# LEVELS OF SELECTED PESTICIDE RESIDUES IN COCOA BEANS FROM THE WESTERN AND CENTRAL REGIONS OF GHANA


**BY**

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## DECLARATION

I hereby declare that this submission is my own work towards the MSc and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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## ABSTRACT

The main objective of this work is to determine the levels of pesticide residues in cocoa beans from Western and Central Regions of Ghana to determine their suitability for consumption using Gas Chromatography with Electron Capture Detector. Twenty samples were collected and analysed. The investigated pesticides were Chloropyrifos, Endosulfan (I and II), Profenefos, Fenvalerate, Bifenthrin, Permethrin (I and II), Cypermethrin (I, II and T), HCH (α, β, γ and δ), Aldrin, Heptachlor – exo – epoxide, Dieldrin, 4,4 – DDD, 4,4 – DDT and Heptachlor. A multiresidue proceedure was used in the extraction. Bond Elute ENVI – 18 and ENVI – Carb/LC – $NH_2$ were used for clean - up. The recoveries ranged between 65% and 123%. The concentration of chloropyrifos was highest among all the pesticides detected with range of 4.39 – 11.87 mg/kg for cocoa beans samples from the Central and 6.67 – 14.00 mg/kg for cocoa bean sample from the Western Region. Aldrin was not detected in samples from both Central and Western Region. Comparing the concentrations of the pesticides with the MRLs, Chloropyrifos (4.39 – 11.87 mg/kg) and Cypermethrin II (0.12 – 1.82 mg/kg) concentrations in all the cocoa beans samples from Central Region exceeded the MRLs set by the EU and Japan. Chloropyrifos (6.67 – 14.00 mg/kg), Permethrin II (0.20 – 2.16 mg/kg) and Cypermethrin II (0.15 – 3.46 mg/kg) in cocoa bean samples from Western Region also exceeded the MRLs set by the EU and Japan. None of the cocoa beans samples from the Central Region exceeded the MRLs set by EU and Japan for Bifenthrin. None of the samples from the Western Region exceeded the MRLs set by the EU for 4, 4 – DDD and 4, 4 – DDT.

## DEDICATION

This thesis is dedicated to my parents Hajia Nura Abdul Rahman and Alhaji Mohammed Haruna Inuwa and my Elder sister Hajia Maame Memuna Adams who have supported me all the way since the beginning of my studies.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| AAB | Acetic Acid Bacteria |
| ACh | Acetylcholine |
| ADI | Acceptable Daily Intake |
| ARfD | Acute Reference Dose |
| ATSDR | Agency for Toxic Substances and Disease Registry |
| BHC | Benzene Hexachloride |
| CCPR | Codex Committee on Pesticide Residue |
| CDC | Centre for Disease Control and Prevention |
| CEC | Cation Exchange Capacity |
| $CEC_1$ | Commission for Environmental Cooperation |
| CFS | Centre for Food Safety |
| ChE | Cholinesterase Enzyme |
| ECD | Electron Capture Detector |
| DDD | Dichlorodiphenyldichloroethane |
| DDT | DichloroDiphenyl Trichloroethane |
| DHHS | Department of Health and Human Services |
| EDSP | Endocrine Disruptor Screening Program |
| EPA | Environmental Protection Agency |
| EU | European Union |
| FAO | Food and Agricultural Organization of the United Nations |
| FDA | Food and Drugs Association |
| GATT | General Agreement on Tariffs and Trade |
| GAP | Good Agricultural Practice |
| GPC | Gel Permeation Chromatography |

| | |
|---|---|
| GUP | General Use Pesticide |
| HCH | Hexachlorocyclohexane |
| HPTE | 2,2-bis(p-hydroxyphenyl)- 1,1,1-trichloroethane |
| IARC | International Agency for Research on Cancer |
| ICCO | International Cocoa Organization |
| IHPA | International HCH and pesticide Association |
| JMPR | Joint FAO/WHO Meeting on Pesticide Residue |
| LAB | Lactic Acid Bacteria |
| LLE | Liquid–liquid extraction |
| LOD | Limit of Determinationor Detection |
| MAF | Ministry of Agricultural and Forestry |
| MCL | Maximum Contaminant Level |
| MHLW | Ministry of Health, Labour and Welfare |
| MRL | Maximum Residue Level |
| NOAEL | No Observed Adverse Effect |
| NPTN | National Pesticides Telecommunications Network |
| NTE | Neuropathy Target Ssterase |
| OCP | Organochlorine Pesticides |
| PAD | Population Adjusted Dose |
| PBA | 3-phenoxybenzoic Acid |
| PIC | Prior Informed Consent |
| POP | Persistent Organic Pollutant |
| POPRC | Persistent Organic Pollutant Review Committee |
| PRDC | Pesticide Residues Problems in Developing Countries |
| RED | Reregistration Eligibility Decision |

| | |
|---|---|
| RfD | Reference Dose |
| RIFA | Red Imported FireAnt |
| SPE | Solid Phase Extraction |
| SPS | Sanitary and Phytosanitary Standard |
| SPME | Solid-Phase Micro-Extraction |
| STDF | Standard and Trade Development Facility |
| TDI | Tolerable Daily Intake |
| UF | Uncertainty Factor |
| UNEP | United Nations Environment Programme |
| USA | United States of America |
| USDA ARS | United States Food and Drugs Association-Agricultural Research Service |
| WHO | World Health Organizations |

# CHAPTER ONE

## 1. INTRODUCTION

Cocoa is of vital importance to the economies of the producing countries in Africa namely, Cameroon, Côte d'Ivoire, Ghana, Nigeria and Togo. In 2008, these countries exported about 1.3 million tonnes of cocoa beans to the EU and about 0.3 million tonnes to the USA, representing about 50% and 9% of total world exports respectively (. The crop contributes major proportions of national foreign exchange earnings and regionally, providing employment to millions of people in Africa (Bateman, 2010). About 60% of the national agricultural labour force in Ghana has been employed by the cocoa industry (Appiah, 2004). Cocoa is still produced predominantly by a large number of resource-poor smallholder farmers. In Ghana alone, there is contribution of about 70 – 100% of annual household incomes for smallholder cocoa farmers (Asamoah and Baah, 2003). Therefore, the Sanitary and Phytosanitary Standard (SPS) regulations of cocoa consuming countries have the potential of constituting a trade barrier, as most cocoa producing countries may not have the capacity to adequately meet these SPS regulations. This will disrupt cocoa trade, limit market access and have a significant economic impact on cocoa producing countries. In light of the above, the International Cocoa Organization (ICCO) Secretariat requested a Project Preparation Grant (PPG) from the Standard and Trade Development Facility (STDF) to engage a consultant to conduct a study to assess the capacity of cocoa producing countries to meet existing international SPS standards (Bateman, 2010).

The following gaps have been identified to be addressed immediately to enhance the capacity of cocoa producing countries to meet international SPS. They are:

quantification of the levels of risk from contaminants affecting the cocoa supply chain; provision of specific information on pesticide science, at all levels in producer countries and infrastructure to monitor and enforce SPS standards. It is proposed that an investment of $5,458,709 would be required to address many of these issues, by strengthening national capacity in five participating countries and developing regional co-operation in SPS. By collaborating with existing in-country and international initiatives for extension and pesticide stewardship, a substantial level of local and counterpart contribution has been identified. The proposed project places emphasis on issues relating to Good Agricultural and Warehouse Practices. It aims to put in place a sound infrastructure to monitor and prevent the occurrence of potentially harmful pesticide residues and other substances (Bateman, 2010).

Cocoa production in Ghana has been confronted with a lot of challenges like diseases and pest infestation over the years which have contributed to Ghana losing its position as the leading cocoa producer in the world (Anim-Kwapong and Frimpong, 2005). Cocoa is easily attacked by black pod disease, cocoa swollen shoot virus (CSSV), and insect pests such as cocoa capsids (*Distinthiella theobromae* and *Salbeligella singularis*). For example, in the 1980s, Ghana's cocoa production reduced due to pest and diseases significantly (PAN, 2001). According to Duguma *et al*, pests and diseases accounts for 30% loss in global yields of cocoa annually, whereas site-specific losses can range from 10 to 80% annually (Duguma *et al.*, 1998). In order to prevent loss due to pests, the applications of pesticides were introduced.

The Government therefore, in the year 2001 initiated a nationwide Cocoa Disease and Pest Control Project (CODAPEC), to help address the two major causes of decline in cocoa production, pests and diseases. Under this programme, cocoa farms across the country were sprayed with insecticides and fungicides at no cost to the farmers. This exercise has resulted in tremendous increases in cocoa production from 340,562 metric tons in the 2001/02 season to 496,846 metric tons in 2002/03 and 736,000 metric tons in the 2003/04 seasons, respectively (Appiah, 2004; ICCO, 2004). The percentage of locally processed beans has also jumped from 20% to 35% with further re-capitalization and expansion programs underway to reach a target of 50% in the near future. However, along with the positive effects of the CODAPEC programme, some negative impacts on the environment have also been caused. For instance the use of pesticides on the farms can lead to the destruction of part of the soil flora and fauna through both physical and chemical deterioration (Cowell and Clift, 1997).

Pesticide application in Ghana is more concentrated on cocoa, oil palm, cereals, vegetables and fruits sectors. Although purchased physical inputs (agrochemicals, seeds and tools) represent less than 30% of the total cost of crop production, the use of pesticides is becoming more widespread. For instance, between 1995 and 2000, about 21 different kinds of pesticides were imported into the country for agricultural purposes (FAO, 2004).

In Ghana, pesticides have also been used in the public health sector for disease vector control and in agriculture to control and destroy completely crop pests for the past several decades (Clarke *et al*., 1997). The majority of pesticides used in agriculture are employed in the forest zones located in the Ashanti, Brong Ahafo, Western, and

Eastern Regions of Ghana (Amoah *et al*., 2006). Improvement of food production in the world depends on pesticides which contribute significantly to controlling and destroying various types of pests. Organochlorine (OCP) and organophosphates (OP) pesticides have been used in Ghana for more than forty years, both for agricultural and public health purposes (Essumang *et al*., 2009). There is the need for continuous monitoring of these pesticides because uncontrolled pesticides use has led to the deaths of animals and humans (Bronstein *et al*., 2007). The uses of pesticides, mode of applications and their abuse in agriculture sector have also been of much interest to the environmental scientists nowadays (Essumang *et al*., 2009). Besides their uses are also the residual effect of these pesticides and uniquely their replicating effect on human health (Essumang *et al*., 2009). Since 1940, Organochlorine pesticides (OCPs) have been used all over the world because of their efficiency in protecting crops and in the fight against the vectors of some endemic diseases such as malaria, typhoid fever and tryponosomiasis. These pesticides, aside having a direct effect on the target organisms, have a short and long term impact on other vertebrates or non-vertebrates and non-target. This development led to the ban of some of them. Despite the ban on the production and the use of some OCPs in industrialized countries, in accordance with the Stockholm Convention in 2001 (Cruz *et al*., 2003; Ennacer *et al*., 2008), these persistent toxic substances continue to cause great damage to the environment and living organisms (Zawiyah *et al*., 2007; Hongtao *et al*., 2008). These OCPs are among the agrochemicals that have been used to a greater extent for long periods in this country (Ntow, 2001; Darko *et al*., 2008; Bempah *et al*., 2011). OCPs and their metabolites have been culpably involved in a wide range of adverse human and environmental effects including reproduction and birth defects (Edwards, 1987), Immune system dysfunction, endocrine disruptions, and cancer (WWF, 1999). These

metabolites and residues of many of these pesticides are very stable, with long half lives in the environment (UNEP, 2002). Moreover a 2008 study on vegetables farmers in southern and Central Regions of Ghana, conducted for the US-based International Food Policy Research Institute, found that 69% farmers surveyed had experienced burning sensations on their skin during application, 47% had experienced headaches after application, 39% reported itchy or watery eyes and third had experienced both dizziness and breathing difficulties (Horna *et al* ., 2008).



Human beings are exposed to the effect of these pesticides by eating foods containing pesticides (William *et al*., 2008). If the amounts of pesticides residue are high in foodstuffs, it can lead to health hazards to consumers (Bempah *et al*., 2011). Therefore there is the pressing need for their control and monitoring in the environment. The harmful effects of pesticide use go beyond the impact on Ghana's farmers, and include the food-consuming population. In the past few years, a number of academic studies have been undertaken investigating pesticides residues in food. They show the presence of pesticide residues in fish, water, sediments, fruits, vegetables, meat and human fluids (blood and breast milk) in Ghana (Darko, 2009). The use of OCPs for agricultural purposes has been banned for about 25years now in Ghana and in the developed world. Moreover, there are evidences of their still usage in many developing nations including Ghana, due to inadequate regulation and management on the production, trade and use of these chemicals (Darko and Acquaah, 2007; Darko *et al*., 2008; Bempah *et al*., 2011). These studies truly confirmed the presence of pesticides residues in those different samples. Some recent works have given evidence of the presence of pesticides residues in surface water, sediments, biota, vegetations in Africa, dairy products, meat, fish (Ntow, 2005; Dem

*et al*., 2007; Ize-Iyamu *et al*., 2007; Darko and Acquaah, 2007, 2008; Darko *et al*., 2008).

There is a limited data and studies on pesticide residue in quality cocoa beans (Botchway, 2000; Ntow, 2001, 2005; Ntow *et al*., 2006; Darko and Acquaah, 2007). In spite of the usage of agrochemicals in the Western and Central Regions of Ghana, there is few or no published work available on the levels of pesticides residues in cocoa beans in these two regions with exception of Owusu-Ansah *et al*, who assessed the level of lindane pesticides residues in cocoa beans from Twifo Praso district in the Central Region (Owusu-Ansah *et al*., 2010). The determination of pesticide residues in cocoa beans can give a suggestion of the extent of the contamination of cocoa beans in Ghana and the world as whole (Kannan *et al*., 1995; Darko *et al*., 2008).

As said above, most of the cocoa produced in Ghana are meant for export and in order for the cocoa products to pass the requirements in terms of the residue levels and meet international standards, it is necessary that the residue levels are determined and monitored. The Japanese have requested Quality Control Division of COCOBOD to determine these pesticides (Permethrin I and II, Fenvalerate I and II, Endosulfan I and II, Chloropyrifos and Imidacloprid) in cocoa beans before the shipment to Japan. Actellic/Tarstar, a cocktail of the organophosphate with the pyrethroid Tarstar (Bifenthrin), imidacloprid and Promecarb have been passed by the Cocoa Research Institute of Ghana (CRIG) (Owusu – Manu, 1996 and 1997) and recommended by the COCOBOD for use by farms. In international circles cocoa beans with pesticide residue level above the stipulated Maximum Residue Limits (MRL) are likely to be rejected. It is because of this rejection that the determination of pesticide residues in food and cash crops like cocoa becomes very important in Ghana.

The above reasons give a justification of this work to be carried out. To be able to assess the levels of pesticide residues in cocoa beans, the following shall be carried out.

1. Determine levels of selected pesticides in cocoa beans from the western and central region of Ghana using Gas Chromatography with Electron Capture Detection (GC - ECD). The pesticides to be determined are Chloropyrifos, Endosulfan I and II, Profenefos, Fenvalerate, Bifenthrin, Permethrin I and II, Cypermethrin I, II and T, Beta HCH, Delta HCH, Gamma HCH, Alpha HCH, Aldrin, Hepta-oxo-epoxide, Dieldrin, 4,4-DDT, 4,4-DDD and Heptachlor.
2. Compare the levels of the pesticides in cocoa beans with some international Allowable Maximum Residue Levels (MRL).

# CHAPTER TWO

# 2. LITERATURE REVIEW

## 2.1 PESTS AND DISEASES OF COCOA

Cocoa production is under threat from insect and fungal infestations and decreasing fertility of the land. Recently, there has been an increase in the more destructive variant of black pod disease and significant loss resulting from capsids that have affected at least 25% of the producers (Padi and Owusu, 2003). For example, in the 1980s, Ghana's cocoa production reduced due to pest and diseases significantly (PAN, 2001). The most important of these are *Phytophthora* pod rot, commonly called "black pod", and locally known as '*akate*'; and the swollen shoot virus, also known locally as '*cocoasasabro*' (Opuku *et al*., 1999). The black pod rot, a fungal disease which appears as characteristic brown necrotic lesions on the pod's surface causes most damage to cocoa. An estimated 20-30% of annual cocoa production is lost to these diseases globally (ICO, 2010) and US$1.5 billion revenue was lost due to these diseases in 2005 alone. Specifically, 450 thousand metric tons annually, while 250, 200 and 50 thousand metric tons are lost to witches broom, capsids and swollen shoot virus (CSSV), respectively. In Latin America, witches broom and frosty pod rot are paramount. The black pod and CSSV are common in West Africa. Capsids, are identified as pests at the turn of the last century and are the main insects that feed on cocoa in Africa (Mahot *et al*., 2005).

### 2.1.1 Black pod disease

The most commonly destructive diseases of the cacao tree are pod rots. A pod rot called black pod is caused by a fungus (Phytophthora) that spreads rapidly on the

pods under conditions of excessive rain and humidity, insufficient sunshine, and temperatures below 21°C (70°F). Control requires timely treatment with copper-containing fungicides. It has been shown that it is possible to reduce the frequency of fungicide application from eight times a year (3-weekly application) to five times a year (4-weekly application) and still achieve good control of the black pod disease (Opoku *et al*., 1998). ). This will help reduce the cost of black pod control and render the method more acceptable to farmers, in addition to reducing the amount of chemical used. The possibility of using phosphorous acid which apparently carries little or no danger of residues or contamination of the environment (Opoku *et al*., 1998) is even more encouraging.



### 2.1.2 Cocoa Swollen Shoot Disease

Cacao swollen-shoot virus (CSSV) is a plant pathogenic virus of the family Caulimoviridae that primarily infects cacao trees. It decreases cacao yield within the first year of infection, and usually kills the tree within a few years. Symptoms vary by strain, but leaf discoloration, stem/root swelling, and die-back generally occur. The virus is transmitted from tree to tree by mealybug vectors. It was first discovered in Ghana in 1936, and is currently endemic in Togo, Ghana and Nigeria (Dzahini-Obiatey *et al*., 2010). Over 200 million trees have already been claimed by this disease, which has prompted Ghana to launch the most ambitious and costly eradication effort of any country in the world against a viral plant disease (Dzahini-Obiatey *et al*., 2010). Cacao swollen shoot virus has had a major impact on cocoa production in Africa. Since its discovery in 1936, it has not been effectively contained (Domfeh *et al*., 2011) despite costly eradication efforts, especially in Ghana (Dzahini-Obiatey *et al*., 2010). With yield losses of 25% and 50% the first and second years,

respectively (Crowdy and Posnette, 1947), and eventual death of the plant, this has been a persistent issue affecting the livelihoods of cocoa farmers

### 2.1.3 Capsids

THE first large-scale trials with γ-BHC against the cocoa capsids *Sahlbergella singularis* Hagl. and *Distantiella theobroma* (Dist.) in Ghana were made in 1954 (Stapely and Hammond, 1959). The level of capsid control achieved, and the resultant crop yields of cocoa, were so outstanding that the use of this insecticide in recent years has been countrywide. Aldrin and endrin have also been used successfully and the control reported by Armstrong (Armstrong, 1959 - 1960) as having been obtained with amounts of insecticide as low as ½ oz active ingredient per acre is ample evidence that capsid strains, tolerant or resistant to chlorinated hydrocarbon insecticides, were unknown in Ghana in 1961. Now, however, capsids resistant to γ-BHC have been found on a plot of Amelonado cocoa at Pankese Cocoa Station, near Nkawkaw.

## 2.2 PESTICIDES

Pesticides are any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest (US EPA, 2007). Pest can be insects, mice and other animals, unwanted plants (weeds), fungi or microorganisms like bacteria and viruses. Though often misunderstood to refer only to insecticides, the term pesticides also applies to herbicides, fungicides and various other substances used to control pest (Nollet, 2000). Under United States law, a pesticide is also any substances intended for use on a plant, regulator, defoliant, or desiccant.

In order to limit losses from pests and diseases, cocoa farmers uses a wide range of pesticides. Prominent among these are: copper sulphate (a fungicide popular in the treatment of black pod infection; Benzene hexachloride (BHC) (an insecticide for the control of cocoa mirids; Aldrin/Dieldren or Aldrex 40 (an insecticide for the control of mealy bugs); Carbamate unden (an insecticides which is effective in controlling cocoa mirids in West African Countries) (Berger, 1975). Although there are benefits to the use of pesticides, some also have drawbacks, such as potential toxicity to humans and other animals. According to the Stockholm Convention on Persistent Organic Pollutants, 9 of the 12 most dangerous and persistent organic chemicals are pesticides (Gilden *et al*., 2010).

Lindane was recommended since 1957 for spraying cocoa as a method for controlling capsids on cocoa in West Africa. From 2008 also, Gammalin 20 (lindane) and Unden 20 (Propoxur) has been applied at monthly intervals from August to December in Ghana to control this same capsids. Although the Organochlorines are banned from importation, there is an evidence of its sales and use in Ghana, (Ntow, 2001). The EPA of Ghana in 2008 discovered 71 tonnes of banned pesticides, most of

which were found at the warehouses of the Ghana Cocoa Board at Anyinam, the Benso Oil Palm Plantation and Twifo Oil Palm Plantation (Daily Graphic, 2008). Studies done in some farming communities in the Ashanti Region of Ghana and some other countries indicate the presence of organochlorine pesticide residues in fish (Osafo and Frempong, 1998). Lindane is listed among the Prior Informed Consent (PIC) pesticides, and so all agricultural uses of Lindane have been banned in 52 countries due to its hazardous nature. Even pharmaceutical uses of lindane have been banned in some countries (PANNA, 2008). Many Organochlorines which over the years have been linked to major health and environmental problems have been banned or are no longer used. Lindane is a neurotoxin that interferes with GABA neurotransmitter function by interacting with the GABAA receptor chloride channel complex at the picrotoxin binding site. Lindane primarily affects the nervous system, liver and kidneys in humans, and may also be a carcinogen or endocrine disruptor (Ntow, 2001). The World Health Organization (WHO) estimates that 1-5 million cases of pesticide poisoning occur every year resulting in 20,000 fatalities among agricultural workers, most of them in developing countries (WHO, 2004). Another estimate is that pesticides cause 14 per cent of all known occupational injuries in agriculture and 10 per cent of all fatal injuries (International Labour Conference, 1999).

### 2.2.1 Pesticide Formulation

Pesticides formulation improves the properties of a chemical for handling, storage, application and may substantially influence effectiveness and safety (Knowles, 1998). The pesticide formulation is a mixture of active and other ingredients (previously called inert ingredients). An active ingredient is a substance that prevents, kills, or

repels a pest or acts as a plant regulator, dessicant, defoliant, synergist, or nitrogen stabilizer (US EPA, 1998). Pesticides come in many different formulations due to variations in the active ingredient's solubility, ability to control the pest, and ease of handling and transport. Synergists are a type of active ingredient that is sometimes added to formulations (US EPA, 1998). They enhance another active ingredient's ability to kill the pest while using the minimum amount of active ingredient, but do not themselves possess pesticidal properties. There are many types of other ingredients: solvents are liquids that dissolve the active ingredient, carriers are liquids or solid chemicals that are added to a pesticide product to aid in the delivery of the active ingredient, and adjuvants often help make the pesticides stick to or spread out on the application surface (US EPA, 1997). Some examples of formulations are Aerosol, Bait, Dust, Dry Flowable, Emulsifiable concentrate, Flowable, Granule, Microencapsulated, Pellet, Ready-to-use, Soluble powder, Ultra-low-volume concentrate, Wettable powder and Water-dispersible granule.

### 2.2.2 Environmental Fate of Pesticides

The environmental fate of pesticides depends on the physical and chemical properties of the pesticides as well as the environmental conditions. The physical and chemical properties of the pesticides determine how likely is to travel through soil (soil mobility), how well it dissolves in water (water solubility), and how likely it is to become airborne (volatility). Once a pesticide has been released into the environment, it can be broken down in the presence of sunlight (photolysis), exposure to water (hydrolysis), exposure to other chemicals (oxidation and reduction), microbial activity (bacteria, fungi, and other microorganisms) and plants or animals ie metabolism (Kellogg, 2000). When pesticide is applied to a crop or soil, it enters a

dynamic ecosystem and immediately begins to be moved from one part of the system to another, degraded in situ or moved out of the system into other systems. It is very important to determine the relative importance of these processes, since pesticides that degrade completely become harmless, and those that move to other systems and persists may do damage the environment. Over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species, including nontarget species, air, water, bottom sediments and food (Miller, 2004).

Although there are benefits using pesticides, incorrect use can counter productively increase pest resistance and can kill the natural enemies of pests, thus the plants itself. Many of the farmers are inadequately informed about potential short and long – term risks, and therefore do not take the necessary precautions in the correct application of such toxic chemicals (Damalas *et al*., 2011).

The current recommended pesticides (insecticides) for cocoa production in Ghana are Promecarb, Confidor (Imidacloprid), Actellic (Primiphos Methyl), Akate Master (Bifenthrin) and Actara (Thiamethoxam) (ICCO, 2010). Also unapproved pesticides are Endosulfan, Chloropyrifos, Lindane, Pyrethroid, Cypermethrin, Primiphos Methyl, Lamda Cyhalothrin and others.

### 2.2.3 Exposure of Pesticides to Humans

People can be exposed to pesticides through different routes including: occupation, in the home, at school and in foods. There are concerns that pesticides used to control pests on food crops are dangerous to people who consume those foods. These concerns are some of the reasons why the organic food movement help in monitoring these agricultural chemicals. Many food crops, including fruits and vegetables, contain pesticides residues after being washed or peeled. Chemicals are no longer

used but that are resistant to breakdown for long periods may remain in soil and water and thus in food (Cornell University, 1999). Some pesticides can remain in the environment for prolonged periods of time. For example, most people in the United states still have detectable levels of DDT in their bodies even though it was banned in the US in 1972 ( Gilden *et al*., 2010). A 2007 systematic review found that "most studies on non – Hodgkin lymphoma and leukemia showed positive associations with pesticide exposure" (Bassil *et al*., 2007). Strong evidence also exists for other negative outcomes from pesticide exposure including neurological, birth defects (Sanborn *et al*., 2007), fatal death, and neuro developmental disorder (Jurewicz and Hanke, 2008).

Acute health problems may occur in workers that handle pesticides, such as abdominal pain, dizziness, headaches, nausea, vomiting, as well as skin and eye problems (Ecobicon, 1996). Pesticides exposure has been associated with various types of cancer, leukaemia, lymphoma, brain, kidney, breast, prostate, pancreas, liver, namely lung and skin cancers (Gilden *et al*., 2010). An increased rate of cancer has been found among farm workers who apply these chemicals (McCauley *et al*., 2006).

The United Nations Codex Alimentarius Commission has recommended international standards for maximum residue limits (MRLs), for individual pesticides in food (Codex Alimentarius Commission, 2007). In the United States, levels of residues that remain on foods are limited to tolerance levels that are established by the U. S. Environmental Protection Agency only (USEPA, 2007). Tolerance levels are obtained using scientific risk assessments methodologies. Pesticide manufacturers are required to conduct toxicological studies, exposure modelling and residue studies before a particular pesticide can be registered. However, the effects are tested for

single pesticides, and there is little information on possible synergistic effects of exposure to multiple pesticide traces in the air, food and water (Rabideau, 2001).

### 2.2.4 Pesticide Monitoring in Ghana

There are two main bodies responsible for pesticides surveillance and monitoring in Ghana. The EPA is the key agency responsible for the registration and management of all chemicals, including pesticides (Mensa-Bonsu, 2006). The Chemicals Control and Management Centre (CCMC) of the EPA is responsible for ensuring the proper labelling, distribution, storage, transportation, use and application of pesticide. It monitors the use of pesticides and takes action against their illegal use and registers and issues licenses for pesticides. There is also the Pesticides Technical Committee which makes recommendations to EPA Board on which pesticides are to be registered or not (Mensa-Bonsu, 2006).

Another agency is the Plant Protection and Regulatory Services Directorate of the Ministry of Food and Agricultural (MOFA), who's Pesticide and Fertilizer Regulatory Division supervises and trains inspectors and extension officers to register and inspect pesticide dealers, conducts training and provides information materials on pesticides.

## 2.3 SELECTED PESTICIDES

Pesticides are often explained according to the type of pest they control. Pesticides can also be considered as either biodegradable pesticides, which will be broken down by microbes and other living beings into harmless compounds, or persistent pesticides, which may take months or years before they are broken down: it was the

persistent of DDT, for example, which led to its accumulation in the food chain and its killing of birds of prey at the top of the food chain. Another way to think about pesticides is to consider those that are chemical pesticides or are derived from a common source or production method (USEPA, 2013). Currently Ghana approves the use of 254 pesticides while 26 have been banned and a small number are restricted (Ghana EPA, 2010). It has long been known that various restricted or banned pesticides are still being used by farmers in Ghana to grow food. A study revealed that lindane and endosulfan, which were restricted to use on cocoa, coffee and maize were being used on vegetables, alongside with DDT which has also been banned (Amoah *et al*., 2006).



### 2.3.1 Organochlorine Pesticides

Organochlorine pesticides (OCPs) are the synthetic organic insecticides that contain carbon, chlorine and hydrogen. They are water soluble and highly lipophilic and so they are highly persistent in an organism and environment (Perry *et al*., 1998). Due to their persistence and low cost, the OCPs such as dichloro-diphenyltrichloroethane (DDT), aldrin and endosulfan had been widely used for pest control. Moreover, residues of these pesticides can be transferred and biomagnified through food chain. Levels of OCPs could be accumulated and can cause adverse health effects in animals at higher trophic levels, including human (Perry *et al*., 1998; Walker *et al.*, 2001 and Cunningham *et al*., 2007).

Organochlorines can cause many acute and chronic illnesses. Exposure to a large dose can cause symptoms of acute poisoning like tremors, headache, dermal irritation, respiratory problems, dizziness, nausea and seizures. Exposure to low doses of

organochlorines is associated with many chronic diseases. Studies have shown there is a correlation between organochlorine exposure and various types of cancer, neurological damage (i. e. neurotoxins), Parkinson's disease, birth defects, respiratory illness, abnormal immune system function, are suspected hormone disruptors, and even low level of exposure in the womb can cause irreversible damage to the reproductive and immune system of the developing fetus. Recent studies have also suggested that even low level of OCP residues (part per billion) may also interfere with the structure or function of the endocrine system and can cause adverse effects to animal reproduction and development (Damstra *et al.*, 2002). OCP contamination in animal tissues was connected to adverse effects on reproductive system such as reduced penis size of American Alligator *alligator mississippiensis* in Lake Apopka, Florida, USA, and abnormality in reproductive functions of Florida red-belly turtles *Chrysemysnelsoni* (Guillette *et al*., 1994; 1996). Since human population may be similarly at risk from these chemicals, it is crucial to monitor the degree of these pesticides residues in foods and enviroments.

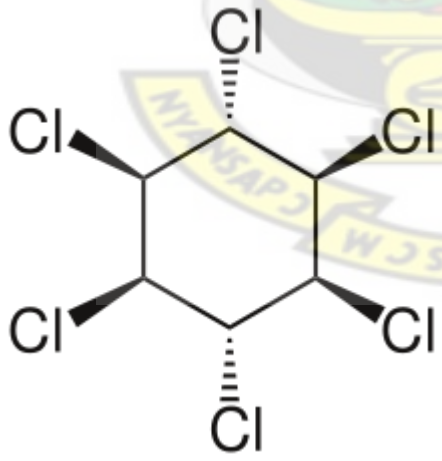


**Fig 2.1: Structure of Gamma HCH**

According to the US EPA, the production and agricultural use of lindane are the primary causes of environmental contaminations (US EPA, 2006). However, the levels of lindane in the environment have been decreasing which is consistent with decreasing agricultural usage patterns (UNEP, 2006). The production of lindane generates large amounts of waste hexachlorocyclohexane isomers, and it is estimated that every ton of lindane manufactured produces about 9 tons of toxic waste (Life after lindane in California, 2008). A process known as cracking is used to convert the waste isomers to lesser toxic molecules in the manufacturing of lindane (UNEP, 2006; IHPA, 2006). An estimated 12 - 30% of it volatilizes into the atmosphere when lindane is used in agriculture where it is subject to long-range transport and can be deposited by rainfall. Lindane found in soil can leach to surface and ground water and can bioccumulate in the food chain (US EPA, 2002), and biotransformation and elimination are relatively rapid when exposure is discontinued (CEC, 2006). Lindane is broken down in soil, sediment and water into less harmful substances by algae, fungi and bacteria and the process is relatively slow and dependent on ambient environmental conditions (ATSDR, 2005). However, the ecological impact of lindane's environmental persistence continues to be debated.

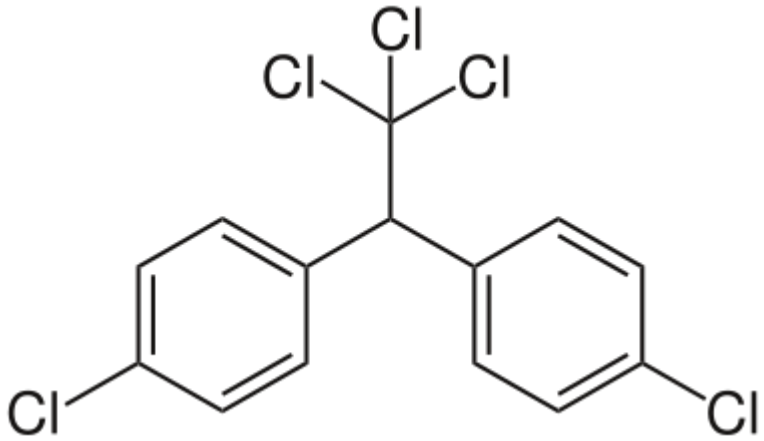




**Fig 2.2: Structure of DDT**

Potential mechanisms of action on humans are genotoxicity and endocrine disruption. DDT may be directly genotoxic and may also induce enzymes to produce other genotoxic intermediates and DNA adducts (Cohn *et al*., 2007). The DDT metabolite, DDE, acts as an antiandrogen (but not as an estrogen) and so is an endocrine disruptor. P, p' – DDT, DDT's main component has little or no androgenic or estrogenic activity whilst the minor component O, p' – DDT has a weak estrogenic activity (Galand *et al*., 1987).

DDT is a persistent organic pollutant that is extremely hydrophobic and strongly absorbed by soil. Its half life can range from 22 days to 30 years. It quickly get absorbed by organisms and soils if it is applied to aquatic ecosystems leaving little DDT dissolved in the water itself. It metabolites (i. e. DDE and DDD) are highly persistent and have similar chemical and physical properties (ATSDR, 2002). The DDT and its metabolites are transported from warmer regions of the world to the Arctic by the phenomenon of global distillation and they can accumulate in the region's food web (The Science and the Environment Bulletin, 1998). They are able

to magnify through food chain with apex predators such as raptor birds concentrating more chemicals than other animals in the same environment.

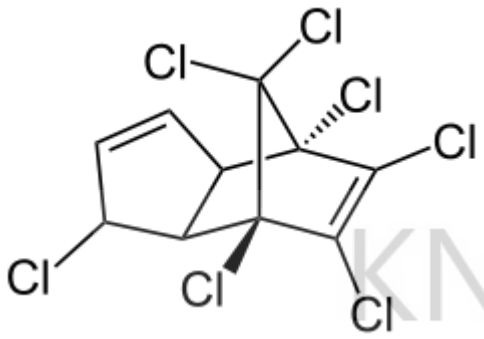


**Fig 2.3; Structure of Heptachlor**

Heptachlor is highly toxic to human and can be absorbed through the skin, lungs and gastrointestinal tract, and as well damage to the liver. Symptoms of poisoning observed in laboratory animals include lethargy, tremors, convulsions, stomach cramps or pain, coma and, death in severe cases as results of respiratory failure. Humans with convulsive disorders or liver damage are at increased risk from exposure.

Heptachlor is persistent organic pollutant (POP) and has a half life of approximately 1.3 – 4.2 days in air, 0.03 – 0.11 years in water and 0.11 – 0.34 years in soil. It is lipophilic so easy to accumulate in the body fat of humans and animals and poorly soluble in water (0.056 mg/L at 25ºC). Heptachlor epoxide is formed when heptachlor breaks down in the environment. Heptachlor epoxide is more liable to be found in the environment, also dissolves more easily in water and is persistent than its parent compound (ATSDR, 2007).

### 2.3.2 Organophosphate Pesticides

An organophosphate (OP) is the general name for esters of phosphoric acid. Phosphates are probably the most pervasive organophosphurus compounds. OPs are very important biochemical and they include DNA and RNA as well as many cofactors that are essential for life. they are the basis of many insecticides, herbicides, and nerve gases. The EPA lists OP as very highly acutely toxic to bees, wildlife and human (EPA, 2003). Recent studies suggest a possible link to adverse effects in the neuro behavioural development of fetuses and children, even at very low levels of exposure. The OP insecticides can act as nerve agents acting on the enzyme acetylcholinesterate. These pesticides are irreversible inactive acetylcholinesterase which is essential to nerve function in insects, humans, and many other animals (Stoytcheva *et al*, 2002).

OP degrades rapidly by hydrolysis on exposure to sunlight, air, and soil, although small amounts can be detected in food and drinking water. Their ability to degrade made them an attractive alternative to the persistent organochlorine pesticides, such as DDT, aldrin and dieldrin. The OPs degrades faster than the OCPs, they have greater acute toxicity, posing risks to people who may be exposed to a larger extent. In 2007, there was a study that was connected to the OP chloropyrifos which have been used before in our cocoa farms, and was found to reduce physical coordination and behavioural problems in children. Again a 2010 study found that OP exposure is associated with an increased risk of Alzheimer's disease (Hayden *et al*., 2010). A study published in Environmental Health Perspective in 2012 found that a prenatal OP exposure had significant impact on birth weight and gestational age (Rauch *et al*., 2012).

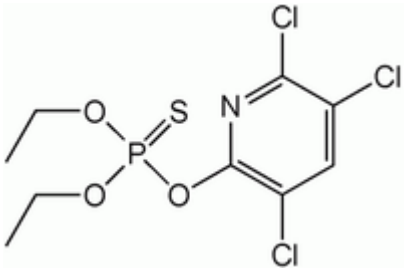


**Fig 2.4: Structure of chloropyrifos**

Subsequently an acute exposure to chloropyrifos results in signs and symptoms typically developing within minutes to hours. Initial signs and symptoms include tearing of the eyes, runny nose, increased saliva and sweat production, nausea, dizziness and headache. An advanced sign include muscle twitching, weakness or tremors, lack of coordination, vomiting, abdominal cramps, diarrhoea, and pupil constriction with blurred or darkened vision (Reigert and Roberts, 1999a; Thompson and Richardson, 2004; Wagner, 1997). Increased heart rate, unconsciousness, loss of control of the urine or bowels, convulsions, respiratory depression and paralysis are observed in the case of severe toxicity (Reigert and Robers, 1999a; Thompson and Richardson, 2004). Also, psychiatric symptoms may be associated with acute exposure, anxiety, depression, memory loss, confusion, stupor, bizarre behavior, and restlessness (Reigert and Roberts, 1999a; Thompson and Richardson, 2004; Wagner, 1997). Different signs and symptoms of chloropyrifos exposure may be experienced by children and their diagnosis of poisoning in general is as well difficult (Reigert and Roberts, 1999a; Wagner, 1997). Usually, reported signs and symptoms in poisonings of children include seizures, pupil constrictions, excess salivation, mental status changes including lethargy and coma. Signs and symptoms observed in adults are less common in children (Reigert and Roberts, 1999a). Exposure to chloropyrifos are limited to acute, high-dose exposures where treatment with therapeutic agents was

used to resolve acute cholinergic toxicity as stated in Organophosphate-Induced Delayed Neuropathy (OPIDN) reports (Richardson, 1995).

More recent studies do not show chronic chloropyrifos exposure causes adverse effects on human health beyond cholinesterase inhibition. An occupational study was conducted to evaluate the potential effect on the central nervous system resulting from chronic, low-level exposure to chloropyrifos to. It was designed with a group of chloropyrifos-manufacturing workers and a control group. The chloropyrifos-exposed workers had significantly higher levels of a chloropyrifos urinary metabolite, 3,5,6-trichloro-2-pyridinol (TCP), and had lower average BuChE levels. There was no substantial difference in neurological symptoms or signs between the two groups, nor was there clinical evidence of adverse effects on the central nervous at baseline or at the 1-year follow-up evaluation (Albers *et al*., 2004).

### 2.3.3 Pyrethroids

Pyrethroids are synthetic chemical insecticides whose chemical structures are adapted from the chemical structures of the pyrethrins, which are modified to increase their stability in sunlight. The use of Pyrethrins and Pyrethroids has increased during the past decade with declining use of OPs, which were found to be more acutely toxic to birds and mammals than pyrethroids. These pyrethroids include Bifenthrin, Cypermethrin, Deltamethrin, Permethrin etc. Cumulative Risk Assessment by EPA in October 2011 on Pyrethrins/pyrethroids shows that exposure from uses of pyrethrin and pyrethroids insecticides do not pose risk to children and adults. $LD_{50}$ of acute toxicity of pyrethroids ranges from low to high, depending on the specific formulation. The low toxicity is attributed to two factors, which are limited absorption of some pyrethroids and rapid absorption and biodegradation in

mammalian liver (Reigart and Roberts, 1999). If pyrethrins are inhaled by humans, it can cause coughing, wheezing, shortness of breath, runny or stuffy nose, chest pain or difficulty in breathing (Sittig, 1991). They have a soil half – life of 12 days and in turn have an extremely low pesticides movement rating because they bind tightly to the soil (Wauchope *et al*., 1992).

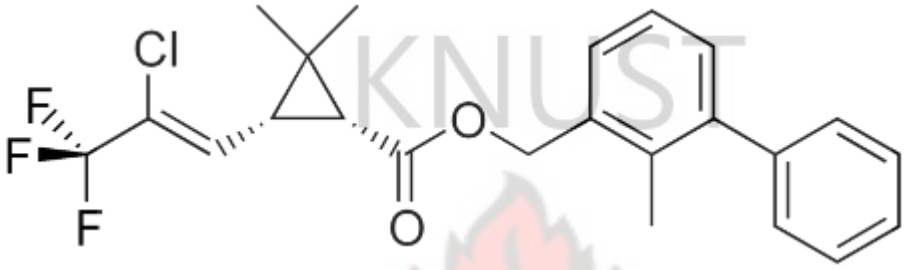


**Fig 2.5: Structure of Bifenthrin**

Like most pyrethroid pesticides, bifenthrin affects the central and peripheral nervous system of insects causing paralysis (Miller and Salgado, 1985). Pyrethroid insecticides effectively paralyze organisms by severely limiting neuro-transmission, thus by acting on the sodium channels to depolarize the pre-synaptic terminals (Salgado *et al*., 1983). The paralysis is often preface by spastic activity of the organism due to the hyper-activity of nerve endings. This spastic activity is caused by sodium channels repeatedly polarizing and depolarizing, mimicking neuro-transmission where none is actually taking place (Huigang *et al*., 2008).

Pyrethroids have also been shown to inhibit ATPase enzyme production (Clark and Matsumura, 1982). This is of primary importance in understanding why aquatic organisms are much more susceptible to pyrethroid insecticides than terrestrial organisms. Moreover, because of its high toxicity to aquatic organisms, bifenthrin products are registered as “restricted use pesticides”, to be sold only to and used by

Certified Pesticide Applicators. According to Mokry and Hoagland, pyrethroids are characterized by greater photostability and greater insecticidal activity than previous pyrethroids (Mokry and Hoagland, 1989). The research that has been conducted on bifenthrin's mode of action on invertebrates or vertebrates indicated that pyrethroid family of pesticides demonstrates very similar effects on invertebrate nervous systems (Miller and Salgado, 1985).



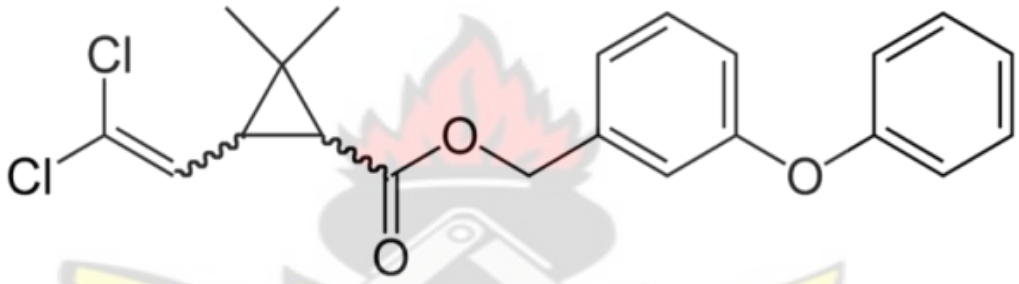


**Fig 2.6: Structure of Permethrin**

Dermal exposure to Permethrin may cause irritation, itching, or paresthesia (a tingly, prickly sensation) at the site of contact and these symptoms rarely last more than 24 hours (Reigart and Roberts, 1999b). Significant exposures may result in pain, redness, or burning sensation (Blondell and Hawkins, 2004). Ingestion of permethrin may cause sore throat, abdominal pain, nausea, and vomiting (Bradberry *et al*., 2005; Blondell and Hawkins, 2004). Moreover, inhalation of permethrin may cause headache, nasal and respiratory, irritation, difficulty breathing, dizziness, nausea or vomiting (Bradberry *et al*., 2005; Blondell and Hawkins, 2004). Inhalation exposures are more likely to result from aerosols, spray droplets, and dust, than from actual vapours because of permethrin's low vapour pressure (Bradberry *et al*., 2005).

The USEPA has determined a reference dose (RfD) and a Population Adjusted Dose (PAD) to be 0.25 mg/kg/day for both acute and chronic dietary exposures to permethrin. These levels are based on a NOAEL (No observed Adverse Effect) of 25 mg/kg/day in rats and an Uncertainty Factor (UF) of 100 (RED for Permethrin, 2007). There is no human data found on chronic effects of permethrin. Experiments with rat and human cancer cell lines indicated that permethrin did not act as an antagonist for estrogens or androgens, nor did it act as an agonist for estrogens or androgens (Garey and Wolff, 1998; Kunimatsu *et al*., 2002). It was concluded that permethrin did not act as a progestin in human cancer cells (Garey and Wolff, 1998; Kunimatsu *et al*., 2002). Moreover, other research on human cancer cell lines implied a potential for permethrin to interfere with estrogenic activity through interface with the progesterone receptor (Kim *et al*., 2005).

A study was conducted involving 196 women who had applied a single, full body and dermal dose of 4% permethrin as a scabies treatment during their second or third trimesters of pregnancy. There was no evidence that exposure to permethrin affected the outcome of the participants' pregnancies (Mytton *et al*., 2007). Another study was conducted involving 113 women using 1% permethrin for treatment of head lice during pregnancy, there was again no indication that exposure to permethrin affected the outcome of their pregnancies (Kennedy *et al*., 2005).

## 2.4 PESTICIDES IN ATMOSPHERE

Most people are aware of and concerned with the health effects of pesticide residues in the water they drink or the food they eat, but many are surprised to learn that pesticides are commonly found in air and rain (Majewski and Capel, 1995). The atmosphere represents the largest most mobile compartment into which a chemical contaminant might be directly released or subsequently moves into, undergo transport, and, in some cases, accumulate (Mackay *et al*., 1997). During and after application, pesticides enter the atmosphere by drift, volatilisation and by wind erosion of particles (soil, vegetation and formulation powders) on which the pesticide is sorbed. The extent to which pesticides enter the air compartment is dependent upon many factors including: the properties of the substance, the amount used, the method of application, the formulation, the weather conditions, the nature of the crops and the soil characteristics (Van Dijk and Guicherit, 1999). Studies have revealed that sometimes more than half of the amount applied is lost into the atmosphere within a few days (Van Den Berg *et al*., 1999: Kubiak, 1999). Many pesticides are detected in the atmosphere throughout the world, but many of these studies have focussed on organochlorine insecticides which are banned in many countries (Majewski and Capel, 1995). There are some studies which have foccussed on current-use pesticides (MC Connel *et al*., 1998: Van Dijk and Guicherit, 1999).

## 2.5 MAXIMUM RESIDUE LIMITS

A maximum residue limit (MRL) is the highest acceptable level of a residue in food. The main purpose of setting a maximum residue limit is to ensure that the best methods of primary production – known as Good Agricultural Practice (GAP) – keep residues in food as low as possible and safe for consumption. MRLs are one tool used to monitor that (GAP) is followed for food production. Violating a MRL does not

necessarily make the food unsafe but is does alert the Ministry of Agricultural and Forestry (MAF) Food Safety or the possibility that the food could have been better produced.

### 2.5.1 Setting a maximum residue limit with holding period

A withholding period is the time between when a residue is applied to a food and when the food can be harvested. For example, if an insecticide is only effective against a pest for 14 days, the insecticides would need to be applied at a similar rate every two weeks until harvest in order to continue to protect the crop. In this case the withholding period is likely to be 14 days and it means fruit should not be harvested until 14 days after the last application of the insecticide. Based on this timescale, if a residue in the crop, for example has less than 0.2mg/kg after 14 days, then a MRL of 0.2 mg/kg are likely to be set. Any violation of this MRL would be from inappropriate use, or applying the insecticide at a rate above that specified on the label.

### 2.5.2 Calculating the acceptable daily intake

The Acceptable Daily Intake (ADI) is an internationally accepted estimate of the quantity of a particular compound in a food which can be consumed by a person on a daily basis over an entire lifetime, without causing any harm to the person. The quantity is derived through tests conducted using different doses of a compound given to laboratory animals. Researchers can observe the first, or slightest, signs of a toxic effect. They then use the dose just below this level – the 'no observed adverse effect level' (NOAEL) to estimate as the ADI.

The difference between animals and humans is taken into account by further dividing the NOAEL by a safety factor of at least 100. The final figure is the estimated ADI for humans. This is expressed as how many milligrams (mg) of the compound can safely be consumed per kilogram (kg) of body weight (bw) every day (mg/kg bw/day) for an entire lifetime. For instance, the maximum residue limit (MRL) for the fungicide iprodione is 5 mg/kg when used on kiwifruit. The ADI is 0.06 mg/kg bw/day. An adult weighing 70 kilograms would need to eat almost one kilogram of kiwifruit per day (all containing iprodione at the MRL – itself very unlikely) every day of their lives; and even then they would be consuming a dose that would be below the ADI and therefore considered to be safe.



### 2.5.3 Health risk if residues in food are greater than the MRL

The MRL is not a health-based exposure limit, and exposure to residues in excess of an MRL does not necessarily imply a risk to health. This is because the use of a pesticide would not be allowed if the proposed MRL resulted in long-term and short-term exposure of pesticide residues in the human diet above safety limits (the Acceptable Daily Intake (ADI) and Acute Reference Dose (ARfD) which are calculated before any pesticide approval is given. The MRL is not linked to the ADI or ARfD, and could result in dietary intakes considerably below these safety levels.

In 1978 anonymous data submitted to the FAO-WHO indicated that the levels of lindane in cocoa beans from Ghana were between 0.0 and 0.3 mg /kg and in between 0.051 - 0.10 mg $kg^{-1}$ in cocoa butter. The residue in cocoa mass was 0.038 mg $kg^{-1}$. The FAO-WHO decided that the maximum residue limit should be 1.0 mg $kg^{-1}$. Codex MRLs are generally set for raw products, and a specific policy has been adopted to avoid, in general, establishing MRLs for processed products. It was noted

that in most of the reported cases of monitoring data provided by the US Food and Drug Administration, a number of processed products had been rejected. In the light of the likely increasing importance of Codex in the enforcement of food standards under GATT, Codex is recommended to review its policy in consideration of potential technical barriers to trade (FAO/WHO, 1991).

## 2.6 ANALYTICAL METHODS

The monitoring of pesticide residues in food is presently a priority area in pesticide research in order to get an extensive evaluation of food quality and to avoid possible risks to human health. In the last 20 years, a large number of multiresidue extraction methods (MRMs) have been developed (Van der Hoff and van Zoonen, 1999), and the most frequently used methods employed is the solvent extraction with acetone (Luke *et al*., 1975), ethyl acetate (Specht *et al*., 1995; Andersson and Palsheden, 1991, 1998; Aguera *et al*., 1993 and Fernandez-Alba *et al*., 1994) which is followed by gas chromatography (GC) with selective detection depending on the type of pesticides. Nonetheless, the appreciable decrease in MRLs in many pesticide/commodity combinations imposed by the European Union (Directive 98/82/CE) in recent years has made necessary and improvement in the limits of detection reached by the current MRMs.

Determination and monitoring of OCPs in different environmental matrices are important for the environment, especially for human health. Accordingly, residue analysis of OCPs in water and soils by developing analytical procedure continue to be an active area of research in recent years (Santos and Galceran, 2004). There are different types of extractions of OCPs, solid phase extractions (SPE) are the oldest

procedures for the extraction of OCPs from aqueous matrices, whilst liquid-liquid extraction (LLE) is probably the most widely used method for the extraction of OCPs from aqueous samples (Barcelo, 1993; Fatoki and Awofolu, 2003; Tahboub *et al*., 2005). LLE needs relatively large volumes of organic solvents and samples and it is time-consuming as well as a SPE method, and hazardous to health and environment. Moreover, because of some complications in the LLE method, the SPE have been used as an alternative method to LLE for the extraction of OCPs from water samples because it uses less solvent and is less time-consuming than LLE. Both LLE and SPE methods has difficulties in automation. However, using large amount of organic solvents can cause environmental pollution and health hazards for laboratory personnel and extra operational costs for waste treatment (Sarafraz-Yazdi and Amiri, 2010).

Depending on the class of pesticides to be quantified, the GC is combined with different kinds of detection methods. Electron Capture Detection (ECD) has usually been employed for OCP analyses. The quantification limits obtained by coupling GC to ECD have been reported to be mostly around 0.1 – 20 ng/g, depending on various factors. The use of ECD usually requires particular attention to be paid to the extract clean-up process (Stefanelli *et al*, 2009). In an analytical method, various extraction and clean-up steps are mixed and matched to achieve maximum analyte recovery with minimum matrix interference at the final measurement step (Bennett *et al*, 1997). Matrix constituents can be co-extracted and later co-eluted with analysed components and can consequently interfere with analyte identification and quantification. Co-extracted compounds, especially lipids, tend to adsorb in GC systems such as injection port and column which can result in poor chromatographic performance

(Hong *et al*., 2004). Moreover, a thorough clean-up minimizes such matrix issues, and so improves sensitivity, permits more consistent and repeatable results, and extends the capillary column lifetime (Navas Diaz *et al*., 1997; Rimkus *et al*., 1996). Various approaches have been attempted to eliminate co-extracted interference from extracts, including freezing, centrifugation, liquid-liquid partitioning, gel permeation chromatography (GPC), Solid-phase extraction (SPE) and solid-phase micro-extraction (SPME).



In most of the published analytical methods, the use of anhydrous sodium sulphate at one or more of the steps is recommended in order to remove water traces from the extraction solvent system. It is mostly during clean-up steps such as solvent partitioning or purification columns (as one of the layers of the column) that the sodium sulphate is used. Methods developed for the determination of pesticides from the same class mostly reach the guideline rates of recovery for all the studied pesticides. However, in multi-residue methods it is very difficult to obtain satisfactory results for all of the analysed compounds because of the diverse physical and chemical properties of the different classes of agricultural chemicals (Bennett *et al*, 1997; Salas *et al*, 2003). As a result the recommended recovery rates are not always reached for all the tested pesticides.

### 2.6.1 Monitoring pesticide residues in food

The Centre for Food Safety (CFS) operates a food surveillance programme and regularly takes food samples, including fruits, vegetables and cereals at import, wholesale and retail levels for testing of pesticide residues. Presently, CFS follows

the testing methods and standards recommended by the Codex Alimentarius Commission (Codex). Codex, established by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) of the United Nations in 1960s, has been the single most important international reference point for consumers, food producers, processors, national food control agencies and the international food trade in developing food associated standards. The Codex Alimentarius, or the food code, is a collection of these standards, codes of practice, guidelines and other recommendations. When there is no relevant Codex standard, CFS will conduct its own risk assessment studies to determine whether the level of pesticide residues detected in food is harmful to human health.



The European Union Legislation on Maximum Residue Levels (MRLs) on Pesticides (Regulation149/2008/EEC) came into effect in September 2008. The Regulation set maximum levels on the amount of pesticides permitted on imported foods including cocoa beans. Consequently, all cocoa beans imported into the EU from September 2008 must conform to the new Regulation. In the U.S.A, the Environmental Protection Agency (EPA) established the Food Quality Protection Act of 1996 which regulates the amount of pesticide residues permitted in food for consumption. The EPA also requires that all approved pesticides are clearly labelled with instructions for proper use, handling, storage and disposal. In Japan, the Ministry of Health, Labour and Welfare (MHLW) established a new legislation that came into effect from May 2006, setting new MRLs for food products (The Japan Food Chemical Research Foundation, 2006).

# CHAPTER THREE

## 3. MATERIALS AND METHODS

### 3.1REAGENTS AND MATERIALS

**TABLE 3.1: REAGENTS AND MATERIALS USED**

| REAGENT | GRADE | SOURCE |
|---|---|---|
| Acetonitrile | Pesticides | Sigma Aldrich, USA. |
| Toluene | Pesticides | SMM Chemicals Pty Ltd |
| Acetone | Pesticides | Labort Fine PVT Ltd, India. |
| Hexane | Pesticides | BDH Laboratory Supplies, England. |
| Sodium Sulphate (anhydrous) | AnalaR | Labort Fine PVT Ltd, India. |
| Sodium chloride | AnalaR | Avondale Laboratory, England. |
| Sodium Hydroxide | AnalaR | BDH Laboratory Supplies, England. |
| Dipotasium hydrogen phosphate | AnalaR | PX PARK Scientific Ltd, UK. |
| Potassium dihydrogen phosphate | AnalaR | BDH Laboratory Supplies, England. |
| Ethyl Acetate | AnalaR | SMM chemicals Pty Ltd, |
| Bond elute C18 SPE cartridge, 1g/6ml | - | Supelco Analytical, USA. |
| ENVI-Car/LC-$NH_2$,500mg/500 mg, 6ml | | Supelco Analytical, USA. |
| Filter Paper, No. 4 | | Whatman International Ltd, England. |

## 3.2 SAMPLING

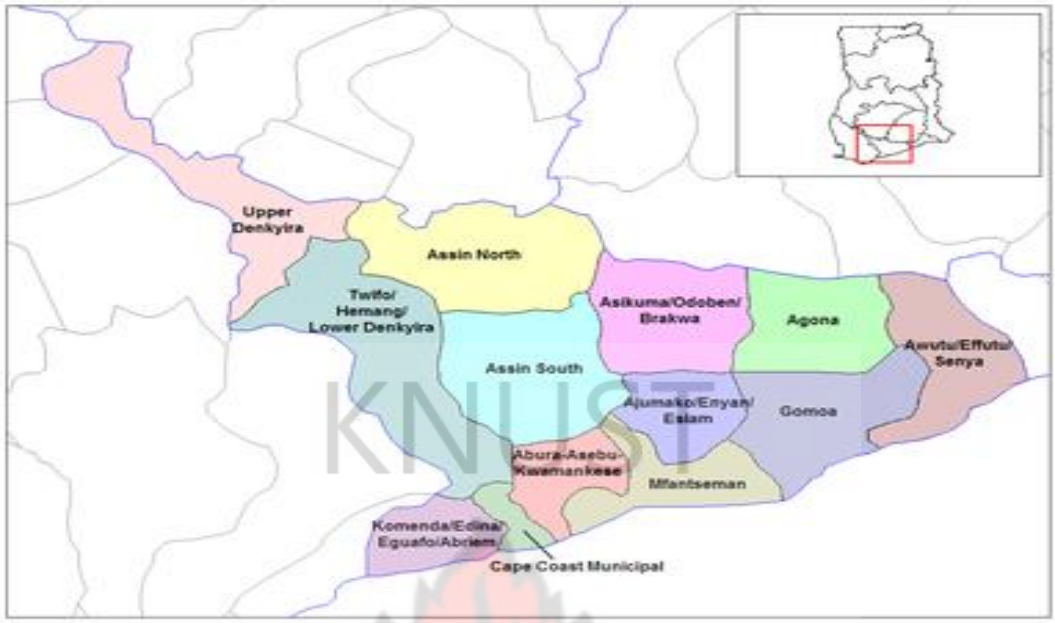


**Figure 3.1: A map of Central Region showing sampling areas**

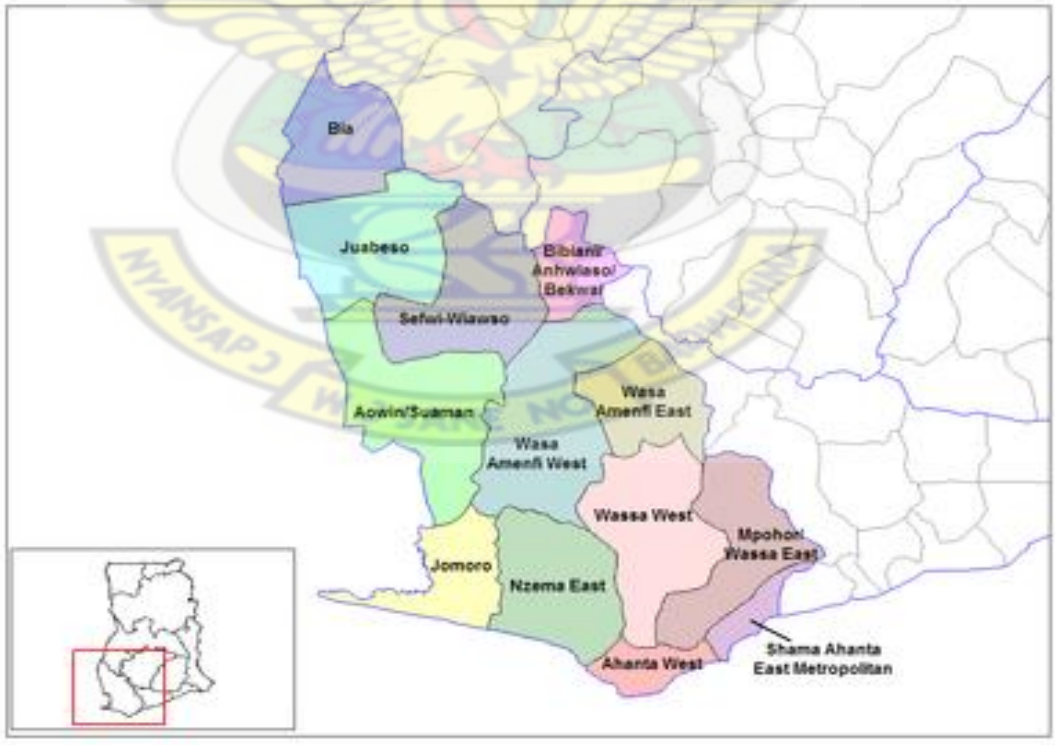


**Figure 3.2: A map of Western Region showing the sampling areas**

Dry cocoa beans were sampled from the main warehouses at the sea ports namely; Apowah (Takoradi) and Tarzan (Tema), where cocoa beans from different districts of the Western and Central Regions of Ghana are received. The consignment was divided into smaller lots of about 30 bags and the split wire was applied as widely as possible to detect foreign matter. The split wire is made of copper metal of dimensions 50 mm long x 30 mm wide. Total of 20 samples were bagged from Central and Western Region of Ghana.



A sampling horn made of aluminium metal was used to draw samples from all sides of each cocoa bag and bulked into a container. The dimension of the sampling horn was 100 mm long x 15 mm internal diameter. The bulked sample was thoroughly mixed and quartered. Two quarters of the opposite sides were rejected. The process was repeated until a final sample of 300 beans was counted. One of the sample bags was sent to the laboratory for analysis (Quality Control Company Division, 1994).

## 3.3 INSTUMENTATION AND APPARATUS

**TABLE 3.2: DETAILS OF EQUIPMENT USED**

| Equipment | Type |
|---|---|
| Gas Chromatograph | Shimadzu GC – 2010 with AOC 20i Autoinjector and AOC 20S Autosampler and Electron Capture Detector |
| Analytical Column | 30m x 0.25mm internal diameter fused silica capillary column coated with VF-5ms (0.25µm film). |
| Centrifuge | Sanyo (MSE) Harrier 18 / 80 |
| Vacuum manifold | 209 x 200 – Aldrich TM round bottom flask |
| Macerator | IKA Ultra Turrrax Homogenizer |
| General laboratory glassware | Round bottomed flasks, volumetric flasks, centrifuge tubes, separating funnels, funnels, measuring cylinders |
| Glass vials | 2 ml |
| Preparation equipment | Blender |
| Rotary film evaporator | Buchi Rotary evaporator (India) |
| Recirculating chiller | Buchi, B-740 |

## 3.4 ANALYSIS

### 3.4.1 Experimental Procedures

Before analysing the samples (before experiment) all glass wares were acid washed and cleansed with distilled water before they were dried in the oven at 200$^{o}$C for about four hours.

### 3.4.2 Preparation of standard solutions

All the pesticides standard stock solution were prepared in ethyl acetate with the aid of an ultrasonic bath, by dissolving a weight of the pesticides which when corrected

for purity will be equivalent to 1000 µg/ml (e.g. 10 mg in 10 ml). This labelled as a Parent Standard Reference for each Pesticides standard e.g Aldrin, ALD 1. For instance the purity of Aldrin is 97%, so 10.30 mg of Aldrin was measured in 10 ml volumetric flask.

These Parent Standard References were diluted for use as fortification standards in the procedural recovery process, and used in the calibration standards in instrument calibration. A summary of the dilutions that were carried out for the fortification and calibration are all presented in Table 3.3 and Table 3.4.



### 3.4.3 Fortification standard preparation

A 1ml of an aliquot of each 1000µg/ml pesticide standard prepared was measured into a 50 ml volumetric flask and adjusted to volume with ethyl acetate giving the resultant mixed standard concentration of 20µg/ml MIX 1. Further dilution was carried out by measuring 2.5ml of MIX 1 into a 25 ml volumetric flask to give a resultant concentration of 2.0µg/ml MIX 2.Another dilution was carried out measuring 2.5 ml aliquot of MIX 2 into 25ml volumetric flasks with a resultant concentration of 0.2 µg/ml MIX 3.

**TABLE 3.3: PREPARATION OF FORTIFICATION STANDARDS SOLUTIONS AND CALIBRATION STANDARDS**

| References of standard solution used | Concentration (µg/ml) | Volume taken (ml) | Final Volume (ml) | Equivalent concentration (µg/ml) | References of standard solution produced |
|---|---|---|---|---|---|
| Each standard | 1000 | 1.0 | 50 | 20 | MIX 1 |
| MIX 1 | 20 | 2.5 | 25 | 2.0 | MIX 2 |
| MIX 2 | 2.0 | 2.5 | 25 | 0.2 | MIX 3 |
| MIX 1 | 20 | 5 | 50 | 2.0 | MIX 4 |



### 3.4.4 Recovery

A 1 ml aliquot of MIX 4 was added to 10 g aliquot of a prepared matrix (1ml = 10µg of each analyte). This is equivalent to a fortification level of 2mg/kg. Extraction and clean – up procedure as described in the methodology were carried out before injection of the fortified samples into the GC. Same chromatographic conditions were used. This was repeated for fortification levels of 1.5 mg/kg and 1.0 mg/kg.

### 3.4.5 Instrument calibration standard preparation

MIX 1 was diluted by measuring 5.0 ml of its aliquot into a 50 ml volumetric flask. It was adjusted to the mark with ethyl acetate giving a resultant concentration of 2.0µg/ml MIX 4.

MIX 4 was diluted by measuring 7.5 ml of its aliquot into a 10 ml volumetric flask. It was adjusted to the mark with ethyl acetate giving a resultant concentration of 1.5µg/ml MIX 5

MIX 4 was further diluted with 5 ml of its aliquot into a 10 ml volumetric flask and adjusted to the mark with ethyl acetate giving a resultant concentration of 1.0µg/ml MIX 6

MIX 4 again was diluted with 2.5 ml of its aliquot into 10 ml volumetric flask and adjusted to the mark with ethyl acetate giving a resultant concentration of 0.5µg/ml MIX 7

MIX 6 was diluted by measuring 2.0 ml of aliquot into 10 ml volumetric flask and adjusted to the mark with ethyl acetate giving a resultant concentration of 0.2µg/ml MIX 8.

**TABLE 3.4:– PREPARATION OF STANDARDCALIBRATION SOLUTIONS**

| References of standard solution used | Concentration (µg/ml) | Volume taken (ml) | Final Volume (ml) | Equivalent concentration (µg/ml) | References of standard solution produced |
|---|---|---|---|---|---|
| MIX 1 | 20 | 5 | 50 | 2.0 | MIX 4 |
| MIX 4 | 2 | 7.5 | 10 | 1.5 | MIX 5 |
| MIX 4 | 2 | 5 | 10 | 1.0 | MIX 6 |
| MIX 4 | 2 | 2.5 | 10 | 0.5 | MIX 7 |
| MIX 6 | 1.0 | 2.0 | 10 | 0.2 | MIX 8 |

### 3.4.6 Preparation of 0.5 mol/l phosphate buffer (pH 7.0)

An amount of 52.7 g of dipotassium hydrogen phosphate and 30.2 g of potassium dihydrogen phosphate were weighed in a 250 ml beaker. Distilled water was used for dissolution and the pH adjusted to 7 with 1 mol/l NaOH and 1 mol/lHCl solution and transferred into a 1.0 litre volumetric flask.

### 3.4.7 Sample preparation



The sample was milled using a Hammer Mill through inn sieve. 10 g was weighed into a 250 ml Nalgene jar. Distilled water (20 ml) was added and left for 15 min. An amount (40 ml) of acetonitrile was added and homogenised for 2 min. It was then centrifuged at 3000 rpm for 3min and filtered through flutted (No. 4) filter paper into a 100 ml volumetric flask. The residue was placed back into the Nalgene jar and 20 ml acetonitrile added, homogenised for 2 min and the dispersing element rinsed with 5 ml acetonitrile into the jar. The suspension was centrifuged at 3000 rpm for 3 min and was filtered into the 100 ml volumetric flask. The residue was rinsed with 15 ml acetonitrile, and the filtrate made up to 100 ml with acetonitrile in the 100 ml volumetric flask. An amount of 20 ml of the filtrate was pipetted into a 250 ml separating funnel. A 10 g of NaCl and 20 ml of 0.5 mol/L phosphate buffer (pH 7.0) was added, shaken for 20 min on an end-to-end shaker left to stand for 10min for longer separation and the aqueous layer was carefully discarded and the acetonitrile layer taken for clean - up.

### 3.4.8 Clean – up step 1 using bond elute C – 18 cartridges

Bond Elute C-18, 1 g/6ml cartridge was conditioned with 10 ml acetonitrile. A receiving flask was placed under the cartridge to collect elute. Approximately 20 ml of the sample extract was loaded into the cartridge and eluted with 2 ml acetonitrile. A 5 g portion of $Na_2SO_4$ was placed on a filter paper in a funnel and the extract dried over it. The container was rinsed with acetonitrile and passed through the $Na_2SO_4$. The sample solution was transferred into 50 ml round bottom flask and evaporated at 40 $^oC$ to near dryness on a Buchi rotary evaporator. Residue was dissolved in 2 ml of 1:3 toluene: acetonitrile mixtures.

### 3.4.9 Clean – up step 2 using ENVI – Carb/LC – $NH_2$

ENVI – Carb/LC – $NH_2$, (500 mg/500 mg)/6 ml cartridge was conditioned with 10 ml of 1:3 toluene: acetonitrile. A 2 ml aliquot of the extract from the previous clean – up step was loaded into the cartridge and eluted. The cartridge was eluted with 20 ml of 1:3 (toluene: acetonitrile). The sample solution was transferred into a 50 ml round bottom flask and evaporated at 40 $^oC$ to approximately 1 ml using rotary evaporator. An amount (10 ml) of acetone was added to the flask and was evaporated to near dryness. It was then re – dissolved in 2 ml ethyl acetate, prior to analysis by GC – ECD. Extracts was then stored in a refrigerator until analysis.

**TABLE 3.5: SUMMARIES OF CHROMATOGRAPHIC CONDITIONS FOR PESTICIDES**

| **APPARATUS:** | |
|---|---|
| **Instrument** | **Description** |
| Gas Chromatograph | Shimadzu GC – 2010 with AOC 20i Autoinjector and AOC 20S Autosampler and Electron Capture Detector |
| Analytical column | 30m x 0.25mm internal diameter fused silica capillary column coated with VF-5ms (0.25µm film). |
| **Temperatures:** | |
| **Item** | **Conditions** |
| Injector | Splitless mode, temperature 225$^{o}$C |
| Oven | 60$^{o}$C / 2 min $\frac{25oC/min}{}$ 180$^{o}$C / 1min $\frac{5oC/min}{}$ 300$^{o}$C |
| Detector – ECD | 300$^{o}$C |
| **Gas;** | |
| **Gas** | **Flow rate** |
| Nitrogen (carrier) | 1ml/min constant flow |

### 3.5 DATA ANALYSIS

20 ml of pipetted sample was concentrated to 2ml. therefore the concentration factor becomes $\frac{20}{2} = 10$

The concentration factor is 10 which is denoted as F

20 ml also was pipetted from 100 ml sample solution before the concentration.

$$\frac{100}{20} = 5$$



$$\left\{\left[\frac{\mathbf{C}}{\mathbf{F}}\right] \times \mathbf{5}\right\}/\mathbf{M}$$

**Eq 1: used to calculate the concentration of pesticides in mg/kg**

Where C = concentration in µg/ml

F = Concentration Factor (10)

M = mass in grams

### 3.6 RECOVERY

The percentage recovery was calculated as:

$$\% \text{ Recovery} = \frac{\text{Amount of analyte recovered}}{\text{Amount of analyte spiked}} \times 100$$

**Equation 2: used to calculate the recoveries of the samples**

The amount of analyte recovered was calculated by subtracting the non spiked sample from the spiked sample.

# CHAPTER FOUR

## 4. RESULTS AND DISCUSSION

Pesticide residues were determined in cocoa beans sampled from the Western and Central Regions of Ghana using GC – ECD. Accuracy of the method was achieved using recovery method at fortifications levels of 2.0 µg/ml, 1.5µg/ml and 1.0µg/ml. Recoveries ranged between 65 % and 123 % (Table 4.1 and Table 4.2). Recoveries vary depending on various parameters such as, the sample processing procedure and analyte concentration. In multiresidue methods it is not usual to obtain satisfactory results for all of the compounds because of the diverse physical and chemical properties of the different classes of agricultural chemicals (Bennett *et al*, 1997; Salas *et al*, 2003).

Twenty one pesticides residues were determined in 20 samples of cocoa beans from two different regions of Ghana namely Central and Western Regions. The pesticides determined were chloropyrifos, endosulfan I, endosulfan II, profenefos, fenvalerate, bifenthrin, permethrin I, permethrin II, cypermethrin I, cypermethrin II, cypermethrin technical, beta HCH, delta HCH, gamma HCH (lindane), alpha HCH, aldrin, heptachlor – exo – epoxide, dieldrin, 4, 4 – DDD, 4,4 – DDT, and heptachlor. Individual standard solutions were prepared from stock standard solution and their individual retention times obtained after injection. The individual retention times which were used to identify the detected pesticides in the cocoa beans are presented in Table 4.3.

**TABLE 4.1: PERCENTAGE RECOVERIES OF PESTICIDES FOR CAPE COAST SAMPLE FROM THE CENTRAL REGION**

| Names of pesticides | Spiked 1 (%) | Spiked 2 (%) | Spiked 3 (%) | mean (%) |
|---|---|---|---|---|
| Chloropyrifos | 101.03 | 97.43 | 99.68 | 102 |
| Endosulfan I | 104.80 | 100.90 | 109.50 | 105 |
| Endosulfan II | | 110.46 | 111.81 | 111 |
| Profenefos | 107.19 | 106.04 | 100.34 | 105 |
| Fenvalerate | 131.07 | 127.52 | 112.37 | 124 |
| Bifenthrin | 103.83 | 85.33 | 105.38 | 98 |
| Permethrin I | | 82.46 | 86.41 | 84 |
| Permethrin II | 96.88 | 92.98 | | 95 |
| Cypermethrin I | 109.93 | 108.33 | | 109 |
| Cypermethrin II | 101.60 | 109.20 | 95.85 | 102 |
| Cypermethrin T | 92.22 | | 90.57 | 91 |
| Beta HCH | 64.55 | 67.47 | 70.7 | 68 |
| Delta HCH | 63.47 | 66.43 | 67.20 | 66 |
| Gamma HCH | 77.20 | 70.20 | 71.47 | 73 |
| Alpha HCH | 59.40 | 67.48 | 69.20 | 65 |
| Aldrin | 85.40 | 67.30 | 84.53 | 79 |
| Dieldrin | 76 | | 73.38 | 75 |
| Heptachlor | 56.88 | 67.52 | 70.70 | 65 |
| Hept-exo-epoxide | | 72.50 | 60.20 | 66 |
| 4,4-DDD | 63.07 | 82.46 | | 73 |
| 4,4-DDT | 60.57 | 65 | 69.40 | 65 |

**TABLE 4.2: PERCENTAGE RECOVERIES OF PESTICIDES IN MENSAKROM SAMPLE FROM WESTERN REGION**

| Names of pesticides | Spike I (%) | Spike 2 (%) | Spike 3 (%) | mean (%) |
|---|---|---|---|---|
| Chloropyrifos | 123.00 | 123.45 | 123.74 | 123 |
| Endosulfan I | 58.61 | 101.12 | 74.05 | 78 |
| Endosulfan II | 110.05 | 110.15 | 103.35 | 108 |
| Profenefos | | 107.77 | 94.82 | 101 |
| Fenvalerate | | 129.25 | 73.75 | 102 |
| Bifenthrin | 97.62 | 108.07 | 88.32 | 98 |
| Permethrin I | 57.73 | 65.58 | 88.73 | 71 |
| Permethrin II | 77.54 | 41.23 | 97.59 | 72 |
| Cypermethrin I | | 110.32 | 105.82 | 108 |
| Cypermethrin II | 64.86 | 50.41 | 76.91 | 65 |
| Cypermethrin T | 35.62 | 97.57 | 79.87 | 71 |
| Beta HCH | 67.52 | 69.71 | 64.64 | 67 |
| Delta HCH | 87.33 | 97 | 107.70 | 97 |
| Gamma HCH | 65.72 | 67.39 | 63.05 | 65 |
| Alpha HCH | 77.75 | 82.25 | | 80 |
| Aldrin | 97.85 | 99.7 | 109.6 | 102 |
| Dieldrin | 52.91 | 72.89 | 77.99 | 68 |
| Heptachlor | 89.22 | 85.22 | 110.52 | 95 |
| Hept-exo-epoxide | 67.22 | 67.90 | 75.86 | 70 |
| 4,4-DDD | 65.77 | 69.78 | 69.83 | 68 |
| 4,4-DDT | 83.50 | 78.85 | 83.96 | 82 |

**TABLE 4.3: SUMMARY OF RETENTION TIMES FOR PESTICIDES**

| STANDARDS | RETENTION TIME (min) |
|---|---|
| Beta HCH | 13.736 |
| Gamma HCH | 13.867 |
| Alpha HCH | 13.953 |
| Delta HCH | 14.596 |
| Chloropyrifos | 16.924 |
| Hepta – exo – epoxide | 18.574 |
| Endosulfan I | 19.621 |
| Profenefos | 20.107 |
| Dieldrin | 20.791 |
| Endosulfan II | 21.535 |
| 4, 4 – DDD | 21.990 |
| DDT | 23.348 |
| Fenvalerate | 24.002 |
| Bifenthrin | 24.995 |
| Heptachlor | 25.292 |
| Permethrin I | 28.524 |
| Permethrin II | 28.772 |
| Cypermethrin I | 30.566 |
| Cypermethrin II | 30.200 |
| Cypermethrin T | 30.690 |
| Aldrin | 17.563 |

For the multiresidue analysis, mixed standard solutions in ethyl acetate were prepared from each pesticide standard by serial dilutions of the stock standard. The chromatogram of standard mix is presented in appendix 1 and 2, and that of a sample is presented in appendix 3.

The HCH isomers (alpha, beta, gamma and delta) were all detected in all the cocoa bean samples from the Central region (Table 4.4). Alpha HCH recorded the highest value of 0.68 mg/kg for cocoa samples from Assin Breku (Table 4.4) and lowest of 0.01 mg/kg for samples from both Atieku and Twifo Hemang (Table 4.4). According to the European Union (EU), the Maximum Residue Level (MRL) value of 0.02 mg/kg was set for the HCH except for the gamma HCH which is 1.0 mg/kg. 100% of the samples had alpha HCH, 77.8% beta HCH and 44.4% (Table 4.6) delta HCH exceeded the EU MRL for samples from the Central Region. Gamma HCH also known as Lindane marketed in Ghana as Gamalin 20 was widely used on cocoa farms, vegetable farms, and for the control of stem borers in maize. Use of Lindane on cocoa was discontinued in Ghana in 2007. Therefore it is not surprising for Lindane to be detected in cocoa beans. It recorded a highest concentration of 0.30 mg/kg for samples from Assin Breku (Table 4.4) and the lowest concentration recorded was 0.14 mg/kg for samples from Twifo Hemang (Table 4.4). The EU and Japan MRL for HCH are 1.0 mg/ kg and 0.1 mg/kg respectively. Therefore none of the samples exceeded the EU value but 77.8% (Table 4.6) of the samples exceeded the MRL for Japan.

Similar work was conducted in Nigeria to determine the levels of total HCH, total DDT and Aldrin in fruits, vegetables and tubers, and all the average levels of these

pesticides were generally low and none were above the Food and Agricultural Organization of the United Nations (FAOs) maximum residue limits. The levels were generally low in all the samples and below the MRL (Adeyeye and Osibanjo, 1999). The high water and low lipid contents of the samples and the lipophilic nature of the pesticides may have contributed to their low residue contents.

The Western Region recorded 0.95 mg/kg for alpha HCH for samples from Kwame Adukrom (Table 4.5), and the lowest concentration of 0.01 mg/kg for delta HCH for samples from Debiso and Anhwiaso (Table 4.5), Nkwanta Bibiani and Mensakrom (Table 4.5). Percentages of 100, 72.72 and 45.45 (Table 4.7) of the samples from the Central Region exceeded the EU MRL for alpha, beta and delta HCHs respectively.

A concentration of 0.29 mg/kg was recorded for gamma HCH for samples from Kwame Adukrom (Table 4.5), and 0.15 mg/kg for samples from Dedemude (Table 4.5). Therefore all samples from Western Region recorded values below the EU MRL for gamma HCH, whereas 81. 81% (Table 4.7) of the samples exceeded the Japanese MRL. Comparing the HCHs recorded for the two regions, samples from the Central Region had most samples with values below the MRL whilst Western Region recorded the highest. The higher level of HCHs may be due to the fact that it was until 2007 HCHs has been used on cocoa in Ghana (Gerken *et al*., 2001). A much higher concentration of HCHs was found in chickens in China as well (Tao *et al*., 2009).

Cocoa beans collected from selected cocoa growing districts in the middle belt of Ghana and the two sea ports at Tema and Takoradi also showed detectable amount of lindane residues; the average level was about 10% of the maximum residue level of 0.1 μg/g permitted by Codex Alimentarius Commission (Botchway, 2000). Nonetheless, many other studies conducted so far have revealed the presence of detectable levels of pesticides especially organochlorines in fruits, vegetables, fish and fish products (Botchway, 2000; Yeboah *et al*., 2004 and Essumang *et al*., 2009).



A 2001 study of pesticide residue levels in farmers at Akomadan in the Ashanti Region discovered other Organochlorines such as hexachlorobenzene and DDE in most blood and breast milk samples taken from the farmers (Asante and Ntow, 2009).

DDT and its metabolites (DDE and DDD) are all organochlorine pesticides. A concentration of 0.31 mg/kg of DDT was detected in samples taken from Agona Swedru (Table 4.4) whereas the lowest concentration of 0.02 mg/kg DDD was recorded for samples from both Twifo Hemang and Cape Coast (Table 4.4).

None of the samples had values exceeding the EU MRL for both DDT and DDD. This could be attributed to the fact that residues have decreased in food since these chemicals were banned in most countries, although trace levels are still detected in many foodstuffs (Yague *et al*., 2001). All the samples had concentrations that exceeded the MRL for Japan and none exceeded the EU MRL considering the DDT. The presence of DDT and DDD in the cocoa beans could be attributed to the fact that some traces of DDT exist. Since it is persistent, it could be in the soil for a long time and be absorbed by cocoa. Studies conducted indicated that the pattern of OCPs in

human fluid showed that DDTs were consistently prevalent in milk and blood, indicating that DDT is a contaminant still in the Ghanaian environment even though it was banned (Ntow, 2001, 2005 and Amoah *et al*., 2006).

**TABLE 4.4: CONCENTRATIONS OF PESTICIDES IN COCOA BEANS SAMPLES FROM DIFFERENT TOWNS OF THE CENTRAL REGION.**

| | TOWNS (mg/kg) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **PESTICIDE** | **AB (mg/kg)** | **AF (mg/kg)** | **AM (mg/kg)** | **AS (mg/kg)** | **BAS (mg/kg)** | **TP (mg/kg)** | **AT (mg/kg)** | **TH (mg/kg)** | **CC (mg/kg)** | **JAPAN MRL** | **EU MRL** |
| Chloropyrifos | 11.87 | 5.77 | 7.80 | 8.83 | 6.27 | 4.39 | 9.00 | 7.51 | 8.30 | 0.05 | 0.1 |
| Endosulfan I | 0.06 | ND | ND | 0.04 | 0.16 | 0.01 | 0.01 | ND | 0.02 | 0.1 | 0.1 |
| Profenefos | 0.12 | 0.08 | 0.07 | 0.04 | 0.09 | 0.06 | 0.08 | 0.08 | 0.07 | | 0.1 |
| Endosulfan II | 0.09 | 0.07 | 0.08 | 0.03 | 0.06 | 0.07 | 0.08 | 0.08 | 0.07 | 0.1 | 0.1 |
| Fenvalerate | 0.35 | 0.20 | 0.24 | 0.34 | 0.13 | 0.15 | 0.27 | 0.29 | 0.26 | | 0.5 |
| Bifenthrin | 0.10 | 0.02 | 0.02 | 0.06 | 0.06 | 0.02 | 0.04 | 0.05 | 0.06 | 0.1 | 0.1 |
| Permethrin I | 0.41 | 0.04 | 0.24 | 0.72 | 0.32 | 0.13 | 0.48 | 0.22 | 0.35 | | 0.1 |
| Permethrin II | 0.12 | ND | 0.26 | 0.16 | 0.95 | 1.02 | 0.24 | 1.58 | 0.16 | | 0.1 |
| Cypermethrin I | 0.04 | 0.03 | 0.03 | 0.04 | 0.06 | 0.03 | 0.33 | 0.08 | 0.16 | 0.03 | 0.1 |
| Cypermethrin II | 0.12 | 0.19 | 0.15 | 0.19 | 0.22 | 0.14 | 1.82 | 0.50 | 0.91 | 0.03 | 0.1 |
| Cypermethrin T | 0.50 | 0.01 | 0.22 | 0.14 | 0.06 | ND | 2.21 | 0.38 | 0.88 | 0.03 | 0.1 |
| Beta HCH | ND | 0.03 | ND | 0.03 | 0.04 | 0.04 | 0.14 | 0.04 | 0.07 | | 0.02 |
| Delta HCH | 0.06 | 0.02 | 0.04 | 0.04 | 0.02 | 0.02 | 0.01 | 0.01 | 0.07 | | 0.02 |
| Gamma HCH | 0.30 | 0.15 | 0.23 | 0.19 | ND | 0.17 | ND | 0.14 | 0.15 | 0.1 | 1.0 |
| Alpha HCH | 0.68 | 0.39 | 0.38 | 0.28 | 0.22 | 0.49 | 0.41 | 0.40 | 0.34 | | 0.02 |
| Aldrin | ND | ND | ND | ND | ND | ND | ND | ND | ND | 0.1 | 0.05 |
| Hepta-exo-epoxide | 0.02 | 0.01 | 0.05 | 0.01 | 0.02 | 0.01 | 0.01 | 0.01 | 0.13 | | 0.02 |
| Dieldrin | 0.07 | 0.04 | 0.04 | ND | 0.01 | 0.04 | 0.05 | 0.04 | 0.04 | 0.1 | 0.05 |
| 4, 4 – DDD | 0.05 | 0.03 | 0.04 | 0.03 | 0.03 | 0.03 | 0.03 | 0.02 | 0.02 | 0.05 | 0.5 |
| 4, 4 – DDT | 0.23 | 0.12 | 0.19 | 0.31 | 0.07 | 0.08 | 0.16 | 0.21 | 0.15 | 0.05 | 0.5 |
| Heptachlor | 0.15 | 2.04 | 2.64 | 0.09 | 0.11 | 0.15 | 2.91 | 2.61 | 0.16 | | 0.02 |

AB –ASSIN BREKU AF – ASSIN FOSU AM – ASSIN MANSO AS – AGONA SWEDRU
BAS – B/ASIKUMA TP – T/PRASO AT – ATIEKU TH – TWIFO HEMANG
CC–CAPECOAST

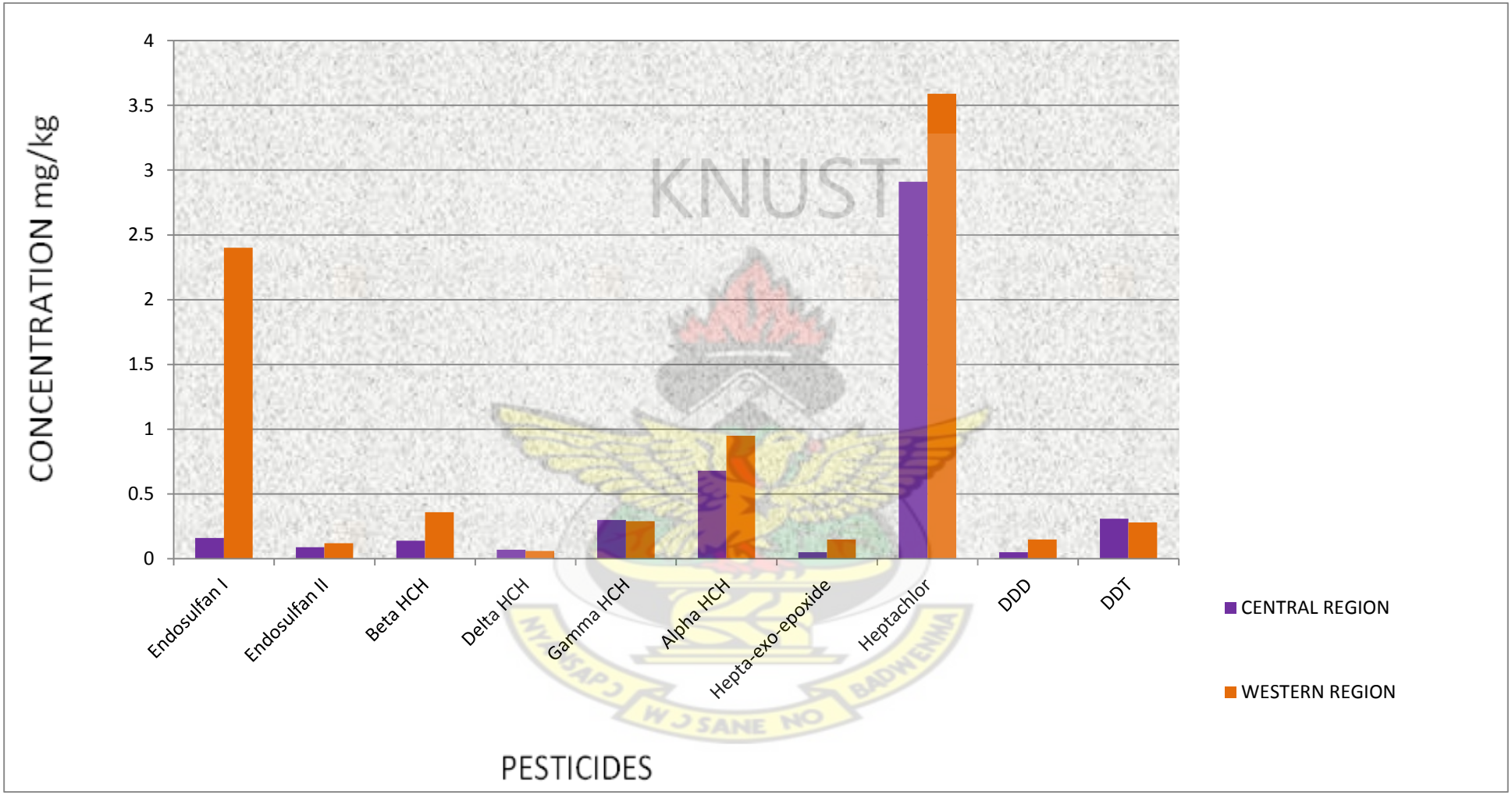


**Figure 4.1: Concentrations of pesticide residues in cocoa beans from Central and Western regions.**

Both DDD and DDT recorded concentrations below the EU – MRL whilst 100% and 18.18% (Table 4.7) of the samples from the Western Region recorded concentrations that exceeded Japanese MRL for DDT and DDD respectively. The presence of DDT and its metabolites in the cocoa beans may be due to the fact that these pesticides have a highly stable, low volatile, non – polar, lipophilic nature, and consequently exhibit considerable environmental persistence with a tendency to bioaccumulate, leading to the contamination of foodstuffs, especially those with high fat content like cocoa beans (Fontcuberta *et al*., 2008; Lazaro *et al*., 1996). Both regions recorded higher concentrations of DDT than DDD, indicating that there is still usage of DDT by Ghanaian farmers though it has been banned for so long.



The proportion of DDT/ (DDE+DDD) has long been used as a rough indicator of the age of DDT residues in the environment. Ratios greater than one suggest relatively recent DDT application (Biddleman *et al*., 2005). B/Asikuma recorded a ratio of 2.333 which indicates that there is a recent DDT application in the cocoa farms though it was banned in 1985 in Ghana. Recent work by Blankson – Arthur et al, indicated the ratio of DDT/DDE to be 2.86 in the liver of grasscutter, which suggest that there is recent application of DDT in the Gomoa area in the Central region of Ghana (Blankson – Arthur *et al.*, 2011).

**TABLE 4.5 CONCENTRATIONS OF PESTICIDES IN COCOA BEANS SAMPLES FROM DIFFERENT TOWNS OF THE WESTERN REGION.**

| | TOWNS (mg/kg) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **PESTICIDES** | **BI** | **SK** | **DD** | **AN** | **SAN** | **FO** | **NB** | **DK** | **DM** | **MK** | **KA** | **JAPAN MRL** | **EU MRL** |
| Chloropyrifos | 14.00 | 8.71 | 8.52 | 6.67 | 9.14 | 9.32 | 8.12 | 7.39 | 6.69 | 8.23 | 9.71 | 0.05 | 0.1 |
| Endosulfan I | 0.04 | 0.07 | 0.05 | 0.05 | 0.06 | 0.05 | 0.05 | 2.40 | 0.04 | 0.04 | 0.04 | 0.1 | 0.1 |
| Profenefos | 0.12 | 0.14 | 0.13 | 0.11 | 0.11 | 0.12 | 0.11 | 0.09 | 0.11 | 0.09 | 0.13 | | 0.1 |
| Endosulfan II | 0.08 | 0.12 | 0.10 | 0.09 | 0.08 | 0.09 | 0.08 | 0.07 | 0.08 | 0.04 | 0.09 | 0.1 | 0.1 |
| Fenvalerate | 0.30 | 0.44 | 0.45 | 0.26 | 0.23 | 0.14 | 0.14 | 0.10 | 0.11 | 0.16 | 0.15 | | 0.5 |
| Bifenthrin | 0.07 | 0.14 | 0.09 | 0.08 | 0.09 | 0.07 | 0.06 | 0.05 | 0.06 | 0.06 | 0.06 | 0.1 | 0.1 |
| Permethrin I | 0.45 | 1.51 | 0.99 | 0.31 | 1.65 | 0.16 | 0.19 | 0.09 | 0.17 | 0.44 | 0.19 | | 0.1 |
| Permethrin II | 1.64 | 0.21 | 1.46 | 0.49 | 2.16 | 0.34 | 0.15 | 0.57 | 0.20 | 0.25 | 0.72 | | 0.1 |
| Cypermethrin I | 0.06 | 0.08 | 0.05 | 0.15 | 0.63 | 0.07 | 0.05 | 0.04 | 0.04 | 0.04 | 0.04 | 0.03 | 0.1 |
| Cypermethrin II | 0.29 | 0.37 | 0.18 | 0.76 | 3.46 | 0.31 | 0.15 | 0.15 | 0.16 | 0.16 | 0.16 | 0.03 | 0.1 |
| Cypermethrin T | 0.24 | 0.54 | 0.71 | 0.79 | 5.79 | 0.25 | 0.11 | 0.11 | 0.07 | 0.24 | 0.09 | 0.03 | 0.1 |
| Beta HCH | 0.07 | 0.36 | 0.03 | 0.02 | 0.06 | 0.05 | ND | 0.28 | 0.03 | 0.05 | ND | | 0.02 |
| Delta HCH | 0.03 | 0.02 | 0.01 | 0.01 | 0.06 | 0.03 | 0.01 | 0.03 | ND | 0.01 | 0.04 | | 0.02 |
| Gamma HCH | 0.20 | ND | 0.19 | 0.20 | 0.18 | 0.23 | 0.26 | ND | 0.15 | 0.18 | 0.29 | 0.1 | 1.0 |
| Alpha HCH | 0.63 | 0.46 | 0.54 | 0.42 | 0.34 | 0.59 | 0.53 | 0.71 | 0.81 | 0.59 | 0.95 | | 0.02 |
| Aldrin | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 0.1 | 0.05 |
| Hepta-exo-epoxide | 0.03 | 0.06 | 0.03 | 0.06 | 0.03 | 0.05 | 0.04 | 0.02 | 0.02 | 0.03 | 0.15 | | 0.02 |
| Dieldrin | 0.06 | 0.07 | 0.05 | 0.06 | 0.04 | 0.05 | 0.03 | 0.04 | 0.05 | 0.04 | 0.07 | 0.1 | 0.05 |
| 4, 4 – DDD | 0.02 | 0.03 | 0.15 | 0.04 | 0.05 | 0.06 | 0.04 | 0.03 | 0.04 | 0.05 | 0.03 | 0.05 | 0.5 |
| 4, 4 – DDT | 0.21 | 0.28 | 0.28 | 0.28 | 0.14 | 0.17 | 0.13 | 0.14 | 0.08 | 0.13 | 0.10 | 0.05 | 0.5 |
| Heptachlor | 3.59 | 0.23 | 2.79 | 2.71 | 0.12 | 2.92 | 0.13 | 0.10 | 2.34 | 0.05 | 0.18 | | 0.02 |

BI – BIBIANI DD – DEBISO D AN – ANHWIASO SAN – S/ANHWIASOFO – FOSUKROM NB-NKWANTA
DK – DENTEKROM DM – DEDEMUDE MK – MENSAKROM KA – KWAME ADUKROM SK – SEHWI KAASE

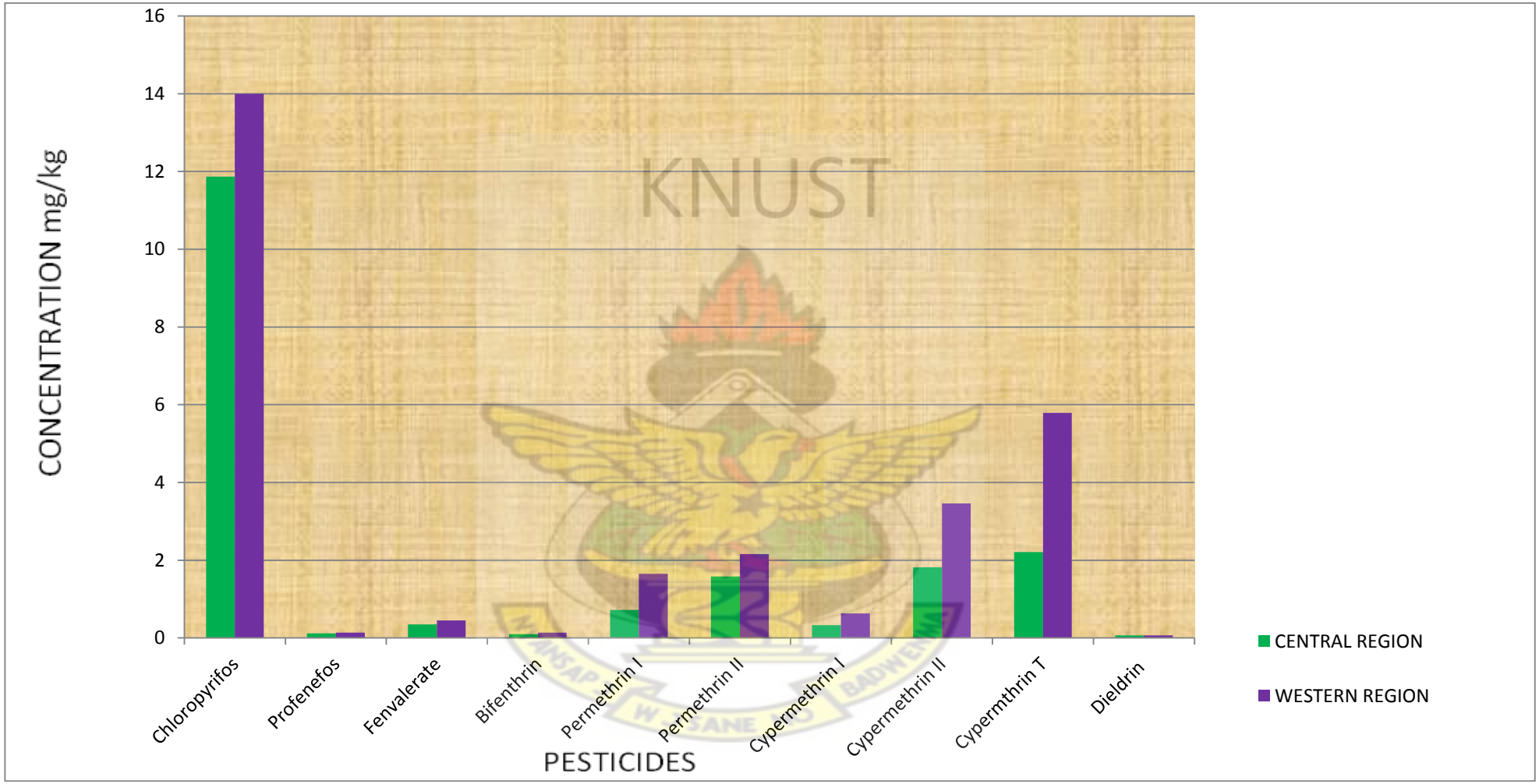


**Figure 4.2: Concentrations of pesticide residues in cocoa beans from Central and Western regions.**

Aldrin was not detected in any samples from the Central Region, whereas dieldrin was detected. The highest concentration of dieldrin was 0.07 mg/kg for samples from Assin Breku while the lowest concentration of 0.01 mg/kg was for samples from B/Asikuma. However, 11.1% (Table 4.6) of cocoa beans samples exceeded the EU – MRL for samples from the Central Region. None of the samples had concentration that exceeded the MRL for Japan for samples from the Central Region. Levels of dieldrin were always greater than those of aldrin probably as a result of metabolic transformation (degradation) of aldrin to the more environmental stable dieldrin or the gradual phasing out of aldrin in cocoa production since the use of aldrin on cocoa was discontinued in 1985.



For samples from the Western Region, the highest concentration of 0.07 mg/kg was obtained for dieldrin in samples from Sehwi Kaase and Kwame Adukrom (Table 4.5). These are cocoa-producing areas and the higher residue levels could be attributed to more intensive use of the pesticides to improve cocoa production. The lowest concentration of 0.03 mg/kg was recorded for samples from Nkwanta Bibiani. Out of all the samples from the Western Region 36.36% exceeded the MRL for EU, and none exceeded the MRL for Japan. The residue levels of OCPs were higher in Indian foods (Kannan *et al*., 1992) compared with most other developing countries like Nigeria and Ghana. The low concentrations or non-detectable levels of aldrin, dieldrin and heptachlor epoxide, indicate a possible phasing out of these persistent organic pollutants.

Heptachlor and its metabolites were also detected in cocoa beans sampled from the Central Region. The highest concentration of heptachlor recorded was 2.91 mg/kg in samples from Atieku (Table 4.4). The lowest concentration of 0.01 mg/kg for hepta–exo–epoxide was obtained for samples from Assin Fosu, Agona Swedru, T/Praso, Atieku and Twifo Hemang.

**TABLE 4.6; PESTICIDES CONCENTRATION RANGE COMPARED WITH EU AND JAPAN MRL LIMITS (CENTRAL REGION)**

| PESTICIDES | RANGES (mg/kg) | % Exceeding EU | % Exceeding Japan |
|---|---|---|---|
| Chloropyrifos | 4.39 – 11.87 | 100 | 100 |
| Endosulfan I | 0.01 – 0.16 | 11.1 | 11.1 |
| Profenefos | 0.04 – 0.12 | 11.1 | |
| Endosulfan II | 0.03 – 0.09 | None | None |
| Fenvalerate | 0.13 – 0.35 | None | None |
| Bifenthrin | 0.02 – 0.06 | None | None |
| Permethrin I | 0.04 – 0.72 | 88.8 | |
| Permethrin II | 0.12 – 1.58 | 88.8 | |
| Cypermethrin I | 0.03 – 0.33 | 22.2 | 66.6 |
| Cypermethrin II | 0.12 – 1.82 | 100 | 100 |
| Cypermethrin T | 0.01 – 2.21 | 66.6 | 77.7 |
| Beta HCH | 0.03 – 0.14 | 77.7 | |
| Delta HCH | 0.01 – 0.07 | 44.4 | |
| Gamma HCH | 0.14 – 0.30 | None | 77.7 |
| Alpha HCH | 0.22 – 0.68 | 100 | |
| Aldrin | ND | ND | ND |
| Hepta-exo-epoxide | 0.01 – 0.13 | 22.2 | |
| Dieldrin | 0.01 – 0.07 | 11.1 | None |
| 4, 4 – DDD | 0.02 – 0.05 | None | None |
| 4, 4 – DDT | 0.07 – 0.31 | None | 100 |
| Heptachlor | 0.09 – 2.91 | 100 | 100 |

All the samples recorded concentrations of heptachlor exceeding the EU – MRL and 22.2% (Table 4.6) of the samples had concentrations of hepta–exo–epoxide exceeding the EU–MRL.

For Western Region, the highest value of heptachlor was recorded for samples from Bibiani with a value of 3.59 mg/kg (Table 4.5). The lowest concentration of 0.02 mg/kg of hepta–exo–epoxide was detected in samples from Dentekrom and Dedemude. However, 100% and 81.81% (Table 4.7) of cocoa beans sampled from the Western Region recorded levels that exceeded the MRL for EU. Heptachlor recorded higher concentrations than its metabolite hepta–exo–epoxide with concentrations as high as 3.59 mg/kg for samples from the Western Region.

Endosulfan (I + II) were present in cocoa beans sampled from the Central Region of Ghana. Endosulfan I recorded the highest concentration of 0.16 mg/kg for samples from B/Asikuma and the lowest concentration of 0.01 mg /kg for samples from T /Praso and Atieku. 22.2% (Table 4.6) of the samples exceeded both the EU and Japan MRL for Endosulfan I. None of the cocoa beans samples exceeded both the EU and Japan MRLs for Endosulfan II.

Cocoa beans sampled from the Western Region contained Endosulfan I and Endosulfan II. The highest concentration was recorded for Endosulfan I with a value of 2.40 mg/kg for samples from the Dentekrom (Table 4.5). Lowest concentration of 0.04 mg/kg of Endosulfan I was recorded for samples from Bibiani, Dedemude and Mensakrom. However, 9.09% (Table 4.7) of the cocoa beans exceeded both the MRLs for EU and Japan for Endosulfan (I and II). Both the Western and Central Regions recorded concentrations lower than the MRLs for EU and Japan.

The Ghanaian Government in 2009 suspended the use of endosulfan which was banned by the EU since 2006 (PAN, 2008). In total, 62 countries have banned the use of endusulfan (Environmental Justice Foundation) as it has been described by the USEPA as highly hazardous (EPA, 2010). It was amazing to be reported in an interview with Osei-Assibey of endosulfan on sale in a shop in Bolgatanga, which is illegal. Therefore there is the need to continue monitoring endosulfan in foods. A study to determine the levels of organochlorine pesticide residues such as Aldrin, endosulfan, endrin and DDT in fruits at five markets in Accra revealded that 23.8% of the fruit samples contained pesticide residue above the accepted Maximum Residue Limit (MRL) whereas 48.7% were below the MRL (Bempah and Donkor, 2010).

Chloropyrifos recorded the highest concentrations in samples from both regions. For Central Region, cocoa beans sampled from Assin Breku recorded as high as 11. 87 mg/kg (Table 4.4). 4.39 mg/kg as the lowest for samples from T/Praso (Table 4.4). All of the samples exceeded the EU and Japan MRLs in case of chloropyrifos. Western Region followed the same trend of high concentration of chloropyrifos. As huge as 14.0 mg/kg was detected for samples from Bibiani (Table 4.5) and 6.67 mg/kg was detected in Anhwiaso. All samples exceeded the MRL set by both EU and Japan.

Similar work was conducted in the water and fish (lagoon tilapia) samples from lagoons in Ghana, and it revealed high levels chloropyrifos among the pesticide analysed. Chlorpyrifos was in a higher concentration, which could be attributed to the fact that it is widely used as agricultural insecticides and also has many uses in households for pest control (Essumang *et al*., 2009). Similar work was again conducted in the Volta Region of Ghana, where revelations of high levels of pesticide residues on foodstuffs has led to an outcry over the inappropriate

use of pesticides on vegetables cultivated in urban and peri-urban areas of Ghana (Kotey *et al*., 2008). In 2006 a survey of sixty farmers from the Volta Region of Ghana revealed inappropriate pesticide application practices (Kotey *et al.,* 2008). The analysis detected the presence of chlorpyrifos, DDT, cypermethrin, and dimethoate in shallots, with levels of chlorpyrifos exceeding the Codex maximum residue level in most samples (Kotey *et al*., 2008). Similarly in this study, chloropyrifos detected in cocoa bean sample exceeded the EU and Japanese MRLs. The detection of chlorpyrifos levels above EU and Japan MRLs in samples has several health implications. Chlorpyrifos is moderately toxic to humans (Howard, 1992; Wauchope *et al*, 1992) and is reported to damage the developing nervous system, specifically targeting the immature brain (Pope, 1999; Barone *et al*, 2000).

For example, in 2006, a consignment of 2,000 metric tonnes of cocoa beans from Ghana was rejected by Japan as a result of the excessive levels of pesticide residues found in the beans (The Statesman, 2006). Residue violations for chlorpyrifos have also been observed on 10 samples of tomatoes grown in the Upper East Region of Ghana (Binney, 2001) and on seven samples of cabbage grown in the Greater Accra Region of Ghana (Odhiambo, 2005). Although there is concern about pesticide residues on produce destined for the international market, targeted monitoring of produce for the domestic market by regulatory agencies is virtually non-existent.

Studies conducted in Brazil demonstrated relatively low environmental persistence but a higher acute toxicity of OP. Therefore, the OP residue in food has been strictly regulated by government in all countries in order to determine whether the concentrations of the pesticides used exceed their maximum residue limits (MRLs) (European Commission Directive, 1993; FAO, 2010).

For fenvalerate, cocoa beans sampled from Assin Breku in the Central Region recorded 0.35 mg/kg as the highest (Table 4.4) and lowest concentration of 0.13 mg/kg from B/Asikuma. Pihlstrom *et al* found significant levels of fenvalerate in the analysis of pesticide residues in fruits and vegetables in Sweden (Pihlstrom *et al*., 2007) as was detected in some cocoa bean samples from Assin Breku. All the samples exceeded the MRL for EU. From the Western Region, 0.45 mg/kg was the highest concentration for fenvalerate and it was a sample from Debiso D (Table 4.5) and the lowest being 0.10 mg/kg sampled from Dentekrom (Table 4.5). All the samples from the Western Region had concentrations above the MRL for EU. All the samples from the two regions all exceeded the MRL for EU.

Levels of Profenefos were determined in samples from the Central Region with the highest value of 0.12 mg/kg which was found in samples from Assin Breku (Table 4.4). Profenefos was not detected in cocoa beans sampled from Assin Fosu and Assin Manso, while samples from Agona Swedru recorded 0.04 mg/kg as the lowest level of profenefos (Table 4.4).

**TABLE 4.7; PESTICIDES CONCENTRATION RANGE COMPARED WITH EU AND JAPAN MRLS (WESTERN REGION)**

| PESTICIDES | RANGES (mg/kg) | % Exceeding EU | % Exceeding Japan |
|---|---|---|---|
| Chloropyrifos | 6.67 – 14.00 | 100 | 100 |
| Endosulfan I | 0.04 –2.40 | 9.1 | 9.1 |
| Profenefos | 0.09 – 0.14 | 81.8 | |
| Endosulfan II | 0.04 – 0.12 | 9.1 | 9.1 |
| Fenvalerate | 0.10 – 0.45 | None | |
| Bifenthrin | 0.05 – 0.14 | 9.1 | 9.1 |
| Permethrin I | 0.09 – 1.65 | 90.9 | 90.9 |
| Permethrin II | 0.15 – 1.64 | 100 | |
| Cypermethrin I | 0.04 – 0.63 | 18.2 | 100 |
| Cypermethrin II | 0.15 – 3.46 | 100 | 100 |
| Cypermethrin T | 0.07 – 5.79 | 81.8 | 100 |
| Beta HCH | 0.02 – 0.36 | 72.7 | |
| Delta HCH | 0.01 – 0.06 | 45.5 | |
| Gamma HCH | 0.15 – 0.29 | None | 100 |
| Alpha HCH | 0.34 – 0.95 | 100 | |
| Aldrin | ND | ND | ND |
| Hepta-exo-epoxide | 0.02 – 0.15 | 81.8 | |
| Dieldrin | 0.03 – 0.07 | 36.4 | None |
| 4, 4 – DDD | 0.02 – 0.15 | None | 18.2 |
| 4, 4 – DDT | 0.08 – 0.28 | None | 100 |
| Heptachlor | 0.05 – 3.59 | 100 | |

All the samples from the Central Region had concentrations below the MRL for EU with the exception of Assin Breku whose concentration was above the EU Value of 0.10 mg/kg. Conversely the levels of Profenefos in cocoa beans from the Western Region were all higher than the MRL for EU of 0.10 mg/kg with the exception of cocoa beans sampled from Dentekrom and Mensakrom (Table 4.5). The levels ranged from 0.09 – 0.14 mg/kg. Comparing the concentrations, Central Region had lower concentrations than Western in terms of levels of Profenefos found in cocoa beans.



Levels of bifenthrin in cocoa beans sampled from the Central Region were all below MRL for EU and Japan of (0.10 mg/kg). The highest value was recorded for Agona Swedru, followed by B/Asikuma and Cape Coast but the values were all below the MRLs for EU and Japan (Table 4.4). All the cocoa beans sampled from the Western Region recorded concentrations of Bifenthrin below MRLs for EU and Japan with the exception of sample from Sehwi Kaase which recorded 0.14 mg/kg (Table 4.5). In general the levels of Bifenthrin in cocoa beans from both the Central Region and Western Region were all below the MRLs.

Permethrin I and Permethrin II were detected in cocoa beans sampled from the Central Region but Permethrin II which was not detected in samples from Assin Fosu. In all, 88.9% of these samples had values that exceeded both the MRL for EU and Japan. The highest concentration recorded was 2.16 mg/kg for samples from S/Anhwiaso. Considering the MRL for EU and Japan, all the samples failed except those from Assin Fosu for both Permethrin I and Permethrin II (Table 4.4). Similar trend was obtained for Western Region where 100% (Table 4.7) of the samples recorded concentrations higher than both MRLs for EU and Japan with the exception of samples from Dentekrom which recorded 0.09 mg/kg of Permethrin I (Table 4.5). Thus from the Western and Central Regions, the levels found were high. In 2003, the

FDA conducted a study of total diet and they detected permethrin residues in only 3% of the 1039 food samples tested. The range of permethrin levels found was 0.0008 - 4.7130 mg/kg (FDA Pesticide Program Residue Monitoring, 2003).

Cypermethrin I, Cypermethrin II and Cypermethrin T were detected in samples from Central Region. 100% and 66.7% (Table 4.6) samples exceeded the value of MRL for EU and Japan for cypermethrin II and cypermethrin T respectively. Just as the case in Cape Coast where a research conducted on pesticides residues in cabbage revealed levels of cypermethrin residue above the respective MRL values (Ato -Armah, 2011). All the samples from Central Region had concentrations of cypermethrin I below the MRL for EU with the exception of Atieku and Cape Coast where values of 0.33 mg/kg and 0.16 mg/kg were recorded respectively (Table 4.4). Similarly cypermethrin I had 55.6% (Table 4.6) of the cocoa beans sampled from the Central Region exceeding the Japanese–MRL.

All the samples from Western Region were above the Japan – MRL for cypermethrin I, II and T. Almost all these samples i.e. 18.18% (Table 4.7) recorded concentrations below the EU – MRL with the exception of Anhwiaso and S/Anhwiaso (Table 4.5) considering cypermethrin I. for cypermethrin T, 81.81% (Table 4.7) exceeded the EU – MRL with the exception of samples from Dedemude and Kwame Adukrom (Table 4.5). All the samples had levels which were above the EU – MRL of 0.10 mg/kg. It is only Cypermethrin I that recorded smaller concentrations which were below the limit in all the two regions. Recently a work conducted by Tahir and others on fruits, apple sample (0.94 mg Kg-1) was found containing cypermethrin residues above the MRL 0.1 mg $Kg^{-1}$ (Tahir *et al*., 2011). In the developed countries many reports are available on the monitoring of pesticide residues in fruits and

vegetables detected above MRLs (Dogheim *et al*, 1999; Blasco *et al*, 2005; Cesnik *et al*, 2006 and Zawiyah *et al*, 2007).

# CHAPTER FIVE

# 5. CONCLUSIONS AND RECOMMENDATIONS

## 5.1 CONCLUSION

The determination of pesticide residues in the environment and in foods is necessary for ensuring that human exposure to contaminants does not exceed acceptable levels for health. This study was undertaken to determine the levels of pesticides in cocoa beans and to compare the levels with some international acceptable MRLs for samples from Central and Western Regions of Ghana. The study revealed the presence of most of the selected pesticide at varying concentrations, with chloropyrifos recording significant concentrations in all the two regions. Aldrin and DDE were not detected in all the samples which could be as a result of gradually fading of the organochlorine in the environment. The Pyrethroids recorded significant concentrations with cypermethrin recording 0.91mg/kg for sample from the Central Region, whereas Permethrin recorded 0.99mg/kg for sample from the Western Region. This could be attributed to the fact that some of these pyrethroid pesticides are registered for use in Ghana for either agricultural use or household purposes (EPA Ghana, 2009). Lastly, almost half of the samples exceeded the EU and Japanese MRLs for both Regions. These compounds are toxic and not environmentally friendly, increased contamination of cocoa beans may pose serious public health problems and loss of income to Ghana.

## 5.2 RECOMMENDATIONS

1. If the use of these pesticides in agriculture and food production cannot be discontinued, then it must be regulated with emphasis on good agricultural practice as any wrong application and handling could lead to serious health effects on man and the environment.

2. Efforts must therefore be continued in the future monitoring in food especially cocoa to forestall any serious problems, and to also build up a database for future regulatory legislation in the country.

3. It is strongly recommended that extensive awareness creation for safe use of pesticides be introduced and impact of pesticide usage in the country be instituted. This will provide information on biological indices of pesticides for effective monitoring of exposure of farmers and farm workers to pesticides.

4. It is also recommended that this work be extended to cover the determination of pesticides in finished products of cocoa since they are cocoa consumables, e.g. milo, chocolate bars, biscuits etc. which are all consumed by the Ghanaian population and the world as a whole.

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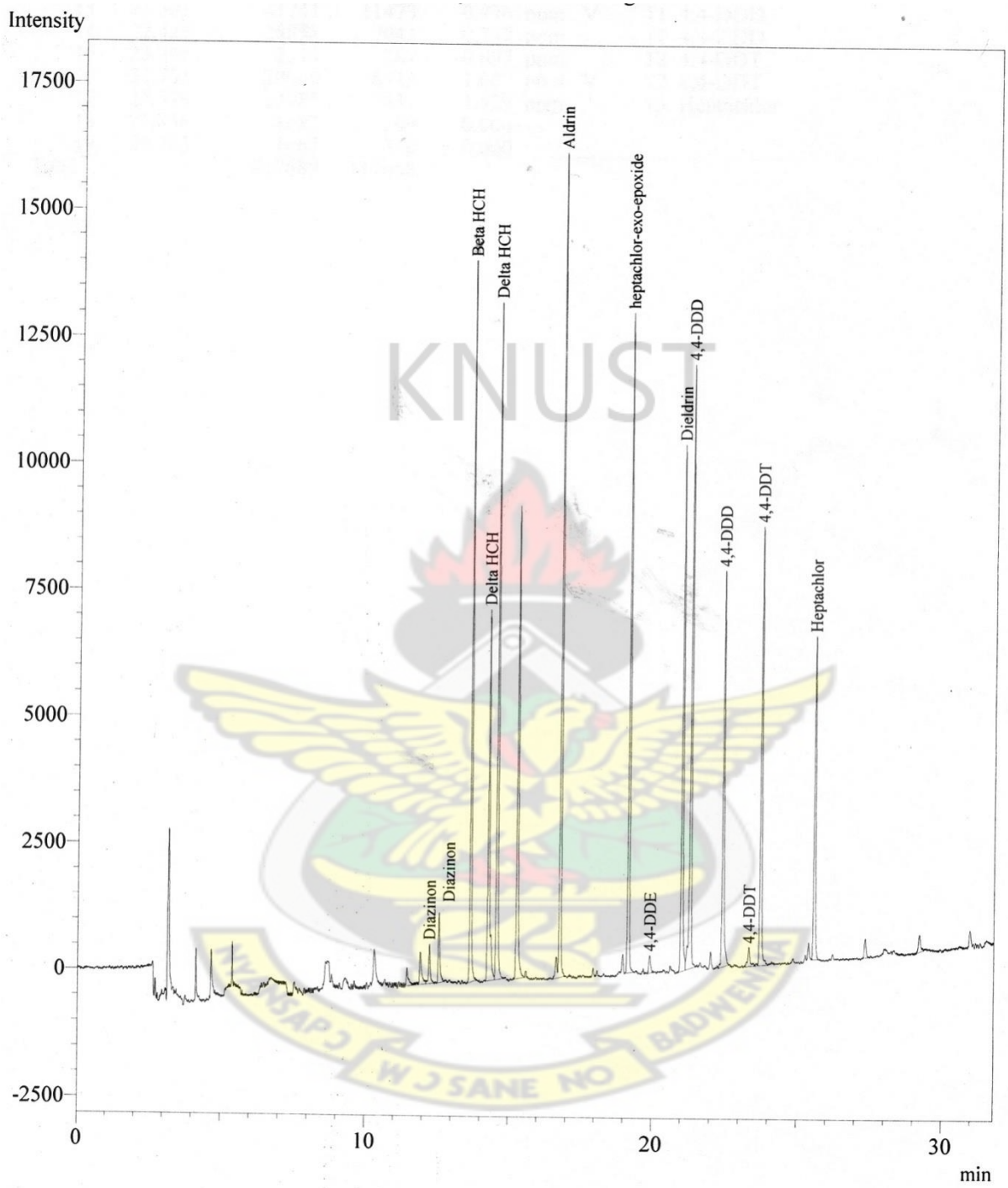
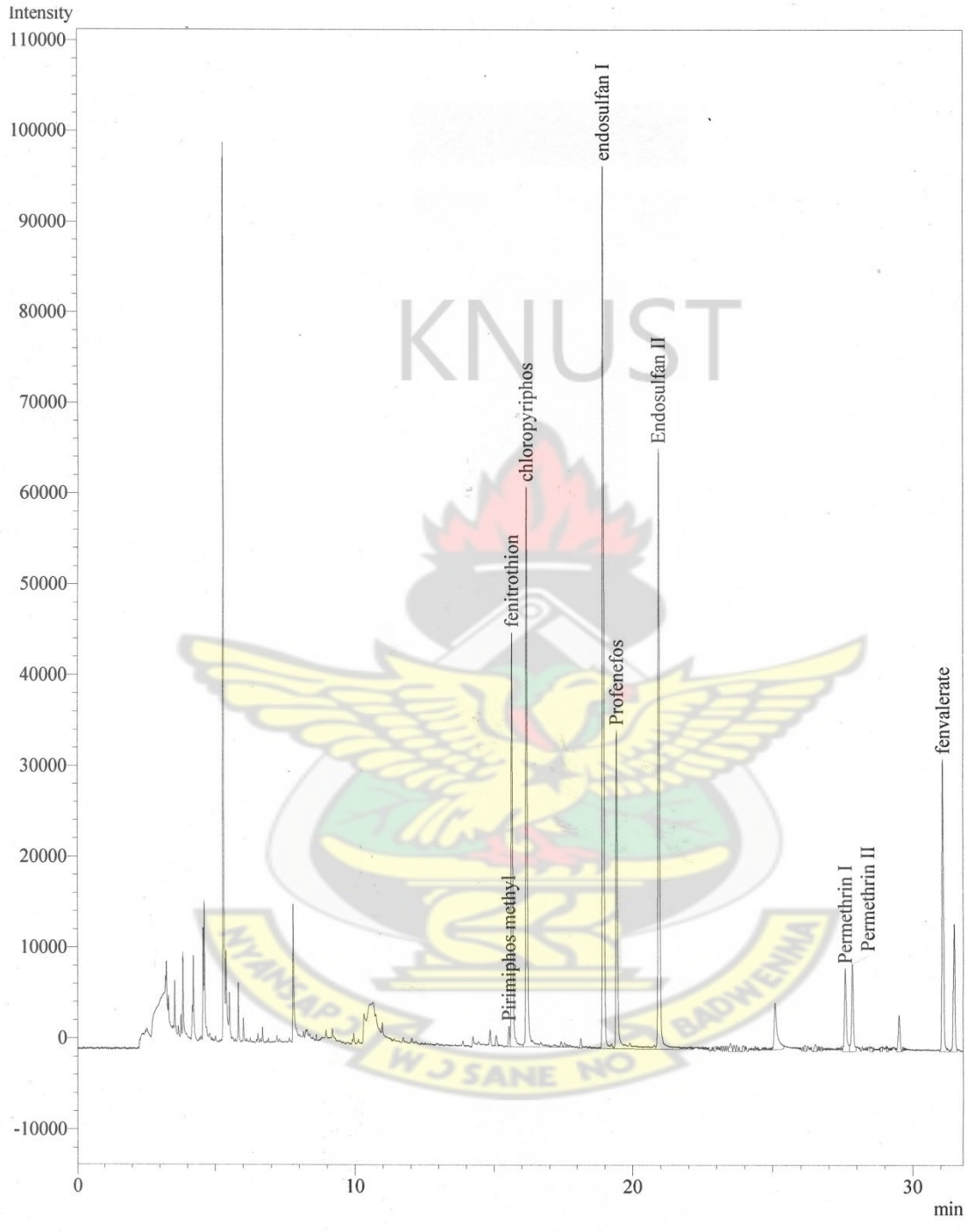
## APPENDIX 1



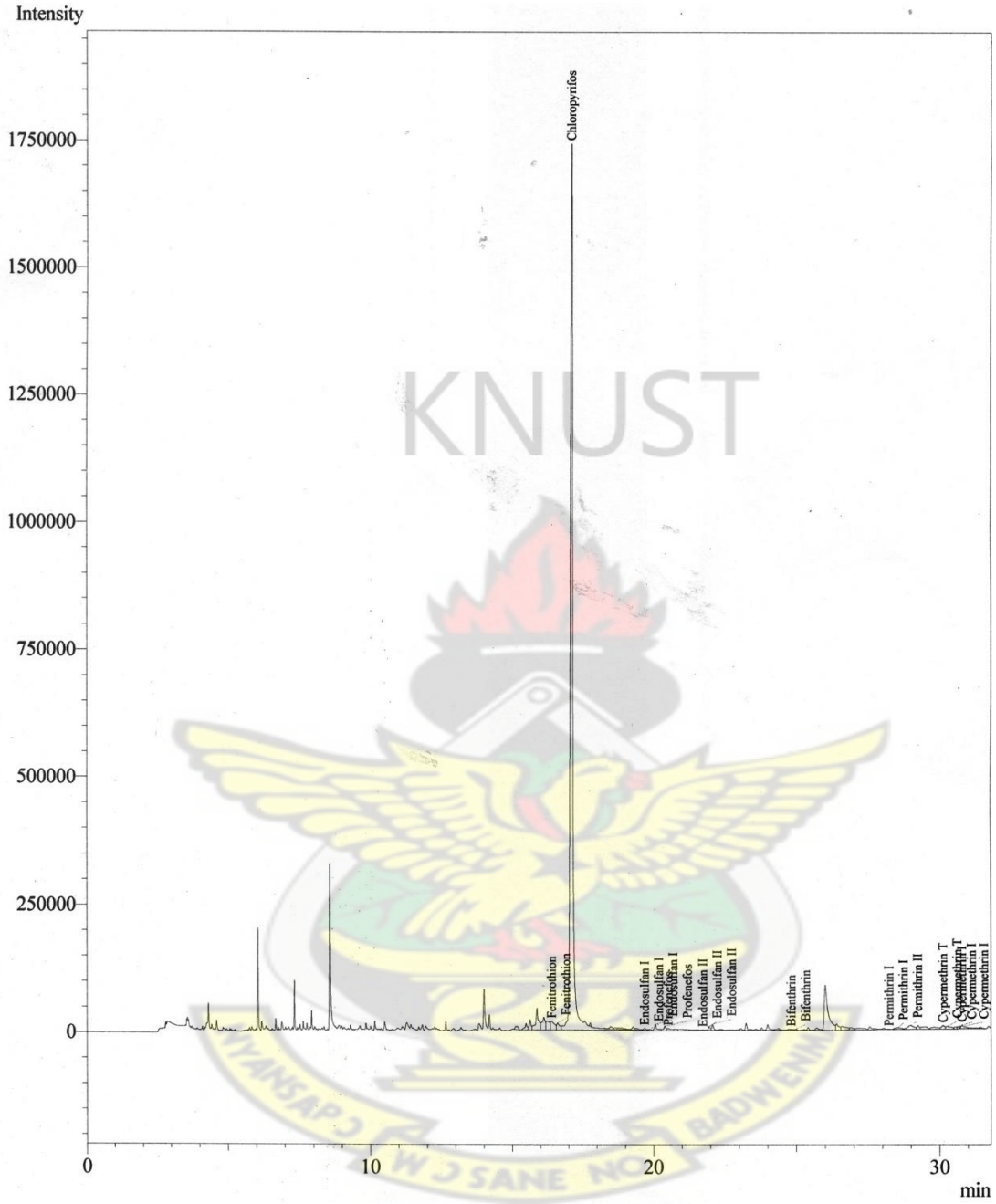

**Chromatogram of a Standard Mix 1**

## APPENDIX 2




**Chromatogram of standard Mix 2**

## APPENDIX 3




**Chromatogram of cocoa beans sample from Bibiani**