KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

KUMASI COLLEGE OF SCIENCE FACULTY OF BIOSCIENCES
DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

HAZARD ANALYSIS OF METALAXYL RESIDUES IN COCOA BEANS

BY

JOEL COX MENKA BANAHENE

(BSc. BIOCHEMISTRY)

AUGUST 2017

HAZARD ANALYSIS OF METALAXYL RESIDUES IN COCOA BEANS

BY

JOEL COX MENKA BANAHENE

(BSc. BIOCHEMISTRY)

THIS THESIS IS SUBMITTED TO THE DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF

MASTER OF SCIENCE IN FOOD QUALITY MANAGEMENT

AUGUST 2017

DECLARATION

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

JOEL COX MENKA BANAHENE (PG4368915)	……..……	……..……. Date
	Signature	
MR. ISAAC W. OFOSU (Supervisor)	……..……	……..……. Date
	Signature	
DR. F.D. WIREKO-MANU (MRS) (Head of Department)	……..……	……..……. Date
	Signature	

DEDICATION

This dissertation is dedicated to Unique Konadu Banahene, Lois Asantewaa Banahene and my unborn child.

ACKNOWLEDGEMENT

To God be the glory great things He has done. My greatest thanks go to the Almighty God for his grace and mercies, love and protection. I am also grateful to my supervisor Mr. Isaac W. Ofosu, without your help, encouragement and patience, I wouldn"t have reached this far. God richly bless you.

I would like to express my gratitude to my mother Elizabeth Konadu Osei, my siblings and most especially my wife Priscilla Egyimah Brefo and my children Unique Konadu Banahene and Lois Asantewaa Banahene for their understanding, co-operation and encouragement which helped me in completion of this project.

I would also like to express my special gratitude and thanks to Dr. Paul A. Agyemang, Research Manager, QCC Ltd for your support and encouragement, the staff at the Pesticide Residue Laboratory of QCC Ltd for their technical assistance and contribution towards this project.

Last but not the least, I am grateful to the Management of Quality Control Company (COCOBOD) for their support and approval to pursue this course.

ABSTRACT

Metalaxyl is a widely used fungicide and one of the important pollutants in the environment, and will result in potential health threat to humans and environmental pollution, so its residues in food production have received more and more attention. Currently, very limited data on the distribution of residues of metalaxyl in agricultural products have been reported. The aim of the study was to determine the distribution of metalaxyl residues in cocoa beans from the cocoa growing regions of Ghana and compare to the current maximum residue limits (MRL) established by Codex, Japan and EU and the proposed EU MRL. Residues of metalaxyl were estimated in two hundred and four (204) samples of fermented, dried and merchantable cocoa beans by GCMS following extraction and clean-up. From the results of the study, 76.96% of the total samples analyzed had no detection of metalaxyl residues, however, 23.04% showed detection which 8.33% had metalaxyl residues lower than the proposed EU (0.02 mg/kg) MRL and 14.71% had metalaxyl residues above the proposed EU MRL. Metalaxyl residues recorded in the samples from the regions were all below the current Codex (0.2 mg/kg), Japan (0.2 mg/kg) and EU (0.1 mg/kg) MRL. However, residues of metalaxyl recorded in the samples from Western north and south, Ashanti and Central region were above the proposed EU (0.02 mg/kg) MRL. The highest mean metalaxyl residue level detected in the samples analyzed was seen in Western south (0.029 mg/kg) followed by Ashanti (0.025 mg/kg), Central (0.024 mg/kg), Western north (0.023 mg/kg), Brong Ahafo (0.019 mg/kg) and Eastern (0.016 mg/kg) region. No significant variation ($p>0.05$) was found between the mean metalaxyl residues recorded in the samples from Ashanti, Brong Ahafo, Central and Eastern region even though there were differences in the metalaxyl residue concentrations. However, signifiant variation ($p<0.05$) was found between the mean metalaxyl residues recorded in the samples from Western south, Western north and Eastern regions.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

WHO	World Health Organization
EFSA	European Food Safety Authority
JMPR	FAO/WHO Joint Meeting on Pesticide Residue
CAC	Codex Alimentarius Commission

EU	European Union
GCMS	Gas Chromatograph Mass Spectrometer
FAO	Food and Agricultural Organization
ICCO	International Cocoa Organization
GAP	Good Agricultural Practices
CODAPEC	Cocoa disease and pest control
MRL	Maximum Residue Level/Limit
GDP	Gross domestic product
ACI	African Cocoa Initiative
ADI	Allowable daily intake
CCPR	Codex Committee on Pesticide Residue
RfD	Reference Dose
US EPA	United State Environmental Protection Agency
HQ	Hazard quotient
COCOBOD	Ghana Cocoa Board
TDI	Tolerable daily intake

CHAPTER ONE

INTRODUCTION

1.1 Background

Most countries in the West African sub-region are sustained by the immense economic role that agriculture plays. As a matter of fact, agriculture contributes significantly to the sustainability of the Ghanaian economy. Ghana‟s labour force derives about 60% employment from agriculture (ISSER, 2010). Cocoa, coffee and rubber are among the crops that dominate in the agriculture sector of Ghana. In Ghana, cocoa is of much interest among these crops and also for the cocoa industry worldwide (Danso-Abbeam *et al*., 2014). About 800,000 smallholder cocoa farmers attracts more than half of Ghana‟s income (Appiah, 2004; Anim-Kwapong and Frimpong, 2004; Ayenor *et al*., 2007; Anang, 2011; Danso-Abbeam *et al*., 2014).

Notwithstanding the contributions derived from cocoa, it is susceptible to the attack of diseases which has resulted in the decline of its production, bearing a negative effect on the economy. Cocoa diseases contribute a significant challenge to farmers. The black pod disease caused by *Phytophthora megakarya* is one of the major fungal diseases in cocoa farming. About 80% losses of cocoa pods have been reported (Dakwa, 1987; Luterbacher and Akrofi, 1993 and Opoku *et al*., 2000), which has resulted in some cocoa farms being abandoned. In the effort to remedy this situation, fungicides are considered important in the integrated black pod disease management plan and have been deemed necessary to avoid over 70% crop losses (ACI, 2012). The advent of pesticide usage by farmers in cocoa farming to maintain high productivity and also control pests and diseases is on the increase. To this effect, farmers indulge in indiscriminate application of pesticides

(Konradsen, 2007 and Sam *et al*., 2008), without realizing the consequences. Safety concerns have been raised on the residue levels of pesticides in cocoa beans and the potential harm it has on humans (Antle and Pingali1, 1994; Pimentel, 2005; Adeogun and Agbongiarhuoyi, 2009; Hou and Wu, 2010 and Adejumo *et al*., 2014). A large number of pesticides in use currently pose considerable short and long term health risks to humans and the environment (WHO, 1990).

In order to regulate the trade of cocoa and ensure the safety of its consumption, standards have been set by FAO/WHO (Moy and Wessel, 2000) and other authorized bodies for the levels of pesticide residues that are permitted in the beans exported. Cocoa beans that are exported internationally are assessed based on the quality in reference to residues of pesticides and other substances. Exports that violate the standards could be disallowed, which may result in loss of revenue by the exporting country.

1.2 Problem statement

The European Union (EU) has made a proposal to the Codex Alimentarius Commission (CAC) to review the current Codex maximum residue limit (MRL) of the pesticide active substance metalaxyl in cocoa beans. This proposal was on the basis that limited data was used to establish the MRL (EFSA, 2013). The proposal entails the review of the existing metalaxyl MRL from 0.2 mg/kg to 0.02 mg/kg in cocoa beans (CAC, 2014). In view of this, Codex Committee on Pesticide Residues (CCPR), a sub-committee of CAC has requested for the occurrence data of metalaxyl residues in cocoa beans from cocoa producing countries and other interested parties to enable a rationale review of the existing Codex MRL. Most of the fungicides used in cocoa farming to mitigate fungal diseases

contain the active substance metalaxyl. Cocoa farmers depend largely on these fungicides to control the destructive fungal diseases.

Lack of data on the occurrence level of metalaxyl residues in cocoa beans poses a significant challenge on the rejection or acceptability of the reviewed metalaxyl MRL. This could lead to the inability of the exporting country to satisfy the requirement of the importing countries with respect to tolerance limit of metalaxyl residue in the cocoa beans which could result in revenue loss. Additionally, lack of data on the prevalence of metalaxyl residues in cocoa beans threatens the use of fungicides containing metalaxyl to mitigate fungal disease as well as increasing the volume of cocoa for export.

The aim of this study is to document the findings on the occurrence level of metalaxyl residues in cocoa beans and to contribute data to help review the MRL.

1.3 Objective of the study

The main objective of the study was to determine the distribution of metalaxyl residues in cocoa beans from the cocoa growing regions of Ghana relative to the current Codex, Japan, and EU and proposed EU MRL.

CHAPTER TWO

LITERATURE REVIEW

2.1 The quality and safety of cocoa

Ghana‟s cocoa has received a tremendous reputation on the international market. This is extremely prized and needs be protected in the short and long term. In order to maintain this

status, it requires relentless vigilance, as there have been some pesticide residue issues underscored by importing countries, particularly Japan. In respect of this, production systems will need to be updated to preserve the status of Ghana‟s cocoa and to stay ahead of the changing regulations and standards demanded on the international market. These proactive systems and structures, which manages quality and safety and also demonstrates that safety is being adequately controlled, will be an increasingly important factor in supplying quality cocoa to the safety-conscious world markets such as Europe, the US and Japan (Cooper and Cudjoe, 2012).

Ghana‟s cocoa has been graded to be richer in theobromine and flavonoids, which attributes a mild and rounded flavor to beans (ICCO, 2010). This has earned Ghana‟s cocoa beans the benchmark for measuring the quality of all cocoa worldwide. International standard for grading cocoa as quality requires a merchantable cocoa to possess these attributes; fermented, thoroughly dried, free from smoke and foreign odor and free from any indication of contamination. In addition, the cocoa beans must be equitably free from living insects, broken beans, and fragments and must be reasonably uniform in size. Other qualities that are demanded by manufacturers include fully fermented and not slaty or purple beans (Ntow *et al.*, 2001).

The rigorous quality control of cocoa beans has earned Ghana an enviable high premium quality placed on its cocoa on the world‟s commodities market (Ntow *et al.*, 2006). This achievement has been meticulously upheld through the effective quality control activities, inspection and monitoring by the Quality Control Company Limited (QCC) of Ghana Cocoa Board at the time of purchase of the beans (COCOBOD Annual Report, 2010).

The QCC undertakes disinfestation (spraying, fogging and fumigation) of cocoa beans, during storage and prior to shipment to ensure the export of insect free cocoa beans. They also carry out pests control activities in all cocoa storage premises to prevent damage to the beans in storage. Additionally, inspection, grading and sealing of cocoa is conducted for the international and local markets. Pesticide residue analyses are also carried out per customers‟ request to ascertain the residual levels of chemicals used on cocoa during production and storage to ensure the safety of the produce. To ensure quality, the cocoa bean undergoes a minimum of three stages of inspection prior to shipment. This assures customers of the quality of the beans (COCOBOD Annual Report, 2010).

Despite all the efforts in maintaining the enviable status in terms of safety and quality, Ghana cocoa is fraught with devastating fungal diseases which accounts for about 40% annual loss (Flood *et al*., 2004).

2.2 Fungal diseases associated with cocoa production

Several fungal diseases including brown root rot, collar rot, cushion gall and black pod attack cocoa in Ghana (Table 2.1). Black pod disease (*Phytophthora* pod rot) is the most significant. This is caused by the agents of two species of *Phytophthora*; *Phytophthora palmivora* and *Phytophthora megakarya* of which the latter is the most virulent as reported by Darkwa (1987) and Luterbarcher and Akrofi (1993). Agents of black pod disease attacks the pods, beans, flower cushions and shoots. The symptoms of the disease include, brown necrotic lesions on the pod‟s surface, rotting of the beans and canker on the stem and these does the most damage to the cocoa (Wood and Lass, 1985 and Opeke, 1987).

Table 2.1: Fungal diseases affecting cocoa in Ghana

Disease	Type of Infection (Causative agent)	Symptoms
Black pod	Fungus (*Phytophthora spp.*)	Rots of the pod as brownish-black colouration. Beans destroyed in immature pods. Could result in die-back
Brown root rot	Fungus (*Fomes noxius*)	Leaves fall prematurely and die-back of twigs occurs. Fungus fruit bodies on root and dead trunks. Soil is affected.
Collar crack	Fungus (*Armillaria mellea*)	Longitudinal cracking of trunk from ground level to about 1.2 m upwards, fills with cream-coloured mycelium
Collar rot	Fungus (*Ustulina zonata*)	Defoliation and death of plants. White fan shaped patches of mycelium are produced underneath bark and roots
Pod rot	Fungus (*Botryodiphlodia theobromae*)	Appears as brown necrotic areas with concentric rings of black spots. Pods are later covered with black sooty powder
White thread blight	Fungus (*Marasmius scandens*)	Leaves are covered and killed in a network of white my celial threads
White rot	Fungus (*Fomes lignosus*)	Premature defoliation, death of twigs, pods are small
Cushion gall	Fungus (*Calonectria rigidiuscula*)	Excessive production of buds at the nodes
Vascularstreak Die – Back	Fungus (*Oncobasidium theobroma*)	Leaves turn yellow and fall prematurely. Smaller branches wither starting from the tips
Mealy pod	Fungus (*Trachysphaera fructigena*)	Pods turn brown, becomes encrusted with white to pinkish mealy growth of the fungus

Source: (Offei, 2000)

The loss in cocoa production due to the fungal diseases in high rainfall times is estimated to about 30%. In 2005 the cocoa bean price lost its forecasted productive market value by an estimated of over 1 billion US dollars in lost revenue on the world commodity market

(ICCO, 2010).

2.3 Control of fungal diseases affecting cocoa

Cocoa production seldom exceeded 400,000 metric tons during the era of the 90‟s. A situation which was ascribed to diverse causes, while the main culprit was the prevalence of cocoa fungal diseases. In order to remedy the situation, the government of Ghana presented the national cocoa diseases and pests control (CODAPEC) programme commonly known as “mass spraying” in the year 2000, to fight the black pod disease and mirids on cocoa farms (Abankwah *et al.*, 2010). The introduction of this intervention presented a chance for farmers and technical personnel have to be trained on the best scientific approaches to control diseases and pests (Adjinah and Opoku, 2010). The application of fungicides on three times (3x) basis during each cocoa season was one of the recommended approaches to mitigate fungal diseases. The Cocoa Research Institute of Ghana (CRIG) approved fungicides to be used to combat the resurgent fungal diseases of cocoa under the CODAPEC programme (Table 2.2).

Table 2.2: Fungicides approved for use on cocoa in Ghana

Fungicide	Active ingredient
Ridomil 72 plus WP	12% metalaxyl + 60% cuprous oxide
Nordox 75 WP	86% cuprous oxide, 14% inert
Funguran OH WP	Cuprous hydroxide
Champion WP	77% cupric hydroxide
Kocide 101 WP	Cupric hydroxide
Fungikill WP	Cupric hydroxide + metalaxyl
Metalm 72 Plus WP	Cuprous oxide + metalaxyl

Source: (COCOBOD, 2010)

In controlling the fungal diseases, the instituted CODAPEC programme covered all cocoa growing districts in the Volta, Brong Ahafo, Ashanti, Eastern and parts of Western region. One of the control measures recommended against the fungal disease was non-chemical means, even though the need for fungicides application to mitigate the disease is inevitable and will continue for years to come. Nonetheless, the possible hazards to humans and environmental risks associated with the usage of pesticides require a thorough re-examination.

2.4 Pesticides use: human health and environmental concerns

Pesticides have an important role in maintaining the yield and quality of cocoa during production, by controlling diseases, controlling insect pests and controlling unwanted weeds. However pesticides need to be applied in a safe and sustainable way that does not threaten the health of the operators using them, present risks to the environment, or result in illegal residues (Ntiamoah and Afrane, 2012). The harmful effects on humans either as acute or chronic can be attributed to a large number of pesticides (Lorenz, 2009). Death or serious illness can result from acute exposure to pesticides (WHO, 1990). The WHO estimates about 355,000 people who die globally each year resulting from accidental severe pesticide poisonings (WHO, 2003). The use of pesticides correlating to human exposure has increasing health impacts ranging from chronic health conditions and diseases like cancer, reproductive, endocrine, immunological, congenital and developmental disorders (UNDP, 1998; Yăněz, 2002; Sanborn *et al*., 2007 and Jurewicz *et al*., 2008). Furthermore, the persistent use of pesticides in the agricultural sector has also brought to light the potential to permeate and reach groundwater (Francisco *et al*., 2002 and Singh *et*

al., 2002). The WHO classification of the toxicity or hazardous potentials of some fungicides used on cocoa in Ghana shown in Table 2.3.

Table 2.3: Hazard classification of fungicides approved for use on cocoa in Ghana

Trade name	Active ingredient	Chemical hazardous class (WHO)
Fungikill	Cupric hydroide + metalaxyl	III
Metalm	Cuprous oxide + metalaxyl	III
Champion	Cuprous hydroxide	
Kocide	Cupric hydroxide	III
Nordox	Cuprous oxide	
Funguran	Cuprous hydroxide	
Ridomil	Metalaxyl cuprous oxide	III

Source: extracted from (Adjinah and Opoku 2010)

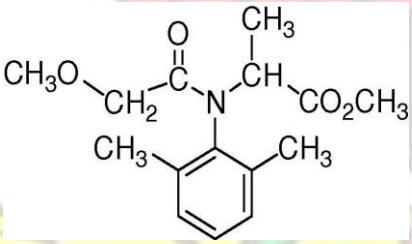
III = slightly hazardous; ***(WHO, 2005)***

2.5 The toxicology of metalaxyl

Metalaxyl [N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-DL-alanine ester] (Figure 1) is a fungicide with equal mixture of two enantiomers (R and S) (FAO, 2005). It has residual and systemic activity recommended for both spraying and soil application (Sukul and Spiteller, 2001). Since its introduction in 1977, it has been widely used for the control of various plant diseases caused by *Pythium spp* and *Phytophthora spp* (Urech *et al.*, 1977).

Upon application, it is absorbed through leaves, stems and roots and transported acropetally within the plant to elicit its protective and curative actions (Tomlin, 2000). The fungicide acts by inhibiting protein synthesis of the fungus to suppress sporangial

formation, mycelial growth and the establishment of new infections (Urech *et al.*, 1977 and Buchenauer, 1990). Metalaxyl is stable to a wide range of pH, light and temperature and thus resulting to its continued use in agriculture. It has a tendency to migrate to deeper soil horizons with a potential to contaminate groundwater, particularly soils with low organnic matter and clay content (Sukul and Spiteller, 2000). Metalaxyl causes severe irritation in rabbits. The dog is the most sensitive species with liver as the major affected target organ. It causes hepatocellular enlargement in rats, while dogs show changes in blood biochemical parameters indicative of hepatocellular damage (JMPR, 2002). The physical and chemical properties of metalaxyl are outlined in Table 4.



(Tomlin, 2000)

Figure 2.1 Chemical structure of metalaxyl

Table 2. 4. Physical and chemical properties of metalaxyl

Property	**Property value**
Molecular formular	$C_{15}H_{21}NO_4$
Molecular weight	279.336 g/mol
Colour	Fine, white powder
Boiling point	295.9 ºC at 760 mm Hg
Melting point	71 - 72 ºC
Density	1.20 g/cm^3 at 20 ºC
Solubility	In ethanol 400, acetone 450, toluene 340, n-hexane 11 and octanol 68 (all g/l at 25 ºC). In water, 8400 mg/l at 22 ºC

Partition coefficient (LogP)	log Kow = 1.65
Vapor pressure	0.293 mPa at 20 °C

Source: extracted from (Hansch et al., 1995; Tomlin, 1997 and O'Neil, 2001)

Metalaxyl is categorized in the class III, slightly hazardous group of fungicides by WHO (JMPR, 2005). The acute oral LD_{50} of metalaxyl is 669 mg/kg body weight and the dermal LD_{50} is greater than 3100 mg/kg body weight in rats (USNLM, 1995). It is slightly toxic through the acute oral and dermal routes of exposure. Rabbits exhibited slight eye and skin irritation, but guinea pigs displayed no sensitization after metalaxyl exposure (Kidd and James, 1992).

2.5.1 Absorption, distribution, metabolism and excretion of metalaxyl

Safety assessment studies are used to predict hazards of pesticides to humans and this can be realized only through selection of appropriate species for study. This is achieved by identifying a laboratory animal species that absorb, distribute, metabolize, and eliminate poisonous substances in ways similar to those in humans (Beasley, 1999). This is an essential component for cogent research on the safe use of toxicants.

In fulfilment of this, a two year study on dogs and other mammals including rats and goats conducted revealed that, metalaxyl was rapidly and extensively absorbed (similar for intravenous and oral elimination profiles), uniformly distributed and does not accumulate, extensively metabolized (<1% parent compound in excreta) and rapidly excreted (70 - 80% in 24 hr) through both urine and faeces (FAO/WHO, 2007). The majority of urinary metabolites were conjugated (glucuronide or sulfate) whereas faecal metabolites were mostly unconjugated. The major metabolite in urine and faeces was N-(2,6-

dimethylphenyl)-N-(hydroxyacetyl) alanine. Three major and one minor metabolic pathway were proposed. One pathway involved hydrolysis of the ether, followed by oxidation of the resulting alcohol, ester hydrolysis, or N-dealkylation of the ester chain. A second pathway involved oxidation of an aromatic methyl to the benzylic acid or ester hydrolysis. The third major pathway was ester hydrolysis, sometimes followed by benzylic acid formation. The minor pathway involved hydroxylation at the meta position of the phenyl ring (USEPA, 1994).

2.6 Human health assessment

Human health assessment is the foundation of risk assessment for chemicals which generally estimates the likelihood of adverse health effects occurring in an individual, subpopulation or population due to exposure to a chemical (such as metalaxyl) (UNEP, 2008). Risk assessment consists of the identification of an agent that causes adverse health effects (hazard identification), evaluation of the nature of the adverse health effects (hazard characterization), evaluation of the intake of the agent (exposure assessment) and finally estimating the occurrence and severity of the adverse health effects (risk characterization) (Mbabazi, 2011).

2.6.1 Hazard identification of metalaxyl

It is the proof of identity of the type and nature of adverse effects that an agent has an intrinsic potential to cause in an organism, system, or sub-population (WHO, 2010). It is the first of four steps in risk assessment. In this step, the different health problems the pesticide could cause are determined by investigating available scientific data about its effect on humans and laboratory animals. Metalaxyl is a moderate eye irritant and has been placed in

toxicity class II which indicates the second-highest degree of acute toxicity for eye irritating effects (USEPA, 1994). However, metalaxyl does not have carcinogenic potentials and has been classified by EPA as Group E carcinogen showing evidence of non-carcinogenicity for humans (Fallis, 2013).

2.6.2 Hazard characterization of metalaxyl

This describes the relationship between the administered dose of, or exposure to, a chemical and the incidence of an adverse health effect (Mbabazi, 2011). It is the second of four steps in risk assessment process. In instances where the adverse effect is presumed to have a threshold (for non-carcinogens), it usually results in the establishment of health-based guidance values, for example, an acceptable daily intake (ADI) or reference dose (RfD) for chemical residues or additives or a tolerable daily intake (TDI) for contaminants (FAO, 2006). Based on this assumption, the 2002 JMPR evaluation of metalaxyl established an ADI of 0 – 0.08 mg/kg body weight for humans with a safety factor of 100 using a two year study in dogs (FAO/WHO, 2007). There is also the assumption that, for carcinogens, no threshold exists and for that matter there is no RfD. Instead, an estimate of the intake associated with a predefined level of risk is considered (USEPA, 1992).

2.6.3 Exposure assessment of metalaxyl

It is the process of estimating the magnitude, frequency and duration of exposure to metalaxyl (hazard) and the number and characteristics of the population exposed (USEPA, 2016). It is the third of four steps in risk assessment process. When a hazard is identified in food, dietary exposure assessment is performed, which takes into account the occurrence and

concentrations of the hazard in the food, the consumption patterns of the foods containing the hazard and the likelihood of consumers eating large amounts of the food in question (high consumers) and of the hazard being present in the food at high levels (Mbabazi, 2011).

2.6.4 Risk characterization of metalaxyl

The final step of the four steps in risk assessment process is to bring the findings in the previous three steps together into an overall risk characterization. Therefore, this step considers the integration of the preceding three steps, which results in an estimate of the magnitude of the public-health problem (Brecher, 2009). Risk posed by non-carcinogenic hazards (metalaxyl) is estimated using the hazard quotient (HQ), which is calculated when there is only one hazardous substance involved. However, cases where multiple hazards are involved in one product, the hazard index (HI), which is the sum of all HQ of the various hazards in the product is used (USEPA, 1999). Conversely, to determine the risk posed by the exposure to carcinogenic hazard, the lifetime risk of cancer is considered. Estimated risk value higher than 10^{-6} (1 in a million, at deminimus) is considered unacceptable (USEPA, 2000). The conclusion drawn from risk characterization informs stakeholders about the hazard, whether its level is acceptable or tolerable and the control measures to introduce if it is above acceptable level (Gillespie *et al*., 2011).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

All reagents used for the study were of pesticide and analytical grade and were purchased from Merck (Darmstadt, Germany). Graphite carbon black (GCB)/Amino propyl (NH_2), Diatomaceous earth column and Florisil sorbents were purchased from Agilent Technologies (Santa Clara, California, USA). The pure standard of metalaxyl (purity ≥98%) was also purchased from Wako Pure Chemicals Industry (Osaka, Japan). Preparation of metalaxyl stock standard solution: 10 mg metalaxyl standard was weighed, then dissolved with acetone to the constant volume of 10 ml; the standard solution with a final concentration of 1000 μg/ml was stored in a refrigerator at 2-8 ℃.

3.1.1 Source of cocoa beans

Fermented dried cocoa beans of merchandize quality were obtained from the Tema and Takoradi ports where cocoa beans from the various cocoa growing regions are received.

3.2 Methods

3.2.1 Sampling

Samples of cocoa beans were randomly taken from all the districts in the seven (7) cocoa growing regions in a monthly interval for three consecutive months during the main cocoa crop season (November 2016 to January 2017). The seven cocoa growing regions comprises of Ashanti (15 districts), Bring Ahafo (9 districts), Central (7 districts), Eastern (10 districts), Western north (14 districts), Western south (12 districts) and Volta (3 districts).

Each sample was taken from lots of about 100 – 400 bags of cocoa beans. The samples were drawn from all sides of each cocoa bag using a sampling horn made of aluminum metal and bulked into a container. The dimension of the sampling horn was 100 mm long × 15 mm internal diameter. The collected sample was mixed thoroughly and quartered. Two opposite side quarters were rejected and the process repeated until a final sample of 1 kg was obtained. The samples were collected in polyethylene bags and transported to the laboratory for analysis.

A total of two hundred and four (204) samples were obtained: Ashanti (45 samples), Brong Ahafo (27 samples), Central (21 samples), Eastern (30 samples), Western north (42 samples), Western south (36 samples) and Volta (3 samples).

3.2.2 Extraction of metalaxyl residue from cocoa beans

The cocoa bean samples were frozen for 24 h at a temperature of -20 ºC. Each sample was milled separately, packed into zip bags, labelled and stored at a temperature of about 10 ºC until analysis. A partially modified and validated residual compositional substances of agricultural chemicals method used for analyzing pesticide residues in cocoa beans was employed (MHLW, 2005). The samples were extracted, purified (clean-up) and analyzed on GCMS (Shimadzu QP2020, Kyoto, Japan) following a described protocol (Appendix 1).

3.2.3 Determination of metalaxyl residues

A 1 µl of the extract was injected into the GCMS at a temperature of 250 ºC. The column oven started from a temperature of 50 ºC and was held for 1 min increasing to 230 ºC at 20 ºC/min ramp rate, with a subsequent increment to 300 ºC at 20 ºC/min ramp and held for 5 min. Helium gas with a purity of 99.999% was used as the carrier gas at a flow rate of 1

ml/min through Agilent VF-1MS of 0.25 µm id × 0.25 µm film and 30 m length capillary column. Injection port was adjusted at 250 °C and splitless injection mode was used. Multi-level calibration ranging from 0.01 to 0.1 µg/ml was used to obtain a calibration curve with a correlation coefficient (R^2) ≥ 0.998. The peak of metalaxyl was identified by the m/z 206, 160, 220 and 249 at the retention time of 9.12 min in comparison with the standard and the concentrations were obtained using peak area by extrapolating from the calibration curve and expressed in mg/kg using equation 1.

$$M = \frac{C}{2} \quad - \quad (1)$$

Where M is the final concentration of metalaxyl residue in the sample (mg/kg) and C is concentration of the sample read from the GCMS results. The limit of detection (LOD) and limit of quantification (LOQ) used for the analysis were 0.003 mg/kg and 0.01 mg/kg respectively. Recovery assays were performed and 75-98% was obtained in the fortification range of 0.1 - 1 mg/kg.

3.2.4 Data analysis

Statistical Package for Social Sciences (SPSS) software version 21 and Microsoft excel 2016 was used to determine the means and standard deviation for the metalaxyl residues detected in the cocoa bean samples. One-way analysis of variance (ANOVA) was used to test for the significant difference and the similarities between metalaxyl residues detected in samples from the regions. Differences were considered significant at $p<0.05$.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Trends of mean metalaxyl residue levels in samples from the regions

In this study, metalaxyl residues were detected in all the regions with the exception of Volta region which had no detection. Concentration of metalaxyl residues ranged from not detected (ND) to 0.029 mg/kg. The mean metalaxyl residue levels detected in the samples from all the regions were lower than the current Codex (0.2 mg/kg) (CAC, 2014), Japan (0.2 mg/kg) (Nawn *et al*., 2009) and EU (0.1 mg/kg) (EFSA, 2013) MRL. However, the mean metalaxyl residue levels in the samples from all the regions were above the proposed EU (0.02 mg/kg) (CAC, 2014) MRL with the exception of Brong Ahafo, Eastern and Volta regions (Table 4.1).

Table 4.1: Mean levels of metalaxyl residues in the regions relative to Codex, Japan and EU and proposed EU MRL.

Region	Metalaxyl residue (mg/kg)	MRL (mg/kg)			
		Codex	Japan	EU	EU proposed
Ashanti	0.025 ± 0.004	0.2	0.2	0.1	0.02
Brong Ahafo	0.019 ± 0.000	0.2	0.2	0.1	0.02
Central	0.024 ± 0.002	0.2	0.2	0.1	0.02
Eastern	0.016 ± 0.000	0.2	0.2	0.1	0.02
Western north	0.023 ± 0.004	0.2	0.2	0.1	0.02
Western south	0.029 ± 0.011	0.2	0.2	0.1	0.02
Volta	ND	0.2	0.2	0.1	0.02

ND – Not Detected

The results from the study showed that there were no significant differences ($p>0.05$) in the mean metalaxyl residue levels recorded in the samples from the Ashanti, Brong Ahafo, Central and Eastern regions even though there were differences in the residue concentrations (Appendix 9). However, a significant difference ($p<0.05$) was found in the mean metalaxyl residue levels recorded in the samples from the Western south, Western north and Eastern regions (Appendix 9). The differences in the concentration of metalaxyl residues detected in the samples as mentioned above could be due to the different agricultural practices adopted by farmers, the prevalence of fungal diseases and also accessibility to the fungicides containing metalaxyl. This assertion is in agreement with Offei *et al*. (2000) who reported that fungal diseases constitutes about 71.43% among the major diseases affecting cocoa in Ghana. Similarly, studies conducted by Adjinah and Opoku (2010) and Afrane and

Ntiamoah (2011) also reported that about 42.86% of fungicides approved for use on cocoa in Ghana contains the active ingredient metalaxyl.

Notably, this report also clearly confirms the claim made.

4.2 Samples from Ashanti region

Forty five (45) samples were analyzed from the Ashanti region, comprising of three (3) samples each from fifteen (15) districts (Appendix 8). Metalaxyl residues were detected in ten (10) of the samples from six (6) districts. The highest and lowest average concentrations of metalaxyl residues were detected in Ampenim (0.03 mg/kg), Juaso (0.021 mg/kg) and Mankranso (0.021 mg/kg) respectively. The levels ranged from ND to 0.03 mg/kg and the mean was 0.025 mg/kg. Metalaxyl residues detected in the samples were lower than the current Codex, Japanese and EU MRL but above the proposed EU MRL (Table 4.2).

Table 4.2: Levels of metalaxyl residues in samples from Ashanti region relative to Codex, Japan and EU and proposed EU MRL.

District	Metalaxyl residue (mg/kg)	MRL (mg/kg)			
		Codex	Japan	EU	EU proposed
Agona	ND	0.2	0.2	0.1	0.02
Ampenim	0.03 ± 0.000	0.2	0.2	0.1	0.02
Antoakrom	ND	0.2	0.2	0.1	0.02
Bekwai	ND	0.2	0.2	0.1	0.02
Effiduase	ND	0.2	0.2	0.1	0.02
Juaso	0.021 ± 0.003	0.2	0.2	0.1	0.02

Konongo	0.026 ± 0.013	0.2	0.2	0.1	0.02
Mankranso	0.021 ± 0.000	0.2	0.2	0.1	0.02
New Edubiase	ND	0.2	0.2	0.1	0.02
Nkawie	0.029 ± 0.006	0.2	0.2	0.1	0.02
Nsokote	ND	0.2	0.2	0.1	0.02
Nyinahin	0.025 ± 0.003	0.2	0.2	0.1	0.02
Obuasi	ND	0.2	0.2	0.1	0.02
Offinso	ND	0.2	0.2	0.1	0.02
Tepa	ND	0.2	0.2	0.1	0.02

ND – Not Detected

Samples from

4.3 Brong Ahafo region

Twenty seven (27) samples from Brong Ahafo region were analyzed for metalaxyl residues, comprising of three (3) samples each from nine (9) districts (Appendix 7).

Metalaxyl residues were detected in two (2) of the samples from one (1) district (Nkrankwanta) with an average concentration of 0.019 mg/kg. The levels ranged from ND to 0.019 mg/kg and the mean was 0.019 mg/kg. The metalaxyl residue detected in the sample was lower than the current Codex, Japan and EU MRL and also the proposed EU MRL (Table 4.3).

Table 4.3: Levels of metalaxyl residue in samples from Brong Ahafo region relative to Codex, Japan and EU and proposed EU MRL

District	Metalaxyl residue (mg/kg)	MRL (mg/kg)			
		Codex	Japan	EU	EU proposed
Asumura	ND	0.2	0.2	0.1	0.02
DormaaAhenkro	ND	0.2	0.2	0.1	0.02
Goaso/Mim	ND	0.2	0.2	0.1	0.02
Hwidiem	ND	0.2	0.2	0.1	0.02
Kasapin	ND	0.2	0.2	0.1	0.02
Kukuom	ND	0.2	0.2	0.1	0.02
Nkrankwanta	0.019 ± 0.007	0.2	0.2	0.1	0.02
Sankore	ND	0.2	0.2	0.1	0.02

Samples from

Sunyani	ND	0.2	0.2	0.1	0.02

ND – Not Detected

4.4 Central region

Twenty one (21) samples from Central region were analyzed for metalaxyl residues, comprising of three (3) samples each from seven (7) districts (Appendix 5). Metalaxyl residues were detected in four (4) of the samples from two (2) districts (Assin Break and Swedru). The highest and lowest average concentrations of metalaxyl residues detected was sampled from Swedru (0.025 mg/kg) and Assin Breku (0.022 mg/kg) respectively. The levels ranged from ND to 0.025 mg/kg and the mean was 0.024 mg/kg. Metalaxyl residues detected in the samples from the two districts were lower than the current Codex, Japan and EU MRL but above the proposed EU MRL (Table 4.4).

Table 4.4 Levels of metalaxyl residues in samples from Central region relative to Codex, Japan and EU and proposed EU MRL.

District	Metalaxyl residue (mg/kg)	MRL (mg/kg)			
		Codex	Japan	EU	EU proposed
Breman Asikuma	ND	0.2	0.2	0.1	0.02
Assin Breku	0.022 ± 0.000	0.2	0.2	0.1	0.02
Cape Coast	ND	0.2	0.2	0.1	0.02
Assin Fosu	ND	0.2	0.2	0.1	0.02
Twifo Nyinase	ND	0.2	0.2	0.1	0.02

Samples from

Twifo Praso	ND	0.2	0.2	0.1	0.02
Swedru	0.025 ± 0.007	0.2	0.2	0.1	0.02

ND – Not Detected

4.5 Eastern region

Thirty (30) samples from Eastern region were analyzed for metalaxyl residues, comprising of three (3) samples each from ten (10) districts (Appendix 4). Metalaxyl residues were detected in two (2) of the samples from Ofoase district. The levels ranged from ND to 0.016 mg/kg and the mean was 0.016 mg/kg. Metalaxyl residue detected in the sample was lower than the current Codex, Japan and EU MRL and also the proposed EU MRL (Table 4.5).

Table 4.5 Levels of metalaxyl residues in samples from Eastern region relative to Codex, Japan and EU and proposed EU MRL.

District	Metalaxyl residue (mg/kg)	MRL (mg/kg)			
		Codex	Japan	EU	EU proposed
Achiase	ND	0.2	0.2	0.1	0.02
Akim Oda	ND	0.2	0.2	0.1	0.02
Akoase	ND	0.2	0.2	0.1	0.02
Asamankese	ND	0.2	0.2	0.1	0.02
Kade	ND	0.2	0.2	0.1	0.02
Kibi	ND	0.2	0.2	0.1	0.02
Koforidua	ND	0.2	0.2	0.1	0.02

Samples from

Nkawkaw	ND	0.2	0.2	0.1	0.02
Ofoase	0.016 ± 0.004	0.2	0.2	0.1	0.02
Suhum	ND	0.2	0.2	0.1	0.02

ND – Not Detected

4.6 Western north region

Forty two (42) samples from Western north region were analyzed for metalaxyl residues, comprising of three (3) samples each from fourteen (14) districts (Appendix 3). Metalaxyl residues were detected in thirteen (13) of the samples from six (6) districts. The highest and lowest average concentration of metalaxyl were sampled from Sefwi Wiawso (0.028 mg/kg) and Adabokrom (0.018 mg/kg) respectively. The levels ranged from ND to 0.028 mg/kg and the mean was 0.023 mg/kg. Metalaxyl residues detected in the samples were lower than the current Codex, Japan and EU MRL but three (3) were above the proposed EU MRL (Table 4.6).

Table 4.6 Levels of metalaxyl residues in samples from Western north region relative to Codex, Japan and EU and proposed EU MRL.

District	Metalaxyl residue (mg/kg)	MRL (mg/kg)			
		Codex	Japan	EU	EU proposed
Adabokrom	0.018 ± 0.005	0.2	0.2	0.1	0.02
Akontombra	0.024 ± 0.008	0.2	0.2	0.1	0.02
Asawinso	ND	0.2	0.2	0.1	0.02
Asempanaye	ND	0.2	0.2	0.1	0.02
Awaso Bekwai	ND	0.2	0.2	0.1	0.02

Samples from					
Bodi	ND	0.2	0.2	0.1	0.02
Bonsu Nkwanta	0.027 ± 0.000	0.2	0.2	0.1	0.02
Debiso	ND	0.2	0.2	0.1	0.02
Bassam	ND	0.2	0.2	0.1	0.02
Fosukrom	ND	0.2	0.2	0.1	0.02
Juabeso	ND	0.2	0.2	0.1	0.02
SefwiAnhwiaso	0.020 ± 0.007	0.2	0.2	0.1	0.02
Sefwi Kaase	0.019 ± 0.001	0.2	0.2	0.1	0.02
Sefwi Wiawso	0.028 ± 0.013	0.2	0.2	0.1	0.02

ND – Not Detected

4.7 Western south region

Thirty six (36) samples from the Western south region were analyzed for metalaxyl residues, comprising of three (3) samples each from twelve (12) districts (Appendix 2). Metalaxyl residues were detected in sixteen (16) of the samples from eight (8) districts. The highest and lowest average concentration of metalaxyl residues were detected in samples from Agona Amenfi (0.048 mg/kg) and Enchi (0.015 mg/kg) respectively. The levels ranged from ND to 0.048 mg/kg and the mean was 0.029 mg/kg. Metalaxyl residues detected in the samples from all the districts were lower than the current Codex, Japan and EU MRL. However, metalaxyl residues detected in seven (7) of the districts were above the proposed EU MRL (Table 4.7).

Table 4.7 Levels of metalaxyl residues in samples from Western south region relative to Codex, Japan and EU and proposed EU MRL.

Samples from

District	Metalaxyl residue (mg/kg)	MRL (mg/kg)			
		Codex	Japan		EU proposed
Agona Amenfi	0.048 ± 0.034	0.2	0.2	0.1	0.02
Asankragua	ND	0.2	0.2	0.1	0.02
Bogoso	ND	0.2	0.2	0.1	0.02
Dadieso	0.021 ± 0.005	0.2	0.2	0.1	0.02
Diaso	0.033 ± 0.000	0.2	0.2	0.1	0.02
Dunkwa	0.021 ± 0.007	0.2	0.2	0.1	0.02
Enchi	0.015 ± 0.000	0.2	0.2	0.1	0.02
Manso Amenfi	ND	0.2	0.2	0.1	0.02
Samreboi	0.035 ± 0.000	0.2	0.2	0.1	0.02
Takoradi	ND	0.2	0.2	0.1	0.02
Tarkwa	0.034 ± 0.008	0.2	0.2	0.1	0.02
Wassa Akropong	0.023 ± 0.000	0.2	0.2	0.1	0.02

ND – Not Detected

4.8 Volta region

Three (3) samples from Volta region were analyzed for metalaxyl residues, comprising of three (3) samples from Hohoe district (Appendix 6). Metalaxyl residues were not detected in any of the samples from the district (Table 4.8).

Table 4.8 Levels of metalaxyl residues in samples from Volta region relative to Codex, Japan and EU and proposed EU MRL.

District	Metalaxyl residue (mg/kg)	MRL (mg/kg)			
		Codex	Japan	EU	EU proposed

Samples from					
Hohoe	ND	0.2	0.2	0.1	0.02

ND – Not Detected

Findings from the study showed that, metalaxyl residues detected in all the samples (100%) were below the current Codex, Japan and EU MRL. This finding is confirmed to a larger extent by the investigation conducted by Okoffo *et al*. (2016) for the presence of some organochlorine pesticides in cocoa beans which revealed that in all the 32 samples, none of the detected pesticide residues exceeded the EU MRL except for one pesticide. This investigation supports the findings from this study where the metalaxyl residues detected in all the samples were below the Codex, Japan and EU MRL. In addition, a recent study conducted by Malhat (2017) showed that metalaxyl residues detected in tomatoes were less than its MRL. This also is clearly in agreement with the findings of this study.

Work done by Frimpong *et al*. (2012) involving 45 samples of cocoa beans investigated for the presence of nine organochlorine pesticides at varying concentrations revealed that in all the 45 samples of cocoa beans, none of the detected organochlorine pesticide residue concentrations exceeded the EU or Japan MRL in cocoa beans. This work supports the findings of this study where none of the 204 cocoa bean samples exceeded the EU or Japan MRL for metalaxyl.

Out of the total number of samples (204) analyzed, 23.04% detected metalaxyl residues which 8.33% of the samples had metalaxyl residues lower than the proposed EU MRL and 14.71% of the samples also had metalaxyl residues above the proposed EU MRL. Study conducted by Nasiri *et al*. (2016) on 60 cucumber samples rather found 41.7% were contaminated with pesticide residues which 31.7% of the samples had pesticide residues below the MRL and 10% of the samples had pesticide residues above the MRL.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The 204 cocoa bean samples investigated for the distribution of metalaxyl residues in the cocoa growing regions of Ghana showed that, Western south (0.029 mg/kg) recorded the highest mean metalaxyl residues followed by Ashanti (0.025 mg/kg), Central (0.024 mg/kg), Western north (0.023 mg/kg), Brong Ahafo (0.019 mg/kg) and Eastern (0.016 mg/kg) in decreasing order. However, samples from Volta region recorded no detection of metalaxyl residues.

Residues of metalaxyl detected in the samples from all the regions were below the current Codex, Japan and EU MRL. On the other hand, the residues of metalaxyl detected in samples from Western north, Western south, Ashanti and Central were above the proposed EU MRL.

There were no significant differences between the mean metalaxyl residue levels recorded in the samples from Ashanti, Brong Ahafo, Central and Eastern region. Nonetheless, significant difference was found between the mean metalaxyl residue levels recorded in the samples from Western north, Western south and Eastern region.

To conclude, the findings from the study suggests that a downward review of the current Codex, Japan, and EU metalaxyl MRL to the proposed EU MRL would have an impact on the sale of cocoa beans from Ashanti, Central, Western north and Western south region to the international market.

5.2 Recommendations

Based on the findings from this study, the following recommendations are made:

i. The cocoa diseases and pest control (CODAPEC) programme should be intensified and also strictly adhered to, in order to deter farmers from engaging in indiscriminate application of fungicides containing metalaxyl which may cause the violation of the international trade standards.

ii. Ghana Cocoa Board should ensure the residue test for metalaxyl is conducted as a quality measure and to also draw inferences from the distribution and trends of its prevalence in all the cocoa growing regions.

iii. Further research should be conducted on the occurrence level of metalaxyl-M in cocoa and other cash crops in Ghana.

REFERENCES

Abankwah, V., Aidoo, R. and Osei, R.K (2010). Socio-Economic Impact of Government Spraying Programme on Cocoa Farmers in Ghana. *Journal of Sustainable Development in Africa*, 12(4): 116–126.

Adejumo, O.A., Ojoko, E.A. and Yusuf, S.A. (2014). Factors influencing choice of pesticides used by grain farmers in Southwest Nigeria. *Journal of Biology, Agriculture and Healthcare*, 4(28): 31–38.

Adeogun, S.O. and Agbongiarhuoyi, A.E. (2009). Assessment of cocoa farmer‟s chemical usage pattern in pest and disease management in Ondo State. *Journal of Innovation and Development Strategy*, 3(2): 27–34.

Adjinah, K.O. and Opoku, I.Y. (2010). The National Cocoa Diseases and Pest Control (CODAPEC): Achievements and Challenges. Ghana Cocoa Board. Myjoyonline.com. Accessed on 15th June 2017.

African Cocoa Initiative PERSUAP – July 23, 2012.

Anang, B.T. (2011). Market structure and competition in the Ghanaian Cocoa Sector after partial liberalization. *Current Research Journal of Social Science*, 3(6): 465–470.

Anim-Kwapong, G.J. and Frimpong, E.B. (2004). Vulnerability and adaptation assessment under the Netherlands Climate Change Studies Assistance Programme Phase 2

(NCCSAP2): vulnerability of agriculture to climate change-impact of climate on cocoa production, vol. 2. Cocoa Research Institute of Ghana.

Antle, J.M. and Pingali, P.L. (1994). Pesticides, Productivity and Farmer Health: A Philippine case study. *American Journal of Agricultural Economics*, 76: 418–430.

Appiah, M.R. (2004). Impact of Cocoa Research Innovations on Poverty Alleviation in Ghana. Ghana Academy of Arts and Science Publishers. pp. 17-19.

Ayenor, G.K., Huis, A.V., Obeng-Ofori, D., Padi, B. and Röling, N.G. (2007). Facilitating the use of alternative capsid control methods towards sustainable production of organic cocoa in Ghana. *International Journal of Tropical Insect Science*, 27(02): 85–94.

Beasley, V. (1999). Absorption, Distribution, Metabolism, and Elimination : Differences among Species (9-Aug-1999). *Veterinary Toxicology*, pp. 1-19.

Brecher, R. W. (2009). Risk assessment. *Toxicologic pathology*, 25, pp. 23–26.

Buchenauer, H., Bowers, W.S., Ebing, W., Martin, D. and Wegler, R. (1990). Physiological reactions in the inhibition of plant pathogenic fungi. In Chemistry of Plant Protection. Part 6: Controlled Release. Biochemical Effects of Pesticides, Inhibition of Plant Pathogenic Fungi, Springer: Heidelberg, 1990; 235–258.

COCOBOD, 2010 Annual Report. Ghana Cocoa Board, Buck Press, pp. 49-50.

Codex Alimentarius Commission (CAC) (2014). „Comments on Draft and proposed draft maximum residue limits for pesticides in foods and feeds at Steps 6and 3, submitted by Cameroon, and European Union". Codex Committee on Pesticide Residues 46th Session Nanjing, P. R. China, 5-10 May 2014. Available at (ftp://ftp.fao.org/codex/meetings/CCPR/CCPR46/CRDs/pr46_crd04x.pdf)

Cooper, J. and Cudjoe, A. (2012). Pesticide use on cocoa in Ghana; an evaluation of the strengths and weaknesses from a food safety perspective; EDES mission100006/CSM/NRI-University of Ghana/010/GHA Publication Number 388, p 12.

Dakwa, J.T. (1987). A serious outbreak of black pod disease in a marginal area of Ghana. In: Proceedings of the 10th Inter. Cocoa Research Conference. Santo Domingo, Dominican Republic. pp. 447-451.

Danso-Abbeam G., Setsoafia, E.D., Gershon I. and Ansah, K. (2014). Modelling farmer"s investment in agrochemicals: the experience of smallholder cocoa farmers in Ghana. *Research in Applied Economics*, 6(4): 1–15.

EFSA (2013). „Reasoned opinion on the review of the existing maximum residue levels (MRLs) for metalaxyl according to Article 12 of Regulation (EC)“, *EFSA Journal*, 11(396): 32-31.

Fallis, A. (2013). Chemicals Evaluated for Carcinogenic Potential. *Journal of Chemical Information and Modeling*, 53(9): 1689–1699.

FAO (2005). Pesticide residues in food — *2004*. Available at: http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Reports_1991-2006/report2004jmpr.pdf.

FAO (2006). Food Safety Risk Analysis: A guide for national food safety authorities. *Food and Nutrition Paper*, 86. pp. 51-57.

FAO/WHO (2007). Pesticide residues in food - 2007. Joint FAO / WHO Meeting on Pesticide Residues, September. pp. 18–27.

Flood, J., Guest, D., Holmes, K.A., Keane, P., Padi, B. and Sulistyowati, E. (2004). Cocoa under attack. In: Flood, J. and Murphy, R. (Eds.), Cocoa Futures: A Source Book of

Some Important Issues Facing the Cocoa Industry. Chinchina, Colombia: CABI-FEDERACAFE, USDA. pp. 33-53.

Francisco, S.B., Baskaran, S. and Kennedy, R.I. (2002). Ecological relative risk (EcoRR): another approach for risk assessment of pesticides in agriculture. *Agriculture Ecosystems and Environment*, 91(1-3): 37-57.

Frimpong, K. S., Yeboah, P. O., Fletcher, J. J., Pwamang, J. and Adomako, D. (2012). Assessment of synthetic pyrethroids pesticides residues in cocoa beans from Ghana. *Food Science*, 49, 9871–9875.

Gillespie, Z., Pulido, O. and Vavasour, E. (2011). Risk assessment approaches for carcinogenic food contaminants. *International Food Risk Analysis Journal*, 1(1): 1–18.

Hansch, C., Leo, A. and Hoekman, D. (1995). Exploring QSAR - Hydrophobic, Electronic, and Steric Constants. American Chemical Society, Washington DC, USA. p. 134

Hou, B. and Wu, L. (2010). Safety impact and farmer awareness of pesticide residues. *Food and Agricultural Immunology*, 21: 191–200.

ICCO (2010). ICCO Quarterly Bulletin of Cocoa Statistics, Vol. XXXVI, No. 4, Cocoa year 2009/2010. Published: 30-11-2010.

Institute of Statistical, Social and Economic Research (ISSER) (2010). The state of the Ghanaian economy. Institute of Statistical Social and Economic Research (ISSER), University of Ghana, Legon, Ghana, p. 21.

JMPR (2002). Pesticide residues in food. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and a WHO Expert Group on Pesticide Residues. FAO Plant Production and Protecion Paper, 176, Rome.

JMPR (2005). Pesticide residues in food. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and a WHO Expert Group on Pesticide Residues. FAO Plant Production and Protecion Paper, 179, Rome.

Jurewicz, J. and Hanke, W. (2008). Prenatal and childhood exposure to pesticides and neurobehavioral development: review of epidemiological studies. *International Journal of Occupational Medicine and Environmental Health*, 21 (2): 121–132.

Kidd, H. and James, D. R. (1992). The Agrochemicals Handbook, Royal Society of Chemistry Information Services. Cambridge, United Kingdom.

Konradsen, F. (2007). Acute pesticide poisoning: a global public health problem. *Danish Medical Bulletin*, 54(1): 58–59.

Lorenz, E. S. (2009). Potential Health Effects of Pesticides. *Agricultural Communications and Marketing*. pp. 1–8.

Luterbacher M.L, Akrofi, A.Y. (1993). The current status and distribution of *Phytophthora megakarya* in Ghana. In: Proceedings of the 11th International Cocoa Research Conference, Yamoussoukro, Cote D'Ivoire. pp. 57-58.

Malhat, F. M. (2017). Persistence of metalaxyl residues on tomato fruit using high performance liquid chromatography and QuEChERS methodology. *Arabian Journal of Chemistry*, 10: 765–768.

Mbabazi, J. (2011). Principles and Methods for the Risk Assessment of Chemicals in Food. *International Journal of Environmental Studies*, 68(2): 251–252.

Ministry of Health Labour and Welfare (2005). Analytical Methods for Residual Compositional Substances of Agricultural Chemicals, Feed Additives, and Veterinary Drugs in Food - (124001), MHLW, Japan. pp. 1–52.

Moy, G.G. and Wessel, J.R. (2000). Codex Standard for Pesticides Residues. In International Standards for Food Safety, Rees, N.; Watson, D., (Eds) Aspen Publishers Inc. Gaithersburg, MD, USA.

Nasiri, A., Amirahmadi, M., Mousavi, Z., Shoeibi, S., Khajeamiri, A. and Kobarfard, F. (2016). A multi residue GC-MS method for determination of 12 pesticides in cucumber. *Iranian Journal of Pharmaceutical Research*, 15(4): 809–816.

Nawn, J. and Sato, S. (2009). Japan MRL Changes in Indoxacarb and other five chemicals. Pharmaceutical and Food Safety Bureau. Ministry of Health, Labour and Welfare, Tokyo, Japan.

Ntiamoah, A. and Afrane, G. (2012). Environmental impacts of cocoa production and processing in Ghana: life cycle assessment approach. *Journal of Cleaner Production*, 16: 1735-1740.

Ntow, W.J., Gijzen, H.J., Kelderman, P. and Drechsel, P. (2006). Farmer perceptions and pesticide use practices in vegetable production in Ghana. *Pest Management Science*, 62(4): 356–365.

Ntow, W.J., Tagoe, L. M., Drechsel, P., Kelderman, P. and Gijzen, H. J. (2001). Organochlorine pesticides in water, sediment, crops, and human fluids in a farming community in Ghana. *Archives of Environmental Contamination and Toxicology*, 40: 557–563.

Offei, S. K., Cornelius, E. W. and Sakyi-Dawson, O. (2000). Crop Diseases in Ghana and their Management. Sponsored by TALIF for College of Agriculture, University of Ghana, Legon, Accra, Smartline Ltd.

Okoffo, E. D., Fosu-Mensah, B. Y. and Gordon, C. (2016). Persistent organochlorine pesticide residues in cocoa beans from Ghana, a concern for public health. *International Journal of Food Contamination*, 3(1): 5.

O'Neil, M.J. (2001). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Edition, Whitehouse Station, New Jersey. p. 1058

Opeke, L.K. (1987). Tropical tree crops. Ibadan, Nigeria: Spectrum Books Ltd. p. 327.

Opoku, I.Y., Appiah A.A., Akrofi, A.Y. and Owusu, G.K. (2000). *Phytophthora megakarya*: a potential threat to the cocoa industry in Ghana. *Ghana Journal of Agricultural Science*, 33: 237-248.

Pimentel, D. (2005). Environmental and socio-economic costs of the applications of pesticides primarily in the United States. *Journal of Environment Development and Sustainability*, 7: 229–252.

Sam, K.G., Andrade, H.H., Pradhan, L., Pradhan, A., Sones, S.J., Rao, P.G. and Sudhakar, C. (2008). Effectiveness of an educational program to promote pesticide safety among pesticide handlers of South India. *International Archives of Occupational and Environmental Health*, 81(6): 787–795.

Sanborn, M., Kerr, K.J., Sanin, L.H., Cole, D.C., Bassil, K.L. and Vakil, C. (2007). Non-cancer health effects of pesticides: systematic review and implications for family doctors. *Canadian Family Physician*, 53 (10): 1712–1720.

Singh, S.K., Raha, P. and Banerjee, H. (2002). Banned Organochlorine Cyclodiene Pesticide in Ground Water in Varanasi, India. *Bulletin of Environmental Contamination and Toxicology*, 76: 935-941.

Sukul, P. and Spiteller, M. (2000). Metalaxyl: persistence, degradation, metabolism and analytical methods. *Reviews of Environmental and Contamination Toxicology,* 164:1-26.

Sukul, P. and Spiteller, M. (2001). Influence of biotic and abiotic factors on dissipating metalaxyl in soil. *Chemosphere*, 45: 941–947.

Tomlin, C.D.S. (1997). The Pesticide Manual - World Compendium. 11th Edition, British Crop Protection Council, Surrey, England. p. 792

Tomlin, C.D.S. (2000). The Pesticide Manual. 12th Edition, British Crop Protection Council, Farnham, Surrey, United Kingdom. p. 654

U.S. National Library of Medicine (1995). Hazardous Substances Databank. Bethesda, M.D. pp 9-10

UNDP (2008). Human development report - consumption for human development. New York: Oxford University Press.

UNEP (2008). Guidance for identifying populations at risk from Mercury exposure. p. 176. DTIE Chemicals Branch and WHO Department of Food Safety. Available at: http://www.who.int/foodsafety/publications/chem/mercuryexposure.pdf

Urech, P.A., Schwinn, F. and Staub, T. (1977). A novel fungicide for the control of late blight, downy mildew, and related soil borne diseases. In Proceedings of the British Crop Protection Conference; 21–24 November 1977. The Boots Company: Nottingham, UK. pp. 623–627.

USEPA (1992). Guidelines for exposure assessment. *Risk Assessment Forum*, 57(104), Washington, DC, EPA/600/Z-92/001.

USEPA (1994). Prevention, Pesticides and Toxic Substances (7508W); EPA-738-F-94013, September 1994. pp. 1-4

USEPA. (1999). Guidelines for carcinogen risk assessment. *Risk Assessment Forum*, Washington, DC, NCEA-F-0644, Review Draft.

USEPA (2000). Revisions to the methodology for deriving ambient water quality criteria for the protection of human health. Available: http://www.epa.gov/ EPA-WATER/2000/November/Day-03/w27924.htm (Accessed: 11th July, 2016).

USEPA (2016). Guideline for Human Exposure Assessment. *Risk Assessment Forum*, pp. 1–199.

WHO (1990). Public health impact of pesticides used in agriculture. Geneva.

WHO (2003). The world health report 2003 - shaping the future. Geneva.

WHO (2005). The WHO recommended classification of pesticides by hazard and guidelines to classification 200-2202 (WHO/PCS/01.5) Available from the International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland.

WHO (2010). Risk Assessment: chemical hazards. *International Programme on Chemical Safety*.

WHO/UNEP (1990). Public health impact of pesticides used in agriculture. World Health Organization, Geneva.

Wood, G.A.R. and Lass, R.A. (1985). Cocoa. 4th Edition. Tropical Agricultural Series, Longmans, London, UK.

Yáñez, L. (2002). Overview of human health and chemical mixtures: problems facing developing countries. *Environmental Health Perspectives*, 110(6): 901-909.

APPENDIX

Appendix 1: Extraction procedure

Ten grams (10 g) of homogenous and representative sample was weighed into a 250 ml Nalgene jar. Distilled water 20 ml was added and allowed to stand for about 15 min to wet the sample and increase the extraction efficiency of the extracting solvent. About 100 ml of acetonitrile was added, homogenized using Kinematica Polytron PT 3100D for 1 min at a speed of 17000 rpm and also centrifuged using Centurion K-3 series from England for 5 min at speed of 2500 rpm. The sample was filtered into a 500 ml round bottom flask and about 50 ml of acetonitrile added to the residue, homogenized and centrifuged per the same conditions in the first round. The sample was then filtered using Whatman No. 4 filter paper from England to top up the filtrate in the first round and concentrated to about 20 ml below 40 ºC using Buchi Rotavapor R-210 from Switzerland to remove the acetonitrile.

Clean-up steps

The extraction process was improved and optimized using clean-up or purification procedures to remove matrix compounds that could be present in the sample extract which may interfere in the GCMS analysis.

Clean-up step 1 using Diatomaceous earth column 20 ml

The sample extract was loaded unto the diatomaceous earth column and allowed to stand for about 5 min to enhance the adsorption of water unto the column bed. About 80 ml of ethyl acetate was measured into the round bottom flask to dissolve any residue, then loaded unto the column and the eluate collected at a flow rate of about 1-3 drops per second using a luer stop valve. The ethyl acetate eluate was concentrated to dryness below 40 ^{o}C using the rotary evaporator. About 25 ml of acetonitrile was added to the concentrate (sample) and re-dissolved with aid of Sonorex Digitech ultrasonic water bath from Germany.

Clean-up step 2 using Graphitized Carbon Black (GCB)/Amino propyl (NH_2) 500 mg/500 mg, 6 ml

A solid phase extraction (SPE) technique was used to remove residual fat (cocoa butter) and colour/pigment in the sample extract using GCB/NH_2 column. The GCB/NH_2 column was conditioned with about 10 ml acetonitrile followed by 5 ml of the sample extract loaded and allowed to flow at a rate of about 1-2 drops per second with the aid of a vacuum manifold. About 20 ml acetonitrile was used to elute the column. The resultant eluate was collected into a 100 ml round bottom flask and concentrated to dryness below 40 ^{o}C using the rotary evaporator. About 2 ml of n-Hexane was added to re-dissolve the sample extract with the aid of an ultrasonic water bath.

Clean-up step 3 using Florisil 1 g/6 ml

The florisil column was conditioned with about 5 ml n-hexane, the sample extract from the previous clean-up step loaded and allowed to flow at a rate of about 1-2 drops per second with the aid of a vacuum manifold. About 5 ml acetone: n-hexane (9:1 v/v) was used to elute

the column and the resultant eluate collected into a 50 ml round bottom flask. The eluate was concentrated to dryness below 40 ºC using rotary evaporator. The concentrate was re-dissolved with about 1 ml of acetone: n-hexane (1:1 v/v) with the aid of an ultrasonic bath and transferred into a 1.5 ml GC vial. The final sample extract in the vial was loaded unto the GCMS for analysis.

Appendix 2: Metalaxyl residues in samples from the districts of western south region

DISTRICT	METALAXYL CONCENTRATION	MEAN METALAXYL CONCENTRATION (mg/kg)
Agona Amenfi	0.087 0.024 0.034	0.048
Asankragua	ND ND ND	ND
Bogoso	ND ND ND	ND
Dadieso	0.019 0.027 0.017	0.021
Diaso	ND 0.033 ND	0.033
Dunkwa	0.014 0.02 0.028	0.021
Enchi	0.015 ND ND	0.015
Manso Amenfi	ND ND ND	ND

Samreboi	ND 0.035 ND	0.035
Takoradi	ND ND ND	ND
Tarkwa	0.038 0.025 0.04	0.034
Wasa Akropong	ND 0.023 ND	0.023

Appendix 3: Metalaxyl residues in samples from the districts of western north region

DISTRICT	METALAXYL CONCENTRATION	MEAN METALAXYL CONCENTRATION (mg/kg)
Adabokrom	0.014 0.021 ND	0.018
Akontonbra	0.018 ND 0.03	0.024
Asawinso	ND ND ND	ND
Asempaneye	ND ND ND	ND
Awaso Bekwai	ND ND ND	ND
Bodi	0.027 ND ND	ND
Bonsu Nkwanta	ND 0.035 0.017	0.027
Debiso	ND ND ND	ND
Essam	ND ND ND	ND

Fosukrom	ND ND ND	ND
Juabeso	ND ND ND	ND
Sefwi Anhwiaso	0.027 0.018 0.014	ND
Sefwi Kaase	ND 0.02 0.018	0.019
Sefwi Wiawso	0.041 0.015 0.027	0.028

Appendix 4: Metalaxyl residues in samples from the districts of eastern region

DISTRICT	METALAXYL CONCENTRATION	MEAN METALAXYL CONCENTRATION (mg/kg)
Achiase	0.014 0.021 ND	0.018
Akim Oda	0.018 ND 0.03	0.024
Akoase	ND ND ND	ND
Asamankese	ND ND ND	ND
Kade	ND ND ND	ND
Kibi	0.027 ND ND	ND
Koforidua	ND 0.035 0.017	0.027

Nkawkaw	ND ND ND	ND
Ofoase	ND ND ND	ND
Suhum	ND ND ND	ND

Appendix 5: Metalaxyl residues in samples from the districts of central region

DISTRICT	METALAXYL CONCENTRATION	MEAN METALAXYL CONCENTRATION (mg/kg)
Breman Asikuma	ND ND ND	ND
Assin Breku	0.022 ND ND	0.022
Cape Coast	ND ND ND	ND

Assin Fosu	ND ND ND	ND
Twifo Nyinase	ND ND ND	ND
Twifo Praso	ND ND ND	ND
Swedru	0.026 0.031 0.018	0.025

Appendix 6: Metalaxyl residues in samples from the districts of Volta region

DISTRICT	METALAXYL CONCENTRAION	MEAN METALAXYL CONCENTRATION (mg/kg)
Hohoe	ND ND ND	ND

Appendix 7: Metalaxyl residues in samples from the districts of Brong Ahafo region

DISTRICT	METALAXYL CONCENTRATION	MEAN METALAXYL CONCENTRATION (mg/kg)
Asumura	ND	ND
	ND	
	ND	
D. Ahenkro	ND	ND
	ND	
	ND	
Goaso/Mim	ND	ND
	ND	
	ND	
Hwidiem	ND	ND
	ND	
	ND	
Kasapin	ND	ND
	ND	
	ND	
Kukuom	ND	ND
	ND	
	ND	
Nkrankwanta	0.014	0.019
	0.024	
	ND	
Sankore	ND	ND
	ND	
	ND	

Sunyani	ND ND ND	ND

Appendix 8: Metalaxyl residues in samples from the districts of Ashanti region

DISTRICT	METALAXYL CON-CENTRATION	MEAN METALAXYL CONCENTRATION (mg/kg)
Agona	ND ND ND	ND
Ampenim	ND ND ND	0.030
Antoakrom	ND ND ND	ND
Bekwai	ND ND ND	ND
Effiduase	ND ND ND	ND
Juaso	0.019 ND 0.023	0.021
Konongo	ND 0.035 0.017	0.026
Mankranso	ND ND 0.021	0.021
New Edubiase	ND ND ND	ND
Nkawie	ND 0.025 0.033	0.029
Nsokote	ND ND ND	ND
Nyinahin	0.027 0.023 ND	0.025

Obuasi	ND ND ND	ND
Offinso	ND ND ND	ND
Tepa	ND ND ND	ND

Appendix 9: One way Anova results

Between-Subjects Factors

		N
Regions	Ashanti	45
	B. Ahafo	27
	Central	
	Eastern	21
	Volta	
	W. North	30
	W. South	3
		42
		36

Descriptive Statistics

Dependent Variable: Metalaxyl (mg/Kg)

Re-gions	Mean	Std. Deviation	N
Ashanti	.005622	.0109696	45

B. Ahafo	.001407	.0052569	27
Central	.004619	.0099924	21
Eastern	.001067	.0041351	30
Volta	.000000	.0000000	3
W. North	.006905	.0112571	42
W. South	.013306	.0188404	36
Total	.005828	.0120608	204

Levene's Test[a] of Equality of Error Variances

Dependent Variable: Metalaxyl (mg/Kg)

F	df1	df2	Sig.
13.263	6	197	.000

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.[a]

a. Design: Intercept + Regions

Tests of Between-Subjects Effects

Dependent Variable : Metalaxyl (mg/Kg)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	.003[a]	6	.001	4.278	.000	.115
Intercept	.002	1	.002	15.567	.000	.073
Regions	.003	6	.001	4.278	.000	.115
	.026					

Error	.036	197	.000			
Total	.030	204				
Corrected Total		203				

Tests of Between-Subjects Effects Dependent Variable : Metalaxyl (mg/Kg)

Source	Noncent. Parameter	Observed Power
Corrected Model	25.667[a]	.979
Intercept	15.567	.975
Regions	25.667	.979
Error		
Total		
Corrected Total		

a. R Squared = .115 (Adjusted R Squared = .088)

b. Computed using alpha = .05

Estimated Marginal Means

Regions

Dependent Variable : Metalaxyl (mg/Kg)

Re-gions	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Ashanti	.006	.002	.002	.009
B. Ahafo	.001	.002	-.003	.006
Central	.005	.003	.000	.010

Eastern	.001	.002	-.003	.005
Volta	1.000E-013	.007	-.013	.013
W. North	.007	.002	.003	.010
W. South	.013	.002	.010	.017

Regions

Multiple Comparisons

Dependent Variable : Metalaxyl (mg/Kg)

LSD

(I) Regions	(J) Regions	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Ashanti	B. Ahafo	.004215	.0028033	.134	-.001314	.009743
	Central	.001003	.0030434	.742	-.004999	.007005
	Eastern Volta	.004556	.0027143	.095	-.000797	.009908
	W. North	.005622	.0068667	.414	-.007920	.019164
	W. South	-.001283	.0024707	.604	-.006155	.003590
		-.007683*	.0025750	.003	-.012761	-.002605
B. Ahafo	Ashanti	-.004215	.0028033	.134	-.009743	.001314
	Central	-.003212	.0033506	.339	-.009819	.003396
	Eastern Volta	.000341	.0030549	.911	-.005684	.006365
	W. North	.001407	.0070083	.841	-.012414	.015228
	W. South	-.005497	.0028406	.054	-.011099	.000105

		-.011898*	.0029318	.000	-.017680	-.006116
	Ashanti	-.001003	.0030434	.742	-.007005	.004999
	B. Ahafo Eastern	.003212	.0033506	.339	-.003396	.009819
	Volta	.003552	.0032765	.280	-.002909	.010014
Central	W. North	.004619	.0071077	.517	-.009398	.018636
	W. South	-.002286	.0030777	.459	-.008355	.003784
		-.008687*	.0031621	.007	-.014922	-.002451
	Ashanti	-.004556	.0027143	.095	-.009908	.000797
	B. Ahafo	-.000341	.0030549	.911	-.006365	.005684
	Central	-.003552	.0032765	.280	-.010014	.002909
Eastern	Volta	.001067	.0069732	.879	-.012685	.014818
	W. North	-.005838*	.0027528	.035	-.011267	-.000409
	W. South	-.012239*	.0028468	.000	-.017853	-.006625
	Ashanti	-.005622	.0068667	.414	-.019164	.007920
	B. Ahafo Central	-.001407	.0070083	.841	-.015228	.012414
	Eastern	-.004619	.0071077	.517	-.018636	.009398
Volta		-.001067	.0069732	.879	-.014818	.012685
	W. North	-.006905	.0068820	.317	-.020477	.006667
	W. South	-.013306	.0069202	.056	-.026953	.000342
	Ashanti	.001283	.0024707	.604	-.003590	.006155
	B. Ahafo Central	.005497	.0028406	.054	-.000105	.011099
	Eastern	.002286	.0030777	.459	-.003784	.008355
W. North	Volta	.005838*	.0027528	.035	.000409	.011267
	W. South					

		.006905	.0068820	.317	-.006667	.020477
		-.006401*	.0026156	.015	-.011559	-.001243
	Ashanti	.007683*	.0025750	.003	.002605	.012761
	B. Ahafo Central	.011898*	.0029318	.000	.006116	.017680
	Eastern	.008687*	.0031621	.007	.002451	.014922
W. South	Volta	.012239*	.0028468	.000	.006625	.017853
	W. North	.013306	.0069202	.056	-.000342	.026953
		.006401*	.0026156	.015	.001243	.011559

Based on observed means.

The error term is Mean Square (Error) = .000.

*. The mean difference is significant at the .05 level.