EFFECT OF PESTICIDE EXPOSURE ON SERUM CHOLINESTERASE LEVEL AND ASTHMA CONTROL AMONG CHILDREN IN NAIVASHA, KENYA

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(U56/64034/2013)

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

AUGUST, 2014
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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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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<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>
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<tr>
<td>CI</td>
<td>Confidence Interval</td>
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<tr>
<td>EPTC</td>
<td>Ethyl Dipropylthiocarbamate</td>
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<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>HIV</td>
<td>Human Immune Deficiency</td>
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<tr>
<td>HOPAK</td>
<td>Hospital Pharmacists Association of Kenya</td>
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<tr>
<td>ISO</td>
<td>International Organization for Standardization</td>
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<tr>
<td>KNH</td>
<td>Kenyatta National Hospital</td>
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<tr>
<td>LD₅₀</td>
<td>Median lethal dose</td>
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<tr>
<td>MNCHLR</td>
<td>Maternal Newborn Child Health Linked Research</td>
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<tr>
<td>M₂</td>
<td>Muscarinic₂ receptors</td>
</tr>
<tr>
<td>NDH</td>
<td>Naivasha District Hospital</td>
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<tr>
<td>NEMA</td>
<td>National Environment management authority</td>
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<tr>
<td>OPs</td>
<td>Organophosphates</td>
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<td>OP/CB</td>
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<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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<td>SD</td>
<td>Standard Deviation</td>
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<td>UoN</td>
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<td>VLDL</td>
<td>Very low density lipoprotein</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.

**Safety Criteria** the mean safety score was set at 3.53, above which the patients were safe and vice versa.

**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 is a risk factor for asthma exacerbations in flower farm regions in the world. Data on low levels of serum cholinesterase among asthmatic children exposed to the effects of pesticides in Kenya is scanty.

Objectives: To compare and identify variables which affect the activity of serum cholinesterases in children exposed and unexposed to pesticides.

Methodology: The design was a comparative cross-sectional study that involved exposed and unexposed children. The study was conducted between May and July, 2014 in Naivasha, Kenya. Patients who met the eligibility criteria were selected using convenient sampling. They were interviewed and serum samples were analysed for cholinesterase levels. Descriptive and inferential data analysis was done. Multi-linear regression was done to identify variables that affected cholinesterase activity.

Results: Five predictor variables were found to be significantly associated with depression of serum cholinesterase levels on multivariable analysis. These included; non-school attendance by children \([\beta = -1676.8, 95\%CI (-3371.6, 18.1), P = 0.052]\), not using household pesticides \([\beta = 96.3, 95\%CI (22.6-170.0), P=0.011]\). Others were; not wearing protective gears \([\beta = -1456.96, 95\%CI (-2594, 1319.82), P = 0.01]\), female sex \([\beta = -695.7, 95\%CI (-1296.2, -95.3), P = 0.024]\) and no break after spraying \([\beta =1105.5, 95\%CI (315.0, 1895.2), P = 0.007]\).

Conclusion: Parents should be encouraged to follow personal protective measures especially wearing of protective gears as this seemed to confer protection from the effects of pesticide exposure.

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 \(^1\), \(^2\). 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 \(^3\), \(^4\). 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 through 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 (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, 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, immunosupression, 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 sub-county.

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

1.4 Objectives
1.4.1 Main objective
It was to measure levels of inhibition of serum acetylcholinesterase (as a biomarker for exposure to organophosphates) and asthma control among 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 Hypothesis
Null: there was no difference between serum cholinesterase levels and asthma control of the exposed and unexposed children.
Alternative: there was a difference between serum cholinesterase levels and asthma control of the exposed and unexposed children.

1.7 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\textsuperscript{(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.

1.8 Significance of the study
The study will assist in timely and proper clinical management of children exposed to pesticides through proper patient education on safety measures.

It will also assist the government to emphasize on integrated pesticide management leading to reduced environmental pollution.

The study creates awareness about exposure of children through their guardians/parents hence health education and proper management within and outside hospital was ensured.

The study will act as a source of reference to the University fraternity to foster research in environmental health.

1.9 Study Limitation
The key limitation of this study was that we did not carry out assessment of hepatic function, nutritional status and genotype which are factors that are known to influence ChE activity. Therefore, it was not possible to relate the interactions between pesticides exposure and these risk factors.
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 revealed that 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\(^{11}\). Studies 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. They 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. 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. 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 to 12% 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.

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.

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. 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. 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.
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 hyperreactivity 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 comparative 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°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 was around 10-15 per cent, on average 12.5 per cent \(^{(14, 19)}\).

At 95 per cent confidence interval, the sample size was:

\[
N = \frac{(a + b)^2 (p_1 q_1 + p_2 q_2)^4}{x^2} \quad \ldots \ldots \ldots \quad (45)
\]

Where:
- \(N\) = sample size in each of the groups
- \(P_1\) = proportions of subjects exposed to pesticides (29.6 per cent (%) in group 1.
- \(Q_1\) = Proportion of unexposed subjects in group 1 \((1-p_1) = (1-0.296)100\% = 70.4\%\)
- \(P_2\) = proportion of subjects exposed to pesticides in group 2 \(= (12.5\%)
- \(Q_2\) = proportion of unexposed subjects in group 2 \((1-p_2) = (1-0.125)100\% = 87.5\%\)
- \(X\) = 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 had 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 Specimen collection and analysis

The patients and caretakers were interviewed with the aid of a structured questionnaire that had been piloted. The questionnaire was designed to obtain information on the exposure status, the parent/caregivers and the use of safety protective measures by the caregivers. Additional information on the location of the child’s school relative to a flower was obtained. The patient’s records such as treatment sheets were used to confirm information obtained from the caregivers.

The Asthma Control Tool Test was used to determine whether the patient had asthma and if it was well controlled (36).

Venous blood was collected from the brachial vein using a 5ml vacutainer [Becton Dickinson and company (BD), Serum Tube, Increased Silica Act Clot Activator, Silicone-Coated Interior, 13x100, 5ml, Red/H, P=100/pack or 1000/case] by the Lewis group (43). The blood was centrifuged and 1.5ml serum was obtained and stored at -21°C for a maximum of 21 days. Frozen serum was transported to the University of Nairobi Clinical Chemistry Laboratory (ISO 9001:2008).

Cholinesterase level was determined using a colorimetric assay as described by Eli Tech Group (44). This assay was dependent on the catalytic hydrolysis by serum cholinesterases on of butyrylthiocholine to form thiocholine iodide. This compound was then reacted with 5, 5 dithiobis-2-nitrobenzoate giving a yellow compound (2-nitrobenzoate-5-mercaptopthiocholine) whose absorbance was measured at a wavelength of 405nm and 37°C. ChE level was expressed as units/litre. The analysis was semi automated and was performed as per the manufacturer’s instructions. The assay was validated before analysis was conducted.
3.7 Data management

3.7.1 Data processing and analysis
All data was entered into an excel spread sheet and exported to STATA version 10 software. All variables were subjected to descriptive data analysis. The Shapiro Wilk test was used to determine which continuous variables were normally distributed. Continuous variables that were not normally distributed were expressed as the median and the interquartile range. Normally distributed variables were expressed as the mean and the standard deviation of the mean. Categorical variables were expressed as proportions. The distribution of the variables across the two arms was compared using the non-parametric Mann Whitney, unpaired t-test and the Chi square test. Linear regression was used to identify the key determinants of ChE levels. Model building was done using a manual forward stepwise approach. P values of 0.05 and less were considered to be statistically 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 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 will also be 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 Eli-Tech analyzer was used to ensure quality. The equipment 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 Eli-Tech systems 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 Participants
Out of 20 hospitals in Naivasha, only 3 were included in the study. These included; Karagita, Finlay’s and Naivasha sub-district hospital. Karuturi hospital had been closed down, hence excluded from the study. From these facilities, out of 80 patients who were screened for eligibility, only 60 were included in the final study. The reasons for exclusion are presented in Figure 1.

![Figure 1: Reasons for patient exclusion](image)

4.3 Baseline characteristics of study participants
The equipment was calibrated and the reagent linearity was set at 1200-8000 U/L. There were more males (63.3%) than females (36.7%), Table 1. This was however, statistically insignificant (P=0.79) on univariable analysis. Age was statistically significant at P=0.015 hence the focus on children aged between 5-12 years in this study. The most represented age group was 9 to 11 years (26.7%), which is the age bracket of early adolescent. All the children were attending school except 2 (3.3%). Out of the children attending school, only 13.3% attended schools that were less than 500m from the flower farms and were considered exposed. Out of the children that were exposed only 1 (3.3%) worked on the farm and this was statistically insignificant (P=1.0).

Most of the guardians who worked on the flower farms were mothers (61.7%), Table 1. All mothers who worked in the flower farms were married to men who also worked in the
farms. Other occupations for mothers included; teaching (3.3%), farming (13.3%), business (15%) and managing homes (11.7%). Only 48.3% fathers worked on the flower farms. The other jobs of fathers were; farmers (10%), drivers (6.7%), Pastors (1.7%), teachers (8.3%) and motor cyclist (10%).

In the exposed arm, 27 (90%) of the guardians were previously exposed to pesticides. This is reflected by the depression of the median ChE level in this group to levels below the baseline; table 2. Further univariable analysis revealed that most guardians had worked in the flower farms for more than 5 years, P=0.00. Consequently, the longer the children were exposed to pesticides, the longer they were managed for asthma, Table 3.

The most commonly used pesticide was diazinon followed by malathion, paraquat, cypermethrin and rodenticides. Pesticides that were used for household pest control included; insecticides (mainly pyrethrins), rodenticides, acaricides, fungicides, miticides, and wood preservatives. Of these pyrethrins were the most common (53.3%). There was a statistically significant association between indoor and outdoor pollution pesticide exposure.

The compliance to safety practices of workers was statistically significant as shown in Table 2 with a P value of 0.000 on univariable analysis.
Table 1: Comparison of the Socio-demographic characteristics of asthmatic patients’ exposed and unexposed to pesticides.

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>EXPOSED ARM N (%)</th>
<th>UN-EXPOSED ARM N (%)</th>
<th>TOTAL N (%)</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
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</tr>
<tr>
<td>Age</td>
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<td>4 (13.3)</td>
<td>13 (21.7)</td>
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<td></td>
<td>7-9</td>
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<td>13 (43.3)</td>
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<td>9-11</td>
<td>11 (36.7)</td>
<td>5 (16.7)</td>
<td>16 (26.7)</td>
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<td>1 (1.7)</td>
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<td>18 (60.0)</td>
<td>38 (63.3)</td>
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<tr>
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<td>Female</td>
<td>10 (33.3)</td>
<td>12 (40.0)</td>
<td>22 (36.7)</td>
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<td>School characteristics</td>
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<tr>
<td>School distance</td>
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<tr>
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<td>&lt; 500m</td>
<td>8 (26.7)</td>
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<td>8 (13.3)</td>
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<tr>
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<td>&gt;500m</td>
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<td>6 (10.0)</td>
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<td>16 (53.3)</td>
<td>30 (100.0)</td>
<td>46 (76.7)</td>
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<td></td>
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<td>15 (50.0)</td>
<td>29 (96.7)</td>
<td>44 (73.3)</td>
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<td>Do not know</td>
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<td>1 (3.3)</td>
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<td>Father</td>
<td>7 (23.3)</td>
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<td>14 (23.3)</td>
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<td>22 (73.3)</td>
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<td>9 (15.0)</td>
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<td>Degree</td>
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<td>1 (3.3)</td>
<td>1 (1.7)</td>
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<td>6 (10.0)</td>
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<td>Farmer</td>
<td>1 (3.3)</td>
<td>3 (10.0)</td>
<td>4 (6.7)</td>
</tr>
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<td>Business man</td>
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<td>4 (13.3)</td>
<td>4 (6.7)</td>
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<td></td>
<td>Motor cyclist</td>
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<td>6 (20.0)</td>
<td>6 (10.0)</td>
</tr>
<tr>
<td></td>
<td>Teacher</td>
<td>0 (0.0)</td>
<td>5 (16.7)</td>
<td>5 (8.3)</td>
</tr>
<tr>
<td></td>
<td>Pastor</td>
<td>0 (0.0)</td>
<td>1 (3.3)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td></td>
<td>Not known</td>
<td>0 (0.0)</td>
<td>5 (16.7)</td>
<td>5 (8.3)</td>
</tr>
<tr>
<td>Mother occupation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Flower farm worker</td>
<td>27 (90.0)</td>
<td>0 (0.0)</td>
<td>27 (45.0)</td>
</tr>
<tr>
<td></td>
<td>House help</td>
<td>1 (3.3)</td>
<td>0 (0.0)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td></td>
<td>Farmer</td>
<td>2 (6.7)</td>
<td>6 (20.0)</td>
<td>8 (13.3)</td>
</tr>
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<td></td>
<td>House wife</td>
<td>0 (0.0)</td>
<td>7 (23.3)</td>
<td>7 (11.7)</td>
</tr>
<tr>
<td></td>
<td>Business lady</td>
<td>0 (0.0)</td>
<td>15 (50.0)</td>
<td>15 (25)</td>
</tr>
<tr>
<td></td>
<td>Teacher</td>
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<td>2 (6.7)</td>
<td>2 (3.3)</td>
</tr>
<tr>
<td>Awareness of health impact</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>21 (70.0)</td>
<td>13 (43.3)</td>
<td>34 (56.7)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>9 (30.0)</td>
<td>17 (56.7)</td>
<td>26 (43.3)</td>
</tr>
<tr>
<td>Knows specific health impact</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Skin problems</td>
<td>16 (53.3)</td>
<td>5 (16.7)</td>
<td>21 (35)</td>
</tr>
<tr>
<td></td>
<td>Chest and skin problems</td>
<td>2 (6.7)</td>
<td>0 (0.0)</td>
<td>2 (3.3)</td>
</tr>
<tr>
<td></td>
<td>Eye problems</td>
<td>3 (10.0)</td>
<td>1 (3.3)</td>
<td>4 (6.7)</td>
</tr>
<tr>
<td></td>
<td>Chest problems</td>
<td>1 (3.3)</td>
<td>0 (0.0)</td>
<td>11 (17)</td>
</tr>
<tr>
<td></td>
<td>N/A</td>
<td>8 (26.7%)</td>
<td>18 (60.0)</td>
<td>26 (43.3)</td>
</tr>
<tr>
<td></td>
<td>infertility</td>
<td>0 (0.0%)</td>
<td>3 (10.0)</td>
<td>3 (5.0)</td>
</tr>
<tr>
<td></td>
<td>Cancer</td>
<td>0 (0.0%)</td>
<td>3 (0.0)</td>
<td>3 (0.0)</td>
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Table 2: Pesticide exposure

<table>
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<tr>
<th>VARIABLE</th>
<th>EXPOSED ARM N (%)</th>
<th>UN-EXPOSED ARM N (%)</th>
<th>TOTAL N (%)</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure of the guardian/parent to pesticides</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Worked on flower farm in the past</td>
<td>Yes</td>
<td>27 (90.0)</td>
<td>3 (10.0)</td>
<td>30 (50.0)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>3 (10.0)</td>
<td>27 (90.0)</td>
<td>30 (50.0)</td>
</tr>
<tr>
<td>Duration worked (yrs)</td>
<td>0.0-0.05</td>
<td>0 (0.0)</td>
<td>1 (3.3)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td></td>
<td>0.5-1</td>
<td>6 (20.0)</td>
<td>0 (0.0)</td>
<td>6 (10.0)</td>
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<tr>
<td></td>
<td>1-2</td>
<td>6 (20.0)</td>
<td>0 (0.0)</td>
<td>6 (10.0)</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>5 (16.7)</td>
<td>0 (0.0)</td>
<td>5 (8.3)</td>
</tr>
<tr>
<td></td>
<td>&gt;5</td>
<td>11 (36.7)</td>
<td>1 (3.3)</td>
<td>12 (20.0)</td>
</tr>
<tr>
<td></td>
<td>N/A</td>
<td>2 (6.7)</td>
<td>27 (90.0)</td>
<td>29 (48.0)</td>
</tr>
<tr>
<td>Currently work in flower farm</td>
<td>Yes</td>
<td>1 (3.3)</td>
<td>0 (0.0)</td>
<td>1 (6.7)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>29 (96.7)</td>
<td>30 (100.0)</td>
<td>59 (98.3)</td>
</tr>
<tr>
<td>Personal protective practices by guardians/parents</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eat while spraying</td>
<td>Yes</td>
<td>3 (10.0)</td>
<td>1 (3.3)</td>
<td>4 (6.7)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>27 (90.0)</td>
<td>7 (23.3)</td>
<td>34 (56.7)</td>
</tr>
<tr>
<td></td>
<td>N/A</td>
<td>0 (0.0)</td>
<td>22 (73.3)</td>
<td>22 (36.7)</td>
</tr>
<tr>
<td>Bath after work</td>
<td>Yes</td>
<td>29 (96.7)</td>
<td>5 (16.7)</td>
<td>34 (56.7)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>1 (3.3)</td>
<td>3 (10.0)</td>
<td>22 (36.7)</td>
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<tr>
<td></td>
<td>N/A</td>
<td>0 (0.0)</td>
<td>22 (73.3)</td>
<td>22 (36.7)</td>
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<tr>
<td>Protective gears</td>
<td>Yes</td>
<td>29 (96.7)</td>
<td>8 (26.7)</td>
<td>37 (61.7)</td>
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<td>No</td>
<td>1 (3.3)</td>
<td>0 (0.0)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td></td>
<td>N/A</td>
<td>0 (0.0)</td>
<td>22 (73.3)</td>
<td>22 (36.7)</td>
</tr>
<tr>
<td>Separate laundry</td>
<td>Yes</td>
<td>19 (63.3)</td>
<td>5 (16.7)</td>
<td>24 (40.0)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>11 (36.7)</td>
<td>3 (10.0)</td>
<td>14 (22.3)</td>
</tr>
<tr>
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<td>N/A</td>
<td>0 (0.0)</td>
<td>22 (73.3)</td>
<td>22 (36.7)</td>
</tr>
<tr>
<td>Follow label instructions</td>
<td>Yes</td>
<td>21 (70.0)</td>
<td>6 (20.0)</td>
<td>27 (45.0)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>9 (30.0)</td>
<td>2 (6.7)</td>
<td>11 (18.3)</td>
</tr>
<tr>
<td></td>
<td>N/A</td>
<td>0 (0.0)</td>
<td>22 (73.3)</td>
<td>22 (36.7)</td>
</tr>
<tr>
<td>Takes break from work after spraying</td>
<td>Yes</td>
<td>29 (96.7)</td>
<td>1 (3.3)</td>
<td>30 (50.0)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>1 (3.3)</td>
<td>7 (23.3)</td>
<td>8 (13.3)</td>
</tr>
<tr>
<td></td>
<td>N/A</td>
<td>0 (0.0)</td>
<td>22 (73.3)</td>
<td>22 (36.7)</td>
</tr>
<tr>
<td>Comply with the concentration recommended by the pesticide label</td>
<td>Yes</td>
<td>28 (93.3)</td>
<td>8 (26.7)</td>
<td>36 (60.0)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>2 (6.7)</td>
<td>0 (0.0)</td>
<td>2 (3.3)</td>
</tr>
<tr>
<td></td>
<td>N/A</td>
<td>0 (0.0)</td>
<td>22 (73.3)</td>
<td>22 (36.7)</td>
</tr>
<tr>
<td>Type of pesticides of types of pesticides used on the farm</td>
<td>Diazinon</td>
<td>4 (13.3)</td>
<td>0 (0.0)</td>
<td>4 (6.7)</td>
</tr>
<tr>
<td></td>
<td>Parathion dichloride</td>
<td>0 (0.0)</td>
<td>1 (3.3)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td></td>
<td>Malathion</td>
<td>26 (86.7)</td>
<td>25 (83.3)</td>
<td>51 (85.0)</td>
</tr>
<tr>
<td></td>
<td>Cypermethrin</td>
<td>0 (0.0)</td>
<td>3 (10.0)</td>
<td>3 (5.0)</td>
</tr>
<tr>
<td></td>
<td>Rat and rat (rodenticides)</td>
<td>0 (0.0)</td>
<td>1 (3.3)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>Knowledge of Type of pesticides used on the farm</td>
<td>Yes</td>
<td>4 (13.3)</td>
<td>5 (16.7)</td>
<td>9 (15.0)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>26 (86.7)</td>
<td>25 (83.3)</td>
<td>51 (85.0)</td>
</tr>
<tr>
<td>Knowledge Specific health impact</td>
<td>Skin problems</td>
<td>2 (6.7)</td>
<td>0 (0.0)</td>
<td>2 (3.3)</td>
</tr>
<tr>
<td></td>
<td>Chest and skin problems</td>
<td>3 (10.0)</td>
<td>1 (3.3)</td>
<td>4 (6.7)</td>
</tr>
<tr>
<td></td>
<td>Eye problems</td>
<td>1 (3.3)</td>
<td>0 (0.0)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td></td>
<td>N/A</td>
<td>8 (26.7)</td>
<td>18 (60.0)</td>
<td>26 (43.3)</td>
</tr>
<tr>
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<td>Infertility</td>
<td>0 (0.0)</td>
<td>3 (10.0)</td>
<td>3 (5.0)</td>
</tr>
<tr>
<td></td>
<td>Cancer</td>
<td>0 (0.0)</td>
<td>3 (10.0)</td>
<td>3 (5.0)</td>
</tr>
<tr>
<td>Indoor pesticide control</td>
<td>Insecticides e.g. pyrethroids</td>
<td>16 (53.3)</td>
<td>16 (53.3)</td>
<td>32 (53.3)</td>
</tr>
<tr>
<td></td>
<td>Acaricides</td>
<td>6 (20.0)</td>
<td>1 (3.3)</td>
<td>7 (11.7)</td>
</tr>
<tr>
<td></td>
<td>Miticides rodenticides</td>
<td>1 (3.3)</td>
<td>0 (0.0)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td></td>
<td>Insecticides and rodenticides</td>
<td>4 (13.3)</td>
<td>4 (13.3)</td>
<td>8 (13.3)</td>
</tr>
<tr>
<td></td>
<td>Insecticides, acaricides, rodenticides</td>
<td>4 (13.3)</td>
<td>0 (0.0)</td>
<td>4 (6.7)</td>
</tr>
<tr>
<td></td>
<td>Insecticides, acaricides</td>
<td>1 (3.3)</td>
<td>0 (0.0)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td></td>
<td>Bush clearing, insecticides</td>
<td>1 (3.3)</td>
<td>1 (3.3)</td>
<td>2 (3.3)</td>
</tr>
</tbody>
</table>
Table 3: Asthma control and management

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>EXPOSED ARM</th>
<th>UN-EXPOSED ARM</th>
<th>TOTAL ARM</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
<td></td>
</tr>
<tr>
<td>Family history of asthma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>2 (6.7)</td>
<td>7 (23.3)</td>
<td>9 (15.0)</td>
<td>0.145</td>
</tr>
<tr>
<td>No</td>
<td>28 (93.3)</td>
<td>23 (76.7)</td>
<td>51 (85.0)</td>
<td></td>
</tr>
<tr>
<td>Asthma control level</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>controlled</td>
<td>21 (70.0)</td>
<td>13 (43.3)</td>
<td>34 (56.7)</td>
<td>0.034</td>
</tr>
<tr>
<td>uncontrolled</td>
<td>9 (30.0)</td>
<td>17 (56.7)</td>
<td>26 (43.3)</td>
<td></td>
</tr>
<tr>
<td>Asthma management period</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 3</td>
<td>0 (0.0)</td>
<td>6 (20.0)</td>
<td>6 (10.0)</td>
<td></td>
</tr>
<tr>
<td>3-6</td>
<td>10 (33.3)</td>
<td>9 (30.0)</td>
<td>19 (31.7)</td>
<td>0.078</td>
</tr>
<tr>
<td>6-12</td>
<td>14 (46.7)</td>
<td>10 (33.3)</td>
<td>24 (40.0)</td>
<td></td>
</tr>
<tr>
<td>12-60</td>
<td>6 (20.0)</td>
<td>5 916.7)</td>
<td>11 (18.3)</td>
<td></td>
</tr>
<tr>
<td>Cormobidities</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>30 (100.0)</td>
<td>30 (100.0)</td>
<td>60 (100.0)</td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>30 (100.0)</td>
<td>30 (100.0)</td>
<td>60 (100.0)</td>
<td></td>
</tr>
</tbody>
</table>

4.4 Cholinesterase levels in asthmatic children

The serum ChE level ranged from 618 U/L to 10575 U/L with a median value of 6155 U/L as shown in Figure 1. Using a cut off of 5320 U/L, the children were dichotomized as having low and high ChE levels. 48 (80%) of the patients had levels that was within the normal value while 12 (20%) children had levels below the normal reference range. In the unexposed arm, one child had a level that was below the reference range while in the exposed arm, they were 11(36.7%), P= 0.001.

Figure 1: Histogram for distribution of serum Cholinesterase
From the box and whisker plot, the exposed arm had a lower median ChE levels than the unexposed pesticide arm (Figure 2). This was reflected in bivariable analysis, which showed the difference in serum levels between the unexposed and exposed arm as 1698.58 U/L. On adjusting for confounding, there was no statistical significance between the arms.

The median serum cholinesterase level of all the children was 6403.7 U/L with a range 618 U/L to 10575 U/L while those of exposed and unexposed were 5828 U/L and 7133 U/L respectively; Figure 1. The data was not normally distributed. Kruskal - Wallis test showed there was a statistically significant difference between the medians of pesticide exposure and unexposed arms P=0.001.

4.5 Regression Analysis for Predictors of serum cholinesterase levels
Linear regression analysis was done to identify variables predictive of serum cholinesterase levels. Bivariable and multivariable analyses were conducted and results were presented in Table 2. They were classified as follows; influence of exposure of the child pesticides, child characteristics, guardians characteristics, household pest management characteristics and the location of the child school near the flower farm. In addition, the association between ChE levels and asthma management were explored. The parsimonious model accounted for 58.8% of the variation in serum ChE activity. Other factors such as genotype and nutrition factors could have explained the remaining variation.
4.5.1 Influence of proximity of the school to the flower farms
On model building, proximity of the school to the flower farm was the most important determinant of ChE levels. It was responsible for 29% of the variation in serum ChE level. Children who attended school had reduced ChE levels even on multivariable analysis regardless of the distance from the flower farm. Children who did not attend school had depressed ChE levels by 1676.8U/L with a P value of 0.05. Children whose schools were located 500m away from the flower farms, also had higher ChE levels, and this was statistically insignificant, P=0.06. Therefore, the distance from the flower farm may have been a determinant of ChE activity.

4.5.2 Effects of exposure of the guardian/parent to pesticides
Children whose parents belong to professions that worked away from flower farms such as pastors, teachers had higher levels of cholinesterases. This was statistically significant on bivariable analysis, P<0.05. The mother’s occupation had an increased effect on ChE levels compared to the father’s occupation. In addition, the longer the parent worked on the flower farm, the lower the child’s ChE levels. Children who were working on the flower farms had lower ChE levels but this was statistically insignificant, P=0.69.

The guardians’ education level had a beneficial effect on the serum ChE levels. Parents who were aware of the health impact of pesticides had higher ChE levels on bivariable analysis but this was statistically insignificant. However, for parents who were able to site the specific health impact of pesticides, there was a statistically beneficial effect on serum ChE activity \[ \beta = 205.9, (2.31, 409.5); P=0.05 \]. On multivariable analysis, parents’ education and their awareness about the health impact of pesticides was statistically insignificant.

The analysis of the effect of protective practices was restricted to parents who worked on flower farm. Amongst children whose parents worked on flower farms, use of protective gear by the parent accounted for 44.4% of the variation in serum ChE level. Parents who washed their clothes separately from their children’s conferred a protective effect although the results were statistically insignificant, P=0.23. Failure to follow pesticide label instructions had a depressed serum ChE levels and this was statistically insignificant, P= 0.66. Surprisingly, failure to take holidays from work on the farm had a protective effect against pesticide exposure and was statistically significant, P=0.007.
Parents’ use of protective gear was the most predictive variable. Decreasing use of household insecticides increased the children’s cholinesterase levels P=0.01.

4.5.3 Influence of exposure of the child’s demographic traits on ChE levels
The child’s age had no influence on cholinesterase level. However, sex was significantly associated with the serum ChE levels both on bivariable and multivariable analysis. On adjusting for confounders, females had a lower serum levels by about 695.7 U/L compared to the males, Table 2.

4.5.4 Effect of association asthma with ChE levels
On bivariable analysis patients who had uncontrolled asthma and a family history of asthma had higher serum ChE levels which was statistically insignificant. The duration of management seemed to have a protective effect on asthmatic patients on bivariable analysis. However, on adjusting for confounders, the serum levels were depressed by 298 U/L with a P value of 0.09 which was statistically insignificant. Hence, this seems to relate to poorly controlled asthma among children in this area.
Table 3: Predictor variables of serum cholinesterase levels among children.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Crude ß-coefficient (95% CI)</th>
<th>p-value</th>
<th>Adjusted ß-coefficient (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pesticide exposure status of the child</td>
<td>1698.58 (961.5, 2435.6)</td>
<td>0.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Child characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age in years</td>
<td>-21.0 (-212.8, 170.9)</td>
<td>0.83</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Age category</td>
<td>75.2 (-296.1, - 446.5)</td>
<td>0.69</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sex</td>
<td>-902.6 [-1764.7, - 40.6]</td>
<td>0.04</td>
<td>-695.7 [-1296.2, - 95.3]</td>
<td>0.024</td>
</tr>
<tr>
<td>School characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child not attending school</td>
<td>-3662.8 [-5861.7, -1464.1]</td>
<td>0.001</td>
<td>-1676.8 (-3371.6, 18.1)</td>
<td>0.052</td>
</tr>
<tr>
<td>Location on a flower farm</td>
<td>293.9 (-607.8, 1195.6)</td>
<td>0.517</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>School distance from the flower farm (&gt;500m)</td>
<td>573.0 (-18.2, 1164.1)</td>
<td>0.06</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Currently working in the flower farm</td>
<td>671.9 (2689.1, 4032.9)</td>
<td>0.69</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Guardian characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education level</td>
<td>573.2 (-1.1, 1147.4)</td>
<td>0.05</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Father occupation</td>
<td>206.8 (84.2, 329.3)</td>
<td>0.001</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mother occupation</td>
<td>356.6 (194.8, 518.3)</td>
<td>0.000</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Exposure of the guardian/parent to pesticides</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Worked on a flower farm in the past</td>
<td>1138.2 (330.1, 946.4)</td>
<td>0.007</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Duration of worked in flower farm (months)</td>
<td>-669.8 [-1120.0, -215.5]</td>
<td>0.005</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Personal protective practices by guardians/parents</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not Eat while spraying</td>
<td>-301.4 (-1684.4, 1081.6)</td>
<td>0.66</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Not bathing after work</td>
<td>288.8 (-1436.8, 2014.4)</td>
<td>0.74</td>
<td>997 (-197.7, 2191.9)</td>
<td>0.10</td>
</tr>
<tr>
<td>Not wearing Protective gears</td>
<td>-5240.3 (-7223.0, -3257.6)</td>
<td>0.00</td>
<td>-1456.9 (-2594.1, 319.8)</td>
<td>0.01</td>
</tr>
<tr>
<td>Separate laundry</td>
<td>532.2 (-331.5, 1395.9)</td>
<td>0.23</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Does not follow label instructions</td>
<td>-205.5 (-1141.3, 730.3)</td>
<td>0.66</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Does not take break from work after spraying</td>
<td>885.4 (-114.7, 1885.5)</td>
<td>0.081</td>
<td>1105.5 (315.0, 1895.2)</td>
<td>0.007</td>
</tr>
<tr>
<td>Not Awareness of health impact</td>
<td>677.2 (-173.9, 1528.27)</td>
<td>0.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Specific health impact</td>
<td>205.9 (2.3, 09.5)</td>
<td>0.05</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Household pesticide management practices</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of pesticide used in the house</td>
<td>158.3 (61.6, 255.0)</td>
<td>0.002</td>
<td>96.3 (22.6, 170.0)</td>
<td>0.011</td>
</tr>
<tr>
<td>Knowledge of type of pesticide used in farm</td>
<td>375.1 (-827.3, 1143.5)</td>
<td>0.53</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Specific pesticide used in the farm asthma</td>
<td>469.6 (-204.3, 1143.5)</td>
<td>0.17</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Family history of asthma</td>
<td>73 (-1133.0, 1578.2)</td>
<td>0.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Asthma control level</td>
<td>816.7 (-25.9, 1659.3)</td>
<td>0.060</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Management period</td>
<td>350.4 (72.1, 628.7)</td>
<td>0.005</td>
<td>-298.11 (-650.2, 54)</td>
<td>0.09</td>
</tr>
</tbody>
</table>
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 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\(^{(19,22,29)}\). 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 (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 findings 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 inhibition are shown. 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 asthmatic attacks. 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 Kenya on people working on flower farms \(^{(7,12,14)}\).

The risk factors of asthma exacerbations were pesticide exposure due to exposure by 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, findings in this study showed no statistical significance between the level of asthma control of the asthmatic child and the exposure status.
This study found that patients who were exposed to pesticides had lower ChE levels which concur with other studies done in Naivasha and Lebanon \(^{(7, 41)}\). The key finding in this study was that use of protective clothing by parents, had a significant effect on ChE levels of the children. Other notable findings were; household insecticide use and not taking break after spraying had a negative effect on ChE levels. The children who were not going to school had significantly reduced ChE levels. It was expected that children who go to school had greater environmental exposure to pesticides and therefore, they would have lower ChE levels. It was noted that proximity to a flower farm did not have a statistical significant effect on serum ChE level. The children who stayed at home were from poorer households and probably did not receive adequate nutrition. This could have explained why staying at home had a negative effect on ChE level. Inadequate nutrition is a known risk factor for reduced serum ChE levels \(^{(37)}\).

It is a well established fact that females have lower ChE levels compared to males because of the hormonal status, genetic status and use of contraception \(^{(42)}\). Similar to other studies, sex was a very strong predictor variable to the levels of depression of serum cholinesterase. This was shown by depressed levels serum ChE in the females by 695.7U/L.

Pesticide exposed patients had low cholinesterase levels. The Kenyan study concurs with this finding (Ohayo-Mitoko, 2000). Children who failed to attend school had depressed cholinesterase activity by 1676.8 U/L. This is explained by the fact that residual pesticides on the skin and clothes were carried home leading to indoor pollution. In addition, the use of household pesticides led to indoor pesticide exposure. Pyrethrins were commonly used and they are known to depress serum ChE levels by exerting oxidative stress on the liver \(^{(11, 12, 19)}\).

Other factors that led to low serum ChE levels were failure to wear protective gears. Similar studies have shown that guardians who practiced safety measures at work place and home had higher serum ChE levels compared to those who did not \(^{(27)}\). Prolonged exposure leads to a higher total pesticide exposure, resulting to low serum ChE levels.

Pesticide exposure causes exacerbation of asthma. This is explained by the fact that the longer the patients stayed on the flower farms the longer the duration they were managed for asthma. Unlike other studies, there was no statistical significance between the level
asthma control and ChE levels (11, 12, 26). This is probably due to the fact that most of the study subjects had the median ChE levels within the normal range.

5.3 Conclusion
Children, whose parents are exposed to pesticides for a long period of time, have reduced ChE levels. This results to long term effects like cancer, birth defects, haematological conditions, respiratory problems like asthma and allergic rhinitis. Therefore, parents should be encouraged to follow personal protective measures especially wearing of protective gear and taking breaks after spraying as these seemed to confer protection from the effects of pesticide exposure.

5.4 Recommendations

5.4.1 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.

5.4.2 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


37. Steven H, Zeisel M and Victoria JD. Micronutrient Information Center. Linus Pauling Institute, Oregon State University; reviewed 2008.


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: Ethics approval letter

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19076 Code 00202
Telegrams: varcity
(254-20) 2726300 Ext 44355

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726308-9
Fax: 722572
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/142

Dr. Wafula Caroline Nasambu
Dept. of Pharmacognosy 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.
Appendix II: Ethics approval letter (continued)

Yours sincerely

PROF. M. L. CHINDIA
SECRETARY, KNH/UCON-ERC

c.c. The Principal, College of Health Sciences, UoN
The Deputy Director CS, KNH
The Chairperson, KNH/UoN-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 Ogongo

Protect to Discover
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 at 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, Kenya. 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 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 I

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
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

9. 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 the pesticide label</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

9. Are you aware of the health impact of exposure to pesticides?

   Yes          No
10. If yes, specify………………………………………………………………
   i.  Chest problems
   ii. Skin problems
   iii. Chest + skin problems
   iv. Eye problems
   v.  N/A
   vi.  Infertility
   vii.  cancer

11. 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

<table>
<thead>
<tr>
<th>Question</th>
<th>Scoring System</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. How does your asthma make you feel today?</td>
<td>Score: 0= I feel very ill today, 1= I feel well today, 2= I feel very well today, 3= I feel well today</td>
</tr>
<tr>
<td>2. How much does your asthma bother you when you run, exercise or play sports?</td>
<td>Score: 0=It bothers me a lot, I can’t do what I want to do, 1=It bothers me and I don’t like it, 2=it bothers me a bit but it is okay, 3=It doesn’t bother me</td>
</tr>
<tr>
<td>3. Do you cough because of asthma?</td>
<td>Score: 0=Yes, always, 1=Yes, most of the time, 2=Yes, some of the time, 4=No, never</td>
</tr>
<tr>
<td>4. Do you wake up during the night because of your asthma?</td>
<td>Score: 0=Yes, always, 1=Yes, Most of the time, 2=Yes, some of the time, 3=No, never</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Question</th>
<th>Scoring System</th>
</tr>
</thead>
<tbody>
<tr>
<td>5. During the past 4 weeks, how many days did your child have any day time symptoms?</td>
<td>Score: 5=Not at all, 4=1-3 days, 3=4-10 days, 2=11-18 days, 1=19-24 days, 0=everyday</td>
</tr>
<tr>
<td>6. During the past 4 weeks, how many days did your child wheeze during the day because of asthma?</td>
<td>Score: 5=Not at all, 4=1-3 days, 3=4-10 days, 2=11-18 days, 1=19-24 days, 0=everyday</td>
</tr>
<tr>
<td>7. During the past 4 weeks, how many days did your child wake up during the night because of asthma?</td>
<td>Score: 5=Not at all, 4=1-3 days, 3=4-10 days, 2=11-18 days, 1=19-24 days, 0=everyday</td>
</tr>
</tbody>
</table>

Total Score (Question 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
Français - FR

**Méthode de dosage**

Méthode de dosage en réactifs et, à petit précurseur impair.

**SIGNIFICATION CLINIQUE**

La cholinestérase sérique (CES) est la forme la plus courante de l'enzymne qui dégrade le neurotransmetteur acétylcholine (ACh) dans le système nerveux central et périphérique. Les cholinesterases hépatiques sont présentes dans les cellules de l'hépatocite et sont impliquées dans le métabolisme de nombreux médicaments et toxiques. La mesure de la CES est couramment utilisée dans le diagnostic des maladies neurologiques et de la toxicologie.

**COMPOSITION DES REACTIFS**

- **Réactif:**
  - composé de la CES à 0,25 U/L.
  - composé de l'acétylcholine à 0,25 mmol/L.
  - composé de l'acétylcholine à 0,25 mmol/L.
- **Reagent:**
  - composé de la CES à 0,25 U/L.
  - composé de l'acétylcholine à 0,25 mmol/L.
- **Water:**
  - composé de l'eau distillée à 0,25 mmol/L.

**PRÉPARATIONS**

- **Reagent:**
  - composé de la CES à 0,25 U/L.
  - composé de l'acétylcholine à 0,25 mmol/L.
- **Reagent:**
  - composé de la CES à 0,25 U/L.
  - composé de l'acétylcholine à 0,25 mmol/L.
- **Water:**
  - composé de l'eau distillée à 0,25 mmol/L.

**MÉTODE**

**Principe**

La méthode est basée sur la mesure de la réduction de l'acétylcholine par la CES. La diminution de l'acétylcholine est mesurée à l'aide d'un détecteur de lumière à spectrophotomètre. La mesure est effectuée à 340 nm. Les résultats sont exprimés en U/L de CES.

**Précautions**

- La mesure doit être effectuée dans un délai de 2 heures après l'arrêt des agents qui inhibent la CES.
- Les résultats peuvent être affectés par les agents qui inhibent la CES, tels que les anticholinestéraseurs.
- Les résultats peuvent être affectés par les agents qui stimulent la CES, tels que les stimulants des nerfs périphériques.
- Les résultats peuvent être affectés par les agents qui réduisent la production de l'acétylcholine, tels que les inhibiteurs de la cholinérgie.

**RÉFÉRENCES**

- **Rendement:**
  - composé de la CES à 0,25 U/L.
  - composé de l'acétylcholine à 0,25 mmol/L.
- **Réactif:**
  - composé de la CES à 0,25 U/L.
  - composé de l'acétylcholine à 0,25 mmol/L.
- **Water:**
  - composé de l'eau distillée à 0,25 mmol/L.

**MÉTÉO**

- **National:**
  - composé de l'acétylcholine à 0,25 mmol/L.
  - composé de l'acétylcholine à 0,25 mmol/L.
- **International:**
  - composé de l'acétylcholine à 0,25 mmol/L.
  - composé de l'acétylcholine à 0,25 mmol/L.
- **Reagent:**
  - composé de la CES à 0,25 U/L.
  - composé de l'acétylcholine à 0,25 mmol/L.
- **Water:**
  - composé de l'eau distillée à 0,25 mmol/L.

**MATERIEL ET MÉTHODE**

- **National:**
  - composé de l'acétylcholine à 0,25 mmol/L.
  - composé de l'acétylcholine à 0,25 mmol/L.
- **International:**
  - composé de l'acétylcholine à 0,25 mmol/L.
  - composé de l'acétylcholine à 0,25 mmol/L.
- **Reagent:**
  - composé de la CES à 0,25 U/L.
  - composé de l'acétylcholine à 0,25 mmol/L.
- **Water:**
  - composé de l'eau distillée à 0,25 mmol/L.