THE EFFECT OF PESTICIDE EXPOSURE ON SERUM CHOLINESTERASE LEVELS AMONG ASTHMATIC CHILDREN IN NAIVASHA

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(U56/64034/2013)

A dissertation submitted in partial fulfilment of the requirements for the award of Master of Pharmacy in Clinical Pharmacy by the University of Nairobi.

AUGUST, 2014-08-30
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DECLARATION

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

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Signature___________________________ Date________________________

Supervisors’ Approval

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DEDICATION

This work is dedicated to the Almighty God, my parents, siblings, husband Dr. Benjamin Esiaba and to our children Tracy and Lindsey for always believing in me.
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I thank my supervisors; Dr. Peter Karimi; Dr. George Wandolo and Dr. Kefa Bosire Ogonyo who despite their busy schedule were always available to read through my work and make the necessary corrections. I am particularly grateful to Dr. P.N. Karimi and Mr. Francis Maina for the help they accorded me while collecting and analyzing my samples.

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## ACRONYMS

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>AChE</td>
<td>acetylcholinesterase</td>
</tr>
<tr>
<td>BuChE</td>
<td>butyrylcholinesterase</td>
</tr>
<tr>
<td>CB</td>
<td>N-methyl-carbamate</td>
</tr>
<tr>
<td>ChE</td>
<td>cholinesterase</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence Interval</td>
</tr>
<tr>
<td>EPTC</td>
<td>Ethyl Dipropylthiocarbamate</td>
</tr>
<tr>
<td>ERC</td>
<td>Ethics and Research Committee</td>
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<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
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<tr>
<td>HOPAK</td>
<td>Hospital Pharmacists Association of Kenya</td>
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<tr>
<td>LD&lt;sub&gt;50&lt;/sub&gt;</td>
<td>median lethal dose</td>
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<tr>
<td>OPs</td>
<td>organophosphate</td>
</tr>
<tr>
<td>KNH</td>
<td>Kenyatta National Hospital</td>
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<tr>
<td>NDH</td>
<td>Naivasha District Hospital</td>
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<tr>
<td>NEMA</td>
<td>National Environment management authority</td>
</tr>
<tr>
<td>PRIME-K</td>
<td>Partnership for Innovative Medical Education in Kenya</td>
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<tr>
<td>RBC</td>
<td>Red Blood Cell</td>
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<tr>
<td>SD</td>
<td>Standard Deviation</td>
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<td>UoN</td>
<td>University of Nairobi</td>
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OPERATIONAL DEFINITION OF TERMS

**Acetylcholinesterase:** A neuronal enzyme, also known as RBC or erythrocyte cholinesterase is an enzyme which hydrolyses acetylcholine esters and is involved in regulating transmission of nerve impulses to effect cells at cholinergic, synaptic and neuromuscular junction. Lack or reduction of this enzyme results in accumulation of acetylcholine at the neuromuscular junction and persistence of muscular contraction at the muscle involved. This is a common feature of bronchospasm seen in bronchial asthma.

**Asthma:** This is a chronic, inflammatory condition characterised by reversible airflow obstruction and airway hyperresponsiveness and it occurs at any age.

**Asthma control:** This is adherence to proper asthma management plans like asthma education, developing management goals related to quality of life, professional consultation, following daily asthmatic treatment plan and understanding difficulties and solutions of asthma.

**β2-agonists:** These are a large group of drugs which mimic the actions of naturally occurring catecholamines like adrenaline, noradrenaline and dopamine by acting on the smooth muscles of vasculature, bronchial tree, intestines and uterus. They are used as bronchodilators and relief medications in asthma management.

**Butyrylcholinesterase:** (also known as pseudocholinesterase, plasma cholinesterase, BCHE, or BuChE) is a non-specific cholinesterase enzyme that hydrolyses many different choline esters. In humans, it is found primarily in the liver and is encoded by the BCHE gene. It is very similar to the neuronal acetylcholinesterase, which is also known as RBC or erythrocyte cholinesterase.

**Caregivers:** includes the biological mother or father, a step mother or guardians who have stayed with the guardian for over three months.

**Corticosteroids:** Also known as steroids and are used as anti-inflammatory medicines prescribed for a wide range of conditions. These are used to control long term effects of airway inflammation in asthmatic patients.
Dissemination plan: The dissemination plan (which is a part of the overall project plan) explains how the project will share outcomes with stakeholders, relevant institutions and organisations, and how it will contribute to the overall dissemination strategy for the programme.

Exposure: this is human contact with the agent (pesticide). In this context it is defined as children whose parents have been working on the flower farm for at least one month and also families that live within a radius of 500m from the flower farm for at least one month.

Unexposed: children whom whose parents have never worked on the flowers farms or those who stay beyond a radius of 500m from the flower farms

Pesticide: A substance used for destroying insects or other organisms harmful to cultivated plants and animals.

Plasma: plasma contains fibrinogen which assists in clotting therefore when it is separated from blood it does not lose the ability to clot.

Plasma Cholinesterase: is a family of enzymes that catalyze the hydrolysis of the neurotransmitter acetylcholine into choline and acetic acid, a reaction necessary to allow a cholinergic neuron to return to its resting state after activation. This is also known as butyrylcholinesterase or pseudocholinesterase.

Primary materials: means physical objects acquired through a process of scholarly investigation from which research data may be derived. It includes ore, biological material, questionnaires or recordings etc.

Red blood cell cholinesterase: this test measures the amount of an enzyme called acetylcholinesterase in red blood cells. It is used to evaluate when a toxicity of organophosphates (a type of pesticide) is suspected.

Serum is the part of blood that remains when fibrinogen is separated from blood.

Serum cholinesterase: it is a blood test that looks at levels of two substances that help the nervous system work properly. They are called acetylcholinesterase and
pseudocholinesterase. Your nerves need these substances to send signals. Acetylcholinesterase is found in nerve tissue and red blood cells. Pseudocholinesterase is found primarily in the liver. **Alternative Names:** Acetylcholinesterase; RBC (or erythrocyte) cholinesterase; Pseudocholinesterase; Plasma cholinesterase; Butyrylcholinesterase; Serum cholinesterase

**Severity of asthma:** this is the level of current clinical control and risks of frequent severe exacerbations (or death) and/or adverse reactions to medications and/or chronic morbidity (including impaired lung function or reduced lung growth in children)." Severe asthma includes 3 groups, each carrying different public health messages and challenges: (1) untreated severe asthma, (2) difficult-to-treat severe asthma, and (3) treatment-resistant severe asthma. The last group includes asthma for which control is not achieved despite the highest level of recommended treatment and asthma for which control can be maintained only with the highest level of recommended treatment.
ABSTRACT

Background: Pesticide exposure has a major concern on health care and general safety profile on public health in the world(1),(2). Pesticides are used for pest control on crops, livestock, commercial purposes, and domestic use. Organophosphates (OPs) in particular have gained popularity worldwide in preference to organochlorines, which are persistent and more damaging to the environment. Farmers and their families are likely to be exposed to agricultural chemicals, even if they are not involved in farm activities. Food, water, and treatment in the home, yard, and school are all potential sources of children’s exposure. Children through biology and their behaviour have a higher chance of exposure to pesticides. Pesticide exposure has been associated with asthma and allergic rhinitis in various studies(3),(4). Exposure can be determined by analysing the levels of serum acetylcholinesterase. This study sought to find if there is a correlation between pesticide exposure and serum cholinesterase levels among asthmatic children in Naivasha.

The study objectives: The main objective of this study was to determine if there is a correlation between pesticide exposure and serum acetylcholinesterase levels among asthmatic children in Naivasha. The specific objectives were to find out the levels of pesticide exposure, level of asthma control, levels of serum ChE inhibition and compared the cholinesterase levels to the level of asthma control among asthmatic children at NDH.

Methodology: A cross sectional study design was used; the target population was sixty children aged between 5 to 12 years old and their parents or guardians in the paediatric wards in Naivasha District. A questionnaires was administered, blood samples were collected and stored in a portable cold chain. At the laboratory, serum was separated from whole blood and frozen at -21 °C in Naivasha Blood Bank. Serum was later transported under freezer packs for analysis at the UoN Clinical Chemistry Unit laboratory. Statistical analysis of data was carried out using Stata version 10 and the results summarized as P-values and the level of significance was tested at 95%CI in tables and charts.

Results: Factors that were found to be significantly associated with cholinesterase levels were distance of school from flower farm (p=0.023), school status of children (p =0.004) and level of asthma control (p=0.037). There was no significant relationship between the child’s cholinesterase levels and sex of the child (p=0.284), working on a flower farm (p=0.614) and the guardian’s previous exposure to pesticides (p =0.0297).
**Conclusion:** There was statistically significant association between pesticide exposure and the mean serum cholinesterase levels (p=0.0405). Further still, children who stayed or schooled near flower farms had low cholinesterase levels (p=0.004). Unsafe work practices predisposed the farmers’ children to health related problems (p=0.000). Factors like age, sex, guardian’s occupation, and working on the farm did not have any association with the serum ChE levels and asthma control

**Recommendations:** A local level policy research for program intervention among flower farm workers using indoor insecticides like pyrethrins should be established to help reduce pesticide exposure among the local people.

This study suggests that intervention measures need to be done to lower pesticide exposure of farmers. It is also suggested that chronic effects of pesticide cited in certain studies such as carcinogenic effects, poor reproductive outcomes, neurologic and respiratory disorders, impairments of the immune system and birth defects should also be investigated in future studies.
CHAPTER ONE: INTRODUCTION

1.1 Background

Organophosphates (OPs) were initially recognized in 1854, but their general toxicity was discovered in the 1930s. Tetraethyl pyrophosphate (TEPP) was the first OP insecticide, developed in Germany during World War Two as a by-product of nerve gas development. OPs are all derived from phosphoric acid. They are generally among the most acutely toxic of all pesticides to vertebrate animals. They are unstable and break down quickly in the environment. OPs are nerve poisons which kill the target pest (usually insects). Most OPs pesticides are insecticides, although there are also a number of related herbicide and fungicide compounds (1). Other pesticides include fungicides, herbicides, carbamates, biologicals and pyrethroids. A study done in Canada, Lebanon and Kenya reported high usage of pesticides by farmers (5), (6), (7).

Food, water, soil, house dust, carpets and treatment in the home, yard, and school are all potential sources of children’s exposure (8). Exposure may be via breast feeding, transplacental, inhalation, and direct skin contact and air pollution. A study done in America showed that the first faeces of newborns and breast milk of exposed mothers had a number of pesticides (9).

In 2008, pesticides were the ninth commonest substance reported to poison control centres, and approximately 45% of all reports of pesticide poisoning in America were children (10). Exposure to OPs is measured by use of cholinesterase levels. This has been confirmed by various studies. In the 1990s in America, about 12% of farm workers had low serum acetylcholinesterase levels (1). In Ecuador, a study revealed that flower farm workers’ children had low levels of acetylcholinesterase enzyme than non-flower farm workers’ children (11). Another study in Zimbabwe, Kwekwe area reported a prevalence of 24.1% with abnormal acetylcholinesterase levels among farm workers in 2011 (12). In Northern Tanzania, a study showed that out of the pesticides used, 24% were acetylcholinesterase inhibitors(13). In Kenya, an East African project revealed 29.6% depression of cholinesterase activity to values below 60% of baseline among the exposed young adults out of whom 14.7% presented with respiratory symptoms (14).
The mechanism of action of organophosphorus and carbamate insecticides involves inhibiting the enzyme acetylcholinesterase in nerve synapses. This leads to accumulation of acetylcholine, the neurotransmitter at the ganglia in the autonomic nervous system and at many synapses in the brain, skeletal neuromuscular junction, at some post-ganglionic nerve endings of the sympathetic nervous system and adrenal medulla (15).

Acute toxic symptoms observed are related to prolonged effects of acetylcholine which include: excessive sweating, salivation and lacrimation, nausea, vomiting, diarrhoea, abdominal cramp, general weakness, headache, poor concentration and tremors. Serious cases may lead to organophosphate - induced delayed neuropathy (OPIDN) (nerve damage). This may begin with burning and tingling sensations and progress to paralysis of the lower limbs. Acute cases can result in respiratory failure and death (16).

The chronic toxic effects of pesticide exposure include cancer, neuro-developmental and behavior effects, neurodegenerative diseases, cardiovascular diseases and birth defects. It can also interfere with parental reproductive health, hence exposed parents may have reduced chances of male birth and increased risk of childhood cancer (8). Evidence has also shown an association of obesity, type 2 diabetes and metabolic disease. Some effects may last a whole life time; while some are passed on to future generations. This makes it hard to assess the extent of the chronic effects of pesticides in any country. In addition, multiple exposures are additive and can lead to respiratory diseases such as chronic obstructive pulmonary disease (COPD), asthma and pneumonia hence the focus of the study on asthmatics (8), (9), (17).

Risk factors associated with exposure to OPs include prenatal, household, malnutrition, immunosuppression, childhood and occupational exposures (maternal and paternal) appear to be the largest risks (10),(11). A study done in the U.S. reported that pesticide exposure affected both individuals who lived in Latin America and the immigrants (1).

Diagnosis of OPs poisoning is based on the history of exposure, signs and symptoms of exposure and laboratory measurements. It also requires a high index of suspicion as it can be misdiagnosed even after acute exposures resulting to irrational management leading to repeated re-exposures unknowingly. Pesticides and/or their metabolites can be measured in samples of blood, soil, food, saliva, water, urine, breast milk, amniotic fluid or
meconium to confirm diagnosis (9), (16). In America, Ecuador, Zimbabwe and Kenya blood samples were used to determine red blood cell cholinesterase levels(1), (7), (12), (14).

Heparinised whole blood sample is submitted for analysis so that both plasma and erythrocyte cholinesterase levels can be determined. However, if the sample is haemolysed, only whole blood cholinesterase is reported(19).

Prevention at local, National and international level policies on human safety and education should be emphasized (10), (16). In addition, integrated pest management (IPM) system should be adapted. IPM is designed to choose environmentally friendly course of action in controlling pests as well as substitution of acetylcholinesterase inhibiting pesticides (19).

Asthma control in these regions is difficult since many pesticides are irritants which directly damage the bronchial mucosa, thus making the airway very sensitive to allergens. They may increase the risk of developing asthma, exacerbate a previous asthmatic condition or even trigger asthma attacks by increasing bronchial hyper-responsiveness (3), (21).

Management of OPs and CB exposure involves skin decontamination, cardiorespiratory support, airway protection and seizure control. Gastric decontamination involves gastric lavage, catharsis, activated charcoal adsorption, syrup ipecac. Antidotal measures like use of atropine and pralidoxime in organophosphates and carbamates are used to preserve life. Atropine is a muscarinic receptor blocker, which blocks the organophosphate-induced over stimulation of central and muscarinic cholinergic nerve terminals. Atropine lowers a subset of organophosphate poisoning symptoms: secretions, bronchoconstriction, bronchospasm, and gastrointestinal toxicity. It does not bind nicotinic receptors; atropine does not affect muscle weakness, including respiratory muscle weakness. Pralidoxime (2-PAM) is a cholinesterase reactivator (oxime), which restores respiratory and skeletal muscle strength. 2-PAM does not cross the blood-brain barrier; hence central effects are not reversed. Seizures are managed by commonly diazepam and lorazepam. Phenytoin can also be used (11), (14), (16).
1.2 Statement of the research problem
Pesticide exposure is one of the leading causes of increased health burden in flower growing areas in the world. It poses acute and chronic toxicities to the families who end up passing over the congenital defects up to four generations long after leaving the exposed area. High morbidity and mortality for families that live in or near the flower farms pose psychological, financial, social burden to the families and the health sector. Lack of public awareness about exposure of pesticides to children magnifies the problem given the limited studies that have been carried out on the flower farms. Consequently, there was need to carry out this study to find out levels of inhibition of serum cholinesterase as a biomarker for exposure to organophosphates among asthmatic children in Naivasha.

1.3 Goal of the study
To determine the relationship between pesticide exposure and asthma among children aged 5-12 years using levels of serum cholinesterase as a biomarker compared to the results of the control.

1.4 Objectives
1.4.1 Main objective
It was to find out levels of inhibition of serum acetylcholinesterase as a biomarker for exposure to organophosphates among asthmatic children in Naivasha.

1.4.2 Specific objective
1. To find out the level of exposure to pesticides among asthmatic children.
2. To find out the level of asthma control among the asthmatic children.
3. To determine the serum acetylcholinesterase levels among asthmatic children.

1.5 Research questions
1. What was the level of pesticide exposure among the asthmatic children?
2. What was the level of asthma control among the asthmatic children?
3. What was the level of serum acetylcholinesterase levels among asthmatic children?
1.6 Justification of the study

Pesticide exposure is one of the leading causes of morbidity and mortality among children, in the world (21). This study mainly focused on organophosphates (OPs) because they are popular worldwide compared to organochlorines, which tend to be persistent and more damaging to the environment. Pesticide exposure cases are associated with lifelong complications and an increased health burden (24). Reports showed that, the main cases admitted in paediatric wards in Naivasha were due to pesticide poisoning. If not well managed, pesticide poisoning led to prolonged hospital stay which affected families financially, socially and psychologically.

Benefits of the study:-

The government of Kenya:
The study contributed to data base and also provided a baseline for larger studies.

Healthcare workers: the study created awareness about exposure of children through their guardians/parents hence health education and proper management within and outside hospital was ensured.

University of Nairobi: the study acted as a reference source to the University fraternity to foster research in environment health.

1.7 Limitations of the study

The study was limited to measuring organophosphate exposure and not other pesticides because of their common use in the region and also as a result of limited capacity for analysis. The study population was children aged 5-12 years old. The study focused mainly on Naivasha as the study site.
CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction
This chapter focuses on relevant studies that have been done in different areas with reference to sources of exposure, acetylcholinesterase levels, asthma control and comparison of ChE concentration and the level of asthma control among children exposed to pesticides.

2.2 Aetiology and mechanisms of organophosphate exposure in children
Soil, water, food, breast milk, house dust, carpets, toys, chemically treated lawns and transplacental exposure are the main etiological causes of exposure in children (8),(25). A study done in Ecuador found out that the children who stayed for the longest duration of time with a plantation worker were four times more likely to have lower enzyme activity than children who never lived with the workers (11). In addition, studies done in Europe and Lebanon reported exposure of minors to pesticides by working on the flower farms (4),(7).

Mechanisms of exposure include inhalation, ingestion, breast feeding, transplacental transfer of pesticides and direct absorption via the skin (4),(25). The group at greatest risk from exposure to pesticides are children (10),(26). This is partly because their exposures are relatively greater than those of other non-occupationally exposed people, and in part because of their greater vulnerability to the effects of pesticides. Children also tend to eat and drink more than adults in relation to their body weight and so take in relatively more residues from fruits and vegetables. Furthermore, children also inhale relatively more air than adults, making them more vulnerable to the effects of spray drift and household insecticides (25), (26).

Studies have shown that those who use more pesticides over a longer period of time had higher total pesticide exposure. Furthermore, similar studies have shown that a farmer who was a pesticide applicator, mixer, loader and who wiped sweat with contaminated piece of fabric, and who had not been given instructions through training association was at risk of having higher pesticide exposure (27).
2.2.1 Prevalence of asthma among flower farm workers

Asthma aetiology is either genetic or environmental. Globally genetic predisposition is at about 50 per cent. A study done in India confirmed that genetic and familial association (28). Environmental factors that exacerbate asthma included pesticides, smoke, pollens etc. Pesticide exposure is the focus of this study. There have been many reports on increased rate of asthma in people occupationally exposed to pesticides (17). Children are more vulnerable to the effects of pesticides. Pesticide exposure is associated with asthma diagnosis before age of five compared to other age groups. These associations are related to immaturity of the respiratory, immune and nervous systems in children(23),(29). In a Lebanese study on school children, a prevalence of 12.4% of chronic respiratory disease due to exposure to pesticides was reported (4). In Denmark, a study done revealed a prevalence of wheezing at 18.2%, asthma 7.7% and bronchitis 23.6% among dairy farm workers (30). In Kenya there are no such statistics reported.

2.3 RBC (erythrocyte) ChE and serum ChE enzyme

This enzyme hydrolyses acetylcholine esters and is involved in regulating transmission of nerve impulses to have an effect on cells at cholinergic, synaptic and neuromuscular junction. Lack or reduction of this enzyme results in accumulation of acetylcholine at the neuromuscular junction and persistence of muscular contraction at the muscle involved. This is a common feature of bronchospasm seen in bronchial asthma. A number of studies have shown that the measurement of erythrocyte cholinesterase is an indirect measure of the enzyme activity that exists in nerve tissue. High values may be found in polycythaemia and in thalassaemia or other congenital blood dyscrasias while low values of erythrocyte cholinesterase not related to OP exposure have been observed in subjects affected with leukaemias or other neoplasms (15),(20),(32),(33).

A study done in Australia showed that serum ChE normal values were 10-15 per cent greater in males than in females. Low values of serum cholinesterase activity not related to OP exposure in the study were associated with liver diseases or drugs affecting the liver, uraemia, cancer, heart failure, allergic reactions, certain collagen diseases, acute infections, Crohn’s Disease, an inflammatory disease of the intestine, chronic anaemia and genetic variants which have a lower activity. In addition, lower values were measured during pregnancy and menstruation in females (20).
Further, the Australian study reported that the levels of red cell and plasma cholinesterase are also depressed by up to 64% by dyes used as food colouring agents like sunset yellow, quinoline and erythrosine and included other causes of decreased plasma ChE levels to be drugs like oral contraceptive agents containing oestrogens, propanolol, ranitidine, anaesthetics such as halothane, penicillin, streptomycin, neostigmine, cyclophosphamide, lithium, phenelzine, bambuterol and glucocorticoids. Prednisolone and corticosteroids in general can also affect plasma cholinesterase activity due to the inhibition of cholinesterase synthesis in the liver by 23 to 69% and that the decrease was related to the initial dose and duration of treatment. Red cell cholinesterase is not affected by corticosteroids including prednisone (14), (20).

Robert and Karr found out that serum cholinesterase activity can be increased in genetic variants, obesity, fatty liver, hypertension, psoriasis, thyrotoxicosis or asthma. They also found out that both red cell and plasma cholinesterase vary between individuals from 10 to 40 %, and normally vary up to 10 to12% in the same individual. In addition, they reported that about 3% of individuals had a genetically inherited low cholinesterase which does not give rise to symptoms or increased risk (10).

Choline (an essential nutrient) is a precursor for synthesis of acetylcholine and cell membranes whose function is to maintain cell integrity, cell signalling, nerve transmission, lipid and fat metabolism. Choline is also required to form the phosphatidylcholine portion of very low density lipoprotein (VLDL) particles. Deficiency of this nutrient leads to fatty liver, cardiovascular diseases, liver cancer due to increased oxidative stress in the liver, neural tube defects in pregnancy and memory loss. Consequently, deficiency of choline leads to depressed acetylcholine concentration (38), (39).

2.3.1 Acetylcholinesterase inhibition by pesticides
Organophosphate (OP) and N-methyl-carbamate (CB) insecticides are widely used in agriculture and are the main inhibitors of AChE (31). Many studies have shown that inhibition of neuronal acetylcholinesterase (AChE) enzyme activity is the main mechanism of OP/CB toxicity. Inhibition of cholinesterase is caused by phosphorylation of the active site of the enzyme by the OP(14),(19),(31).
AChE plays a critical role in regulating nerve transmissions in the central and peripheral nervous systems. It is found in blood in two different forms; AChE associated with red blood cell membranes and butyrylcholinesterase (BuChE) present in serum. Inhibitions of these two forms are considered as markers of early biologic effects related to OP/CB exposure. However, AChE inhibition is considered to be a better marker of toxicity. Butyrylcholinesterase inhibition is a more sensitive marker of exposure because it is inhibited more effectively than AChE by most OP/CBs including chlorpyrifos, diazinon, and Malathion (14),(19),(31).

Hoffman in his study found out that once the enzyme is sufficiently inhibited, acetylcholine accumulates at the synapse and disrupts the normal response to discrete nerve impulses. Plasma cholinesterase reactivates with a half life of about 12 days immediately exposure has ceased. Erythrocyte cholinesterase regeneration depends on the replacement of erythrocytes in the peripheral blood at 1% per day as new erythrocytes are released from the bone marrow (19), (31).

Depressions of plasma pseudocholinesterase and/or RBC AChE activities indicate excessive organophosphate absorption. The enzyme depression is obvious within a few minutes or hours after absorption of a significant amount of organophosphate. Certain organophosphates may selectively inhibit this enzyme. Depression of the plasma enzyme generally persists for several days to a few weeks. The RBC enzyme activity may take several days to reach its minimum and usually remains depressed longer, sometimes 1-3 months, until new enzyme replaces that inactivated by organophosphate (19),(32).

2.4 Asthma control among flower farm workers

Exposure to either sensitizing or irritant chemicals in the workplace may lead to work-related asthma. When occupational asthma appears after exposure to an inhaled irritant at work, it is termed irritant-induced occupational asthma. This makes it hard to control asthma (29).

Many pesticides are irritants which directly damage the bronchial mucosa, thus making the airway very sensitive to allergens (29). They may increase the risk of developing asthma, exacerbate a previous asthmatic condition or even trigger asthma attacks by
increasing bronchial hyper-responsiveness (3),(21). A Lebanese study on asthma found out that exposure to pesticides worsens asthmatic attacks. It also confirmed that asthmatic episodes are specifically linked to organophosphates and organochlorates due to their anticholinesterase activity and their effect on neuronal M2 receptor. In addition, the study showed that pyrethroid derivatives also cause asthma-like episodes (7).

A systematic review study showed the association of asthma and organophosphates among children of flower farm workers (33). Other risk factors of the asthma in the study included obesity, a familial predisposition, positive allergen-specific immunoglobulin E (IgE) test, viral respiratory illnesses, lower socioeconomic status and exposure to aeroallergens like pesticides, cigarette smoke. Zhang et al suggests that those children who are genetically predisposed to asthma are likely to have an even higher risk if they are overweight beyond infancy (34).

Exposure to pesticides makes it hard to control asthma as all the classes of pesticides can trigger or exacerbate asthmatic attacks. Herbicides (glyphosate, chlorophenoxy and atrazine), insecticides (pyrethrum, pyrethrins, permethrin,cypermethrin, cyfluthrin), organophosphates(chlorpyrifos, diazinon, malathion, methyl parathion) and fungicides cause asthma by either weakening the respiratory muscles or by acting as allergens (3),(7),(21),(35).

Prevention of asthma exacerbations includes ongoing monitoring of patients’ symptoms and sustained optimal control of the disease. There are existing tools, which are validated for continuous monitoring of asthma by WHO. In Kenya, the levels of asthma control are classified as shown in appendix V. The Kenyan guidelines for asthma management has a tool customised for use by children or by their caregivers as shown in appendix 3, (36).

2.5 Relationship between ChE levels and asthma control in children

A Lebanese study showed that organophosphates and organochlorates have anticholinesterase activity and are known to cause asthma episodes. The study revealed that OPs cause airway hyper reactivity in the absence of AChE inhibition by decreasing neuronal M2 receptor function. It also revealed that pyrethroid derivatives are also associated with asthma-like episodes (7). A different study done in Iran showed an association between work-place exposure and asthma episodes (17).
CHAPTER THREE: STUDY DESIGN AND METHODOLOGY

3.1 Introduction
This chapter describes the study design and methodology in details.

3.2 Study area
This study was part of a wider Partnership for Innovative Medical Education in Kenya-Medical Education (PRIME K) Maternal Newborn and Child Health Linked Research topic: “The effect of pesticide exposure on serum cholinesterase levels among asthmatic children in Naivasha” and was carried out at the paediatric ward at Naivasha District, Kenya.

Naivasha District hospital is a level 4 hospital located in Sokoni location, Lakeview sub location in Nakuru County. It is about 100 km North West of Nairobi. It has bed capacity of 143 and 17 cots. The services offered include antiretroviral therapy, Curative In-patient Services, Family Planning, HIV Counselling and Testing and Immunization.

3.3 Study design
A cross sectional study design was used. In this study, the researcher reached out to the population of interest through the paediatric filtration clinic, identified the exposed from non exposed cases at a point in time, interviewed respondents, collected blood samples, separated serum from whole blood, batched up the serum samples and froze them at \(-21^\circ C\) at Naivasha Blood Bank laboratory. Serum Cholinesterase levels of children living in or near flower farms and those who stay in non flower farm areas was analyzed at UoN Clinical Chemistry Unit laboratory.

3.4 Target population
The target population was asthmatic male and female paediatric patients aged 5-12 years old, who were either exposed or unexposed to pesticides and their parents or guardians in Naivasha District.

3.4.1 Inclusion criteria
Both male and female children exposed or unexposed to organophosphates aged 5-12 years old presenting to the hospital with an asthmatic attack and whose guardians/parent gave consent to participating in the study.
3.4.2 Exclusion criteria

These included:

- Children above 13 years and below 5 years old.
- Other conditions respiratory tract infections like tuberculosis
- Children who had stayed in Naivasha for less than three months.
- Children on chronic steroid use, contraceptives
- Children suffering from leukaemia, liver disease, severe anaemia
- Children or guardians who refused to give consent to participate in the study.

3.5 Sampling

3.5.1 Sampling technique

NDH paediatric unit (both out/inpatient) was the focus of the Study. Simple randomized technique was employed where all children who presented with asthma; were included in the study.

3.5.2 Sampling Size.

Patient sample size

A study done in Kenya showed a prevalence of exposed people was 29.6 per cent and had their cholinesterase activity depressed to values below 60 per cent of baseline. The baseline for the unexposed group is around 10-15 per cent, on average 12.5 per cent (14), (19).

At 95 per cent confidence interval, the sample size will be:

\[
N = \frac{(a + b)^2}{x^2} (p1q1 + p2q2)\]

Where:

- \(N\) = sample size in each of the groups
- \(P1\) = proportions of subjects exposed to pesticides (29.6 per cent (%)) in group 1.
- \(Q1\) = Proportion of unexposed subjects in group1 (1-p1) = (1-0.296)100% = 70.4%
- \(P2\) = proportion of subjects exposed to pesticides in group 2 = (12.5%)
- \(Q2\) = proportion of unexposed subjects in group 2 (1-p2) = (1-0.125)100% = 87.5%
- \(X\) = mean difference between two samples the investigator wishes to detect = (29.6% - 12.5%) = 17.1%.
a = conventional multiplier of alpha = 1.96
b = conventional multiplier for power = 0.842
Thus;
N = 30.44
Hence each group will have a sample size of approximately 30.

3.6 Data collection method and instruments of data collection

3.6.1 Research instrument
Data was collected using structured questionnaires (Appendix V part 1). The parent or guardian of the child was interviewed, the data on the knowledge about pesticide exposure and the social demographic characteristics and the information was filled in the questionnaire. The results helped correlate control of asthma and the levels of serum ChE levels among children 5-12 years old.

3.6.2 Researchers
The investigator co-ordinated the study and administered the questionnaire. The research assistant was a Medical officer who drew blood from the participants. Two clinical officers working at the paediatric department dispatched the consent forms and collection of blood samples. One laboratory technologist helped separate serum from whole blood. A clinical Chemist specialist analyzed the final serum levels at the UoN clinical chemistry unit laboratory.

3.6.3 Pilot Study or Pre-Testing
The questionnaires were proof read by a research expert then piloted in the study area before data collection was done.

3.6.4 Validity
To ensure quality of the data collected, the piloted questionnaires were evaluated from the respondents’ perspective, any ambiguities were addressed and the questionnaires edited.
3.6.5 Reliability
The same questionnaires were used to collect data among the exposed patients and non-exposed patients hence the results were generalizable to the Naivasha population.

3.6.6 Laboratory procedures
The skin was swabbed with an alcohol based cotton swab and this was followed by collection of 5ml venous blood using the red top bottles which were frozen to 4 degrees centigrade in a cold chain. 1.5ml of Serum was then separated from whole blood and frozen at -21°C at the Naivasha Blood bank laboratory. The serum was batched up for 28 days and then transported to UoN Clinical Chemistry Unit Laboratory under cold chain using cooler boxes. The samples were then analyzed as shown in appendix VI.

3.7 Data management

3.7.1 Data processing and analysis
The questionnaire was coded and data entered into an Excel spreadsheet. Data analysis was carried out using Stata version 12. Categorical data was summarized using frequencies and percentages while continuous data was summarized using means. Continuous data was categorized and the chi square test was used to determine the association between categorical predictor and outcome variables. Where the assumptions to carry out the chi square test were not all met, Fischer’s exact test was used. All p-values less than 0.05 were considered to be statically significant.

3.7.2 The Storage of Research Data and Primary Materials
The primary investigator was permitted to retain copies of data and materials for his/her own use, however original data and materials was controlled as outlined in and subject to external legislative requirements and the University’s policies and procedures (40).

3.7.3 Retention of Research Data and Primary Materials
The paper records were converted to an electronic format and were retained until the research process was over. The primary materials were kept under lock and key until the dissertation was handed over to the examiners.
3.7.4 Remains of samples after laboratory analysis

The remains of the samples were only stored for a maximum of three months (end of study). This is because during storage, AChE enzyme activity may take several days to reach its minimum and usually remains depressed longer, sometimes 1-3 months, until new enzyme replaces that inactivated by organophosphate. Hence the backup samples were stored for three months at UoN Clinical Chemistry Unit laboratory then destroyed as per regulations. The remaining specimens were destroyed.

3.7.5 Dissemination plan

This will be done by presenting papers at conferences or seminars, producing posters, and publishing in journals and books in collaboration with Prime K. The study was also included in the UoN electronic archives. In doing so, the study will be available worldwide for free download to anyone who has access to the Internet.

3.7.6 Data quality control

Before commencement of data collection, the research assistants involved were recruited and trained on ethical conduct of interviews, collection of blood samples and separation of serum from whole blood and filling of the structured questionnaires. The analysis equipment was calibrated, pretested and data compared with sampled physical data to ascertain its validity. A blank was used as a baseline.

3.7.7 Data Quality Assurance

The right analytical information system Roche/Hitachi/902/912 analyzer was used to ensure quality. The Roche method was validated before any test was run. The users of this equipment were experts from UoN Clinical Chemistry Unit laboratory trained to use it according to US users’ specifications. The materials and the laboratory procedure used were according to Roche Method specification as shown in appendix VI.

3.8 Ethical considerations:

3.8.1 Informed consent

Ethical approval was sought from the UoN/KNH Ethics Committee (Appendix I). Subsequently permission to carry out the study in Naivasha was sought from the administrators of the respective hospitals. Informed consent was obtained from all the children, guardians or parents. The data collected was treated with confidentiality and no
names were written on the questionnaire in order to guarantee the confidentiality of the participants. Coding of the questionnaire was done. No inducement was given to the participants.

Potential participants (children, guardians or parents) were informed about the study through an oral presentation in a private room by the investigators regarding the objective of the study, procedure that was carried out, potential hazards and rights of the participants. Participants were required to understand and sign a consent form summarizing the discussion prior to admission to the study (Appendix III and IV). A copy of the signed informed consent statement was to participants while a second copy was retained by the investigator.

3.8.2 Participant recruitment
This was done through on-site screening at the paediatric filtration clinic and enrolment done by health care workers and the investigators at paediatric clinic and casualty areas at Naivasha County hospital. The informed consent was administered to the participants by the research investigators.

3.8.3 Withdrawal from study
Participants were informed that they were free to drop-out from the study at any time if they were not interested, the data and sample obtained was destroyed according to NEMA (see acronyms) rules and also according to the ethics code with no further reference. Every effort was made to obtain a complete follow up for any withdrawal.

3.8.4 Compensation
Participants were not compensated on account of their participation in the study.
CHAPTER 4: RESULTS

4.1 Introduction
This chapter discusses the analysis of the results guided on the objectives. They include socio-demographic characteristics, exposure status and level of asthma control, and comparison of serum cholinesterase levels and the levels of asthma control among asthmatic children in Naivasha. The analysis was tested at a level of significance of 0.05.

4.2.1 Socio-demographic characteristics of children
There were more males (63.3%) than females (36.7%) (Table1). Test subjects had slightly more males (53.3%) whereas the control subjects had more females (55%) (Figure1).

Figure 1: Distribution of male and female in the test and control samples.

The most represented age group was 9-13 years, which is the age bracket of early adolescent. Majority (96.7%) were attending school and therefore capable of traversing wide areas which may predispose them to come in contact with the pesticides directly or indirectly. The results showed that most of the children (40%) had been managed for duration of 6-12 months since the first diagnosis. Family history of asthma was associated with only 15% of the children in the study.
Table 1: Socio-demographic characteristics of asthmatic children

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No.</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>38</td>
<td>63.3</td>
</tr>
<tr>
<td>Female</td>
<td>22</td>
<td>36.7</td>
</tr>
<tr>
<td><strong>Age in years</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 - &lt; 7</td>
<td>15</td>
<td>25</td>
</tr>
<tr>
<td>7 - &lt; 9</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>9 - &lt; 11</td>
<td>19</td>
<td>31.7</td>
</tr>
<tr>
<td>11-13</td>
<td>14</td>
<td>23.3</td>
</tr>
<tr>
<td><strong>School status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>58</td>
<td>96.7</td>
</tr>
<tr>
<td>No</td>
<td>2</td>
<td>3.3</td>
</tr>
<tr>
<td><strong>Period of asthma management in months</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 3</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>3 - 6</td>
<td>19</td>
<td>31.7</td>
</tr>
<tr>
<td>6 – 12</td>
<td>24</td>
<td>40</td>
</tr>
<tr>
<td>12-60</td>
<td>11</td>
<td>18.3</td>
</tr>
<tr>
<td><strong>Family history of asthma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>No</td>
<td>51</td>
<td>85</td>
</tr>
</tbody>
</table>

4.2.2 Socio-demographic characteristics of guardians

Most of the guardians were mothers (61.7%) who worked in the flower farms followed by fathers (Table 2). The other occupation for the mothers included teaching, farming, business and managing homes. Comparatively, most fathers (48.3%) worked in the flower farms while others were farmers, drivers, and motor cyclist respectively. Only one guardian had no formal education and the majority (58.3%) had attained secondary education. Degree was the highest level of education.
Table 2: socio-demographic characteristics of guardians

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No.</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Guardian</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Father</td>
<td>14</td>
<td>23.3</td>
</tr>
<tr>
<td>Mother</td>
<td>37</td>
<td>61.7</td>
</tr>
<tr>
<td>Mother + Father</td>
<td>9</td>
<td>9.0</td>
</tr>
<tr>
<td><strong>Father’s occupation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flower worker</td>
<td>29</td>
<td>48.3</td>
</tr>
<tr>
<td>Farmer</td>
<td>6</td>
<td>10.0</td>
</tr>
<tr>
<td>Driver</td>
<td>4</td>
<td>6.7</td>
</tr>
<tr>
<td>Business man</td>
<td>4</td>
<td>6.7</td>
</tr>
<tr>
<td>Motor cyclist</td>
<td>6</td>
<td>10.0</td>
</tr>
<tr>
<td>Teacher</td>
<td>5</td>
<td>8.3</td>
</tr>
<tr>
<td>Pastor</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td>Not known</td>
<td>5</td>
<td>8.3</td>
</tr>
<tr>
<td><strong>Mother’s occupation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flower farm worker</td>
<td>27</td>
<td>45.0</td>
</tr>
<tr>
<td>House help</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td>Farmer</td>
<td>8</td>
<td>13.3</td>
</tr>
<tr>
<td>House wife</td>
<td>7</td>
<td>11.7</td>
</tr>
<tr>
<td>Business lady</td>
<td>15</td>
<td>15.0</td>
</tr>
<tr>
<td>Teacher</td>
<td>2</td>
<td>3.3</td>
</tr>
<tr>
<td><strong>Level of education</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No formal education</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td>Primary</td>
<td>18</td>
<td>30</td>
</tr>
<tr>
<td>Secondary</td>
<td>35</td>
<td>58.3</td>
</tr>
<tr>
<td><strong>Tertiary</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Certificate</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diploma</td>
<td>2</td>
<td>3.3</td>
</tr>
<tr>
<td>Degree</td>
<td>4</td>
<td>6.7</td>
</tr>
</tbody>
</table>

4.3 The level of exposure to pesticides among asthmatic children

4.3.1 Pesticide utilization

Slightly more than half of the guardians (53%) reported using insecticides as a method of pest control (table 3). Other methods applied included use of rodenticides, acaricides, bush clearing and miticides respectively. A few of them (1.7%) used combination of the above methods to control pests. Thirteen percent of the guardians did not use any pesticides for pest control in their homes hence had no direct indoor exposure.
Table 3: Pesticide utilization

<table>
<thead>
<tr>
<th>Method</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insecticides</td>
<td>32</td>
<td>53.3</td>
</tr>
<tr>
<td>Acaricides</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Miticides</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td>Rodenticides</td>
<td>8</td>
<td>13.3</td>
</tr>
<tr>
<td>Insecticides and rodenticides</td>
<td>4</td>
<td>6.7</td>
</tr>
<tr>
<td>Insecticides, acaricides and rodenticides</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td>Insecticides and acaricides</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td>Bush clearing and insecticides</td>
<td>2</td>
<td>3.3</td>
</tr>
<tr>
<td>Not known</td>
<td>8</td>
<td>13.3</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>100</td>
</tr>
</tbody>
</table>

4.3.2 Safety practices of the guardians

Safety practices of the guardians were assessed using a series of questions that sought to find out if they used protective measures during the application of pesticides. Each question was given a score of 1. The maximum possible score was 7 points. The mean safety score was 3.53 points. An analysis of the safety score by exposure status of the guardians showed that guardians who had worked in flower farms had higher mean safety scores than those who had not and this difference was statistically significant.

Table 4: Safety practices of guardians

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Number</th>
<th>Mean safety score</th>
<th>95%CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>30</td>
<td>5.17</td>
<td>(1.93 - 4.45)</td>
<td>0.0000</td>
</tr>
<tr>
<td>No</td>
<td>30</td>
<td>1.9</td>
<td>(0.92 - 2.88)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>3.53</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.3.3 Criteria of exposure to pesticides among children

Children were exposed to pesticides through direct working on the flower farm (1.7%), proximity of school to a flower farm <500m (13.3%) and previous exposure in a learning institution (17%) (Table5).
Table 5: Sources of exposure to pesticides among children

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No.</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distance of school from flower farm in meters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 500</td>
<td>8</td>
<td>13.3</td>
</tr>
<tr>
<td>&gt;500</td>
<td>52</td>
<td>86.7</td>
</tr>
<tr>
<td>Previously attended school near a flower farm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>No</td>
<td>43</td>
<td>71.7</td>
</tr>
<tr>
<td>Not known</td>
<td>16</td>
<td>26.6</td>
</tr>
<tr>
<td>Children working on the farm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td>No</td>
<td>59</td>
<td>98.3</td>
</tr>
</tbody>
</table>

4.3.4 Criteria of exposure to pesticides among guardians

Fifty percent of the guardians of the test children were exposed to pesticides while the rest were not (table 6). The duration of exposure to pesticides was between up to sixty months. The longest duration of exposure was rated at 20% (> 24-60 months). Slightly less than half (48.3%) of them were either not exposed or did not know the duration of exposure to the pesticides.

Table 6: Level of exposure to pesticides among guardians

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No.</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure to pesticides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>No</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>Duration of exposure to pesticides in months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;3</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td>3-6</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>&gt;6-12</td>
<td>7</td>
<td>11.7</td>
</tr>
<tr>
<td>&gt;12-24</td>
<td>5</td>
<td>8.3</td>
</tr>
<tr>
<td>&gt;24-60</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>Not known</td>
<td>29</td>
<td>48.3</td>
</tr>
</tbody>
</table>
4.4 Level of Asthma control among the asthmatic children.

4.4.1 The effect of education to Asthma control

Participants who had well controlled asthma constituted 56.7% (table 7). Majority of the guardians (58.3%) had secondary education and most of their children had asthma under control. Those who attained primary education (30%) had majority of cases under control. Further still majority of those with degree education had most of their children’s asthma uncontrolled. Comparison of these categories reveal that there was no significant association between the guardian’s education status and the level of asthma control of the child (Fischer’s exact test, p=0.288).

Table 7: Relationship between guardian’s education levels and asthma control

<table>
<thead>
<tr>
<th>Status of Asthma control</th>
<th>Education level of the guardian</th>
<th>Fischer’s exact test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not known</td>
<td>Primary</td>
</tr>
<tr>
<td>Under control</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1(1.7%)</td>
<td>13(21.6%)</td>
</tr>
<tr>
<td>Not controlled</td>
<td>0(0%)</td>
<td>5(8.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>1(1.7%)</td>
<td>18(30%)</td>
</tr>
</tbody>
</table>

4.4.2 The effect of exposure status and asthma control

There were equal numbers of children who were exposed and non-exposed. Among the exposed group, more children had controlled asthma than those who had uncontrolled asthma (31.7% and 18.3% respectively) while in the later group, asthma control was equal in both cases 15(25%). There was no statistically significant association between the level of asthma control of the child and the exposure status ($\chi^2$=1.09, p=0.297).

Table 8: Relationship between asthma control and exposure status

<table>
<thead>
<tr>
<th>Asthma control</th>
<th>Previous exposure</th>
<th>Chi test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exposed</td>
<td>Non exposed</td>
</tr>
<tr>
<td>Under control</td>
<td>19(31.7%)</td>
<td>15(25.0%)</td>
</tr>
<tr>
<td>Not controlled</td>
<td>11(18.3%)</td>
<td>15(25%)</td>
</tr>
</tbody>
</table>
| Total          | 30 (50%)  | 30 (50%)  | 60 (100%) | $\chi^2=1.09$, p = 0.297
4.5 The serum cholinesterase levels among asthmatic children

4.5.1 Pesticide exposure and Cholinesterase level

The mean serum cholinesterase level (table 9) of all the children was 6403.7 with a range 618-10575 while those of exposed and unexposed were 5834.6 and 6972.8 respectively. There was statistically significant association between pesticide exposure and mean serum cholinesterase levels (t= 2.0960, p= 0.0405).

Table 9.0: Relationship between pesticide exposure and mean serum cholinesterase levels

<table>
<thead>
<tr>
<th>Exposure status</th>
<th>Number</th>
<th>Mean ChE level</th>
<th>95% CI</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposed</td>
<td>30</td>
<td>5834.6</td>
<td>(5236.0 - 6433.1)</td>
<td>t=2.0960</td>
</tr>
<tr>
<td>Non exposed</td>
<td>30</td>
<td>6972.8</td>
<td>(6404.1 - 7541.6)</td>
<td>P =0.0405</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>6403.717</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.5.2 Association between age and serum cholinesterase levels

The popular age group (31.7%) was in the range of (9-<11) years while the rest {(5-<7), (7-<9), (11-13)} were 25%, 20% and 23.3% respectively (table10). The age categories were further cross tabulated against the level of serum ChE either as low or normal. Few of the children (3.3%) were in age categories of (5-<7) and (11-13) years respectively while the rest (8.4%) were in the low levels of serum ChE group. The normal ChE group had most of them (23.3%) in the age range of (9-<11) years while the category with the least cholinesterase levels (15%) was (7-< 9). This showed that there was no association between the age and ChE levels. There was no statistically significant association between the age of the child and the cholinesterase levels ($\chi^2 =1.36$, p=0.714)

Table 10: Relationship between age and serum ChE levels

<table>
<thead>
<tr>
<th>Cholinesterase levels</th>
<th>Age (years)</th>
<th>Chi test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5-&lt;7</td>
<td>7-&lt;9</td>
</tr>
<tr>
<td>Low</td>
<td>2(3.3%)</td>
<td>3(5%)</td>
</tr>
<tr>
<td>Normal</td>
<td>13(21.7%)</td>
<td>9(15%)</td>
</tr>
<tr>
<td>Total</td>
<td>15 (25%)</td>
<td>12(20%)</td>
</tr>
</tbody>
</table>
4.5.3 Association between serum cholinesterase levels and sex of the children

There were more males (63.3%) than females (36.7%) in the study (table 11). More males (53.3%) than females (26.7%) had normal cholinesterase levels while there was no difference in prevalence in the low ChE category. There was no statistically significant association between the gender of the child and the cholinesterase levels ($\chi^2 = 1.15$, p=0.284).

Table 11: Relationship between serum ChE levels and sex of children

<table>
<thead>
<tr>
<th>Cholinesterase levels</th>
<th>Sex</th>
<th>Chi test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Low</td>
<td>6(10%)</td>
<td>6(10%)</td>
</tr>
<tr>
<td>Normal</td>
<td>32(53.3%)</td>
<td>16(26.7%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>38 (63.3%)</strong></td>
<td><strong>22(36.7%)</strong></td>
</tr>
</tbody>
</table>

4.5.4 Association between school attendance status and serum cholinesterase levels

Majority of the study participants reported that they attended school (96.7%), out of these, 16.7% had low cholinesterase levels and 80% had high cholinesterase levels. Only two students (3.3%) did not attend school and both of them had low cholinesterase levels. The association between the school status and the cholinesterase levels was determined. There was a statistically significant association between the school status of the child and the cholinesterase levels ($\chi^2 = 8.28$, p=0.004).

Table 12: Relationship between school attendance status and cholinesterase level

<table>
<thead>
<tr>
<th>Cholinesterase levels</th>
<th>School attendance status</th>
<th>Chi test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Attends school</td>
<td>Does not attend school</td>
</tr>
<tr>
<td>Low</td>
<td>10(16.7%)</td>
<td>2(3.3%)</td>
</tr>
<tr>
<td>Normal</td>
<td>48(80%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>58 (96.7%)</strong></td>
<td><strong>2(3.3%)</strong></td>
</tr>
</tbody>
</table>

4.5.4.1 Association between school distance and serum cholinesterase level

The distance of the school from the flower farm is important because it is one of the factors that determine exposure of children to pesticide exposure. Most children (86.6%) attended schools that were far away from the flower farms. Of these, 13.3% had low cholinesterase levels and 73.3% had normal cholinesterase levels. The number of children who attended schools that were near flower farms was (13.3%). Equal numbers of these
had low and normal cholinesterase levels (6.7%). The association between the distance of the school from the flower farm and the cholinesterase levels was statistically significant ($\chi^2 = 5.19, p=0.023$).

**Table 13: Relationship between distance of school from flower farm and serum cholinesterase level**

<table>
<thead>
<tr>
<th>Cholinesterase levels</th>
<th>Distance of school from flower farm</th>
<th>Chi test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;500m</td>
<td>&gt;500m</td>
</tr>
<tr>
<td>Low</td>
<td>4(6.7%)</td>
<td>8(13.3%)</td>
</tr>
<tr>
<td>Normal</td>
<td>4(6.6%)</td>
<td>44(73.4%)</td>
</tr>
<tr>
<td>Total</td>
<td>8(13.3%)</td>
<td>52(86.7%)</td>
</tr>
</tbody>
</table>

**4.5.5 Association between working on farm and serum cholinesterase levels**

A small population of the guardians (1.7%) worked on the flower farm while 98.3% did not (table 14). All the 12 children whose guardians did not work on the flower farm had low cholinesterase levels. Few of their children had normal ChE levels (1.7%) compared to the majority of them (78.3%). There was no statistically significant association between working on the farm and the serum cholinesterase levels ($\chi^2 = 1.09, p=0.614$).

**Table 14: Relationship between working on farm and serum ChE levels**

<table>
<thead>
<tr>
<th>Cholinesterase levels</th>
<th>Status of working</th>
<th>Chi test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Works in a farm</td>
<td>Does not work in a flower farm</td>
</tr>
<tr>
<td>Low</td>
<td>0(0%)</td>
<td>12 (20%)</td>
</tr>
<tr>
<td>Normal</td>
<td>1(1.7%)</td>
<td>47 (78.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>1(1.7%)</td>
<td>59 (78.3%)</td>
</tr>
</tbody>
</table>

**4.5.6 Association between asthma control and cholinesterase levels**

Slightly more than half of the children (56.7%) had the asthma under control (table 15). Out these, 16.7% had low serum cholinesterase level and 40% were within the normal range. Comparison of serum ChE in the poorly controlled asthma category revealed that majority of the children (40%) had normal levels while rest had very low levels (3.3%). The ratio of asthma controlled to uncontrolled was 1:1 in the normal ChE category. There was a significant association between the level of asthma control and the cholinesterase levels ($\chi^2 = 4.34, p=0.037$).
Table 15: Cross tabulation of asthma control and serum ChE levels

<table>
<thead>
<tr>
<th>Cholinesterase levels</th>
<th>Level of asthma control</th>
<th>Total</th>
<th>Chi test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Under control</td>
<td>Poorly controlled</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>10(16.7%)</td>
<td>2(3.3%)</td>
<td>12(20%)</td>
</tr>
<tr>
<td>Normal</td>
<td>24(40%)</td>
<td>24(40%)</td>
<td>48(80%)</td>
</tr>
<tr>
<td>Total</td>
<td>34 (56.7%)</td>
<td>26(43.3%)</td>
<td>60 (100%)</td>
</tr>
</tbody>
</table>

\[ \chi^2 = 4.34, \ p=0.037 \]

4.6 Summary of results

Factors that were found to be significantly associated with serum cholinesterase levels were distance of school from flower farms, school status of the child and level of asthma control. There was no significant relationship between the child’s cholinesterase levels and gender of the child, working on a flower farm and the guardian’s previous exposure to pesticides.
CHAPTER FIVE: DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Introduction
This chapter involves the discussion of results, described in chapter 4 in comparison to other studies done in relation to pesticide exposure, serum ChE concentrations and asthmatic children between 5-12 years old. Conclusion and recommendations are also included in this chapter.

5.2 Discussion
Asthma is caused by genetic or environmental factors. This study revealed that only a few of the children had a family history of asthma compared to global prevalence associated with genetic pre-disposition(28). Early in life, genetic factors contribute to the risk of severe lower respiratory tract viral infection as well as later development of wheezing and asthma(26). Predisposing factors to asthma attack include pesticides, smoke, allergens, infections, microbes, and stress. Pesticides and pesticides have been found to be associated with increased frequency of asthma attacks since they are irritants and directly damage the mucosa, thus making the airway very sensitive (14), (19),(28)(29).

A study done in Canada, Lebanon and Kenya reported an association between organophosphates and asthma (5), (6), (7). The commonest OPs used in this area were Malathion, diazinon and chlorpyrifos. A herbicide known as paraquat was also used(14), (19), (28). Pesticides may increase the risk of developing asthma, exacerbate a previous asthmatic condition or even trigger asthma attacks by increasing bronchial hyper-responsiveness (3),(21). Mechanisms of exposure to pesticides include inhalation, ingestion, breast feeding, transplacental transfer and direct absorption through the skin (17).

Children are usually more vulnerable to the effects of pesticides and asthma diagnosis is made before the age of five because respiratory, immune and nervous systems in children are still developing hence are more vulnerable to pesticides (19),(22),(29). In addition, their exposures are relatively greater than those of other non-occupationally exposed people because they tend to eat and drink more than adults in relation to their body weight and so take in relatively more residues from fruits and vegetables. Furthermore, they
inhale relatively more air than adults, making them more vulnerable to the effects of spray drift and household insecticides. Studies have also shown that a breathing rate approximately double that of adults, in the first 12 years of life and together with relatively greater lung surface area means the amount of airborne residues reaching the lung surface in a 3-month old child is likely to be about 3-4 times that in adults (24), (26).

Males were more than females and this is similar to other studies done whose findings showed a high prevalence of asthma among the male sex (29). Pesticide exposure is associated with asthma diagnosis before age of five compared to other age groups due to the fact that respiratory, immune and nervous systems in children are still developing hence are more vulnerable to pesticides (22),(29). However, these study participants were between 5 and 13 years (30).

Sources of exposure among the children included direct working on the flower farms, living or schooling near farms within a distance less than 500m and direct inhalation of household insecticides like cypermethrins. Studies have shown that people who live within the vicinity of flower farms are more likely to be exposed to agricultural chemicals (26). Indirect exposure by their guardians was through poor safety practises as revealed in this study. Majority of the parents who were exposed were within the safety margin unlike the unexposed and some of them went home with contaminated clothing which caused indoor pollution. The findings are consistent with previous studies conducted in Zimbabwe, Lebanon and Kenya (7), (12),(14). The results indicate that the health and safety programmes in the commercial farms in these areas are inadequate. Other studies have also shown poor safety practises in personal hygiene and proper use of personal protective equipment by flower workers (15), (27).

Studies have shown that those who use more pesticides over a longer period of time had higher total pesticide exposure. In this study, most guardians had worked on the farms for more than six months. Those who were exposed to fungicides, herbicides, organophosphates and pyrethrins had higher total pesticide exposure. Furthermore, similar studies have shown that a farmer who was a pesticide applicator, mixer, loader and who wiped sweat with contaminated piece of fabric, and who had not been given instructions through training was at risk of having higher pesticide exposure (27). Higher total pesticide exposure has an effect on serum acetylcholinesterase concentration.
The results generally revealed a lower mean cholinesterase level among the exposed patients compared to the controls. This is explained by the fact that all the test children were exposed by location of residence or school and by their guardians who work on flower farms. Overexposure to organophosphate and carbamate insecticides can result in cholinesterase inhibition. These pesticides combine with acetylcholinesterase at nerve endings in the brain and nervous system, and with other types of cholinesterase found in the blood. This allows acetylcholine to build up, while protective levels of the cholinesterase enzyme decrease. The more cholinesterase levels decrease, the more likely symptoms of poisoning from cholinesterase inhibiting pesticides are to show. This results to repeated and unchecked firing of electrical signals which can cause uncontrolled, rapid twitching of some muscles, paralyzed breathing, convulsions, and in extreme cases, death (11), (39). This therefore, suggests why the patients who were exposed to organophosphates had frequent attacks of asthma. A few of them were exposed to pyrethroids like cypermethrin.

Organophosphorus and carbamate insecticides are toxic to insects and mammals by virtue of their ability to inactivate the enzyme acetylcholinesterase. Presence of cholinergic effects, oxidative stress and hyperglycemia has been reported by many authors as one of the adverse effects in poisoning by OP. Oxidative stress induced by organophosphate leads to disturbances in the function of different organs and tissues. In sub-chronic or chronic OP exposition induction of oxidative stress has been reported, as the main mechanism of its toxicity (19). Thus, this study found that most patients were aware of the effect of pesticides to their health. The guardians reported respiratory, skin, eye, cancer and infertility problems as some of the effects caused by exposure to pesticides. These effects have been reported in Lebanon, Kwekwe area and the Kenyan working in flower farms (7), (12), (14).

The risk factors of asthma exacerbations were pesticide exposure due to exposure of the parents or location of stay or school and generally low socioeconomic status (34). Parents carry the sensitizers on their clothes back home hence leading to indoor pollution. However, an analysis of the safety score by exposure status of the guardians showed that guardians who had worked in flower farms had higher mean safety scores because of on the job training than those who had not and this difference was statistically significant (10), (11). A study done in the U.S. reported that pesticide exposure affected both
individuals who lived in Latin America and the immigrants (1). However, the findings in this study showed no statistically significant association between the level of asthma control of the asthmatic child and the exposure status.

Pesticide causes exacerbation of asthma. Organophosphate pesticide-induced asthma (irritant induced asthma) is mediated by inhibition of acetyl-cholinesterase (AChE), the enzyme that degrades acetylcholine. However, organophosphates can produce bronchial hyper-responsiveness in the absence of AChE inhibition by causing a loss of parasympathetic pre-junctional muscarinic M2 receptor function. The level of asthma control among children was done as per the Kenyan guidelines. The level of education of the guardian and their occupation did not show any statistically significant association with the level of asthma control compared to other studies(3),(7),(17),(21),(36).

There was statistically significant association between pesticide exposure and mean serum cholinesterase levels. Oxidative stress increases production of reactive oxygen species has been implicated in the toxicity of many pesticides. A similar study done on rats revealed that with a mixture of organophosphates and pyrethroids found significant increase in thiobarbituric acid reactive substances, which might be associated with decreased levels of reduced glutathione, superoxide dismutase, catalyse, glutathione S-transferase and acetylcholinesterase activities and protein content in rat brain Hernandez AF et Al, Mostafalou S et al and Henneberger PK et al and found the same trends in their studies(3),(17),(21). The mean serum ChE concentration was generally lower in the test arm compared to the control. Further still, the means of both arms was generally lower in comparison to the average normal value. This indicates that the population in this area may be exposed directly or indirectly. The residents were probably exposed through inhalation of the pesticides arising from dispersion of the chemicals in the environs by wind or through traversing through the flower farms. Assessment of the general knowledge of the participants showed evidence of use of insecticides like organophosphates and pyrethroids; hence the effect of pesticide exposure is evident on the concentration of serum cholinesterase among the asthmatic children.

The factors affecting severity of asthma include working on the flower farm age, sex, school status and school distance from the flower farms. The factors have a strong association with the serum ChE levels were school status and the school distance from the flower farm. These trends were similar to a Lebanese study on school children which
reported a prevalence of 12.4% of chronic respiratory disease due to exposure to pesticides (7). Studies have shown that prolonged stay on or near commercial fields using pesticides is associated with a high prevalence of asthmatic conditions. However, there was no significant relationship between the child’s cholinesterase levels and sex, age, or guardian attributes which was consistent with the findings of a study done in Iran and Denmark (17), (30).

Kenya has current guidelines that govern asthma management, within which there is a criteria for assessing the level of asthma control among children (36). Analysis of asthma control and serum ChE levels showed an association. This result is similar to other studies done in Lebanon, Kwekwe area in Zimbabwe and Kenyan (7), (12), (14). A Lebanese study showed that organophosphates and organochlorates have anticholinesterase activity and are known to cause asthma episodes. In addition, the study revealed that OPs can also cause airway hyperreactivity in the absence of AChE inhibition by decreasing neuronal M2 receptor function. The same study further showed that pyrethroid derivatives are also associated with asthma-like episodes (7). This is evident in this study as most of the guardians used pyrethroids as a method of indoor pest control amongst other methods.

5.2 Conclusion

There was statistically significant association between pesticide exposure and the mean serum cholinesterase levels. Further still, children who stayed or schooled near a flower farms had low cholinesterase levels. Unsafe work practices predisposed the farmers’ children to health related problems. Factors like age, sex, guardian’s occupation, and working on the farm did not have any association with the serum ChE levels and asthma control.
5.3 Recommendations

5.3.1 Recommendations for policy and Practice

A local level policy research for program intervention among flower farmer workers using indoor insecticides like pyrethrins should be established to help reduce pesticide exposure among the local people. In addition, integrated pest management (IPM) system should be adapted. IPM is designed to choose environmentally friendly course of action in controlling pests as well as substitution of acetylcholinesterase inhibiting pesticides.

5.3.2 Recommendations for research

This study suggests that intervention measures need to be done to lower pesticide exposure of farmers. It is also suggested that chronic effects of pesticide cited in certain studies such as carcinogenic effects, poor reproductive outcomes, neurologic and respiratory disorders, impairments of the immune system and birth defects should also be investigated in future studies.
REFERENCES


APPENDICES

Appendix I: Funding Information
This study is part of a wider Partnership for Innovative Medical Education in Kenya-Medical Education (PRIME K) Maternal Newborn and Child Health Linked Research topic; ‘‘The effect of pesticide exposure on serum cholinesterase levels among asthmatic children in Naivasha’’ and was carried out at the paediatric ward at Naivasha District, Kenya.

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)

The PRIME-K program aims 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.
Appendix II: Proposal approval letter

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Ref: KNH-ERC/A/142

Dr. Wafula Caroline Nasambu
Dept of Pharmaceutics and Pharmacy Practice
School of Pharmacy
University of Nairobi

Dear Dr. Wafula

RESEARCH PROPOSAL: THE EFFECT OF PESTICIDE EXPOSURE ON RED BLOOD CELL CHOLINESTERASE LEVELS AMONG ASTHMATIC CHILDREN IN NAIVASHA (P60/2/2014)

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and approved your above proposal. The approval periods are 15th May 2014 to 14th May 2015.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.

b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.

c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN ERC within 72 hours of notification.

d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.

e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period.

Attach a comprehensive progress report to support the renewal.

f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.

g) Submission of an executive summary report within 90 days upon completion of the study.

This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and or plagiarism.

For more details consult the KNH/UoN ERC website www.uonbi.ac.ke/activities/KNHUoN.

Protect to Discover
Appendix II: Proposal approval letter (continued)

Yours sincerely

[Signature]

PROF. M. L. CHINDIA
SECRETARY, KNUON-ERC

c.c. The Principal, College of Health Sciences, UoN
The Deputy Director CS, KNH
The Chairperson, KNUON-ERC
The Assistant Director, Health Information, KNH
The Dean, School of Pharmacy, UoN
The Chairman, Dept. of Pharmaceutics and Pharmacy Practice, UoN
Supervisors: Dr. Peter N. Karimi, Dr. Kefa Bosire Ongayo
Appendix III: Informed consent form for caregivers in Naivasha District

Title of the study: The effect of pesticide exposure on serum cholinesterase levels among asthmatic children in Naivasha.

Introduction: The study aims to determine the serum ChE levels in children aged 5-12 years presenting with asthma from households of flower farm work and non-flower farm workers. This study will provide useful information on the ChE levels as a biomarker of exposure, find out the levels of pesticide exposure and the results will be compared to the level of asthma control among children in Naivasha District. Consequently this will provide basis for policy making on health safety among families of flower farm workers.

Purpose of the study: the purpose of the study is to find out the effect of pesticide exposure among children presenting with asthma.

Procedure to be followed: With your permission, I will ask you some questions about pesticide exposure in relation to asthma. I will also use your file to obtain some information on your child’s illness history and history of pesticide exposure. In addition, I will withdraw some blood along with other routine hospital tests to be analysed in Nairobi Lancet Laboratory. All information will be handled with confidentiality and will only be used for the purpose of this study.

Risks: The study will involve venous blood withdrawal and it might be a bit uncomfortable to the child.

Benefits: No direct benefit to you is anticipated other than the knowledge obtained that may be used to reduce pesticide exposure and as a result improve on asthma control hence this will lead to reduced health burden, mortality and school absenteeism among children in the study area.

Confidentiality: All information obtained from you will be kept in confidence. Numbers will be used and at no point will your participation in this study be revealed.

Participant selection

The participants in this study are the care providers who are directly involved in care of asthmatic children aged 5-12 years old. You have been purposively selected to participate in this study because you have an asthmatic child within the mentioned age bracket.

Voluntarism

Your participation in this study is voluntary. You may choose not to participate in the study.

You may withdraw consent at any time and decide not to continue participating in the study.
**Confidentiality**

No names or personal information will be collected at any stage during the study. A coded number will be assigned to the questionnaire as opposed to the name. Interviews will be conducted in private. Any information that will be collected during the study will be kept confidential and not shared with a 3rd party unless your consent is sought. The data collected from the study will be stored under lock and key and presented as a thesis towards the Master of Pharmacy degree.

**Information on researchers**

If you require any additional information regarding the researcher, please contact:

**Institution:** Department of Pharmaceutics and Pharmacy Practice, School of Pharmacy, University of Nairobi, P.O BOX 30197-00400, Nairobi.

**Investigator:** Dr.WafuLa Caroline Nasambu, P.O.Box 30196-00100, NAIROBI, Email address; wafulacaroline@yahoo.com Mobile no. 0723245127.

**Supervisors:**

1. Dr. Peter N. Karimi, M. Pharm (MSc), MBA; Department of Pharmaceutics and Pharmacy practice, University of Nairobi; Mobile no. 0722436019
2. Dr. George Wandolo MB.Ch, B, Msc. (Chemical Pathology)
   Department of Human Pathology (clinical Chemistry Unit), University of Nairobi; Mobile No. 0721-563947
3. Dr.Kefa Bosire Ogonyo, M.Pharm (Pharmaceutical Analysis), Department of Pharmacology and Pharmacognosy, University of Nairobi, Mobile no. 07135421

**Information on the UoN/KNH Ethics and Research Committee**

If you would like to contact of the University of Nairobi/Kenyatta National Hospital Ethics and Research Committee regarding any aspects of this study, the contact details are:

University of Nairobi/Kenyatta National Hospital Ethics and Research Committee
Telephone 2726300 Ext. 44102

**Study approval**

The study proposal has been reviewed and approved by the University of Nairobi/Kenyatta National Hospital Ethics and Research Committee.
Signature of Research Participant
I have read the above information. I have been given the opportunity to ask questions and the questions have been answered satisfactorily. I agree to participate in the study.
Name of participant: ______________________________________________
Signature of participant: _________________________________________
Date: _______________________________________

Signature of Investigator
I have explained the research to the participant and answered his/her questions to the best of my ability. I confirm that consent has been given freely.
Name of Investigator: _______________________________________________
Signature of Investigator: _________________________________________
Date: ____________________________
Appendix IV: Child assent form (7-12 years)

I am Dr. Caroline Wafula from the University of Nairobi. I am doing a study to figure out (the effect of Pesticide exposure on Serum Cholinesterase levels among asthmatic Children in Naivasha). We are asking you to take part in the research study because (your parent recommended you for this study).

For this research, we will (ask you some questions about to find out the effect of pesticide exposure among children presenting with asthma). We will keep all your answers private, and will not show them to (parent(s)/guardian, friends or teacher). Only people working on the study will see them.

We don’t think that any big problems will happen to you as part of this study, but you might feel some slight pain when blood will be withdrawn from your hand.

Benefits: No direct benefit to you is anticipated other than the knowledge obtained that may be used to reduce pesticide exposure and as a result improve on asthma control hence this will lead to reduced health burden, mortality and school absenteeism among children in the study area. You can feel good about helping us to (make things better for other kids who might have problems at their home and school.)

You should know that:

- You do not have to be in this study if you do not want to. You won’t get into any trouble with (parent/guardian, your doctor, the school or me) if you say no.
- You may stop being in the study at any time. (If there is a question you don’t want to answer, just leave it blank.)
- Your parent(s)/guardian(s) were asked if it is OK for you to be in this study. Even if they say it’s OK, it is still your choice whether or not to take part.
- You can ask any questions you have, now or later. If you think of a question later, you or your parents can contact the following researchers or institution;

Information on researchers

If you require any additional information regarding the researcher, please contact:

Institution: Department of Pharmaceutics and Pharmacy Practice, School of Pharmacy, University of Nairobi, P.O BOX 30197-00400, Nairobi.
Investigator: Dr. Wafula Caroline Nasambu, P.O.Box 30196-00100, NAIROBI, Email address; wafulacaroline@yahoo.com, Mobile no. 0723245127.

Supervisors:
1. Dr. Peter N. Karimi, M. Pharm, MSc, MBA; Department of Pharmaceutics and Pharmacy practice, University of Nairobi; Mobile no. 0722436019
2. Dr. Kefa Bosire Ogonyo, M. Pharm (Pharmaceutical Analysis)
Department of Pharmacology and Pharmacognosy, University of Nairobi
Mobile no. 0713542111

Information on the UoN/KNH Ethics and Research Committee
If you would like to contact of the University of Nairobi/Kenyatta National Hospital Ethics and Research Committee regarding any aspects of this study, the contact details are:
University of Nairobi/Kenyatta National Hospital Ethics and Research Committee
Telephone 2726300 Ext. 44102

Study approval
The study proposal has been reviewed and approved by the University of Nairobi/Kenyatta National Hospital Ethics and Research Committee.

Sign this form only if you:
• have understood what you will be doing for this study,
• have had all your questions answered,
• have talked to your parent(s)/legal guardian about this project, and
• agree to take part in this research

_______________________________________
Your Signature

_______________________________________
Printed Name

_______________________________________
Date

_______________________________________
Name of Parent(s) or Legal Guardian(s)

_______________________________________
Researcher explaining study

Signature

_______________________________________
Printed Name

_______________________________________
Date
Appendix V: Questionnaire 1

Biodata

Study no.: ___________________ Date: ___________________
Data collector’s Initials: __________ Prescription code number: _________

Child’s Sociodemographic characteristic

Date of Birth: ______________ Age (years): _________________
Gender: Male Female

Does child go to school? Yes No
1. Is the school located near a flower farm? Yes No
2. If yes: specify the distance < 500m > 500m
3. If no, has child ever attended school near flower farm? Yes No N/A
4. Does the child work on the farm? Yes No

Guardian’s Sociodemographic Characteristics

1. Guardian: Father Mother mother and father
2. Guardian’s Occupation:
   i. Flower farm worker
   ii. House help
   iii. Farmer
   iv. Driver
   v. House wife
   vi. Business man/lady
   vii. Motor cyclist
   viii. Teacher
   ix. pastor
   x. N/A
3. Has the guardian ever worked on a flower farm? Yes No
4. If yes, specify duration: _________________________________
   0-0.5yrs 0.5-1year 1-2 years 2-5years > 5years N/A
5. How long has the child been managed for asthma? < 3 months 3 – 6 months 6-12 months >1-5yrs >5yrs
6. Other co-morbidities No Yes
7. If yes, specify……………………………………………………………………………………………………...
   Sickle cell anaemia N/A
8. Highest educational level of guardian:
No formal Education  Primary  Secondary  Tertiary:  Degree  
Diploma  certificate

9. How do you keep pests away from your household?
   a) By use of insecticides e.g. pyrethroids like doom
   b) By use of acaricides to kill ticks
   c) fungicides,
   d) miticides,
   e) rodenticides,
   f) wood preservatives
   g) insecticides + rodenticides
   h) insecticides + acaricides + rodenticides
   i) acaricides + rodenticides
   j) insecticides + acaricides
   k) bush clearing + insecticides
   l) N/A

10. What are some of the safety measures practiced by workers?

<table>
<thead>
<tr>
<th>item</th>
<th>Safety measure</th>
<th>Yes</th>
<th>No</th>
<th>N/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>a.</td>
<td>Wears protective clothes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b.</td>
<td>Clothes are cleaned in the same Laundry with other family member clothes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c.</td>
<td>Follow label instructions and agronomist guiding while working on the farms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d.</td>
<td>Have a re-entry period in the farm after applying pesticides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e.</td>
<td>Farmer smokes, eats, drinks, or chews gum during application of pesticides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d.</td>
<td>Have a water bath After application of pesticides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e.</td>
<td>Comply with the concentration recommended by</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
11. Are you aware of the health impact of exposure to pesticides?
   Yes  No

12. If yes, specify………………………………………………………………………………
   i. Chest problems
   ii. Skin problems
   iii. Chest + skin problems
   iv. Eye problems
   v. N/A
   vi. Infertility
   vii. Cancer

13. Do you have a family history of asthma?
   Yes  No

**Types of pesticides used on the farm**

1. Do you know the type of pesticides use on the farm?
   Yes  No

2. If yes, specify………………………………………………………………………………
   i. Diazinon
   ii. Cyclone (active ingredient paraquat dichloride)
   iii. N/A
   iv. Malathion
   v. Ectomin (cypermethrin; synthetic pyrethroid)
   vi. Rat and rat (rodenticides)

**Levels of serum ChE inhibition in children**

1. RBC ChE inhibition level in the child

   ________________________________________________________________
### Childhood Asthma Control Test

**Ask your child to answer these questions**

1. How does your asthma make you feel today?

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>I feel very ill today</td>
</tr>
<tr>
<td>1</td>
<td>I feel well today</td>
</tr>
<tr>
<td>2</td>
<td>I feel very well today</td>
</tr>
</tbody>
</table>

2. How much does your asthma bother you when you run, exercise or play sports?

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>It bothers me a lot, I can’t do what I want to do</td>
</tr>
<tr>
<td>1</td>
<td>It bothers me and I don’t like it</td>
</tr>
<tr>
<td>2</td>
<td>It bothers me a bit but it is okay</td>
</tr>
<tr>
<td>3</td>
<td>It doesn’t bother me</td>
</tr>
</tbody>
</table>

3. Do you cough because of asthma?

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Yes, always</td>
</tr>
<tr>
<td>1</td>
<td>Yes, most of the time</td>
</tr>
<tr>
<td>2</td>
<td>Yes, some of the time</td>
</tr>
<tr>
<td>3</td>
<td>No, never</td>
</tr>
</tbody>
</table>

4. Do you wake up during the night because of your asthma?

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Yes, always</td>
</tr>
<tr>
<td>1</td>
<td>Yes, Most of the time</td>
</tr>
<tr>
<td>2</td>
<td>Yes, some of the time</td>
</tr>
<tr>
<td>3</td>
<td>No, never</td>
</tr>
</tbody>
</table>

### Please complete the following questions on your own.

5. During the past 4 weeks, how many days did your child have any day time symptoms?

<table>
<thead>
<tr>
<th>Score</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Not at all</td>
</tr>
<tr>
<td>4</td>
<td>1-3 days</td>
</tr>
<tr>
<td>3</td>
<td>4-10 days</td>
</tr>
<tr>
<td>2</td>
<td>11-18 days</td>
</tr>
<tr>
<td>1</td>
<td>19-24 days</td>
</tr>
<tr>
<td>0</td>
<td>Everyday</td>
</tr>
</tbody>
</table>

6. During the past 4 weeks, how many days did your child wheeze during the day because of asthma?

<table>
<thead>
<tr>
<th>Score</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Not at all</td>
</tr>
<tr>
<td>4</td>
<td>1-3 days</td>
</tr>
<tr>
<td>3</td>
<td>4-10 days</td>
</tr>
<tr>
<td>2</td>
<td>11-18 days</td>
</tr>
<tr>
<td>1</td>
<td>19-24 days</td>
</tr>
<tr>
<td>0</td>
<td>Everyday</td>
</tr>
</tbody>
</table>

7. During the past 4 weeks, how many days did your child wake up during the night because of asthma?

<table>
<thead>
<tr>
<th>Score</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Not at all</td>
</tr>
<tr>
<td>4</td>
<td>1-3 days</td>
</tr>
<tr>
<td>3</td>
<td>4-10 days</td>
</tr>
<tr>
<td>2</td>
<td>11-18 days</td>
</tr>
<tr>
<td>1</td>
<td>19-24 days</td>
</tr>
<tr>
<td>0</td>
<td>Everyday</td>
</tr>
</tbody>
</table>

Total Score (Questions 1-7)

**NB:** **SCORE 20 OR MORE**: child’s asthma under control

**SCORE 19 OR LESS**: Child’s asthma may not be as well controlled as it should be.
Appendix: VI Procedure for determining levels of ChE inhibition

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Bottle</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>11577763</td>
<td>216</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>REAGENT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>→ 4 x 20 mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>REAGENT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>→ 4 x 4.5 mL</td>
</tr>
</tbody>
</table>

Some analyzers and kits shown may not be available in all countries. For additional system applications, contact your local Roche Diagnostics representative.

**Precautions and warnings**

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Disposal of all waste material should be in accordance with local guidelines. Safety data sheet available for professional user on request.

This kit contains components classified as follows according to the European directive 1999/45/EC:

- B1 T – Toxic
- R32/35, R36, S1, S22, S45

Toxic by inhalation and if swallowed. Danger of cumulative effects. Keep locked up. Do not breathe dust. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Contact phone: all countries: +49-6021-7590, USA: +1-800-428-2336

**Reagent handling**

**NOTE:** The white, opaque bottles are adequate protection from light for the R1 reagent.

**NOTE:** If there is any indication that moisture has penetrated the seal on Bottle 2, contact Roche Diagnostics Customer Technical Support.

R1: Dissolve the contents of one Bottle 1 with 20 mL distilled or deionized water. Store in an amber glass bottle or opaque plastic bottle.

R2: Dissolve the contents of one Bottle 2 with 4.5 mL distilled or deionized water. Mix by gentle inversion. Use adapter to transfer into the R5 empty bottle supplied. Remove any bubbles from the surface of the working solution prior to use.

**Storage and stability**

Unopened kit components: up to the stated expiration date at 2-8°C.

R1 Working Solution: 42 days opened and refrigerated on the analyzer, when protected from light.

R5 Working Solution: 7 days opened and refrigerated on the analyzer.

**Specimen collection and preparation**

For specimen collection and preparation only use suitable tubes or collection containers. Only the specimens listed below were tested and found acceptable.

- Serum: Serum is the preferred specimen; avoid hemolysis.
- Plasma:Whole Blood: EDTA does not inhibit cholinesterase activity. Use of other anticoagulants is not recommended. Avoid hemolyzed plasma.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer. Centrifuge samples containing precipitates before performing the assay.

Whole Blood: Prepare the whole blood sample as follows:

**NOTE:** If calculation of RBC cholinesterase activity is desired, the hemocrit value must be determined on the whole blood sample prior to separation for plasma determination. Refer to Calculation section for details concerning RBC cholinesterase calculation.

1. Mix whole blood thoroughly by inversion.
2. Determine hemocrit value for whole blood sample.
3. Prepare a whole blood hemolysate:
   a. Mix whole blood thoroughly by inversion.
   b. Into a clean, dry test tube, pipette 1.8 mL of distilled water.
c. Add 0.2 mL of the whole blood sample.

d. Mix until hemolysis is complete.

4. Determine the cholinesterase activity in the hemolysate. Multiply the results by 10 to account for the dilution of the whole blood.

Stability: serum: 7 days at 4 °C

6 hours at 20-25 °C

Assay whole blood hemolysate as rapidly as possible (within 4 hours).

Materials provided

- See "Reagents - working solutions" section for reagents.

Materials required (but not provided)

- Calibrator: C.f.a.s. (Calibrator for automated systems), Cat. No. 10759320 190, 10759320 360 (for USA)
- Controls: Precinorm U, Cat. No. 10717143 122, Precinorm U plus, Cat. No. 12149435 122, 12149435 160 (for USA); Precipath U, Cat. No. 10717178 122, Precipath U plus, Cat. No. 12149443 122, 12149443 160 (for USA), PreciControl ClinChem Multi 1, Cat. No. 05177002 190, 05947826 160 (for USA), PreciControl ClinChem Multi 2, Cat. No. 05177216 190, 05947774 190 (for USA)
- 0.9 % NaCl
- General laboratory equipment

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions. The performance of applications not validated by Roche is not warranted and must be defined by the user.

Calibration

Traceability: This method has been standardized manually against the Roche method.

S1: 0.9 % NaCl

S2: C.f.a.s. (Calibrator for automated systems)

Calibration frequency

Two-point calibration is recommended:

- after reagent lot change
- as required following quality control procedures

US users only

Roche/Hitachi 902/912 analyzers:

Like the K-factor determined at installation.

Calibration frequency

A blank calibration is recommended:

- after reagent lot change
- as required following quality control procedures

Quality control

For quality control, use control materials as listed in the Materials required section. Other suitable control material can be used in addition.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte activity of each sample. Conversion factor: U/L x 0.0167 = μkat/L

Calculation of erythrocyte (RBC) activity

After plasma (PI) and whole blood (WB) activities have been determined, erythrocyte (RBC) activity is calculated as follows:

Roche/Hitachi

Since the compartmentalization of cholinesterase is given by the equation:

\[ WB = (RBC \times \text{Hct}^\ast) + [\text{PI} \times (1 - \text{Hct}^\ast)] \]

solving this equation for RBC we obtain:

\[ RBC = \frac{WB - [\text{PI} \times (1 - \text{Hct}^\ast)]}{\text{Hct}^\ast} \]

*Hematocrit is expressed as decimal equivalent, e.g. 44 % = 0.44.

Limitations - interference

Criterion: Recovery within ± 10 % of initial value.

Serum/Plasma

Icterus: No significant interference up to an I index of 60 (approximate unconjugated bilirubin concentration: 60 mg/dL, 600 μmol/L). Hemolysis: Significant interference. RBCs contain acetylcholinesterase, thereby causing contamination.

Lipemia (Intra/Plasma): No significant interference up to an L index of 1000. There is poor correlation between L index (corresponds to turbidity) and triglycerides concentration.

For diagnostic purposes, the results should always be assessed in conjunction with the patient’s medical history, clinical examinations and other findings. In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi analyzers. Refer to the latest version of the Carry-over wash lists, and the operator manual for further instructions. US users refer to the Special Wash Programming document (located on MyLabOnline website) and the operator manual for special wash instructions.

Where required, special wash/carry-over wash programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

Serum: 25-10000 U/L

Specimen dilution

Serum/Plasma

On instruments without rerun function, manually dilute samples with 0.9 % NaCl. Multiply the result by the appropriate dilution factor.

Roche/Hitachi 912/920/920+ MODULAR P analyzers:

Serum/Plasma

Determine samples with higher activities via the rerun function. For samples with higher activities the rerun function decreases the sample volume by a factor of 1.5. The results are automatically multiplied by this factor.

Lower limits of measurement

Lower detection limit of the test

25 U/L

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Expected values

<table>
<thead>
<tr>
<th>Serum</th>
<th>Plasma</th>
<th>Whole Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>3167-6333 U/L</td>
<td>1700-6778 U/L</td>
<td>6021-9166 U/L</td>
</tr>
</tbody>
</table>

Calculated RBC Activity:

11188-16998 U/L

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol. Repeatability (n = 21), Intermediate precision (3 aliquots per run, 1 run per day, 21 days). The following results were obtained: