statement to the editor concerning materials in a manuscript that might be considered redundant or duplicative. For additional clarification, please contact the Editor-in-Chief.

Scientific Integrity

EHP requires assurances that animals used in a study have been treated humanely and with regard for the alleviation of suffering. Research involving humans must have been conducted according to the Common Rule (http://ori.dhhs.gov/education/products/ucla/chapter2/page04b.htm). Research involving humans also must be approved by an appropriate institutional review board and comply with all relevant national, state, and local regulations. For research conducted outside the United States and thus exempt from U.S. federal regulations, authors must perform the research in accordance with principles of the Declaration of Helsinki (http://www.wma.net/en/30publications/10policies/b3/index.html). Approval and compliance with research requirements regarding human subjects and information regarding informed consent procedures must be noted in the Methods section of manuscripts concerning human subjects research.

EHP is sometimes confronted with issues regarding potential research misconduct, such as plagiarism or data fabrication. Authors should be aware that all papers submitted to *EHP* are screened routinely for plagiarism, defined as “the appropriation of another person’s ideas, processes, results, or words without giving appropriate credit” (American Medical Association.

2007. *AMA Manual of Style: A Guide for Authors and Editors*, 10th edition. New York:Oxford University Press). Instances of documented plagiarism and allegations of data fabrication will be brought to the attention of the authors' host institutions. Documented cases of plagiarism or data fabrication could lead to a 3-year ban on future publication in*EHP* by the authors, a published Expression of Concern and/or retraction of the paper.

Dual-Use Research

EHP anticipates receiving submissions on research that, based on current understanding, can be reasonably anticipated to provide knowledge, products, or technologies that could be directly misapplied by others to pose a threat to public health and safety, agriculture, plants, animals, the environment, or material (also known as dual-use research). Papers flagged for dual-use issues by *EHP* editors will undergo an additional level of review concerning the implications to society of publishing such a paper. It is possible that the editors of *EHP* may not be technically qualified to evaluate such cases independently; thus, *EHP* reserves the right to seek expert advice in cases where such concerns may be evident. Authors should be aware that *EHP* could determine that the risks to public health and safety of publishing the paper outweigh the benefits of publishing, even though the paper has otherwise been deemed acceptable for publication.

Competing Financial Interests

EHP has a policy of full disclosure. Authors must declare all actual or potential competing financial interests involving people or organizations that might reasonably be perceived as relevant. Disclosure of competing interests does not imply the information in the article is questionable or that conclusions are biased. Decisions to publish or reject an article will not be based solely on a declaration of a competing interest.

For each manuscript, authors must submit a Competing Financial Interests Declaration (CFID) form (available online at http://ehp.niehs.nih.gov/cfi.pdf). Papers will not be processed for peer review unless a CFID form has been submitted. Authors of Correspondence, Editorials, and Book Reviews also are required to submit a CFID form.

Authors must disclose all actual or potential competing financial interests occurring within the last 3 years, including but not limited to:

- Grant support

- Employment (past, present, or firm offer of future)
- Patents (pending or applied)
- Payment for expert witness or testimony
- Personal financial interests by the authors, immediate family members, or institutional affiliations that may gain or lose financially through publication of the article
- Forms of compensation, including travel funding, consultancies, board positions, patent and royalty arrangements, stock shares, or bonds. Diversified mutual funds or investment trusts do not constitute a competing financial interest. Authors should carefully examine the wording of documents such as grants and contracts to determine whether there might be an actual or potential competing interest.

Employment of any author by a for-profit or nonprofit foundation or advocacy group or working as a consultant also must be declared.

As a condition of review and publication, authors must further certify that their freedom to design, conduct, interpret, and publish research is not compromised by any controlling sponsor.

A statement of disclosure consistent with the information contained in the CFID form must be included in the Acknowledgments section of the manuscript submitted to the journal. If there are no actual or potential competing financial interests, a declaration of "no competing financial interests" must be included in the Acknowledgments of the manuscript.

Editors and reviewers also should disclose to the Editor-in-Chief any actual or potential competing interests, both financial and nonfinancial, that have occurred within the last 3 years and could reasonably be perceived as relevant. Competing nonfinancial interests include former or current mentor–student relationships, faculty appointments in the same department or organization, familial relationships, service on advisory boards that oversee the research under review, collaborations, or membership in organizations that hold ideological views that are contradictory to the theme or topic under review.

EHP relies on the integrity of all authors to provide accurate disclosure statements. However, authors can expect scrutiny of their statements by the editors, reviewers, and readership. Alleged inaccuracies of declared competing interests should be addressed to the Editor-in-Chief. *EHP* will impose a 3-year ban on publication in *EHP* by any authors found to have

willfully failed to disclose a competing financial interest. A paper may also be retracted or an Expression of Concern published and appended to the online version of the article.

\Manuscript Preparation

Article Length

All words in the main text, title pages, abstract, tables, and references count toward*EHP* word limits. In addition, each figure is counted as 250 additional words. Manuscripts that do not conform to the word limits may be returned to the author(s) for revision before the review process is initiated. Depending on the topic and potential impact of a paper, the Editor-in-Chief reserves the right to waive word limits. Authors should consider placing some types of information such as lengthy descriptions of previously published methods into Supplemental Material; however, these methods must be summarized briefly in the text of the paper. Information included in Supplemental Material does not count toward the word limit. The judicious use of references also may help meet the following word limits:

- Correspondence: ≤ 750 words
- Commentaries: ≤ 5,000 words
- Research Articles: ≤ 7,000 words
- Emerging Issue Reviews: ≤ 5,000 words
- Substantive Reviews: ≤ 10,000 words
- Quantitative Reviews and Meta-Analyses: ≤ 10,000 words
- Meeting Reports: ≤ 5,000 words
- Grand Rounds: ≤ 6,000 words
- Case Reports: ≤ 6,000 words

Parts of a Manuscript

Title Pages

The title pages should include the following items in the order shown, beginning on the first page of the manuscript:

- Manuscript title, not to exceed 20 words (titles generally should not contain abbreviations or numerical values, with the possible exception of abbreviated study names [e.g., NHANES])
- Names of the authors spelled out in full
- Full addresses of the institutions where the work was performed
- Affiliations of all authors
- Name of and contact information for corresponding author to whom page proofs should be sent, including complete address for express mail service, telephone and fax numbers, and e-mail address
- A short running title, not to exceed 50 characters and spaces
- 5–10 key words for indexing purposes
- Acknowledgments, including grant information
- A competing financial interests declaration, not to exceed 50 words
- A list of relevant abbreviations and definitions used in the manuscript.

Abstract

All papers must include a structured abstract, which is not to exceed 250 words and should not contain references. No information should be reported in the abstract that does not appear in the text of the manuscript. In general we recommend that authors indicate study names or sources of data that are integral to the study in the title or abstract. Conclusions should mention the impact of the work to environmental health sciences. Headings to be used in the structured abstracts vary by article type as described below:

- Commentaries and Meeting Reports: Background, Objectives, Discussion, Conclusions
- Research Articles: Background, Objectives, Methods, Results, Conclusions
- Substantive Reviews and Emerging Issue Reviews: Background, Objectives, Methods, Discussion, Conclusions
- Quantitative Reviews and Meta-Analyses: Background, Objectives, Methods, Results, Conclusions
- Grand Rounds and Case Reports: Context (the relevance to environmental exposures and environmental health), Case Presentation, Discussion, Relevance to Clinical or Professional Practice.

Main Text

The organization of the text will vary by article type and roughly reflects the structure of the abstract with some exceptions as described below:

- Commentaries and Meeting Reports: Introduction (comprising the Background and Objectives stated in the abstract), Discussion, Conclusions
- Research Articles: Introduction (comprising the Background and Objectives stated in the abstract), Methods, Results, Discussion, Conclusions. Concise subheadings (not to exceed 8 words each) may be used to designate major topics within each of these sections; do not include tables and figures in these headings.
- Reviews: Introduction (comprising the Background and Objectives stated in the abstract), Methods (including data sources), Results, Discussion, Conclusions
- Grand Rounds and Case Reports: Context (the relevance to environmental exposures and environmental health), Case Presentation, Discussion, Conclusions.

References, Tables, Figures, and Supplemental Material

The following items should be provided after the main text of the paper in this order: References, Tables, Figure Legends, Figures, Supplemental Material. The References, Tables, and Figure Legends must each begin on a new page of the manuscript. Figures and Supplemental Material should be provided as separate files. Additional information concerning each of these sections is provided in "*EHP* Style" below.

Conformance to *EHP* Style Guidelines

Manuscripts submitted to *EHP* must conform to all *EHP* style requirements as described in "*EHP* Style" below. Authors should take special note of requirements for citations and references, figures, and tables. Manuscripts that do not conform to style requirements may be returned to the authors for modification before the initiation of the peer-review process. This step will cause a significant delay in the review and possible acceptance of the manuscript. All manuscripts must be submitted to *EHP* in English.

Manuscript Formatting

Manuscript pages must be numbered consecutively, beginning with the title page, and lines should be numbered in the original submission and all subsequent revisions. The manuscript should be prepared using Times New Roman font at 12-point size. The manuscript must be

double-spaced, with all margins set at 1 inch. Authors should note that page charges are calculated based on the number of manuscript (Microsoft Word) pages.

For additional information, see the *AMA Manual of Style: A Guide for Authors and Editors*, 10th edition (American Medical Association 2007). A basic source for spelling is *Merriam-Webster's Collegiate Dictionary*, 11th edition.

Resources for assistance with research, presentation, and language are available from the following organizations:

- International Committee of Medical Journal Editors [*Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication* (http://www.icmje.org/)]
- AuthorAID (http://www.authoraid.info/).

EHP Style

Plain Language

EHP covers all disciplines engaged in the broad field of environmental health sciences. Therefore, authors should write in a clear and simple manner, in the active voice, and avoid unnecessary jargon, so the article is understandable to readers in other disciplines and to those whose first language is not English. In deference to the breadth of the journal's readership, please define terms that may not be universally recognized among all environmental health scientists.

Results should be presented in a clear and unambiguous manner. Clearly define all outcomes, exposures, predictors, confounders, and covariates, and describe the methods or assays used to characterize study data. Comparison groups or reference conditions should be clearly indicated when reporting measures of association or effect and when reporting *p*-values for statistical tests comparing outcomes or effects between groups.

We recommend against the use of "-fold" terminology because it can be difficult to determine whether it is being used to describe relative versus absolute differences or changes between groups or conditions.

Whenever possible, provide an estimate of variability or precision when reporting measures of association or central tendency (e.g., confidence intervals, standard deviations, interquartile ranges), regardless of whether *p*-values are also reported for these estimates.

Abbreviations

All abbreviations, including abbreviations for elements (e.g., Fe, Cu) and chemical compounds [e.g., polychlorinated biphenyls (PCBs), carbon dioxide (CO_2)], should be defined in the text on first use with abbreviations used thereafter.

Units of measure should be abbreviated only when a specific amount is given (e.g., "concentration of 10 ng/mL" versus "units of nanograms per milliliter").

In-Text Citations and Reference Lists

References and citations must be formatted according to *EHP* style as described below. This will reduce copyediting time and the number of author queries included in page proofs. Authors should double-check all references for accuracy and completeness of information, spelling, diacritical marks, symbols, subscripts/superscripts, and italics. Authors are fully responsible for the accuracy of their references.

In-Text Citations

All in-text citations must be in name/date form. Place the citation immediately after the textual information cited, placing name and date within parentheses without a comma. EndNote (http://www.endnote.com/) is a useful source for *EHP* reference style.

- Single author: (Wing 2002)
- Two authors: (Wing and Wolf 2000)
- Three or more authors: Use first author's last name plus "et al." (Wing et al. 2008)
- Multiple sources cited at one time: List publications alphabetically by author in the citation. Separate publications by the same author(s) with commas and those by different

authors with semicolons: (Aldridge et al. 2005; Jameson et al. 2006; Levin et al. 2007; Slotkin 2004a, 2004b; Slotkin et al. 2008)

- Multiple sources cited at one time with different first authors but same last name and date: Use first author's last name plus initial(s) (Smith A 2000; Smith J 2000).

Provide references for any quotations used in the text. For example:

According to Rubin et al. (2001), "it is only with a multidisciplinary and collaborative approach that the environmental and public health significance of *Pfiesteria*will be fully understood."

All manuscripts submitted but not yet accepted, unpublished data, and personal communications—any items that must be cited but are not accessible to the public—must appear in the text in parentheses but should not be listed in the references: (Ramsdell JS, Moeller PDR, personal communication); (Reeves MK, unpublished data).

Reference List

Authors are fully responsible for the accuracy of their references. The list of references should begin on a new page after the Conclusions of the manuscript. All references must include:

- Author/editor last name plus initials (for six or fewer authors; if there are more than six authors, use "et al." after the sixth) or authoring agency
- Year of publication
- Full title of article or chapter (lower case)
- Title of journal (abbreviated according to BIOSIS, *Index Medicus*, or PubMed) or book/proceedings in title case
- For books and meeting reports, city/state/country of publication and name of publisher
- Volume and inclusive page numbers
- DOI number, if available, with online publication date; this information is required for articles published online only.

If you are uncertain what to include, please include all information.

List references alphabetically by the last name of the first author. If the first author has more than one publication, list references in alphabetical order (letter by letter) of subsequent authors. If the first author shares the last name with another first author (Smith JM vs. Smith RB), alphabetize

by initials. If you list more than one publication by the same author/group of authors, arrange publications by date, early to late. If you list more than one publication published in the same year by the same author/group of authors, use a, b, c, d, and so on to distinguish the publications.

Sample alphabetical list:

Slotkin TA. 2004a. Cholinergic systems in brain development and disruption by neurotoxicants: nicotine, environmental tobacco smoke, organophosphates. ToxicolApplPharmacol 198:132–151.

Slotkin TA. 2004b. Guidelines for developmental neurotoxicity and their impact on organophosphate pesticides: a personal view from an academic perspective. Neurotoxicology 25:631–640.

Slotkin TA. 2005. Developmental neurotoxicity of organophosphates: a case study of chlorpyrifos. In: Toxicity of Organophosphate and Carbamate Pesticides (Gupta RC, ed). San Diego:Elsevier Academic Press, 293–314.

Slotkin TA, MacKillop EA, Ryde IT, Tate CA, Seidler FJ. 2007. Screening for developmental neurotoxicity using PC12 cells: comparisons of organophosphates with a carbamate, an organochlorine and divalent nickel. Environ Health Perspect 115:93–101.

Slotkin TA, Persons D, Slepetis RJ, Taylor D, Bartolome J. 1984. Control of nucleic acid and protein synthesis in developing brain, kidney, and heart of the neonatal rat: effects of a difluoromethylornithine, a specific, irreversible inhibitor of ornithine decarboxylase. Teratology 30:211–224.

Slotkin TA, Seidler FJ. 2007. Comparative developmental neurotoxicity of organophosphates in vivo: transcriptional responses of pathways for brain cell development, cell signaling, cytotoxicity and neurotransmitter systems. Brain Res Bull 72:232–274.

Types of references

Journal article—conventional reference

Lewin SW, Arthur JR, Riemersma RA, Nicol F, Walker SW, Millar EM, et al. 2002. Selenium

supplementation acting through the induction of thioredoxinreductase and glutathione peroxidase protects the human endothelial cell. BiochimBiophysActa 1593:85–92.

Journal article—DOI reference

Fanshawe TR, Diggle PJ, Rushton S, Sanderson R, Lurz PWW, Glinianaia SV, et al. 2007. Modelling spatio-temporal variation in exposure to particulate matter: a two-stage approach.Environmetrics; doi:10.1002/env.889 [Online 17 December 2007].

Journal article—conventional reference and DOI reference

Berglund M, Lind B, Björnberg KA, Palm B, Einarsson Ö, Vahter M. 2005. Inter-individual variations of human mercury exposure biomarkers: a cross-sectional assessment. Environ Health 4:20; doi:10.1186/1476-069X-4-20 [Online 3 October 2005].

Journal article, "in press"

Theppeang K, Glass TA, Bandeen-Roche K, Todd AC, Rohde CA, Schwartz BS. In press. Sex and race/ethnicity differences in lead dose biomarkers: predictors of lead in blood, tibia, and patella in older, community-dwelling adults in an urban setting. Am J Public Health.

Chapter in edited book

Clark K, Cousins I, MacKay D, Yamada K. 2003.Observed concentrations in the environment. In: The Handbook of Environmental Chemistry, Vol 3, Part Q: Phthalate Esters (Staples CA, ed). New York:Springer, 125–177.

Agency as author

Institute of Laboratory Animal Resources. 1996. Guide for the Care and Use of Laboratory Animals. 7th ed. Washington, DC:National Academy Press.

Proceedings

Zaslavsky I, Pezzoli K, Valentine D, Lin A, Sarabia H, Ellisman MH, et al. 2006. Integrating GIS and portal technologies for assessing environmental health impacts of Hurricane Katrina. In: Proceedings from the Second International Conference on Environmental Science and Technology, 19–22 August 2006, Houston, TX, Vol 2 (Starrett SK, Hong J, Lyon WG, eds). Houston, TX:American Science Press, 385–390.

Web site

NTP (National Toxicology Program). 2008. NTP-CERHR Monograph on the Potential Human

Reproductive and Developmental Effects of Bisphenol A. NIH Publication no. 08-5994. Available:http://cerhr.niehs.nih.gov/evaluations/chemicals/bisphenol/bisphenol.pdf [accessed 24 June 2010].

Footnotes

Do not use footnotes. Place all textual information within the manuscript and all references in the proper form both in text and in the reference list.

Preparing Tables and Figures

Tables

Each table must begin on a new page after the References. Tables must be numbered with Arabic numerals, followed by a brief title (not to exceed 25 words). Tables should contain no more than three layers of column headings, and the entire table should fit on one journal page or less. Large tables (> 2 manuscript pages) may be published online as Supplemental Material. A column heading must be provided for each column. Rather than placing additional column heads in the middle of a table, a new table should be created. For tables spanning > 1 page in the Microsoft Word version of the manuscript, authors should indicate that the table is continued from the previous page [e.g., "Table 1 (cont.)"], and all column headings should be repeated at the top of the table on each new page. List abbreviations and definitions under each table. Type footnotes directly after the abbreviations, beginning on the next line. General footnotes to tables must be indicated by lowercase superscript letters beginning with "a" for each table. Footnotes indicating statistical significance must be identified in the following order: asterisks (*, **), number signs ($^{\#}$, $^{\#\#}$), and daggers (†, ††). The comparison to which the *p*-value applies must be clearly indicated (e.g., "compared with untreated controls"). For presentation of data in tables, please use the "±" symbol for arithmetic mean and standard deviation or standard error (e.g., "mean ± SE") and parentheses for the standard error when presented with the geometric mean [e.g., "GM (SE)"]. Please present number and percent as "*n* (%)" (i.e., in one column separated with one space).

Figure Legends

Figure legends should be provided on a new page after tables. Each figure legend should include a title for the entire figure and descriptors for each panel [e.g., "Figure 1. Incidence of hepatocellular adenomas (*A*) and carcinomas (*B*) in mice exposed to DEHP"]. Define error bars and any abbreviations not defined in the text. Footnotes indicating statistical significance must be

identified in the following order: asterisks (*, **), number signs ($^{\#}$, $^{\#\#}$), and daggers (†, ††). The comparison to which the *p*-value applies must be clearly indicated (e.g., "compared with controls from the corresponding age group"). Type footnotes directly after the abbreviations beginning on the next line.

Figures

Each figure must be provided as a separate file in one of the following formats: TIFF, JPG, EPS, or PDF. Do not embed figures in the main text (Microsoft Word) file. Each figure must be labeled with the figure number. For TIFF or JPG format, the resolution should be 300 dpi for color images, 600 dpi for grayscale images, and 1200 dpi for line art (black-and-white art). JPG files should be saved on the "highest quality" setting. Color images should be RGB and saved at a minimum of 8 bits per channel. Because figures may be reduced or enlarged to fit our layouts, sufficient resolution is essential. Vector images should be saved as editable EPS files. Any images embedded in the EPS should also be included in a separate file. Do not convert text to path outlines before submission.

Graphics must fit standard letter-size paper (8.5 × 11 inches, portrait orientation). Multiple panels within a figure also must fit on a single page. All letters, numbers, and lines must be clearly legible and easy to differentiate. Provide a key defining representational elements (e.g., dotted/dashed lines, symbols) for each figure. All axes must be clearly labeled, giving both the measure and the unit of measurement where applicable. Consistency among terms and styles (including symbols and colors) used in figures is desirable. For example, if "luteinizing hormone" is abbreviated "LH" in the text, "LH" should be used in figures; if a black circle represents the control in Figure 1, a black circle (or a black bar) should be used for controls in all other figures. Photomicrographs should include a scale bar in each image, and the length should be specified in the typed figure legend (e.g., "bar = 10 μm").

EHP editors reserve the right to request that complex figures (e.g., figures with multiple panels showing information in a variety of formats, or that include panels related to different experiments) be divided into separate figures for publication. Questions concerning figures should be directed to EHPmanuscripts@niehs.nih.gov.

Image Integrity

Adjusting an image for brightness and contrast is acceptable if it is applied to the entire image. Background data of gels and blots must not be removed. The final image must accurately represent the original data.

In-Text Citations and Reference Lists

References and citations must be formatted according to *EHP* style as described below. This will reduce copyediting time and the number of author queries included in page proofs. Authors should double-check all references for accuracy and completeness of information, spelling, diacritical marks, symbols, subscripts/superscripts, and italics. Authors are fully responsible for the accuracy of their references.

Supplemental Material

EHP welcomes reasonable amounts of material suitable for inclusion as online documentation for submitted manuscripts. Examples are bioinformatic data, formulae, statistical derivations, full gene data and analysis, additional high-resolution microscopic data, kinetic analyses, and other supporting tables, figures, or videos. The submitted manuscripts must be able to stand alone in the absence of Supplemental Material. All information included as Supplemental Material should be directly relevant to the article and cited in the main body of the paper. The principal methodological approach must be clearly described in the main body of the paper and not relegated to Supplemental Material.

Supplemental Material must not exceed a total of four tables or figures. Text, exclusive of figure legends, tables, and references, must not exceed a total of 750 words. If the Supplemental Material exceeds this limit, the author must request a waiver from the Editor-in-Chief before the paper is submitted to the journal. Authors may provide a separate (ideally permanent) web repository for information that is not included in the Supplemental Material file if they believe it would be of interest to readers. This material should be clearly identified as not peer reviewed. This information should be cited in the text and included in the reference list (formatted as a web site).

Information included as Supplemental Material does not count toward the word count for the paper. Supplemental Material must be uploaded as a separate single PDF file and labeled as such. Although Supplemental Material is published online only, it will be peer reviewed along with the manuscript and thus must meet the same rigorous standards.

The Supplemental Material PDF file that you provide will be linked with your paper through a common DOI number. We use Supplemental Material files "as is" (i.e.,*EHP* will not copyedit or reformat the file). Therefore, please carefully check the text and figures to confirm that they are complete and accurate.

1. Provide a single Supplemental Material file in PDF format to ensure that the formatting of the file is exactly the way you want it, without the possibility of inadvertent changes in formatting as the file is converted from a .doc file to a PDF.
2. Begin the file with a title page that indicates "Supplemental Material" followed by the title of the paper and the author list.
3. Include page numbers, but remove all line numbers before generating the PDF file.
4. Provide a Table of Contents (on or after the title page) if the Supplemental Material comprises multiple tables, figures, and/or sections of text.
5. Place figure legends below corresponding figures. Landscape (versus portrait) layout may be used when needed.
6. Tables or figures included in the Supplemental Material should be labeled as Supplemental Material, Table 1; Supplemental Material, Figure 1; and so on.
7. When referring to Supplemental Material in the main manuscript, indicate the table, figure, or section as follows: See Supplemental Material; see Supplemental Material, Table 1; see Supplemental Material, p. 6; see Supplemental Material, Part 2.
8. A separate reference list must be included in the Supplemental Material file for any sources cited in the Supplemental Material, even if they are cited in the main paper.

Public Databases

Manuscripts using microarrays must follow the Minimum Information About a Microarray Experiment (MIAME) guidelines developed by the Microarray Gene Expression Data (MGED) Society (http://www.mged.org/miame). On acceptance, all integral data supporting the article's conclusions should be submitted to either the ArrayExpress (http://www.ebi.ac.uk/arrayexpress) or GEO (http://www.ncbi.nlm.nih.gov/geo/) database.

Manuscript Submission

Manuscript Central

Manuscripts submitted to *EHP* will be processed using Manuscript Central, an online manuscript submission and tracking program (http://mc.manuscriptcentral.com/ehp).

Initial Submission of a Manuscript

Authors may either log in or select the "Create a New Account" icon to create a new account. To determine if an account exists, e-mail EHPmanuscripts@niehs.nih.gov. Once logged in to the Manuscript Central site, authors must select the "Author Center" link. From this point, the system will guide the user through the submission process. Online help is available at all times during the process via the "Get Help Now" button in the upper right corner of the screen. Users may also exit and reenter the submission process at any time before completing a manuscript submission.

After completing an online submission, authors must submit a CFID form as soon as possible. This form can be found by selecting the "Instructions & Forms" link in the Author Center. The assigned manuscript number should be noted on the form. Authors should complete and sign the form, then submit a scanned document by e-mail to EHPmanuscripts@niehs.nih.gov. Completed forms may also be faxed to 919-541-0273 or mailed to:

Editor-in-Chief
Environmental Health Perspectives
National Institute of Environmental Health Sciences
Mail Drop K3-01
PO Box 12233
Research Triangle Park, NC 27709-2233
USA

Authors can monitor the progress of submissions at any time by logging in to the Author Center using their ID and password. Forgotten passwords may be obtained by entering your e-mail address in the "Password Help" section of *EHP*'s Manuscript Central homepage. If an account exists, instructions for resetting the password will be e-mailed to the user.

Manuscripts may be submitted only via the online system. Manuscripts submitted by other methods (e.g., hard copy, e-mail) will not be processed.

Required Cover Letter

A cover letter must accompany the manuscript and include the following points:

- Assurances that the manuscript *a*) is an original work, *b*) has not been previously published whole or in part, and *c*) is not under consideration for publication elsewhere
- A statement that animals used in research have been treated humanely according to institutional guidelines, with due consideration to the alleviation of distress and discomfort. The source of those guidelines must be provided
- A statement that participation of human subjects did not occur until after informed consent was obtained
- Confirmation that all authors have disclosed any actual or potential competing interests regarding the submitted article and the nature of those interests (the required CFID form is available at http://ehp.niehs.nih.gov/cfi.pdf)
- If applicable, written permission from any copyright holder (usually the publisher) to reproduce figures, tables, questionnaires, or a substantial block of text in both print and electronic forms
- A statement indicating that all authors *a*) have read the manuscript, *b*) agree the work is ready for submission to a journal, and *c*) accept responsibility for the manuscript's contents
- The names and e-mail addresses of up to six possible preferred reviewers, as well as up to six nonpreferred reviewers for the manuscript
- If applicable, a statement concerning previous publication of a manuscript or materials that might be considered redundant or duplicative.

Peer Review

Manuscripts are assessed for originality, scientific quality, environmental health significance, clarity of presentation, and conciseness. Scientific quality and environmental significance have a higher weight than the other criteria.

At least two peer reviewers will be solicited for comments on the manuscript, and authors will not know the identity of the reviewers. Peer review is conducted electronically to accelerate the process, and each reviewer is asked to complete the review of each version of the paper within three weeks. Authors may nominate up to six preferred reviewers for the manuscript. Providing the names and contact information, including e-mail addresses, is strongly encouraged. Authors may also identify up to six nonpreferred reviewers.

After editorial consideration, a decision letter and reviewers' comments will be e-mailed to authors. If a revision of the manuscript is required, authors must submit the revised manuscript to *EHP* within 6 weeks of the request. If authors fail to meet this deadline, the submission will be canceled unless the authors have obtained prior permission for an extension from the Editor-in-Chief. Authors must submit both the revised manuscript and a letter responding to reviewers' comments.

Resubmission of a Revised Manuscript

If *EHP* requests revisions or accepts the manuscript, authors will need to submit all of the following through Manuscript Central (http://mc.manuscriptcentral.com/ehp):

- All text, tables, and figure legends must be in one Microsoft Word document. Please ensure any symbols and/or equations appear correctly on printed copies and that all figures and tables are cited in the body text in numerical order.
- Each revised figure must be submitted as a separate file in one of the following formats: TIFF, JPG, EPS, or PDF. Each figure must be labeled with the correct figure number for the revised manuscript. Additional information on formatting and content requirements is provided in "Preparing Tables and Figures" above.
- Authors should submit a cover letter with point-by-point responses to the reviewers' comments, a copy of the revised manuscript with changes tracked in Microsoft Word, and a clean version of the revised manuscript with all changes accepted. In order to expedite the processing of revised manuscripts, it is important to be as specific as possible in responding to reviewers' comments. Authors should copy the editors' and/or reviewers' comments into the response letter and respond to each comment individually, including the specific changes made in response to each comment (if any) and where the changes are located in the revised draft. As in the original submission, the revised manuscripts must have numbered lines to facilitate locating specific text or changes.
- Revised manuscripts must conform to *EHP* length requirements, even if additional material is added to the manuscript in response to reviewer requests. Authors may want to consider moving text, tables, and figures to Supplemental Material to reduce the length of the manuscript, provided such material is not necessary for most readers to follow or interpret the findings. Authors should consult with the Associate Editor for their paper for

additional guidance if needed. Papers that substantially exceed *EHP* word limits may be returned to authors for additional revisions to reduce their length before acceptance.

Publication Sequence

Ahead of Print

Authors will be notified their paper has been accepted provisionally, at which point they may be asked to respond to additional post-review requests from the *EHP* Editor-in-Chief or Science Editor. Authors also are asked at this point to conduct their own final review of their paper to confirm it is ready for Ahead of Print publication. Final acceptance will occur after papers have undergone in-house editorial review for scientific content and accuracy and compliance with *EHP* formatting and CFI requirements.

Papers are not copyedited until they are prepared for print publication. *EHP* publishes unedited PDF versions of articles online as Ahead of Print articles (http://www.ehponline.org/) within 24 hours of final acceptance unless a prepublication embargo period is agreed upon in advance (for more information on embargoes, see "Press Releases and Embargo Policy" below). Additionally, unedited abstracts are published online at PubMed (http://www.ncbi.nlm.nih/gov/pubmed/) and at http://www.ehponline.org/.

Ahead of Print articles are citable using the assigned DOI (Digital Object Identifier) number for the article. The DOI number enables the article to be immediately referenced and establishes publication priority. The PDF version of Ahead of Print articles will be replaced with the copyedited, formatted version as soon as possible, but the DOI number will remain with the copyedited article. In addition to the DOI number, the copyedited article will include assigned volume and page numbers that will allow full conventional citation.

Copyediting/Page Proofs

To prepare each paper for print publication, *EHP* staff will convert electronic material to a desktop publishing format and copyedit the manuscript. The copyedited version, with embedded author queries, will be converted to a PDF version (page proofs) and sent to authors by e-mail. The authors can use free Acrobat Reader software (http://get.adobe.com/reader/otherversions/) to review the proofs. Authors should return corrected page proofs to the responsible editor by e-mail or fax (919-541-0273). There are two methods of correcting and returning proofs:

- Authors may use Comment and Markup Tools in Acrobat and e-mail the corrected proof.
- Authors may print the proof and write corrections directly on the printed copy; then return the corrected proof by fax (919-541-0273), or scan the marked proof and return it by e-mail. Authors using this method should also include with the page proofs a list of itemized changes (including their locations).

The copyedited proofs of an article may be slightly different from the Ahead of Print version as a result of the editing process, but no substantive changes will be allowed. Any significant changes at this stage of processing will require a correction to be published at the end of the article. Extensive changes cannot be made at the proof stage; only minor changes, such as spelling, grammar, clarification, and referencing, should be requested. If new information has become available after acceptance of the manuscript, an addendum in proof can be included with the permission of the Editor-in-Chief. Articles will usually be published in the print version in order of acceptance as journal space permits.

Page and Color Figure Charges

On acceptance of the manuscript, authors will be required to pay page charges at the current rate of $35 per accepted Microsoft Word manuscript page (excluding the first three pages). Authors will also be charged $500 for the first color figure and $100 for each additional color figure.

Publication Date of Record

Manuscripts accepted for publication in *EHP* will appear online within 24 hours of final acceptance unless they have been embargoed. The date the article is posted on the web site will be considered the publication date of record.

Copyright, Reproduction, and Citation

EHP is a publication of the U.S. Government. Publication of *EHP* lies in the public domain and is therefore without copyright. All text from *EHP* may be reprinted freely. Use of materials published in *EHP* should be acknowledged (for example, "Reproduced with permission from *Environmental Health Perspectives*"); pertinent reference information should be provided for the article from which the material was reproduced. Articles from *EHP*, especially the News section, may contain photographs or figures copyrighted by other commercial organizations or

individuals that may not be used without obtaining prior approval from the holder of the copyright. For further information, contact *EHP* Permissions (ehponline@niehs.nih.gov).

Press Releases and Embargo Policy

Authors are responsible for arranging media outreach with their own press offices in conjunction with *EHP*. *EHP* will happily tailor publication dates to suit the needs of authors and their press officers. We recommend an embargo period of at least 2 full working days for any paper that receives a press release. This gives members of the media time to prepare stories and contact corresponding authors for additional information.

Authors whose papers have been provisionally accepted for publication should contact Susan Booker, *EHP* News Editor, at booker@niehs.nih.gov to coordinate embargo and publication dates. Authors or press officers should also provide *EHP*a copy of their final press release.

Upon final acceptance *EHP* will send press officers a PDF copy of the article to be distributed to media who request it. All pre-press materials will be clearly identified as embargoed and will include the embargo date and time established by *EHP* in conjunction with the authors. Authors must adhere to *EHP*'s embargo policy, and authors and media alike are responsible for ensuring that all third parties with whom they share pre-press materials honor the embargo.

Types of References

Journal article—conventional reference
Waalkes MP, Liu J, Diwan BA. 2007. Transplacental arsenic carcinogenesis in mice. ToxicolApplPharmacol 222:271–280.

Journal article—DOI reference
Latendresse JR, Bucci TJ, Olson G, Mellick P, Weiss C, Thorn B, et al. 2009. Genistein and ethinylestradiol dietary exposures in multigenerational and chronic studies induce similar poliferative lesions in mammary gland of male Sprague-Dawley rats. ReprodToxicol; doi:10.1016/j.reprotox.2009.04.006 [Online 19 April 2009].

Journal article—conventional reference and DOI reference
Glas AM, Floore A, Delahaye LJ, Witteveen AT, Pover RC, Bakx N, et al. 2006. Converting a

breast cancer microarray signature into a high-throughput diagnostic test. BMC Genomics 7:278; doi:10.1186/1471-2164-7-278 [Online 30 October 2006].

Journal article, "in press"

Holmes AK, Maisonet M, Rubin C, Kieszak S, Barr DB, Calafat AM, et al. In press.A pilot study of exposures to endocrine-disrupting compounds in pregnant women and children from the United Kingdom.Int J Child Adolesc Health.

Article in non-English language

Rateau JG, Broillard M, Morgant G, Aymard P. 1986.Etude experimental chez le lapin de l'effet de la cholestyraminedans le traitement des diarrheesinfectieusesd'orginecholerique [in French].ActualiteTherapeut 22:289–296.

Magazine article

Grant M. 1997.The cell from hell. People, 19 May:101–103.

Newspaper article

Clabby C. 2001. Study details how centuries of fishing depleted sea life. News and Observer (Raleigh, NC) 27 July: B1.

Book

Luna LG. 1968.Manual of Histopathologic Staining Methods of the Armed Forces Institute of Pathology. 3rd ed. New York:McGraw-Hill.

Book, edited

Gross TL, Ihrke PJ, Walder EJ, eds. 1992.Veterinary Dermatopathology. St. Louis, MO:Mosby Year Book.

Chapter in edited book

Gurevitch J, Hedges LV. 1993. Meta-analysis: combining the results of independent experiments. In: The Design and Analysis of Ecological Experiments (Scheiner SM, Gurevitch J, eds). New York:Chapman& Hall, 378–398.

Book chapter, "in press"

McCoy KA, Guillette LJ.In press.Endocrine disruptors. In: Amphibian Biology. Vol 8.

Conservation and Decline of Amphibians (Heatwole HF, ed). Chipping Norton, New South Wales, Australia:Surrey Beatty & Sons.

Agency monograph

IARC (International Agency for Research on Cancer). 1993. Cadmium and cadmium compounds. IARC MonogrEvalCarcinog Risk Hum 58:119–237.

Agency as author

CDC (Centers for Disease Control and Prevention). 2005. Fourth National Report on Human Exposure to Environmental Chemicals. Atlanta, GA:Centers for Disease Control and Prevention. Available:http://www.cdc.gov/exposurereport/ [accessed 14 January 2010].

Proceedings

Ibrahim K. 1994.The status of marine turtle conservation in Peninsular Malaysia. In: Proceedings of the first ASEAN Symposium Workshop on Marine Turtle Conservation, 6–10 December 1993, Manila, Philippines (Nacu A, Trono R, Palma JA, Torres D, Agas F Jr, eds). Manila, Philippines:ASEAN, 87–103.

Technical paper

NTP. 2006. Toxicology and Carcinogenesis Studies of Bromodichloromethane (CAS No. 75-27-4) in Male F344/N Rats and Female B6C3F_1 Mice (Drinking Water Studies). TR 532. Research Triangle Park, NC:National Toxicology Program.

Dissertation/thesis

Gelobter M. 1993. Race, Class, and Outdoor Air Pollution: The Dynamics of Environmental Discrimination from 1970 to 1990 [PhD Dissertation]. Berkeley, CA:University of California, Berkeley.

Software manual

SAS Institute Inc. 2001.SAS/STAT Guide for Personal Computers, Version 8. Cary, NC:SAS Institute, Inc.

Web site

CDC (Centers for Disease Control and Prevention). 2003. National Health and Nutrition Examination Survey Homepage. Available:http://www.cdc.gov/nchs/nhanes.htm [accessed 6 August 2008].

Online database

National Center for Biotechnology Information. 2011. PubMed.
Available: http://www.ncbi.nlm.nih.gov/pubmed/ [accessed 14 July 2011].

Abstract

Barbeito AG, Guelfi N, Varga MR, Pehar M, Beckman J, Barbeito L, et al. 2005. Chronic low-level lead exposure increases survival of G93A SOD-1 transgenic mice [Abstract]. In: Amyotrophic Lateral Sclerosis: Beyond the Motor Neuron.
Available: http://iibce.edu.uy/ALSmeeting/abstract.htm [accessed 14 April 2008].

Federal regulation

U.S. Environmental Protection Agency. 2001. National primary drinking water regulations. Arsenic and clarifications to compliance and new source contaminants monitoring.Final rule.Fed Reg 66:6076–7066.

Executive order; federal regulation

Clinton WJ. 2000. Executive Order 13148. Greening of the government through leadership in environmental management.Fed Reg 65:24595–24606.

U.S. Government document

U.S. Environmental Protection Agency. 2004. Air Quality Criteria for Particulate Matter. EPA/600/P-99/002aF. Research Triangle Park, NC: U.S. Environmental Protection Agency.

State document

State of Maryland. 1998. Water Quality Improvement Act of 1998. Annapolis, MD:General Assembly.

Law

Food Quality Protection Act of 1996. 1996. Public Law 104-170.

Court case

Leach *v*. E.I. du Pont de Nemours & Co. 2002. Civil Action No. 01-C-608, 2002 WL 1270121. Circuit Court of Wood County, West Virginia, 10 April 2002.

Abbreviations

All nonstandard abbreviations [e.g., organochlorine (OC) pesticides, limit of detection (LOD), polymerase chain reaction (PCR)] and abbreviations for elements (e.g., Fe, Cu, Ag) and chemical compounds [e.g., polychlorinated biphenyls (PCBs), carbon dioxide (CO_2)] should be defined in the text on first use and abbreviated thereafter.

Standard abbreviations, which do not need to be defined, are shown below. Units of measure should be abbreviated only when a specific amount is given (e.g., "concentration of 10 ng/mL" versus "units of nanograms per milliliter").

Å	angstrom
amu	atomic mass unit
ATP	adenosine 5´- triphosphate
BW	body weight
°C	degrees Celsius
cm	centimeter
cm^2	square centimeter
cm^3	cubic centimeter
Da	dalton
df	degrees of freedom
DNA	deoxyribonucleic acid
EDTA	ethylenediamine-tetraacetic acid
ELISA	enzyme-linked immunoadsorbent assay
ft	foot
g	gram
g	gravity (10,000 x *g*)
gal	gallon
Gy	gray (unit of absorbed dose of ionizing radiation)
ha	hectare
HEPES	*N*-2-hydroxyethylpiperazine-*N*´-2-ethane sulfonic acid
HPLC	high-performance liquid chromatography
hr	hour
Hz	hertz
i.d.	inside diameter
IM	intramuscular
in.	inch
IU	international unit
J	joule
kDa	kilodalton

kg	kilogram
km	kilometer
K_m	Michaelis constant
L	liter
lb	pound
ln	natural logarithm
M	molar
m	meter
m^2	square meter
m^3	cubic meter
mCi	millicurie
µg	microgram
mg	milligram
mi	mile
µL	microliter
min	minute
mL	milliliter
mM	millimolar
mm	millimeter
mol	mole
mRNA	messenger RNA
n	number
ng	nanogram
nL	nanoliter
nmol	nanomole
o.d.	outside diameter
pg	picogram
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
RNA	ribonucleic acid
RNase	ribonuclease
SD	standard deviation
SDS/PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SE	standard error, standard error of the mean
sec	second
U	unit
V	volt

vol/vol	volume/volume
W	watt
wt	weight
wt/vol	weight/volume
yd	yard

APPENDIX D – CONSENT FORM

Consent to participate in a survey of investigating health effects due to occupational and environmental pesticide exposures on male farm residents in the rural Western Cape

1. **Title of research project**

Male reproductive effects due to pesticide exposure in the Western Cape, South Africa

2. Names of the researchers

Mohamed Aqiel Dalvie (BSc, Honours, MSc, PhD)
Algernon Africa (BTech)
Vicky Major (
Leslie London (MBChB, Honours, MD)
Eugene Cairncross (BSc, Honours, PhD)

3. Purpose of research

The University of Cape Town is conducting this survey to investigate the reproductive health effects of pesticides on young boys and men in the Western Cape. This will be of benefit to men and boys living in farming areas and who are exposed to pesticides either at work or in the environment.

4. Description of the research project

We will conduct tests on one day. Your son will be required to produce a urine and blood sample and undergo a physical examination 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 about your son, his general medical health, genital health history and lifetime environmental exposure to pesticides.

b) **Urine sample:** Your son has to 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 10 ml blood from a vein on your son's arm. The blood will be analysed for pesticides and for the levels of hormones.

d) **Physical examination:** A doctor will assess your son's reproductive health.

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 pesticides and reproductive hormones 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 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

A doctor will examine your son's reproductive health.

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

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

7. Costs to you resulting form participation in the study

The study is offered at no cost to you.

8. Confidentiality of information collected

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

9. Documentation of the consent

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

10. Contact person.

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

Name of person: MA Dalvie (The principal investigator) - telephone 021 4066610
Name of person: Lamees Emjedi (Ethics administrator) - telephone 021 4066492

11. Voluntary nature of participation

Your son's participation in this project is voluntary. Subsequent to your consent, you may refuse your son 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 for my son to participate in the study.

_____ _____

Printed name of parent/ participant (adolescent or adult) **signature**

_____ _____

Date

_____ _____ **Interviewers**

(print) **signature** **Date**

_____ _____

Witness (print) **signature** **Date**

Date:_____

Study Number _____

APPENDIX E – ETHICAL APPROVAL LETTER