UNIVERSITY OF GHANA

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COLLEGE OF HEALTH SCIENCES

PESTICIDES EXPOSURE IN INDIVIDUAL FARMERS FROM A COCOA GROWING COMMUNITY - NEW EDUBIASE

BY

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DECLARATION

I, Justice Dogbey hereby confirm that this dissertation represents my own work apart from references to other people's work which have been duly acknowledged, the contribution of my supervisors to the dissertation was consistent with normal supervisory practice.

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DEDICATION

I dedicate this project work to my mum Stella Tay for her encouragement throughout my study and my elder brother Julius Dogbey who have been supportive during my study and carrying out this project work.

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ABSTRACT

Background: Pesticides are used by individuals especially farmers to prevent crop damage by harmful insects or plant pests. In the process of preventing and eradicating pests, individuals are occupationally and environmentally exposed to pesticides. Although pesticides help in preventing crop destruction by pests, they are toxic, and exposure can result in a number of health effects. This study aimed at evaluating pesticide residues in human urine samples of farmers who apply pesticide and a household member who is a farmer but does not apply pesticide from a cocoa growing community at New Edubiase.

Method: An analytical cross-sectional study, with laboratory analysis using GC-ECD, GC-PFPD to detect pesticide residues in urine samples of participants. Mann-Whitney U test was used to determine the frequency, percentage and median concentrations.

Results: About 59.7% of the participants were farmers who apply pesticides and 40.3% were members of the farmers' household who were farmers but do not apply pesticide. According to the field survey, pesticides used frequently by the farmers were Bifenthrin, thiamethoxam and, imidacloprid. The major pesticide detected in the urine samples of the participants was chlorpyrifos with a median concentration of 0.349mg/kg (IQR 0.218-0.484). Other pesticide residues detected in the urine samples of the participants were Organophosphate (Dimethoate, Fonofos), Organochlorine (Gamma HCH) and Pyrethroid (Lambda cyhalothrin, Cyfluthrin, Cypermethrin, Bifenthrin). The results presented showed that, there is no statistically significant association in median concentration of pesticides in urine sample among the farmers who apply pesticides and members of the farmers' household who are farmers but do not apply pesticides.

Conclusion: Pesticides exposure was observed among both farmers who apply pesticide and members of the farmers' household who are farmers but do not apply pesticide, and the higher frequency of existence of chlorpyrifos could be as a result of excessive and frequent usage of pesticides. The study observation shows that Bifenthrin, thiamethoxam and imidacloprid are the pesticides mostly used by the farmers; however, results of the laboratory analysis did not confirm their presence in the urine samples of participants.

Keywords: Pesticides, Concentration, Farmers, Urine

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LIST OF ACRONYMS

Acronym	Meaning
AChE	Acetylcholinesterase
CA	Carbamate
DDT	Dichlorodiphenyltrichloroethane
DDE	Dichlorodiphenyltrichloroet
EPA	Environmental Protection Agency
GC-ECD	Gas Chromatography-Electron Capture Detector
GC-MS	Gas Chromatography-Mass Spectrometry
GC-PFPD	Gas Chromatography Pulse Flame-photometric Detector
GSA	Ghana Standard Authority
HPLC	High-Performance Liquid Chromatography
LC50	Lethal Concentration
LC-MS	Liquid Chromatography-Mass Spectrometer
LOD	Limit of Detection
MRM	Multi-Residue Method
MSPD	Matrix Solid Phase Dispersion
WHO	World Health Organisation
OC	Organochlorine
OP	Organophosphate
PPE	Personal Protective Equipment
IQR	Interquartile Range

CHAPTER ONE

INTRODUCTION

1.1 Background

In most developing countries, agriculture plays an important role in the economic performance and development of the country, and Ghana is not an exception. Agriculture serves as a major source of foreign exchange earnings, and also in providing employment to a large portion of the population, especially the less privileged in the society (Omorogiuwa, Zivkovic, & Ademoh, 2014). In Ghana, all kinds of crops are cultivated all year across the various parts of the country, including cash crops such as cocoa, cashew nut, fruit crops such as pineapple, mangoes and oranges, vegetables such as tomatoes, carrots and cereals and grains such as millet, maize, and rice. Due to the heavy destruction caused by pests to crops in the farm, there is usually a reduction in crops produced to sustain the individuals, leading to unfavourable effects on the national economy (Burrack & Chapman, 2013; Kibira et al., 2010). In the process of preventing and eradicating pests over the years, pesticide application in Ghana especially in the farming communities has been on the rise and the quantity of pesticides used in the fields across the nation is enormous (Mattah, 2015).

Pesticides are substances (mostly synthetic) used to repel, kill, or control the activities of insects or plants that are regarded to be pests. Pesticides are formulated using active and inactive compounds, the active compound is mostly responsible for the toxicity of the target organism. The active ingredient in some pesticide must be converted into a lethal substance (metabolites) by the insect or the plant to which the pesticide is being applied (Cloyd, 2012). These metabolites are the product of chemical reactions that occur naturally within cells during metabolism. Pesticides are very prevalent, and exposure of the general population to these chemical is nearly impossible to avoid (Elvis Asare, 2015). Most of our daily diets are contaminated with some levels of pesticide residues due to their extensive use in agriculture and food production. Improper use of pesticides lead to occupational, environmental exposure, and risk to food security (Nakano et al., 2016). Pesticides exposure has been linked to a wide range of negative health effects in humans, with short-term effects such as headaches, nausea and chronic diseases like cancer and endocrine disruption (Nakano et al., 2016). The extensive application of pesticides, deposit some quantity of pesticides' residue on food crops which are mostly eaten without washing and also pesticide residues enter into farmers who are not well protected during the application process. In a quest to reduce the exposure to pesticide residues in food, to prevent the general population from negative health effect, and to ensure Good Agriculture Practices (GAP), Maximum Residue Levels (MRLs) has been set by many countries for pesticide residues for each crop (Nakano et al., 2016).

Many researches have pointed to the health effects of pesticides, for which some are unclear, but their use has been linked to conditions such as cancer, diabetes, neurological effects and other potential effects including destruction to the human endocrine, nervous and reproductive systems (Damalas & Eleftherohorinos, 2011; Sanborn et al., 2007). The frequent use of pesticides has been deeply rooted in our lives especially farmers as they frequently get their benefits due to the significant increase in productivity and also conserving food supply. According to the US Environmental Protection Agency (EPA), pesticides are usually the most effective way to prevent disease causing organisms. Pesticides are used to protect our buildings and other properties from structural destruction by pests such as cockroaches, bed bugs, termites and ants.

They can also prevent disease outbreaks by controlling insect and rodent populations. Regardless of the several benefits of pesticide usage in agricultural and other areas, they can also have unfavourable effects on human health. Most synthetic pesticides contain chemicals compound such as organophosphates, carbamates, pyrethroids, and sulfonylureas (Lozowicka et al., 2014).

Majority of pesticides are non-biodegradable, and normally enter into the food chain and cause health problems to humans and animals due to bioaccumulation. Occupational and environmental exposure of pesticides to humans may occur during farming activities, and through consumption of food crops or inhalation of contaminated air. The absence of pesticides could lead to a decline in crop production and this could affect food prices by increasing as much as 75% (Popp, 2013). The persistent circulation and settling of pesticides in the biosphere and various objects for a period of time, and entering all kinds of organism and objects are well known characteristics of pesticide since their existence. Due to toxic nature of most pesticides, frequent inhalation or ingestion by human even in the smallest quantity can result in accumulation in body tissues with serious adverse health effects (Handa, 1999). Carbamate and organophosphorus pesticides have been reported to be potential acetylcholinesterase inhibitors (Office of Pesticide Programs, 2000). Inhibition of acetylcholinesterase, an enzyme used in muscle relaxation, results in accumulation of acetylcholine causing continuous and excessive muscle stimulation (Boettger & McClintock, 2012). Carbamate insecticides are derivatives of carbonic acids which are mostly used in agriculture activities and their mode of action is inhibiting the vital enzyme acetylcholinesterase. Depending mainly on the chemical stability and dose, time and mode of application, pesticide residues may occur in crops and derived products (Muccio, 2006). The characteristics of pesticide in the agricultural produce is of great interest, since their persistence, disappearance or partial transformations determine their usefulness or their potential effects to the environment (Bergmann, 1989;Chang et al., 2005).

1.2 Problem Statement

Pesticides are hazardous, and their exposure has been of a great concern globally as a result of their health effects on the human population. Studies conducted on food crops in parts of Ashanti Region showed a trace amount of residue levels of pesticide (Akomea-Frempong, 2017), which can eventually enter the human body. Also, various studies have been carried out on the exposure of pesticide and the use of personal protective equipment in cocoa farming areas and the results showed high risk of pesticide exposure (Okoffo, 2016). Symptoms of acute poisoning of pesticides could occur during or after the period of exposure, considering the type of pesticide used and the manner of contact (Latif, 2012). Farmers may use a higher quantity of pesticide than recommended quantities to kill pests without being aware of the negative impact and the protective measures. The presence of pesticide residues indicates their characteristic of long-term persistence in the environment (Taha & Salghi, 2013). Individuals are most likely exposed to huge amounts pesticides in the communities especially farmers as they may frequently get in contact and/or breathe in pesticides. While farmers can be directly exposed during application, other household members can be exposed indirectly through entry into sprayed farms, consumption of contaminated food, water, and handling of pesticides' containers. The consequences of pesticide exposure could lead to both short and long-term human health effect, among farmers and the general public. Chronic exposure of pesticide is a potential cause of cancers, reproductive and endocrine disruption, neurological damage, and immune system dysfunction (Miah, Hoque, Paul, & Rahman, 2014). A research conducted on 50 pesticides and over 30,000 licensed pesticide applicators associated exposure of seven pesticides that contain chlorinated compounds including two herbicides, two organophosphate insecticides, and two organochlorines to increased risk of diabetes (National Institutes of Health, 2008). This study aims to determine the levels of pesticide concentration in individuals in New Edubiase, a cocoa growing community.

1.3 Conceptual Framework

Pesticides are absorbed into the human body by ingesting, breathing, or through dermal exposure. Individuals, especially farmers who actually apply the pesticides are mostly exposed during the application process (mixing, loading, and spraying). There is likelihood that an individual who is directly exposed during the self-application may have a higher concentration of pesticide in their body as they are indirectly exposed as well. Individuals who do not take part in pesticide application may also get exposed without even being aware due to indirect exposure from the residual pesticides in food, water, the home or outdoor environment (Teitelbaum, 2002), and contact with applicators clothes. Age, education and the use of Personal Protective Equipment (PPE) also have an effect on exposure and level of pesticide concentration in the individuals' body. Older individuals are likely to absorb and accumulate more pesticides into their bodies because they are more susceptible and also may have been exposed for a longer period (National Pesticide Information Center, 2011), whiles the use of PPE reduces the exposure level of pesticide in the individual applying the pesticide and the education also influences the knowledge of the use of pesticides and the hazards that come with it.

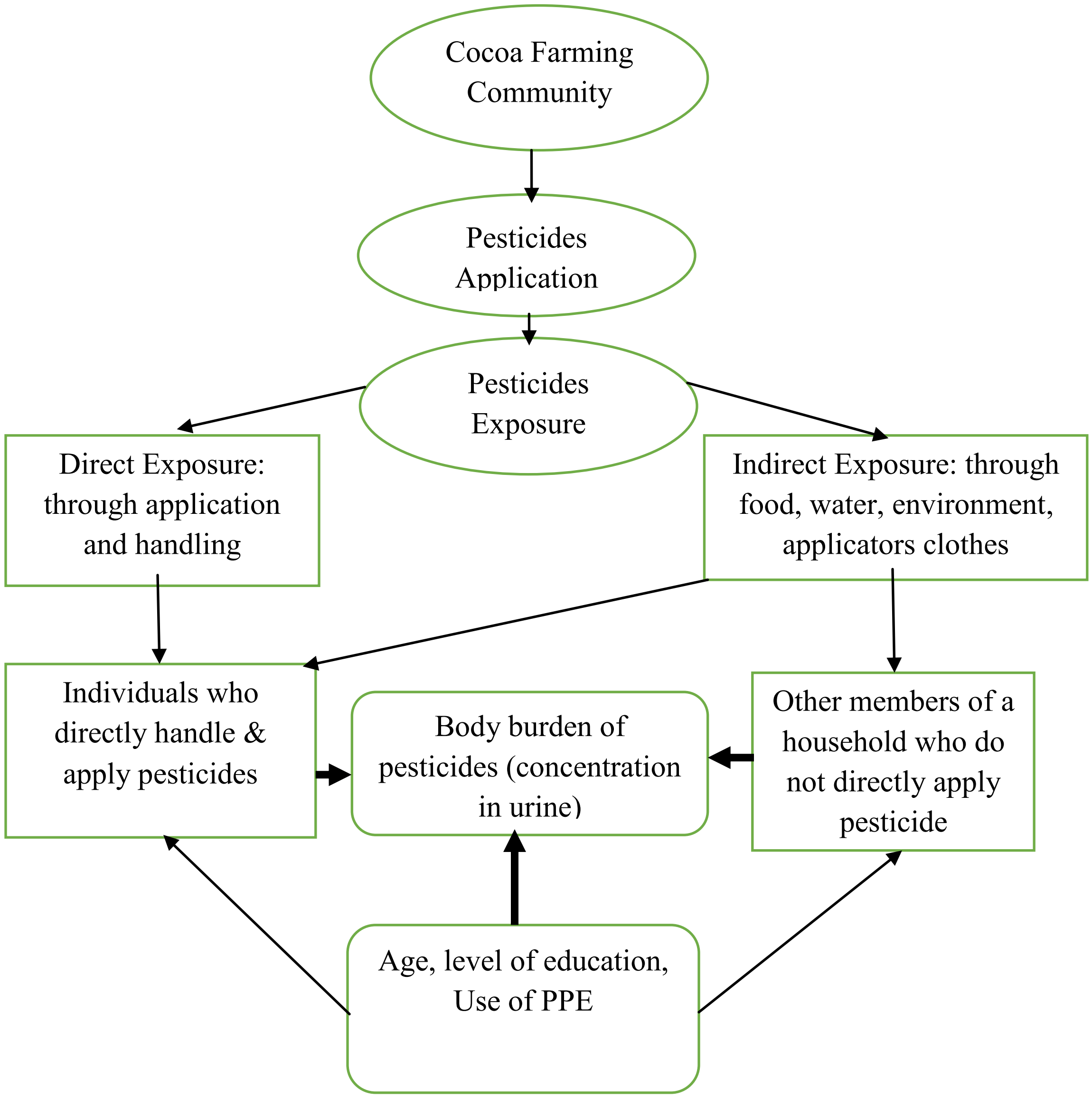

Figure 1.1: Conceptual Framework

1.4 Justification for the study

The measurement of the level of pesticide concentration in urine of human populations is a good biomarkers in assessing the extent of exposure and in evaluating the hazard (Taha & Salghi, 2013). The focus of the research on pesticide exposure is mostly on farmers who are involved in the direct application. However, since indirect exposure could occur through several means, it is important that assessment of pesticide exposure is done not only in those involved in the direct application, but other members of the farmers' household who are likely to be exposed through indirect means; around pesticides application areas, contact with equipment used in application, pesticide containers, ingestion of food and water with pesticide residues. The outcomes will be available to the concerned authorities who may use it to come up with appropriate measures on the use of pesticides and also to familiarise the public on pesticide residues in food crops, soil, water bodies, environment and its aftereffect.

1.5 Research Questions

Local cocoa farmers spray pesticides on their cocoa plants for genuinely good reasons but are usually not aware of the side effects and/or protective measures they could adopt. In most cases, the farms contain not only cocoa but vegetables and other food crops which are planted at subsistence level.

The study seeks to address the following research questions;

i. Are there pesticides exposures among individual farmers in a cocoa growing community?
ii. What are the levels of pesticides exposure among farmers directly involved in pesticides application and farmers who are not directly involved in pesticide application?

iii. What are the differences in concentration of pesticide residues in farmers directly involved in application compared to farmers not directly involved in an application?

1.6 Objectives

1.6.1 General Objective

i. To determine level of pesticides concentration in individual farmers in New Edubiase, a cocoa growing community.

1.6.2 Specific Objectives

1. To determine the concentration of pesticide level in the individual farmers who personally apply pesticides.
2. To determine the concentration of pesticides level in members of the farmer's household who are farmers but do not personally apply pesticides.
3. To compare pesticides concentration levels in individual farmers who personally apply pesticides and members of the farmers' household who are farmers but do not personally apply pesticide.

CHAPTER TWO

LITERATURE REVIEW

2.1 Pesticides

Pesticides are chemical or biological substances used in protecting farm crops and other areas of agriculture against pest destruction. Pesticides are classified under different chemicals with frequently used ones being organochlorines, organophosphates, carbamates, and pyrethroids (Ogah & Coker, 2012). The most common group of pesticides used are herbicides, insecticides, fungicides, fumigants and rodenticides (Sanborn, 2002). The widely use of pesticides for agriculture purposes have been known throughout the world to protect crops, aside pesticides benefit to agriculture, they are harmful not only to target organisms but also to non-target organisms, animals and man. The widespread of pesticides use does not only pollute our surroundings but threatens the health of the general public. Pesticides are part of urgent pollutants to be monitored due to their availability in foods, waters, and soil, which is considered to be a potential hazard to human health (Chuanjiang et al., 2010). Insecticides are known to be the most toxic to the environment compared to fungicides and herbicides (Yadav, 2010) with exceptions to certain herbicides considered to be more toxic and hazardous to the environment (Yadav, 2010).

Most harmful types of pesticide are distinguished on the basis of either being water or fat soluble. Substances that are water soluble can easily be transported out of the target area into the immediate surrounding whiles fat-soluble substances are readily absorbed in insects, fish, and other living organisms, often resulting in extended persistence in food chains (Richardson, 2002).

The concentration of pesticide residues that remains indicates the quantity of pesticide applied, the time elapsed since application, and the rapid nature in which the pesticide disperses in the environment. Residue levels present on crops are also affected by the application area of the pesticide, such as in the soil or on the plant, and when they are used in the life cycle of the plant, such as the plants' seedling stage or time of harvest (States & Accounting, 2003). Individuals of the general public who do not apply pesticide are likely to have multiple routes of exposure depending on the individuals' location during pesticide applications, whether a household was treated with pesticides, the occupations of the individuals in household, the dissipation nature of active ingredient and its stability in the environment (Alavanja, 2013). Excessive intake of pesticides by human can lead to acute intoxication while long time exposure can result in chronic poisoning. The majority of extreme pesticide poisoning results from acute exposure to organophosphate and carbamate insecticides (Lorenz, 2009). A study conducted on 'Raid' insecticide about its toxicity and bioaccumulation in Wister rats, showed that mortality in animal gradually increased with increasing concentrations while growth in weight decreased (Achudume, 2010).

Table 2.1 Pesticide classifications by target pests

Types of Pesticide	Target pests/Function	Examples
Insecticides	Kill insects and other arthropods	Aldicarb
Fungicides	Kill fungi (including, molds, and rust)	Azoxystrobin
Bacteriacides	Kill bacteria or acts against bacteria	Copper complexes
Herbicides	Kill weeds and other unwanted plants	Atrazine
Rodenticides	Control mice and other rodents	Warfarin
Algaecides	Control or kill growth of algae	Copper sulfate
Repellents	Repel pests by its taste or smell	Methiocarb
Larvicides	Inhibits growth of larvae	Methoprene
Virucides	Acts against viruses	Scytovirin
Acaricides	Kill mites that feed on plants and animals	Bifenazate
Nematicides	Kill nematodes that act as parasites of plants	Aldicarb

2.1.1 Organochlorine (OC)

Organochlorine (OC) pesticides have been in existence from the 1940s through the 1960s, they are chlorinated hydrocarbons used in agriculture activities and mosquito control. Organochlorines are made up of the following compounds, Dichlorodiphenyltrichloroet (DDT), methoxychlor, dieldrin, chlordane, toxaphene, mirex, kepone, lindane, and benzene hexachloride (US EPA, 2010). The lipophilic bio accumulative organochlorine (OC) insecticides that were widely used in the mid-20th century were gradually replaced with Organophosphates (OPs), carbamates, and pyrethroids due to their less toxicity nature to the environment and do not accumulate in the food chain to the same extent as the OCs (Alavanja et al., 2013).

Human exposure to organochlorine pesticides could be as a result of accidental inhalation by entering an area where pesticide application has been carried out. Pesticides can also enter into fish, dairy products, and other fatty foods that get contaminated with the chemicals. Organochlorine pesticides build-up in the environment and travel long distances in both underground and surface runoff due to their persistent nature (US EPA, 2010). Organochlorine insecticides are still being used in most developing countries though they have been banned for agricultural and domestic use due to their negative environmental impact and chemical stability (Sanborn et al., 2002). In spite of the ban on organochlorines, they are still being imported into Ghana, and there is prove of their existence in the ecosystem (Darko & Acquaah, 2007).

2.1.2 Organophosphate (OP) and Carbamate (CA)

Organophosphates (OP) have similar chemical structure as organochlorines, but their detailed structure, properties and uses are significantly different. Carbamates (CA) are also similarly considered as such. Due to the ban on organochlorines, OPs and CAs became the widely used insecticides. There is reduction in the use of OPs and CAs, especially for OPs, with the exception of developing countries where they are still frequently used (Moretto, 2014). Organophosphate (OP) and Carbamate (CA) insecticides are two groups of pesticides that are mostly treated alongside because they act on the same molecular target, neural acetylcholinesterase (AChE), in both mammals and insects (Moretto, 2014). Exposure to carbamates is commonly through inhalation, ingestion and through the skin, which are considered to be less-toxic route. For instance, carbofuran has a rat oral LD50 of 5mg/kg, compared to a rat dermal LD50 of 120mg/kg, which makes the oral route approximately 24 times more toxic when ingested (Roberts & Reigart, 2013).

2.1.3 Pyrethroids

Pyrethroids are synthetic compounds of naturally found pyrethrins obtained from the flowers of a species of chrysanthemum (Chrysanthemum cinerariaefolium). Pyrethrin compounds have two properties desired for pest control; tend to be more potent and stable in the environment. Pyrethroid pesticides are commonly used for agriculture purposes, insects control, domestic and in veterinary care and are chemically stable compared to the natural pyrethrum (Bateman, 2015). During the past decade, there have been increased use of Pyrethrins and Pyrethroids with a decline in the use of Organophosphates, which were found to be more acutely toxic to birds and mammals than pyrethroids (Mohammed, 2015). Some representative pyrethroids include Bifenthrin, Permethrin, Resmethrin, Fenvalerate, and Cyfluthrin.

Bifenthrin is a synthesized pyrethroid compound used controlling a wide range of foliar insect pests, termites, and wood-infesting insects. It is an insecticide used for both indoor and outdoor in controlling bed bugs, commercial and industrial lawns and agricultural crops including vegetables and cocoa. Synthetic pyrethroids are considered neurotoxicants with tremors and tumors being the toxicological endpoints of prime concern for this pyrethroid active ingredient (Dong, 1995). Their mechanism of action for neurotoxicity of pyrethroids result from interference with the sodium gate in the nerve membrane of mammals by prolonging the open phase of the sodium channels gate when a nerve cell is excited (Stackelberg, 2012). Exposure of bifenthrin can occur in human through contaminated food and water with residues whiles occupational exposure could occur through inhalation and skin contact during application of bifenthrin, or after entering an area within 24 hours of bifentthrin application. Mild health effects such as skin tingling or eye irritation are mostly reported due to exposure to bifenthrin.

2.1.4 Neonicotinoids

Neonicotinoids are synthetic derivatives of nicotine, the tobacco toxin which are the recent class of insecticides used in the agricultural sector to protect crops. Due to their comparatively low toxicity, they have been widely used in agricultural activities. Nicotine as pesticide has been in use for over 200 years but due to its rapid degradation, neonicotinoids were made to be persistent. Neonicotinoid normally targets the nervous system of targeted insect, binding with its nicotinic receptors and interrupting the sending of nerve impulses (Pesticide Action Network Asia and Pacific, 2011).

Neonicotinoids contain different active ingredients such as acetamiprid, clothianidin, imidacloprid, and thiamethoxam. Neoniconinoids are mostly systemic insecticides and their residues will be deposited on food crops as they do not break down rapidly and are unchanged by processing. Studies show the presence of imidacloprid in many food crops such as ginger, vegetables, potatoes, wine, fruit, and fruit juices. The first neonicotinoid to be introduced was imidacloprid and it was used on over 140 crops in over 120 countries around the world (Pesticide Action Network Asia and Pacific, 2011). Though Imidacloprid has a very low toxicity via dermal contact and moderate toxicity when ingested, its toxicity during inhalation is variable (Kumar, 2013). It is routinely used to kill fleas present on pet animals, termites, and bees.

Thiamethoxam is a neonicotinoid-based insecticide that is readily converted into the metabolite clothianidin, which the active ingredient contained in a number of pesticides (Cloyd, 2012). Clothianidin is also rapidly absorbed by plant roots because it has the ability to dissolve in fats, oils, and lipids. Subchronic and chronic studies show that thiamethoxam targets four primary organs; the liver, kidney, hematopoietic system, and testes (Hurley, 2010).

Table 1.2 Typical uses of pesticides

Activity	Uses
Agriculture	Control of multiple crop pests in any stage
Public health	Control of disease vectors such as malaria, dengue, Chagas disease, onchocerciasis, plague, yellow fever, filariasis, trypanosomiasis, schistosomias, and typhus.
	Control of pests (rodents) and eradication of plantations whose final product is a prohibited drug
Home	Incorporated in products such as cosmetics, shampoos, soaps and insect repellents. They are used in the washing and drying of carpets, household disinfectants and care products for pets and plants, and the use of insecticides
Treatment of structure	Treatment of public and private buildings, offices, hospitals, hotels, cinemas, theatres, restaurants, schools, supermarkets, department stores, sports facilities, food warehouses and the rail industry and sea and air
Industries	In the manufacture of refrigerators, electrical equipment, paints, resins, adhesives, pastes, waxes, tents, sails for sailing, sports nets, mats, carpets and tapestries, the timber industry, packaging materials food, cardboard and paper multiple products. In the food industry for the preservation of fresh foods such as meat, etc.
Maintenance of green areas	Treatment of parks, gardens, playgrounds, golf courses, highways, railways, platforms, towers, high voltage lines and poles

*source: (Garcia, Ascencio, Oyarzun, Hernandez, & Alavarado, 2012)

2.2 Pesticides Exposure & Health Effects

Individual exposure to pesticides occurs primarily through our daily activities including the food we eat and drinking water contaminated with pesticide residues. In a study by Ansah,(2015), chlorpyrifos was detected in cocoa beans. Amoah et al.,(2006) also reported 78% of chlorpyrifos was detected in lettuce samples analysed. Pesticides could easily be passed on in the food chain and accumulated in the body of the livestock fed with contaminated food crops and water (

Damalas & Eleftherohorinos, 2011; Wang, 2009). According to a study conducted on evaluation of pesticide in human blood by Latif et al.,(2012) shows quantity of pesticide residue to be alarming, with chlorpyrifos being the predominant. Substantial exposure to pesticides can also occur within or around the home (Environmental contamination), and chlorpyrifos is suspected to be cause of exposure to non-farmers as it is mostly used in homes against cockroaches, mosquitoes and termites (Latif et al., 2012). About 40 to 60% of pesticides are usually deposited in the soil and 5 to 30% are within the atmosphere when they are applied to the farmland, grassland, and forest (Wang et al., 2009). Concerning the adverse effects of pesticides on the environment, most of these effects depend on the toxicity of the pesticide to the target organism. The protective measures taken by the applicator during pesticide application could determine toxicity effect on the exposed individual as improper protective measures could cause exposure. The quantity of pesticide applied and its adsorption ability determine its abundance in the environment and the effect on the target and non-target organisms. Also the weather conditions after application, and the stability of the pesticide in the environment determines the rate of degradation and persistent in the target and non-target organism (Damalas & Eleftherohorinos, 2011). Organochlorines are known to be persistent in the environment and are considered to be harmful agrochemical due to their function as endocrine disruptor, acute toxicity and bioaccumulation (Latif et al., 2012).

2.3 Community awareness of pesticide effects

Agrochemicals are used extensively during farming activities to control pests' destruction. However, any inappropriate use of insecticides could lead to contamination of the environment by the residue. Therefore, pesticide residue is becoming a serious food safety concern to the general public and government (Chang et al., 2005). Pesticides exposure is mostly through the three most common exposure routes: ingestion, inhalation and dermal absorption. Exposure is usually linked to several routine procedures involving pesticides, such as handling, mixing, loading, cleaning contaminated application equipment, cleaning up spills, and re-entering pesticides application area not long after spraying (Herzfeld & Sargent, 2011).

Farmers, consumers as well as many officials in charge of pesticide management may be inadequately aware of the adverse effects of pesticides. As a by-product of pesticide use, farmers and their relatives are usually affected with health problems due to exposure to pesticide. Most farmers store pesticides inappropriately, storing drinking water in pesticide containers and washing other clothes together with pesticide-contaminated clothes.

Poverty and education are factors that also contribute to pesticide exposure in various ways. Some farmers especially the poor farmers are unable to afford protective and spraying equipment and the uneducated ones are unable to read instructions on the pesticides containers about proper use. Reports show that farmers in the Gaza strip used pesticides extensively, although they are well informed on the adverse health effect of pesticides, the safety practices and use of protective equipment was very poor (Yassin, 2002). Another study conducted reported that nearly 60% of farmers from Pampaimadu in Sri Lanka were knowledgeable about practices on crop protection with 6% of the farmers having broad knowledge towards the set standards on crop protection measures, however most of the farmers depended on agro-chemicals to control pests and diseases but at 35% higher concentrations than set standard (Nagenthirarajah & Thiruchelvam, 2008). According Damalas and Eleftherohorinos, (2011), most pesticide users are not well-informed about the potential short and long-term risks of exposure and the appropriate precautions to take during application of such toxic chemicals. Therefore, it is very important to create awareness to help farming communities reduce exposure to agricultural chemicals such as pesticide. Due to the growing concern on the health risk involved in the use of pesticide in Ghana, the recommended pesticides for use by cocoa farmers are Confidor (Imidacloprid), Akate Master (Bifenthrin), Acati power (Thiamethoxam) and Actara (Thiamethoxam).

2.4 Methods used for analysis of pesticide residues

Several methods have been used in analysing pesticide residues, with those used for methomyl and acephate pesticide residue analysis include; Gas chromatography with tandem mass spectrometric detection (GC-MS/MS) (Chuanjiang et al., 2010) high performance liquid chromatography (HPLC) with diode array detector (DAD) and matrix solid-phase dispersion (MSPD) (Wang et al., 2009), liquid chromatography mass spectrometer (LC-MS). This study will use GC/MS.

2.5 Limitations of the study

Financial resource was a major limitation of this study as the cost per urine sample analysis was expensive. This affected the sample size used, as larger sample size is important in quantitative studies to enable statistical test to identify significant relationships on the exposure of pesticides among the farmers' population in New Edubiase.

CHAPTER THREE

METHODOLOGY

3.1 Description of Study Area

New Edubiase is the District capital of Adansi South which is about 92km from Kumasi on the main Kumasi-Bekwai-Cape Coast trunk road in the Ashanti Region of Ghana. According to the 2010 Population and Housing Census, Adansi South District population is 115,378. Males constitute 50.3 percent and females represent 49.7 percent (Ghana Statistical Service, 2012).

The main occupation of the people in the District is agriculture involving the production of crops, rearing of animals, trading in agricultural produce and products and carpentry. The major food crops produced in the district are rice, cassava, plantain, maize, and vegetables. The major tree crops include cocoa, oil palm, and citrus. Production of cocoa is dominant. It substantially employs a greater number of people in the district and thus a determinant in the income distribution in the area.

3.2 Study Design

This study is an analytical case-control study to determine levels of pesticide concentration in urine samples of farmers who apply pesticides and other members of their household who do not apply pesticides. Urine samples were collected in June 2018 from the respondents after a brief interview have been conducted with the help of interpreter using a pre-design questionnaire. The levels of pesticide concentration were determined using Gas-chromatography (GC) and data analysed using MS Excel 2016 and Stata software 2015.

3.3 Study Variables

Dependent variables: Concentration of pesticides in urine

Independent variables: Direct exposure (through application and handling), Indirect exposure (through contaminated food, water, entry into the sprayed farm).

Confounders: Age, level of education, use of PPE.

3.4 Study Population

Inclusion criteria: Adult (18 years and above).

Exclusion criteria: Not willing, Pregnant women or unable to understand or follow study procedures

3.5 Sample size

Sixty-two (62) participants were selected for the study (37 of farmers who apply pesticide and 25 of farmers' household who are farmers but do not apply pesticide) participants were selected based on previous study by Bedi, (2015), Payán-Rentería et al., (2012) and Taha and Salghi, (2013). The 62 sample size was considered due to cost of laboratory analysis per sample.

3.6 Data Collection/Sampling Technique

The study was carried-out among members of households including farmers who apply pesticides and other members who do not apply pesticides in New Edubiase. The agriculture extension officer and the chief farmer were contacted, and the list of farmers who apply pesticide were obtained. A Random sample generator using excel, was used to select the participants and the chief farmer informed the selected participants about the study and to converge at the community centre with household members who do not spray. Participants were interviewed with the help of an interpreter using questionnaires to collect demographic data. Specimen collection was a one spot sampling, 40ml sterile urine containers labelled with the participants' identification code and date of sample collection, were provided for the participants. Urine sample was collected one half to two thirds full in a sterile screw-top container between the hours of 6am to 10am. The containers (sample) lid were screw (air-tight) and were placed in a clean container with ice cubes at 4 ºC preventing external contamination and protection against damage to the sample and transported by road to the laboratory for analysis for levels of pesticide concentration.

3.7 Urine Sample Preparation, Extraction, and Analysis

10ml of urine sample was pipetted into a test tube and acidified with 1ml of molar sulphuric acid to hydrolyse the sample. Sample extraction was carried out by adding 10mL chloroform to the urine sample and shaken gently using the centrifuge for 5min. The layers were allowed to separate, and the chloroform extract was drained out. The combined organic phase was dried by passing it through anhydrous Sodium Sulphate (Na_2SO_4) to remove the moisture into an evaporating dish. The extraction procedure was repeated twice.

Clean-up (extracts purification) was done with Solid Phase Extraction (Silica SPE) cartridge. A silica cartridge (1000mg/6ml) containing 2g layer of sodium sulphate was conditioned with chloroform and the extracts loaded onto the cartridge and then collected into 100ml pear-shaped flask. The column was eluted with 20ml chloroform and the eluent collected concentrated to dryness using the rotary evaporator below 40 ºC. The extracts were reconstituted in 1mL ethyl acetate and transferred into 2mL auto sampler vials for quantitation by Varian CP-3800 GC-ECD and GC-PFPD. All solvents used were analytical grade with purity greater than 98%, different sets of calibration spiking standards in acetonitrile with various range of analyte concentrations were prepared. Gases used by the instrumentation have a minimum purity of 99.99%.

3.8 Instrumentation

A varian CP-3800 Gas Chromatograph (Varian Associates Inc. USA) equipped with an on-column injector and electron capture detector (ECD) and pulse flame photometric detectors (PFPD) were used for GC analysis. Samples extract of 1 µL aliquots was injected and the separation performed on a silica gel capillary column. The pesticide residues were determined by comparing the measured relative retention times to known standards. Using the peak area, the external standard method was used to quantitatively determine the pesticide residue levels. Measurements were carried out within the linear range of the detectors. The peak areas whose retention times matched with the known standards were extrapolated on their corresponding calibration curves to determine the concentration.

3.9 Instrumental Conditions

Varian Saturn CP-3800 GC-ECD with a CombiPAL Auto-sampler comprising an analytical column of 30m + 10m EZ Guard x 0.25mm internal diameter fused silica capillary coated with VF-5ms (0.25µm film) from Varian Inc was used for confirmation of organochlorine and synthetic pyrethroids pesticide analysis. The injection port temperature was set at 270 ºC, in a split less mode with injection volume 1 µL. The detector-ECD temperature was 300 ºC. Oven temperature was programmed as follows; initial temperature 70 ºC/2min, increase at 25 ºC/min to 180 ºC/1min, and lastly increased at 5 ºC/min to 300 ºC. Nitrogen was used as the carrier gas at a constant flow rate of 1mL/min with make-up of 29ml/min.

For the detection of the organophosphorus, Varian Saturn CP-3800 GC-PFPD with a CombiPAL Auto-sampler comprising an analytical column of 30m x 0.25mm internal diameter fused silica capillary coated with VF-1701ms (0.25µm film) from Varian Inc. was used. The injection port temperature was set at 270ºC, in a splitless mode with injection volume 1µL. The detector-PFPD temperature was 280ºC. Temperature of the oven was set as follows; initial temperature 70 ºC/2min, increase at 25 ºC/min to 200 ºC/1min, and lastly increased at 20 ºC/min to 250 ºC. Nitrogen was used as the carrier gas at a constant flow rate of 2mL/min, Air 1, H2 and Air 2 at flow rate of 17, 14 and 10 respectively.

3.10 Training of Research Assistant

The research assistant was fluent in Twi and English language. He was trained on how to simply explain the questionnaires, ethics and informed consent to the study participants and to adhere to the ethical procedures of the study.

3.11 Ethical considerations

Ethical clearance was acquired from the Ghana Health Service ethical review board and attached as appropriate leaders of the communities including the heads of the households where the study was conducted were contacted to seek permission before carrying out the study.

The objectives and procedures of the study including possible risks or benefits associated with participating in the study were well explained to the participants in English and the local language during recruitment. Individual farmers qualified for the study were selected after obtaining written informed consent from them. Questionnaires were issued to individual farmers who agreed to participate and satisfy the inclusion criteria. They were made aware that they have the right to stop at any point in the study. All responses obtained from the participants were treated confidential and saved in a restricted place. The electronic data were saved with a password to prevent unauthorised access.

CHAPTER FOUR

RESULTS

4.1 Demographic characteristics

Table 4.1 presents the demographic characteristics of the study participants. A total of sixty-two (62) volunteers were recruited for the study. Out of the sixty-two participants, 59.7%(37) were farmers who personally mix or apply pesticides, 40.3%(25) were members of the household who were also farmers but were not directly involved in pesticide mixing or cocoa spraying activities. 51.6%(32) of study participants were males and 48.39%(30) were females. The youngest participant was 22 years, and 40.3%(25) were between 22 to 49 years. Approximately half, 59.7%(37) were over 49 years. 93.6%(58) out of the total sample size had lived at their present location for over 10 years.

Majority of the participants 74.2%(46) had some form of education. 25.8%(16) had no formal education. All the study participants were cocoa farmers with 87.1%(54) of the participants cultivating other crops other than cocoa. Almost all participants, 96.8%(60) lived away from their farms, and only 3.2%(2) lived on their farms.

Table 4.1 Demographic characteristics

Case	Frequency N	Percentage %
Pesticides applicators	37	59.68
Non-pesticides applicators	25	40.32
Sex		
Male	32	51.61
Female	30	48.39
Age in years* (mean ± SD)	51(±15)	
22-49 years	25	40.32
>49 years	37	59.68
Period lived in the town		
Less than a one year	1	1.61
1 to 5 years	2	3.23
6 to 10 years	1	1.61
Over 10 years	58	93.55
Highest level of education		
No formal education	16	25.81
Primary	11	17.74
JHS	17	27.42
SHS	11	17.74
Tertiary	7	11.29
A cocoa farmer		
Yes	62	100
Grow any other crop apart from cocoa		
Yes	54	87.10
No	8	12.90
Apply pesticides		
Yes	37	59.68
No	25	40.32
Live on the farm		
Yes	2	3.23
No	60	96.77

SD=Standard Deviation

4.2 Pesticides commonly used by the participants

Cocoa farmers in the community mostly use different pesticides in controlling pest destruction. Presented in table 4.2 are the types of pesticide commonly used by the farmers.

Table 2.2 Types of pesticides and active ingredients used by farmers

Classification	Common name	Active Ingredient	Pesticide Group	Reg. status	Crops	Hazard class
Insecticide	Confidor	Imidaclopride	Neonicotinoid	FRE	Cocoa	II
	Akate master	Bifenthrin	Pyrethroid	FRE	Cocoa	II
	Akate power	Thiamethoxam	Neonicotinoid	FRE	Coca	
	Bufalo	Acetamipride	Neonicotinoid	FRE	Vegetable and Fruit	III
	Actara	Thiamethoxam	Neonicotinoid	FRE	Banana	III
	Karate	Lambda cyhalothrin	Pyrethroid	FRE	Vegetable	II
Herbicide	Gramazon	Paraquate dichloride		UNREG	Weeds	
	Adwumawura	Glyphosphate	Organophosphate	FRE	Vegetable and Cereal	III

FRE= Fully Registered, UNREG= Unregistered, Reg.= Registration Class II: moderately hazardous. Class III: slightly hazardous

*hazard class according to World Health Organization toxicity classes

4.3 Characteristics of farmers who personally applied pesticides

Table 4.3 represents participants who personally apply pesticides. All 37 said they personally mix, load and handle pesticides. Approximately half, 54.1%(20) used Personal Protective Equipment (PPE) during handling and application whiles 46.0%(17) did not use any form of PPE. With regards to cleaning of equipment used in applying pesticides, most 75.7%(28)

sprayers clean their applicator equipment after each spraying activity. 16.2%(6) clean weekly and 8.1%(3) of participants never clean equipment after use. Majority, 80.6%(29) do not wear PPE when cleaning equipment. Almost all sprayers 94.6%(35) wash their hands, and 81.1%(30) bath or shower before continuing any other activities in the farm after use of pesticides. Majority, 81.1%(30) of sprayers do not protect their nose whiles spraying, compared to 18.9%(7) protected their nose by wearing nose mask. Majority 97.3%(36) wash clothes worn during pesticide application and only 2.7%(1) do not. 59.5%(22) of the sprayers tend to have symptoms such as headache, nausea, difficulty breathing after applying pesticides whiles 40.5%(15) do not have any of such symptoms after spraying.

In terms of duration for applying pesticides on the farm, a large percentage of the participants 62.2% sprayed between 4 to 6 hours, followed by 29.7% who applied between 7-10 hours. With regards to the number of years the farmers have been engaged in spraying, 2.7%, 27.0%, 32.4%, 10.8% and 27.0% representing less than a year, 1 to 5, 6 to 10, 10 to 15 and over 15 years spent applying pesticides respectively. Most of the participants representing 61.1%(22) applied pesticides between 1 to 3 times in a month, 19.4%(7) apply 4 to 6 times and 19.4%(7) also apply more than 10 times in a month. Half of the participants' population applied pesticide less than a week, 11.1%(4) participants applied between 1 to 2 weeks, 25.0%(9) between 3 to 4 weeks and 13.9%(5) of the participants applied over a month before the study was conducted. Out of the 37 participants who personally apply pesticides, 62.2%(23) stored pesticides at home and 37.8%(14) do not store pesticides at home.

Table 4.3 Characteristics of farmers who personally apply pesticides

Characteristics	Yes N (%)	No N (%)
Farmer personally mixed, load or handle pesticide	37 (100)	0 (0)
Use/wear PPE during mixing, load or handling	19 (51.4)	18 (48.7)
Use/wear PPE during application	20 (54.1)	17 (46.0)
Store pesticide at home	23 (62.2)	14 (37.8)
Wear any PPE when cleaning equipment	7 (19.4)	29 (80.6)
Wash your hands after working with pesticide	35 (94.6)	2 (5.4)
Bath/shower before continuing other activities in the farm after pesticide use	30 (81.1)	7 (18.9)
Protect nose before carrying out other activities in the farm after working with pesticide	7 (18.9)	30 (81.1)
Wash clothes used in pesticide application separately	36 (97.3)	1 (2.7)
Have symptoms (headache, nausea, difficulty breathing, skin irritation, blurred vision) after pesticide application	22 (59.5)	15 (40.5)
Hours spent applying pesticide on the farm		
1 to 3 hours	3 (8.1)	
4 to 6 hours	23 (62.2)	
7 to 10 hours	11 (29.7)	
Duration farmer have been applying pesticide		
Less than a year	1 (2.7)	
1 to 5 years	10 (27.0)	
6 to 10 years	12 (32.4)	
11 to 15 years	4 (10.8)	
Over 15 years	10 (27.0)	
Number of times pesticide used in a month		
1 to 3 times	22 (61.1)	
4 to 6 times	7 (19.4)	
Over 10 times	7 (19.4)	
Personally clean pesticide applicator equipment after use		
Never	3 (8.1)	
Weekly	6 (16.2)	
After each spray	28 (75.7)	
Last time pesticide was applied		
Less than a week	18 (50.0)	
1 to 2 weeks	4 (11.1)	
3 to 4 weeks	9 (25.0)	
Over a month	5 (13.9)	

4.4 Pesticides frequently used by farmers for spraying crops

Referring to figure 4.1, currently, Bifenthrin (Akatemaster), Thiamethoxam (Actara), imidacloprid (Confidor), (Akate power), (Commandor), and (Sidacor) are the major pesticides used by the farmers in controlling pests. Among the various pesticides used by the farmers, confidor, akate master, akate power, commandor, actara, sidacor and other pesticides representing 72.97%, 70.27%, 48.65%, 21.62%, 51.35%, 51.35% and 29.73% with confidor and akate master being the mostly used pesticides.

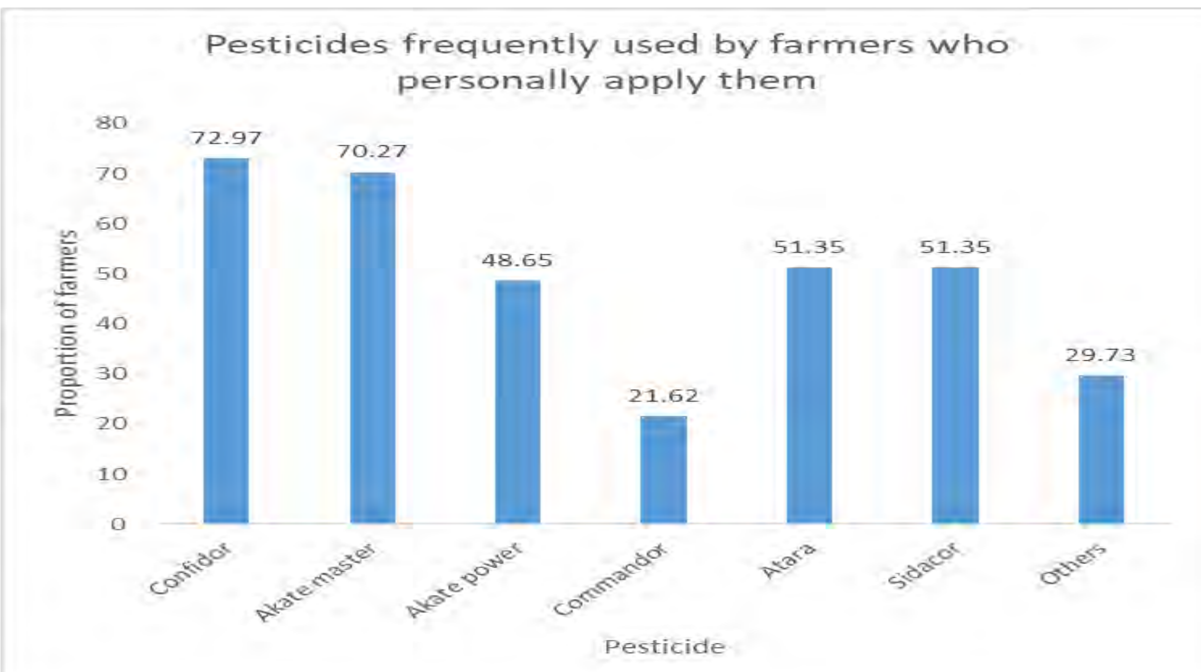

Figure 4.1: Pesticides frequently used among farmers who personally apply pesticides

4.5 Personal Protective Equipment frequently used among farmers who personally apply pesticides

Figure 4.2 represents the participants and the type of protective equipment used during application of pesticides. Majority of the participants 59.46% protect their feet and the smallest population of participants protected their face or eyes representing 37.84% and 40.54% representing the protection of hand, body, and nose (inhalation) each.

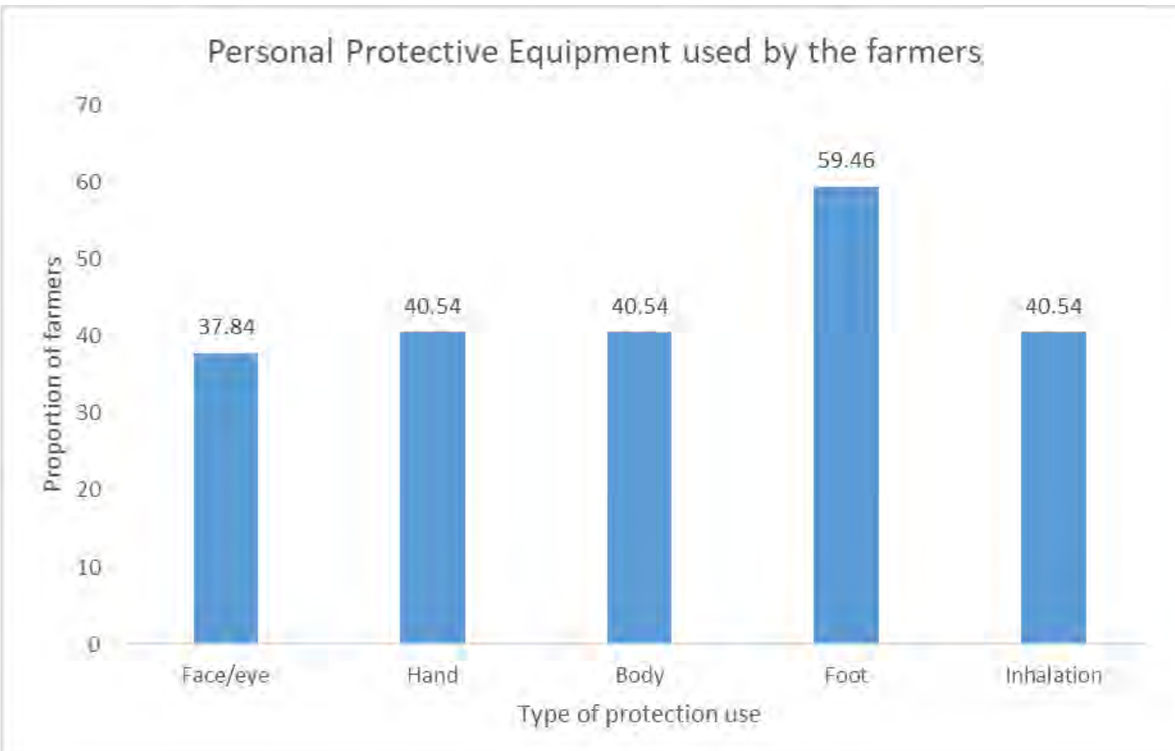

Figure 4.2: Personal Protective Equipment frequently used among farmers who personally apply pesticides

4.6 Pesticides Concentration in Urine Samples of study Participants

From the laboratory analysis conducted on the participants' urine samples, eight (8) active ingredients of pesticide origin were detected. The Limit of Detection (LOD) for the GC/MS according to GSA was 0.01mg/kg. Chlorpyrifos was detected in majority 96.7%(60) of participants, with median concentration of 0.349mg/kg (IQR 0.218-0.484), Lambda cyhalothrin was detected in 9.7%(6) of the participants with a median concentration of 0.011mg/kg (IQR 0.011-0.014), dimethoate was detected in 4.8%(3) participants with median concentration of 0.629mg/kg (IQR 0.134-13.6), cypermethrin was detected in 3.2%(2) participants with a median concentration of 0.265mg/kg (IQR 0.087-0.443), 1.6%(1) participant each were detected fonofos, bifenthrin, gamma HCH, and cyfluthrin with median concentrations of 0.011mg/kg(IQR 0.011-0.011), 0.234mg/kg (IQR 0.235-0.235), 0.012mg/kg (IQR 0.012-0.012) and 0.013mg/kg (IQR 0.013-0.013) respectively as represented in Table 4.4.

Table 4.4 Pesticides concentration in urine of study participants.

Pesticides detected	**N (%)**	**LOD (mg/kg)**	**Median conc. (IQR) (mg/kg)**
Chlorpyrifos	60 (96.8)	0.01	0.349 (0.218 0.484)
Lambda cyhalothrin	6 (9.7)	0.01	0.011 (0.011 0.014)
Dimethoate	3 (4.8)	0.01	0.629 (0.134 13.6)
Cypermethrin	2 (3.2)	0.01	0.265 (0.087 0.443)
Fonofos	1 (1.6)	0.01	0.011 (0.011 0.011)
Bifenthrin	1 (1.6)	0.01	0.235 (0.235 0.235)
Gamma HCH	1 (1.6)	0.01	0.012 (0.012 0.012)
Cyfluthrin	1 (1.6)	0.01	0.013 (0.013 0.013)

IQR=Interquartile Range; LOD=Limit of Detection

4.7 Concentration curve showing distribution of chlopyrifos among Pesticide Applicators and Non-applicators.

Figure 4.3 shows the chlopyrifos distribution among farmers who personally apply pesticide and farmers who do not personally apply pesticide. This graph shows the concentration level between pesticide applicators and non-applicators against the frequency of exposure among both groups. The distribution shows that the maximum concentration level of chlopyrifos in the study participants was 1.51mg/kg which was detected in non-applicators. According to the distribution, it was shown that chlopyrifos was detected in all the participants who apply pesticide and no detection in two of the participants who do not apply pesticide.

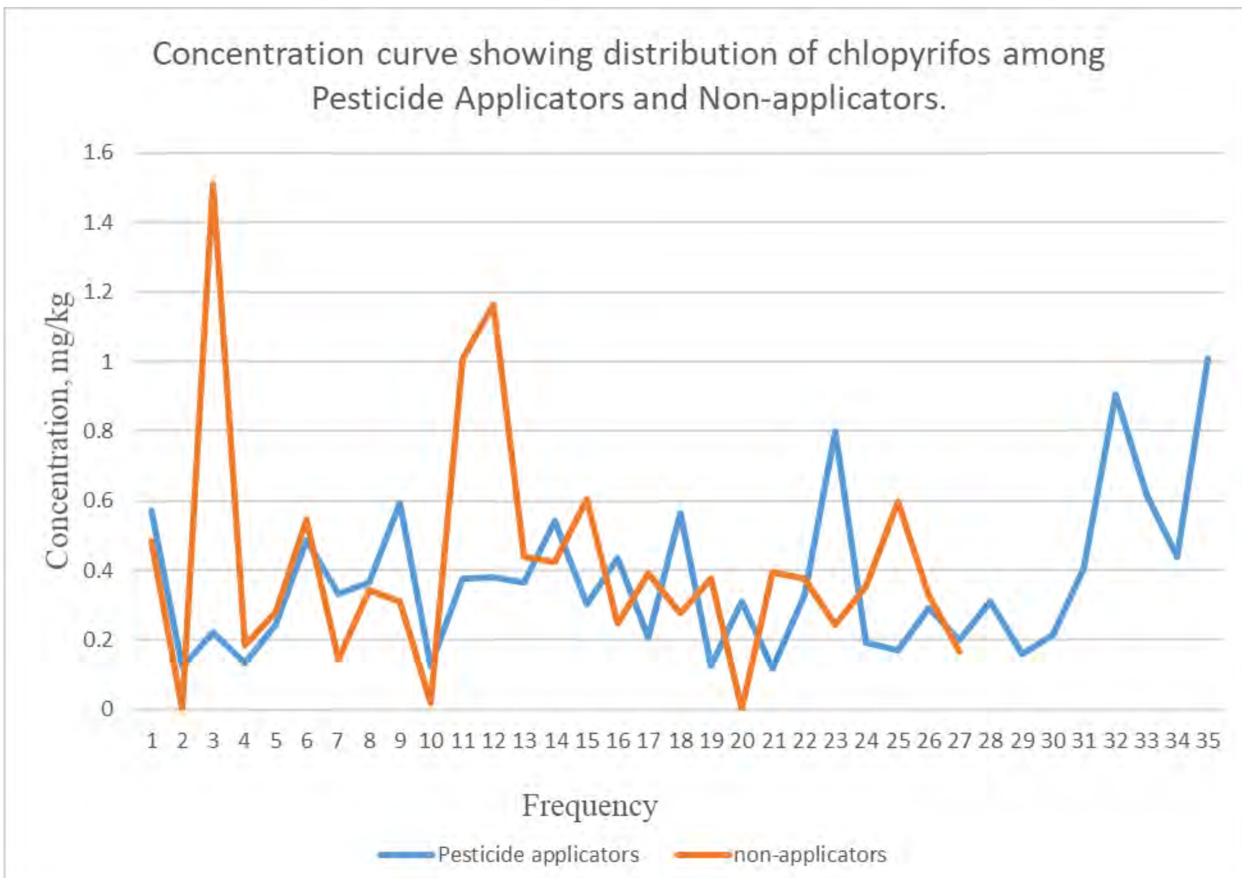

Figure 2.3: Concentration curve showing distribution of chlopyrifos among Pesticide Applicators and Non-applicators.

4.8 Comparison of pesticides concentration in urine of farmers who apply pesticides and household members who are farmers but do not apply pesticides.

Table 4.5 shows median concentrations of pesticide residues and their interquartile ranges in urine of farmers who apply pesticides and household members who are farmers but do not apply pesticides. The LOD was 0.01mg/kg, therefore any residue below the LOD is recorded as Not Detected (ND). In accordance with the results, chlorpyrifos was detected in both farmers who apply pesticide and farmers who do not apply pesticide, lambda cyhalothrin, dimethoate, fonofos, and cyfluthrin were detected in only farmers who apply pesticide, and cypermethrin, bifenthrin and gamma HCH were detected in only farmers who do not apply pesticide. out of total 60 participants with residue of chlorpyrifos detected in their urine sample, 37 participants (100%) were farmers who apply pesticide, 23 participants (92.0%) were farmers who do not apply, with a median concentration of 0.329mg/kg (IQR 0.199-0.438) and 0.376mg/kg (IQR 0.279-0.545), respectively. Lambda cyhalothrin was detected in the urine sample of 6(16.2%) of farmers who apply pesticide with a median concentration of 0.011mg/kg (IQR 0.011-0.014) and no detection in farmers who do not apply. Dimethoate with median concentration of 0.629mg/kg (IQR 0.134-13.6) was detected in 3(8.1%) of farmers who apply pesticide with no detection in farmers who do not apply pesticide. No detection of cypermethrin in farmers who apply pesticide, and 2(8.0%) of farmers who do not apply pesticide had 0.265mg/kg (IQR 0.087-0.443) median concentration of cypermethrin in their urine samples. Fofonos and cyfluthrin were each detected in 2 farmers who apply pesticides with no detection in farmers who do not apply pesticide with median concentration of 0.011mg/kg (IQR 0.011-0.011) and 0.013mg/kg (IQR 0.013-0.013) respectively. Bifenthrin and gamma HCH with median concentration of 0.235mg/kg (IQR 0.235-0.235) and 0.012mg/kg (IQR 0.012-0.012) respectively were detected in

2 farmers who do not apply pesticides with no detection in farmers who apply pesticides. Comparing the concentration level of pesticide in farmers who apply pesticide and those who do not apply pesticide, there was no differences in median concentration of chlorpyrifos, p>0.05.

Table 4.5 Comparison of pesticides concentration in urine sample of farmers who apply pesticide and household members who are farmers but do not apply pesticides.

	Farmers who apply pesticide		Farmers who do not apply pesticide			
Pesticide Detected	**N**	**Median conc. (IQR) (mg/kg)**	**N**	**Median conc. (IQR) (mg/kg)**	**LOD (mg/kg)**	**P value**
Chlorpyrifos	37	0.329 (0.199 0.438)	23	0.376 (0.279 0.545)	0.01	0.178
Lambda cyhalothrin	6	0.011 (0.011 0.014)	0	-ND-	0.01	
Dimethoate	3	0.629 (0.134 13.6)	0	-ND-	0.01	
Cypermethrin	0	-ND-	2	0.265 (0.087 0.443)	0.01	
Fonofos	1	0.011 (0.011 0.011)	0	-ND-	0.01	
Bifenthrin	0	-ND-	1	0.235 (0.235 0.235)	0.01	
Gamma HCH	0	-ND-	1	0.012 (0.012 0.012)	0.01	
Cyfluthrin	1	0.013 (0.013 0.013)	0	-ND-	0.01	

IQR=Interquartile Range; ND=Not Detected; LOD=Limit of Detection, *Blanks are where no statistical comparison could be made.

4.9 Relationship between sex and pesticides exposure

Table 4.6 shows median concentrations of pesticide residues and interquartile range based on the gender of participants. Considering the results, chlorpyrifos and dimethoate were detected in both male and female farmers, lambda cyhalothrin, fonofos and cyfluthrin were detected in only male farmers, and cypermethrin, gamma HCH and bifenthrin were detected in only female farmers. Out of total 60 participants with a residue of chlorpyrifos detected in their urine samples, 32(53.3%) participants were male, 28(46.7%) participants were female with median concentrations of 0.332mg/kg (IQR 0.203-0.516) and 0.370mg/kg (IQR 0.264-0.461) respectively, respectively, 6 participants with a residue of lambda cyhalothrin detected in their urine samples with a median concentration of 0.0111mg/kg (IQR 0.011-0.014) were all male. Dimethoate was detected in 2 male farmers and 1 female farmer with a median concentration of 6.862mg/kg (IQR 0.134-13.600) and 0.629mg/kg (IQR 0.629-0.629) respectively. Cypermethrin was detected in a 2 female farmers with median concentration of 0.265mg/kg (IQR 0.087-0.443) and no detection among male farmers, fonofos with a concentration of 0.011mg/kg (IQR 0.011-0.011) was detected in a male farmer with no detection among female farmers, bifenthrin and gamma HCH with median concentrations of 0.235mg/kg (IQR 0.235-0.235) and 0.012mg/kg (IQR 0.012-0.012) respectively were each detected in 2 female farmers with no detection among male farmers. Cyfluthrin residue with median concentration of 0.013mg/kg (IQR 0.013-0.013) was detected in 1 male farmer with no detection in female farmers. There were no statistically significant differences in the median concentration for chlorpyrifos and dimethoate among male and female farmers, $p>0.05$.

Table 4.6 Relationship between sex and pesticides exposure

	Male		Female		LOD (mg/kg)	P value
	N	Median conc. (IQR)	N	Median conc. (IQR)		
Chlorpyrifos	32	0.332 (0.203 0.516)	28	0.370 (0.264 0.461)	0.01	0.589
Lambda cyhalothrin	6	0.011 (0.011 0.014)	0	-ND-	0.01	
Dimethoate	2	6.867 (0.134 13.600)	1	0.629 (0.629 0.629)	0.01	1
Cypermethrin	0	-ND-	2	0.265 (0.087 0.443)	0.01	
Fonofos	1	0.011 (0.011 0.011)	0	-ND-	0.01	
Bifenthrin	0	-ND-	1	0.235 (0.235 0.235)	0.01	
Gamma HCH	0	-ND-	1	0.012 (0.012 0.012)	0.01	
Cyfluthrin	1	0.013 (0.013 0.013)	0	-ND-	0.01	

IQR=Interquartile Range; ND=Not Detected, LOD=Limit of Detection, *Blanks are where no statistical comparison could be made.

4.10 Relationship between PPE use (PPE users and Non-PPE users) and pesticide concentrations in urine of farmers who apply pesticides

Table 4.7 shows median concentrations of pesticide residues and interquartile range of detected pesticide residues among farmers who apply pesticide using PPE (PPE users) and farmers who apply pesticide without the use of PPE (non-PPE users). Four pesticide residues were detected among farmers who use PPE and those who do not use PPE. Out of total 37 farmers with residue of chlorpyrifos detected in their urine sample, 20 use PPE and 17 do not use PPE, with a median concentration of 0.332mg/kg (IQR 0.211-0.515) and 0.312mg/kg(IQR 0.169-0.406), respectively. Lambda cyhalothrin with median concentrations of 0.012mg/kg (IQR 0.011-0.0135) and 0.011mg/kg (IQR 0.011-0.011) was detected in 6 of the farmers who apply pesticide, in which 5 are PPE users and 1 participant do not use PPE respectively. Dimethoate with median concentration of 0.134mg/kg (IQR 0.134-0.134) and 7.115mg/kg (IQR 0.629-13.6) was detected in 1 PPE user and 2 non-PPE users respectively. Cyfluthrin with concentration of 0.013mg/kg (IQR 0.013-0.013) was detected in a PPE user with no detection among non-PPE users. Comparing the use of PPE among farmers who apply pesticides, there were no differences in median concentration for chlorpyrifos, lambda cyhalothrin, and dimethoate, $p>0.05$.

Table 4.7 Relationship between PPE use (PPE users and Non-PPE users) and pesticide concentrations in urine sample of farmers who apply pesticides.

	PPE users		Non-PPE users		LOD (mg/kg)	P value
	N	Median conc. (IQR)	N	Median conc. (IQR)		
Chlorpyrifos	20	0.332 (0.211 0.515)	17	0.312 (0.169 0.406)	0.01	0.637
Lambda cyhalothrin	5	0.012 (0.011 0.0135)	1	0.011 (0.011 0.011)	0.01	0.380
Dimethoate	1	0.134 (0.134 0.134)	2	7.115 (0.629 13.6)	0.01	0.221
Cyfluthrin	1	0.013 (0.013 0.013)	0	-ND-	0.01	

IQR=Interquartile Range; ND=Not Detected, LOD=Limit of Detection, *Blanks are where no statistical comparison could be made.

CHAPTER FIVE

DISCUSSION

5.1 Pesticides used by farmers in the community

Studies conducted by Ansah, (2015) and Mohammed, (2015) on assessment of pesticides in cocoa beans showed that various types of pesticides are used by farmers in cocoa growing communities throughout Ghana. Overall, seven types of pesticides are used by the farmers to protect their crops in the study area. Three pesticides (imidacloprid, bifenthrin, and thiamethoxam) are commonly used by all farmers. The frequent use of these major pesticides observed in this study could be as a result of the presumption that they are non-toxic or less toxic to humans and are easily degraded in the environment. Also, the availability of these pesticides due to choice and supply by the Ministry of Agriculture and the cost of purchase is another factor leading to their frequent use among the farming community.

5.2 Use of PPE by farmers in the community

The use of PPE especially for face and eye protection among the population of farmers who apply pesticide was minimal. A study conducted by Yassin et al.,(2002) shows the frequent use of pesticides in Gaza strip and the awareness of their health effect, safety practices were very poor. 37.84% of the farmers who apply pesticide protect their faces during pesticide application, 59.46% of the farmers protected their feet and 40.54% representing farmers who protect their hands, body, and nose (inhalation) each during pesticide application. Results of the study did not show statistically significant association between median concentration and the use of PPE among farmers who apply pesticide.

5.3 Concentration levels of pesticide residues in urine samples

In this study, eight (8) pesticide residues were detected in the urine samples of the participants. The pesticide residues detected are classified as Organophosphate (Chlorpyrifos, Dimethoate, Fonofos), Organochlorine (Gamma HCH) and Pyrethroid (Lambda cyhalothrin, Cyfluthrin, Cypermethrin, Bifenthrin). Out of the 62 study participants, pesticide residues were detected in 96.8%(60) with the exception of 3.2%(2) farmers who do not apply pesticide. Chlorpyrifos was the major pesticide residue detected during the study, with 96.8% participants having detectable levels in urine sample with a median concentration of 0.349mg/kg and the high exposure level is expected to be as a result of frequent usage in both farming activities and as household insecticide (Hill et al., 1995). Six (6) (9.7%) of the participants were detected to have residue of lambda cyhalothrin in their urine samples with a median concentration of 0.011mg/kg. Lambda cyhalothrin is pyrethroid which are considered to be chemically stable than the natural pyrethrum (Bateman, 2015). Dimethoate, cypermethrin, fonofos, bifenthrin, gamma HCH and cyfluthrin were detected with a median concentration of 0.629mg/kg, 0.265mg/kg, 0.011mg/kg, 0.235mg/kg, 0.012mg/kg, and 0.013mg/kg, respectively. Although, Bifenthrin, thiamethoxam and imidacloprid were the most frequently used pesticides among the farmers, they were not detected in the urine samples of the participants, which could be as a result of their rapid degradation nature.

In comparing farmers who personally apply pesticide and farmers who do not personally apply pesticide, there was no statistically significant difference in the median concentration of pesticide residues ($p>0.05$). This could be associated with entering pesticide treated areas without allowing stipulated wait period, drift from neighbouring farms and contact with residues on farm crops (Damalas & Koutroubas, 2016).

Chlorpyrifos was detected in most of the urine samples analysed for pesticide exposure among farmers who apply pesticide and members of the farmers' household who are farmers but do not apply pesticide, this confirms the study conducted by Latif et al.,(2012), where chlorpyrifos was the most detected pesticide residue in the blood samples analysed among agro professionals and non-agro professionals. In this study, the median concentration of chlopyrifos was higher in farmers who do not apply pesticide and the maximum concentration level among the individual study participants was detected in non-applicators as compared to pesticide applicators. Chlorpyrifos is an insecticide suspected to be an endocrine disruptor and causes change to hormonal homeostasis (Sifakis, Mparmpas, Soldin, & Tsatsakis, 2011), it is moderately toxic and persistent in nature. A different study conducted in USA among adults observed 82% of the population to have high level of concentration of chlorpyrifos in their urine samples (Hill et al., 1995). The results of this study is an indication that chlorpyrifos is still being used in most farming communities and its presence indicates their characteristic of long-term persistence in the environment (Taha & Salghi, 2013). According to Ansah, (2015) and Amoah, (2006), chlorpyrifos was detected in cocoa beans and lettuce samples respectively, with Amoah et al., (2006) reporting 78% of detection in the samples analysed. The concentration levels detected in this study could be as a result of transport and bioaccmulation of pesticide residues in the food chain (Yadav, 2010). Chlorpyrifos residue concentration detected in this study could have serious health implications to individuals exposed, as organophosphate pesticides have been reported to be potential acetylcholinesterase inhibitors (Office of Pesticide Programs, 2000).

In comparing male and female participants, and among farmers who personally apply pesticides using PPE and farmers who do not use PPE during application of pesticide there was no statistically significant differences in concentration, $p>0.05$.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Eight (8) pesticides residues, Organophosphate (Chlorpyrifos, Dimethoate, Fonofos), Organochlorine (Gamma HCH) and Pyrethroid (Lambda cyhalothrin, Cyfluthrin, Cypermethrin, Bifenthrin) were detected in the urine samples of the participants at varying concentrations. It was observed that three pesticides are mostly used by the farmers in the study area; Bifenthrin, thiamethoxam and imidacloprid which are approved and registered pesticides under the Ghana Cocoa Board. However, the results of the laboratory analysis did not confirm their presence in the urine sample of participants.

The study also found that participants examined from both groups (farmers who apply pesticide and members of the farmers' household who are farmers but do not apply pesticide) have been exposed possibly through improper practices such as improper handling, application and improper use of personal safety protective equipment. It is concluded in this study that the higher frequency of existence of chlorpyrifos could be as a result of excessive and frequent usage and also the high concentration level in farmers who do not apply pesticides could be as a result of not protecting themselves whiles in the areas where pesticide application is being carried out by the applicators. The study highlighted the presence of pesticides in the individuals in the cocoa growing communities. The high frequency of chlorpyrifos indicates that there is a possibility of being present in the environment and could have serious health implication on individuals in the community.

6.2 Recommendations

- Further biomonitoring studies should be conducted on farmers to determine pesticides residue especially the newly introduced pesticides (Bifenthrin, thiamethoxam and imidacloprid) expected to be non-persistent in the environment to confirm if they are truly non-persistent as they were not detected in this study.
- Further studies should be conducted on farmers (sprayers and non-sprayers) and non-farmers to determine pesticides exposure in the farming community.
- A case study should be carried out in cocoa growing communities to determine the health effect of chlorpyrifos on individuals exposed.

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APPENDIX

APPENDIX 1: PARTICIPANTS INTRODUCTION LETTER

I am a master's student of the School of Public Health, University of Ghana, legon. As part of the programme, we are to carry out research work. My work is to determine the level of pesticide residue in individuals in a cocoa growing community (New Edubiase). It is hoped that the findings of this study will help identify accumulated level of pesticide residues in the individuals in New Edubiase. The risk to you by participating is minimal. The only discomfort associated with this study is the discomfort of sharing of personal information but be assured that confidentiality and anonymity will be used. Participation is completely voluntary and you are free to opt out if you wish not to continue with this study.

Who to contact

In cases of any questions regarding the research, you can contact:

• GHS/ Ethical Review Committee administrator, Hannah Frimpong (mobile: 0507041223)

• School of Public health, University of Ghana, Legon.

or

• Justice Dogbey

Mobile number: 0246018759/ 0501374544

Email: justicedogbey1@gmail.com

Before taking Consent

Do you have any questions you wish to ask about the study? Yes/ No

(If yes, please, indicate the questions below) …………………………………………………

APPENDIX 2: STATEMENT OF CONSENT

I ………………………………………………., declare that the purpose, procedures to be followed, risks and benefits of the study have been read/ had been explained and every question(s) have been answered. I hereby agree to participate in this study.

Signature/Thumbprint of participant................................ Date.........../............../..........

Statement by the Researcher

I, undersigned, have explained this consent form to the subject in the language that he/she understands on information regarding this study. I agree to answer any future questions concerning the study and also adhere to the approved protocol.

Signature ………………………… Date………../………………/…………

APPENDIX 3: QUESTIONNAIRE

My name is Justice Dogbey from the School of Public Health, University of Ghana. We need your assistance to carry out a research work on the level of pesticide residues in your body. This study will provide you information on the accumulated pesticide residue level in relation to the use of pesticide during farming in your community. Your participation is important to the achievement of the study. All information that you provide us will be treated with caution and will not be released to anyone but researchers conducting the study. Confidential information will be stored in locked files accessible only to study staff.

We would administer a questionnaire and also take urine sample from you. You are free to skip any question in the form or stop at any point of the interview/procedure.

Please do you have any questions about the study?

Thank you for accepting to participate in this important research project.

A: General Information

Participants code: …………………………………………...………

Name of participant:…………………………………………………

Contact information:…………………………………………………..

Name of interviewer:………………………………….………………

Date of interview: ………………………………………………………

Place of interview: …………………………………………………….

B: Personal Information

Date of birth: …../……./……. (DD/MM/YY) Age:………………

Sex ☐ Male ☐ Female

Highest level of education. ☐ No formal education ☐ Primary ☐ JHS ☐ SHS

How long have you lived in the town? ☐ less than a year ☐ 1-5yr ☐ 6-10yrs ☐ over 10yrs

C: Occupational Information

1. Are you a cocoa farmer? ☐ Yes ☐ No
2. Apart from cocoa, do you grow any other crops? ☐ Yes ☐ No
3. Do you personally apply pesticide? ☐ Yes ☐ No **if NO, continue from question 21**
4. How many hours do you spend applying pesticide on the farm? ☐ less than 1hr ☐ 1 to 3hrs ☐ 4 to 6hrs ☐ 7 to 10hrs
5. How long have you being applying pesticide? ☐Less than a year ☐1 to 5yrs ☐6 to 10yrs ☐11 to 15yrs ☐ >16yrs
6. What are the types of pesticides you use?

Confidor	☐
Akate master	☐
Akate power	☐
Commandor	☐
Atara	☐
Sidacor	☐

Others (Please name them) ………………..

7. How many times do you use pesticide in a month? ☐ 1 to 3 ☐4 to 6 ☐7 to 10 ☐>10

8. Do you personally mixed, load or handled? ☐ Yes ☐ No

9. Do you use/wear any personal protective equipment (PPE) during mixing, loading or handling? ☐ Yes ☐ No

10. When was the last time you applied pesticide?.............

11. Do you use/wear any personal protective equipment (PPE) during application?

    ☐ Yes ☐ No

12. If yes, Tick (√) what applies

Face/Eye protection e.g. safety glasses, face shield	
Hand protection e.g. safety gloves	
Body protection e.g. overall coat, apron	
Foot protection e.g. safety boot	
Inhalation protection e.g. nose masks	

13. Do you store pesticides at home? ☐ Yes ☐ No

14. How often do personally clean your pesticide applicator equipment after using it?

    ☐ Never ☐ weekly ☐ monthly ☐ after each spray

    ☐ others (specify please) ………….

15. Do you wear any personal protective equipment when cleaning your equipment?

    ☐ Yes ☐ No

16. After working with pesticides, do you usually wash your hands before eating? ☐ Yes ☐ No

17. After working with pesticides, do you usually bathe or shower before continuing with other activities? ☐ Yes ☐ No

18. After working with pesticides, do you usually protect your nose before carrying out other activities in the farm? ☐ Yes ☐ No

19. Are the clothes you use when working with pesticides usually washed separately? ☐ Yes ☐ No

20. Do you tend to have symptoms (such as headaches, nausea, difficulty breathing, etc.) after applying pesticides? ☐ Yes ☐ No

21. Have you personally mixed, loaded, handled or applied pesticides? ☐ Yes ☐ No

22. Do you wash clothes worn in applying pesticide? ☐ Yes ☐ No

23. Are you around the area (farm) when pesticides are being applied? ☐ Yes ☐ No

24. Do you live on the farm? ☐ Yes ☐ No