KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

COLLEGE OF SCIENCE

FACULTY OF BIOSCIENCE

DEPARTMENT OF ENVIRONMENTAL SCIENCE

CONCENTRATION OF PESTICIDE RESIDUES IN FERMENTED DRIED COCOA BEANS IN ASUKESE AND ITS ENVIRONS IN THE TANO NORTH DISTRICT OF BRONG AHAFO REGION, GHANA

BY

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INDEX NO.: PG 3112209

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September, 2011

Concentration of Pesticide Residues in Fermented Dried Cocoa Beans in Asukese and its Environs in the Tano North District of Brong Ahafo Region, Ghana

By

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A thesis submitted to the Department of Environmental Science,
Kwame Nkrumah University of Science and Technology,
in partial fulfilment of the requirements for the degree
of
MASTER OF SCIENCE (ENVIRONMENTAL SCIENCE)
Faculty of Bioscience, College of Science

September, 2011

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DECLARATION

It is hereby declared that this thesis is the outcome of research work undertaken by the author, any assistance obtained has been duly acknowledged. It is neither in part nor whole been presented for another degree elsewhere.

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DATE.....

DEDICATION

I dedicate this thesis to the Almighty God, my parents, cocoa farmers of Asukese and all my well wishers for their love and support.

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ACKNOWLEDGEMENT

In the accomplishment of this study, I wish to express my heartfelt gratitude to the almighty God for his grace and favour upon me throughout the period of this work.

I am also very thankful to Dr. J. Issifu Adam for his vital comments and suggestions as he supervised the work and Mr. Agorku Eric Selorm of Chemistry Department of KNUST, for assisting in vetting this work.

My gratitude also goes to Dr. George B. Crentsil, (Ag. Executive Director of Ghana Standards Board (GSB) for his immeasurable assistance offered me at his outfit.

Many thanks to Mr. Paul Osei-fosu and his team at pesticide residue laboratory of Ghana Standards Board (GSB) for assisting me in the samples preparations and GC/MS analysis of the pesticide residues.

My final thanks go to cocoa farmers of Asukese who willingly allowed me to take samples of cocoa beans from their farms. To Kwaku Ani, I say God bless you for your invaluable assistance.

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ABSTRACT

The concentrations of pesticide residues in fermented dried cocoa beans in ten inorganic sampling sites and one organic sampling site in Asukese and its environs were determined using partially modified multi-residue method for Agricultural chemicals by GC/MS. The Organochlorine pesticides residues found in samples of cocoa beans were aldrin, p,p-DDD, p,p-DDE, p,p-DDT, endosulfan Sulphate, beta-endosulfan, alpha-endosulfan, chlorpyrifos and dimethoate. Aldrin, p,p-DDD, p,p-DDE, p,p-DDT and endosulfan are insecticides banned for use in the cocoa industry by the European Union (EU) and Japan. The mean concentration of aldrin was found to be above the EU MRL of 0.05 mg/Kg, ADI of 0.0001 mg/Kg bw/day and the no observable adverse effect level (NOAEL) of 0.01 mg/Kg bw/day. P,p-DDD, p,p-DDE, and p,p-DDT were within their various MRLs but above the Acceptable Daily Intake of 0.001 mg/Kg bw/day. The Organophosphate Pesticides residues registered in cocoa samples were Ethophosphos, Fenitrothion, Malathion and Parathion. Fenitrothion and Parathion insecticides have been banned for use in the cocoa industry by the EU and Japan. Parathion, malathion and ethoprophos recorded mean residue concentration higher than their various MRLs except fenitrothion which recorded highest mean concentration lower than the EU MRL and ADI of 0.2 mg/kg and 0.002 mg/Kg bw /day respectively. The pyrethroids pesticide residue recorded in the cocoa beans samples analysed from all the 11 sites were fenvalerate, Deltamethrin, Cypermethrin and Permethrin. The highest mean concentration of fenvalerate (0.0898 mg/Kg) was found to be above the EU MRL and ADI but lower than the NOAEL value of 1.7 mg/Kg bw/day. The highest mean concentration of cypermethrin occurring in a sample was slightly higher than the EU MRL and would be unacceptable at the EU markets. The highest mean concentration of deltamethrin was 0.0035 mg/Kg which is far below the EU MRL and the Acceptable Daily Intake. Permethrin mean concentration of 0.0144 mg/Kg was moderately higher than the EU MRL and the ADI value of 0.05 mg/Kg bw/day. The study conducted showed no significant difference between pesticide residues in inorganic cocoa farms and the organic cocoa farms (Control) in Tano North of Brong Ahafo as indicated in the statistical results. Residues of Chemicals approved for use in the cocoa sector under the CODAPEC (mass Cocoa Spraying exercise) were not detected in the fermented dried cocoa beans samples analysed.

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ABBREVIATIONS

ADI - Acceptable Daily Intake
AI, ai - active ingredient
AOEL - Acceptable Operator Exposure Level
ARfD - acute reference dose as active substance
CCPR - Codex Committee on Pesticide Residues
CNS - central nervous system
CXL - Codex Maximum Residue Limit (Codex MRL
DDA - 2,2-bis(4-chlorophernyl)-acetic acid.
DDD - Dichlorodiphenyldichloroethane
DDE - Dichlorodiphenyldichloroethylene
DDT - Dichlorodiphenyltrichloroethane
FAO - Food and Agricultural Organization of the United Nations
GAP - Good Agricultural Practice in the use of pesticides
GC - Gas chromatography
HCB - hexachlorobenzene
HCH - Hexachlorocyclohexane
HPLC - high performance liquid chromatography (sometimes high pressure ~)
IPM - integrated pest management
IUPAC - International Union of Pure and applied chemistry
J**MPR** - Joint FAO/WHO Meeting on Pesticide Residues
JMPR - Joint FAO/WHO Meeting on Pesticide Residues (Codex Alimentarius)
Kow - octanol-water partition coeffi mg/L Milligram per liter
LD50 - median lethal dose; dosis letalis media
LOAEL - lowest observable adverse effect level
LOD - limit of determination - has also been used for "limit of detection"
LOEL - lowest observable effect level
LOQ - limit of quantification
MRLs - Maximum residue limits/level
ND - Not Detected
NOAEC - no observed adverse effect concentration
ng/ml - nanogram per millimeters
NOAEL - no observed adverse effect level
NOED - no observed effect dose
NOEL - no observed effect level
o, p-isomer - ortho, para-isomer
OCs - Organochlorines
OP - organophosphorous pesticide
p, p - isomer para, para-isomer
POPs - Persistent organic pollutants
TMDI - theoretical maximum daily intake
tMRL - temporary maximum residue limit
γ- isomer - gamma isomer
µg/Kg - Microgram per kilogram
µg/L - Microgram per liter
µL - microliters

CHAPTER ONE

INTRODUCTION

1.0 BACKGROUND

Ghana is an important cocoa producing country. In 2006/2007 season, Ghana produced 720 thousand tons of the world's total of 3.5 millions tons representing 20.7% to come second after the largest cocoa producer, Cote d'Ivoire (http://www.icco.org, accessed 6th Feb., 2011). Cocoa forms the basis of Ghana's economy, accounting for 36% of GDP in 2001. Cocoa exports in 2001 contributed 16% ($ 246 .7 million) to total exports. About 23% of the total area, or 5, 300,000 ha (13,096,000 acres) was cultivated in 1998 (Wikipedia, 2001, Ghana Agriculture).

Cocoa beans are very useful and constitute the main ingredients for the production of all types of chocolates. Recent research suggests that cocoa bean contains healthful catechins or polyphenols of the flavonol group. A meta-analysis of medical trials on cocoa's effect on blood pressure suggests cocoa flavonols may increase blood flow and reduced systolic and diastolic blood pressure numbers (Science Daily, 2007). Cocoa beans contain several flavanols that may reduce cardiovascular disease risks (Science Daily, 2007).

Pest and diseases cause very serious yield losses to cocoa production worldwide. While non-chemical means of managing cocoa pests and diseases are widely recommended, the need for agro-chemicals to manage cocoa pests and diseases is unavoidable and will continue for years to come (Asante, 1997).

However, the effects of continued exposure of users of pesticides, environmental risks, issues of pest resistance and possible hazards for consumers require a re-examination of the benefits of pesticide application and an examination of the risk involved. Hence the introduction of Good Agricultural practices (GAP) to considerably mitigate, if not eliminate, the problems associated with the excessive and unnecessary application of pesticides (Bateman, 2009). Technologies are presently available for the safe use of pesticides on cocoa and awareness on their correct and proper use needs to be stimulated. However, introducing GAP to illiterate smallholder farmers in the world cocoa economy is a major challenge (http//www.icco.org, accessed 6th Feb., 2011). Pesticides have been used on cocoa for more than 50 years, with notable early research carried out independently in the former West African Cocoa Research Institute now the Cocoa Research Institute of Ghana, Nigeria, Brazil, Cameroon, Costa Rica, Cote d'ivoire, Indonesia, Malaysia and Togo (Bateman, 2010). By the early 1970s a number of effective control techniques had become established and there was little incentive for change until environmental awareness increased in the 1990s. Most notable amongst these were concerns over the use of lindane for the control of cocoa insect pests; this was eventually phased out, but in some countries, not until the early 21st century. Many farmers believe that pesticides work at least against some cocoa pest problems, and continue to use them, depending on the pest and country (Bateman, 2009). Pesticides have been used in the public health sector for disease vector control and in agriculture to control and eradicate crop pests for the past several decades in Ghana (Clarke and Levy, 1997).

The majority of pesticides used in agriculture in Ghana are employed in the forest zones located in the Ashanti, Brong Ahafo, Western, and Eastern Regions of Ghana (Amoah *et al*., 2006).

Cocoa farmers use a wide range of pesticides to limit losses from pests and diseases in cocoa agriculture. Prominent among these are: copper sulphate, a fungicide popular in the treatment of black pod infection, Benzene Hexachloride (BHC) an insecticide for control of cocoa mirids, Aldrin/ Dieldrin or Aldrex 40 (to control mealy bugs); Carbamate unden, an insecticide which is effective in controlling cocoa mirids in West African countries (Berger and Aro, 1975). Others are Kokotine, Apeco, Perenox, Arkotine, Didimac 25, Basudin and Brestan. Pesticide use is associated with risk and can be hazardous if not handled properly. Cocoa farmers using pesticides containing Aldrin, Gamma BHC, Cuprous Oxide, Copper Sulphate, etc face constant exposure to these pesticides (Fajewonyomi, 1995).

As part of Ghana's determination to maintain high position in the cocoa production, COCOBOD in 2001, initiated a national programme which provides free spraying on cocoa farms to control the spread of black pod diseases and pests which has contributed to declining cocoa yield over the previous decades. The Cocoa Diseases and Pests Control Exercise Committee (CODAPEC) was formed to ensure the effective implementation of the project (CRIG, 2008). The committee was mandated to oversee the implementation of the mass cocoa spraying programme. The programme aimed at providing free assistance to farmers in controlling cocoa pests and diseases such as black pod that had reduced the yield of cocoa farms over the years. COCOBOD recommended four-times spraying in a year, within July, August, September and November.

The Cocoa Research Institute of Ghana (CRIG) recommended the insecticides and fungicides to be used to the CODAPEC for effective and efficient control of pests and black pod on cocoa. The insecticides are confidor and cocoster whilst fungicides are champion, kocides, fungaram and ridomil. The exercise involves spraying of farms in all cocoa growing areas against the black pod diseases and CODAPEC was instituted to manage the project. The project simply known as the mass cocoa spraying exercise was code-named CODAPEC. The mass cocoa spraying exercise was introduced in the country more than 50 years ago, which increased cocoa production by 500.00 metric tons and when the exercise was stopped the crop production sank to 300,000 metric tons. Cocoa production went up to 700,000 metric tons when the mass cocoa spraying exercise was re-introduced a few years ago (ISSER, 2008). The mass cocoa spraying exercise is aimed at ensuring that the correct amount of the chemicals are used to spray the farms to control diseases like capsid, blackpod and swollen shoot to increase cocoa production in Ghana and to limit the incidence of pesticide residues in cocoa beans and cocoa products (Antwi, 2010).

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1.1. PROBLEM STATEMENT

In Ghana information on pesticide residue levels in crops and vegetable produced locally is scarce. Earlier studies have assessed the level of banned pesticides such as lindane pesticide residues in cocoa beans (Owusu-Ansa *et al*., 2010). The Cocoa Association of Nigeria (CAN) raised an alarm over fears that cocoa seeds from Nigeria may be rejected at the international market due to high residue of pesticides (Saka, 2010). Cocoa from Nigeria was banned in 1988 and agreement had to be signed in London to produce

quality cocoa for the international market (Saka, 2010). Pesticides use has been on the increase worldwide. This trend resulted from the need for new methods and improved techniques to produce food for the ever increasing world's population. Excessive use of pesticides in food production has led to pesticide residues in cocoa products such as chocolate (Edward, 2006).

Cocoa consumers seek cocoa of high quality, containing minimal pesticide residues. This led to the introduction of Maximum Residue Limits (MRLs), which restrict the allowable pesticide content in cocoa and cocoa products to protect consumers. It is noteworthy that Japan introduced new legislation on MRLs in 2006, while the European Union (EU) introduced new regulations on MRLs in 2008 (Bateman, 2009). In Ghana, Ghana Standard Board is the appointed competent Authority for the analysis of pesticides residue level in fruits and vegetable meant for the European Union (EU) and also cocoa beans to Japan.

A response to the urgent need to reduce the impact of pesticides on human health and the environment led to the enactment of pesticides control and management act, 1996 Act 528 and introduction of mass cocoa spraying to regulate the use of pesticides. The environmental and toxicological impacts of pesticides are highly dependent not only on the parent compound, but also on their metabolites (Kulkami and Mitra, 1990). With the establishment of cocoa processing industries to add value to cocoa and eventual consumption of cocoa locally, it is imperative for effective monitoring of pesticide residues in cocoa beans at the production point to ensure conformity to standards to protect local consumers. Many farming communities in Ghana including Asukese are not adequately informed about the hazards associated with the chemicals. Asukese is a

cocoa farming community located in Tano North District of Brong Ahafo. Asukese and its surrounding communities are covered by the mass cocoa spraying exercise (CODAPEC).

1.2. **JUSTIFICATION FOR THE STUDY**

Recent changes to legislation in the European Union (EU) and Japan have concentrated minds over crop protection practices in cocoa and other commodity crops. From the 1st September, 2008, assessment of the quality of cocoa imported into the EU included measurement of traces of substances that have been used upstream in the supply chain, including pesticides used on farms or in storage (Bateman, 2010). The crop protection activities of farmers and middlemen will therefore be of increasing concern to all in the cocoa trade, some of whom may have a limited working knowledge of pesticide science. Pesticides have a poor public image and are known to present dangers to both people and the environment. Nevertheless cocoa, like other tropical crops, is often ravaged by insects, diseases and other pests that must be controlled effectively as well as safely. Pesticides can provide useful control solutions, but must be approved for use on the basis of Good Agricultural Practices (GAP). Unfortunately up-to-date GAP has not yet been established in many cocoa growing areas. Pesticides usage generally is fraught with problems of undesirable side effects and food chain involvement. Many pesticides pose substantial short and long term health risk (WHO, 1990). They are known to disturb the biochemical and physiological functions of erythrocytes and lymphocytes (Banerjee, 1999). This has led to the prescription of tolerances such as Maximum Residue Level (MRL) and Acceptable Daily Intake (ADI) as well as No Observable Adverse Effect

Level (NOAEL) for various pesticides in food and water, especially by the Codex Alimentarius Commission (CODEX Alimentarius, 2004) and other designated authorities in several developed countries of the world like U.S Environmental Protection Agency (U.S EPA, 1997 www. ep.gov/pesticides/op accessed on 19th March, 2011).

Although MRL, ADI and NOAEL for pesticides in cocoa are tested in the international market, it is however not tested at the point of production in Ghana. Determination of pesticide residues in cocoa at the points of production will position Ghana very well in the international market regarding pesticide use being demanded by the European Union (EU) which threatens Ghana's dream of increasing the volume of premium cocoa exports. The study will also be useful in protecting local cocoa industries and consumers of cocoa products in Ghana.

This work is aimed at documenting findings on the topic to serve as basis for further research. It is also hoped that the findings of the research would be useful to the Cocoa Research Institute of Ghana (CRIG) and the Cocoa Diseases and Pests Control Exercise Committee (CODAPEC) regarding using approved and correct amount of chemicals to spray cocoa farms.

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1.3. **OBJECTIVE OF THE STUDY**

1.3.1. **Main Objective**

The main objective of the study is to determine the concentration of pesticide residues in fermented dried cocoa beans in Asukese and its environs in the Tano North District of Brong Ahafo Region, Ghana.

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1.3.2. **Specific Objectives**

The specific objectives of the research are:

1. To find out what pesticides are approved for use by the cocoa spraying gangs in Asukese and its environs under CODAPEC programme.

2. To extract pesticides residues from fermented dried cocoa beans.

3. To determine the concentration of Active Ingredients of pesticides in the pesticides extract from the fermented dried cocoa bean samples by Gas Chromatography (GC) and Mass Spectrometry (MS).

4. Compare the active ingredients of pesticides in the cocoa beans to the pesticide residues standards such as MRL, ADI, and NOAEL of European Union (EU).

CHAPTER TWO

LITERATURE REVIEW

2.0. COCOA

Cocoa (*Theobroma cacao)* beans are the dried and fully fermented fatty seeds from which cocoa solids and cocoa butter are extracted (http://www.freepatentsonline.com15395635, accessed 10th Feb., 2011). They are the ingredients of chocolate, as well as many mesoamerican foods such as mole sauce and tejat. A cocoa pod or fruit has a rough leathery rind about 3 cm thick. It is filled with sweet mucilaginous pulp enclosing 30 to 50 large seeds that are fairly soft and white to pale lavender in colour. While seeds are usually white they become violet or reddish brown during the fermentation and drying process (http://www.freepatentsonline.com15 395635 accessed 10th Feb., 2011). The cocoa tree is native to the Americas. It may have originated in the foothills of the Andes in the Amazon and Orinoco basins of South America where today, examples of wild cocoa still can be found. It was first cultivated by the olmecs at least 1500 BC in central America (http://www.thinibble.com/reveiws/chocolate/the history of chocolate.as p accessed 10th Feb., 2011). Cocoa trees will grow in a limited geographical zone of approximately 20 degrees to the north and south of the Equator. Nearly 70% of the world cocoa is grown in West Africa. The cocoa plant was first given its botanical name by the Swedish natural scientist Carolus Linnaeus in his original classification of the plant Kingdom, who called *Theobroma* ("food of the gods") *cacao.*

Plate 1. *Theobroma cacao* tree in Asukese.

The largest cocoa bean-producing countries in the world including Ghana are given in table 1 below. The table gives the production estimates for the 2006/2007 season from the International Cocoa Organization. The percentage is the proportion of the world's total of 3.5 million tonnes for the period.

Table 1. Cocoa production estimates for 2006/2007 season.

Country	Amount produced	Percentage of world production
Cote d'Ivoire	1.3 millions tons	37.4%
Ghana	720 thousand tons	20.7%
Indonesia	440 thousand tons	12.7%
Cameroon	175 thousand tons	5.0%
Nigeria	160 thousand tons	4.6%
Brazil	155 thousand tons	4.5%
Ecuador	118 thousand tons	3.4%
Dominican Republic	47 thousand tons	1.4%
Malaysia	30 thousand tons	0.9%

http://icco.org. accessed on 22nd Feb., 2011

2.1. COCOA ECONOMY IN GHANA

There are different stories of how Ghana's "black gold" cocoa was introduced to Ghana. It was an indigenous plant in the rain forests of central and southern America, and so rare and expensive that only the royalty of Inca or Aztecs were permitted to eat it (www.freepatentsonline.com- accessed 6th Feb., 2011). Cultivating the plant by commoners was forbidden, and it was considered traitorous to export the plant.

The Portuguese and Spanish both stole cocoa plants to grow elsewhere. Ghanaian oral history has it that cocoa beans were first introduced to Ghana in the eighteenth century by Tetteh Quarshie. Thereafter, the cultivation of cocoa increased steadily until Ghana became the world's largest cocoa producer, supplying more than one-third of world production by the mid-1960s. By the early 1980s production was less than half that of two decades before; market conditions were aggravated by a drop of nearly 75% in world cocoa prices between 1977 and 1982 (myjoyonline, accessed Wed., 28th April, 2010). Cocoa has been the main export crop and a major source of foreign exchange and domestic income earner since its introduction in Ghana. Until 1977 and for 66 years (1910 to 1977), Ghana was the world's leading producer of cocoa with the market shares ranging from 30-40% (myjoyonline, accessed wed, 28th April 2010). Records indicate that production increased from a level of 36.3 MT in 1891 to an all time peak of about 557,000 MT in 1964/65 giving Ghana a global output share of about 33% and leading cocoa Producer (myjoyonline, accessed wed, 28th April, 2010).

In 1983/84, cocoa production totaled 158,000 tons, the lowest since independence; by 1999, production had rebounded to about 409,000 tons to come second after Cote d'Ivoire (http://wikipedia.org/wiki/ Ghana-agriculture). The Ghana Cocoa Marketing Board purchases and exports the cocoa crop (http://icco.org.(accessed 22nd Feb; 2011).

Cocoa forms the backbone of Ghana's economy accounting for 36% GDP in 2001. Cocoa exports in 2001 contributed 16+% ($246.7 million) to total exports. About 23% of the total area, or 5,300,000 hacters (13,096,000 acres), was cultivated in 1998 (http://wikipedia.org/wiki/ghana agriculture).

2.2. BENEFITS IN COCOA BEANS

Cocoa beans are used for the production of all types of chocolate. Azlecs and Mayans cultivated cocoa tree and mixed the beans with water, added vanilla, chilis and spices and used as drink (Science Daily, 2007).

Recent research suggests that cocoa beans contain health catechins or polyphenol group. Flavonol are a sub-class of natural compounds called flavonoilds, which are found in plants.

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Research materials are rich with proof that polyphenols with antioxidant properties, such as those in the non-fat parts of cocoa beans, benefits the circulatory system. A meta-analysis trial on cocoa's effect on blood pressure suggests cocoa flavonols may increase blood flow and reduce systolic and diastolic blood pressure numbers (Science Daily, 2007).

Cocoa beans contain several flavanols that may reduce cardiovascular disease risks. The flavanols are found in unprocessed cocoa and even in some processed cocoa products. A study by Dr. Romina Di Giuseppe at Catholic University in Campobosso, Italy, suggests that chocolate reduces inflammation which causes heart disease in the first place, because flavonoids lower C-reactive protein (CRP) in the blood (Science Daily, 2007). A high level of magnesium in cocoa beans contributes to its elevated stature as a heart-healthy food. Magnesium plays a role in reducing bad, LDL, cholesterol. It seems unlikely, but Kathleen M. Zelman, writes that cocoa can also aid the body's insulin sensitivity and improve blood sugar numbers. This could play a part in staving off Type II diabetes (Science Daily, 2007).

The phenylethylamine in dark chocolate acts as a mood booster. Chocolate is often referred to as the feel-good treat. Cocoa beans contain phyenylethylamine (PEA), an antidepressant that stimulates the body's adrenaline and dopamine levels for a dose of happy feelings. Researchers at Rush University center for creative Development in Chicago studied the effects of having a low level of phenylethylamine (PEA) and discovered that its deficit may be a cause of depression. Patients responded positively to PEA treatment. The PEA in chocolate is most likely responsible for its mood-lifting reputation (Science Daily, 2007). It is also speculated that epicatechin, a photochemical in cocoa, is being studied by researchers such as Dr. Norman Hollenberg at Harvard Medical School as a proven deterrent of cancer, diabetes and heart disease (Science Daily, 2007).

Another beneficial compound in cocoa is pentameric procyanidin. Scientists at the Lombardi comprehensive cancer center at Georgetown University Medical center are speculating that compounds in cocoa are inhibitors of breast cancer cell growth. These compound test results could one day lead to prevention and better cancer treatment (Cacao web accessed on 17th June, 2011). A 15- year study of elderly men published in the Archives of Internal Medicine in 2006 found a 50 percent reduction in cardiovascular mortality and 47 percent reduction in all-causes mortality for the men regularly consuming the most cocoa, compared to those consuming the least cocoa from all sources (Buijsse *et al*., 2006).

2.3. COCOA PESTS AND DISEASES

Even though cocoa is useful both as food and for health, like other crops, it is attacked by a number of pests including fungal diseases, insects and rodents (Table 2), some of which dramatically spread, and are sometimes described as invasive species (Entwistle, 1972)

Fungal diseases are a principal constraint to world cacao production and on a global scale the greatest losses result from black pod rots caused by *Phytophthora* spp. Two basidiomycete fungal diseases such as witches' broom and frosty pod rot pose a special threat to liverlihoods in Latin America. Black pod rots currently cause the greatest loss of production, but estimates of severity perhaps underemphasize the potential importance of frosty pod rot: *Moniliophthora roreri.*

Some cocoa problems have a world-wide distribution; others are restricted to individual cocoa growing regions in the Americas, Africa and S.E. Asia (Entwistle, 1972).

Table 2. Estimates of severity of cocoa diseases

Pest / Disease		Region	Potential crop loss ('000 tonnes)
Black pod rots	Phytophthora spp. e.g. P. megakarya	Ubiquitous W. Africa	450
Witches' broom disease	Moniliophthora (Crinipellis) perniciosa	Latin America	250
Frosty pod rot	Moniliophthora roreri	Latin America	30
Capsids (Miridae)	Sahlbergella spp. Distantiella theobroma Helopeltis spp. Monalonian spp.	W. Africa Africa & Asia Latin America	200
Swollen shoot virus (CSSV)	Vector believed to be mealy-bug: Planococcoides njalensis	W. Africa	50
Vertebrates (depends on region)	Woodpeckers, squirrels, rats and larger mammals	Ubiquitous (but many different spp.)	Various estimates of losses, typically between 1 - 20%
Cocoa pod borer	Conopomorpha crammerella	SE Asia	40
Vascular streak die-back (VSD)	Oncobasidium theobromae	SE Asia	30
Other diseases including: root diseases & minor pod diseases	Many spp. including Ceratocystis & Roselinia spp. Botroidiplodia theobromae	Depends on Sp.	n/a

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Insect pests of the cocoa tree, including termites, stemborers, etc	Various spp. including: Zeuzera sp. (S.E. Asia) Eulophonotus sp. (Africa)	Locally-serious in many cocoa growing areas.	n/a
Pests of young cocoa	Many spp, - often polyphagous insects	Ubiquitous	n/a
Weeds (especially in young cocoa)	Many spp (includes mistletoes on mature trees)	Ubiquitous	n/a
Storage insect pests: - Beetles - Warehouse moths	Many spp. including: Cryptolestes ferrugineus, Ephestia spp.	Ubiquitous	n/a

Entwistle, (1972).

2.4. COCOA PESTS AND DISEASES IN GHANA

The cocoa industry in Ghana which is one of its major export commodities contributing 26.3% of export revenue and 26% agricultural growth in 2006 is afflicted by two major plant diseases and one major group of insect pests (ISSER, 2008).

The diseases are Cocoa swollen shoot virus Disease (CSSVD) and the Black Pod disease (ISSER, 2008). The major pests of cocoa are the mirids (Capsids), *Distantiella theobroma* and *Sahlbergella singularis*. The Economic importance of these diseases and pests are derived from their adverse effects on per hectare yields and consequent losses to the industry's aggregate cocoa output (Asante, 1997).

2.4.1. Black pod disease

The black pod is a fungal disease cause by two *Phythophthora* species, *P. palmivora* and *P. megakarya*. Prior to the occurance of the P. *palmivora* in 1985, evidence from field studies indicated that losses due *P. palmivora* infection ranged from 16 to 23% (Asare-Nyako, 1974).

Though losses as high as 38% were reported (Asare-Nyako, 1974), losses through *P. megakarya* infection is however much more drastic with 70-90% losses or 100% in some cases.

Plate 2. *P. palmivora*:Asukese

Plate 3. *P. megakarya*:Asukese

Chemical control involves the coating of pod surface with recommended fungicides which stops the germination of fungal spores (Opoku *et al.*, 2007). Spraying of cocoa against black pod begins in the rainy season at 3-4 weekly intervals or at any time a farmer sports 1-2 infected pods (CRIG, 2008). Currently research efforts are towards the

possible use of phosphoric acid, a fully systematic fungicide for the control of *P. megakarya* (Opoku and Owusu, 1995) and the breeding of resistant planting material.

2.4.2. Cocoa Swollen Shoot Virus Disease (CSSVD)

Cocoa swollen short virus Disease (CSSVD) is caused by a virus which is transmitted by a mealy bug. It was originally discovered in Ghana in 1936. The cocoa swollen shoot disease is undoubtedly the single most important threat to the Ghanaian cocoa industry. It is largely responsible for Ghana's loss of her position as the world's leading cocoa producer (Thresh and Owusu, 1986). The economic importance of the CSSVD lies in its debilitating and destructive effect on the cocoa tree sometimes within as short period as three years (Thresh and Owusu, 1986). The basic method of controlling vector-borne viruses of tree crops is by eradicating sources of infection. Such measures have been used extensively in Ghana since 1944 in attempts to control swollen shoot virus disease. The cutting out of infected trees is usually preceded by surveys in which infected trees are identified by spotters and the farm marked out (Thresh and Owusu, 1986).

2.4.3. West African miridae (capsids)

Capsids are the most important pests of economic significance to cocoa in Ghana. The two main species responsible for crop losses are *Sahlbergella singularis* and *Distantiela theobroma* (plate 4). These insects are capable of reducing yields of healthy farms to less than 25% of their potential in one year (Thresh and Owusu, 1986). Seedlings may completely fail to become established due to the presence of capsids. Even when seedlings are not killed outright, capsids delay cocoa coming into bearing several years. On national scale, Owusu (1984) found out that about 25% of acreage under cocoa was

badly affected by capsids causing annual losses of about 100,000 tons of dry cocoa at the time.

Plate 4. ***Sahlbergella singularis*** **(left): geographically the more widespread species. Right:** ***Distantiella theobroma***

Plate 5. Bathycoelia thalassina (Shield bugs)

Although less damaging than mirids, shield bugs such as *B. thalassina* feed on developing pods via their very long stylets - resulting in damage to the beans themselves (plate 5). Pods become distorted and Entwistle (1972) reported that up to 40% pod loss may occasionally occur with Amazon and hybrid cocoa.

2.4.4 **Rodents and other vertebrate**

Rodents such as squirrels, rats and woodpeckers cause damage to cocoa pods in Ghana (plate 6 and plate 7).

Plate 6. Damage probably caused by squirrel (left) and a rat (right)

Woodpecker hole

Plate 7. woodpecker hole

Various chemical formulation (Pesticides) are used to fight cocoa pests and diseases which bring losses to the cocoa industry.

2.5. PESTICIDES AND THEIR PROPERTIES

2.5.1 What is pesticide?

According to Bateman (2010), the term pesticide can be defined simply as any substance which is used to control a pest, at any stage in crop production, storage or transport. The major pesticides types given by Bateman (2010) include: Fungicide-for crop diseases such as black pod, Insecticides for killing or repelling insects and Herbicides-for killing

weeds. Insecticides-Control insect pests, but they may be acaricides which is used for controlling mites.

Nematicides: for controlling Nematodes (eelworms), rodenticides: kill rats and mice and other pesticides-type include molluscides that kill slugs and snails and bactericides, but they are not usually used on cocoa. Some substances have multiple actions eg. Metam is a fungicide, herbicide and nematicide.

Pesticides are often referred to according to the type of pest they control. Another way to think about pesticides is to consider those that are chemical pesticides or one derived from a common source or production method. Other categories are biopesticides, antimicrobials, and pest control devices (US EPA, 2001).

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2.5.2. Chemical insecticides

Some examples of chemically-related pesticides as given by US EPA (2001) are as follows:

Organophosphate insecticides-These insecticides affect the nervous system by disrupting the enzyme that regulates acytelcholine, a neurotransmitter. They were developed during the early 19th century, but their effects on insects, which are similar to their effects on humans, were discovered in 1932. Some are very poisonous and were used in worldwar II as nerve agents. However, they are persistent in the environment (US EPA, 2001).

Carbamate pesticides affect the nervous system by disrupting an enzyme that regulates acetylcholine, a neurotransmitter. The enzyme effects are usually reversible. There are several subgroups within the carbamates (US EPA, 2001).

Organochlorine Insecticides were used commonly in the past, but many have been removed from the market due to their health and environmental effects and their persistence (e.g DDT and chlordane) (US EPA, 2001).

Pyrethroid insecticides were developed as a synthetic version of the naturally occurring insecticide pyrethrin, which is found in chrysanthemums. They have been modified to increase their stability in the environment. Some synthetic pyrethroids are toxic to the nervous system.

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2.5.3 Biopesticides

Biopesticides are certain types of pesticides derived from such natural materials as animals, plants, bacteria and certain minerals (US EPA, 2001), for example, canola oil and baking Soda have pesticidal applications and are considered biopesticides. At the end of 2001, US EPA had registered 195 biopesticide active ingredients and 780 products. Biopesticides fall into three major classes as follows: Microbial pesticides consist of a microorganism such as a bacterium, fungus, virus or protozoan as the active ingredient. Microbial pesticides can control many different kinds of pests, although each separate active ingredient is relatively specific for its target pest(s). For example, there are fungi that control certain weeds, and other fungi that kill specific insects. The most widely used microbial pesticides are subspecies and strains of *Bacillus thuringiensis* (US EPA, 2001).

Plant-Incorporated-Protestants (PIPs) are pesticidal substances that plants produce from genetic material that has been added to the plant. For example, scientists can take the

gene for the Bt pesticidal protein, and introduce the gene into the plant's own genetic material.

Then the plant; instead of the Bt bacterium, manufactures the substance that destroys the pest. The protein and its genetic material but not the plant itself, are regulated by U S. EPA (US EPA, 2001).

Biochemical pesticides are naturally occurring substances that control pest by non-toxic mechanisms. Conventional pesticides, by contrast, are generally synthetic materials that directly kill or inactivate the pest. Biochemical pesticides include substances, such as insect sex pheromones that interfere with mating as well as various scented plant extracts that attract insect pests to traps (Bateman, 2010).

Pesticides are used to help protect crops from pests such as weeds, fungus and bacteria. Farmers use pesticides to help produce food by protecting the crops which also leaves residues in the food crop.

2.6. PESTICIDES AND RESIDUES

Pesticides have been used in public health sector for disease vector control and agriculture to control and eradicate crop pests for the past several decades in Ghana (Clarke and Levy, 1997). Endosulfan, marketed as thiodan, is widely used in cotton growing areas on vegetable farms, and on coffee plantations. Organochlorine pesticides such as DDT, lindane and endolsulfan are also employed to control ectoparasites of farm animals and pests in Ghana (Menlah, 2008). In the public health arena, pesticides have been used by the onchocerciasis programme in the Volta Basin for control of black flies (*Simulium* spp) which transmit Onchocerciasis to human beings and for control of

domestic pests, eg cockroaches, flies, mosquitoes, ectoparasites including ticks, and other insects. Pesticides have also been used to control black flies along the banks of the Tano and Pra Rivers (Menlah, 2008). Cocoa farmers use a wide range of pesticides to limit losses from pests and diseases in cocoa agriculture. Prominent among these are: copper sulphate (a fungicide popular in the treatment of black pod infection. Benzene Hexachloride BHC (an insecticide for control cocoa Mirids); aldrin / dieldrin or aldrex 40 (to control mealy bugs); carbamate unden, (an insecticide which is effective in controlling cocoa mirids in West African countries) (Berger and Aro, 1975)

Others are kokotine, apeco, perenox, arkotine, didimac 25, basudin and brestan. Pesticide use is associated with risk and can be hazardous if not handled properly. Cocoa farmers using pesticides containing aldrin, gamma BHC, cuprous oxide, coppers sulphate, etc. face constant exposure to these pesticides (Fajewonyomi, 1995). Since 1957 when Lindane was recommended, spraying with synthetic insecticides has been the only effective method for controlling capsids on cocoa in West Africa. Presently, spray treatment with Gammalin 20 (Lindane) at 280g a.i/ha or 1.4 litres/ha and unden 20 (propoxur) at 210g a.i/ha or 1.1 litres/ha applied at monthly intervals from August to December, is the only protection measure recommended in Ghana (Menlah, 2008) Although the organochlorines are banned from importation, sales and use in Ghana, there are evidence of their continued usage and presence in the ecosystem.

Work already done in some farming communities in the Ashanti Region of Ghana and some other countries indicate the presence of organochlorine pesticide residues in fish (Osofo and Frempong, 1998), vegetables, water, sediments, mothers milk, and blood samples (Menlah, 2008) U.S EPA on 11th Jan, 2011 proposed phaseout of fluoride-base pesticide (Schor, 2011, accessed on 16th Feb, 2011). US EPA (2001) propose to start

gradually banning pesticides often used on cocoa beans and dried fruits that degrades to fluoride, a move closely linked to the Obama administration's decision to curb the maximum levels of fluoride in drinking water out of concern for children's health (Schor, 2011, accessed on 16th Feb, 2011).

A research by Rob (2002) found traces of a highly toxic chemical sprayed on most of the world's cacao crops present in most of the chocolate and Easter eggs-on sale in Britain.

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Lindane, a highly poisonous organochlorine, is sprayed on crops of cacao plants grown in Ghana and other West African countries to control mirid bugs, which cause the plants to wilt. It has been linked with breast cancer, non-Hodgkins hymphoma and a plastic anaemia in humans. It is also a suspected gender-bender that could disrupt the body's hormones. Such is the concern over lindane that the European Union agreed in 2000 to ban its sale and use (Rob, 2002).

An investigation by Britain's pesticides safety Directorate found Lindane residues in three-quarters of chocolate samples on sale in British supermarkets. "Many of us will be surprised to be given a pesticide which causes breast cancer for Easter, but that is what will be in many of the Easter eggs being eagerly eaten today", said Dr. Richard Dixon, head of research at Friends of the Earth Scotland (Bateman, 2010). Lindane traces have been found in thorntons, Nestle and Tesco own-brand chocolate, as well as luxury plain chocolate made by swiss.

2.7. HEALTH HAZARDS OF PESTICIDES

Pesticides are specifically formulated to be toxic to living organisms, and as such, are usually hazardous to humans. Most pesticides used today are acutely toxic to humans. Pesticides cause poisonings and deaths every year and are responsible for about one out of every sixteen calls to poison control centers (Litovitz *et al.,* 1996). Chronic health effects have also been reported from pesticides, including neurological effects, reproductive problems, interference with infant development, and cancer.

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2.7.1 Acute impacts

Acute pesticide poisonings frequently involve organophosphate pesticides, or sometimes their close relatives, the n-methyl carbamates. These pesticides were originally derived from chemical warfare agents developed during World War II. Some common organophosphates in use today include chlorpyrifos (Dursban), diazinon, azinphos-methyl (Guthion), malathion, and methyl-parathion. Aldicarb (Temik) and carbaryl (Sevin) are common n-methyl carbamates. They kill by blocking the enzyme that breaks down a critical nerve-impulse-transmitting chemical known as acetylcholine. The result is that certain nerve impulses are over-expressed, resulting in an array of acute toxic symptoms (Blondell, 1997). Symptoms of organophosphate or carbamate poisoning include blurred vision, salivation, diarrhoea, nausea, vomiting, wheezing, and sometimes seizures, coma, and death. Mild to moderate pesticide poisoning mimics gastroenteritis, bronchitis, or intrinsic asthma, and even astute clinicians may not link these symptoms to pesticides (Blondell, 1997). The American Association of Poison Control Centers reported 97,278 calls about pesticide poisonings in 1996.

2.7.2. Chronic impacts

Laura (1996) is reported to have seen illnesses, more children being born ill, more families that miss work because every day they have more problems, headaches, and depression which may be due to chemicals.

Chronic effects of pesticide exposure may include adverse effects on neurological function, cancer, reproductive harm, reduced growth and development, and birth defects. Much of the evidence of chronic effects is based on studies of adult workers who are exposed to a mixture of chemicals every day, making it difficult to pinpoint specific pesticides (Laura, 1996). The effects of individual pesticides during specific periods of foetal life, infancy, and early development have been studied in laboratory animals. Little research on the chronic effects of pesticides has been done directly on children, and even less on farm children.

2.8. THE NATIONAL COCOA DISEASES AND PESTS CONTROL

As part of efforts to arrest the decline in cocoa production, the Government of Ghana through cocoa Board initiated a National Cocoa Diseases and Pest Control (CODAPEC) programme, popularly known as "Mass Spraying" to assist all cocoa farmers in the country to combat the capsid/mirid and the black pod disease. Other objectives were to train farmers and technical personnel on the cultural and chemical methods of pests and disease control, educate and train local sprayers on safe pesticides usage. This measure according to Antwi (2010), is also aimed at ensuring that the correct amount of the chemicals are used to spray the farms to control diseases like capsid, black pod and swollen shoot in order to increase cocoa production. Ghana COCOBOD identified that

due to the high cost of fungicides and insecticides, maintenance of cocoa farms was becoming a burden on farmers, so most of them felt reluctant to maintain their farms to the extent of abandoning them. This according to Himme and Snoeck (2001) led to a sharp decline in cocoa production in Ghana from the 1980s to the beginning of the year 2000. As part of Ghana's determination to maintain high position in cocoa production, COCOBOD, in 2001, was equipped to initiate a national programme which provides free spraying on cocoa farms to control the spread of black pod diseases and pests which has contributed to declining cocoa yield over the previous decades. The aim of the project was to facilitate increased production of cocoa that would also translate into increasing farm income to enhance the living standard of farmers. The CODAPEC programme was introduced in 2001/2002 cocoa season. Currently, 72 political districts covering all the cocoa growing areas are benefiting from the programme; 21 districts from the black pod disease only, 35 districts from mirids only and 16 from both programmes. District Task force (DTF) and local Task Force (LTF) have been formed in each operational district and unit centre, respectively. The DTF represents the project Management at the District level and is in charge of Gang recruitment, storage and distribution of inputs and logistics to the Gang Areas, and general supervision.

The LTF on the other hand, represents the Project Management at the society (Village) level and is responsible for the planning and execution of the programme at the local level (Antwi, 2010).

The blackpod control programme covers all cocoa districts in the Volta, Brong Ahafo and parts of Western, Ashanti and Eastern Regions. Spraying against mirids on the other hand, covers the central, Eastern, and parts of Western and Ashanti Regions (Antwi, 2010).

Each farm is sprayed three times between June and October in the case of black pod and twice between August and December in case of mirids. Spraying gangs are established in each unit centre. Each gang of ten (in case of black pod programme) and six (in case of mirid programme) has a supervisor who is responsible for the general supervision of the programme at unit level.

Eight fungicide types, Ridomil Gold 66 plus WP (cuprous oxide + mefenoxam), Metalm 72 plus Wp (Cuprous oxide + metalaxyl), nordox 75 WG 765(Cuprous oxide), funguran-OH WP (Cupric Hydroxide), Champion WP (Cuprice hydroxide) and kocide 2000 WP (Cupric hydroxide), fungikill WP (Cupric Hydroxide + metalaxyl) and Agro-comet WP (Cuprous oxide + metalaxyl) are recommended for spraying against the black pod.

Similarly three insecticide types, confidor (Imidacloprid), Akate master (Bifenthrin) and Actara (Thiamethoxam) are being used against capsids (Antwi, 2010).

As a result of the CODAPEC programme, the black pod diseases incidence and mirid infestation have reduced significantly as shown by field evidence and by farmers' testimonies (Antwi, 2010). Hitherto, losses due to black pod were about 60 to 100% while losses due to mirid were between 25% and 35%.

Production figures show that yield per ha has increased substantially in virtually all the districts across the country. Consequently, cocoa production in Ghana went up from 380,000MT at the inception of the programme to almost 500,000MT in 2002/2003 and reached an all time high of 740,458 MT in the 2005/2006 (Ghana Commercial Bank, 2006).

The renewed enthusiasm of farmers following the introduction of CODAPEC has rekindled cocoa cultivation, new farms have been established and old ones rehabilitated.

According to the seed production unit of COCOBOD, the demand for planting materials has gone up substantially since the CODAPEC programme begun (Ghana Statistical Service, 2007)

2.9. SAFETY AND RESIDUES

Pesticide residues is a matter of great concern since members of the general public perceive a risk but feel it is a matter over which they have little control (Bateman, 2009). In response, authorities attempt to regulate by setting standards and monitoring exposure (Hamilton and Crossly, 2004). This is achieved by legislation and enforcement of the legislation. Two important measures are especially prominent in legislation which includes; Acceptable Daily Intake (ADI) and measures and limits of actual residues based on field studies which includes maximum residue levels (MRLs). It is a practical specification for food producers for a given crop. Testing for residues is carried out following internationally agreed and validated methods and good laboratory practice (GLP) standards apply in some countries. Procedures include extraction and clean-up from samples, followed by analysis using various instruments, depending on the residue being analyzed.

Analysis technique include: gas chromatography (GC), gas-liquid chromatography (GLC), gel permeation chromatography (GPC), high-pressure liquid chromatography (HPLC) and various mass spectrometry techniques, so such laboratories are expensive to set-up and maintain (Bateman, 2009)

2.10 MAXIMUM RESIDUE LEVEL (MRL)

Maximum residue level **(**MRL) is the maximum concentration of pesticide residue likely to occur in or on a specific food commodity after the pesticide has been used under Good Agricultural Practice (GAP). MRLs are not necessarily safety limits, but primarily a check that GAP is being followed and are intended to assist international trade in produce treated with pesticides (GRO- Cocoa, 2006). Limit of Detection (LOD) is the lowest concentration of a pesticide residue that can be measured by routine analysis. Continuing progress in analytical methods means residues can be detected at even smaller concentrations.

Import Tolerance described in European Commission (EC) No. 396/200 as an MRL set to meet the needs of international trade where the use of the active on a commodity is not authorized in the EU or a different level is appropriate because the existing EU MRL was set for reasons other than public health (GRO- Cocoa, 2006). Pesticide residues on crops are monitored with reference to minimum residue limits (MRL) and are based on analysis of quantity of a given Active ingredient (AI) remaining on food product samples. The MRL for a given crop or active ingredient is usually determined by measurement, during a number (in order of 10) of field trials, where the crop has been treated according to Good Agricultural Practice and appropriate Pre-harvest interval has elapsed (Bateman, 2009).

For many pesticides, however, MRL is set at the limit of determination (LOD) since only major crops have been evaluated and understanding of Acceptable Daily Intake (ADI) is incomplete. LOD can be considered a measure of presence or absence, but true residues may not be quantifiable at very low levels. For this reason the limit of

quantification (LOQ) is often quoted in preference (GRO-Cocoa, 2006). For substances that are not included in EU regulation, a default MRL of 0.01 mg/Kg normally applies. It follows that adoption of GAP at the farm level must be a priority and includes the withdrawal of obsolete pesticides. With increasingly sensitive detection equipment, a certain amount of pesticide residue will often be measurable following field use. In the current regulatory environment, it would be wise for cocoa producers to focus on pest control agents that are permitted for use in major importing countries (GRO-Cocoa, 2006)

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2.10.2 Acceptable Daily Intake (ADI)

This is the amount of an active ingredient (Active) that can be consumed daily over a life-time without harm, expressed in mg/Kg body weight of the consumer and based on toxicological evaluations (GRO-Cocoa, 2006).

Acute Reference Dose (ARfD) refers to an estimate of amount of an active, expressed in mg/Kg body weight of the consumer that can be ingested over a short period of time (1 meal or 1 day) without appreciable health risk (GRO-Cocoa, 2006).

A pesticide can only be approved for use if the risk to consumers, based on potential exposure, is acceptable. The limit set for a pesticidal active ingredient (AI), the ADI, is an estimate of the amount that can be consumed daily, for a lifetime, without harm to the person. The term acceptable is considered to involve a 100 fold safety factor from a measure called the No Observed Effect Level (NOEL) obtained in laboratory studies, which is 10 times lower than the Lowest Observable Effect Level (LOEL) (Bateman, 2009)

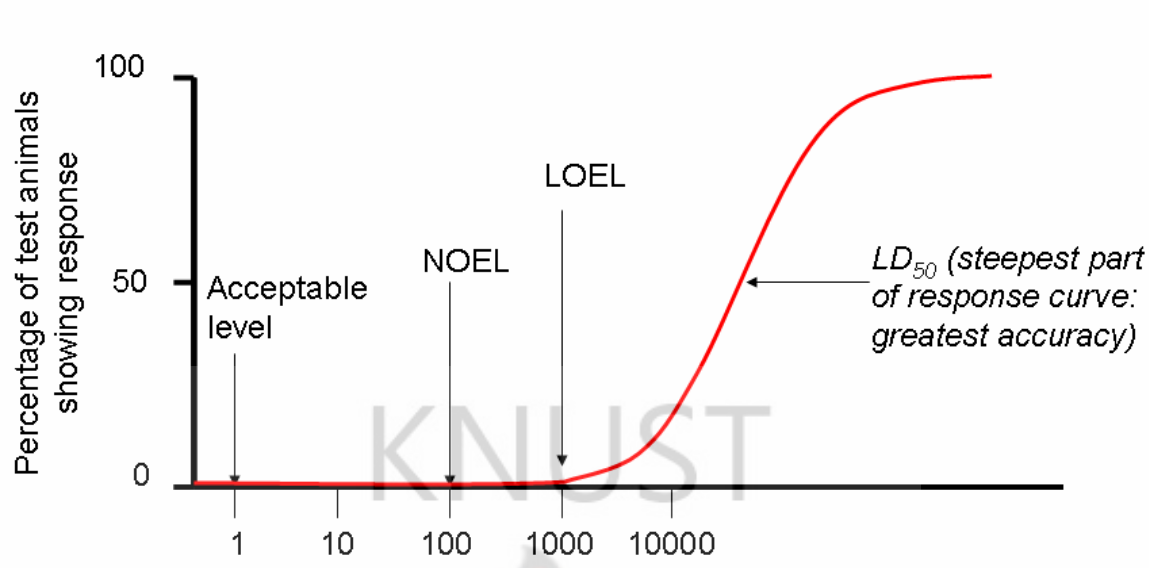

Fig. 1. Relationship between ADI, NOEL, LOEL and LD50

Data from laboratory studies is expressed as a dose usually mg/Kg body weight and it is necessary to extrapolate these data for human exposure be it dermal toxicity for AOEL or ADI for dietary safety. Dietary intake is often based on the National Estimated Dietary Intake (NEDI), but there may be substantial variations between infants, children and adults even after adjusting for body weight (GRO-Cocoa, 2006).

2.11. PESTICIDE BREAKDOWN

After application, pesticides are degraded by chemical and physical processes in the environment such as sunlight, soil and water (abiotic degradation) or metabolized within living organisms. Breakdown of a pesticide in the environment can be thought of as following a decay curve. This is a function of the chemicals' half-life, which is the time (usually in days) required for half of the applied pesticide to become converted into degradation products which may in turn be biologically active and have substantial half-lives (Bateman, 2009).

The rate of break-down depends on many factors, not least the chemical stability of the pesticide in question, but factors such as temperature and pH are extremely important, so the half life may be expressed as a range. The most important mode of pesticide degradation is oxidation by activated oxygen (ozone and hydroxyl radicals generated by sunlight, hydrogen peroxide generated in plants) rather than oxygen in the atmosphere. Allowing sufficient time to elapse between application and harvest enables any residue to degrade to acceptable levels (i.e the MRL) and the Pre- Harvest Interval (PHI) has a built-in safety factor. Reducing the dosage reduces the time to which acceptable levels are reached, but pest control may be impaired. Excessive residues occur with short harvest intervals, overdosing, or worst of all both of these (Bateman, 2009).

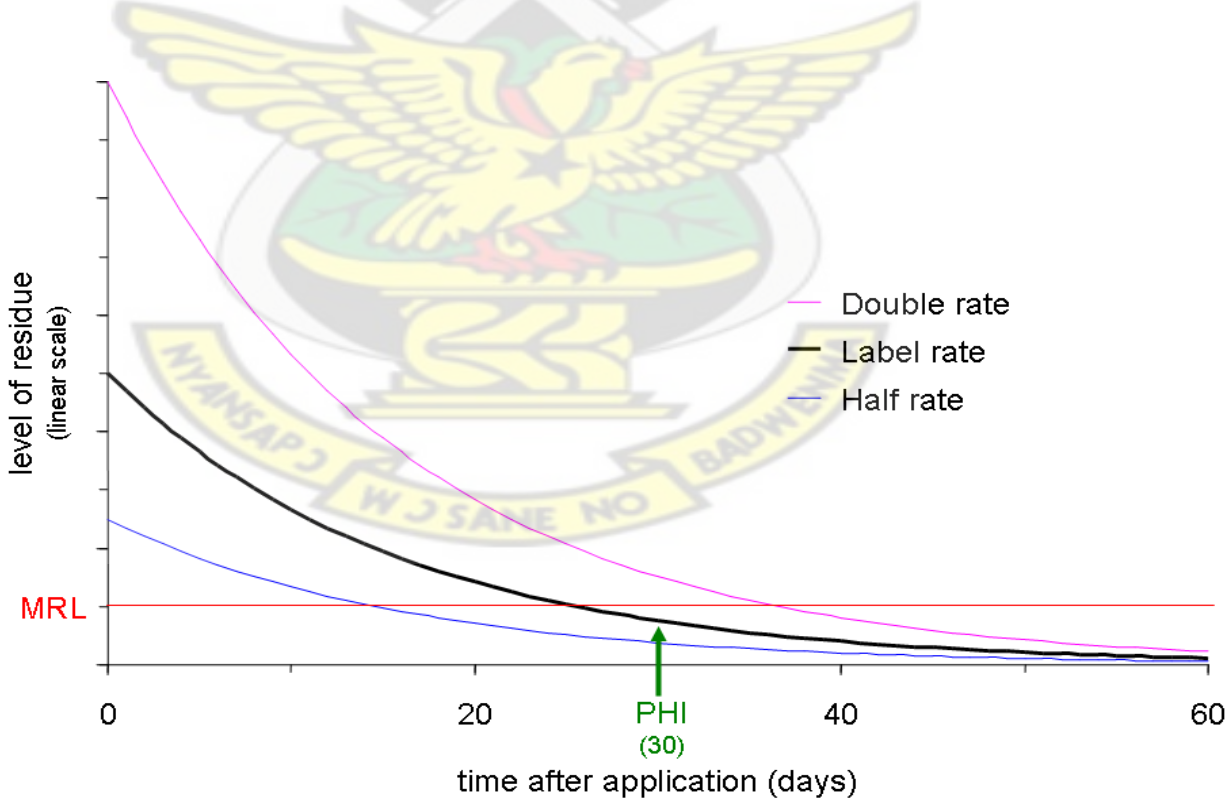

Fig. 2. Breakdown of a pesticide after application

The curves illustrated are modelled on the basis of an 'industry default half-life' of 10 days (supported by limited data); all axes are linear

2.12. IMPLICATIONS FOR APPLICATION AND ENVIRONMENTAL IMPACT

Improved application techniques are an especially promising way of mitigating residues and lowering environmental impact, but unfortunately research in this field has been very limited (Cropping, 2004).

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Targeted dose-transfer can increase Pest mortality for a given level of application to the crop, while maintaining equivalent pest control (Bateman, 2003)

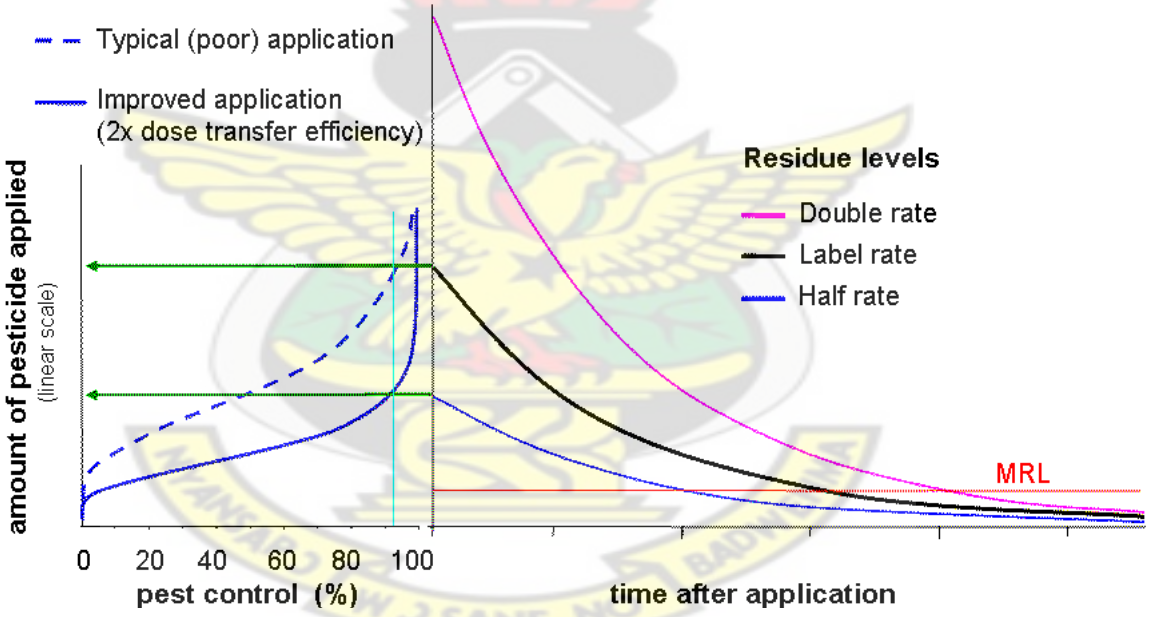

Fig. 3. Breakdown curves (as above) juxtaposed to rotated dosage response curves for indicative Standard and improved application methods against a target pest (Bateman, 2003).

Typical label rates allow for sub-optimal application methods. If spraying can be improved, the benefits may include reduced environmental load of pesticide residues and savings for the farmer.

2.13. PESTICIDE RESIDUES REGULATIONS

The International Cocoa Organization (ICCO) reminded those in the cocoa and related industries that the EU legislation on Pesticide Residues will apply to all beans entering the European Union from 1st September, 2008 (Bateman, 2009). Japan introduced new legislation on Pesticide residues in 2006 (Bateman, 2009). This led to the prescription of tolerance such as maximum residue Level (MRL) and Acceptable Daily intake (ADI) as well as No Observable Adverse Effect Level (NOAEL) for various Pesticides in food and water especially by the Codex Alimentarius Commission (CODEX Alimentarius, 2004 http://www. Codexalimentarius.net/ accessed 14th Feb; 2011) and other designated Authorities in several developed countries of the world. All consignments of cocoa beans being imported into the EU must conform to the provisions of Regulation 149/2008/EEC from 1st September, 2008.

Assessment of the quality of the imported cocoa will include measurement of substances that have been used upstream in the supply chain, including pesticides used on farms or in storage. The crop protection activities of farmers and middlemen will therefore be of great concern to all in the cocoa trade, some of whom may have a limited working knowledge of Pesticide science (Bateman, 2009). Regulation 149/2008/EEC of January, 2008 relates to a large number of products, of which cocoa is one and amends EC 396/2005 on maximum residue levels of pesticides in products of plant and animal

origin intended for human and animal consumption. The objective of this act is to ensure that pesticide residues in foodstuffs do not constitute an unacceptable risk for consumer and animal health (Bateman, 2009).

2.14. BIOLOGICAL CONTROL METHODS, ORGANIC PRODUCTION AND THE SEARCH FOR SUSTAINABILITY

Cocoa that is certified as being organic carries a substantial price premium. One of the issues for organic cocoa may be the withdrawal of permission to use copper fungicides which are already on restricted list (IFOAM: www: Ifoam.org accessed on 13th May, 2011).

In the EU, it was proposed that the use of copper should be below 8Kg/ha /year after 2002, and the international federation of organic Agriculture movements proposed that it should be withdrawn altogether by 2010 (IFOAM: www: Ifoam.org accessed on 13th May, 2011).

This probably represents a maximum of 5 sprays per season, which probably approaches the economically viable limit at normal application rates and cocoa prices. Research efforts have focused on biology- based technologies such as the use of pheromones to lure insects such as mirids and pod borers. Biological control (biocontrol) has been promoted frequently and, amongst the various strategies, which is of potential impotence are Classical Biological Control, Conservation of natural enemies and Biopesticides in cocoa (Bateman, 2009).

Biopesticides are one example of inundative biological control in which beneficial microbial organisms are often applied in the same way as their chemical equivalents.

One of the principles of Organic Agriculture (OA) is of course to minimize external inputs, but with many tropical crops-not least cocoa-crop losses can be extremely high (>50%) if pests remain unchecked (Bateman, 2009).

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CHAPTER THREE

MATERIALS AND METHODS

3.0 SELECTION OF STUDY SITES

The study was carried out in Asukese and its environs, a cocoa farming community located in Tano North District in Brong Ahafo Region of Ghana (Fig.5 and Fig. 6). Tano North is one of the 16 districts in which the Government of Ghana through cocoa Board initiated a National Cocoa Disease and Pest Control (CODAPEC) programme popularly known as 'mass spraying" to assist all cocoa farmers in the country to combat the capsid/Mirid and Black Pod disease as part of efforts to arrest the decline in cocoa production in 2001/2002 (Antwi, 2010). The black pod control programme covers all cocoa districts in the Volta, Brong Ahafo and parts of Western, Ashanti and Eastern regions.

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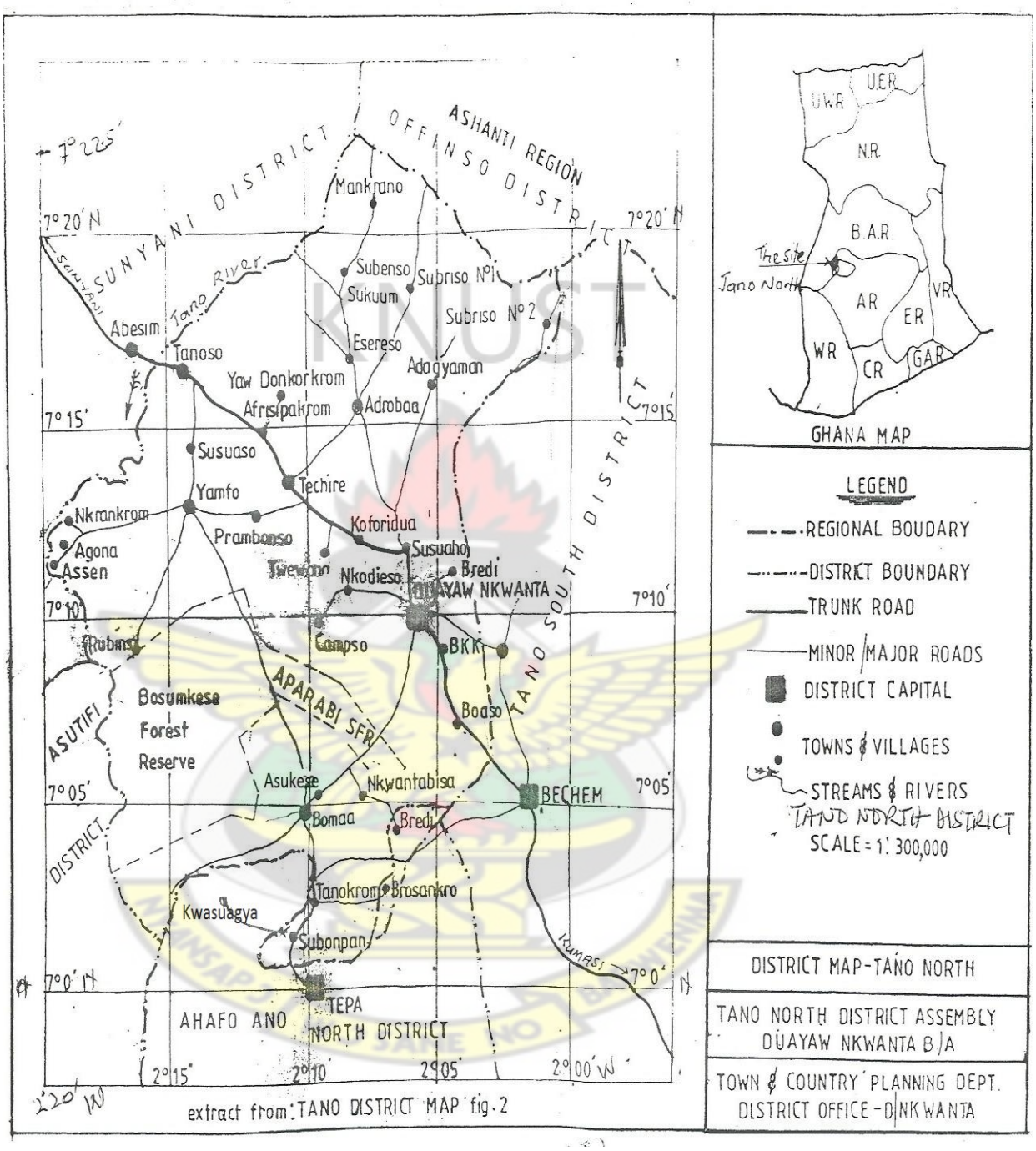

Fig. 4. Map of Tano North District

Fig. 5. Map of Asukese and its Environs

3.1. SAMPLE COLLECTION

Cocoa beans samples were collected from eleven study sites in Asukese and its environs in the Tano North Distirct. Each study site was made up of four cocoa farms. Ten sites were inorganic cocoa farms covered by CODAPEC programme while the eleventh site was made up of organic cocoa farms. The study was conducted between October 2010 and April, 2011. At each site, ten cocoa pods were taken from ten different cocoa trees from each cocoa farm in November 2010. A pod was taken from each tenth cocoa tree in a linear fashion. A total of 40 cocoa pods were collected in November, 2010 at each site. In all 440 cocoa pods were sampled in the 11 sites.

The pods were opened with a machete and the pulp and cocoa seeds were removed and the rind discarded. The pulp and the beans from each site were placed in bins and labelled appropriately and left to stand for seven days. During this time, the beans and

pulp underwent "sweating" where the thick pulp liquefied as it fermented. The fermented beans were air dried for a period of 21 days by spreading them on a large surface and constantly raking them.

One kilogram of dried cocoa beans from each sampling site were neatly packed into polyethylene bags and transported to pesticide residue analysis laboratory of the Ghana Standards Board, Accra, for preparation and subsequent residue analysis by Gas chromatography and mass spectrometry. This was replicated in January and March, 2011 and the means computed.

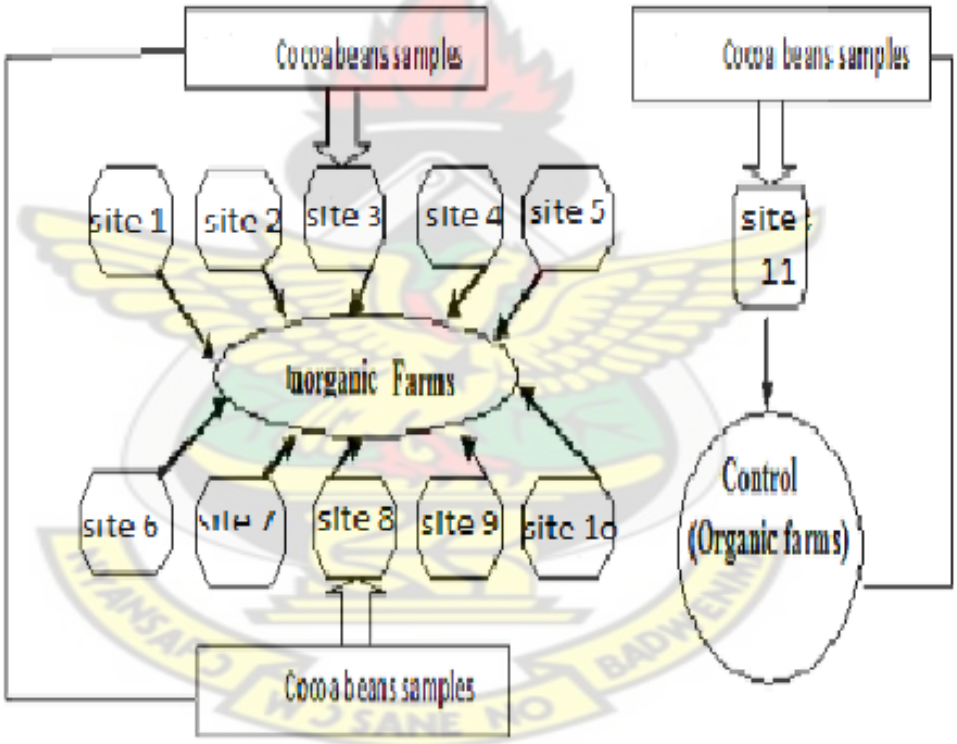

Fig. 6. Sampling design

3.2 LABORATORY PROCEDURES

3.2.1 Reagents

The reagents such as Acetonitrile, toluene, acetone, and n-hexane were of pesticide analysis grade, anhydrous magnesium sulphate and sodium chloride were of analytical grade (walko pure chemical industries, Osaka, Japan). Pesticide standards certified (wako; Kanto Chemical Co., Tokyo, Japan) was used. Each compound was dissolved in acetone to make a l mg/ml stock standard solution. Mixed-compound intermediate solutions were prepared from stock solutions at concentrations ranging from 40 to 100ug/ml. Two groups of spiking solutions were prepared from intermediate solutions containing approximately 140 compounds at the concentration of 5ug/ml. Spiking solutions were used for fortifying the cocoa samples and also for the calculation after appropriate dilution.

3.3. QUICK EASY CHEAP EFFECTIVE RUGGED SAFE (QuEChERS)

The fermented dried cocoa bean samples were prepared and analyzed using Quick Easy cheap Effective Rugged Safe (QuEChERS) method (Anastassiades, *et al.,* 2003). QuEChERS is the acronym for highly beneficial analytical approach that vastly simplifies the analysis of multiple pesticide residues in fruit vegetables, cereals and processed products thereof. The method is a multi-class, multi-residue method that can analyze for more than 100 pesticides, including the pyrethroids, in a variety of matrices. It is developed to avoid all time consuming step, to be cheap. The QuEChERS procedures entails a number of simple analytical steps and is thus fast and easy to

perform and little susceptible to errors. QuEChERS provide high recoveries for a very broad scope of pesticides belonging to various chemical classes and the final extract being dissolved in acetonitrile gives full flexibility in the choice of the determinative analysis technique.

In brief the procedure entailed the following steps:

- Weighing of 10g of cocoa sample into a beaker of water.
- Then 10ml acetonitrile and internal standard were added.
- The mixture was agitated intensively for a few minutes.
- NaCl, $MgSO_4$, and buffering salts for phase-separation and pH adjustment were added.
- The mixture was agitated intensively and centrifuged for raw extract.
- An aliquot of the upper organic phase was taken and subjected to dispersive solid phase extraction (d-SPE) cleanup by mixing it with 4g $MgSO_4$, and a sorbent to remove water and undesired co-extractives.
- The mixture was agitated shortly and centrifuged for final extract.
- The final extract was analyzed directly by GC-MS for pesticide residues in the cocoa extract.

3.3.1. **Blank extracts for the preparation of calibration solution**.

Matrix matched calibration was used to minimize errors associated with matrix induced enhancement or suppression effects during GC determination.

The Blank sample was treated the same way as any other sample, but no internal standard (ISTD) was added during extraction and cleanup.

The Department of Food Safety of the Ministry of Health, Labour and Welfare of Japan's Standard mix reference material (mixed cocoa standard) was used for the analysis of organochlorines, organophosphates and pyrethroids insecticide residues.

The standard was serially diluted and the retention times determined for each concentration. The calibration curves were then constructed for the analysis of the residues in the cocoa beans samples.

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3.3.1. SAMPLE PREPARATION

The dry samples of cocoa beans were separately milled and this step was carried out two times for each sample to achieve better extracts (plate 8).

Plate 8. Milling of cocoa beans

Ten grams (10g) of each sample was weighed into a beaker and 20ml of distilled water was added and made to stand for 15 minutes to dissolve water soluble pesticides if any. This was followed by stirring. Then 50 ml of Acetonitrile was added and macerated for

two minutes to dissolve non-water soluble pesticides in the samples. The spike and blank were then prepared using mixed cocoa standard of concentration 1mg/Kg and together with the samples were centrifuged for 3 minutes. The samples, blank and the spike were then topped to 100ml mark using acetonitrile.

3.3.3. DISPERSIVE SOLID PHASE EXTRACTION AND MOBILE PHASE SEPARATION

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Twenty millilitres (20 ml) of each sample was pipetted into a pp-single use centrifugation tube containing 500mg Primary Secondary Amine (PSA) (Buffer) and 3.36g magnesium sulphate. The tube was shaken vigorously for 10 seconds and centrifuged for 10 minutes. The mixture was then filtered into a separating funnel. The extract was allowed to settle for 10 minutes, separating into organic and aqueous solutions. The aqueous portion was drained off and the organic solution was drained into C18 cartridges for liquid or mobile phase separation. This was done by opening the under tap of the separating funnel. Co-extracted fat and waxes may negatively affect the ruggedness of the GC analysis and so the co-extracted fats and waxes in the samples were separated from the extracts to a large extent by putting them in the freezer for one hour. After a short centrifugation, the required amount of the still cold extract was withdrawn.

The mobile phase separation took care of all particles and foreign materials. The mobile phase separation set-up was connected to a pump which was switched on/off to achieve an increase in flow of samples out of the cartridges. This was achieved using C18 silica

based reversed phase sorbents together with PSA and magnesium sulphate in the dispersive solid phase extraction step.

The extracts were then filtered with sodium sulphate and chaired charcoal to remove water and concentrated afterwards using rotary pump.

The concentrated sample was washed by using ultrasonic barge with 10ml, 7ml and 5ml of Toluene acetone (3+1) and then concentrated again and reconstituted with ethyl acetate (1+9). The concentration of the sample represented by the test solution was 1g/ml.

After centrifugation, 1ml of the cleaned extract was transferred into a 2ml screw cap vial and pH was quickly adjusted by adding a 5% formic acid solution in acetonitrile (vol/vol) for GC-MS analysis.

3.4. GAS CHROMATOGRAPHIC AND MASS SPECTROMETRY DETECTION (GC-MS)

The GC-MS analysis was performed by a varian model star 3400 gas chromatograph equipped with electronic flow control (EFC) and fitted with a Saturn II ion trap mass spectrometer (plate 3.1). The GC chromatographic column consisted of BPx5 capillary column (SGE GMbH, Darmstadt, Germany), length 30m, internal diameter (I.D) 0.32mm and containing 5% phenyl-polysilphenlen-siloxane with a Phase thickness of 0.5um connected to the splitless injector. The carrier gas was helium (99.999%).

The oven temperature programme of GC was to hold the temperature initially at 60°C for 6 min to a final temperature of 280°C at a rate of 20°C per minute and then held at this temperature (280°C) for 8 minutes. A column head pressure of 11 p.s.i and an

injector temperature of 300°C were used. The injector was operated by manual holder into splitless mode (SPI/1077) for 6 min, the lapse of time for SPME fibre desorption was set at a fixed constant temperature of 300°C. The GC transfer line was maintained at continual 300°C.

The mass spectrometer was operated in the electron-impact ionization (EI) scan mode with a source temperature of 300°C. Ionization mode was obtained at fixed mode. The electron energy was 70ev and the filament current 10A. The manifold temperature was set at 1800°C. The electron multiplier voltage was established at 1800 volts. The amplitude voltage (AM) was 4.0volts. The external vent I was turned on. Chromatograms were acquired in 'Scan' mode, scanning the mass range from m/z 50 to m/z 300 (with scan rate 1000 milliseconds), with a background mass of m/z 45 segments acquire time for 25 min. In order to improve the peak identification, three fragment ions were monitored from the spectrum of each compound to quantify the response in the selected-ion monitoring (SIM) mode.

The mass spectrum of m/z 125, 236 and 294 for organochlorines retention time were 17.038 ± 0.2 min) and m/z 159, 270 and 272 for organophosphates and pyrethroids (Internal standard, Retention time 17.518 ± 0.2 min) were ion monitored as references.

The residues were identified by comparing the retention time of sample peaks with that of the standard.

Plate 9. GC/MS Analysis of pesticides

3.5. STATISTICAL ANALYSIS

The mean concentrations of residues were determined statistically using SPSS and compared to the European Union and Japanese maximum residue limits (MRLs) as a measure of safety to consumers and international cocoa trade. Least Significance Difference (LSD) at 5% was used to determine the significance of the differences among the means of the different sampling sites. LSD was established using ANOVA.

CHAPTER FOUR

RESULTS

4.0 ANALYTICAL TEST REPORT

The Analytical Test Report indicated that beta-HCH, delta-HCH, cyfluthrin, methoxychlor, pirimiphos-methyl, chlorfenvinphos, bifenthrin, methamidophos, phorate, diazinon, fonofos, lindane, heptachlor, allethrin, gamma chlordane, dieldrin, endrin, lambda-cyhalothrin were not detected in the fermented dried cocoa beans samples from all the 11 sampling sites including the control (organic).

The organochlorine insecticides detected in cocoa beans were aldrin, p,p-DDD, p,p-DDE, P,P-DDT, endosulfan sulphate, beta-endosulfan, alpha-endosulfan, and chlorpyrifos (Table 4.0). The organophosphate insecticides detected in cocoa beans were ethoprophos, fenitrothion, malathion, parathion, and profenofos (Table 4.0 and Appendix 5). The pyrethroid insecticides detected in cocoa beans were fenvalerate, deltamethrin, cypermethrin, and permethrin (Table 4.0 and Appendix 5).

The Limit of Detection (LOD) of organochlorines, organophosphates and synthetic pyrethroids were 0.005 mg/Kg, 0.010 mg/Kg and 0.010 mg/Kg respectively.

The mean concentration of insecticide residues in Cocoa samples juxtaposed to EU MRL, ADI and NOAEL are presented in Table 4.0

Table 3. Mean concentration of insecticides residues in fermented dried cocoa beans from Asukese and its environs in 2011

Location	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11	Lsd (0.05)	Cv%
Ethoprophos	ND	0.0573	ND	0.0033	0.0044	0.0134	0.0045	0.0051	0.0061	0.0047	0.0062	0.0009	5.34
Dimethoate	0.0153	ND	ND	0.0143	0.0153	0.0220	0.0240	0.0173	0.0191	0.0247	0.0237	0.0016	5.97
Permethrin	ND	0.0041	ND	0.0045	0.0144	0.0014	0.0047	0.0105	0.0147	0.0055	ND	0.0006	6.63
Fenitrothion	0.0737	0.0963	0.0591	0.0620	0.0537	0.0583	0.1591	0.0137	0.0781	0.0791	0.1143	0.0015	1.12
Malathion	ND	ND	0.0161	0.0537	0.0257	0.0227	0.0133	0.0171	0.0217	0.0517	0.0000	0.0014	3.98
Chlorpyrifos	0.0141	0.0210	0..0217	0.0110	0.0237	0.0133	0.0323	0.0130	0.0251	0.0337	0.0141	0.0022	6.44
Aldrin	ND	0.5614	ND	0.6368	ND	ND	ND	ND	ND	ND	ND	0.0355	0.04
Parathion	ND	ND	0.0071	0.0151	0.0161	0.0071	0.0074	0.0162	0.0161	0.0191	0.0107	0.0003	1.61
Profenofos	ND	0.0581	0.0511	ND	ND	ND	ND	ND	ND	ND	ND	0.0002	0.99
PP-DDE	0.0207	0.0253	0.0181	0.0146	0.0216	0.0242	0.0217	0.0314	0.0212	0.0153	0.0278	0.0002	0.62
Alpha-endosulfan	ND	ND	0.0012	0.0056	0.0112	0.0051	0.0217	0.0046	0.0057	0.0071	0.0047	0.0015	14.46
Beta-endosulf	ND	ND	ND	0.0031	ND	ND	ND	ND	ND	ND	ND	0.0000	3.07
Endosulfan sulfate	ND	ND	ND	0.0071	ND	ND	ND	ND	ND	ND	ND	0.0000	1.35
PP-DDD	0.0103	0.0147	0.0119	0.0084	0.0071	0.0087	0.0177	0.0177	0.0167	0.0181	0.0177	0.0000	0.23
PP-DDT	0.0184	0.0168	0.0952	0.0198	0.0183	0.0182	0.0187	0.0189	0.0192	0.0234	0.0154	0.0002	0.53
Cyprmethrin	ND	ND	0.1758	ND	ND	ND	ND	ND	ND	ND	ND	0.0675	0.05
Fenvalerate	ND	0.0878	0.0214	0.0114	0.0765	0.0789	0.0779	0.0898	0.0688	0.0888	0.0547	0.0000	0.10
Deltamethrin	ND	ND	ND	0.0035	ND	ND	ND	ND	ND	ND	ND	0.0000	2.61

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Table 4. Mean concentration of oganochlorine insecticides residues in cocoa samples from Asukese in 2011 juxtaposed to EU MRL, ADI, and NOAEL

Pesticide	Concentration Range (mg/Kg)		Means + standard Deviation	Lsd (0.05)	Cv%	Eu MRL (mg/Kg)	A D I (Mg/Kg bw/day)	NOAEL (mg/Kg bw/day)
	Minimum	Maximum						
Aldrin	< 0.005	0.6368	0.10893± 0.02423	0.0355	0.04	0.05(max)	0.0001	0.0010
P,P'-DDD	< 0.005	0.0181	0.01355± 0.00434	0.0000	0.23	0.50(max)	0.0010	0.0100
P,P'-DDE	< 0.005	0.0314	0.02199± 0.00505	0.0002	0.62	0.50(max)	0.0010	0.0100
P,P'-DDT	< 0.005	0.0952	0.02566± 0.02315	0.0002	0.53	0.50(max)	0.0010	0.0100
Endosulfan sulphate	< 0.005	0.0071	0.00065± 0.00214	0.0000	0.35	0.10(max)	0.0060	0.0600
Beta-endosulfan	< 0.005	0.0031	0.00028± 0.00093	0.0000	3.07	0.10(max)	0.0060	0.0600
Alpha-endosulfan	< 0.005	0.0217	0.00608± 0.00612	0.0015	14.46	0.10(max)	0.0060	0.0600
Chlorpyrifos	< 0.005	0.0337	0.02027± 0.00791	0.0022	6.44	0.10(max)	0.0030	0.0300

4.1. MEAN AND LSD ALL PAIRWISE COMPARISONS OF ACTIVE INGREDIENTS (AI) OF ORGANOCHLORINES INSECTICIDES BY TREATMENT

The average concentration of aldrin in the cocoa beans samples analyzed ranged between 0.5614 mg/Kg and 0.6368 mg/Kg. The highest concentration of aldrin of 0.6368 mg/Kg occurred at site 4 and the least concentration of aldrin of 0.5614 mg/Kg at site 2. The other remaining sites recorded no concentration of aldrin.

Statistically, there was difference observed between the Eleven (11) different Sites. ($P<0.05$).

P,p-DDD concentration in cocoa beans analyzed across all the sampling sites ranged between 0.0071 mg/Kg and 0.0181 mg/Kg. The highest concentration of p,p-DDD of 0.0181 mg/Kg was recorded at sampling site 10 while the lowest concentration of p,p-DDD of 0.0071 mg/Kg was detected at site 5. Statistically analysis indicated that, there were significant difference observed between all the various sampling sites ($P<0.05$).

P,p-DDE concentration ranged between 0.0153 mg/Kg and 0.0314 mg/Kg.

The highest average concentration of p,p-DDE of 0.0314 mg/Kg was recorded at sampling site 8 while the lowest concentration of 0.0153 mg/Kg was registered at site 10. All the sampling sties registered p,p-DDE concentration. Differences were statistically observed between the various sampling sites as indicated by the 'P' value which was less than 0.05 ($P<0.05$).

The concentration of p,p-DDT in samples of cocoa ranged from 0.0154 mg/Kg and 0.0952 mg/Kg. The maximum p,p-DDT of 0.0952 mg/Kg was recorded in samples from sampling site 3 while the minimum p,p-DDT residue of 0.0154 mg/Kg was registered in sampling site 11. The statistical analysis gave a 'p' value less than 0.05 ($P<0.05$) showing a significant differences between p,p-DDT concentrations in the various individual sampling sites.

Endosulfan sulphate concentration of 0.0071 mg/Kg was recorded at sampling site 4 only. All the other sampling sites recorded no detectable Endosulfan sulphate concentration.

Statistical analysis revealed that, there were significant difference observed between the individual sites ($P<0.05$).

Beta-endosulfan concentration of 0.0031 mg/Kg was recorded only in samples from sampling site 4. Samples from all other sampling sites recorded no detectable concentration of beta-endosulfan. Statistically, there were significant difference observed between the various sites ($P<0.05$).

The highest concentration of alpha-endosulfan of 0.0217 mg/Kg was recorded in the samples of cocoa beans from site 7 and the lowest concentration of 0.0012 mg/Kg was registered at site 3 while sites 1 and 2 recorded no detectable Alpha-endosulfan residue. Statistically, there were significant difference observed between the various sampling sites ($P<0.05$).

The range of chlorpyrifos concentration in cocoa beans across the sampling sites ranged between 0.0110mg/Kg and 0.0337 mg/Kg.

Sampling site 10 registered the highest average chlorpyrifos concentration of 0.0337 mg/Kg while site 4 recorded the least chlorpyrifos concentration of 0.0110 mg/Kg. Statistically, significant difference were observed between the individual sites ($P<0.05$)

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Table 5. Mean concentration of organophosphate insecticides residues in cocoa samples from Asukese in 2011 juxtaposed to EU MRL, ADI, and NOAEL

Pesticide	Concentration Range (mg/Kg)		Means + standard Deviation	Lsd (0.05)	Cv%	Eu MRL (mg/Kg)	A D I (Mg/Kg bw/day)	NOAEL (mg/Kg bw/day)
	Minimum	Maximum						
Ethoprohos	< 0.010	0.0573	0.00955± 0.01623	0.0009	5.34	0.02(max)	0.0003	0.0300
Dimethoate	< 0.005	0.0247	0.01597± 0.00873	0.0016	5.97	0.05(max)	0.0200	0.2000
Parathion	< 0.010	0.0191	0.01045± 0.00666	0.0003	1.61	0.10(max)	0.0050	0.0500
Fenitrothion	< 0.010	0.1143	0.07704± 0.03737	0.0015	1.12	0.20(max)	0.0020	0.0200
Malathion	< 0.010	0.0537	0.02018± 0.01858	0.0014	3.98	0.05(max)	0.0010	0.0500
Profenofos	< 0.010	0.0581	0.00993± 0.02214	0.0002	0.99	0.01(max)	0.0001	0.0072

4.2. MEAN AND LSD ALL PAIRWISE COMPARISONS OF ACTIVE INGREDIENTS (AI) OF ORGANOPHOSPHATES INSECTICIDES BY TREATMENT

The range of Ethoprophos concentration in cocoa beans across the sampling sites ranged between 0.0033 mg/Kg and 0.0573 mg/Kg (Appendix 5).

Sampling site 2 recorded the highest average Ethoprophos concentration value of 0.0573 while sites 1 and 3 registered no Ethoprophos compounds. Site 4 recorded the least average Ethoprophos concentration of 0.0033 mg/Kg. Statistically, significant difference was observed between the various sites ($P < 0.05$) (Appendix 6)

Dimethoate concentration in the sampling site 10 recorded the highest concentration value of 0.0143 mg/Kg whilst Site 4 recorded the lowest concentration of 0.0143

mg/Kg. Sites 2 and 3 however recorded no Dimethoate Concentration. Statistically, there was significant difference observed between the individual sites ($P < 0.05$).

The highest concentration of parathion of 0.0191 mg/Kg was recorded at site 10 while the lowest of 0.0071 mg/Kg was recorded at sites 3 and 6. Samples from sites 1 and 2 registered no concentration of Parathion.

Statistical analysis revealed that, there were significant difference between the eleven (11) different sites ($P<0.05$).

The highest average concentration of fenitrothion was recorded at site 11 with average concentration value of 0.1143 mg/Kg. The lowest concentration of fenitrothion occurred at site 8 with a concentration value of 0.0137 mg/Kg. There was no site which did not record concentration of fenitrothion.

The "P" value was less than 0.05 ($P < 0.05$) indicating significant difference in fenitrothion concentration between the various sites that were under study.

Site 4 recorded the highest average concentration of malathion of 0.0537 mg/Kg while sites 7 recorded the lowest average concentration of malathion of 0.0133 mg/Kg. Sites 1, 2 and 11 had not concentration of malathion.

There were significant difference in concentration of malathion between the various sites since the 'P' value was less than 0.05 ($P < 0.05$).

Site 2 recorded the highest concentration of profenofos of 0.0581/mg/Kg followed by site 3 which registered 0.0511 mg/Kg of profenofos concentration.

The "P' value obtained from the statistical analysis was less than 0.05 ($P<0.05$) indicating a significant difference between the two sites.

Table 6. Mean concentration of pyrethroid insecticides residues in cocoa samples from Asukese in 2011 juxtaposed to EU MRL, ADI, and NOAEL

Pesticide	Concentration Range (mg/Kg)		Means + standard Deviation	Lsd (0.05)	Cv%	Eu MRL (mg/Kg)	A D I (Mg/Kg bw/day)	NOAEL (mg/Kg bw/day)
	Minimum	Maximum						
Cypermethrin	< 0.010	0.1758	0.01598± 0.05301	0.0675	0.05	0.10(max)	0.0500	5.0000
Fenvalerate	< 0.010	0.0898	0.05964± 0.03316	0.0000	0.10	0.05(max)	0.0200	1.7000
Permethrin	< 0.010	0.0147	0.00544± 0.00548	0.0006	6.63	0.01(LOD)	0.050	5.0000
Deltamethrin	< 0.010	0.0035	0.00032± 0.00106	0.0000	2.61	0.05(max)	0.0100	1.0000

4.3 MEAN AND LSD ALL PAIRWISE COMPARISONS OF ACTIVE INGREDIENTS (AI) OF PYRETHROIDS INSECTICIDES BY TREATMENT

Cypermethrin concentration of 0.1758 mg/Kg was recorded only at sampling site 3. There was however no detectable concentration of cypermethrin in the other 10 sampling sites.

Statistically, there were significant differences observed between the various sites ($P < 0.05$).

The concentration of fenvalerate ranged from 0.0114 mg/Kg to 0.0898 mg/Kg.

The highest fenvalerate residue concentration was recorded at site 8 while the lowest residue was detected at site 4. Site 1 however did not register fenvalerate residue concentration. The "P" value obtained for fenvalerate statistical analysis was less than 0.05 signifying a significant difference observed between the various sites.

The average concentration of permethrin ranged between 0.0014 mg/Kg and 0.0147 mg/Kg with the highest average concentration obtained at site 9 and the lowest obtained at site 6. Site 1 and 11 registered no concentration of permethrin. Statistical analysis

revealed significant difference in permethrin concentration between the various sampling sites ($P < 0.05$).

Deltamethrin concentration of 0.0035 mg/Kg was recorded only at site 4 while all other sites recorded no Deltamethrin concentration (Appendix 5).

Statistically, there were significant differences observed between the various sites ($P < 0.05$) (Appendix 6).

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4.4. COMPARISON OF MEAN CONCENTRATION OF ORGANOCHLORINE RESIDUES DETECTED IN COCOA BEANS FROM ASUKESE IN 2011 WITH EU MRLs.

The average concentration of aldrin found in samples of sites 2 and 4 were above the EU MRL of 0.05 mg/Kg.

The Concentration of p,p-DDD detected in the cocoa bean samples from all the sampling sites were found to be below the EU MRL of 0.50 mg/Kg.

P,p-DDE concentration found in the sampling sites were all below the EU MRL of 0.5 mg/Kg.

The concentrations of p,p-DDT detected in the various sampling sites were found to be below the EU MRL of 0.50 mg/Kg.

The endosulfan sulfate concentration found in cocoa beans from sampling site 4 was below the EU MRL of 0.1 mg/Kg.

The concentration of beta-endosulfan found in site 4 was below the European Union (EU) Maximum Residue Limit (MRL) of 0.1 mg/Kg.

Alpha–endosulfan concentration detected in the cocoa beans samples from all the sampling sites were within the EU MRL of 0.1 mg/Kg.

The average concentration of chlorpyrifos found in all the sites including the control were below the EU MRL of 0.1 mg/Kg and the Japan MRL of 0.05 mg/Kg.

4.5. COMPARISON OF MEAN CONCENTRATION OF ORGANOPHOSPHATE RESIDUES DETECTED IN COCOA BEANS FROM ASUKESE IN 2011 WITH EU MRLs

The mean concentration of Ethoprophos found in all the sites were below the European Union (EU) Maximum Residue Limit (MRL) of 0.02 mg/Kg except sampling site 2 which recorded ethoprophos concentration above the EU Maximum Residue Limit of 0.02 mg/Kg.

The average concentrations of Dimethoate found in all the sampling sites were below the European Union (EU) MRL of 0.05 mg/kg.

The average concentrations of parathion in cocoa beans analyzed from all the sampling sites were found to be below the European Union MRL of 0.1 mg/Kg.

The average concentrations of fenitrothion found in all the sites were below MRL of the EU of 0.2 mg/Kg. However, concentrations at sites 7 and 11 were found to be above the Japanese MRL of 0.1 mg/Kg.

The average concentration of malathion found in sites 3, 5, 6, 7, 8 and 9 were below the EU MRL of 0.05 mg/Kg while sites 4 and 10 were above the EU MRL of 0.05 mg/Kg.

The average concentrations of Profenofos in the sampling sites which registered it were above the EU MRL of 0.01 mg/Kg.

4.6. COMPARISON OF MEAN CONCENTRATION OF PYRETHROID RESIDUES DETECTED IN COCOA BEANS FROM ASUKESE IN 2011 WITH EU MRLs

Cypermethrin concentration found in cocoa beans was above the EU MRL of 0.10mg/Kg and that of the Japanese MRL of 0.03 mg/Kg.

Fenvalerate concentration in cocoa bean samples from all the sampling sites were found to be above the EU MRL of 0.05 mg/Kg and that of the Japanese MRL of 0.01 mg/Kg

The average concentration of permethrin in sample site 5, 8 and 9 were found to be above the limit of Detection (LOD) of 0.01 mg/Kg. All other sites registered concentration of permethrin below the LOD.

Deltamethrin concentration in cocoa bean samples were below the EU MRL of 0.05 mg/Kg and that of Japanese MRL of 0.05 mg/Kg.

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CHAPTER FIVE

DISCUSSION

5.0. LEVELS OF ORGANOCHLORINE INSECTICIDES RESIDUES IN SAMPLES OF FERMENTED DRIED COCOA BEANS

The Organochlorine (OC) insecticide residue found in samples of cocoa beans were aldrin, p,p-DDD p,p -DDE, p,p-DDT, endosulfan sulphate, beta-endosulfan, alpha-endosulfan, and chlorpyrifos. Aldrin, p,p-DDD, p,p-DDE, p,p-DDE, p,p-DDT and endosulfan sulphate are WHO/EPA category II insecticides banned for use in the cocoa industry by the European Union (EU) and Japan (Bateman, 2010).

The Presence of p,p-DDD, p,p-DDE, p,p-DDD, endosulfan sulphate, beta-endosufan and alpha-endosulfan in the cocoa beans analyzed showed that DDT and endosulfan are still being used or were used some years back and are still present in the soil and in the cocoa trees which is an indication of the persistent nature of insecticides. The sources of endosulfan and DDTs could have originated form historical application rather than new input, suggesting the efficient management by CODAPEC in restricting the application of organochlorine inseticides such as endosulfan and DDTs. The low concentrations or non-detectable levels of aldrin, dieldrin, heptachlor, trans-heptachlor epoxide, cis-heptachlor epoxide, trans-nanochlor, and trans-chlordane, indicate a possible phasing out of these persistent organic pollutants.

The findings agree with an earlier study by Menlah (2008) that although the organochlorines are banned from importation, sales and use in Ghana, there are evidence of their continued usage and presence in the ecosystem. In a study to analysed pesticides

and pathogen contamination of vegetables in Ghana's urban markets, Amoah *et al.*, 2006) documented that chlorpyrifos (Dursban) was detected on 78% of lettuce, Lindane on 31%, endosulfan on 36%, Lambda cyhalothrin (Karate) on 11% and DDT on 36%.

Similar work already done in some farming communities in the Ashanti Region of Ghana and some other countries indicates the presence of organochlorine insecticide residues in fish (Osofo and Frempong, 1998) vegetables, water, sediments, mother's milk, and blood samples (Menlah, 2008).

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Edwards (2006) found traces of highly toxic chemical sprayed on most of the world's cacao crops present in most of the chocolate and Easter eggs-on sale in Britain.

The pesticide levels found in the present study were comparable to a study by Osafo and Frempong (1998). Although DDT is banned for agricultural use in Ghana, it was detected in sediment samples, along with its metabolite, DDE and the study demonstrated the well-known environmental persistence of this substance, even in tropical environments (Kidd *et al*., 2001), justifying its prohibition from agricultural use in Ghana. The DDT concentration in the sediment, however, was lower than the DDE level indicating a high degradation rate (Jiries, 2002).

The mean concentration of aldrin was found to be above the EU MRL of 0.05mg/Kg, ADI of 0.0001mg/Kg bw/day and the no observable adverse effect level (NOAEL) of 0.01 mg/Kg bw/day. The presence of aldrin residue in fermented dried cocoa at levels beyond the EU MRL makes it unacceptable on the European Union market and residues above the Acceptable Daily Intake (ADI) is a threat to public health and steps need to be taken to prevent residue accumulation in the cocoa beans.

P,p-DDD, p,p-DDE, and p,p-DDT were within their various MRLs but above the Acceptable Daily Intake of 0.001mg/Kg bw/day.

Endosulfan sulphate, beta-endosufan and alpha-endosulfan were all below their various MRLs, however endosulfan sulphate and alpha-endosulfan were above the Acceptable Daily Intake set at 0.006 mg/Kg bw/day. Endosulfan has endocrine disrupting potential and does induce oxidative stress leading to inhibition of cellular respiration (Ho-Yong, 2004). It has been implicated in a decrease of semen quality, as well as increase in testicular and prostate cancer (Pastore *et al*., 1997).

Chlorpyrifos has permitted MRL in some markets, but not others and the MRL is only temporary MRL which will be phased out in 2-3 years (Bateman, 2010).

The mean highest concentration of chlorpyrifos was within its MRL but above the Acceptable Daily Intake (ADI) of 0.003 mg/Kg bw/day and the no observable adverse effect level (NOAEL) of 0.03 mg/Kg bw/day for humans.

Chlorpyrifos concentration level detected in cocoa samples is acceptable in the international market on the basis of its MRL but would have serious public health implications. Chlorpyrifos has the potential for both acute toxicity at larger amounts as well as neurological effects on fetuses and children even at very small amounts. FAO/EPA classifies chlorpyrifos as class II moderately toxic pesticide. Chlorpyrifos poisoning has been described by New Zealand Scientists as the likely cause of death of several tourists in Thailand who developed myocarditis in 2011 (www.Cvent.com/events/toxins-2011/event accessed on 20th June, 2011).

Dimethoate is one of the strategic recorded organophosphate insecticides for use in the cocoa industry. It belongs to WHO/EPA toxicity Class II. EU MRL of Dimethoate remains TMRL and its status should be regularly checked before use.

Dimethoate mean concentration found in the samples of cocoa was below the EU MRL of 0.05mg/kg but above the Acceptable Daily Intake ADI of 0.02 mg/Kg bw/day. The no observable adverse effect level of dimethoate for humans is set at 0.2 mg/Kg bw/day which is higher than that of the dimethoate concentration detected.

Dimethoate concentration level in the cocoa samples analysed are acceptable at the international market. It is however; very important that steps are taken to ensure that there is no further accumulation of dimethoate in the cocoa beans

Dimethoate is an anticholinesterase which disables cholinesterase an enzyme essential for central nervous system function.

This study further agrees with a study conducted by Ntow *et al.,* (2001), which assessed the accumulation of persistent organochlorine contaminants in milk and serum of farmers in Ghana. The levels of DDTs in the breast milk samples were found to correlate positively with the age of the milk sample of female donors. DDTs residues detected in blood serum were significantly higher ($p < 0.005$) in males than females and there was association between breast milk and serum residues in females. When the daily intakes of DDTs of infants through human breast milk were estimated, some individual farmers in the case of DDTs accumulated OCs in breast milk above the threshold (tolerable daily intake guidelines proposed by Health Canada) for adverse effects, which may raise concern on children health.

The measured concentrations of organochlorines, organophosphates, and the pyrethroids insecticides were all lower than some of the similar published studies in Africa (Kishimba *et al.,* 2004., Westbom *et al.,* 2008), Asia Hu *et al*., 2009; Keithmaleesatti *et al.,* 2009; Wang *et al*., 2009, Zhang *et al.,* 2011) and Europe (Covaci *et al*., 2001). The detection of lower levels of DDT than its metabolite and DDE, in the samples implies

that the presences of these contaminants in the farms might be due to past usage of the pesticides on the farms. This finding agrees with that of Darko *et al*. (2008) who studied organochlorine insecticides in the fish and sediments of Lake Bosomtwi, in Ghana.

5.1. LEVELS OF ORGANOPHOSPHATE INSECTICIDES RESIDUES CONCENTRATION IN SAMPLES OF FERMENTED DRIED COCOA BEANS

The Organophosphate insecticides residues registered in fermented dried cocoa samples were ethoprophos, fenitrothion, malathion and parathion. Fenitrothion and parathion are WHO Category II insecticides. They are moderately hazardous with acute oral LD>50<500 (WHO, 1992) which have been banned for use in the cocoa industry by the EU and Japan (Bateman, 2010). Fenitrothion highest mean concentration in cocoa samples was 0.1143 mg/Kg (Table 4.0 and Fig. 4.1) which is lower than the EU MRL of 0.2 mg/Kg. The residue level of fenitrothion would be acceptable in the European Union Markets at least for now, however steps must be taken to eliminate it completely since fenitrothion is a banned chemical in the cocoa industry. It can cause cholinesterase inhibition in humans, that is, it can over stimulates the nervous system causing nausea, dizziness, confusion, and at very high exposures such as accidents or major spills, respiratory paralysis and death may occour (www.epa.gov/pesticides/op).

Parathion mean highest concentration of 0.0191 mg/Kg was slightly higher than the EU MRL of 0.10 mg/Kg. It is however much higher than the Acceptable Daily Intake and the no observable adverse effect level values of 0.005 mg/Kg bw/day and 0.05 mg/Kg bw/day for humans respectively.

These values of residue would not be acceptable at the international cocoa market since parathion use is not permitted in the cocoa industry. It would be accepted if the EU has cause to believe that the residue is not due to recently used chemical but were persistently available in the environment (Bateman, 2010). Steps must be taken to eliminate Parathion residue from cocoa beans. Parathion is a cholinesterase inhibitor. It generally disrupts the nervous systems by inhibiting the acetylcholinesterase. It is absorbed via the skin mucous membranes, and orally. Absorbed Parathion is rapidly metabolized to paraoxon. Paraoxon exposure can result in headaches, convulsions, poor vision, vomiting, abdominal pain, severe diarrhoea, unconsciousness, tremor, dyspnoea, and finally lung-edema as well as respiratory arrest, symptoms of poisoning are known to last for extended periods of time, sometimes months (US EPA, 2007).

Parathion is considered to be a possible human carcinogen (US EPA, 2007). Studies have shown that parathion is toxic to foetuses, but does not cause birth defects (Pesticide information profiles-parathion, 1993).

Parathion is very toxic to bees, fish, birds, and other forms of wildlife (Pesticide information profiles-parathion, 1993). Parathion can be replaced by many safer and less toxic alternatives.

Malathion mean concentration in cocoa samples was found to be slightly above the EU MRL of 0.05 mg/Kg and the Acceptable Daily Intake of 0.001 mg/Kg bw/day. Malathion is a chemical permitted for use in cocoa industry but with great caution.

It has permitted MRL in some markets, but not others and may be phased out within 2-3 years (Bateman, 2010). Cocoa products with this level of malathion concentration may be rejected at the EU market. Steps must be taken to eliminate it entirely. Malathion itself is of low toxicity, however, absorption or ingestion into the human body readily

results in its metabolism to malaoxon, which is substantially more toxic (Edwards, 2006) Chronic exposure to low levels of malathion have been hypothesized to impair memory, but this is disputed. Possible symptoms include skin and eye irritation, cramps, nausea, diarrhoea, excessive sweating, seizures and even death.

Ethoprophos concentration in cocoa samples was above the EU MRL of 0.02 mg/Kg, the Acceptable Daily Intake of 0.0003 mgKg, and the No observable adverse effect levels of 0.030 mgKg. Cocoa beans with high Ethoprohos residues would be unacceptable at the EU market. Profenofos highest mean residue concentration of 0.0581 mgKg was higher than the EU MRL, ADI, and the NOAEL of 0.01 mgKg, 0.0001 mgKg bw/day, and 0.0072 mgKg bw/day respectively.

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5.2. LEVELS OF PYRETHROID INSECTICIDE RESIDUES IN SAMPLES OF FERMENTED DRIED COCOA BEANS

The pyrethroids insecticide residue recorded in the cocoa beans samples analyzed from all the 11 sites were fenvalerate, Deltamethrin, Cypermethrin and Permethrin (Table 4.0) Fenvalerate mean highest concentration of 0.0898 mg/Kg was found to be above the EU MRL of 0.05 mg/Kg and ADI of 0.020 mg/Kg but lower than the NOAEL value of 1.7 mg/Kg bw/day. Fenvalerate insecticide is to be used with great caution. It has permitted MRL in some markets but not others and belongs to WHO/EPA toxicity class II.

Residue of fenvalerate above the EU MRL of 0.05 mg/Kg would not be acceptable in the International market. Steps therefore need to be taken to reduce fenvalerate accumulation in cocoa beans.

Cypermethrin and deltamethrin are recorded insecticides approved for use in cocoa industry. They do not belong to WHO/EPA toxicity class 1 and have tMRLs in most markets. The highest mean concentration of cypermethrin occurring in a sample was slightly higher than the EU MRL of 0.10 mg/Kg and would be unacceptable at the EU markets. This could results in the rejection of cocoa produce in Ghana on the EU market. The concentration of cypermethrin of 0.1758 mg/Kg was found to be above the ADI of 0.050 mg/Kg bw/day and could have undesirable consequences on human health. Excessive exposure of cypermethrin can cause nausea, headache, muscle weakness, salivation, shortness of breath and seizures (Baselt, 2008). In humans cypermethrin is deactivated by enzymatic hydrohysis to several carboxylic acid metabolites which are eliminated in the urine (Baselt, 2008). Many products containing cypermethrin are classified as restricted use pesticides (RUP) by the FAO/EPA because of cypermethrin's toxicity to fish (Baselt, 2008).

Deltamethrin mean highest concentration recorded in cocoa samples was 0.0035 mg/Kg which is far below the EU MRL of 0.05 mg/Kg and the Acceptable Daily Intake of 0.01 mg/kg bw/day. This level of deltamethrin concentration is not problematic on the EU market, because Deltamethrin is an approved Active ingredient for use in the cocoa sector.

Permethrin mean concentration of 0.0144 mg/Kg was moderately higher than the EU MRL of 0.01 mg/Kg and the ADI value of 0.05 mg/Kg bw/day. Permethrin is not permitted for use in the cocoa sector by the EU. Cocoa products with permethrin residues constitutes unacceptable product in the international market.

Residues of pesticides approved for use in the cocoa sector under the CODAPEC (mass Cocoa Spraying exercise) were not recorded in the samples of cocoa beans analyzed in all the 11 sampling sites. CODAPEC fungicides such as ridomil Gold 66 plus WP (cuprous oxide + mefenoxam), metalm 72 plus Wp (Cuprous oxide + metalaxyl), nordox 75 WG(Cuprous oxide), funguran-OH WP (cupric hydroxide), champion WP (cuprice hydroxide) and kocide 2000 WP (Cupric hydroxide), fungikill WP (cupric hydroxide + metalaxyl) and agro-comet WP (cuprous oxide + metalaxyl) did not record any residue in the fermented dried cocoa beans.

Similarly CODAPEC insecticides such as confidor (imidacloprid), akate master (bifenthrin) and actara (thiamethoxam) did not record any residue in the fermented dried cocoa beans. Farmers may have privately sprayed other unapproved pesticides on their cocoa farms.

The study conducted showed no significant difference between pesticide residues in Inorganic cocoa farms and the organic cocoa farms (Control) in Tano North of Brong Ahafo as indicated in the statistical results (Appendix 6). Out of the eighteen active ingredients of pesticides detected in the cocoa samples, the organic cocoa farms recorded fifteen active ingredients (Appendix 5). The above might be due to the persistence of these insecticides in the environment such as in the soil and in the cocoa trees following long periods of pesticides use in agriculture.

The study is in line with literature that; the bulk of pesticides residues are generally confined to the upper 1-2 inches of soil and although vertical transport of pesticides

through soil by water is limited, water can wash away the soil particles that contain pesticide residue.

This study agrees with that of Owusu-Ansah *et al.*, (2010) that the following factors may have contributed to the low levels of pesticide residues in the sampled cocoa beans: biological degradation as a result of microbial metabolism of pesticides, which is often the main source of pesticide degradation in soils. It occurs when fungi, bacteria, and other microorganisms in the soil use pesticides as food or other energy source, or consume the pesticides along with other sources of food or energy. Soil organic matter content, moisture, temperature, aeration, and pH all affect microbial degradation (Owusu-Ansah *et al*., 2010).

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CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.0. **CONCLUSIONS**

The study revealed that fermented dry cocoa beans in Asukese and its environs contain residues of banned organochlorine insecticides. Aldrin, p,p-DDD, p,p-DDE, p,p-DDT and endosulfan which are WHO/EPA category II insecticides banned for use in the cocoa sector by the EU were detected in the fermented dry cocoa beans analysed (Appendix 5).

The study found out that ethoprophos, permethrin, fenitrothion, malathion, aldrin, profenofos, cypermethrin, and fenvalerate insecticide recorded residue concentrations 0.0573 mg/Kg, 0.1143 mg/Kg, 0.0537 mg/Kg, 0.6368 mg/Kg, 0.0581 mg/Kg,0.1758 mg /Kg and 0.0898 mg/Kg respectively which are higher than their various EU MRLS in all the samples or at least one of the samples. Ethoprophos, fenitrothion, malathion, aldrin, cypermethrin, and fenvalerate insecticides residues were above their various Acceptable Daily Intakes (ADIs) and No Observable Adverse Effect Level (NOAEL).

P,p-DDT, Chlorpyrifos, p,p-DDE, Dimethoate, endosulfan, parathion, p,p-DDD, Permethrin and Deltamethrin residues were however within their various MRLs and NOAELs .

Residues of pesticides approved for use in the cocoa sector under the CODAPEC (mass Cocoa Spraying exercise) such as Ridomil Gold 66 plus WP (cuprous oxide +

mefenoxam) Metalm 72 plus Wp (Cuprous oxide + metalaxyl), nordox 75 WG (Cuprous oxide), funguran-OH WP (Cupric Hydroxide), Champion WP (Cuprice hydroxide) and kocide 2000 WP (Cupric hydroxide), fungikill WP (Cupric Hydroxide + metalaxyl) and Agro-comet WP(Cuprous oxide + metalaxyl) for spraying against the black pod disease were not recorded in the samples of cocoa beans analysed in all the 11 sampling sites. Similarly, CODAPEC insecticides which include confidor (Imidacloprid), Akate master (Bifenthrin) and Actara (Thiamethoxam) for miridae (capsids) were also not detected in the samples of cocoa beans analysed in all the 11 sampling sites (Appendix 5).

The study conducted showed no significant difference between pesticide residues in inorganic cocoa farms and the organic cocoa farms (Control) in Asukese and its environs (Appendix 6). Out of the Eighteen Active ingredients of pesticides detected in the fermented dry cocoa beans samples, the organic cocoa farms recorded fifteen active ingredients including ethoprophos, dimethoate, fenitrothion, malathion, chlorpyrifos, parathion, p'p DDE, alpha endosulfan,, p'p DDD, p'p DDT, and fenvalerate.

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6.1. RECOMMENDATIONS

1. Good Agricultural practice (GAP) must be introduced and encouraged among farmers in the cocoa sector.

2. Rational and scientific pesticide use such as pesticide-use skills at the farm and extension service levels must be intensified.

3. It is also suggested that, COCOBOD identify a positive list of strategic cocoa pesticides and recommend for specific important pests and stages in the supply chain. Extra special care is needed for pesticides used against storage pests, in warehouses and in cocoa transport.

4. Cocoa farmers must be educated on basic pesticide science to create awareness of the problem of pesticide residue limits.

5. CODAPEC must institute a monitoring mechanism to make sure that farmers who privately spray their farms do so with approved cocoa pesticides.

6. The Government of Ghana through EPA would have to enforce the restriction of the importation, sale and use of obsolete and banned pesticides in the country.

7. Pesticide residue levels of cocoa should be tested at the point of production to ascertain its quality.

8. Further research should be carried out to determine the concentration of pesticide residues in the soil, rivers and in the cocoa trees.

9. The Crop Research Division of Council for Scientific and Industrial Research (CSIR) in collaboration with Cocoa Research Institute of Ghana (CRIG) should research into Integrated Pest Management for cocoa to solve the problem of pesticide residues bedevilling the cocoa sector in Ghana.

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APPENDICES

Appendix 1:

Lists of strategic / recorded pesticides for use in cocoa

All these AIs:

Are known to be on 91/414/EEC Annex 1(s Y, or pending-P); See box F1.

EU MRL s (Mg. Kg-1) remain tMRLs and their stats should be checked regularly; those listed here refer to Cocoa fermented beans) as in Reg. (EC) no 396/2005.

Have shown demonstrable efficacy in at least one regional cocoa growing country

Do no belong to WHO/EPA toxicity Class 1.

(i) black pod diseases:

Active ingredients	Mo A group	EU status s	EU MRL	JP MRL
Benalaxyl	A1	Y*	0.1	0.01
Copper hydroxide	M1	Y	Cuions:	α
Copper Oxide	M1	Y	50.0	α
Copper oxychloride	M1	Y		α
Fosety 1 aluminium	P	Y	2.0	0.05
Metalaxyl-M (mefenoxam)	A1	Y	0.1	0.02

(ii) insects

Active ingredients		Mo A group	EU status s	EU MRL	JP MRL
As sprays (mostle against mirids					
Acetamiprid		4A	Y	0.1	0.01
Beta-cyfluthrin	β	3	Y	0.1	0.1

Cypermenthrin (α isomer- β)	3	Y*	0.1	0.03
Deltamethrin β	3	Y	0.05	0.05 α
Dimethoate	1B	Y	0.05	0.05
Imidacloprid	4A	P	0.05	0.05
Lambda-cyhalothrin	3	Y*	0.05	0.01
Thiamethoxam	4A	Y	0.05	0.02

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Termite Control

Fipronil y β	2	P	0.005y	0.01

(iii) Weeds

Active ingredients	MoA group	EU status	EU	MRL JPMRL
2,4-D dimethylamine salt	0	Y*	0.1	0.01
Glyphosate trimesium	G	Y	0.1	0.2
Glyphosate isoprophylamine	G	Y	0.1	0.2

(IV) Store produce	MoA group	EU status	EU	MRL JPMRL
Active ingredients	24	Y	0.05	0.01(as
Aluminium phosphide hydrogen	24	Y	0.05	

Phosphide)				
Pyrethrins (pyrethrum) for fogging	3	Y	0.5	1.0
Pyrethroids (treating sacks, etc.)	3	if yes as above		

* High residue levels have been found in imported produce to the EU and/or Japan

α No MRL given in Japan and copper is exemptin the USA: see box B1

β Registered (Widely used) for cocoa pod borer control in Indonesia

y Fipronil (Sum fipronil + sulfone metabolite (MB46136) expressed as fipronil) includes deltamethrin (as total)

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Appendix 2:

Compounds to be used with great CAUTION (limited time span, restricted markets, etc)

These AIs:

Have permitted MRls in some markets, but not others and/or

Many of these are Temporary (tMRLs) and are likely to be phased out within 2-3 years, but

Have shown demonstrable efficacy in at least one regional cocoa growing country

Do not belong to WHO/EPA toxicity Class 1

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(i) Black pod diseases

Active ingredients	MoA group	EU status	EU MRL	JPMRL
Metalyxyl (unresolved) (See Box B1)	A1	Y Until 06/2010 (All isomers)	0.1(T)	

(ii) Insects

Active Ingredients	MoA group	EU status	EU MRL	JPMRL
Bifenthrin	3	N	0.1	0.1
Diazinon	1B	N	0.02	0.05
Chlorpyrifos (ethyl) β	1B	Y*	0.1	0.05
Fenitrothion	1B	N	0.2	0.1
Fenvalerate	3	N*	0.05	0.1
Fenobucarb (BPMC)	1A	N*Ø	0.05	0.02
Isoprocarb (MIPC)	1A	N Ø	0.01	0.01

Malathion	1B	N*	0.5	0.5
Pirimiphos methyl	1B	Y*□	0.05	0.05

(iii) weeds

Active ingredients	MoA group	EU status	EU MRL	JPMRL
Picloram	0	Y	0.01(T)	

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(iv) Stored produce	MoA group	EU status	EU MRL	JPMRL
Bioresmethrin	3	N	0.01	0.1
Methyl bromide (as inorganic bromide ion)	8	Pμ	0.01	

* High residue levels have been found in imported produce to the EU and/or Japan

B Registered for cocoa pod borer control in Indonesia

□□Use of pirimiphos methyl in cocoa is not defended by syngenta. Zero tolerance (LOD) for this A1 in Australia; EU MRL is 0.05 mg. kg-1

M Restricted under the Montreal Protocol

Appendix 3:

List of experimental control agents for possible future inclusion in the cocoa sector

All these AIs:

are known to be on 91/414/EEC Annex 1(Y, or pending-P)

are subject to current or recent field testing and may well conform to criteria in category 3A, when it is established that they conform to criteria in box F1

do not belong to WHO /EPA toxicity Class 1.

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(i) Black pod diseases

Active ingredients	MoA group	EU status	EU MRL	JPMRL
Dimethomorph	F5	Y	0.05	
Iprovalicarb	F5	Y	0.1	
Mandipropamid	F5	P	0.02	

Other MoA groups to consider testing:

B3, B5, C 3(Strobilurins) C 4 (Qil fungicides) U5

MCAs such as Trichoderma spp.

(ii) Insects

Active ingredients	MoA group	EU status	EU MRL	JPMRL
Emamectin benzoate	6	p	0.02	
Other neo-nicotinoids: e.g				
Thiacloprid	4A	Y	0.05	
Clothianidin	4A	P	0.05	0.02

IGRs:

Novaluron	15	P	0.01	0.02
Teflubenzuron	15	Y	0.05	0.02
Spiromesifen	23	P	0.02	
Spirotetramat	23	P	0.1	

b. Cocoa pod borer

emamectin benzoate	6	P	0.02	
IGRs: novaluron, teflubenzuron	15		if Y (as above)	
Methoxygenozide	18	Y	0.05	
Granulosis viruses?	MCA	-		

(iii) Weeds

Active ingredients	MoA group	EU status	EU MRL	JPMRL

Safer contact herbicides required

(iv) stored produce

Active ingredients	MoA group	EU status	EU MRL	JPMRL
Sulferyl fluoride	8		0.02	

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Appendix 4:

Pesticides that MUST NOT BE USED for cocoa

Active ingredients	MoA group	EU, MRL status § and notes
Insecticides		
acephate	1B	N
amitraz	19	N Ĵ
aldrin	2	N Class 1
azinphos-methyl	1B	N Class 1
cabaryl	1A	N
carbofuran	1A	N Class 1 as spray formulation
carbosulfan	1A	N
cartap	4C	N
cyhalothrin (unresolved)	3	N α
cyhexatin (acaricide)	12B	N Ĵ
DDT	3	N Φ (
dichlorvos (DDVP)	1B	N Class 1
dieldrin	2	N Clas1
dioxacarb	1A	N
endosulfan	2	N (MRL 0.1 mg/kg) * Class 1
lindane, gamma BHC, HCH	2	N * Φ
methyl-parathion (parathion-methyl)	1B	N * Φ Class 1
methomyl	1A	N β Class 1

monocrotophos	1B	N Φ Class 1
profenfos	1B	N
promecarb	1A	N Class 1
propoxur	1A	N
terbufos	1B	N Class 1
Herbicides		
Ametryn	C1	N
atrazine	C1	N
diuron	O	N*
fomesafen	E	N
MSMA (methyl arsenic acid)	Z	N
2,4,5-T	O	N Ĵ
Fungicides		
benomyl	B1	N δ
captafol	M4	N Φ Ĵ
hexaconazole	G1	N
pyrifenox	G1	N
triadimefon	G1	N
tridemorph	G2	N
zineb	M3	N
Stored produce		
allethrin (esbiothrin)	3	N
fenitrothion	1B	N

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isoprocarb (MIPC)	1A	not listed ø
permethrin	3	N
resmethrin	3	N
tetramethrin	3	N

* High residue levels have been found, within the last 5 years, in imported produce to the EU

and/or Japan

Cocoa growers are strongly advised to stop using any products containing any AI listed here.

Where they have been used in the past for cocoa pests, there should be satisfactory substitutes for them now recommended.

They include:

- obsolete and banned compounds (e.g. aldrin, lindane).

α Note: as with metalaxyl, unresolved cyhalothrin is not included on Annex 1, but the isomer lambda-cyhalothrin (used for mirid control) is included.

§ compounds not included on 91/414/EEC Annex 1 and are not thought to be essential for cocoa production.

Ĵ Compounds specifically listed at LOD for cocoa in Japan

Φ Pesticides listed in the PIC Convention

ø P pesticides are used outside the EU but for which no toxicological data and no MRLs have been notified for inclusion in 396/2005/EC Annex III (neither by the member states, in the form of import tolerances, nor by third countries). Such compounds may have a clear purpose outside Europe (e.g. fenobucarb and isoprocarb: which are widely

used for control of hemipteran pests of rice in Asia, and have also been applied to cocoa in certaincountries).

β Also breakdown product of thiodicarb

δ Breaks down into the permitted compound carbendazim

These lists may not be exhaustive: they have been based on ICCO records and the findings of the ECA/CABI/CAOBISCO project (Global Research on Cocoa, June 2008).

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Appendix 5:

Statistix - 30 Day Trial Version 9.0 7/1/2011, 2:51:22 AM

Completely Randomized AOV for ethoproph

Source	DF	SS	MS	F	P
treatment	10	0.00791	7.913E-04	3043.33	0.0000
Error	22	0.00001	2.600E-07		
Total	32	0.00792			

Grand Mean 9.56E-03 CV 5.34

Completely Randomized AOV for dimethoat

Source	DF	SS	MS	F	P
treatment	10	0.00228	2.282E-04	250.71	0.0000
Error	22	0.00002	9.103E-07		
Total	32	0.00230			

Grand Mean 0.0160 CV 5.97

Completely Randomized AOV for permethrin

Source	DF	SS	MS	F	P
treatment	10	8.957E-04	8.957E-05	692.24	0.0000
Error	22	2.847E-06	1.294E-07		
Total	32	8.986E-04			

Grand Mean 5.43E-03 CV 6.63

Completely Randomized AOV for fenitroth

Source	DF	SS	MS	F	P
treatment	10	0.04194	0.00419	5632.88	0.0000
Error	22	0.00002	7.445E-07		
Total	32	0.04196			

Grand Mean 0.0770 CV 1.12

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Completely Randomized AOV for malathion

Source	DF	SS	MS	F	P
treatment	10	0.01034	0.00103	1605.29	0.0000
Error	22	0.00001	6.439E-07		
Total	32	0.01035			

Grand Mean 0.0202 CV 3.98

Completely Randomized AOV for chlorpyri

Source	DF	SS	MS	F	P
treatment	10	0.00188	1.876E-04	110.03	0.0000
Error	22	0.00004	1.705E-06		
Total	32	0.00191			

Grand Mean 0.0203 CV 6.44

Completely Randomized AOV for aldrin

Source	DF	SS	MS	F	P
treatment	10	1.77052	0.17705	1.1E+08	0.0000
Error	22	3.333E-08	1.515E-09		
Total	32	1.77052			

Grand Mean 0.1089 CV 0.04

Completely Randomized AOV for parathion

Source	DF	SS	MS	F	P
treatment	10	0.00134	1.335E-04	4722.03	0.0000
Error	22	6.221E-07	2.828E-08		
Total	32	0.00134			

Grand Mean 0.0105 CV 1.61

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Completely Randomized AOV for profenofo

Source	DF	SS	MS	F	P
treatment	10	0.01473	0.00147	151864	0.0000
Error	22	2.133E-07	9.697E-09		
Total	32	0.01473			

Grand Mean 9.93E-03 CV 0.99

Completely Randomized AOV for pp

Source	DF	SS	MS	F	P
treatment	10	7.624E-04	7.624E-05	4102.82	0.0000
Error	22	4.088E-07	1.858E-08		
Total	32	7.628E-04			

Grand Mean 0.0220 CV 0.62

Completely Randomized AOV for alpha

Source	DF	SS	MS	F	P
treatment	10	0.00112	1.119E-04	144.77	0.0000
Error	22	0.00002	7.734E-07		
Total	32	0.00114			

Grand Mean 6.08E-03 CV 14.46

Completely Randomized AOV for beta

Source	DF	SS	MS	F	P
treatment	10	2.649E-05	2.649E-06	34969.0	0.0000
Error	22	1.667E-09	7.576E-11		
Total	32	2.649E-05			

Grand Mean 2.83E-04 CV 3.07

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Completely Randomized AOV for endosulfa

Source	DF	SS	MS	F	P
treatment	10	1.381E-04	1.381E-05	182329	0.0000
Error	22	1.667E-09	7.576E-11		
Total	32	1.381E-04			

Grand Mean 6.47E-04 CV 1.35

Completely Randomized AOV for PP~01

Source	DF	SS	MS	F	P
treatment	10	5.514E-04	5.514E-05	57952.0	0.0000
Error	22	2.093E-08	9.515E-10		
Total	32	5.514E-04			

Grand Mean 0.0135 CV 0.23

Completely Randomized AOV for PPDDT

Source	DF	SS	MS	F	P
treatment	10	0.01605	0.00160	86462.1	0.0000
Error	22	4.083E-07	1.856E-08		
Total	32	0.01605			

Grand Mean 0.0257 CV 0.53

Completely Randomized AOV for cyprmethr

Source	DF	SS	MS	F	P
treatment	10	0.08430	0.00843	1.1E+08	0.0000
Error	22	1.667E-09	7.576E-11		
Total	32	0.08430			

Grand Mean 0.0160 CV 0.05

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Completely Randomized AOV for fenvalera

Source	DF	SS	MS	F	P
treatment	10	0.03299	0.00330	1012595	0.0000
Error	22	7.167E-08	3.258E-09		
Total	32	0.03299			

Grand Mean 0.0596 CV 0.10

Completely Randomized AOV for deltameth

Source	DF	SS	MS	F	P
treatment	10	3.298E-05	3.298E-06	31659.4	0.0000
Error	16	1.667E-09	1.041E-10		
Total	26	3.298E-05			

Grand Mean 3.91E-04 CV 2.61

Appendix 6

Statistics - 30 Day Trial Version 9.0 7/1/2011, 2:52:01 AM

LSD All-Pairwise Comparisons Test of ethoproph by treatment

treatment	Mean	Homogeneous Groups
site 2	0.0573	A
site 6	0.0134	B
site 11	6.17E-03	C
site 9	6.13E-03	C
site 8	5.13E-03	D
site 10	4.70E-03	D
site 7	4.50E-03	D
site 5	4.40E-03	D
site 4	3.33E-03	E
site 1	0.0000	F
site 3	0.0000	F

Alpha 0.05 Standard Error for Comparison 4.163E-04

Critical T Value 2.074 Critical Value for Comparison 8.634E-04

There are 6 groups (A, B, etc.) in which the means are not significantly different from one another.

LSD All-Pairwise Comparisons Test of dimethoat by treatment

treatment	Mean	Homogeneous Groups
site 10	0.0247	A

site 7	0.0240	A
site 11	0.0237	A
site 6	0.0220	B
site 9	0.0191	C
site 8	0.0173	D
site 1	0.0153	E
site 5	0.0153	E
site 4	0.0143	E
site 2	0.0000	F
site 3	0.0000	F

Alpha 0.05 Standard Error for Comparison 7.790E-04

Critical T Value 2.074 Critical Value for Comparison 1.616E-03

There are 6 groups (A, B, etc.) in which the means

are not significantly different from one another.

LSD All-Pairwise Comparisons Test of permethrin by treatment

treatment	**Mean**	**Homogeneous Groups**
site 9	0.0147	A
site 5	0.0144	A
site 8	0.0105	B
site 10	5.47E-03	C
site 7	4.73E-03	D
site 4	4.53E-03	DE
site 2	4.10E-03	E
site 6	1.36E-03	F
site 1	0.0000	G
site 11	0.0000	G

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site 3 0.0000 G

Alpha 0.05 Standard Error for Comparison 2.937E-04

Critical T Value 2.074 Critical Value for Comparison 6.091E-04

There are 7 groups (A, B, etc.) in which the means

are not significantly different from one another.

LSD All-Pairwise Comparisons Test of fenitroth by treatment

treatment	**Mean**	**Homogeneous Groups**
site 7	0.1591	A
site 11	0.1143	B
site 2	0.0963	C
site 10	0.0791	D
site 9	0.0781	D
site 1	0.0737	E
site 4	0.0620	F
site 3	0.0591	G
site 6	0.0583	G
site 5	0.0537	H
site 8	0.0137	I

Alpha 0.05 Standard Error for Comparison 7.045E-04

Critical T Value 2.074 Critical Value for Comparison 1.461E-03

There are 9 groups (A, B, etc.) in which the means

are not significantly different from one another.

LSD All-Pairwise Comparisons Test of malathion by treatment

treatment	Mean	Homogeneous Groups
site 4	0.0537	A
site 10	0.0517	B
site 5	0.0257	C
site 6	0.0227	D
site 9	0.0217	D
site 8	0.0171	E
site 3	0.0161	E
site 7	0.0133	F
site 1	0.0000	G
site 11	0.0000	G
site 2	0.0000	G

Alpha 0.05 Standard Error for Comparison 6.552E-04

Critical T Value 2.074 Critical Value for Comparison 1.358E-03

There are 7 groups (A, B, etc.) in which the means

are not significantly different from one another.

LSD All-Pairwise Comparisons Test of chlorpyri by treatment

treatment	Mean	Homogeneous Groups
site 10	0.0337	A
site 7	0.0323	A
site 9	0.0251	B
site 5	0.0237	BC
site 3	0.0217	CD
site 2	0.0210	D
site 1	0.0141	E
site 11	0.0141	E

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site 6	0.0133	E
site 8	0.0130	EF
site 4	0.0110	F

Alpha	0.05	Standard Error for Comparison	1.066E-03
Critical T Value	2.074	Critical Value for Comparison	2.211E-03

There are 6 groups (A, B, etc.) in which the means
are not significantly different from one another.

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Descriptive Statistics of aldrin by treatment

treatment	**Mean**
site 1	0.0000
site 10	0.0000
site 11	0.0000
site 2	0.5614
site 3	0.0000
site 4	0.6368
site 5	0.0000
site 6	0.0000
site 7	0.0000
site 8	0.0000
site 9	0.0000

LSD All-Pairwise Comparisons Test of parathion by treatment

treatment	Mean	Homogeneous Groups
site 10	0.0191	A
site 8	0.0162	B
site 5	0.0161	B
site 9	0.0161	B
site 4	0.0151	C
site 11	0.0107	D
site 7	7.37E-03	E
site 3	7.13E-03	E
site 6	7.11E-03	E
site 1	0.0000	F
site 2	0.0000	F

Alpha 0.05 Standard Error for Comparison 1.373E-04

Critical T Value 2.074 Critical Value for Comparison 2.848E-04

There are 6 groups (A, B, etc.) in which the means

are not significantly different from one another.

LSD All-Pairwise Comparisons Test of profenofo by treatment

treatment	Mean	Homogeneous Groups
site 2	0.0581	A
site 3	0.0511	B
site 1	0.0000	C
site 10	0.0000	C
site 11	0.0000	C
site 4	0.0000	C

site 5 0.0000 C

site 6 0.0000 C

site 7 0.0000 C

site 8 0.0000 C

site 9 0.0000 C

Alpha 0.05 Standard Error for Comparison 8.040E-05

Critical T Value 2.074 Critical Value for Comparison 1.667E-04

There are 3 groups (A, B, etc.) in which the means

are not significantly different from one another.

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LSD All-Pairwise Comparisons Test of pp by treatment

treatment	**Mean**	**Homogeneous Groups**
site 8	0.0314	A
site 11	0.0278	B
site 2	0.0253	C
site 6	0.0242	D
site 7	0.0217	E
site 5	0.0216	E
site 9	0.0212	F
site 1	0.0207	G
site 3	0.0181	H
site 10	0.0153	I
site 4	0.0146	J

Alpha 0.05 Standard Error for Comparison 1.113E-04

Critical T Value 2.074 Critical Value for Comparison 2.308E-04

There are 10 groups (A, B, etc.) in which the means

are not significantly different from one another.

LSD All-Pairwise Comparisons Test of alpha by treatment

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treatment	**Mean**	**Homogeneous Groups**
site 7	0.0217	A
site 5	0.0112	B
site 10	7.12E-03	C
site 9	5.72E-03	CD
site 4	5.63E-03	CD
site 6	5.12E-03	D
site 11	4.72E-03	D
site 8	4.62E-03	D
site 3	1.16E-03	E
site 1	0.0000	E
site 2	0.0000	E

Alpha 0.05 Standard Error for Comparison 7.181E-04

Critical T Value 2.074 Critical Value for Comparison 1.489E-03

There are 5 groups (A, B, etc.) in which the means

are not significantly different from one another.

LSD All-Pairwise Comparisons Test of beta by treatment

treatment	**Mean**	**Homogeneous Groups**

site 4	3.12E-03	A
site 1	0.0000	B
site 10	0.0000	B
site 11	0.0000	B
site 2	0.0000	B
site 3	0.0000	B
site 5	0.0000	B
site 6	0.0000	B
site 7	0.0000	B
site 8	0.0000	B
site 9	0.0000	B

Alpha	0.05	Standard Error for Comparison	7.107E-06
Critical T Value	2.074	Critical Value for Comparison	1.473E-05

There are 2 groups (A and B) in which the means
are not significantly different from one another.

LSD All-Pairwise Comparisons Test of endosulfa by treatment

treatment	**Mean**	**Homogeneous Groups**
site 4	7.12E-03	A
site 1	0.0000	B
site 10	0.0000	B
site 11	0.0000	B
site 2	0.0000	B
site 3	0.0000	B
site 5	0.0000	B
site 6	0.0000	B
site 7	0.0000	B

site 8	0.0000	B
site 9	0.0000	B

Alpha 0.05 Standard Error for Comparison 7.107E-06

Critical T Value 2.074 Critical Value for Comparison 1.473E-05

There are 2 groups (A and B) in which the means

are not significantly different from one another.

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LSD All-Pairwise Comparisons Test of PP~01 by treatment

treatment	**Mean**	**Homogeneous Groups**
site 10	0.0181	A
site 11	0.0177	B
site 8	0.0177	B
site 7	0.0171	C
site 9	0.0167	D
site 2	0.0147	E
site 3	0.0119	F
site 1	0.0103	G
site 6	8.72E-03	H
site 4	8.42E-03	I
site 5	7.12E-03	J

Alpha 0.05 Standard Error for Comparison 2.519E-05

Critical T Value 2.074 Critical Value for Comparison 5.223E-05

There are 10 groups (A, B, etc.) in which the means

are not significantly different from one another.

LSD All-Pairwise Comparisons Test of PPDDT by treatment

treatment	Mean	Homogeneous Groups
site 3	0.0952	A
site 10	0.0234	B
site 4	0.0198	C
site 9	0.0192	D
site 8	0.0189	E
site 7	0.0187	E
site 1	0.0184	F
site 5	0.0183	F
site 6	0.0182	F
site 2	0.0168	G
site 11	0.0154	H

Alpha 0.05 Standard Error for Comparison 1.112E-04

Critical T Value 2.074 Critical Value for Comparison 2.307E-04

There are 8 groups (A, B, etc.) in which the means

are not significantly different from one another.

Descriptive Statistics of cyprmethr by treatment

treatment	Mean
site 1	0.0000
site 10	0.0000
site 11	0.0000
site 2	0.0000
site 3	0.1758
site 4	0.0000
site 5	0.0000

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site 6	0.0000
site 7	0.0000
site 8	0.0000
site 9	0.0000

LSD All-Pairwise Comparisons Test of fenvalera by treatment

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treatment	Mean	Homogeneous Groups
site 8	0.0898	A
site 10	0.0888	B
site 2	0.0878	C
site 6	0.0789	D
site 7	0.0779	E
site 5	0.0765	F
site 9	0.0688	G
site 11	0.0547	H
site 3	0.0214	I
site 4	0.0114	J
site 1	0.0000	K

Alpha	0.05	Standard Error for Comparison	4.660E-05
Critical T Value	2.074	Critical Value for Comparison	9.665E-05

All 11 means are significantly different from one another.

LSD All-Pairwise Comparisons Test of deltameth by treatment

treatment	Mean	Homogeneous Groups
site 4	3.52E-03	A
site 1	0.0000	B
site 10	0.0000	B
site 11	0.0000	B
site 2	0.0000	B
site 3	0.0000	B
site 5	0.0000	B
site 6	0.0000	B
site 7	0.0000	B
site 8	0.0000	B
site 9	0.0000	B

Alpha 0.05 Standard Error for Comparison 8.333E-06 TO 1.020E-05

Critical T Value 2.120 Critical Value for Comparison 1.767E-05 TO 2.164E-05

There are 2 groups (A and B) in which the means are not significantly different from one another.

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LSD All-Pairwise Comparisons Test of ethoproph by treatment

treatment	Mean	Homogeneous Groups

site 4	4.17E-03	A
site 1	0.0000	B
site 10	0.0000	B
site 11	0.0000	B
site 2	0.0000	B
site 3	0.0000	B
site 5	0.0000	B
site 6	0.0000	B
site 7	0.0000	B
site 8	0.0000	B
site 9	0.0000	B

Alpha	0.05	Standard Error for Comparison	7.107E-05
Critical T Value	2.074	Critical Value for Comparison	1.474E-04

There are 2 groups (A and B) in which the means
are not significantly different from one another.

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