CHRONIC PESTICIDE POISONING IN WOMEN OF REPRODUCTIVE AGE AND THE EFFECTS ON REPRODUCTIVE OUTCOMES AT NAIVASHA COUNTY HOSPITAL, MARCH 2016

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A dissertation; submitted in partial fulfilment of the requirements for the award of the degree of Master of Medicine in Obstetrics and Gynaecology, School of Medicine, University of Nairobi.

APRIL, 2016

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DECLARATION

I hereby declare that this dissertation is my original work and has not been presented to any other academic instituition for evaluation and examination to the best of my knowledge.

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Supervisors' Approval

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Acknowledgement

1.The funding was from the Linked-Strengthening Maternal, Newborn and Child Health Research Training in Kenya. The grant is linked to Partnership for Innovative Medical Education in Kenya (PRIME-K). The project was supported by Award Number 5R24TW008907 from the US National Institutes of Health. The content is solely the responsibility of the authors and does not necessarily represent the official views of the US National Institutes of Health

2.The Clinical Chemistry Laboratory, School of Medicine, University of Nairobi for the laboratory support.

ABBREVIATIONS

AChE	Acetylcholinesterase
ANC	Antenatal clinic
APH	Antepartum haemorrhage
ERC	Ethics and Research Committee
FP	Family Planning
KNH	Kenyatta National Hospital
LBW	Low birth weight
MCH	Maternal and Child Health
MEPI	Medical Education Partnership Initiative
NCH	Naivasha County Hospital
NBU	New Born Unit
OP	Organophosphate
PNC	Postnatal clinic
PPH	Post partum haemorrhage
PPROM	Preterm premature rupture of membranes
PRIME-K	Partnership in Innovative Medical Education in Kenya
UNICEF	United Nations Children's Fund
UoN	University of Nairobi
WRA	Women of Reproductive Age
WHO	World Health Organization

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DEFINITION

Abortion Rate	Number of abortions/Number of pregnancy x100
Delayed Fertility	Inability to conceive despite 12 months of regular and unprotected intercourse in women below 30 years or 6 months in those above 30 years of age.
Pesticide poisoning	Acetylcholinesterase activity < 75%
Pregnancy	Period of amenorrhea coupled with positive pregnancy detection test (PDT) or clinical evidence of developing fetus.
Abortion	Fetal loss at less than 20 weeks of gestation
Premature delivery	Birth of a fetus between 21^{st} and 36^{th} week gestation
Rate of prematurity	Number of premature births/Number of pregnancy x100 *****includes abortions,stillbirths,premature births and term births
Stillbirth	Fetus born after the 21^{st} week of gestation or weighing above 500g but without any evidence of life at birth.
Stillbirth Rate	Number of stillbirth/Number of pregnancy x100
Term delivery	Birth of a fetus after 37^{th} week of gestation
TORCHES	Toxoplasmosis, Rubella, Cytomegalovirus, Herpes, Syphilis

1.0 Abstract

Background:Pesticide exposure is a major health hazard among agricultural farm workers. Countries that rely on agriculture as economic mainstay, like Kenya, may therefore have a big part of their population facing this potential danger. Many studies have shown various health hazards associated with chronic pesticide poisoning especially the organophosphate and carbamate groups, which are the most commonly used. These include carcinogenicity, neurotoxicity and adverse reproductive effects in both males and females (1). Naivasha is one of the regions in Kenya where heavy commercial agriculture is practised and with heavy pesticide use. A study in 1994 showed Naivasha leading in pesticide poisoning in Kenya, with 36% of the workers having chronic poisoning (2). Considering the high level of poisoning and the known adverse reproductive effects, there is need to establish the prevalence of this problem in the women of reproductive age .

Research Question: What is the magnitude of pesticide poisoning among women of reproductive age working in the flower farms in Naivasha?

Broad Objective: To determine the prevalences of pesticide exposure and adverse reproductive outcomes among women of reproductive age working in Naivasha flower farms.

Study Design: A Cross-sectional study, carried out at Naivasha County Hospital, among women of reproductive age (WRA), working on the farms and coming for MCH services.

Sample Size: 181 women attending the MCH clinic were interviewed about their previous obstetric performance. Data was collected using questionnaires and blood analysis done to determine their levels of serum acetylcholinesterase activity.

Results: AChE activity depression prevalence was found at 22.7% in the study population with actual poisoning being low at only 3.9%. Lower levels of AChE activity were found to be associated with incidence of abortion and neonatal deaths. Women who reported to have had abortions had a significantly lower mean AChE activity of 4533.3 U/L compared to 5460 U/L in those who had not and 4588.75 U/L in women who had had neonatal deaths compared to 5475.6 U/L. Other outcomes were not significantly associated with AChE activity levels.

Conclusions Pesticide exposure is a significant problem among the flower farm workers, requiring enhanced protective mechanisms. This exposure is associated with adverse health effects-reproductive, respiratory and allergic skin conditions

Recommendations: Get baseline AChE levels for farm workers at time of employement then 3 monthly follow ups. Enhance protection of workers by addressing all the weak areas such as enhancing awareness. Prospective study to objectively assess level of AChE depression in this population.

Study Relevance: The findings will raise awareness about the magnitude of this problem and hence inform policy regarding work place safety.

2.0 Introduction

Pesticides can be classified into five classes; insecticides, fungicides, herbicides, nematocides, and rodenticides. Insecticides are the main class of pesticides and it has three chemical classes which include organophosphates, carbamates and thiocarbamates. Pesticides are widely used to prevent and control pests, diseases, weeds, fungi and nematodes in plants. Organophophates and carbamates are the classes most commonly used, although practically, there is use of varied pesticide categories whose adverse effects may be synergistic or antagonistic. Crop losses due to these pests are estimated to be 10-30% in developed countries and upto 75% in developing countries (3), hence the dire need for use of the pesticides. In the United States, approximately 40 billion dollars worth of increased agricultural production is realized due to pesticide use annually; at the same time, the human health and environmental costs arising from this use is estimated at about 9.6 billion dollars (4). Therefore, while their use helps to minimise huge losses, it also brings along numerous challenges such as adverse health effects.

Organophosphates(OP) were first discovered in 1854, but it was not until in the 1930s that their toxicity was noticed. They are nerve poisons which kill the target pests and also among the most potent acute toxins to vertebrate animals. The mode of action of organophosphate and carbamate insecticides is by inhibition of acetylcholinesterase leading to accumulation of acetylcholine, the neurotransmitter at autonomic nervous system synapses in the brain, skeletal neuromuscular junction and adrenal medulla. This gives rise to most of their acute poisoning manifestations (5). Poisoning occurs when these chemicals affect non target organisms such as humans and wildlife. Generally, it is deemed that the poisoning is from the active ingredients of the pesticides, though it may also be from associated impurities, solvents, carriers, emulsifiers and other constituents of the formulated pesticides.

Acute poisoning occurs when one is exposed to high doses of the pesticide at once and is seen amongst those with accidental poisoning especially in children, in cases of suicide or in pesticide formulators. Chronic poisoning occurs when exposure occurs at
low levels of the pesticides, repeatedly over long periods of time, usually at least 90 days or more. It is commonly seen among pesticide manufacturers and farmworkers as well as exposure through the food chain. Food chain exposure occurs due to pesticide residues

in food, water, soil and plants, plus off-target drift in the air. Exposure may be through inhalation, ingestion and skin contact (6).

Acute organophosphate poisoning presents dramatically with symptoms like sweating, lacrimation, salivation and diarrhoea-hence is easier to recognize. Chronic poisoning may manifest gradually over a long period of time and some manifestations may not be directly linked to the pesticides. The effects may also last a life time with some being passed on to future generations. Hence it is hard to assess the exact extent of the effects of chronic pesticide poisoning due to the highly varied manifestations and timimg of these manifestations. It is therefore likely that the burden of pesticide poisoning is so huge both on the health sector as a whole and on the affected individuals (7,8). It poses psychological, financial, and social burden both to the families and the health sector.

Chronic poisoning has been shown to be associated with cancer, neuro-developmental and behavioral effects, neurodegenerative diseases, cardiovascular diseases, birth defects and adverse reproductive effects plus respiratory diseases such as chronic obstructive pulmonary disease (COPD), asthma and pneumonia. Obesity, type 2 diabetes and metabolic disease have been observed as well (9).

The focus for this study will be on the reproductive effects. Exposure to pesticides during any stage of the reproductive cycle can have serious and devastating effects on both mother and the unborn child. Periods of rapid growth, especially in utero, are the most sensitive to these chemicals with the most critical period being 3 months before to 1 month after conception (9,10). It is however, a challenge to assess fetal exposure to the pesticides in early pregnancy because this would need amnioscentesis to obtain amniotic fluid which is the best medium to characterize direct fetal exposure (11). Following delivery, it may be relatively easier to do the aseessment by studying the placentae to reveal any in utero exposure (12).

Despite these difficulties, a number of studies have shown that preconception chronic pesticide exposure of either mother or father may cause adverse pregnancy outcomes. These include early pregnancy losses, preterm deliveries, still births, neural tube defects such as anencephaly and other birth defects.There may also be deviation from the normal

male-to female birth ratio with fewer males being born than normal. The exposed foetuses have higher risks of low birth weight (LBW), oncogenicity, mutagenicity, carcinogenicity and neurotoxicity leading to various mental disorders (13,14,15,16).

Postnatally, exposure to some pesticides have been associated with shorter durations of brestfeeding due to their interference with mother's milk production as a result of oestrogenic effects (17,18). Certain protective biological products (like enzymes) do not develop for several months after birth hence neonatal exposure may lead to accumulation of these chemicals in the brain. This is confounded by the blood-brain-barrier that is not well developed in infants thereby allowing the pesticides to cross over into the fetal brain (19).

3.0 Literature Review

Several studies have shown significant pesticide poisoning among farm workers globally, with various associated adverse effects. Evidence has shown association between chronic occupational exposure and poisoning with lung damage and respiratory failure, haematopoietic cancers, soft tissue sarcomas, aplastic anaemia and related blood dyscrasias.(2)

Various adverse reproductive outcomes have been shown such as male infertility, delayed conception (sub-fertility), early pregnancy losses, preterm premature rupture of membranes (PPROM), preterm births, stillbirths, low birth weight (LBW), neonatal deaths and lack of breast milk. Post-conception exposures have been associated with a various negative reproductive outcomes, including late spontaneous abortions (20). One study found that occupational poisoning during the first and second trimesters was associated with increase in stillbirths due to all causes, while poisoning in first two months of gestation contributed to an even higher risk of stillbirth due to congenital anomalies (21).

These reproductive effects may be seen regardless of which partner had the exposure-the female or the male partner. A study done in Bogota area, Colombia, showed definite poisoning among farm workers with increased incidence of adverse reproductive outcomes in the female workers as well as the spouses of the male workers. The abortion rate was 8.8% after the population was exposed to pesticides for periods ranging from 6 months and above compared to 4.3% in the same group before pesticide exposure (22).

However, it is important to point out that most of the studies have had limitations in showing direct cause-effect association. This is largely due to the fact that most of the chronic effects manifest after years or decades hence there is a challenge in associating them to the exposure. It may be easier to show this cause-effect relationship with acute pesticide poisoning due to short interval between exposure and outcome. There have been many cases reported of adverse pregnancy outcomes from such acute poisoning. WHO estimates that there may be 3 million serious acute poisoning cases annually (5). These are mainly cases of attempted suicide and are obtained from hospital data hence may only represent a fraction of the actual situation.

A survey of self reported minor poisoning carried out in the Asian region, estimated that there could be as many as 25 million agricultural workers in the developing world suffering an episode of poisoning each year (2). In Canada in 2007, more than 6000 cases of acute pesticide poisoning occurred (23). In Africa, a study done in South Africa in 2006 showed an increase in abortion rate (spontaneous) among female farmers exposed to pesticides at 6.7% above general population (20). Another study in Zimbambwe, Kwekwe district, in 2006 showed organophosphate poisoning prevalence of 24.1% among farm workers, with acetylcholinesterase (AChE) activity depressed below 75% (24). Developing countries use less than 20% of the global pesticide production but account for upto 70% of the total cases of acute poisoning in the working place (25).

In 2012, Yan SM et al (26), did a meta analysis on all articles showing relationship between pesticide exposure and adverse pregnancy outcomes published worldwide from the year 1990 to Feb 2012. A total of 12 articles were collected and analysed. It concluded that pesticide exposure occurred in farmers and increased the risks of spontaneous abortion (combined Odds Ratio[OR] 1.52), delayed conception (OR 1.43), premature birth (OR 1.33), still births (OR 1.9), birth defects (OR 2.02), LBW (OR 1.62) and neonatal death (OR 2.18).

The exact global extent of long term risks, however, still remains poorly defined and many gaps still exist. Research is still ongoing to establish connections between perinatal pesticide exposure and certain diseases. The diseases which have been somehow linked but are still under further studies include allergies and hay fever (27), neurodegenerative diseases such as Parkinson's and Alzheimer's diseases (28), neurodevelopmental delays (29, 30), neurobehavioural problems (31), hyperglycemia (32), obesity, diabetes and depression (33). Inspite of the existing gaps, statistically significant association has been shown by other studies between pre- and perinatal pesticide exposures and several adverse outcomes in the children born (34, 35, 36, 37, 38, 39).

In the East Africa region, a study by Ngowi A.V. et al in 2007 looking at pesticide use practices, perceptions, cost and health effects among the smallholder vegetable farmers in Northern Tanzania found out that upto 68% of the farmers reported feeling sick after routine application of pesticides (40). In Uganda, Jackline Bonabana in 2008 conducted a

study to assess the health and environmental benefits of reduced pesticide use and estimated that about 700,000 of Uganadan population were at risk of pesticide poisoning annually (41).

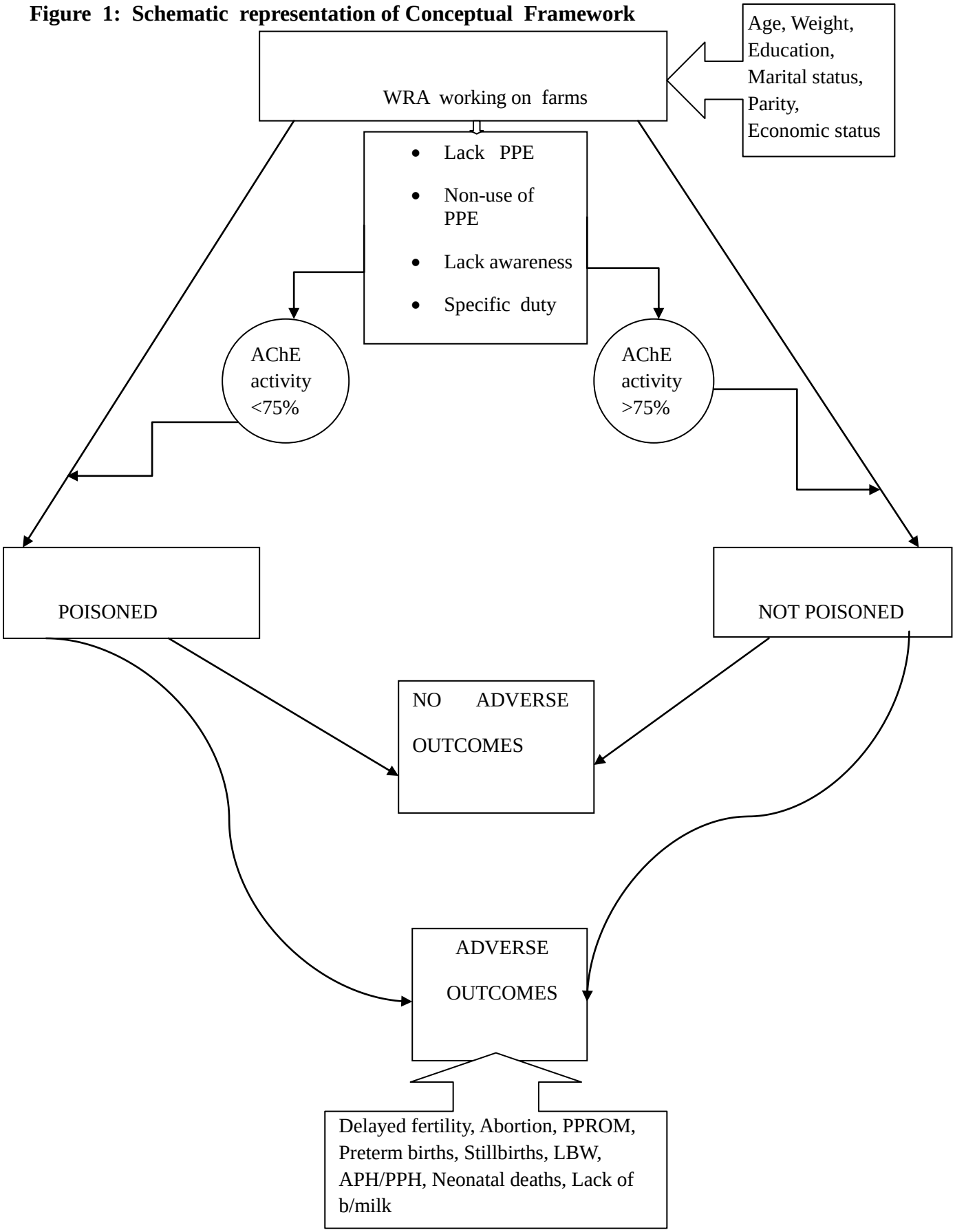
Locally, a study done by Ojwang S.B et al over a four year period from 1983-1986 showed significant accumulation of organochlorine pesticide residues in maternal tissues. It showed significant accumulation in maternal blood, adipose tissue, breast milk and cord blood of their infants and at almost equal amounts (42). Another one in Naivasha in 1994 by Ohayo-Mitoko to assess the extent of pesticide poisoning among exposed farm workers showed clear evidence between exposure and poisoning. It measured serum AChE levels whose inhibition/depression is a clear indicator of organophosphate and carbamate poisoning, which form the main pesticides in use in Kenya. It compared levels in exposed farm workers (pesticide applicators) and non exposed workers (from rural agricultural areas) covering four agricultural zones namely: Naivasha flower farms, Homa Bay cotton growers,Wundanyi vegetable growers and Migori tobacco growers (3). It showed marked AChE inhibition in the exposed group at 33% compared with non exposed at 4%. Naivasha was highest at 36%, Homabay 35%, Wundanyi 33% and Migori 26% with 115 out of 390 cases having 60% individual inhibition.

The above findings are similar to those of another study in 2014 by Wafula C.N et al which showed AChE inhibition rate of 18% among children passively exposed to pesticides by their contact with parents who work on the farms in Naivasha(43). Macharia I. et al in 2015 published the results of a prospective cohort study done between 2005 and 2008 to find the risk factors of pesticide poisoning among farmers in certain districts in Central and Eastern Kenya. It found out that over the 4 years, poisoning prevalence increased in most regions where use of protective gear was not common. However, ironically, use of gumboots was associated with increased poisoning probably due to pesticide pouring into them hence ensuring continued absorption for the duration they are worn (44).

There is therefore, great need to assess the impact of this poisoning locally and this study looked at its prevalence particularly in the women of reproductive age (WRA) and its effects on their reproductive performance

4.0 Conceptual Framework

Figure 1: Schematic representation of Conceptual Framework



KEY:

WRA- Women of reproductive age

PPE- Personal Protective Equipment

AChE- Acetylcholinesterase enzyme

PPROM- Preterm premature rupture of membranes

LBW- Low birth weight

APH- Antepartum haemorrhage

PPH- Postpartum haemorrhage

B/milk- Breastmilk

4.1 Narrative of Conceptual Framework

Women of reproductive age working on the farms may be exposed to the pesticides due to a number of reasons such as lack of awareness of the dangers (knowledge gap), lack of personal protective equipment (poor working environment), non-use of PPE (negative attitude), or unavoidably despite taking all the precautions (inadvertent). Exposure was considered significant if it lowerered AChE activity to below 75%, which actually denotes pesticide poisoning. Hence based on the levels of AChE activity, the participants were categorised into two groups namely Poisoned and Not poisoned.

Regardless of exposure status, the women may still suffer from some of the adverse pregnancy outcomes. This may be due to other confounding factors such as chromosomal abnormalities, hormonal insufficiency, genital tract abnormalities, infections including TORCHES, nutritional factors. Others may be undiagnosed maternal haematological conditions such as anaemia and bleeding disorders.

Assessment was therefore done of the occurrence of these outcomes in both exposed and non-exposed arms and comparissons done at the end.

5.0 Justification of the study

Despite the risks associated with pesticide exposure and poisonng as shown in the studies highlighted earlier, no study has been done to look at the magnitude of the problem in the flower farm workers of reproductive age in Naivasha.

This study's findings and recommendations should serve to raise awareness about the magnitude of the problem and inform government policy plus regulatory frameworks regarding workplace safety. Secondly, should further inform the owners of the farms on the need for improved personal protective equipment (PPE) recommendations and use at the work place. Finally, it should help improve the quality of life and health status among populations in similar conditions.

6.0 Research Question

What is the magnitude of chronic pesticide poisoning among women of reproductive age working on the flower farms in Naivasha?

7.0 OBJECTIVES

7.1 Broad objective

To determine the prevalence of pesticide poisoning among women workers in the flower farms in Naivasha presenting at Naivasha County Hospital.

7.2 Specific objectives

1.To determine the prevalence of pesticide poisoning among the female workers in the flower farms.

2.To determine the factors associated with chronic pesticide poisoning (abnormal acetylcholinesterase activity) among the exposed women working on the flower farms.

3.To determine the prevalence of adverse reproductive outcomes.

4.To determine the socio-demographic characteristics of women presenting at Naivasha County Hospital MCH (FP, ANC, PNC) who are flower farm workers.

8.0 METHODOLOGY

8.1 Study Design

This was a health instituition based cross-sectional study. The main aim was to assess the prevalence of chronic pesticide poisoning among women of reproductive age working on the farms, factors associated with such poisoning and the prevalence of adverse reproductive outcomes among this group of women.

8.2 Justification of the study design

The choice of cross-sectional study design was due to the fact that from the onset of the study, we did not know who had the outcome of interest and who did not-which would have been the basis of a case-control study that is commonly used to study "associations" between exposure and outcome. The only thing in common for the study population here was seeking services at Naivasha District Hospital's MCH and working in the flower farms while both the exposure(pesticide poisoning) and outcome(adverse reproductive outcomes) status were to be determined at the same time by this study. It was designed to give both the prevalences of poisoning as well as adverse reproductive outcomes in this given population.

8.3 Study Site

Study was done at Naivasha Couty Hospital MCH (FP, ANC, PNC) clinic. This is a hospital set within an area of large scale commercial farming, employing many workers and majority of whom are women of reproductive age. It is the nearest referral hospital for the farm workers who may require some specialised treatment which is not available in the satellite clinics within the farms. It is a level 4 hospital located in Naivasha town in Nakuru county. It is lies about 100km North west of Nairobi, with a bed capacity of 180.

The hospital runs its MCH clinics daily on weekdays and on average, about 40 clients are attended to per day, averaging 800 clients per month and 9,600 annually. The clinics are run by six nurses offering antenatal care, well baby services and family planning services. They are assisted by upto four surbodinate staff, offering supportive services. They offer contraceptive services such as pills, depo injections, intrauterine contraceptive devices (IUCDs), implants and barrier methods. Cases requiring tubal ligation are booked and procedure done by medical officers weekly. High risk cases are referred to the specialist

Antenatal clinics (ANC), Gynaecological outpatient clinics (GOPC) as well as Paediatric outpatient clinics (POPC) to be seen by medical officers and consultants.

8.4 Study population

The study populatlion were women of reproductive age presenting at the MCH clinics of Naivasha County Hospital for routine care such as family planning, antenatal and postnatal care services.

8.5 Inclusion criteria

Patients were recruited into the study if they met the following criteria: aged 15 to 49 years, had worked on the farms for at least three months and had had previous confirmed pregnancy either clinically or by pregnancy detection test (PDT). In addition, they must have given informed, voluntary consent before being recruited.

8.6 Exclusion criteria

Patients were excluded from the study if they had underlying chronic medical conditions such as hypertension, diabetes, organ disorders and connective tissue or immune diseases. Those who declined to give consent were also excluded even if they would have otherwise qualified to participate**.`**

8.7 Sample Size

A study done in 1994 in Naivasha area showed exposure(p) levels of upto 36%(3). At 95% confidence interval and absolute precision (d) of 7%, using the formula by Dobson (45):

Sample size(n)=$Z_{1-\alpha/2}^2 p(1-p)/d^2$

Where:

- $Z_{1-\alpha/2}$ is standard normal variate and at 5% type 1 error ($p<0.05$), it is 1.96
- p = expected proportion in the population with the characteristic of interest from previous studies/ pilot studies = pesticide exposure level in Naivasha of 36%
- d = absolute error/precision = 7%

therefore,

n=$1.96^2(0.36)(0.64) / 0.07^2$=181=181 participants

8.8 Sampling technique

All clients coming to the MCH (FP, ANC, PNC) clinics were sequentially requested to participate in the study. Those who consented and met the inclusion criteria were recruited into the study until the required sample size was achieved. Therefore any client attending MCH clinics during the recruitment duration stood equal chance of participating in the study.

8.9 Recruitment method

The study participants were recruited from the clients attending the MCH clinic at Naivasha County Hospital. Only those who worked on the farms were recruited. The clients were talked to as they waited to be attended by the clinic staff. Each client was talked to individually in privacy by the principal investigator/research assistant. The nature and purpose of the study was explained and those who accepted were asked to sign consent form and hence became participants. Once recruited, a mark was put on their clinic cards to avoid repeat recruitment.

8.10 Data Variables

8.10.1 Dependent Variables

The main outcome of interest which is the dependent variable was the prevalence of poisoning with the farm pesticides by virtue of being workers on the farms and occurrence of the adverse reproductive outcomes. These adverse reproductive outcomes included delayed conception, abortions, preterm deliveries, still births, neonatal deaths and occurence of obstetrical complications such as antepartun and postpartum haemorrhage.

8.10.2 Independent Variables

The independent variables include those factors that may affect the probability of the above outcomes occurring, either by increasing or reducing it. These include specific job descriptions, duration of employment, hours worked per week (exposure duration), availability and use of PPE, level of awareness of the pesticide hazards (knowledge, attitude and practices of the farm workers) and availability of interventions to minimise effects of accidental exposures. Others are the demographic and socio-economic characteristics such as age, weight, marital status, parity, level of education and economic status.

9.0 Data Collection and Management

9.1 Research Instrument

Data was collected by use of pre-tested interviewer administered questionnaire after obtaining consent from the participants. The questionnaire obtained information regarding exposure status by asking about the specific duties performed on the farms, hours of work per week, and the duration one has worked on the farms. Levels of protection against pesticide exposure was assessed by asking about the availability and use of protective gears/procedures. Possible adverse effects of the exposure was assessed by asking about the obstetric history with regard to delayed conception, abortions, PPROM, preterm births, still births, LBW, NBU admissions, neonatal deaths, shortened breast milk production and maternal obstetrical complications such as APH and PPH.

5mls of venous blood samples were collected from the brachial vein using a 5ml vacutainer. It was centrifuged and 1.5ml of serum obtained from it. This was then stored at $4^{o}C$ and the frozen serum later transported to the University of Nairobi's Clinical Chemistry Laboratory for analysis of the Acetylcholinesterase enzyme activity levels.

A 2X2 contingent table was used to compare the prevalence of the various adverse reproductive outcomes in those poisoned and those not poisoned. Those poisoned and had adverse outcomes were considered True Positives (TP) while those not poisoned and did not have the adverse outcomes were considered True Negatives (TN). Those poisoned but did not have the adverse outcomes were considered False Negatives (FN) while those without poisoning but had the outcomes were False Positives (FP).

AChE test results for each participant was included in the questionnaire under **No 16** for each participant.

9.1.1 Reliability/Quality Control

The research assistants underwent a one day training on the study protocol and procedures-including contents of the questionnaire and how to correctly fill them. The training also encompassed how to interact with the participants and communication skills so as to ensure clarity of communication between them and the participants.

The questionnaire was pre-tested on 18 interviwees from the study population (10% of sample size) to assess the flow of questions and their clarity to the interviewee plus their ability to collect the required information. The pre-tested questionnaires were analysed and necessary modifications made to clear any ambiguities before producing the final copies. The pretesting also ensured the assistants were competent in carrying out the research.

The principal investigator closely supervised the process of data collection- scrutinizing filled questionnaires promptly to ensure completeness and consistency. Double recruitment was avoided by putting a mark on the antenatal cards/books of those already recruited. Cases of double recruitment detected were withdrawn and discarded.

9.1.2 Quality Assurance

Blood sample collection was done by a qualified phlebotomists from the laboratory of the Naivasha County Referral Hospital, who then oversaw the handling and storage of the specimens before they were promptly transported to Nairobi for analysis.

Procedure for sample collection

The skin was sterilized with a spirit swab then 5mls of venous blood collected from the brachial vein using 5ml plain vacutainer by phlebotomists/laboratory technologister after the interview. The samples were left to clot after which serum was separated at the Naivasha County Hospital Laboratory by the laboratory technologists, cooled and kept at 2-8 ^{o}C, then transported to the University of Nairobi's Clinical Chemistry Laboratory by principal researcher in cool boxes to maintain the frozen state, within 7 days of collection. Analysis was done by the laboratory technologists at the Clinical Chemistry lab of the Unversity of Nairobi's Medical School.

The tests were run using Kinetic colorimetric determination of cholinesterase activity according to the DGKC recommendations in serum by SENTINEL DIAGNOSTICS on COBAS MIRA analyzer machine to determine the levels of AChE. Manufacturer's instructions on sample collection, processing and analysis were followed and appropriate quality control (QC) and quality assuarance (QA) protocols followed (see appendix V).

9.2 Data Analysis and Interpretation of results

Filled forms were accessible only to the investigator and were kept safely for future use. Data was entered and managed in Microsoft Access 2013 database. Statistical analysis was conducted in SPSS version 21.0 software. The study polulation was described using socio-deomographic and environmental variables. Categorical variables was summarized into percentages while continuous data was presented as means (standard deviations) or medians (interquartile ranges). Serum AChE activity levels <75% were considered to have poisoning while those above 75% were considered not to have poisoning. Prevalence of chronic pesticide poisoning was calculated as a percentage of participants with serum AChE activity levels <75% presented with 95% confidence interval (CI).

Prevalence of previous adverse reproductive outcomes which included delayed conception, abortions, preterm deliveries, still births, maternal obstetrical complications (APH and PPH), New Born Unit (NBU) admissions, shortened breast milk production and neonatal deaths were presented as percentages with 95% CI. The extent of the adverse outcomes were compared between the poisoned and the non poisoned groups to determine if there was any added risk of these adverse outcomes among the poisoned population. Comparison with poisoning was done using Chi square test of associations for categorical variables and Student's t or Mann Whitney U tests for continuous data. Odds ratios was calculated and presented as estimates of relative risk of adverse reproductive outcomes associated with poisoning. Multivariate analysis using logistic regression analysis was done to determine whether poisoning was independently associated with adverse reproductive outcomes while controlling for confounding factors. Statistical significance will be set at p values of 0.05. From *Table 1* above, OR = ad / bc.

10.0 Ethical considerations:

10.1 Approval

The proposal was submitted to the KNH/UoN Ethical Review Board for review and approval. Once approved, further consent was sought and obtained from Naivasha County Hospital management before commencing the study.

10.2 Informed Consent

Participants were individually informed about the study through an oral explanation regarding the purpose and procedure of the study. Potential risks and rights of the participants was clearly explained by the investigators. Participants were then given a consent form written in the language they understand which they were required to read through then sign as sign of their willingness to participate in the study. They were, however, free to withdraw from the study at any stage or decline to answer any questions in the questionnaire without any penalties.

For those below 18 years, consent was given by the guardians after being provided with all the relevant information as provided above, regarding the study.

10.2.1 Confidentiality

Confidentiality was strictly assured to the participant, with identity and other details kept secret to the principal investigator. Names and medical identity numbers did not appear on the questionnaires and any other documents associated with the study. Each participant was assigned a serial number for identification purposes during the study so as to link them with the results and enable follow up where necessary. Completed questionnaires were handled by the principal investigator only and the information obtained was only used for the purposes of the study.

10.2.2 Risks and Benefits

There were minimal risks to the participants during the study especially with the collection of blood samples for laboratory analysis. Such risks included infection and the pain from needle pricks. These risks were clearly explained to the participants prior to being enrolled in the study.

Participants were not compensated for their participation in the study. Participation was voluntary, without any direct individual benefits or incentives attached. However, the findings so obtained were shared with the relevant authorities to help in formulation of appropriate policies which would be beneficial to the participants.

10.3 Information Sharing

The findings of the study were submitted to the Department of Obstetrics and Gynaecology, University of Nairobi in partial fulfilment of the requirements for the award of the degree of Master of Medicine, Obstetrics and Gynaecology, to the funding organization Medical Education Partnership Initiative (MEPI) and Partnership in Innovative Medical Education in Kenya (PRIME-K), to the relevant authorities involved in policy formulation and implementation such as the administration of Naivasha County Referral Hospital and Nakuru County Government. They would also be presented in different meetings, workshops and seminars as need may arise as well as be published in a peer reviewed scientific journal.

11.0 RESULTS

A total of 200 participants were recruited into the study. These were women of reproductive age who were attending MCH clinic services at Naivasha County Referral Hospital(NCRH). All of them were working at the commercial horticultural/flower farms found in Naivasha. Blood samples were collected from all of the 200 who participated, 14 serum samples were found to share serial numbers hence were disqualified. Out of the remaining 186, five questionnaires had some missing data and were therefore also disqualified from the study. Therefore, a total of 181 participants' data and samples were used in this study. This was in keeping with the required sample size of 181.

Table 1

Socio-demographic characteristics of WRA working in flower farms in Naivasha

Variable	**Frequency (%) n=181**
Mean age (SD)	26.8 (4.5)
Mean Body weight (Kg)	62.6 (8.5)
Marital status	
Single	113 (62.4)
Married	47 (27.1)
Separated/Widowed	19 (10.5)
Education	
None	3 (1.7)
Primary	94 (51.9)
Secondary	74 (40.9)
Tertiary	10 (5.5)
Resident of Naivasha	
Yes	178 (98.3)
No	3 (1.7)
Ever lived in flower farms	
Yes	46 (25.4)
No	135 (74.6)

The mean age of the participants was 26.8 years which is the average age of the women seeking services at NCRH,with standard deviation of 4.5 years. Average body weight is 62.6 Kilograms(Kg) with stanadard of deviation of 8.5 Kilograms. Most of the women of reproductive age working on the farms being seen at NCRH are single,72.9%,with 27.1% being married. Out of those single, a larger percentage(62.4%) had never been into any marriage while the rest(10.5%) were either widowed or separated. Majority of the

participants(98.3%) had some form of education, with only 1.7% being illiterate. Of those with education, most had basic primary level education with only 5.5% attaining tertiary education.

Table 2

Occupational history of WRA working in flower farms in Naivasha, March 2016

Variable	Frequency (%) n=181
Occupation at the farms	
Weeding	40 (26.7)
Grading	18 (12.0)
Packaging	3 (2.0)
Picking	27 (18.0)
Spraying	2 (1.3)
Office work	9 (6.0)
General work	51 (34.0)
Working hours/ week	
<10	18 (12.0)
10-20	4 (2.7)
31-40	1 (0.7)
>40	127 (84.7)
Did you use protective gear	
Yes	132 (88.0)
No	18 (12.0)
Protective gear	
Caps	24 (18.2)
Goggles	5 (3.8)
Mask	63 (47.7)
Overall	116 (87.9)
Gloves	72 (54.5)
Gum boots	124 (93.9)
Does your partner work on the farm	
Yes	57(31.4)
No	62 (34.3)
N/A	62(34.3)

This table shows the details of specific duties performed on the farms and the working environment. A big percentage of the participants,60%, work in direct contact with the farm produce-either weeding, spraying, picking, grading or packaging the produce.

Most of the workers work for over 40 hours per week(84.7%) which acceptable according to labour laws in Kenya and there is good use of protective gear whereby majority use gumboots and overall.

Table 3

Average duration of work in the Naivasha flower farms among WRA

	Overall	<75%	>=75%	P value
Duration at the farm (Years)	3.6 (3.3)	5.9 (4.8)	3.5 (3.2)	0.129

The average duration that has been spent working on the farms is 3.6 years, with those poisoned having longer periods of 5.9 years while those non poisoned being 3.5years

Table 4

Medical history of WRA working in flower farms in Naivasha

Variable	**Frequency (%) n=181**
Do you suffer any recurrent/chronic health problem	
Yes	57 (31.5)
No	124 (68.5)
Chronic health problem	
Asthma	15 (26.3)
Body itchiness	1 (1.8)
Chest problems	6 (10.5)
Chronic eye itchiness	3 (5.3)
Chronic lacrimation	1 (1.8)
Chronic sneezing	3 (5.3)
Eczema	3 (5.3)
Exertional dypnoea	1 (1.8)
Rhinitis	5 (8.8)
Skin condition	11 (19.3)
Skin puriritis	6 (10.5)
Not specified	2 (3.5)

A third of the workers have had one or more health complications with majority having asthma (26.3%) and skin conditions at 17.5%. Awareness level was noted to be very high about dangers of pesticide poisoning with 80.7% relating their health problems to their occupational exposure.

Table 5

Prevalence of pesticide poisoning as indicated by serum AChE Enzyme Activity in WRA working in flower farms in Naivasha, March 2016

Variable	Mean (SD)/ n (%)	95% CI	Average duration worked in the farms(yrs)
AChE			
Mean (SD)	5413.9 (1192.5)	5241.5 – 5588.4	
Activity levels, (% of normal)			
<75%	7 (3.9)	1.1-7.2	5.9 (4.8)
75 to <80%	7 (3.9)	1.1-7.2	2.8 (1.1)
80 to <85%	4 (2.2)	0.6-4.4	7.0
85 to <90%	15 (8.3)	4.4-12.2	3.5 (2.1)
90 to <95%	8 (4.4)	1.7-7.7	4.3 (3.3)
≥95%	140 (77.3)	71.3-83.4	3.5 (3.4)

Mean AChE activity lies within the normal range of 4900-11,900 U/L for this study-at 5,413.9U/L. Majority of the participants showed no poisoning with only 3.9% having AChE activity below 75% which is the indicator of poisoning. Many exhibit depressed enzyme activity (less than 100% but above 75%) which indicate exposure to pesticides but do not meet the threshold of poisoning. Enzyme inhibition is evident in 22.7% of the participants but poisoning is in 3.9%.

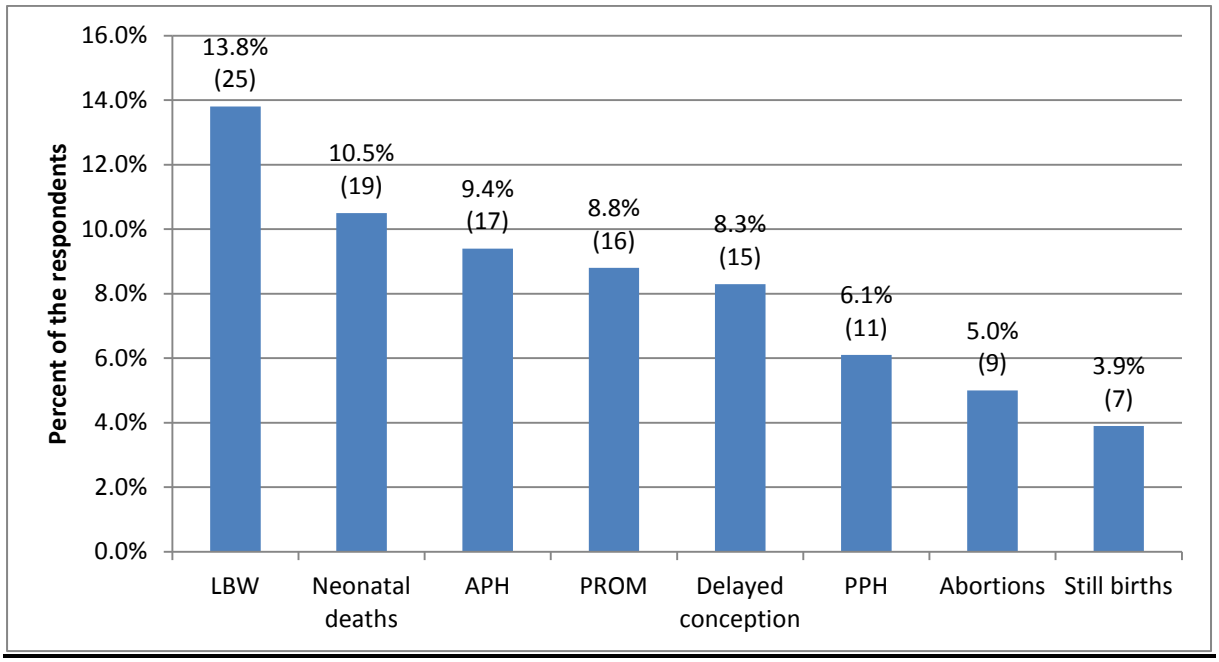
Table 6

Factors associated with pesticide poisoning among WRA working in Naivasha flower farms, March 2016

Variable	AChE<75%	AChE>75%	P value
Mean age (SD)	27.9 (3.9)	26.7 (4.5)	0.502
Mean Body weight (Kg)	61.1 (5.8)	62.7 (8.6)	0.642
Marital status			
Single	5 (71.4)	108 (62.1)	0.649
Married	2 (28.6)	47 (27.0)	
Separated/Widowed	0	19 (10.9)	
Education			
None	0	3 (1.7)	0.775
Primary	3 (42.9)	91 (52.3)	
Secondary	4 (57.1)	70 (40.2)	
Tertiary	0	10 (5.7)	
Resident of Naivasha			
Yes	7 (100.0)	171 (98.3)	0.726
No	0	3 (1.7)	
Ever lived in flower farms			
Yes	4 (57.1)	42 (24.1)	0.049
No	3 (42.9)	132 (75.9)	
Occupation at the farms			
Weeding	3 (50.0)	37 (24.7)	0.454
Grading	1 (16.7)	17 (11.3)	
Packaging	0	3 (2.0)	
Picking	1 (16.7)	26 (17.3)	
Spraying	1 (16.7)	7 (4.7)	
Office work	0	9 (6.0)	
General work	0	51 (34.0)	
Working hours/ week			
<10	0	18 (12.4)	<0.001
10-20	2 (40.0)	2 (1.4)	
31-40	0	1 (0.7)	
>40	3 (60.0)	124 (85.5)	
Did you use protective gear			
Yes	5 (71.4)	127 (73.0)	0.927
No	2 (28.6)	47 (27.0)	
Protective gear			
Caps	2 (28.6)	22 (12.6)	0.223
Googles	0	5 (2.9)	0.649
Mask	2 (28.6)	62 (35.6)	0.702
Overall	5 (71.4)	123 (70.7)	0.966
Gloves	4 (57.1)	71 (40.8)	0.390
Gum boots	5 (71.4)	131 (75.3)	0.817
Does your partner work			
Yes	5 (71.4)	107 (61.5)	0.596
No	2 (28.6)	67 (38.5)	

This endeavours to do a comparison between the two arms-poisoned versus the non poisoned with regard to sociodemographic data and occupational history. However, due to the small number of those with poisoning, this comparison seems not to yield any statistically significant conclusions. Most of the sociodemographic factors are similar across entire population.

Figure 2: Prevalence of adverse reproductive outcomes among the WRA working in flower farms in Naivasha in March 2016 (n=181)



Low birth weight (LBW) is the most common adverse reproductive outcome seen in this population at 13.8%, followed by neonatal deaths at 10.5. There was no case of preterm birth though 8.8% experienced PROM

Table 7

Obstetric outcomes in WRA working in Naivasha flower farms compared with their AChE activity levels

Variable	Obstetric outcome		P value
	Present Mean AChE (SD)	**Absent Mean AChE (SD)**	
PROM	5263.7 (663.2)	5428.4 (1232.1)	0.599
APH	5391.2 (1235.0)	5416 (1191.9)	0.935
PPH	5398.4 (1171.3)	5414.9 (1197.2)	0.965
LBW	5460 (1233.9)	5410 (1192.6)	0.847
Abortion	4533.3 (1046.3)	5460.0 (1184.4)	**0.023**
Still births	5590.3 (933.1)	5406.8 (1203.3)	0.691
Neonatal deaths	4887.5 (1425.9)	5475.6 (1151.5)	**0.042**

Lower levels of AChE activity were found to be associated with incidence of abortion and neonatal deaths. Women who reported to have had abortions had a significantly lower mean AChE, 4533.3 U/L compared to 5460 U/L in those who had never had abortions (p=0.023). Similarly, there was a lower AChE activity level (4588.75 U/L) in women who had ever had neonatal deaths than those who had never had the experience (5475.6 U/L), p=0.042. All the other outcomes were not significantly associated with AChE activity levels.

Table 8

Impact of male exposure on occurrence of adverse reproductive outcomes in WRA working in Naivasha flower farms

Variable	Partner works on farms		P value
	Yes	No	
PROM	4 (9.8)	3 (7.2)	0.553
APH	5 (12.5)	1 (4.3)	0.068
PPH	4 (9.8)	0	0.007
LBW	5 (13.4)	5 (14.5)	0.835
Abortion	34(92.9)	45 (98.6)	0.087
Still births	1 (1.8)	3 (7.2)	0.064
Neonatal deaths	4 (10.7)	5 (10.1)	0.903

Male exposure is associated with increased incidences of PPH while the other outcomes are independent of male exposure, statistically

Table 9

Contraceptive use and Delay in conception among WRA working in Naivasha flower farms

Variable	Frequency (%) n=181
Were you treated for delayed conception	
Yes	11 (73.3)
No	4 (26.7)
How were you treated	
>3 hospital admissions annually	2 (13.3)
Changed family planning method	4 (26.7)
Given medication	2 (13.3)
Herbal treatment	3 (20.0)
Have you used contraceptives	
Yes	132 (72.9)
No	49 (27.1)
Contraceptives	
Barriers (condoms)	3 (1.7)
BTL	1 (0.6)
Injectables	73 (40.3)
Implants	21 (11.6)
Oral contraceptives	56 (30.9)
IUCD	0 (0.0)

Out of the 15 participants who reported delayed conception, 11 sought medical intervention of some sort. Majority of the women use contraceptives, including some of the single ones which is a proof of them having sexual partners. Depo provera is the most preferred mode of contraception.

Table 10

Social History of the WRA working in the flower farms in Naivasha

Variable	Frequency (%) n=181
Where do you get drinking water	
River	5 (2.8)
Well	16 (8.8)
Borehole	150 (82.9)
Tap water	52 (28.7)
Rain water	13 (7.2)
Lake	1 (0.6)
Purchased mineral water	5 (2.8)
Where do you get groceries	
Farm	18 (9.9)
Gilgil market	1 (0.6)
Kabati market	1 (0.6)
Kamere shopping centre	1 (0.6)
Kinungi market	1 (0.4)
Market place	61 (33.7)
Naivasha market	54 (29.8)
Roadside	6 (3.3)
Supermarket	2 (1.1)
Vipanda	29 (16.0)
Missing	8 (4.4)

Risks of ecological exposure via food chain is low as shown from the above table. Only a very small percentage,11.6%, use water from rivers and wells which be contaminated by running surface water carring pesticides from the farms. Furthermore groceries from the large farms are consumed by only 9.9%

12.0 DISCUSSION

Seven out of the one hundred and eighty one participants had AChE activity below 75%, giving pesticide poisoning prevalence of 3.9% though enzyme inhibition was seen in 22.7%. The sociodemographic data was similar across the entire study population hence no statistically significant sociodemographic risk factors were noted. However, duration of work was a significant factor with the poisoned having longer durations of work on the farms and their jobs involved direct handling of farm produce. Low birth weight was the most prevalent adverse obstetric outcome with stil births being the least. Majority of the participants were single (72.7%) and most had at least basic primary education (98.3%).

This study compared participants' AChE activity level against the normal range which was 4900-11900U/L for this study. The lower limit of 4900U/L was used to calculate the percentage activity. It revealed enzyme inhibition rate of 22.7% which compares to a previous study done in Kenya at several agricultural regions which showed highest AChE depression among farm workers in Naivasha with 36% of the workers having depressed AChE activity(3). Another study done in 2014 showed passive exposure among children whose parents work on the farms at 18%(41).Despite the high prevalence of exposure at 22.7%, actual poisoning was found to be low at only 3.9% having marked depression of less than 75%. This is much lower than the findings in a study in 2006 in Kwekwe district of Zimbabwe which was 24.1%(24).

The women with levels depicting poisoning were noted to have worked much longer on the farms with average work duration of 5.9 years compared to 3.5 years in the non poisoned population (Table 3). Average body weight was, however, slightly lower in the poisoned group at 61.1Kg compared to 62.7Kg in the non exposed which is contrary to the known fact that higher body fat content increases risk of organophosphate poisoning. This difference was nevertheless found to be statistically insignificant with p-value of 0.642(Table 2). The poisoned were largely those involved with actual handling of the farm produce such as weeding, spraying, picking and grading flowers as compared to those working in offices or other duties with no direct contact with the farm produce. They were also residents of Naivasha hence there could have been confounding effect from inhalational exposure via polluted air. Of significance is that they were all literate 57.1% having secondary school education. None of the illiterate ones were in this category. They were also noted to have

been using protective gear fairly adequately hence underprotection seems not to be a factor in their poisoning. Hence the only outstanding factor for this poisoning seemed to be comparatively longer duration of working on the farms(Table 3).

Pesticide poisoning is associated with several health effects with respiratory system most afflicted as well as reproductive performance. Adverse reproductive outcomes were noted with almost equal magnitude regardless of the AChE activity levels with the exception of abortion and neonatal deaths which were slightly higher in those with pesticide poisoning. The most prevalent adverse effect was low birth weight with still births being the least common. The figures are almost comparable to general population and not associated with low AChE activity levels hence no particularly risk attributable to pesticide exposure (Table 4). For example, the LBW rate of 13.8% is comparable to world statistics of 15.5% according to WHO/UNICEF report of 2004. While comparing these reproductive outcomes with levels of pesticide exposure, only abortion and neonatal death were found to be related to lower levels of AChE activity. This correlates with other studies done in Bogota(22) and South Africa(20).

Mean age of the women of reproductive age working on the farms is 26.8 years, which is a prime reproductive age. Seven participants (3.9%) were aged below 20 years with 2 being underage at 17 years. Almost two thirds (62.4%) were single ladies and a further 10.5% being widowed or separated. This points towards a low socioeconomic status in majority of the workers since most of them belong to vulnerable population faced with hardships(Table 6).Literacy level was found to be high among the participants with only 1.7% having no education(Table 6). Another important finding is that about 75% of the workers lived outside the farms and this could be a protective mechanism against environmental exposure outside work hours such as by polluted air. The high literacy levels could translate to being more informed about the potential risks of pesticide poisoning hence being cautious while handling them.

A third of the women had chronic health problems with respiratory system being most affected at 26.5% of the 31.5% having asthma and another 10.5% having non specific chest complications. Skin conditions were also of high prevalence with 28% having skin related diseases. Most of these conditions were allergic in nature, both in the respiratory system and on skin(Table 3). This is despite the high prevalence of protective gear use of such as

overall(87.9%), gloves(54.5%), masks(47.7%) and gum boots(93.9%)(Table 3). This could be due to improper or inconsistent use of the variuos protective gear.

13.0 Study Limitations

The study's findings had the following limitations:

1.Inability to prove direct causal-effect link between pesticide exposure and reproductive outcomes.

2. Inability to identify the exact chemicals in use on the farms

3. Other occupational factors such as prolonged standing or strenous manual work may be confounders to the occurrence outcomes under study

4.Contribution from certain undiagnosed individual medical conditions such as chromosomal abnormalities, hormonal insufficiency, genital tract abnormalities, infections (TORCHES), nutritional status and maternal haematological conditions (anaemia, bleeding disorders)

5.Lack of baseline AChE levels with which to objectively calculate percentage depression due to poisoning.

14.0 Study Strength

The study gives important preliminary information about this crucial health issue especially with regard to WRA and their reproduction in the local set up. These findings can form the basis of a more elaborate study designed to establish why the flower farm workers still suffer from health complications despite use of protective gear. It seems gaps still exist which need to be addressed.

14.0 CONCLUSION

1. Women working on the farms have inhibition of AChE giving high prevalence of pesticide exposure despite use of protective gear.
2. The prevalence of severe AChE depression showing poisoning is however low at only 3.9%.
3. The exposure is associated with adverse reproductive outcomes such as increased abortions and neonatal deaths as well as allergic respiratory and skin conditions.

RECOMMENDATIONS

1.There is need to enhance protective measures for the farm workers by identifying the existing weaknesses such as inadequate provision or worker ignorance and addressing them.

2.Those being newly employed into the farms should have their baseline AChE activity levels determined and this should be regularly rechecked at 3-monthly intervals to keep track of their safety on the farms.

3.There is need for a prospective study to determine the prevalence of poisoning more objectively by comparing the post exposure AChE levels with baseline values.

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18.0 APPENDICES:

Appendix I: Questionnaire **Serial No.....**

Instructions to the respondents

1.Answer the following questions by putting a tick where applicable or by a statement as per the question.

2.The information provided will be treated with utmost confidentiality.

A.Bio Data

1. Age_____(years) 2. Weight_____(kg) 3. Level of Education_____

4.Marital Status__(a)Married (b)Single (c)Separated (d)Divorced (e)Widowed

If yes to (c), (d), or (e), please state year_____

5.Employed___(Yes) , (No)

If Yes, state occupation_____

6.Resident in Naivasha__(Yes),(No)

If Yes, since when_____(month,year)

7.Have you ever lived on the farms___(Yes),(No)

If Yes, from _____(month, year) to_____(month, year)

B.Occupational History

8.(a)Have you ever worked on the farms____(Yes),(No)

If Yes, from _____(month, year) to_____(month, year)

(b)If Yes to 8(a);

(i) describe each specific work you do/did and the duration for each

_____

_____

_____

_____

(ii) how many hours/week do you work_____

(c)If Yes to 8(a), do/did you use protective gear__(Yes),(No). If Yes, specify below;

(i) Caps [ ] (ii) Goggles [ ] (iii) Mask [ ] (iv) Overall [ ] (v) Gloves [ ]

(vi) Gum boots [ ] (vii) Other, specify_____

_____

_____

9. (a)Does your partner work_____(Yes, No)

If Yes, where _____

what work_____

how long_____

(b) Incase he has changed jobs, please describe the other jobs he has done and the durations_____

_____

_____

_____

_____

_____

C.Past Medical History

10. (a)Do you suffer any recurrent/chronic health problem(s) _____(Yes) (No)

(b)If Yes to (a), which one(s)_____

_____

_____

(c)IF Yes to (a), do you think it is work related_____

D.Obstetric History

11.Please fill the table below regarding your previous pregnancies.

No.	Month/Year	Gestational age	Outcome/ APH/PPROM	Hosp/ Home	Birth Weight (g)	Sex	Pueperium (PPH)	Fate

12.(a)Have you had difficulty conceiving__Yes/No

(b) If yes, when and how long_____

_____

_____

_____

_____

_____

_____

_____

(c)If yes to (a), were you treated_____Yes/No

(d)If yes to (c), how_____

13.Have you used any contraceptives_____Yes/No

If Yes, please fill the table below.

CONTRACEPTIVE	FROM (YEAR)	TO (YEAR)

E.Social History

14.Where do you get your drinking water?

(a)River. Which one?_____

(b)Well

(c)Borehole

(d)Other,Specify_____

15.Where do you get your groceries?

16. **Laboratory Result**

AChE Activity (%)	

JUSTIFICATION FOR THE INCLUSION OF ALL THE QUESTIONS IN THE QUESTINNAIRE

Qn No.	**Question**	**Justification**
1.	Age	-Provides sociodemographic characteristics of the study population-answering specific objective 5. -Prevalence of the pesticide poisoning and adverse reproductive outcomes shall be stratified according to the various age groups to assess impact of age factor.
2.	Weight	-Is a sociodemographic characteristic hence answering specific objective 5. -Most of the pesticides accumulate in the fatty tissues hence the higher the weight, the higher the risk. Hence it will also answer specific objective 2.
3.	Educational level	-A sociodemographic characteristic, answering specific objective 5 -Educational level may also determine level of knowledge about dangers of the pesticides hence level of precaution taken. It therefore goes ahead to answer specific objective 2.
4.	Marital status	-A sociodemographic characteristic, answering specific objective 5 -Difficulty in conception needs to be considered in a situation of regular, adequate and unprotected intercourse- which is most likely to occur in marriage situation. Hence it helps to evaluate the prevalence of delayed conception as an adverse reproductive outcome thereby answering specific objective 4

5.	Employment	-A sociodemographic characteristic hence answering objective 5 -Various jobs have various risk levels hence there is need to stratify the poisoning levels with the various jobs or unemployment hence answer objective 2.
6.	Residence in Naivasha	-Being resident may confer more risks via the environment or food chain ontop of risk conferred occupationally, There is need to assess the levels of poisoning among residents compared to non residents. This answers objective 2.
7.	Farm residence	Residing on the farm would theoretically confer higher risks than just being in the larger town. This is due to closer proximity to the point of pesticide application hence continued poisoning via inhalation. This answers specific objective 2.
8.	Working on the farms	-Working on the farms may confer higher risks than just being resident of Naivaaha. Hence the need to assess this this notion as a response to specific objective 2
9.	Spouse (husband) working on the farms	-There may be double risk of poor reproductive outcomes if both spouses work on the farms, since even husband's exposure alone has been shown to affect wife's reproductive performance. This answers objective 2.
10.	Suffering recurrent/chronic medcal conditins	-It woul help in filtering those to be excluded since having any of these could contribute to the occurrence of the poor reprodutive effects thereby being a confounder. -It also aims to assess the knowledge level of the participants as to whether they know that the ir work could have certain health-related effects such as chest complications and chronic rhinitis.

11.	Past obstetric history	-This is the main aim of this study, aiming to establish the prevalence of adverse reproductive outcomes hence answering specific objective 4.
12.	Difficulty with conception	-This is one of the poor adverse reproductive outcomes under study hence is a direct answer to specific objective 4. It shall be evaluated in conjuction with Qn 4.
13.	Use of contraception	-Contraceptive use is one of the factors that can cause delay in conception hence this information shall help in evaluating the response to Qn 12 above.
14.	Source of domestic water	-Aims to assess risk of poisoning via consumption of contaminated water hence providing an answer to specific objective 2.

Appendix II: Consent Form

Title of the study: CHRONIC PESTICIDE POISONING IN WOMEN OF REPRODUCTIVE AGE AND ITS EFFECTS ON REPRODUCTIVE OUTCOMES.

Institution: Department of Obstetrics and Gynaecology, School of Medicine, College of Health Sciences, University of Nairobi, P.O BOX 30197-00400, NAIROBI.

Investigator: Dr.Raute Nick Maurice Ochieng', M.B.Ch.B (Nbi)
P.O. BOX 1289-40200, KISII
Registration no: H58/82933/2012
Contact: 0720703776

Supervisors:1: Prof. Eunice Cheserem, M.B.Ch.B, MMed (Obs/Gyn);
Department of Obstetrics and Gynaecology,
University of Nairobi;

2: Dr. Kizito M. Lubano, M.B.Ch.B, MMed (Obs/Gyn);
Department of Obstetrics and Gynaecology,
University of Nairobi.

Introduction: The study aims to establish if pesticide poisoning has any adverse reproductive effects on women of reproductive age in Naivasha region. This poisoning may mostly be directly by working on the farms but can also be indirectly through contaminated food (especially the farm products) and water. The adverse effects under examination include delayed conception, abortion, PPROM, preterm delivery, still births, LBW, birth defects, maternal haemorrhage, neonatal deaths and shortened breast milk production. The outcome of this study will help in policy formulation to ensure farm worker safety, safe farm produce and safe ecosystem.

Purpose of the study: the purpose of the study is to find out the prevalence of chronic pesticide poisoning among women of reproductive age and the effects on their reproductive performance.

Procedure to be followed: With your permission, you will be asked some questions about pesticide poisoning, to find out if you could have been poisoned or not. The

questions will also seek to establish the outcomes of your previous pregnancies. Finally, little amount of blood shall be collected for laboratory analysis to establish your level of exposure to pesticides. All information will be handled with confidentiality and will only be used for the purpose of this study.

Risks: Risks of bleeding and infection that may be associated with blood collection shall be minimised by use of a trained phlebotomist who will observe strict aseptic technique.

Benefits: No direct benefit to the patients is anticipated other than the knowledge obtained that may be used to improve the safety standards on the farms and so protect the vulnerable.

Confidentiality: All information obtained will be kept in confidence. Code numbers will be used and at no point will patient identities be revealed. Completed questionnaires will only be handled for research by principal investigator and research assistants.

The study protocol has been reviewed by an Ethics committee and can be availed to you should you wish to know the details. I shall be available to clarify any issues that may enable you understand the study well. Incase of any enquiry, please contact me (Principal Investigator) on 0720703776.

Principal Investigator.....

Signature.....

Date.....

Subject's statement

I, the undersigned, have read the foregoing information and voluntarily agree to participate in this study. All my questions have been answered to my satisfaction. The confidentiality of the information I shall give has been assured to me and also the freedom to withdraw from the study anytime should I wish. If I get any further questions about the study, I can ask the investigator and any questions on my right as research subject can be directed to the University of Nairobi Ethics and Research Committee on 020 2726300. I am entitled to

receive a copy of this consent form if I so desire.

Name of participant.....

Signature.....

Date.....

*For participants below 18 years old, see below:

I, the undersigned guardian, have read the foregoing information and voluntarily agree to allow the undermentioned participant to participate in this study. All our questions have been answered to our satisfaction. The confidentiality of the information she shall give has been assured to us and also the freedom to withdraw from the study anytime should she wish. If she gets any further questions about the study, she can ask the investigator and any questions on her right as research subject can be directed to the University of Nairobi Ethics and Research Committee on 020 2726300. She is entitled to receive a copy of this consent form if she so desires.

Name of participant.....

Name of guardian.....

Guardian's Signature.....

Date.....

Idhini ya Kushiriki katika Utafiti

Mada ya Utafiti: AUTHIRIKAJI WA MUDA MREFU NA SUMU ZA MASHAMBANI KWA AKINA MAMA NA ATHARI ZAKE KWA UZAZI

Idara: Chuo Kikuu cha Nairobi, College of Health Sciences,

Obstetrics and Gynaecology Department, S. L.P 30197-00400, NAIROBI.

Mchunguzi Mkuu: Dkt Raute Nick Maurice Ochieng',

S. L.P. 1289-40200,KISII.

Namabari ya usajili Chuoni: H58/82933/2012.

Nambari ya simu: 0720703776

Waalimu: **1.** Professa Eunice Cheserem, M.B.Ch.B, MMed (Obs/Gyn);

Idara ya Obstetrics and Gynaecology,

Chuo Kikuu cha Nairobi.

2. Dkt. Kizito M. Lubano, M.B.Ch.B, MMed (Obs/Gyn);

Idara ya Obstetrics and Gynaecology,

Chuo Kikuu cha Nairobi.

Utangulizi

Utafiti huu unalenga kuangalia kama sumu kutoka kwa madawa ya matumizi mashambani yana athari zozote mbaya za kiuzazi kwa akina mama wanaofanya kazi kwenye hayo mashamba katika eneo la Naivasha. Sumu hizi zinaweza patikana kwa kufanya kazi shambani au kutokana na kula vyakula ambavyo vimenyunyiziwa dawa na pia kunywa maji yenye madawa hayo. Athari zitakazoangaziwa ni kama vile ugumu wa kushika mimba, uavyaji mimba, kutoboka maji ya uzazi kabla ya mtoto kukomaa, kuzaa kabla mtoto kukomaa, kuzaa mtoto ambaye amefariki, kuzaa mtoto mwenye uzito wa chini, kuzaa mtoto mwenye hitilafu za kimaumbile, mama kuvuja damu nyingi wakati wa kuzaa, mtoto kufariki kabla ya kumaliza wiki nne baada ya kuzaliwa na maziwa ya mama kukauka mapema. Ripoti ya huu utafiti itasaidia kuweka mikakati ya kuhakikisha mazingira salama ya kufanyia kazi mashambani na kuishi pamoja na mazao salama kutoka mashambani.

Madhumuni ya utafiti huu

Kiini cha utafiti huu ni kuangalia kiasi cha kupata sumu hizo kwa akina mama na athari zake kwa uzazi.

Utaratibu wa utafiti huu

Ukikubali, utaulizwa maswali fulani kuhusu sumu za madawa ya shambani ili kuangalia kama kuna uwezekano kuwa umepata hizo sumu au la. Maswali hayo pia yanalenga kuangalia afya yako kwa mambo ya uzazi kwa muda uliopita. Pia damu kidogo itatolewa ili ichunguzwe katika maabara kuangalia kama kuna sumu kwa damu yako na kama iko, tujue kiwango chake. Habari zote utakazopeana zitahifadhiwa na kuwekwa kwa siri ila zitatumiwa tu kwa minajili ya utafiti huu.

Maadhara

Kuna uwezekano wa kutokwa na damu nyingi au kuambukizwa ugonjwa wakati wa kutolewa damu lakini haya yote yatathibitiwa kwa kutumia mtaalamu aliyehitimu na ana ujuzi mkubwa wa kazi hiyo.

Manufaa

Hakuna manufaa ya moja kwa moja utakayopata kutokana na kushiriki kwa huu utafiti ila matokeo yake yanaweza tumiwa kuboresha hali ya utenda kazi katika mashamba ili kulinda afya ya wafanyi kazi.

Siri

Habari zote zitahifadhiwa kwa siri kubwa. Nambari za siri zitatumiwa kutambulisha washiriki wa utafiti wala majina yao hayatatumiwa. Fomu zitakazojazwa zitahifadhiwa na mtafiti mkuu na wasaidizi wake pekee.

Utartibu wa utafiti huu umekaguliwa na Kamati ya Maadili ya utafiti na unaweza pewa uisome iwapo utataka. Mimi nitakuwepo kukuelezea mambo yoyote zaidi ambayo huenda ukataka kujua kuhusu huu utafiti. Kwa maswali yoyote, nipigie (Mtafiti mkuu) kwa namabari 0720703776.

Mtafiti mkuu……

Sahihi……..

Tarehe……

Idhini ya Mshirika

Nimeelezewa kuhusu utafiti huu na nikakubali kwa hiari yangu binafsi, kushiriki. Maswali yangu yamejibiwa kikamilifu na nimehakikishiwa kuwa habari nitakazotoa zitahifadhiwa kwa siri. Pia nimehakikishiwa kuwa niko hiari kujiondoa kwa utafiti huu wakati wowote. Maswali yoyote nitakayopata, naweza kumuuliza mtafiti mkuu na yale kuhusu haki zangu kama mshiriki, naweza uliza Kamati ya Maadili na Utafiti katika Chuo Kikuu cha Nairobi-nambari ya simu 0202726300. Nikitaka nakala ya idhini hii, nitapewa.

Mshiriki…….....

Sahihi……..

Tarehe……..

*Ikiwa mshiriki hatahitimu umri wa miaka 18, angalia sehemu ifuatayo.

Nimeelezewa kuhusu utafiti huu na nikakubali kwa niaba ya mshiriki, kushiriki. Maswali yetu yamejibiwa kikamilifu na nimehakikishiwa kuwa habari atakazotoa zitahifadhiwa kwa siri. Pia nimehakikishiwa kuwa yuko hiari kujiondoa kwa utafiti huu wakati wowote. Maswali yoyote atakayopata, naweza kumuuliza mtafiti mkuu na yale kuhusu haki zake kama mshiriki, naweza uliza Kamati ya Maadili na Utafiti katika Chuo Kikuu cha Nairobi-nambari ya simu 0202726300. Akitaka nakala ya idhini hii, atapewa.

Mshiriki……..

Msimamizi wa mshiriki.....

Sahihi ya msimamizi……..

Tarehe……..

Appendix III: FUNDING INFORMATION:

The study is part of a bigger study to be funded by Medical Education Partnership Initiative (MEPI) and Partnership for Innovative Medical Education in Kenya (PRIME-K). The broader study topic is "ECOLOGICAL STUDY ON USE OF PESTICIDES AND ITS IMPACT ON MATERNAL, NEONATAL AND CHILD HEALTH " It shall be done by a multi-disciplinary team with each looking at different aspects of Maternal, Neonatal and Child health effects.

MEPI/PRIME-K is made up of a partnership involving the Universities of Nairobi in Kenya and the Universities of Washington and Maryland Baltimore in the United States of America (USA). Its programs aim to provide opportunities for multidisciplinary teams of post graduate students to carry out research that will enhance the clinical and research capacity at the University of Nairobi and thus improve health care delivery in Kenya.

Funding for this study is expected to be 100% by PRIME-K as per the budget.

EN

REF 17019H

Cholinesterase Liquid

Kinetic colorimetric determination of cholinesterase (CHE) activity according to the DGKC recommendations in serum and plasma

IVD

CE

REAGENT 1: 2 x 50 mL - **REAGENT 2:** 1 x 20 mL

STANDARD/CALIBRATOR: the term refers to the standard / the calibrator

REAGENT: the term refers to the single reagent **CONTROL:** the term refers to the control

SUMMARY

There are two cholinesterases (CHE and ACHE) differing in substrate specificity, tissue of origin and biological role. The term cholinesterase (ACHE), also known as acetylcholine acetylhydrolase, is found in erythrocytes, in the lungs, spleen and in grey matter of the brain. The pseudocholinesterase (CHE), also referred to as acylcholine acylhydrolase, is found in serum, the liver, pancreas, heart and in the white matter of brain. The assay of serum cholinesterase (CHE) is useful to diagnose: liver disorders, hepatitis, cirrhosis, carcinoma with metastasis, sensitivity to succinylcholine administration and pesticide poisoning. Levels decrease in all of the diseases above.

PRINCIPLE

The method uses butyrylthiocholine as the specific substrate for cholinesterase (CHE). Cholinesterase catalyses the hydrolysis of butyrylthiocholine substrate forming butyrate and thiocholine. Thiocholine reduces hexacyanoferrate (III) to hexacyanoferrate (II). The decrease in absorbance is directly proportional to the CHE activity in the sample.

REAGENTS

The components of the kit, stored at 2-8 °C in unopened vials, are stable up to the expiry date indicated on the package.
Components of the kit and concentration of reactive ingredients:

REAGENT 1
pyrophosphate buffer 92 mmol/L pH 7.6, hexacyanoferrate (III) 2.5 mmol/L

REAGENT 2
butyrylthiocholine 91 mmol/L

PREPARATION OF REAGENT SOLUTIONS

REAGENT 1 and **REAGENT 2**: Ready to use.

STABILITY

On Board: 30 days, if contamination is avoided.
Calibration: 15 days. Repeat the calibration at any variation in the reagent lot.

CALIBRATION

For the calibration, use the following material:

Cln Chem Cal REF 16550 4x3 mL
Multiparametric lyophilised calibration serum. For use, follow the instructions contained in the kit.

QUALITY CONTROL

Use the following control materials to verify test accuracy:

Cln Chem Control 1 REF 16150 6x5 mL
Multiparametric lyophilised control serum. For use, follow the instructions contained in the kit.

Cln Chem Control 2 REF 16250 6x5 mL
Multiparametric lyophilised control serum. For use, follow the instructions contained in the kit.

STANDARDIZATION

DGKC (Deutsche Gesellschaft für Klinische Chemie).

SAMPLE

Fresh serum, plasma (EDTA, heparin) not hemolyzed and promptly separated from the red blood cells. Do not use sodium fluoride as an anticoagulant because it inhibits cholinesterase.
Collect samples in accordance with the NCCLS procedure reported in bibliography.[1]

STABILITY OF THE SAMPLE

7 days at 2-8 °C or 3 months at -20 °C.

WARNING AND PRECAUTIONS

- For in vitro diagnostic use.
- Do not use components beyond the expiration date.
- Do not mix materials from different kit lot numbers.
- Safety Data Sheets are available at www.sentineldiagnostics.com or contact your local representative.
- ⚠ **CAUTION:** This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and be handled in accordance with the OSHA Standard on Bloodborne Pathogens[2]. Biosafety Level 2[3] or other appropriate biosafety practices[4,5] should be used for materials that contain or are suspected of containing infectious agents.
- This product contains sodium azide; for a specific listing, refer to the REAGENTS section of this package insert. Contact with acids liberates very toxic gas. This material and its container must be disposed of in a safe way.

REAGENT 2

WARNING: contains
BUTYRYL-THIOCHOLINE IODIDE, MALEIC ACID

Hazard statements:
- H317 May cause an allergic skin reaction.

Precautionary statements:
- P272 Contaminated work clothing should not be allowed out of the workplace.
- P280 Wear protective gloves/protective clothing/eye protection/face protection.
- P333+P313 If skin irritation or rash occurs: Get medical advice/attention.
- P501 Dispose of contents/container in accordance with applicable regulations.

INSTRUMENTATION AND MATERIALS REQUIRED BUT NOT PROVIDED

General laboratory equipment.

ANALYTICAL PROCEDURE

Wavelength:	415 nm (main) 660 nm (reference)
Pathlength:	1 cm
Temperature:	37 °C
Sample/REAGENT 1/REAGENT 2:	1/50/10
Reaction:	Kinetic (decrease)

Allow reagents to reach working temperature before using. A proportional variation of the indicated reaction volumes does not change the results.

EXAMPLE OF ANALYTICAL PROCEDURE ON AUTOMATED INTRUMENTS

Time 0
Calibrator/Controls/Sample = 4 µL
REAGENT 1 = 200 µL

After 300 sec
Addition of REAGENT 2 = 40 µL

After 100 sec
Reading 1

After 100 sec
Reading 2

CALCULATION

1. Plot a calibration curve on a graph paper by tracing absorbance (y axis) according to corresponding U/L activity (x axis) for each Calibrator.
2. Indicate on the calibration curve the absorbance value obtained for Samples and Controls.
3. Extrapolate the U/L value for Samples and Controls from the calibration curve.

CONVERSION FACTOR

CHE [U/L] x 0.001 = CHE [kU/L]
CHE [U/L] x 0.01667 = CHE [µkat/L]

REFERENCE VALUES

Adults (37 °C): **4900 - 11900 U/L**
In infants up to 6 months of age, cholinesterase activity is 40% to 50% higher than in adults. In young women, the enzyme activity is approximately 64% to 74% of that in adults males. The activity decreases during pregnancy.

It is recommended that each laboratory establish its own expected range. For diagnostic purposes, results obtained should always be evaluated taking into consideration the patient's history and all other clinical findings.

PERFORMANCES AUTOMATIC ANALYZER

SENSITIVITY:

105 U/L. Sensitivity was calculated on 20x3 replicates x run of normal saline and reported as the "mean zero value + 3 SD".

INTRA-ASSAY PRECISION:

It was determined on 21 replicates of two controls (L1/L2) and of two human sera (L3/L4). The results were as follows:

U/L	L1	L2	L3	L4
mean	7122	4304	8610	5722
SD	127.1	53.0	101.0	67.9
CV%	1.8	1.2	1.2	1.2

INTER-ASSAY PRECISION:

was determined from 22x2x2 tests (day x run x rep) on each control (3 levels - L1/L2/L3). The results were as follows:

	mean	Total Imprecision		Between Days		Repeatability	
	U/L	SD	CV%	SD	CV%	SD	CV%
L1	5060	110.6	2.2	0.0	0.0	108.2	2.1
L2	6966	168.9	2.4	0.0	0.0	101.4	1.5
L3	8212	205.7	2.5	7.4	0.1	110.4	1.3

ACCURACY:

this test (y) was compared with a commercially available method (x).
The results were as follows:
N = 198, r = 0.998, y = 1.11x + 384.58

INTERFERENCES:

the test is not affected by the presence of ascorbic acid up to **20** mg/dL, haemoglobin up to **1000** mg/dL, bilirubin up to **60** mg/dL and lipids up to **1000** mg/dL.

ANALYTICAL RANGE:

150 - 22000 U/L.
Samples with values higher than 22000 U/L must be diluted 1:10 with normal saline and the result multiplied by 10.

WASTE MANAGEMENT

Reagents must be disposed of in accordance with local regulations.

BIBLIOGRAPHY

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Explanation of symbols

IVD	In vitro Diagnostic Medical Device
REF	Catalogue number
LOT	Batch code
Cont.	Contents of kit
	Caution, consult accompanying documents
	Consult instructions for use
	Use by (last day of the month)
XXX	Contains sufficient for <n> tests
	Temperature limitation
	Manufacturer

Note: changes in comparison to the previous version are indicated by a vertical bar in the text margin.