RELATIONSHIP BETWEEN URINARY LEVELS OF ORGANOPHOSPHATE METABOLITES AND PESTICIDE EXPOSURES AMONG SCHOOL BOYS IN THE RURAL WESTERN CAPE, SOUTH AFRICA

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PREAMBLE

Declaration

I, **Regina Ntsubise Molomo**, hereby affirm that the work on this dissertation is my original work (except where acknowledgement indicate otherwise) and it has never been submitted for another degree in University of Cape Town or any other university globally.

I empower the UCT to reproduce this work for research purposes.

Signature: Signed by candidate

Date: January 2020

Dedication

I thank God and Mother Mary for the guidance, support and strength they gave me to complete this work together with my parents, siblings, friends and family.

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Abstract

Background: Biomonitoring of pesticides is an objective measure of short-term pesticide exposure as it measures possible exposure in the human body. Current evidence on the relationship between demographic, socio-economic and pesticide exposure risk factors and urinary levels of organophosphate (OP) pesticide metabolites among children is generally incomplete and conflicting in some cases. There is therefore a need for further research.

Objectives: This study investigated the relationship between socio-economic, demographic and reported pesticide exposure related activities and characteristics in relation to urinary levels of three dialkyl phosphate (DAP) metabolites (diethyl phosphate (DEP), dimethyl phosphate (DMP) and dimethyl triphosphate (DMTP)) among boys living in the rural areas of the Western Cape, South Africa.

Methods: This was an analysis of data collected during a cross- sectional study of 183 boys from three agricultural intense areas in the Western Cape of South Africa between April 2007 and March 2008. Measurements included a questionnaire on demographic, socio-economic and pesticide exposure risk factors and analysis of spot urine samples for DAP metabolites.

Results: Most of the boys (70%) lived on farms with a median age of 12 years (range: 5.0 - 19.5 years). The median concentrations of DAP, DEP, DMP and DMTP were 68.3 ng/ml (IQR= 27.9; 129.5), 5.5 ng/ml, 32.6 ng/ml and 16.7 ng/ml, respectively. The sum of the three DAP levels was inversely associated with age. Children older than 14 years had less DAP levels (β = -68.1; 95% CI: -136.8,0.6) than children 9 years and younger. DAP levels also varied significantly with residential area, with the levels highest in Grabouw (apple farming), followed by Hex River Valley (grape farming) (β= -52.1; 95% CI: -97.9, -6.3) then Piketberg (wheat farming) (β= -54.2; 95% CI:-98.8, -9.7). Other weaker and non-significant associations with increased DAP levels were found with increased household income, member of household work with pesticides, living on a farm, drinking water from an open water source and eating crops from the vineyard and or garden.

Conclusion: The study found younger age and living in and around an apple and grape farms, to be associated with increased urinary DAP concentrations among the school children provide evidence that younger age and residential area can be associated with increased urinary DAP concentrations among boys. Additionally, there are other household and behavioural characteristics that are associated with elevated urinary DAP levels. Further studies with larger sample sizes and longitudinal designs to improve the statistical power and the associations found are recommended. The study provided more insight to incomplete and inconclusive evidence of previous studies.

Keywords: Organophosphorus pesticides, pesticide biomonitoring, urinary dialkyl phosphates, rural boys, socio-economic factors, farm boys and non-farm boys.

Table of Contents

TABLE OF TABLES

PART A: RESEARCH PROTOCOL

PART B: STRUCTURED LITERATURE REVIEW

PART C: MANUSCRIPT

APPENDICES

PART A: RESEARCH PROTOCOL

1.Introduction

1.1 Background

Global population growth stimulates the need for massive food production and subsequently excessive pesticide use (1). From 1940 to 2001, 2.26 million tons of pesticides were reported to be in market (2) and currently, approximately 2.8 million tons of pesticides are used globally (3). Among such, 25% of pesticides use is from developing countries (3), with South Africa the largest consumer in sub- Saharan Africa. South Africa currently imports nearly 3000 pesticide active ingredients to adhere to changing trade standards and meet yearly food demand (4).

Pesticides are complex mixtures of active ingredients used to control or kill pests (5, 6). They can pose human health risks, especially when used frequently (7). Some factors that lead to frequent use of pesticide are pesticide resistance, pesticide treadmill, pest resurgence and selective pressure (7). Human beings can be exposed to pesticides through either ingestion, inhalation, dermal contact or eye contact (8-11).

Most affected population are farmers, farm workers, children, adults, women and elderly in neighbouring areas to the farms (8-10, 12). Among all exposed persons, children and pregnant women are the most at risk due to their behaviour and compromised immune system (9, 13, 14). Children are mostly exposed to pesticides through take home pesticides, diet and residential pesticide use (10, 13, 15-17). In children, pesticides have been associated with a number of adverse health effects including impaired neurodevelopment, behavioural problems, asthma and reproductive disorders (18, 19).

To investigate effects of pesticides on human health, it is important to understand which exposures and factors determine their adsorption into the body. Hence, this analysis seek to understand the relationship between urinary levels of pesticide residues, socio-economic, demographic factors and reported pesticide exposures, from data of a study investing the health effects of pesticides among school boys from rural communities in Western Cape (20, 21).

2. Literature Review

2.1 Organophosphate Pesticide use

Pesticides are widely used in the world to increase food production. In 2010, approximately 2 million tons of pesticides were used worldwide, with China the major contributing country, followed by the USA (22). In Africa, about 4% of global market share of pesticides were used, even though most people depend on farming for their living (22). One of the major pesticide users in Sub- Saharan Africa is South Africa (4).

The agricultural sector accounts for approximately 2.2 % of South Africa's economy (23). To increase produce, profits and to keep up with changing trade standards, most farmers abundantly use pesticides (1). For this, pesticide use in the agricultural sector continuously increase with years (24). For instance, pesticide use increased from 4623 tons, 7495 tons to 20947 tons in 1994, 1999 and 2009 respectively (25, 26).

Western Cape, one of the top agricultural intense provinces in South Africa, is the leading province in OP pesticides use (4, 27). Grapes, pome fruits and wheat farming are leading farming sectors in this province and OP pesticides such as chlorpyrifos, azinphos-methyl and prothiofos are commonly used to control for unwanted pests (28). Residues of these pesticides have also been detected in food samples (local and imported wheat samples) (27, 29), surface water, groundwater and soil samples (28, 30).

Pesticides previously detected in drinking water especially from boreholes in the rural Western Cape raise a public health concern regarding health of exposed persons especially farm workers, farmers and people residing on farms or communities in areas neighbouring farms in the Western Cape (31). Pesticide residues can also be transported to residential places through pesticide spray drifts and expose children and other residents to unwanted pesticide exposure (7).

2.2 Organophosphates

Organophosphates are toxic chemicals that are used in agriculture to control for insects as they are neurotoxic (5). In humans, OP neurotoxicity occurs through inhibition of the enzyme acetylcholinesterase, which breaks down the neurotransmitter acetylcholine, leading to its accumulation at synaptic clefts and continued neural activity which can cause increased blood pressure, cardiac rhythm and muscle contractions and ultimately death (32). Short term health effects of organophosphates include skin and eye irritation, headaches and dizziness (33, 34). Long term health effects include asthma, diabetes and cancer (33, 35, 36).

Organophosphate pesticides are commonly absorbed through the skin, but can also enter human body through inhalation, eye contact and ingestion (37). Once in the human body, organophosphates are broken down into various metabolites including specific metabolites and one or more of the non- specific dialkyl phosphates (DAPs): dimethyl phosphate (DMP), dimethyl thiophosphate (DMTP), dimethyl dithiophosphate (DMDTP), diethyl phosphate (DEP), diethyl thiophosphate (DETP) and diethyl dithiophosphate (DEDTP) (9, 15, 16, 38). These metabolites are usually detected in urine samples (39). Organophosphate metabolites concentrations are depended on various factors and exposure factors can differ from place to place (4). However, DMDTP and DETP metabolites that are the least detected non-specific metabolites compared to other metabolites as shown in Table 1. High levels of DAP metabolites in the body have been associated with adverse effects on the central nervous, reproductive and endocrine systems (40, 41).

Table 1: Urinary organophosphate metabolites concentrations detected from exposed populations

Study source	**Location, Period and study sample**	**units**	**Metabolites**	**Mean**	**Detection frequency %**
Ye et al. (42)	**Location:** Canada **Period:** 2010 **Participants:** 3466 people	nmol/g	DMP DMTP DEP	27.5 17.2 17.5	76.4 66.7 77.9
Hefferman et al. (43)	**Location:** Australia **Period:** 2012-2013 **Participants:** 2400 people of all age groups	µg/L	DMP DMTP DMDTP DEP DETP	13.6 10.6 0.4 6.2 1.3	100 100 75.0 100 83
Ye et al. (44)	**Location:** Canada **Period:** 2007-2009 **Participants:** 5604 people of all ages	nmol/L	DMP DMTP DEP	23.5 14.3 14.9	77.4 67 78.6
Ye et al. (42)	**Location:** Canada **Period:** 2010 **Participants:** 980, 12-19 years old children	nmol/g	DMP DMTP DEP	27.1 14.0 16.8	82.3 68.6 82
Roca et al.(45)	**Location:** Spain **Period:** 2010 **Participants:** 125, 6-11 years old children	µg/L	DMP DMTP DMDTP DEP DETP	8.6 5.4 1.0 4.0 1.7	18 39 9 79 36

2.3 Pesticide exposure among children

Children are more vulnerable to hazardous environmental exposures than adults because of their daily behaviour, diet, activities, underdeveloped body system and detoxification mechanism that are still going through physiological development (9). Their frequent engagement with the ground and soil, where pesticide residues settle, and hand to mouth activity increase their environmental risk (9, 13). Moreover, they consume more food (predominantly fruits , vegetables and water) per their unit weight compared to adults' food consumption per unit weight (9, 13). Additional factors that can increase children's pesticide exposure include residential pesticide application, take home pesticides, residential proximity to agricultural fields pesticide runoffs and spray drifts (7). Table 2 display a summary of reported children pesticide exposures and urinary DAP metabolites concentrations.

Table 2: Studies investigating the relationship between urinary levels of OP pesticide residues and demographic, socio- economic and reported pesticide exposures in children

Study source	Organophosphates metabolites analysed	Location, Population, age	Pesticide Exposure sources	Biological matrix	Results
Sinha S and Banda V (39)	Six dialkyl phosphates metabolites (DAPs)	**Location:** Hyderabad India **Population:** 377 children aged 6 to 15 years old (188 boys and 189 girls)	Food (fruits, vegetables and conventional diets)	Urine (spot samples in the morning and 6-10 voids during the day)	Dimethyl phosphate metabolites median in 6-10 years old girls was almost like that of boys, with 0.4µmol/L in girls and 0.55µmol/L for boys. DEs median was also almost the same for girls and boys, 0.97µmol/L for girls and 0.95µmol for boys. In 11-15 years, DMs for girls (0.7µmol/L) was slightly higher than of boys (0.59µmol/L) and DEs median was almost the same for both girls and boys. DAPs metabolites levels in11- 15 years girls were higher than of boys at the same age group. This was related to more fruits that girls consume per day compared to boys (2.69µmol/L vs 1.85µmol/L, $p<0.001$)
Coronado et al. (12)	Six urinary DAP metabolites, Chlorpyrifos, Malathion, Diazinon, Parathion, AZM and Phosmet	**Location:** USA **Period:** 2005-2006 **Study sample:** 100 farmworker's children and 100 non-farmworker's children aged 2-6 years	Take home pesticide exposure (house dust samples n=109 and vehicle dust samples n=109)	Urine (n=579 with three spot urine samples per child on each visit)	Farmworker children (16.5µg/L, 95 % CI: 9.5, 28.4) had higher mean DMTP levels than non- farm worker children (7.5µg/L, 95% CI:5.9,9.5). Median dimethyl concentrations were higher in farmworkers and their children during thinning and harvesting seasons and low during non-spray seasons. Pesticide concentrations were higher in farmworkers house and vehicle dusts compared to non-farmworker's house and vehicle dust. 20 % pesticide metabolites concentration reduction in both children and guardians was observed with each mile distance from the nearest farm/ field.
Bradman et al. (46)	Six dialkyl phosphates metabolites	**Location:** USA **Period:** 1999-2003 **Study sample:** 417 children in CHAMACOS cohort,	Take home pesticide exposure	Urine (n=1192)	Most participants had at least one DAP detected with a detection frequency of over 90%. DMAP metabolites levels were three-folds higher at 24 months and two folds higher at 12 months compared to 6 months

		aged 6,12 and 24 months			participants. DMTP was the most detected DMAP metabolite (92.1%) and DETP was most detected DEAP metabolite (72.1%). Proximity of home to agricultural fields and farmworkers' occupational status were related to children's pesticide exposures.
Tamaro et al. (47)	Six DAP metabolites	**Location:** Washington, USA **Period:** April 2005 to February 2006 **Population Sample:** 100 farmworkers and 100 non-farmworkers and their children ages 2 to 6 years	Take home pesticide exposure: house dust and vehicle dust	Urine, saliva and blood (n=119 urine samples)	DMAP geometric mean in children was higher than concentrations of DEP and DETP combined. Farmworker children had eight-fold higher molar concentrations than DEP and DEPT during thinning season and twenty-two-fold higher DMAP than DEP and DEPT during harvesting season. Farmworker children presented two-fold higher DMAP concentrations than non-farmworker children during thinning season. Higher concentrations of organophosphates were observed from farmworker house and vehicle dust compared to non-farmworker vehicle and house dust.
Holme et al. (48)	Six dialkyl phosphates metabolites	**Location:** Washington State, USA **Period:** June 2005 to February 2006 **Study participants:** 100 farmworkers children and 100 non-farmworker children, ages 1- 7 years	Diet (fruits, vegetables and apple juice consumption)	Urine samples (n=555)	Among farmworker's children, median DMAP levels ranged between 0.077nmol/ml in the non -spray season to 0.205nmol/ml in spraying season (thinning and harvest seasons). Among non-farmworker's children, median DMAP levels were lower (0.095nmol/ml) in thinning season, 0.107nmol/ml in non-spray season and slightly higher (0.197nmol/ml) in harvest season. DMAP levels were high during harvesting season in both farmworker and non-farmworker children. There was a significant positive association between fruit and vegetables consumption, and DMAP and DEAP levels.
Bradman et al. (49)	Six urinary DAP metabolites and pyrethroid metabolites	**Location:** California, USA **Period:** 2006 **Study participants:** 40 Mexican American children, 3-6 years 20 children residing in urban community 20 children from agricultural community	Organic diet and conventional non-organic diet	Urine samples	Organic diet was significantly associated with reduction of urinary concentrations of DAPs. Detection frequency for all DAPs was greater than 50%. Geometric means of DMs (105.4) and DAPs (149.6) reduced to DMs (54.1) and DAPs (90.2) after /during consumption of organic diet. Among all children,
Ye M et al. (44)	Six DAP metabolites and pyrethroid metabolites	**Location:** Canada **Study participants:** 5604 participants aged 6-79 years Stratified: 6-11 years, 12-19 years and 20-79 years	Diet	Urine samples	Total DAPs concentration was 1.43 times (95%CI: 1.26-1.61) among participants with high fruit consumption (3rd tertile) compared to those that consume less fruits (1st tertile). Total DAPs concentrations were 1.33 (95%CI: 1.16, 1.52) times more in the group that consumed more vegetables compared to those that consumed less. Children aged 6-11 years had higher geometric mean (152.03nmol/g, 95% CI 135.65, 170.40) of DAPs than adolescents (83.81nmol/g, 95% CI 72.53,96.85) and adults (90.49nmol/g, 95% CI 81.11,100.95).

2.3.1 Exposure from take home pesticides

Pesticides can be tracked from work places on parents' work clothes, shoes, skin or hair to their residential places (16). Para occupational pesticide exposure can be perpetuated by absence of wash rooms at work and/ or absence of personal protective equipment (PPE) (10, 15, 16). Mixing or laundering children's clothes with contaminated parents' work clothes can also lead to dermal pesticide exposure to children (17).

Three studies that examined the relationship between parental occupation and children's urinary OP metabolites concentrations have found significant association between the two factors. The USA study found that farmworkers' children (16.5µg/L, 95 % CI: 9.5, 28.4) had higher mean DMTP levels than non- farmworkers' children (7.5µg/L, 95% CI:5.9,9.5) (12). Similarly, another study in USA also found farmworker children presenting two-fold higher DMAP concentrations than non-farmworker children ($p<0.001$) (47). This relationship was also observed by Thompson et al. that found 198nmole/L of DM metabolites in farmworkers' children compared to 75nmole/L found in non-farmworkers' children (50).

2.3.2 Pesticide exposure from residential pesticide application

Pesticide application in household interiors, on family lawns, family gardens and pets increase children's pesticide exposures (9, 16, 51). The Canadian study found higher Pyrethroid metabolites (3.94nmol/g creatinine) in Canadian people (5604 people) that lived in households that use pesticides in their households or on their lawns and 3.40nmol/g creatinine in those that lived in households that do not use pesticides (44). Additionally, frequency of pesticide application and methods of application also determines pesticide exposures and urinary OP metabolites levels in children (9, 52). For instance, spraying of pesticides directly on crawling pests can increase pesticide exposures to individuals living in such households versus fumigating the whole house (52).

2.3.3 Pesticide exposure from dietary intake

Organophosphates can be found in food as; most fruits and vegetables are treated with pesticides. In Canada, people who consumed more fruits and vegetables had total urinary DAPs concentrations 1.33 (95%CI: 1.16, 1.52) times higher compared to those who consumed less (44). Among these participants, children's (6–11 years) urinary DAP geometric mean (152.03nmol/g, 95% CI 135.65, 170.40) was higher than of adolescents (83.31nmol/g) and adults (90.49nmol/g) as 6-11 years children consumed more fruits than adolescents and adults (44).

Moreover, another study observed reduction of urinary organophosphates metabolites when conventional food was replaced with organic food (49). Bradman et al. observed reduction of DAP metabolites among California children after organic food was introduced to their diet. Geometric means of DMs and total DAPs decreased from 105.4 and 149.6 ng/mL to 54.1 and 90.2 ng/mL, respectively after /during consumption of organic diet of all children (49).

2.4 Aim and objectives

Aim

This study aims at determining the relationship between reported pesticide exposures, socio-demographic factors and urinary levels of organophosphate (OP) metabolites among boys living in the rural areas of Western Cape, South Africa.

Objectives

1. To determine the urinary levels of OP metabolites among participating boys from Hex River Valley, Grabouw and Piketberg
2. To determine reported recent and short-term pesticide exposures among participating boys from Hex River Valley, Grabouw and Piketberg
3. To identify socio-economic, demographic factors and other explanatory factors of pesticide exposure among participants.
4. To determine the relationship between urinary levels of organophosphates metabolites and self-reported pesticide exposure, socio-economic factors, demographic factors and other explanatory factors of pesticide exposures of participating boys.

2.5 Justification

There is growing concern that pesticide (organophosphates, carbamates, organochlorines and pyrethroids) exposures among children are associated with adverse health effects such as inhibition of brain acetylcholinesterase and impaired neurodevelopmental outcomes including poor working memory and verbal comprehension in children (33). In addition, most affected children are those in low income or developing countries, where there is already overburden of infectious and non-communicable diseases. Overburden of diseases result in increased child mortality rates, hence a need for health promotion interventions to intervene. So, this study seeks to investigate relationship between reported pesticide exposures and organophosphate metabolites in children’s urine samples and propose effective interventions to reduce pesticide related morbidity and mortality rates.

3. Methods and Materials

3.1 Study Design

This was an analysis of data collected during a cross-sectional study of 183 boys conducted between April 2007 and March 2008 in three agricultural intensive areas in Western Cape, South Africa. The main study investigated the effects of agricultural pesticides exposure on growth and pubertal development of boys and adolescents in the rural Western Cape region of South Africa (20, 21). This analysis investigated the relationship between urinary levels of pesticides, self-reported pesticide exposures and socio-demographic factors.

3.2 Study Setting

The study was conducted on farm and non-farm school-going boys from the Hex River Valley, Grabouw and Piketberg where pesticides (chlorpyrifos, azinphos-methyl and prothiofos and other non- organophosphate pesticides) were detected in environmental media in a previous study (28). Grape, apple and wheat and fruit farming are intensive respectively in Hex River Valley, Grabouw and Piketberg (20).

3.3 Study Population and sampling

During the main study, 269 boys aged 5 to 19 years from primary and secondary schools (n=8) were recruited from the three study areas. Farm boys (boys residing on farms or around farms) living in Hex River Valley, Piketberg and Grabouw and non- farm boys (those living in within one to three kilometres away from farms) were targeted for the study. The sampling frame was the list of primary and secondary schools provided by the Western Cape Department of Education. The most accessible primary and secondary schools with students living in farms and neighbouring farm communities were selected for the study. Prior commencement of the study, school principals were approached by the main investigator for their approval to execute the study in their schools. Parents were also consulted, by means of letters distributed at schools to provide provisional written permission for their children to be recruited in the study.

Among boys whose parents gave permission (n = 492), all 94 boys not living on a farm and 180 boys (60 in each area) out of 398 living on farms were recruited (21). They were then stratified according to their residential area and age (5 to 9 years (prepubertal), >9 to 11 years (early puberty), >11 to 14 years (mid-late puberty) and greater than 14 years (post-puberty).

At schools where the number of consenting boys living on farms exceeded the number to be included in the study, a systematic random selection was conducted. Selected boys and their parents (n = 274) were invited to participate in the study on specified dates. Five participants were excluded from the study because their parents or guardians did not participate and eventually remaining 269 boys were included in the main study but only urine samples of 183 boys (Appendix A) were available in the current analysis (21).

3.4 Study Instruments

3.4.1 Questionnaires

Children's guardians (children's care givers) were interviewed face to face by trained interviewers in their preferred language. Interviewers captured responses onto electronic questionnaires preloaded on mobile phones using mobile technology (Mobile Researcher and /or Clyral). Captured responses were then transferred to the central website through the internet, where they were easily accessed by the principal investigator. Stata 13 [Stata Corporation, Texas, USA] and Microsoft Excel® were then used for further data management and analysis.

Questionnaires that were translated into participants' preferred language (either Xhosa or Afrikaans), were then back translated into English during data capturing. Questionnaires (Appendix B) had among others, sections on socio-demographic factors and pesticide exposures. Pesticide exposure questions included: domestic use of pesticides including frequency of use; guardians' occupation; take home exposures and the and the participant's involvement in agricultural and pesticide application activities.

Other environmental pesticide exposures questions included place of residency, proximity of home to pesticide spraying, and mode of pesticide application practiced in farms. Domestic water sources, use of empty pesticide containers and pesticide exposure through diet (vegetables, soya and meat intake) were also measured. Questionnaires were administered only to guardians. Questions on socio-demographic factors included parents' education and marital status; family income, age and medical history.

3.4.2 Urinary levels of organophosphate pesticides

Spot urine samples of boys were collected by the field nurse from April to May 2008. Only one sample was collected per child. Colourless 50mL plastic urine containers sealed with plastic caps were used for urine sample collections. Samples were then stored in dry ice and transported to School of Public Health and Family Medicine, where they were stored at -20 ºC before being analysed by the Clinical Pharmacology Laboratory, UCT. Only three metabolites; Diethyl phosphate (DEP), Dimethyl thiophosphate (DMTP) and Dimethyl phosphate (DMP) were detected. Urinary DAP metabolites were not adjusted for creatinine or specific gravity as these methods were not established by the collaborating laboratory that conducted the analysis.

Analysis of the three urinary metabolites were done with a validated liquid chromatography tandem mass spectrometry assay developed at the Clinical Pharmacology Laboratory at UCT. Samples were then processed with a protein precipitation extraction method using Isoniazid-d4 and Acetyl Isoniazid-d4 as internal standards, followed by high performance liquid chromatography with MS/MS detection using an AB SCIEX API 3000 instrument as described in detail by Monate (20). The level of detection for all three urinary metabolites was 0.78ng/mL (20).

3.5. Data Analysis

3.5.1 Data management and quality assurance

Mobile technology (Mobile Researcher and /or Clyral) was used for data capturing. The technology enabled quick sharing and access of study data, so that immediate actions could be taken in case any queries were raised after data was shared through central web. This ensured that interviewers obtained all relevant information before leaving the study venue and reduced missing data. Collected information was also compared to generated checklist on study envelopes to ensure all significant information was collected. Data were then cleaned using STATA.

3.5.2 Statistical Analysis

The dataset was explored for any missing or incorrect data entry. Data was explored using frequency tables. Continuous exposure variables, like income were categorized using tertiles, medians or quartiles. OP pesticide metabolites were then tested for normality using box and whisker plot. Log transformation of data not normally distributed did not improve normality and therefore untransformed data are represented. Bivariate analyses were conducted to explore relationship between urinary DAP metabolites and potential exposure factors (like age, socio-economic status and residential places). Tests used in bivariate analyses included Wilcoxon sum rank test, Fisher's exact test (where frequency was <5 or the assumption of a large sample size was not met) and simple linear regression.

Relevant OP exposure factors to be included in multivariable analysis were identified based on bivariate testing (where associations with a significance of $p \leq 0.1$ were found), non-co-linearity and through iterative model building. Sensitivity analysis was then conducted by running the model with only fam boys.

The results of the sum of three DAP metabolites are represented as they are similar and representatives of the combined results of the three individual metabolites (DEP, DMP and DMTP) which are reported individually in appendix F, table 10.

3.6 Potential biases, reliability and validity of instruments

To minimize measurement, selection and confounding bias, trained staff piloted measurements to ensure reliability, and questionnaires collected information on all potential confounders. In addition, questions were kept simple and easy to understand to reduce recall and reporting biases.

3.7 Pilot Study

Study questionnaires were distributed randomly to pilot study participants at the UCT School of Public Health and Family Medicine to determine flaws in the structure of questionnaires. A pilot study was also conducted on three boys from the study areas before the main study commenced.

4. Ethics

This thesis will be conducted in accordance with the University of Cape Town Human Research Ethics committee.

4.1 Ethics Approval

The main study was approved by University of Cape Town Human Research Ethics Committee in 2005, REC REF: 279/2005 (Appendix C) and Department of Education prior commencement.

4.2 Consent

Department of Education gave permission for the study to be conducted. School principals and School boards also gave permission for the study to be carried within their leadership territory. After approval by school leaders, guardians/ parents were also asked for their consent to participate (Appendix E) and thereafter visited schools for interviews. Study consent forms were in English, Xhosa and Afrikaans and guardians were given an opportunity to select consent forms with their preferred languages. Interviewer, in the presence of witnesses, interpreted forms to people that were illiterate. Guardians either used their personal signatures or thumb print to sign off consent forms. Guardians were permitted to withdraw at any point as the consent form had stated and interviewers ensured that participants fully understand the consent form before signing a form.

4.3 Respect for persons

All collected information was only accessible to the research team and confidentiality of participant was reserved. In a case that medical concerns were pin pointed during the study, such people were referred to health facilities.

4.4 Privacy

Interviews were carried in enclosed spaces where participants felt comfortable. Only the interviewer, participant and parent/guardians were allowed in the interviewing room.

4.5 Confidentiality

Only participants' initials were used for triangulation of data and all information was held in strict confidentiality records of the University of Cape Town. All files were locked in filling cabinets. For this research, only summary data will be used for public presentations and confidentiality will be maintained through execution of the study by proper handling of soft records and unique coding of data.

4.6 Beneficence

No financial incentives were awarded to participants. Data will contribute towards implementation strategies and, health and environmental policies.

4.7 Non- maleficence

The study had minimal harm to the participants. Only prick needles were used for blood collection.

4.8 Envisioned outcomes

This study will:

- Predict future pesticide exposures of children.
- Generate recommendations for policies on interventions that can reduce children's pesticide exposure.
- Ensure future training of rural farming communities on safe pesticide use, handling and storage.

4.9 Communication Strategy

- This study will be published, and a final report will be made available to the University of Cape Town and University stakeholders.

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PART B: STRUCTURED LITERATURE REVIEW

1.Introduction

1.1 Background

Organophosphates (OPs) were first manufactured in the 1930's by the reaction of alcohols and phosphoric acid (1). They were initially used as insecticides, but the German Military used them as weapons (neurotoxins) during second World War 1939-1941(2). The use of organophosphates pesticides became more popular in the 1980's after some organochlorines were banned due to their persistence in the environment and are still currently widely used worldwide (3). Currently, OPs are mainly used for agricultural purposes to control pests and for public health protection against vector borne diseases like Malaria (2).

Existing data show an increase in the use of OP pesticides regardless of their toxic effects to humans, the environment and animals. From 1940 to 2001, 2.26 million tons of pesticides have been reported to be used (4) and by the year 2009, approximately 2.8 million tons pesticides were used globally (5). About 25% of pesticides are used in developing countries (5) and, South Africa is the largest user in Sub- Saharan Africa (6-8). In 2010, the South African Department of agriculture, fisheries and forestry reported that South Africa was importing nearly 3000 excess pesticides to adhere to changing trade standards and meet yearly food demands (9). These pesticides include acephate, azinphos-methyl, chlorpyrifos, phosmet, malathion, parathion and diazinon, which are classified as organophosphates (10).

Organophosphate pesticides exposure is associated with several health effects including neurotoxic effects, cancer, asthma and reproductive health effects (8, 11-14). Some OP pesticides have been identified as endocrine disrupting chemicals, due to their ability to interfere with the endocrine system by either mimicking hormones or altering production of hormones (15, 16). Commonly used endocrine disrupting OP pesticides include pirimiphos-methyl which is an anti-androgenic and chlorpyrifos which is a weakly estrogenic chemical (17-19). Health effects associated with endocrine disrupting properties of OP pesticides include altered male and female reproduction, asthma and poor neurobehavioral outcomes (11, 12, 20). Human beings get exposed to pesticides through ingestion, dermal contact, inhalation and / eye contact (21-24). Most affected populations include those living or working on farms or near farms on which OP pesticides are applied (24-26). Among all exposed persons, children and pregnant women are the most at risk due to their vulnerable physiological systems (22, 27, 28).

In order to investigate adverse health effects due to pesticide exposure, it is important to understand which factors predict pesticide exposure in different study populations. Hence, this study seeks to understand the relationship between urinary levels of pesticide residues, socio-economic, demographic factors and reported pesticide exposures in the Western Cape, one of the most agricultural intense provinces in South Africa.

1.2 Objective of the literature Review

This literature review will give an overview of organophosphate pesticide use in the Western Cape; describe the presence of OP pesticides in the environment and in rural residents especially children; describe organophosphorus pesticides toxicity and review previous studies that have investigated factors determining OP pesticide absorption into the body.

1.3 Search Strategy

Electronic sources were used for this literature review. PubMed and Google scholar were used as main search engine. Additional databases used were Scopus and Medline. Search terms for the general review included organophosphate pesticides, pesticide usage, pesticide use in South Africa, pesticide exposure and children, OP pesticide toxicity, OP pesticides in the environment, organophosphorus pesticides and health effects, dialkyl phosphates metabolites, dimethyl phosphate (DMP), dimethyl thiophosphate (DMTP), dimethyl dithiophosphate (DMDTP), diethyl phosphate (DEP), diethyl thiophosphate (DETP) and diethyl dithiophosphate (DEDTP). Search terms used for literature review of previous studies were urinary organophosphate pesticide metabolites, pesticide biomonitoring and children, take home pesticides and dietary pesticide exposures. Reviews included publications in English and focused on both laboratory, epidemiological and systematic reviews. Non-published sources, such as dissertations were also used to obtain additional information. Reference lists from both published and non- published sources were used to search for additional articles.

2. Literature Review

2.1 Organophosphates Pesticide use

Pesticides are widely used in the world to increase food production. In 2010, approximately 2 million tonnes of pesticides were used worldwide, with China the major contributing country, followed by the USA (29). In Africa, approximately 4% of global market share of pesticides were used, even though most people depend on farming for their living (29). One of the major pesticide users in Sub- Saharan Africa is South Africa (9).

Agricultural sector accounts for approximately 2.2 % of South Africa's economy (30). To increase produce, profits and to keep up with changing trade standards, most farmers use pesticides intensively (31). Pesticide use in agricultural sector has continuously increased with years (32). For instance, pesticides use increased from 4623 tons, 7495 tons to 20947 tons in 1994, 1999 and 2009 respectively (6, 33).

The Western Cape is one of the most agricultural intense provinces in South Africa (7, 9, 34) and one of the highest users of OP pesticides. Grapes, pome fruits and wheat farming are leading farming sectors in this province and OP pesticides such as chlorpyrifos, azinphos-methyl and prothiofos are commonly used to control for weeds and unwanted pests (35). Residues of these pesticides have also been detected in food samples (local and imported wheat samples) (34, 36), surface water, groundwater and soil samples (7, 37).

Pesticides previously detected in drinking water especially from boreholes in the rural Western Cape raise a public health concern regarding health of exposed persons especially farm workers, farmers and people residing on farms or people residing in areas neighbouring farms in the Western Cape (38). Pesticide residues can also be transported to residential places through pesticide spray drifts and expose children and other residents to unwanted pesticide exposure.

2.2 Toxicity of organophosphate compounds and absorption into the human body

Organophosphates are used in agriculture to control for insects as they are neurotoxic (39). In humans, OP neurotoxicity occurs through inhibition of the enzyme acetylcholinesterase, which breaks down the neurotransmitter acetylcholine (40), leading to its accumulation at synaptic clefts and continued neural activity which can cause increased blood pressure, cardiac rhythm, muscle contractions and ultimately death (40). Short term health effects of organophosphates can range from skin and eye irritation, headaches, dizziness, nausea to acute pesticide poisoning (13, 41). Long term health effects include asthma, diabetes, cancer and Guillain-Barre Syndrome (13, 42-45)

Organophosphate pesticides, like other pesticides, are commonly absorbed through the skin, but can also enter human body through inhalation, eye contact and ingestion (21-24). Skin contact can occur when cleaning empty pesticide containers for re-use, pesticide spraying without using personal protective clothes (PPE) or swimming in pesticide contaminated dams or rivers (46). Exposure via ingestion can happen through consumption of contaminated fresh vegetables and fruits from farms, using pesticide contaminated water to wash fruits or crops before consumption or drinking contaminated water (47). Exposure through inhalation can occur when exposed to airborne spray drifts (48).

Once in the body, organophosphates are broken down into various metabolites including specific metabolites and one or more of six non-specific dialkyl phosphates (DAPs), dimethyl phosphate (DMP), dimethyl thiophosphate (DMTP), dimethyl dithiophosphate (DMDTP), diethyl phosphate (DEP), diethyl thiophosphate (DETP) and diethyl dithiophosphate (DEDTP) (22, 25, 26, 49). These metabolites are then excreted in urine (47).

There are several factors influencing pesticide toxicity in human. Usage of large quantities of pesticides, frequency, mode of application and route of exposure determines the presence and toxicity of OP pesticides on the environment and therefore exposure of human beings (48). The chemical properties of pesticides such as their ability to bioaccumulate, their persistent in the environment, water solubility, volatility, photolysis and hydrolysis are additional factors that determine the presence of pesticides in the environment (50). Table 1 present chemical properties and acute health effects of organophosphate pesticides commonly used in South Africa. Table 1 shows that although these pesticides are of low acute toxicity, they can all bioaccumulate, may persist in the environment and are associated with various health effects. Apart from physicochemical properties of pesticides, an exposed individual’s immune system also determines toxicity of pesticides in that individual’s body, hence children, with underdeveloped immune system, are more susceptible to environmental hazards than adults (22)

Table 1: Chemical properties of organophosphates that determine their fate and toxicity (50-52).

Pesticide	Chemical formula	Specific and non- specific metabolites	Chemical Properties	Acute toxicity classification	Human health effects
Malathion	$C_{10}H_{19}O_6PS_2$	• Malathion monocarboxylic acid • Malathion dicarboxylic acid • Iso malathion • Malaoxon • DMP, DMTP, DMDTP	• Moderately soluble in water • Moderately bio accumulative (Octanol-water partition coefficient at pH 7, 20^{o}C- Log P 2.75) • Low volatility • Stable aqueous photolysis at pH 7 • Not persistent to aqueous Hydrolysis at 20 ^{o}C, pH 7	Class III	Possible adrenal gland, neurotoxicant, thyroid and liver toxicant. Acetylcholinesterase inhibition Inhibit catecholamine secretion
Parathion	$C_{10}H_{14}NO_5PS$	• Para nitrophenol • DEP, DETP, DEDTP • DMP, DMTP, DMDTP	• Less soluble in water • Bio accumulative (Octanol-water partition coefficient at pH 7, 20^{o}C- Log P 3.83) • Low volatility • Stable aqueous photolysis at pH 7 • Persistent to aqueous Hydrolysis at 20 ^{o}C, pH 7	Class III	Mutagenic; eye, skin and respiratory irritant; highly toxic to reproduction and development systems
Azinphos-methyl	$C_{10}H_{12}N_3O_3PS_2$	• Cysteinylmethyl benzazimide sulfone • Methylsulfonylmethyl benzazimide • Desmethyl isoazinphos-methyl • DM, DMTP, DMDTP	• Low solubility in water • Moderately bio accumulative (Octanol-water partition coefficient at pH 7, 20^{o}C- Log P 2.96) • Moderately fast aqueous photolysis at pH7 • Moderately persistent to aqueous Hydrolysis at 20 ^{o}C, pH 7	Class III	Neurotoxicant and cholinesterase inhibitor
Prothiofos	$C_{11}H_{15}Cl_2O_2PS_2$	• 4-chloroprothiofos • Etaphos • 2,4-dichlorophenol	• Less soluble in water • Highly bio accumulative (Octanol-water partition coefficient at pH 7, 20^{o}C- Log P 5.67) • Moderately volatile • Fast aqueous photolysis at pH 7	Class III	Neurotoxicant; cholinesterase inhibitor; skin and respiratory tract irritant

			• Stable to aqueous Hydrolysis at 20 °C, pH 7		
Chlorpyrifos	$C_9H_{11}Cl_3NO_3PS$	• 3,5,6- trichloro-2-pyridinol • 2,3,5- trichloro-6-methoxypyridine • 3,5,6-trichloro-2-pyridyl • DEP, DETP, DEDTP	• Low aqueous solubility • Highly bio accumulative (Octanol-water partition coefficient at pH 7, 20°C- Log P 4.7) • Moderately volatile • Slow aqueous photolysis at pH7 • Moderately persistent to aqueous Hydrolysis at 20 °C, pH 7	Class III	Neurotoxicant, cholinesterase inhibitor, reproductive developmental effects, highly toxic by ingestion

2.3 Pesticide exposure among children

Children are more vulnerable to hazardous environmental exposures than adults because of their daily behaviour, diet, activities, underdeveloped body system and detoxification mechanisms, that are still going through physiological development (22). Their proximity to the ground, frequent engagement with the ground and soil, where pesticide residues normally settle and their hand to mouth activity also increase their environmental risks (22, 27). Furthermore, they consume more food (predominately fruits, vegetables and water) per their unit weight compared to adults' food consumption per unit weight (22, 27). They can be exposed to organophosphates through dermal contact with soiled articles, ingestion, inhalation and eye contact. After entry into body system, organophosphates metabolites can be detected in urine (47). Additional factors that can increase children's pesticide exposure include demographic and socio-economic factors (age, gender, care- giver's education level, occupation and household income), season, residential pesticide application; residential proximity to agricultural fields, spray drifts and run-offs (47, 53-56).

2.4 Non- Systematic review of studies on the relationship between urinary levels of OP pesticide residues and socio-demographic and reported pesticide exposures in children

Table 2 summarises the epidemiological and environmental studies (2010-2019) that have examined determinants of urinary levels of OP pesticide metabolites in children. Sixteen studies were reviewed, including nine studies from USA, two studies from Chile and one, each from Canada, India, Thailand and Italy. In addition, one multi-national study that looked at six countries in Europe was also reviewed.

Factors associated with urinary levels of OP pesticides are summarised below.

2.4.1 Demographic, socio-economic and environmental factors that can contribute to children's pesticide exposure

Age

There were ten studies that investigated relationship between age and organophosphate pesticide biomonitoring (26, 47, 53, 54, 56-61). Seven of those found an association between age and OP pesticide metabolites (26, 47, 53, 54, 56, 59, 60), while others did not find any association (57, 58, 61).

Four studies found higher levels of OP metabolites associated with younger children compared to older children (26, 53, 59, 60). Ye et al. found higher DAP urinary concentrations (geometric mean = 152.03 nmol/g) among 6- 11 years old children compared to (83.81 nmol/g) of 12-19 years old children in Canada (53). Similarly, Muñoz-Quezada M et al. found higher diethylakylphosphate (DEAP) urine concentrations among younger children than older children (p = 0.03) and higher urinary parathion metabolites (Para nitrophenol, PNP) per one year decrease in age (β = -0.06, p = 0.035) in Chilean children with age range of 5-13 years (60). However, there was no significant association of 3,5,6-trichloro-2-pyridinol (TCPy), Dimethyl Alkyl Phosphates (DMAPs) with age (60). Higher urinary OP metabolites among younger children could be attributable to younger children spending more time on the floor, eating food from floor and having less developed physiological mechanisms to clear pesticides than older children (21).

Three studies observed increasing OP metabolites with increase in age. Sinha and Banda found higher DAP metabolites amongst 11-12 years old females (median = 2.69µmol/L) than in 6-10 years old females (median = 2.13µmol/L) in Hyderabad, India (47). Likewise, in California, USA, Bradman et al. found three-fold higher dimethyl alkyl phosphates (DMAP) metabolites in 24 months and two-fold DMAP metabolites in 12 months compared to six months old children (56). High DAP and DMAP concentrations among older children was associated with diet (47, 56). Older children were found to be consuming more fruits and vegetables than younger children (47). Additionally, in Washington State, USA, Coronado et al. found significantly higher phosmet levels in older people (35 years and older) than youth (less than 25 years) (54). Although most studies that investigated association between urinary OP metabolites and age found a significant association, Holme et al. and Thompson et al. studies in Washington State, USA and Rohitrattana et al. study in Thailand did not observe any association between the two (57, 58, 61).

Child Sex

The literature review provided some evidence that gender might be a factor determining urinary levels of organophosphate metabolites in exposed children. Four out of ten studies that investigated association between urinary organophosphate metabolites and gender, found an association between the two factors (47, 53, 56, 60). In India, Sinha and Banda found that girls in 6-10 years (median = 2.13µmol/L) and 11-15 years (median = 2.69µmol/L) old groups had higher DAP urinary metabolites than 6-10 (median = 1.9μmol/L) and 11-15 (median = 1.85μmol/L) years old boys (47). Higher DAP urinary metabolites in girls was due to more fruits that girls consumed per day compared to boy's fruits consumption (47). Likewise, Ye et al. observed that Canadian females had higher DAP (geometric mean = 112.86nmol/g creatinine) than males (76.86nmol/g creatinine) and this was associated with higher consumption of vegetables in females compared to males as well (53). Bradman et al. also observed significantly higher DMAP levels in six months old females compared to boys of the same age group (geometric mean = 22nmol/L vs 15nmol/L, $p<0.05$) and this was positively associated with differences of fruits and vegetables intake by the two groups (56).

As for Chilean children, Muñoz-Quezada et al. found that boys had higher DEAP urine concentrations than girls ($p = 0.01$) and this was also attributed to fruits consumption at school (60). Other six studies that did not find association between gender and urinary OP metabolites are Holme et al., Thompson et al., Coronado et al., Hyland et al., Muñoz-Quezada et al. and Rohitrattana et al. (26, 54, 57-59, 61) .

Parents' Education

The literature review provided little evidence that parental education can affect urinary levels of organophosphate metabolites in children. Only one study out of five studies found an association between OP metabolites and education, and this was an unexpected positive association between parents' educational level and pirimiphos metabolite (2- diethylamino-6-methylpyrimidin-4-0l, DEAMPY) urinary concentration ($p<0.05$) (55). A possible explanation provided by the authors was that highly educated parents (those that attended university) were able to afford and provide their children with fruits and vegetables, which may have pesticide residues on them (55, 62).

Parental Training

Three studies in the review investigated the effect of pesticide training on urinary OP metabolite levels (60, 63, 64). Griffith et al. reported lower child to adult ratio of urinary DMTP concentrations post the pesticide education intervention compared to pre- intervention concentrations in Washington, USA (63). This study reported a 2.7 fold organophosphate metabolites decrease in intervention group versus 1.7 fold decrease in control group (63). A decrease in both intervention and control groups could indicate differences in seasonal use of OP pesticides (63). Baseline samples for both groups could have been collected during the thinning season when OP pesticides were highly applied, and farmworkers spent more time in farms and post intervention samples during the season when farmworkers spent less time in fields (63). Salvatore et al. in California, USA also found slightly lower DAP geometric mean of 73.3nmol/L in children after the pesticide handling education and training program compared to 84.6nmol/L that was detected at baseline (64). Even though Muñoz- Quezada et al. did not find any association between training and urinary OP metabolites, they observed an increase in awareness and knowledge of pesticide risk among 26 children and 26 parents of Chile, after an educational intervention (60).

Household Income

One study in USA (Washington State) found children from low-income household to have higher urinary levels of OP metabolites compared to children from higher income households. Coronado et al. found higher urinary azinphos-methyl and phosmet levels in children from low income households compared to those from high income households (54). High urinary OP metabolite concentrations observed among low income families could be associated not only with poor housing conditions, high residential pesticide use or pests' infestation but also with inability of low income households to afford organic foods compared to middle- or high-income households (62, 65).

Parental occupation

Parental occupation can introduce take home pesticide exposures to children living in the same households with farm workers (26). Para occupational pesticide exposure can be worsened by lack of wash rooms at work for workers to change work clothes and/ or lack of access to personal protective equipment (PPE) (24-26). Pesticides residues are then tracked from work to residential places on clothes, shoes, skin and hair (26). Six studies examined the relationship between parental occupation and urinary OP metabolites (57, 58, 66, 67), including Coronado et al. that observed higher mean DMTP levels in farmworkers' children (16.5µg/L, 95 % CI: 9.5, 28.4) than non- farm worker' children (7.5µg/L, 95% CI:5.9,9.5) in Washington State, USA (54). Similarly, Rohitrattana et al. found increasing TCPy ($p = 0.02$), DEP (0.003) and

DETP (p = 0.002) levels among children of rice farm workers (farms in which pesticides were applied frequently) in Pathum Thani Province, Thailand, compared to those whose parents worked in aquaculture (61). Moreover, Holme et al. (mean = 0.205nmol/mL versus 0.107nmol/mL), Tamaro et al. (two-fold higher DMAP concentrations in farmworkers' children versus non-farmworkers' children) and Thompson at al. (median = 198nmol/L versus 75nmol/L) also observed higher levels of dimethyl metabolites in farmworkers' children versus non- farmworkers' children (57, 58, 66).

Season

Season of the year plays a vital role in pesticide exposure as agricultural pesticides use is seasonal. All five studies that investigated the effects of season on pesticide exposures observed high urinary pesticide metabolites and household pesticides residues during thinning and harvesting season (spraying season) compared to non- spray season (56-58, 66, 68). Holme et al. observed DMAP median of 0.077nmol/ml in non- spray season and 0.205nmol/ml in both thinning and harvesting season in Washington State USA (57). Still in Washington State, USA, Thompson et al. observed higher DM urinary concentrations in farmworker's children (median = 198nmol/L) than non-farmworker's children (median = 75nmol/L) during the thinning season (spray season) but during the non- spray season, their urinary concentrations were almost equal among farm and non-farmworker's children (58). Bradman et al. also observed higher DMAP urinary levels in children during spraying season (geometric mean = 25nmol/L) compared to non- spray season (geometric mean = 13nmol/L) in California, USA (56). Difference between urinary OP metabolites across the year suggest that season of the year plays a vital role in OP pesticide exposures.

2.4.2 Pesticide exposure from residential pesticide application

Pesticides used on lawns, family vegetable gardens, pets and in household interiors can expose children to high levels of OP pesticide residues (26). Two studies, one in Canada and the other in Chile, found higher levels of OP metabolites among children whose households used pesticides than those living in households that do not use pesticides. Ye et al. found 3.94nmol/g creatinine of Pyrethroid in Canadian people (5604 people) that lived in households that use pesticides either within households or lawns and 3.40nmol/g creatinine in those that lived in families that do not use pesticides (53). In addition, the Muñoz-Quezada et al. study (190 children) in Chile, found that children in houses treated with fenitrothion had urinary DMAP levels 3.5 times higher compared to those living in non- treated houses (59). Both studies provide evidence that urinary levels of pesticides can be dependent on household practices and types of pesticide used in homes.

2.4.3 Proximity of home to pesticide spraying

Pesticide drift or volatilization is an additional source of pesticide exposure for people residing near farms or on farms (48). Five studies maintain that children living near agricultural fields are at a greater risk of high pesticide exposures than those that live far from fields or in cities(54, 56, 59-61). Bradman et al. observed higher urinary levels of DMAP and DEAP metabolites in 12 months old American children living within 60 meters from the farm compared to those living more than 60 meters from the nearest farm in Salinas, California (56). Children that lived within 60 meters from the agricultural farm had 60nmol/L DMAP urinary metabolites while those that lived beyond 60 meters from the farm had 25nmol/L (56). As for DEAP, those living within 60 meters from the farm had 107% higher DEAP metabolites compared to their counterparts (56). Likewise, Washington State study by Coronado et al. observed 20% overall DMTP concentration reduction with every mile distance increase from agricultural fields (54). Two studies, one in Chile (p=0.013) and the other in Thailand (p=0.03) observed a significantly higher TCPy urinary levels among children that lived close to the farms compared to those that did not live on farms or lived far from farms (60, 61). Additionally, another study in Chile found that children that lived less than 500 meters from the farm were 2.5 times more likely to have higher urinary DMAP levels than children that lived more than 500 meters from the farms (59).

Though there is strong evidence on association between proximity of residential places and high urinary OP levels, Bradman et al. and Thompson et al. did not find any association between the two factors (58, 65). However, Bradman et al. trust that children that live closer to farms would be more exposed to pesticides than children that live far from farms because of pesticide volatilization or pesticides drifts from farms (65).

2.4.4 Pesticide exposure from dietary intake

Organophosphates can be found in food, as, most fruits, vegetables and grains are treated with pesticides (47, 52). Ye et al. found that in Canada, people that consumed more fruits and vegetables had total urinary DAP concentrations 1.33 (95%CI: 1.16, 1.52) times more compared to those that consumed less (53). Similarly, Sinha and Banda found that in India, 11-15 years girls had higher DAPs metabolites levels than boys of the same age group. Girls presented median of 2.69µmol/L DAP urinary concentration while boys had median of 1.85µmol/L DAPs concentration (47). These was related to girls consuming more fruits (233.4g/day) than boys (214.2g/day) (47).

Moreover, two other studies observed a reduction of urinary organophosphate metabolites when conventional food was replaced with organic food. Bradman et al. observed reduction of DAP metabolites among California children after organic food was introduced to their diet. Geometric means of DM (105.4) and DAP (149.6) reduced to DM (54.1) and DAP (90.2) after /during consumption of organic diet by all children (65). Hyland et al. found that TCPy levels reduced from median of 2.99ng/mL to 1.77ng/mL and from median of 1.04ng/mL malathion dicarboxylic acid (MDA) to <0.02ng/mL after or during consumption of organic food compared to consumption of conventional food by children aged 4-15 years in USA (52). Overall, reduction of -69.5 % (95% CI: -76.6%, -60.2%; p <0.01) of DAP, -83.9% (95% CI: -88.0%, -78.4%; p<0.01) DM and -26% (95% CI: -43.7%, -2.6%; p<0.01) diethylphosphate (DE) (52) was during consumption of organic food.

Table 2: Studies investigating the relationship between urinary levels of OP pesticide residues and demographic, socio-economic and reported pesticide exposures in children.

Study source	**Pesticides, metabolites analysed and biomarkers**	**Location, Population, age**	**Reported Pesticide Exposure**	**Reported Socio-demographic and environmental factors**	**Results**
Sinha S and Banda V (47)	Six Dialkylphosphates metabolites (DAPs) **Biomarker:** Urine (spot samples in the morning and 6-10 voids during the day)	**Location:** Hyderabad, India **Population:** 377 children aged 6 to 15 years old (188 boys and 189 girls)	Food (fruits, vegetables and conventional diets) and water	Body weight, age of children, parents' age, occupation, annual family income, length of stay at current residence, pesticide product name, household pesticide use	Dimethyl phosphate metabolites median in 6-10 years old girls was almost like that of boys, with 0.4µmol/L in girls and 0.55µmol/L for boys. DEs median was also almost the same for girls and boys, 0.97µmol/L for girls and 0.95µmol for boys. In 11-15 years, DMs for girls (0.7µmol/L) was slightly higher than of boys (0.59µmol/L) and DEs median was nearly the same for both girls and boys. DAPs metabolites levels in11- 15 years girls were higher than of boys at the same age group. This was related to more fruits that girls (2.69µmol/L) consume per day compared to boys (1.85µmol/L) p<0.001
Coronado et al. (54)	Six dialkylphosphates metabolites, Chlorpyrifos, Malathion, Diazinon,	**Location:** USA **Period:** 2005-2006	Take home pesticide exposure	Gender, occupation, household	Farmworker children (16.5µg/L, 95 % CI: 9.5, 28.4) had higher mean DMTP levels than non- farm worker children

	Parathion, AZM and Phosmet **Biomarker:** Urine (n=579 with three spot urine samples per child on each visit)	**Study sample:** 100 farmworkers' children and 100 non-farmworkers' children aged 2-6 years	(house dust samples n=109 and vehicle dust samples n=109)	income, marital status, type of household dwelling, number of children	(7.5µg/L, 95% CI:5.9,9.5). Median dimethyl concentrations were higher in farmworkers and their children during thinning and harvesting seasons and low during non-spray seasons. Pesticide concentrations were constantly higher in farmworkers house and vehicle dusts compared to non-farmworker's house and vehicle dust. Approximately 20 % pesticide metabolites concentration reduction in both children and guardians was observed with each mile distance from the nearest farm/ field. Azinphos-methyl residues were higher in farmworkers' household dust (643ng/g) than in non- farmworkers' household dust(121ng/g). Azinphos-methyl and phosmet residues levels were associated with income and birthplace.
Thompson et al. (58)	Azinophos-methyl, phosmet, diazinon, malathion, chlorpyrifos, six organophosphate metabolites. **Biomarker**: Urine (n=579 samples)	**Location:** USA **Period:** 2005-2006 **Study sample:** 100 farmworkers' children and 100 non-farmworkers' children aged 2-6 years	Vehicle and house dust	Eating behaviours, parents' occupation, child behavioural practices, family pesticide use, proximity of family to agricultural fields	Farm workers had high pesticide residues in both house dust and vehicle dust compared to that of non-farmworkers. Farmworkers' children had higher DMs (198nmole/L) than that of non- farmworkers' children (75nmole/L) during the thinning season. Non- farm workers households, children's DM metabolites increased to 191nmole/L during the harvest season. Azinphos-methyl, chlorpyrifos, phosmet and malathion were higher in farmworkers household and vehicle dust than residues detected in non-farmworkers households and vehicles.
Bradman et al. (56)	Six dialkylphosphates metabolites **Biomarker**: Urine (n=1192)	**Location:** USA **Period**: 1999-2003 **Study sample:** 417 children in CHAMACOS cohort, aged 6,12 and 24 months	Take home pesticide exposure	Age, sex, children's behaviour, diet, household pesticide use, work status and proximity of homes to fields	Most participants had at least one DAP detected with a detection frequency of over 90%. DMAP metabolites levels were three-folds higher at 24 months and two folds higher at 12 months compared to 6 months participants. DMTP was the commonly detected DMAP metabolite (92.1%) and DETP was most detected DEAP metabolite (72.1%). Proximity to agricultural fields and occupational status of farmworkers were associated with children's pesticide exposures.
Tamaro et al. (66)	Six DAPs metabolites, Azinphos-methyl, chlorpyrifos, phosmet, malathion and diazion. **Biomarker:** Urine (n=119 urine samples)	**Location:** Washington, USA **Period:** April 2005 to February 2006 **Population Sample:** 100 farmworkers and 100 non-farmworkers and their children ages 2 to 6 years	Take home pesticide exposure: house dust and vehicle dust	Parents' occupation, agricultural season and age	DMAP geometric mean in children was higher than concentrations of DEP and DETP combined. Farmworker children had eight-fold higher molar concentrations than DEP and DETP during thinning season and twenty-two-fold higher DMAP than DEP and DETP during harvesting season. Farmworker children presented two-fold higher DMAP concentrations than non-farmworker children during thinning season. Higher concentrations of Azinphos-methyl, phosmet and chlorpyrifos were observed from farmworker house and vehicle dust compared to non-farmworker vehicle and house dust.

Holme et al.(57)	Six Dialkylphosphates metabolites **Biomarker:** Urine samples (n=555)	**Location:** Washington State, USA **Period:** June 2005 to February 2006 **Study participants:** 100 farmworkers' children and 100 non-farmworkers' children, ages 1- 7 years	Diet (fruits, vegetables and apple juice consumption)	Age, gender, type of household dwelling, marital status, household income and number of children in household	Among farmworker's children, median DMAP levels were 0.077nmol/ml in the non -spray season and 0.205nmol/ml in both thinning and harvest seasons. Amongst non-farmworkers' children, median DMAP levels were lower (0.095nmol/ml) in thinning season, 0.107nmol/ml in non-spray season and slightly higher (0.197nmol/ml) in harvest season. DMAP levels were high during harvesting season in both farmworkers' and non-farmworkers' children. There was also a significant positive association between fruit and vegetables consumption, and DMAP and DEAP levels.
Bradman et al. (65)	Six dialkylphosphates metabolites and pyrethroid metabolites **Biomarker**: Urine samples	**Location:** California, USA **Period:** 2006 **Study participants:** 40 Mexican American children, 3-6 years 20 children residing in urban community 20 children from agricultural community	Organic diet and conventional non-organic diet	Household characteristics, parental occupation, recent pesticide application, diet, pesticide active ingredient and proximity of home to agricultural farms	Organic diet was significantly associated with reduction of urinary concentrations of DAPs. Detection frequency for all DAPs was greater than 50%. Geometric means of DMs (105.4) and DAPs (149.6) reduced to DMs (54.1) and DAPs (90.2) after /during consumption of organic diet. Among all children,
Ye M et al. (53)	Six DAPs metabolites and pyrethroid metabolites **Biomarker**: Urine samples	**Location:** Canada, USA **Study participants:** 5604 participants aged 6-79 years Stratified: 6-11 years, 12-19 years and 20-79 years	Diet	Age, gender, race/ ethnicity, body mass index, education level, household pesticide use and household income	Total DAPs concentration was 1.43 times (95%CI: 1.26-1.61) among participants with high fruit consumption (3^{rd} tertile) compared to those that consume less fruits (1^{st} tertile). Total DAPs concentrations were 1.33 (95%CI: 1.16, 1.52) times more in the group that consumed more vegetables compared to those that consumed less. Children aged 6-11 years had higher geometric mean (152.03nmol/g, 95% CI 135.65, 170.40) of DAPs than adolescents (83.81nmol/g, 95% CI 72.53,96.85) and adults (90.49nmol/g, 95% CI 81.11,100.95).
Hyland et al. (52)	Chlorpyrifos, Malathion, Azinphos-methyl, chlorpyrifos-methyl, dichlorvos, dicrotophos, dimethoate, fenitrothion, fenthion, isazofos-methyl, malathion, methidathion, methyl parathion, naled, oxydemeton-methyl, phosmet, pirimiphos-methyl, temephos, tetrachlorvinphos, trichlorfon, Chlorethoxyphos, chlorpyrifos, coumaphos, diazinon,	**Location:** USA **Period**: February-May 2017 **Study participants:** 80 participants; children aged 4-15 years and adults aged 36-52 years	Conventional and organic diet	Age and household pesticide use	Overall, reductions in DAP, DM and DE levels were observed after introduction of organic food both in children and adults. During the organic diet phase, urinary levels of total DE, total DM, and total DAP metabolites decreased by −26.0% (95% CI: −43.7%, −2.6%; p <0.01), −83.9% (95% CI: −88.0%, −78.4%; p < 0.01), and −69.5% (95% CI: −76.6%, −60.2%; p < 0.01), respectively. In children, MDA reduced form median 1.04ng/mL during consumption of conventional foods to less than 0.02ng/mL MDA levels after consumption of organic food. TCPy decreased from median of 2.99ng/mL levels to 1.77ng/L after

	disulfoton, ethion, parathion, phorate, sulfotepp, terbufos, Allethrin, cyhalothrin, cypermethrin deltamethrin, fenpropathrin, permethrin, trialomethrin, Cyfluthrin, cis-Cypermethrin, cis-cyfluthrin, cis-permethrin, trans-Cypermethrin, trans-cyfluthrin, trans-permethrin, Imidacloprid, Clothianidin, Thiabendazole, Boscalid, Iprodione, 2,4-Dichlorophenoxyacetic acid and six non- specific organophosphates. **Biomarker:** Urine samples				introducing organic foods to children's diet.
Griffith et al. (63)	Six dialkylphosphates metabolites, azinphos-methyl, phosmet, Malathion **Biomarkers**: Urine samples	**Location:** USA **Study participants:** approximately 200 farmworkers and 3-5 years old children	Household dust	Age, ethnicity, education level and household income	Higher urinary organophosphates metabolites were higher during the thinning season (when pesticides are used). Child to adult ratio decreased by 2.7-fold in intervention group than in control group which decreased by 1.7 folds.
Muñoz-Quezada M et al. (60)	3,5,6-trichloro-2-pyridinol (TCPy), 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMPY), malathion dicarboxylic acid (MDA), p-nitrophenol (PNP), non-specific diethylakylphosphates (DEAPs) and dimethylalkylphosphates (DMAPs) **Biomarkers:** Urine samples	**Location:** Maule, Chile **Study participants:** 48 school children, aged 5- 13 years	Diet (fruits) and proximity of home/ school to agricultural farms	Age, sex, educational level, parents' occupation, produce cleaning practices before consumption of fruits and vegetables	DEAP urine concentrations were higher among children that consume more fruits at school (p=0.03), younger age (p=0.03) and in males (p=0.01). TCPy was higher among children whose homes where less than 200m away from farms (β=1.68, p=0.013). Children attending school Bu (that shared its courtyard with common space adjacent to fields) had higher IMPY urinary concentrations (β=1.32, p=0.000) than those that attend school that was 200m away from agricultural fields. Compared to girls, boys had higher IMPY concentrations (β=0.46, p=0.020). Additionally, higher PNP concentrations were observed in younger children (β=-0.06, p=0.035). However, the community outreach and education program on pesticides did not reduce/ decrease children's urinary OP levels.
Muñoz-Quezada M et al. (59)	Dialkylphosphate metabolites, chlorpyrifos, phosmet, azynphos-methyl, dimethoate and diazinon. **Biomarkers:** Urine samples	**Location:** Talca, Chile **Study participants:** 190 children aged 2-12 years	Diet (fruits and vegetables), water, household dust, water	Age, gender, urban/ rural place, parents' education level, parents' occupation, type of pesticide used at home and proximity of home from farm	Urinary DEAP was associated with chlorpyrifos in consumed fruits (p= 0.0001), living in a rural area (p= 0.02) and age less than 9 years (p= 0.004). DMAP was associated with presence of phosmet residues in fruits (0.0001), proximity of home from agricultural farm (p= 0.002), household fenitrothion use (p= 0.009) and the season (p=0.0000). Children who lived close to the farm (<500m from the farm) were 2.5 times more likely to have higher urinary DMAP that those that don't. Children living in homes that were treated with fentrothion were 3.5 times more likely to have high urinary DMAP

					urinary levels than those whose families don't. No OP pesticide residues were detected in soil and drinking water samples.
Rohitrattan J. eta al (61)	Six dialkyl phosphates metabolites and 3,5,6-trichloro-2-pyridinol (TCPy) **Biomarkers:** Urine Samples	**Location:** Pathum Thani Province, Thailand **Study participants:** 53 children, aged 6-8 years	Residential area	Parental occupation, proximity of home to agricultural farm, residential pesticide use, sex, age, BMI	Increasing levels of TCPy levels were significantly related to proximity of home to the rice farm (p=0.03). Children of parents that are working on a farm had higher urinary TCPy metabolites concentrations than children of non- farmworkers (p=0.02). children that played on farms had statistically significant TCPy levels than children that do not play in agricultural fields (p= 0.03). DEP (p= 0.003) and DETP (p= 0.002) concentrations among participants from rice farming area had higher urinary concentrations levels than those that lived from aqua cultural farming.
Bravo N. et al. (55)	3,5,6-trichloro-2-pyridinol (TCPy), 2-diethylamino-6-methylpyrimidin-4-01 (DEAMPY), 3-nitrophenol (PNP), 3-phenoxybenzoic acid (3-PBA), 4-fluoro-3-phenoxybenzoic acid (4-F-3 PBA), 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMPY), malathion dicarboxylic acid (MDA) and six dialkylphosphates metabolites. **Biomarkers:** urine samples	**Location:** Trieste, Italy **Study participants:** 199 children, aged 7 years.	Diet	Gender, BMI, parents' educational level, smoking, breast feeding	Children with parents with higher educational level had higher urinary DEAMPY metabolites (p<0.05) than those with parents with lower educational level. Most abundant pesticides were DEAMPY (4.5ng/ml), PNP (1.5 ng/ml) and TCPY (0.41ng/ml). Fish consumption was not significantly associated with urinary OP levels and PTR concentrations.
Salvatore A. et al. (64)	Allethrin, bifenthrin, cyfluthrin, cypermethrin, deltamethrin, esfenvalerate, sumithrin, trans-permethrin, carbaryl, carbofuran, chlorpyrifos, diazinon, diazinon-oxon, malathion, methidathion, phosmet, dacthal, iprodione,piperonyl butoxide. Six Dialkylphosphates metabolites **Biomarker:** urine samples	**Location:** Salinas Valley, California, USA **Study participants:** 106 children, ≤ 4 years old.	Take home pesticide exposure: Household dust	Household pesticide use, income, occupational status of parents, child's fruit and vegetable consumption, household composition, age, proximity of home to agricultural fields, sex and poverty status.	DAP metabolites between control and intervention group were not significantly different. However, children in control group had higher DAP metabolites levels at follow up (DAP mean of 91.5nmol/l) compared to pre intervention stage (63.3nmol/l). As for the intervention group, slightly lower DAP metabolites levels were observed at follow up compared to baseline stage, with DAP geometric mean of 73.3nmol/l at follow up and 84.6nmol/l at pre- intervention stage. Carbaryl dacthal and diazinon were associated with living ≤¼ mile from an agricultural field (p <0.1).

Papadopoulou E et al. (62)	Poly brominated diphenyl ethers, polychlorinated biphenyls (PCBs), per-and polyfluoroalkyl substances (PFAS), Mercury, phthalate metabolites, phenolic compounds, and organophosphate pesticide (OP)metabolites. **Biomarker:** urine samples	**Location:** United Kingdom, France Spain, Lithuania, Norway and Greece. **Time:** 2013- 2015 Study **Participants:** 818 mothers and 1, 288 children aged 6- 11 years	Diet	Education, smoking and diet	Consumption of fruits was associated with high levels of urinary OP metabolites in both children and their mothers. Children that had fruits > 14 times per week had 88% DETP more compared to other children who had fruits < 14 times a week. 50% increase in DEP and DMTP was also observed for children who had fruits >14 times per week.

2.5 Conclusion

The vast majority (n=14) of the 16 studies that have investigated OP biomonitoring among children were conducted in developed countries, with only two studies in developing countries (that is India and Thailand). There is therefore a need for data from developing countries, including South Africa. There is evidence that demographic and socio-economic factors such as age, gender, education and household income, and pesticide exposures such as residential proximity to the farm, diet, residential pesticide use and season of the year are associated with urinary levels of OP metabolites in children, but there are few studies on certain factors and some conflicting evidence. Hence a need for further research.

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PART C: MANUSCRIPT

Preparation for submission

Journal

Prepared to be submitted for publication in the Science of the Total Environment Journal. The journal's instructions for authors have been followed, except for tables instructions.

Short running head

Urinary Organophosphate pesticides metabolites levels and pesticide exposures.

Competing financial interest declaration

None

Abbreviations

OP- Organophosphate

DEP- Diethyl phosphate

DMP- Dimethyl phosphate
DMTP- Dimethyl thiophosphate
DAP- Dialkyl phosphate
∑DAP- Sum of three DAP metabolites (DEP, DMP and DMTP)
WC- Western Cape
µg/L- Microgram per litre

Abstract

Background: Biomonitoring of pesticides is an objective measure of short-term pesticide exposure as it measures possible exposure in the human body. Current evidence on the relationship between demographic, socio-economic and pesticide exposure risk factors and urinary levels of organophosphate (OP) pesticide metabolites among children is generally incomplete and conflicting in some cases. There is therefore a need for further research.

Objectives: This study investigated the relationship between socio-economic, demographic and reported pesticide exposure related activities and characteristics in relation to urinary levels of dialkyl phosphate (DAP) metabolites (diethyl phosphate (DEP), dimethyl phosphate (DMP) and dimethyl triphosphate (DMTP)) among boys living in the rural areas of the Western Cape, South Africa.

Methods: This was an analysis of data collected during a cross- sectional study of 183 boys from three agricultural intense areas in the Western Cape of South Africa between April 2007 and March 2008. Measurements included a questionnaire on demographic, socio-economic and pesticide exposure risk factors and analysis of spot urine samples for DAP metabolites.

Results: Most of the boys (70%) lived on farms with a median age of 12 years (range: 5.0 - 19.5 years). The median concentrations of DAP, DEP, DMP and DMTP were 68.3 ng/ml (IQR= 27.9; 129.5), 5.5 ng/ml, 32.6 ng/ml and 16.7 ng/ml, respectively. The sum of the three DAP levels was inversely associated with age. Children older than 14 years had less DAP levels (β = -68.1; 95% CI: -136.8,0.6) than children 9 years and younger. DAP levels also varied significantly with area, with the levels highest in Grabouw (apple farming), followed by Hex River Valley (grape farming) (β= -52.1; 95% CI: -97.9, -6.3) then Piketberg (wheat farming) (β= -54.2; 95% CI:-98.8, -9.7). Other weaker and non-significant associations with increased DAP levels were found with increased household income, member of household work with pesticides, living on a farm, drinking water from an open water source and eating crops from the vineyard and or garden.

Conclusion: The study found younger age and living in and around an apple and grape farms, to be associated with increased urinary DAP concentrations among the school children provide evidence that younger age and residential area can be associated with increased urinary DAP concentrations among boys. Additionally, there are other household and behavioural characteristics that are associated with elevated urinary DAP levels. Further studies with larger sample sizes and longitudinal designs to improve the statistical power and the associations found are recommended. The study provided more insight to incomplete and inconclusive evidence of previous studies.

Keywords: Organophosphorus pesticides, pesticide biomonitoring, urinary dialkyl phosphates, rural boys, socio-economic factors, farm boys and non-farm boys.

1. Introduction

Organophosphates (OPs) are widely used in agriculture globally and in South Africa. They have been detected in surface water, ground water, food products and in biological samples of rural residents in the Western Cape (WC), an important agricultural province in South Africa (1-8). Exposure to OPs has been associated with a number of acute and chronic health effects (8-14) and their presence in the environment is therefore a concern for public health as large number of people are exposed including vulnerable groups such as pregnant women and children.

Organophosphates are toxic chemicals widely used for their positive benefits in crop productivity, reduction of vector borne diseases and general control of pests (15). They kill or eliminate insects by inhibiting acetylcholinesterase which is essential for neurotransmission (16). They are not only toxic to pests but to humans as well. Organophosphate pesticide exposure is associated with several health effects such as poor neuro behavioural outcomes (17). The most important health effects associated with OP pesticides are neurotoxic effects, but some OPs such as pirimiphos-methyl and chlorpyrifos have been identified as endocrine disrupting chemicals (18, 19), because of their ability to interfere with the endocrine system by either mimicking hormones or altering production of hormones (20).

Exposure to pesticides can occur through residues present in food and water, contact with contaminated surfaces and air drift from spraying (1, 21-24). Most affected populations include those living or working on farms or near farms on which OP pesticides are applied and children. Children's proximity to the ground, frequent engagement with the ground and soil, where pesticide residues normally settle and their hand to mouth activity increase their environmental risk (22, 25). Furthermore, they consume more food (predominately fruits, vegetables and water) per their unit weight compared to adults' food consumption per unit weight (25).

Biomonitoring of pesticides is an objective measure of pesticide exposure as it measures the internal dose present in body fluid such as pesticide metabolites in urine. A number of studies have investigated the associations of determinants of pesticide exposure (such as age, gender, education, income, parental occupation, living on farm, dietary intake, household pesticide application and season) and urinary levels of OP pesticide metabolites among children but the vast majority were conducted in high income countries (26-37), with only two in low and middle income countries (LMICs) (38, 39). There is therefore a need for data from LMICs including South Africa because rural communities exposed to pesticides in low- and middle-income countries like South Africa are more vulnerable to adverse effects of pesticides due to poverty, lack of resources and poor policy implementation (40).

The evidence is generally incomplete as very few studies in low- and middle- income countries have investigated multiple factors of pesticide exposure. The evidence is also inconsistent for instance different associations are found for age in several studies. Better understanding of factors relevant for pesticide exposure and their adsorption into the body is needed for further research to investigate adverse health effects due to pesticide exposure. Hence, this study seeks to provide further insight into the relationship between urinary levels of OP's pesticide residues and socio- economic, demographic factors and reported pesticide exposures in the Western Cape, one of the most agricultural intense provinces in South Africa.

2. Methods and Materials

2.1 Study Design

This was an analysis of data collected during a cross-sectional study of 183 boys conducted between April 2007 and March 2008 in three agricultural intensive areas in Western Cape, South Africa. The main study investigated the effects of agricultural pesticides exposure on growth and pubertal development of boys and adolescents in the rural Western Cape region of South Africa (6, 41). This analysis investigated the relationship between urinary levels of pesticides, self-reported pesticide exposures and socio-demographic factors.

2.2 Study Setting

The study was conducted on farm and non-farm school-going boys from the Hex River Valley, Grabouw and Piketberg where pesticides (such as chlorpyrifos, azinphos-methyl and prothiofos) were detected in environmental media in a previous study (2). Grape, fruit and wheat farming are intensive respectively in Hex River Valley, Grabouw and Piketberg (41).

2.3 Study Population and sampling

During the main study, 269 boys aged 5 to 19 years from primary and secondary schools (n=8) were recruited from the three study areas. Farm boys (boys residing on farms or around farms) living in Hex River Valley, Piketberg and Grabouw and non- farm boys (those living in within one to three kilometres away from farms) were targeted for the study. The sampling frame was the list of primary and secondary schools provided by the Western Cape Department of Education. The most accessible primary and secondary schools with students living in farms and neighbouring farm communities were selected for the study. Prior commencement of the study, school principals were approached by the main investigator for their approval to execute the study in their schools. Parents were also consulted, by means of letters distributed at schools to provide provisional written permission for their children to be recruited in the study.

Among boys whose parents gave permission (n = 492), all 94 boys not living on a farm and 180 boys (60 in each area) out of 398 living on farms were recruited (6). They were then stratified according to their residential area and age (5 to 9 years (prepubertal), >9 to 11 years (early puberty), >11 to 14 years (mid-late puberty) and greater than 14 years (post-puberty).

At schools where the number of consenting boys living on farms exceeded the number to be included in the study, a systematic random selection was conducted. Selected boys and their parents (n = 274) were invited to participate in the study on specified dates. Five participants were excluded from the study because their parents or guardians did not participate and eventually remaining 269 boys were included in the main study but only urine samples of 183 boys (Appendix A) were available in the current analysis (6).

2.4 Study Instruments

2.4.1 Questionnaires

Children's guardians (children's care givers) were interviewed face to face by trained interviewers in their preferred language. Interviewers captured responses onto electronic questionnaires preloaded on mobile phones using mobile technology (Mobile Researcher and /or Clyral). Captured responses were then transferred to the central website through the internet, where they were easily accessed by the principal investigator. Stata 13 [Stata Corporation, Texas, USA] and Microsoft Excel® were then used for further data management and analysis.

Questionnaires that were translated into participants' preferred language (either Xhosa or Afrikaans), were then back translated into English during data capturing. Questionnaires (Appendix B) had among others, sections on socio-demographic factors and pesticide exposures. Pesticide exposure questions included: domestic use of pesticides including frequency of use; guardians' occupation; take home exposures and the and the participant's involvement in agricultural and pesticide application activities.

Other environmental pesticide exposures questions included place of residency, proximity of home to pesticide spraying, and mode of pesticide application practiced in farms. Domestic water sources, use of empty pesticide containers and pesticide exposure through diet (vegetables, soya and meat intake) were also measured. Questionnaires were administered only to guardians. Questions on socio-demographic factors included parents' education and marital status; family income, age and medical history.

2.4.2 Urinary levels of organophosphate pesticides

Spot urine samples of boys were collected by the field nurse from April to May 2008. Only one sample was collected per child. Colourless 50mL plastic urine containers sealed with plastic caps were used for urine sample collections. Samples were then stored in dry ice and transported to School of Public Health and Family Medicine, where they were stored at -20 ºC before being analysed by the Clinical Pharmacology Laboratory, UCT. Only three metabolites; Diethyl phosphate (DEP), Dimethyl thiophosphate (DMTP) and Dimethyl phosphate (DMP) were detected. Urinary DAP metabolites were not adjusted for creatinine or specific gravity as these methods were not established by the collaborating laboratory that conducted the analysis.

Analysis of the three urinary metabolites were done with a validated liquid chromatography tandem mass spectrometry assay developed at the Clinical Pharmacology Laboratory at UCT as described in details by Monate (41). The level of detection for three urinary DAP metabolites was 0.78ng/mL (41).

2.5 Statistical Analysis

The dataset was explored for any missing or incorrect data entry. Data was explored using frequency tables. Continuous exposure variables, like income were categorized using tertiles, medians or quartiles. OP pesticide metabolites were then tested for normality using box and whisker plot. Log transformation of data not normally distributed did not improve normality and therefore untransformed data are represented. Bivariate analyses were conducted to explore relationship between urinary DAP metabolites and potential exposure factors (like age, socio-economic status and residential places). Tests used in bivariate analyses included Wilcoxon sum rank test, Fisher's exact test (where frequency was <5 or the assumption of a large sample size was not met) and simple linear regression.

Relevant OP exposure factors to be included in multivariable analysis were identified based on bivariate testing (where associations with a significance of $p \leq 0.1$ were found), non-co-linearity and through iterative model building. Sensitivity analysis was then conducted by running the model with only fam boys.

The results of the sum of the three DAP metabolites are represented as they are similar and representatives of the combined results of the three individual metabolites (DEP, DMP and DMTP) which are reported individually in appendix F, table 10.

2.6. Ethics

The University of Cape Town's Faculty of Health Sciences Research Ethics Committee approved the main study (REC REF: 279/2005) (Appendix C) as well as this sub-study (HREC Ref: 473/2019, Appendix D). Approval was also obtained by the Department of Education in South Africa to conduct the study at schools. Consent (Appendix E) was obtained from the parents/legal guardians of participating boys and the boys before conducting the tests on them.

3. Results

3.1 Study participation

Among the 183 boys from whom urine samples were collected, 70% were farm boys and 30% non-farm boys. Boys that gave urine samples were not statistically different with respect to socio-demographic and exposure factors from those that did not give urine samples (n=86), except for their age (Appendix F, Table 9).

3.2 Socio demographic characteristics

The demographic and socio- economic characteristics of participants are shown in Table 1. There was representation of boys in the different age categories with a median age of 12 years. There was also similar representation of boys from the different study areas. Only 30% of the parents completed high school and 3 % had tertiary education. The monthly median household income level of R 1890 (131.16 USD) was also low. Only 1 participant had previously been hospitalised with pesticide related illnesses.

Table 1: Socio-demographic characteristics of the study population

Characteristics (N)	**n (%)**	**Median (IQR)**
Demographics, socio-economic and health		
Age (Years) (183)		12 (10, 13)
≤ 9	22 (12.0)	
>9-11	47 (25.7)	
>11-14	87 (47.5)	
>14	27 (14.8)	
Area of Residence (183)		
Grabouw	57 (31.2)	
Hex River Valley	59 (32.2)	
Piketberg	67 (36.6)	
Parent Education Level		
Schooling (181)		
Grade 1-6	83 (43.9)	
Grade 7-8	43 (23.8)	
Grade 9-12	55 (30.4)	
Monthly Household Income (178)		R1890.00 (R1238, R2500)
≤ R1550 (107.6 USD)	72 (40.5)	
> R1550 – R2449 (107.6 USD-170.0 USD)	53 (29.8)	
≥ R2450 (170.0 USD)	53 (29.8)	
Children pesticide Poisoning (181)		
Ever poisoned	1 (0.6)	

3.3 Reported pesticide exposures

Parental occupation, residential pesticide exposures, household activities and children's recreational activities of participants are presented in Table 2. Eighty percent the participant's parents were farm workers and a small proportion were pesticide applicators (10%) and washed contaminated work clothes at home (3%).

About half (52%) of the parents reported that they were living within 50m from the nearest spraying area with some (17 %) living on a farm but more than 50m away from the nearest spraying area. About a third of the parents (30%) reported that pesticide spray drifts reach their home. About half (55%) of the parents reported that pesticides are used at home and about a third (28%) reported that it is used every week.

Some of the children were reported to swim in dams/ rivers either on or close to farms (31%), and helped on a farm (12%) and a small percentage were reported to mix pesticides (<1%) and/ or came in to contact with empty pesticide containers at work (4%).

Table 2: Reported pesticide exposures among study participants

Characteristics (N)	**n (%)**
Para- occupational exposure/ take-home exposure	
Parent Work in farm (123)	98 (79.7)
Current Parental job description (123)	
Farmworker applicator (Supervisor, sprayer, mixer)	12 (9.8)
Farmworker non-applicator	86 (69.9)
Non- farmworker	25 (20.3)
Member of Household Work with pesticide (183)	18 (9.8)
Pesticide contaminated clothes washed at home (183)	6 (3.3)
Reported pesticide exposures	
Live on farm (183)	127 (69.4)
Reported distance of home to spraying (meters) (183)	
0-50m	96 (52.5)
51-100m	11 (6.0)
>100m	20 (10.9)
Live in town	56 (30.6)
Reported spraying of pesticides on farm (183)	125 (68.3)
Pesticide drift enters the house (183)	54 (29.5)
Children play on farm after spraying (183)	33 (18.0)
Sources of drinking water (173)	
Open (Borehole, river, farm dam, rainwater tank)	62 (35.8)
Closed (Municipal water, mountain water)	111 (64.2)
Recreational Water Sources (171)	
Open (Borehole, river, farm dam, rainwater tank)	59 (34.5)
Closed (Municipal water, mountain water)	112 (65.5)
Household Pesticide use (183)	100 (54.6)
Frequency of pesticide application in households (Monthly)	
Weekly	52 (28.4)
Monthly	48 (26.2)
Never	83 (45.4)
Recreational Activities (183)	
Swimming in dams/rivers	56 (30.6)
Location of the river or dam	
On the farm	32 (17.5)
< 100 m from farm	20 (10.9)
>100 m from farm	4 (2.2)
Never swim	127 (67.4)
Helps on the farm	22 (12.0)

3.4 Dietary factors

Reported dietary factors are presented in Table 3. Nearly half (49%) of the parents reported that their sons ate crops from their garden and about a third (33%) eat crops from the spraying area. The majority (more than 95%) of parents reported that their sons eat meat and/or vegetables at home and most reported that their sons eat soya (73%) and nuts (59%) at home.

Table 3: Reported dietary factors among study participants

Characteristics (183)	**n (%)**	**Median (IQR)**
Diet		
Eats crops from vineyard	60(32.8)	
Eats fruits or vegetables from gardens	89(48.6)	
Consumption of fish or meat	181(98.9)	
Weekly consumption of fish or meat		4 times per week (3, 6 times per week)
Never to rarely (≤1 time per week)	12 (6.7)	
Occasionally (2-3 times per week)	76 (41.5)	
Often (4-6 times per week)	53 (29)	
Daily (7 times per week)	42 (23)	
Consumption of vegetable	176(96.2)	
Weekly consumption of Vegetables		4 times per week (2, 7 times per week)
Never to rarely (≤1 time per week)	31 (16.9)	
Occasionally (2-3 times per week)	59 (32.2)	
Often (4-6 times per week)	31 (16.9)	
Daily (7 times per week)	62 (33.9)	
Eats soya	134(73.2)	
Weekly consumption of Soya		2 times per week (0, 4 times per week)
Never to rarely (≤1 time per week)	75 (41)	
Occasionally (2-3 times per week)	54 (29.5)	
Often (4-6 times per week)	50 (27.3)	
Daily (7 times per week)	4 (2.2)	
Nuts consumption	108(59.0)	
Weekly consumption of Nuts		Once a week (0, once a week)
Never to rarely (≤1 time per week)	142 (77.6)	
Occasionally (2-3 times per week)	28 (15.3)	
Often (4-6 times per week)	10 (5.5)	
Daily (7 times per week)	3 (1.6)	

3.5 Urinary levels of the three Dialkyl phosphate metabolites (DAPs)

Three DAP urinary metabolites (DEP, DMP and DMTP) were detected in majority of the urine samples (Table 4). At least one DAP was detected in each sample. All three metabolites were detected in 90% of the samples. Dimethyl thiophosphate (DMTP) was the most detected metabolite, followed by DMP.

Table 4: The sum of three DAPs urinary levels (ng/mL) among boys					
Organophosphate metabolites	**N**	**Overall**		**Farm boys**	**Non-farm boys**
		Median (IQR)	**Max**	**Median (IQR)**	
ΣDAP	183	68.3 (27.9 -129.5)	734.4	77.8 (35.3 -163.0)	54.5 (16.0 - 86.9)
DEP	183	5.5 (2.2 - 13.6)	244.0	6.6 (2.8 - 14.9)	2.4 (1.1 - 10.0)
DMP	183	32.6 (12.6 - 62.8)	406.0	37.8 (15.9 - 73.9)	25.0 (7.2 - 57.0)
DMTP	183	16.7 (7.6 - 43.4)	399.0	23.0 (8.6 - 56.0)	13.7 (6.0 - 28.5)

Samples below limit of detection (DL) were assigned a value calculated by dividing the DL value (0.78 ng/ml) by square root of 2 (42).
IQR= Interquartile range
ΣDAP: sum of 3 dialkyl phosphates metabolites; DEP: diethyl phosphate; DMP: dimethyl phosphate; DMTP: dimethyl thiophosphate
19 participants had urinary levels below DL of DEP, 2 participants below DL of DMP and 1 participant below DL of DMTP

3.6 Association between urinary levels of DAP metabolites and demographic, socio economic, self-reported pesticide exposures and diet.

Table 5 summarise the relationship between the sum of the DAP metabolites and socio-demographic, socio-economic, self-reported pesticide exposures and diet. Age and area of residence had the strongest associations with the sum of the three DAPs with household income having the weakest association. The sum of three DAPs decreased in a dose dependant manner with age. Boys at >14 years had 68 ng/ml less DAPs than boys younger than 9 years (95% CI: -136.765, 0.603; p=0.05). The sum of the three DAPs also varied significantly with area, with the levels highest in Grabouw (apple farming) followed by Hex River Valley (grape farming) (β= -52.1; 95% CI: -97.9, -6.3) then Piketberg (wheat farming) (β= -54.2; 95% CI: -98.8, -9.7). The sum of the three DAPs also increased with income but there was no dose-response and the association was not significant. Other weaker and non-significant associations include member of household work with pesticides, living on farm and eating crops from the vineyard and /or garden. There was also an unexpected negative association between household pesticide use and DAP levels.

Table 6 summarise the relationship between the sum of three DAP metabolites and socio-demographic, socio-economic, self-reported pesticide exposures and diet only among farm boys. The results were similar to that of Table 5 apart from the fact that water source had a non-significant association with DAP levels. The sum of three DAPs was higher among boys that used water from open water sources compared to those using water from closed water sources.

Table 5: Summary of Multiple Linear Regression model between sum of DAP metabolites and various explanatory variables among all school going boys[1].		
Characteristics (n = 178)	**Association with ∑DAP**	
	Beta (95% CI)	***p-value***
Age (years)		
≤ 9		
≥9-11	-57.625 (-120.658, 5.409)	0.073
>11-14	-60.180 (-116.671, -3.688)	0.037
>14	-68.081 (-136.765, 0.603)	0.052
Area of residence		
Grabouw		
Hex River Valley	-52.118 (-97.943, -6.294)	0.026
Piketberg	-54.231 (-98.761, -9.702)	0.017
Monthly household income		
≤ R1550 (107.6 USD)		
> R1550 – R2449 (107.6 USD-170.0 USD)	39.087 (-3.777, 81.951)	0.074
≥ R2450 (170.0 USD)	30.983 (-18.072, 80.037)	0.214
Member of household work with pesticide		
No		
Yes	41.630 (-18.310, 101.571)	0.172
Live in farm		
No		
Yes	29.013 (-20.339, 78.364)	0.247
Household pesticide use		
No		
Yes	-27.705 (-70.448, 15.038)	0.202
Eating crops from vineyard		
No		
Yes	19.894 (-24.570, 64.358)	0.378
Eating crops from garden		
No		
Yes	28.027 (-21.455, 77.508)	0.265

[1] All explanatory variables in this table were included in the multiple linear regression model all together, thus adjusting for one another.

Table 6: Summary of Multiple Linear Regression model between sum of DAP metabolites and various explanatory variables among school going boys living on a farm.

Characteristics (118)	**Association with ∑DAP**	
	Beta (95% CI)	***p-value***
Age (years)		
≤ 9		
≥9-11	-52.244 (-131.829, 27.341)	0.196
>11-14	-50.197 (-123.728, 23.335)	0.179
>14	-80.261 (-169.827, 9.305)	0.078
Area of residence		
Grabouw		
Hex River Valley	-31.096 (-93.511, 31.319)	0.325
Piketberg	-23.409 (-100.703, 53.885)	0.549
Member of household work with pesticide		
No		
Yes	60.898 (-12.241, 134.037)	0.102
Reported distance of home to spraying (meters)		
>100m		
51-100m	-96.597 (-202.598, 9.404)	0.074
0-50m	1.624 (-81.259, 84.506)	0.969
Pesticide drift enters the house		
No		
Yes	-25.785 (-83.217, 31.646)	0.375
Household pesticide use		
No		
Yes	-9.777 (-67.071, 47.516)	0.736
Sources of drinking water		
Closed (Municipal water, mountain water)		
Open (Borehole, river, farm dam, rain water tank)	46.167 (-4.580, 96.913)	0.074
Eating crops from vineyard		
No		
Yes	4.729 (-57.060, 66.518)	0.880
Eating crops from garden		
No		
Yes	28.130 (-36.433, 92.693)	0.390

4. Discussion

This study provided valuable data on socio- demographic, household and behavioural characteristics associated with increased urinary levels of the sum of three DAP metabolites. This contributes to an area of limited data in the international literature, especially in low-and-middle income countries. Among all characteristics explored, the boy's age and area of residence demonstrated the most independent significant association with urinary DAP levels compared to exposures such as household income, member of house work with pesticides, living on farm, eating crops from the vineyard and/ or garden and open drinking water source that all had weaker associations. Additionally, there was an unexpected inverse association between household pesticide use and DAP levels.

The sum of the three DAPs (median = 68.3 ng/ml ≈170nmol/L) observed in this study is slightly less than the total DAP (median = 212.98 nmol/L) observed in the USA among 4 - 15 years children (43).Pesticide levels among non-farm residents and non-applicators among rural communities in the Western Cape have previously been found to be higher than other settings (3, 5, 8). High DAP levels among those living on farms and those not on farms could be due to exposures from pesticide spray drifts, contact with contaminated surfaces, and from drinking water from open water sources and eating crops contaminated with pesticides.

A decrease in DAP levels with an increase with age in a dose dependent manner was consistent with a study from Canada (26) and two studies from Chile (34, 35) that observed statistically significant higher OP urinary metabolites in younger children (6-11years) than in older children (older than 11 year).This could be attributed to younger children (6-11years) consuming a significantly higher proportion of vegetables and fruits in their diet than older children (older than 11years) (25). Additionally, younger children spend more time on floors, eat food from floors and have less developed physiological mechanism to detoxify and clear pesticides than older children (21). Increase in DAP levels with increasing household income requires further exploration in a larger study sample with a wider income range. Even though most previous studies, including one from India, Canada and two from America did not find any relationship between household income and urinary OP metabolites, a Danish study that enrolled 144 children observed increased DAP concentrations with increasing monthly household income, which is consistent with this study (44). This relationship might be explained by dietary choices related to socio- economic status, as income determines fruits and vegetables intake (44).

Higher DAP concentrations were observed in children living in Grabouw, a predominately fruit farming area, compared to those from Piketberg, a predominantly wheat farming area and Hex River Valley, a predominantly grape farming area (1, 45) . This is consistent with the USA study that found higher levels of OP exposures among farmworkers that worked in pome fruit (apples or pears) farms compared to those that worked on other types of crops (32). Additionally, more pesticides, including chlorpyrifos, were detected in surface waters of Grabouw, indicating more frequent pesticide applications in this area, compared to Piketberg and Hex River Valley in a recent study (1).

Consumption of fruits such as apples treated with pesticides (27), specifically chlorpyrifos and phosmet, which have been associated with high urinary OP metabolite levels among children

(34). This could explain relatively higher urinary DAP levels observed among boys that consumed crops from the vineyard (β= 19.9; 95% CI -24.570, 64.4) compared to those that did not in this study. These observations suggest that farming choices/ activities and behaviours and diet also play a vital role in pesticide exposures.

The non-significant increase in urinary DAP levels in children that lived with a household member working with pesticides compared to those that did not is consistent with studies in the USA and Thailand (28, 29, 31, 32, 35, 39, 46), indicating that take home exposures such as contaminated clothes is an important exposure route. The reason for the statistically non-significant result in the current study, which is in contrast to previous studies could be due to a lower sample size and also lower prevalence of households with farm applicators in the current study (10%) compared to previous studies (25%-45%) (35, 39).

The finding that the sum of the three DAPs tended to be higher for children living on farms compared to those that did not, is consistent with previous studies in South Africa, Thailand, Chile and USA (6, 30, 32, 34, 35, 39, 41, 46). High DAP levels among those living on farms could be due to exposures from pesticide spray drifts. This result was however statistically significant in previous studies and not in this study despite the high proportion of those living on farms (69%) in the study sample. There could be possible misclassification of exposure with boys categorised into farm or non-farm groups based on their residence and therefore not accounting for exposure at schools. This misclassification would reduce the association between DAPs and farm residence.

Distance of home to pesticide spraying was not found to be associated with higher urinary levels of DAP, which is inconsistent with previous studies. This null association could be due to the fact that distance to spraying was self- reported in the current study and not physically measured as in other studies (32). The unexpected negative association between household pesticide application and DAP levels, is contrary to the results of a Canadian study (26), and could be due to information bias from incorrect reporting of household pesticide use.

Additional limitations to those discussed above include the lack of correction for urinary dilution and the measurement of DAP metabolites in one single urine sample. The lack of correction of urinary dilution is likely to increase the variation in urinary levels of DAPs measured and to reduce the strength of associations found (47). Urine samples were also collected in April and May not during the peak spraying season (October-February) and DAP levels are representing only short term exposure, as organophosphate pesticides are eliminated from the body within a few days (26, 29). However, OPs and environmental DAPs persist in environment for a long period, thus repeated exposures are likely (48). A longitudinal study design in which OP measurements are repeated a few times per year would significantly strengthen a future study.

Despite the limitations, this study has several strengths. Firstly, individual pesticide exposures of participants were characterised using biomonitoring. Secondly, the study sample had sound exposure variance including farm boys from three intensive agricultural areas and non- farm boys from neighbouring areas. Thirdly, the study had more data on socio economic and pesticide exposures.

5. Conclusions

This study provided evidence that socio-demographic factors such as younger age and living in or around an apple farm (Grabow), are associated with increased urinary DAP levels among school children. There was also some evidence that characteristics such as member of household work with pesticides, living on farm, drinking water from an open water source and eating crops from the vineyard and /or garden are also associated with increased urinary levels of DAPs. The study also adds to the growing body of literature on multiple determinants of OP pesticide exposures in low- and middle-income countries like South Africa.

Future studies with larger sample size may provide a larger power in understanding the relationship between urinary levels and children's household and behavioural characteristics.

Stringent legislation and implementation on reducing pesticide exposure in nearby communities, especially children are recommended.

6. Acknowledgements

The National Research Foundation (SA) is acknowledged for funding the study and for bursary support of the first author and the National Manpower Development Secretariat (NMDS, Lesotho) for additional bursary support.

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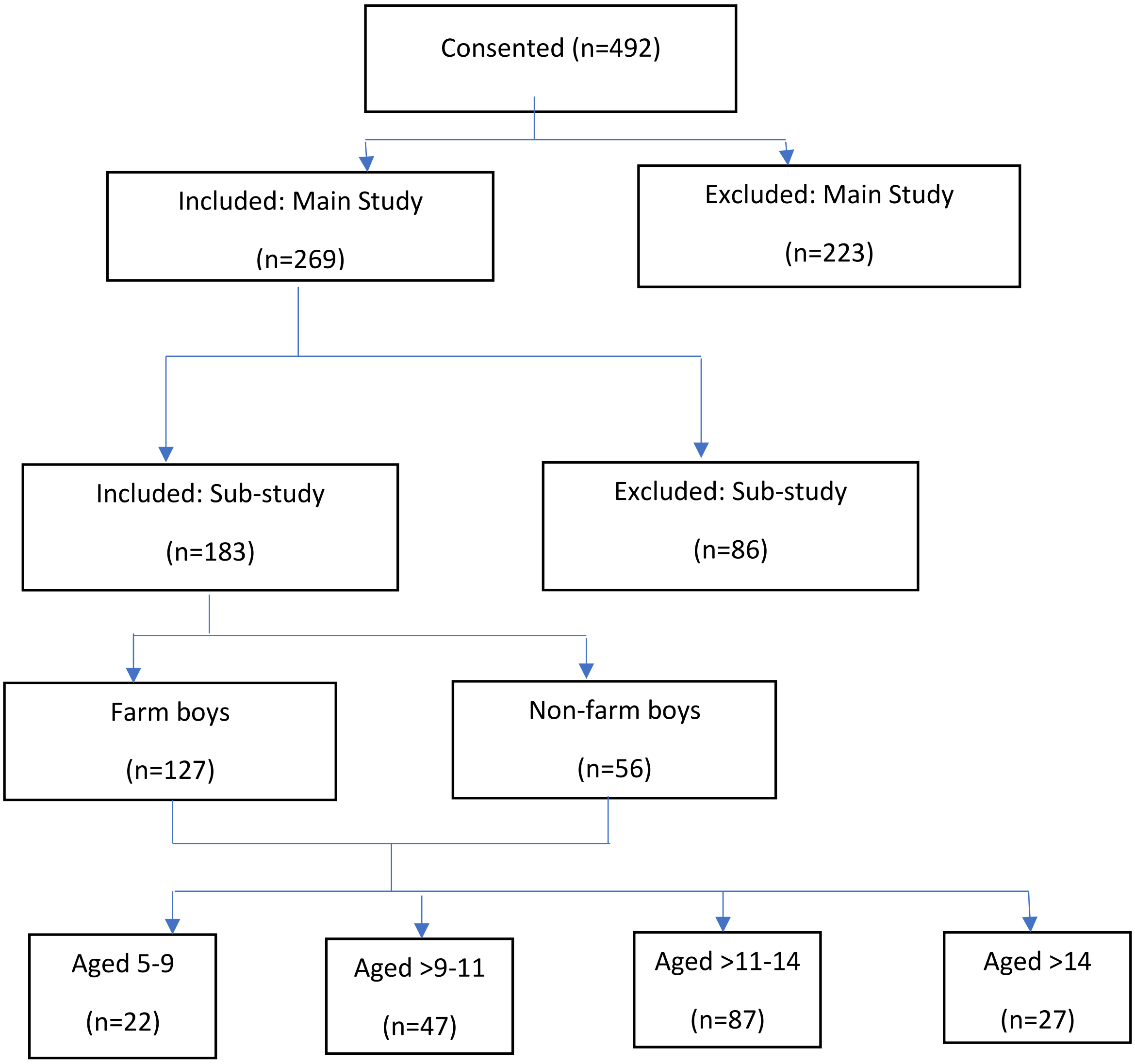
APPENDICES

Appendix A: Study flow- diagram



Appendix B: Child Questionnaire

(Pesticide exposure among farm and neighbouring 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

Q 1	Relationship to participants	Mother, father, other (circle which one is applicable) If other, specify ________________________
Q2	Highest Standard/Grade passed at school:	___________
Q3	Diplomas/Tertiary Education:	(Y/N)
Q4	Employment status	Yes, no, student, retired, other If employed, Job Title: ____________________ If farm worker, Exposure group (Supervisor, Sprayer/Mixer, Non- Sprayer Farmworker, Non-Farmworker): __________
Q5	Marital Status	Married, living with someone as married, widowed, divorced, separated, single with girl friend, single with no girlfriend
Q6	What is your monthly household income (in Rands)?	_________________
Q7	How often do your family go hungry or have no food to eat?	Never _____, Seldom____, Sometimes______, Often_______

Details of the child (Son)

Q8	Date of birth	______________
Q9	Age	______________
Q10	Birth weight	________(kg)
Q11	Current standard/grade at school:	_______
Q12	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. LIVING HISTORY

Please answer the following questions regarding the places where your son lives.

B1	Where does he live currently?	_____ (Name of town or city)
B2	For how long has he been living there?	_____ (years, months)
B3	Is his home located on a farm, town or city?	_______________
B4	If the place was on a farm, what kind of farm	_______________
B5	If his home is located on a farm, how far from the house is the nearest Vineyard/field?	________ (meters)

B6	Are pesticides sprayed on the vineyard/field during the year?	____ (yes, no, DN) **IF No (go to B8), 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)
B7	Does the pesticides spraying come into the house?	____ (yes, no, DN)
B8	Does your son come into contact with pesticides outside the house while spraying occurs (for e.g. playing near spraying area)?	______ (yes, no)
B9	Does your son go into the field/vineyards soon after spraying or come into contact with sprayed surfaces?	____ (yes, no)
B10	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)
B11	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)
B12	Does your son play or swim 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)
B13	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)
B14	Is he involved in spraying or mixing pesticides?	_____ (yes, no)
B15	Does he work in the pesticide store?	_______ (yes, no)

B16	Does your son come into contact with empty pesticide containers?	___ (yes, no) If yes, how __________________________ (for eg play, drinking water, burning)
B17	Does your son eat from the crops in the vineyard/field soon after spraying?	______ (yes, no)

C. HOUSEHOLD PESTICIDE EXPOSURE

C1	Do you use any pesticides in your garden or in your home (e.g. doom, rat poison,fleas)?	_____ (yes, no)
C2	If yes, for how long have you been using pesticides at home?	__________ (number of years)
C3	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)
C4	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)
C5	Does any person in the house work with pesticides?	**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)
C6	Does your son eat fruit or vegetables from your garden	______ (yes, no)
C7	Do you use empty pesticide containers at home for domestic purposes	If yes, what do you use them for? ___________________________ Since when have you been using empty containers at home ________ (year)

D. DIET

D1	Does your son eat meat/fish?	______ (Yes, No)
D2	How many times a week does he eat meat/fish	_______

D3	In his lifetime, how many times a week did he eat meat/fish	______
D4	Does he eat vegetables?	___ (Yes, No)
D5	How many times a week does he eat vegetables	_______
D6	How many times a week does he eat soy products	_____
D7	In his lifetime, how many times a week did he eat vegetables	_______
D8	In his lifetime, how many times a week did he eat soy products	_____
D9	Does your son like to eat nuts?	__ How many times a week does he eat nuts? _____
D10	In his lifetime, how many times a week did he eat nuts?	___
D11	Was he on soya milk after birth?	___ For how long? ______
D12	Does your son eat meals provided by the school?	If yes, what do they provide? ________________ Please specify the meals_______________

Appendix C: Ethics approval of the main study

UNIVERSITY OF CAPE TOWN

Research Ethics Committee
E52 Room 24,Old Main Building Groote Schuur Hospital, Observatory, 7925
Queries : Lamees Emjedi
Tel : (021) 406-6338 Fax: 406-6411
E-mail : lemjedi@curie.uct.ac.za

12 August 2005

REC REF: 279/2005

Dr MA Dalvie
Public Health & Family Medicine

Dear Dr Dalvie

ENDOCRINE DISRUPTING EFFECTS OF PESTICIDES AMONGST MALE FARM RESIDENTS IN THE WESTERN CAPE

Thank you for submitting your study to the Research Ethics Committee for review.

*It is a pleasure to inform you that the Ethics Committee has **formally approved** the above-mentioned study on the 4 August 2005.*

The REC requests the following changes be made to the documentation and that amended copies be submitted:

- *Adolescent to sign assent (include a form).*
- *Change contact person of REC as Mr Fula has left.*

Please quote the REC. REF in all your correspondence.

Yours sincerely

PROF T. ZABOW
CHAIRPERSON

UNIVERSITY OF CAPE TOWN

Health Sciences Faculty
Research Ethics Committee
Room E53-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
e-mail: preaward@curie.uct..ac.za

07 September 2005

REC REF: 279/2005

Dr MA Dalvie
School of Public Health and Family Medicine

Dear Dr Dalvie

ENDOCRINE DISRUPTING EFFECTS OF PESTICIDES AMONGST MALE FARM RESIDENTS IN THE WESTERN CAPE

Thank you for your letter to the Research Ethics Committee dated 20 July.

It is a pleasure to inform you that the Ethics Committee has formally approved the changes made to the consent form:

Adding the word, "adolescent or adult" in various parts of the consent form text
Changing the name of Ethics Administrator

Please quote the REC. REF in all your correspondence.

Yours sincerely

PROF T. ZABOW
CHAIRPERSON

Last saved by kbonani

Appendix D: Ethics approval of this study

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E53-46 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6626
Email: shuretta.thomas@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

30 July 2019

HREC REF: 473/2019

Prof Aqiel Dalvie
Environmental and Occupational Health
Public Health & Family Medicine
Falmouth Building

Dear A/Prof Dalvie

PROJECT TITLE: RELATIONSHIP BETWEEN URINARY LEVELS OF ORGANOPHOSPHATES METABOLITES AND PESTICIDE EXPOSURE AMONG RURAL SCHOOL BOYS OF THE WESTERN CAPE. (SUB-STUDY LINKED TO: 279/2005) (MASTERS CANDIDATE: MS N MOLOMO)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30 July 2020.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

Please quote the HREC REF in all your correspondence.

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

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

The HREC acknowledge that the student, Ntsubise Molomo will also be involved in this study.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

HREC 473/2019

Appendix E: 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 from participation in the study
The study is offered at no cost to you.

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

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

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

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

11. Voluntary nature of participation
Your 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 F: Supplementary analysis material

Table 7: List of socio demographic and exposure variables		
Variable Name	**Original Measurement Scale**	**Units/ categorisation**
Age	Numerical-continuous	Years
Parent education level	Categorical	Yes/ No
Household Income	Continuous	Rands
Pesticide poising	Categorical	Yes/No
Parent job description	Categorical	Supervisor, Sprayer/Mixer, Non-sprayer farm worker & Non-farm worker
Member of household working with pesticide	Categorical	Yes/No
Pesticide contaminated clothes washed at home	Categorical	Yes/No
Live on farm	Categorical	Yes/No
Reported distance of home to spraying	Numerical	Meters
Pesticide drift entering the house	Categorical	Yes/No/ Don't Know
Children plays in farm after spraying	Categorical	Yes/No
Source of drinking water	Categorical	Municipal water/ Mountain/Borehole, spring/ River/Farm dam/ Rain Water tank/ other
Household pesticide use	Categorical	Yes/No
Frequency of pesticide application in households	Categorical	Everyday/ 3 times a week/ once a week/ once a month/ less than a month
Use of empty pesticide containers	Categorical	Yes/No
Children swim in dams	Categorical	Yes/No
Location of the river that children swim	Categorical	On farm/ Just outside farm/more than 100m away/ out of town
Children helps on farm	Categorical	Yes/No
Frequency of help provided	Categorical	Everyday/ twice a week/ once a week/once a month/ school holiday
Child involved in mixing pesticide	Categorical	Yes/No
Child in contact with empty pesticide containers during work	Categorical	Yes/No
Child eat crops from farm/vineyard	Categorical	Yes/No
Child eat vegetables from family garden	Categorical	Yes/No
Consumption of fish/meat	Categorical	Yes/No
Consumption of Vegetables	Categorical	Yes/No
Consumption of Soya	Categorical	Yes/No
Consumption of Nuts	Categorical	Yes/No

Table 8: Socio-demographic characteristics, reported pesticide exposures and dietary pesticide exposures of children that gave urine samples and those that did not.					
Children that gave urine samples			**Children that did not give urine samples**		
Characteristics (N)	**n (%)**	**Median (Interquartile range)**	**Characteristics (N)**	**n (%)**	**Median (Interquartile range)**
Demographics, socio-economic and health			**Demographics, socio-economic and health**		
Age (Years) (183)		12 (10, 13)	Age (Years) (86)		10 (9, 13)
≤ 9	22 (12.02 %)	9 (8,9)	≤ 9	19 (22.09%)	8.67 (8.33, 8.75)
>9-11	47 (25.68%)	10 (9.33, 11)	>9-11	30 (34.88%)	9.5 (9.167, 10.083)
>11-14	87 (47.54%)	13 (12, 13)	>11-14	32 (37.21%)	12.5 (12, 13.209)
>14	27 (14.75%)	15 (15,17)	>14	5 (5.81%)	14.67 (14.17, 15)
Area of Residence (183)			Area of Residence (86)		
Grabouw	57 (31.15%)		Grabouw	43 (50.00%)	
Worcester	59 (32.24 %)		Worcester	18 (20.93%)	
Piketberg	67 (36.61%)		Piketberg	25 (29.07%)	
Parent Education Level			Parent Education Level		
Schooling (181)		Grade 7 (Grade 5, Grade 9)	Schooling (84)		Grade 8 (Grade 5, Grade 10)
Grade 1-6	83 (43.86%)		Grade 1-6	30 (35.71%)	
Grade 7-8	43 (23.76%)		Grade 7-8	26 (30.95%)	
Grade 9-12	55 (30.39%)		Grade 9-12	28 (33.33)	
Tertiary Education (183)			Tertiary Education (86)		
Tertiary	5 (2.73%)		Tertiary	5 (5.81%)	
No Tertiary	178 (97.27%)		No Tertiary	81 (94.19%)	
Household Income (178)		R1890.00 (R1238, R2500)	Household Income (84)		R2050 (R1450, R3400)
≤ R1550	72 (40.45%)		≤ R1550	30 (35.71%)	
> R1550 – R2449	53 (29.78%)		> R1550 – R2449	26 (30.95%)	
≥ R2450	53 (29.78%)		≥ R2450	28 (33.33%)	
Pesticide Poisoning (181)			Pesticide Poisoning (86)		
Ever poisoned: Yes	1 (0.55%)		Ever poisoned: Yes	1 (1.16%)	
No	180 (99.45%)		No	85 (98.84%)	
Para- Occupational Exposure/ take-home exposure			**Para- Occupational Exposure/ take-home exposure**		

Parent Work in farm (123)			Parent Work in farm (53)		
Yes	98 (79.67%)		Yes	36 (67.92%)	
No	25 (20.33%)		No	17 (32.08%)	
Current Parental job description (123)			Current Parental job description (53)		
Farmworker applicator (Supervisor, sprayer, mixer)	12 (9.76%)		Farmworker applicator (Supervisor, sprayer, mixer)	6 (11.32%)	
Farmworker non-applicator	86 (69.92%)		Farmworker non-applicator	30 (56.60%)	
Non- farmworker	25 (20.33%)		Non- farmworker	17 (32.08%)	
Member of Household Work with pesticide (183)			Member of Household Work with pesticide (86)		
Yes	18 (9.84%)		Yes	4 (4.65%)	
No	165 (90.16%)		No	82 (95.35%)	
Pesticide contaminated clothes washed at home (183)			Pesticide contaminated clothes washed at home (183)		
Yes	6 (3.28%)		Yes	0 (0.00%)	
No	177 (96.72%)		No	86 (100.00%)	
Reported Pesticide Exposures			**Reported Pesticide Exposures**		
Live on farm (183)			Live on farm (86)		
Yes	127 (69.40%)		Yes	48 (55.81%)	
No	56 (30.60%)		No	38 (44.19%)	
Reported distance of home to spraying (meters) (183)			Reported distance of home to spraying (meters) (86)		
0-50m	96 (52.46%)		0-50m	38 (44.19%)	
51-100m	11 (6.01%)		51-100m	4 (4.65%)	
>100m	20 (10.93)		>100m	5 (5.81%)	
Live in town	56 (30.60%)		Live in town	39 (45.35%)	
Reported spraying of pesticides on farm (183)			Reported spraying of pesticides on farm (86)		
Yes	125 (68.31%)		Yes	46 (53.49%)	
No	58 (31.69%)		No	40 (46.51%)	
Pesticide drift enters the house (183)			Pesticide drift enters the house (86)		
Yes	54 (29.51%)		Yes	22 (25.58%)	
No	129 (70.49%)		No	64 (74.42%)	
Children plays on farm after spraying (183)			Children plays on farm after spraying (86)		
Yes	33 (18.03%)		Yes	17 (19.77%)	
No	150 (81.97%)		No	69 (80. 23%)	

Sources of drinking water (173)			Sources of drinking water (78)		
Open (Borehole, river, farm dam, rain water tank)	62 (35.84%)		Open (Borehole, river, farm dam, rain water tank)	24 (30.77%)	
Closed (Municipal water, mountain water)	111 (64.16%)		Closed (Municipal water, mountain water)	54 (69.23%)	
Recreational Water Sources (171)			Recreational Water Sources (77)		
Open (Borehole, river, farm dam, rain water tank)	59 (34.50%)		Open (Borehole, river, farm dam, rain water tank)	24 (31.17%)	
Closed (Municipal water, mountain water)	112 (65.50%)		Closed (Municipal water, mountain water)	53 (68.83%)	
Household Pesticide use (183)			Household Pesticide use (86)		
Yes	100 (54.64%)		Yes	52 (60.47%)	
No	83 (45.36%)		No	34 (39.53%)	
Frequency of pesticide application in households (Monthly)			Frequency of pesticide application in households (Monthly) (86)		
Weekly	52 (28.42%)		Weekly	26 (30.23%)	
Monthly	48 (26.22%)		Monthly	26 (30.23%)	
Never	83 (45.36%)		Never	34 (39.53%)	
Use of empty pesticide containers at home (183)			Use of empty pesticide containers at home (86)		
Yes	4 (2.19%)		Yes	3 (3.49%)	
No	171 (97.81%)		No	83 (96.51%)	
Recreational Activities (183)			**Recreational Activities (86)**		
Swimming in dams/rivers			Swimming in dams/rivers		
Yes	56 (30.60%)		Yes	21 (24.42%)	
No	127 (69.40%)		No	65 (75.58%)	
Location of the river or dam			Location of the river or dam		
On the farm	32 (17.49%)		On the farm	13 (15.12%)	
< 100 m from farm	20 (10.92%)		< 100 m from farm	2 (2.33%)	
>100 m from farm	4 (2.19%)		>100 m from farm	6 (6.98%)	
Never swim	127 (67.40%)		Never swim	65 (75.58%)	
Helps on the farm			Helps on the farm		
Yes	22 (12.02%)		Yes	9 (10.47%)	
No	161 (87.98)		No	77 (89.53%)	
Frequency of help provided			Frequency of help provided		
Everyday	1 (0.55%)		Once a week	1 (1.16%)	

Twice a week	2 (1.09%)		During vacation	8 (9.30%)	
Once a week	2 (1.09%)		Never help	77 (89.53%)	
Once a month	1 (0.55%)				
During vacation	16 (8.74%)				
Never help	161 (87.98%)				
Child involved in mixing pesticide			Child involved in mixing pesticide		
Yes	1 (0.55%)		Yes	1 (1.16%)	
No	182 (99.45%)		No	85 (98.84%)	
Child in contact with empty pesticide containers during work			Child in contact with empty pesticide containers during work		
Yes	8 (4.37%)		Yes	4 (4.65%)	
No	175 (95.63%)		No	82 (95.35%)	
Diet (183)			**Diet (86)**		
Eats crops from vineyard			Eats crops from vineyard		
Yes	60(32.79%)		Yes	25 (29.07%)	
No	123(67.21%)		No	61 (70.93%)	
Eats fruits or vegetables from gardens			Eats fruits or vegetables from gardens		
Yes	89(48.63%)		Yes	33 (38.37%)	
No	94(51.37%)		No	53 (61.63%)	
Consumption of fish or meat			Consumption of fish or meat		
Yes	181(98.91%)		Yes	84 (97.67%)	
No	2(1.09%)		No	2 (2.33%)	
Consumption of vegetable			Consumption of vegetable		
Yes	176(96.17%)		Yes	81 (94.19%)	
No	7(3.83%)		No	5 (5.81%)	
Eats soya			Eats soya		
Yes	134(73.22%)		Yes	60 (69.77%)	
No	49(26.78%)		No	26 (30.23%)	
Nuts consumption			Nuts consumption		
Yes	108(59.02%)		Yes	53 (61.63%)	
No	75(40.98%)		No	33 (38.37%)	

Table 9: Difference between children that provided urine samples and those that did not (Fishers exact test)	
Characteristics (N)	**p- value**
Demographics, socio-economic and health	
Age (Years) (183)	
Those that provided urine samples	0.042
Those that did not provide urine samples	
Area of Residence (183)	
Those that provided urine samples	0.270
Those that did not provide urine samples	
Parent Education Level	
Schooling (181)	
Those that provided urine samples	0.072
Those that did not provide urine samples	
Tertiary Education (183)	
Those that provided urine samples	-
Those that did not provide urine samples	
Household Income (178)	
Those that provided urine samples	0.259
Those that did not provide urine samples	
Pesticide Poisoning (181)	
Those that provided urine samples	0.541
Those that did not provide urine samples	
Para- Occupational Exposure/ take-home exposure	
Parent Work in farm (123)	
Those that provided urine samples	-
Those that did not provide urine samples	
Current Parental job description (123)	
Those that provided urine samples	0.789
Those that did not provide urine samples	
Member of Household Work with pesticide (183)	
Those that provided urine samples	0.232
Those that did not provide urine samples	
Pesticide contaminated clothes washed at home (183)	
Those that provided urine samples	0.181
Those that did not provide urine samples	
Reported Pesticide Exposures	
Live on farm (183)	
Those that provided urine samples	-
Those that did not provide urine samples	
Reported distance of home to spraying (meters) (183)	
Those that provided urine samples	0.240
Those that did not provide urine samples	
Reported spraying of pesticides on farm (183)	
Those that provided urine samples	-
Those that did not provide urine samples	
Pesticide drift enters the house (183)	

Those that provided urine samples	0.563
Those that did not provide urine samples	
Children plays on farm after spraying (183)	
Those that provided urine samples	0.739
Those that did not provide urine samples	
Sources of drinking water (173)	
Those that provided urine samples	0.475
Those that did not provide urine samples	
Recreational Water Sources (171)	
Those that provided urine samples	0.664
Those that did not provide urine samples	
Household Pesticide use (183)	
Those that provided urine samples	-
Those that did not provide urine samples	
Frequency of pesticide application in households (Monthly)	
Those that provided urine samples	0.775
Those that did not provide urine samples	
Use of empty pesticide containers at home (183)	
Those that provided urine samples	0.683
Those that did not provide urine samples	
Recreational Activities (183)	
Swimming in dams/rivers	
Those that provided urine samples	0.315
Those that did not provide urine samples	
Location of the river or dam	
Those that provided urine samples	0.315
Those that did not provide urine samples	
Helps on the farm	
Those that provided urine samples	0.839
Those that did not provide urine samples	
Frequency of help provided	
Those that provided urine samples	0.839
Those that did not provide urine samples	
Child involved in mixing pesticide	
Those that provided urine samples	0.538
Those that did not provide urine samples	
Child in contact with empty pesticide containers during work	
Those that provided urine samples	1.000
Those that did not provide urine samples	
Diet (183)	
Eats crops from vineyard	
Those that provided urine samples	0.576
Those that did not provide urine samples	
Eats fruits or vegetables from gardens	
Those that provided urine samples	0.118
Those that did not provide urine samples	
Consumption of fish or meat	

Those that provided urine samples	-
Those that did not provide urine samples	
Consumption of vegetable	
Those that provided urine samples	-
Those that did not provide urine samples	
Eats soya	
Those that provided urine samples	-
Those that did not provide urine samples	
Nuts consumption	
Those that provided urine samples	-
Those that did not provide urine samples	

Table 10: Association between organophosphate metabolites and demographic, socio-economic, self-reported pesticide exposures and diet

Characteristics	∑DEP (median (ng/mL), IQR)	B coefficient	P-Value	∑DMP (median (ng/mL), 1QR)	B coefficient	P-Value	∑DMTP (median(ng/mL), IQR)	B coefficient	P-Value	∑DAP (median (ng/mL), IQR)	B coefficient	P-Value
Demographics, socio-economic and health												
Age (Years) (183)	5.54 (2.16, 13.60)	-0.390	0.633	32.60 (12.60, 62.80)	-1.776	0.442	16.70 (7.58, 43.40)	-5.102	**0.004**	68.32 (27.92, 129.52)	-7.268	**0.057**
≤ 9	8.27 (2.16, 18.50)	-2.114	0.339	50.15 (13.70, 87.30)	-8.441	0.177	29.70 (12.10, 113.00)	-17.269	**0.0003**	107.03 (24.19, 222.40)	-27.825	**0.007**
≥9-11	5.54 (2.22, 13.50)			35.70 (13.40, 57.00)			25.00 (8.23, 56.20)			73.90 (30.19, 130.52)		
>11-14	5.49 (2.52, 11.80)			34.10 (12.60, 66.30)			23.40 (7.88, 42.30)			77.08 (29.30, 126.97)		
>14	2.74 (1.87, 14.90)			18.20 (9.38, 40.70)			10.30 (6.56, 16.10)			39.88 (24.77, 76.34)		
Area of Residence (183)												
Grabouw	5.69 (2.60; 13.80)	2.622	0.264	43.80 (15.70; 76.20)	-16.552	0.012	29.30 (10.70; 61.40)	-9.450	0.067	79.45 (32.78; 149.50)	-23.380	0.033
Worcester	5.60 (2.19; 8.51)			38. 30 (16.00; 69.50)			15.20 (6.78; 30.30)			61.84 (30.35; 115.03)		
Piketberg	4.76 (1.73; 15.30)			29.30 (8.95; 55.20)			16.70 (6.89; 48.40)			72.36 (16.92; 126.50)		
Parent Education Level												
Schooling (181)												
Grade 1-6	5.83 (2.29; 14.90)	-2.801	0.217	31.90(10.20; 62.80)	-1.830	0.776	16.40 (6.89; 43.40)	3.991	0.366	71.72 (27.31; 128.14)	-0.641	0.951
Grade 7-8	6.10 (3.53; 13.90)			40.40 (17.40; 63.60)			28.40 (12.30; 58.60)			86.43 (37.36; 140.10)		
Grade 9-12	3.93 (1.12; 11.10)			29.30 (11.20; 62.60)			14.40 (6.42; 36.40)			54.43 (22.05; 105.84)		
Household Income (178)	5.54 (2.16, 13.60)	-0.001	0.165	32.60 (12.60, 62.80)	0.0009	0.729	16.70 (7.58, 43.40)	0.0009	0.657	68.32 (27.92, 129.52)	0.0005	0.905
≤ R1550	5.15 (2.19; 14.40)	-3.925	0.097	31.05 (10.60; 55.75)	8.598	0.194	15.65 (7.73; 40.25)	10.176	0.050	66.74 (26.35; 117.43)	14.848	0.178
> R1550 – R2449	7.29 (3.93; 14.90)			44.70 (23.80; 87.30)			26.00 (11.00;56.30)			78.27 (43.80; 167.51)		
≥ R2450	3.94 (1.73; 10.90)			26.10 (14.90; 62.60)			16.10 (7.27; 38.30)			51.39 (22.89; 103.04)		
Para- Occupational Exposure/ take-home exposure												
Parent Work in farm (123)												
No	3.86 (1.24; 11.20)	6.377	0.272	31.90 (10.20; 55.60)	33.169	0.064	13.10 (5.38; 29.80)	7.884	0.590	61.01 (16.40; 87.36)	47.430	0.115
Yes	6.35 (2.59; 13.90)			40.80 (17.10; 75.50)			18.40 (8.63; 48.30)			74.33 (36.71; 163.00)		
Current Parental job description (123)												

Non- farmworker	3.86 (1.24; 11.20)	3.861	0.374	31.90 (10.20; 55.60)	8.617	0.523	13.10 (5.38; 29.80)	-1.341	0.902	61.01(16.40; 87.36)	11.137	0.622
Farmworker non-applicator	7.21 (2.52; 14.90)			42.80 (17.10; 87.30)			18.35 (8.63; 48.90)			77.60 (36.85;167.51)		
Farmworker applicator (Supervisor, sprayer, mixer)	3.73 (3.12; 7.65)			28.45 (13.69;53.75)			20.50 (10.94; 35.35)			53.51 (27.59; 108.85)		
Member of Household Work with pesticide (183)												
No	5.49 (2.10; 12.40)	0.060	0.993	31.60 (12.30; 62.80)	24.224	0.186	16.20 (7.58; 40.30)	45.497	0.001	66.10 (28.01; 126.97)	69.781	0.021
Yes	5.82 (4.02; 15.30)			39.65 (17.10; 58.10)			35.15 (10.60; 82.80)			87.69 (24.77; 168.00)		
Pesticide contaminated clothes washed at home (183)												
No	5.54 (2.19; 13.30)	-2.523	0.816	32.60 (12.30; 62.80)	40.897	0.182	16.70 (7.88; 43.40)	29.911	0.209	68.32 (28.01; 128.14)	68.286	0.180
Yes	8.96 (2.11; 17.20)			27.35 (17.10;136.00)			22.35 (4.93; 167.00)			56.81 (22.89; 338.00)		
Reported Pesticide Exposures												
Live on farm (183)												
No	2.37 (1.14; 10.01)	8.416	0.043	24.95 (7.24; 56.95)	28.908	0.014	13.65 (5.95; 28.50)	17.166	0.062	54.48 (16.04; 86.90)	54.490	0.005
Yes	6.55 (2.84; 14.90)			37.80 (15.90; 73.90)			23.00 (8.63; 55.90)			77.77 (35.29; 163.00)		
Reported distance of home to spraying (meters) (183)												
Live in town	2.37 (1.14; 10.01)	3.354	0.018	24.95 (7.24; 56.95)	11.881	0.003	13.65 (5.95; 28.50)	5.238	0.095	54.48 (16.04; 86.90)	20.474	0.002
>100m	6.31 (2.55; 13.25)			41.20 (14.40; 56.15)			38.95 (7.95; 65.45)			89.87(31.65; 131.82)		
51-100m	5.90 (1.12; 17.80)			17.10 (8.45; 44.20)			14.90 (6.20; 32.70)			43.80 (22.58;103.04)		
0-50m	6.63(3.00; 15.00)			39.35 (17.30; 86.40)			22.00 (9.51; 48.60)			78.82 (36.78; 167.75)		
Reported spraying of pesticides on farm (183)												
No	2.52 (1.23; 9.33)	8.586	0.038	24.95 (7.29; 58.30)	26.993	0.021	13.65 (6.00; 27.70)	18.142	0.046	54.48 (16.40; 87.36)	53.721	0.005
Yes	6.70 (2.84; 14.90)			37.80 (15.90; 71.40)			24.50 (9.29; 55.90)			77.77 (35.29;160.28)		
Pesticide drift enters the house (183)												
No	4.81(1.87; 11.20)	1.450	0.732	32.60 (10.80; 61.70)	24.323	0.041	16.20 (7.13; 41.10)	5.823	0.532	65.16 (24.19; 126.50)	31.596	0.111
Yes	7.22 (3.10; 15.10)			34.10 (17.40; 87.30)			23.75 (9.93; 43.90)			72.13 (32.78; 164.08)		
Children plays on farm after spraying (183)												

No	5.51(2.13; 13.50)	-3.733	0.457	32.50 (12.60; 61.70)	26.379	0.062	16.70 (7.88; 41.10)	16.784	0.128	69.69(27.92; 121.10)	39.430	0.094
Yes	5.60 (2.45; 14.90)			37.30 (13.20; 87.30)			21.00 (7.58; 82.80)			68.32 (28.04; 181.00)		
Sources of drinking water (173)												
Closed (Municipal water, mountain water)	4.53 (1.80; 10.80)	4.219	0.314	29.50 (8.95; 58.40)	36.064	0.001	15.30 (6.56; 36.40)	21.775	0.016	61.01(18.68; 103.04)	62.058	0.001
Open (Borehole, river, farm dam, rain water tank)	6.03 (3.93; 16.70)			36.75 (16.00; 104.00)			27.65 (9.93; 58.60)			89.87 (35.29; 199.33)		
Recreational Water Sources (171)												
Closed (Municipal water, mountain water)	4.55 (1.81; 10.75)	4.614	0.281	29.50 (9.57; 58.70)	33.878	0.003	15.40 (6.59; 36.60)	20.630	0.024	61.27 (18.94; 104.72)	59.122	0.002
Open (Borehole, river, farm dam, rain water tank)	6.15 (3.53; 17.20)			35.70 (15.90; 87.30)			25.00 (9.29; 58.60)			79.23 (30.35; 199.33)		
Household Pesticide use (183)												
No	5.26 (2.09; 15.10)	-6.935	0.073	35.70 (12.30; 61.70)	2.461	0.823	16.20 (7.15; 40.20)	15.904	0.061	73.90 (26.88; 121.10)	11.430	0.531
Yes	5.62 (2.33; 11.70)			31.75 (12.80; 64.95)			20.60 (9.05; 47.75)			61.74 (27.98; 141.60)		
Frequency of pesticide application in households (183)												
Never	5.26 (2.09; 15.10)	-4.164	0.068	35.70 (12.30; 61.70)	-4.823	0.457	16.20 (7.15; 40.20)	5.786	0.251	73.90 (26.88; 121.10)	-3.202	0.767
Monthly	5.87 (2.21;11.70)			45.10 (16.40; 98.70)			25.60 (9.38; 60.85)			84.62 (35.07; 167.75)		
Weekly	5.15 (2.49;11.65)			26.05 (9.68; 50.85)			14.80 (7.97; 43.10)			48.74 (26.04; 107.45)		
Recreational Activities (183)												
Swimming in dams/rivers												
No	5.69 (2.11; 13.60)	-1.142	0.785	32.60 (11.20; 66.30)	3.213	0.787	16.70 (7.88; 40.30)	-5.251	0.569	72.36 (27.31; 130.30)	-3.180	0.872
Yes	5.23 (2.50; 12.40)			34.45 (16.55; 61.40)			17.50 (7.23; 45.35)			62.83 (28.02; 128.23)		
Location of the river or dam												
Never swim	5.69 (2.11; 13.60)	-0.793	0.621	32.60 (11.20; 66.30)	3.101	0.495	16.70 (7.88; 40.30)	-1.144	0.746	72.36 (27.31; 130.30)	1.164	0.877
>100 m from farm	3.46 (1.40; 12.23)			43.90 (23.70; 67.25)			19.35 (9.05; 35.90)			68.56 (46.50; 103.02)		
< 100 m from farm	5.71 (3.38; 8.47)			33.30 (13.03; 48.35)			18.25 (6.47; 43.65)			80.88 (22.29; 126.34)		
On the farm	5.23 (2.50; 14.50)			34.95 (14.15; 63.15)			17.45 (7.23; 52.25)			55.32 (28.02; 139.51)		

Helps on the farm												
No	5.54 (2.11; 13.50)	8.255	0.164	32.60 (12.60; 67.50)	-24.239	0.148	16.70 (7.88; 43.40)	-8.510	0.515	68.32 (28.01; 129.52)	-24.494	0.380
Yes	5.23(2.84; 18.50)			33.65 (13.00; 47.10)			18.35 (6.78; 43.40)			68.44 (27.92; 126.94)		
Diet (183)												
Eats crops from vineyard												
No	4.97 (2.09; 11.20)	-2.430	0.555	31.60 (11.20; 61.70)	18.368	0.114	15.30 (7.15; 38.40)	24.408	0.007	65.16 (24.77; 115.50)	40.346	0.036
Yes	6.72 (2.81; 15.10)			38.05 (15.95; 76.85)			30.60 (9.28; 59.60)			75.47 (37.59; 174.50)		
Eats fruits or vegetables from gardens												
No	4.89 (1.82; 11.60)	0.881	0.820	29.50 (11.20; 58.40)	22.502	0.039	14.15 (6.78; 35.80)	24.259	0.004	61.27 (21.99; 106.40)	47.643	0.008
Yes	5.90 (2.84; 13.90)			37.80 (13.70; 73.70)			26.00 (10.50; 56.30)			78.40 (35.29; 164.08)		
Consumption of fish or meat												
Low frequency	5.64 (2.26; 13.40)	-0.927	0.687	37.80 (13.55; 68.15)	-3.288	0.614	23.95 (8.30; 46.30)	-2.008	0.692	72.13 (29.11; 139.30)	-6.223	0.566
Medium frequency	7.30 (2.09; 16.00)			31.75 (11.60; 67.50)			13.25 (9.88; 40.20)			60.76 (26.88; 151.40)		
High frequency	3.86 (2.16; 9.39)			32.40 (13.00; 59.00)			18.00 (7.18; 39.30)			67.03 (28.01; 111.80)		
Consumption of vegetables												
Low frequency	6.41(2.62; 14.30)	-3.843	0.103	31.75 (13.15; 70.45)	-5.952	0.374	16.10 (7.37; 46.30)	-5.183	0.319	78.33 (31.24; 164.51)	-14.978	0.177
Medium frequency	3.86 (1.42; 8.34)			29.30 (11.60; 57.00)			15.30 (6.20; 29.40)			56.53 (22.72; 90.04)		
High frequency	7.02 (2.84; 15.30)			43.10 (13.80; 68.70)			30.05 (9.34; 48.40)			84.87 (39.88; 140.10)		
Soya consumption												
Low frequency	4.76 (1.80; 9.14)	-1.217	0.612	29.10 (10.80; 60.00)	7.103	0.296	13.40 (6.42;31.00)	10.865	0.039	57.57 (16.92; 103.04)	16.750	0.137
Medium frequency	5.83 (2.11; 13.30)			41.80 (15.90; 73.90)			24.90 (9.46; 55.90)			76.93 (28.01; 140.10)		
High frequency	5.60 (3.07; 15.10)			32.40 (9.97; 63.50)			20.20 (7.88; 47.20)			77.08 (32.78; 138.49)		
Nuts consumption												
Low frequency	5.15 (2.00; 14.25)	-1.254	0.610	31.75 (10.50; 57.55)	0.676	0.923	18.20 (6.70; 43.90)	1.1977	0.825	67.67 (25.10; 124.62)	0.620	0.957
Medium frequency	6.00 (2.29; 13.90)			41.25 (16.60; 73.90)			18.40 (9.46; 47.20)			72.13 (36.71; 160.28)		
High frequency	4.67 (2.44; 10.70)			29.50 (11.00; 63.50)			16.00 (7.18; 34.50)			57.57 (22.05; 115.03)		

Appendix G: Instructions to authors

SCIENCE OF THE TOTAL ENVIRONMENT

An International Journal for Scientific Research into the Environment and its Relationship with Humankind

AUTHOR INFORMATION PACK

TABLE OF CONTENTS

ISSN: 0048-9697

DESCRIPTION

Science of the Total Environment is an international multi-disciplinary journal for publication of original research on the **total environment**, which includes the **atmosphere**, **hydrosphere**, **biosphere**, **lithosphere**, and **anthroposphere**. totalenvironment.gif

AUDIENCE

Environmental Scientists, Environmental Toxicologists, Ecologists, Chemical/Environmental Engineers, Environmental Health Scientists and Epidemiologists, Risk Scientists, Environmental Science Managers and Administrators.

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INTRODUCTION

Aims and Scope

Science of the Total Environment is an international journal for publication of original research on the **total environment**, which includes the **atmosphere**, **hydrosphere**, **biosphere**, **lithosphere**, and **anthroposphere**.

totalenvironment.gif-Total Environment

The total environment is characterized where these five spheres overlap. Studies that focus on at least two or three of these will be given primary consideration. Papers reporting results from only one sphere will not be considered. Field studies are given priority over laboratory studies. The total environment is studied when data are collected and described from these five spheres. By definition total environment studies must be multidisciplinary. Examples of data from the five spheres are given below:

stoten-banners.jpg-The five spheres of the total environment

Subject areas may include, but are not limited to:

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- Environmental remediation
- Environmental sources, processes and global cycling
- Groundwater hydrogeochemistry and modeling
- Human health risk assessment and management
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- Noise in the environment
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- Plant science and toxicology
- Remote sensing
- Stress ecology in marine, freshwater and terrestrial ecosystems
- Trace metals and organics in biogeochemical cycles
- Waste and water treatment

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