Investigation of a Hazardous Metabolite, Ethylenethiourea, in Ethylene Bis-Dithiocarbamates, Fresh and Processed Tomatoes: Case of Mwea Division, Kirinyaga District

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

To my sister, Julia Kariuki, for the assistance she extended to me when I enrolled for the course. To her I say, 'may God bless you'.

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LIST OF ABREVIATIONS AND ACRONYMS

AIDS	Acquired Immune Deficiency Syndrome
BMR	Basal Metabolic Rate
DCs	Dithiocarbamates
EBDC	Ethylenebis-dithiocarbamate
EBIS	Ethylene Bis Isothiocyanate Sulphide
EPA	Environmental Protection Agency of USA
ETU	Ethylene thiourea
EU	European Union
EUREP GAP	European –Retail Good Agricultural Practices
FAO	Food and Agriculture Organization
HIV	Human Immuno-deficiency Virus
HPLC	High Performance Liquid Chromatography
IHA	Italian Health Authority
JMPR	Joint Meeting on Pesticide Residues
LD_{50}	Lethal $Dose_{50}$
MRLs	Maximum Residue Levels
NIOSH	National Institute for Occupational Safety and Health
nm	nanometer
NRCC	National Research Council Criteria
OSHA	Occupational Safety and Health Administration

PBDC	Propylene Ethylenebis-dithiocarbamate
PCPB	Pest Control Products Board
PHI	Pre-Harvest Interval
ppm	parts per million
T_3	Tri-iodothyronine
T_4	Thyroxine
μl	microlitre
UNEP	United Nations Environmental Programme
USEPA	United States Environmental Protection Agency
UV	Ultra Violet
WHO	World Health Organization

LIST OF CHEMICAL SYNONYMS

Mancozeb	Manganese ethylene bis(dithiocarbamate)
Maneb	Manganese ethylene bis(dithiocarbamate)
Metiram	Zinc ethylene bis(dithiocarbamate)-poly-ethylene bis(thiuramdisulfide) complex
Nabam	Disodium ethylene bis(dithiocarbamate)
Zineb	Zinc ethylene bis(dithiocarbamate)

ABSTRACT

Ethylenethiourea (ETU), an EBDC metabolite with adverse health effects was studied. The overall objective of the study was to investigate how the fungicide formulation and value chain of tomatoes influence dietary exposure to ETU. The set-up involved a case study survey using semi-structured questionnaire in Mwea Division, field experimentation, stratified random sampling of DCs and copper formulation from retail outlets and fresh tomatoes from open-air markets. The effect of washing of surface fungicide deposits on ETU levels was also done alongside the combined effect of washing and cooking. ETU extraction from samples was by 20:80 methanol:water and filtration with hyflo supercel. Samples were analyzed under a reversed phase HPLC with Lichrosorb[R]100 RP8 (5µm) column and a UV detector. Sancozeb[R] (mancozeb) had 4.7 % ETU, which was higher than 0.5% standard of IHA and that of EU. No ETU was detected in copper and propineb formulations. Tomatoes sprayed with Oshothane[R] (mancozeb) and Antracol[R] (propineb) had 10.20 mg/kg and 0.61mg/kg of ETU respectively. With prior washing t-test indicated no significant differences ($P>0.05$) in ETU levels in raw and cooked tomatoes. Conversely, independent t-test, cooked-washed and cooked-unwashed indicated significant differences ($P<0.05$) between means. Canned tomato products, namely puree, paste and ketch-up showed mixed levels of ETU. The research findings showed that dietary exposure to ETU can be reduced by proper choice of fungicide and washing of tomatoes before consumption.

Chapter 1

INTRODUCTION

1.1 Background

Ethylene bis-dithiocarbamate (EBDCs) and propylene bis-dithiocarbamates (PBDCs) are the two main classes of dithiocarbamates used as anti-fungals in agriculture. According to Kontou *et al* (2004), EBDCs: maneb, zineb, mancozeb, metiram and naban, are organic, non-systemic fungicides widely used to protect tomato crops against various diseases and a maximum residue limit of 3mg/kg (expressed as CS_2) has been set in the European Community (EU 1998) on a wide variety of horticultural crops, including tomato crop.

In Kenya, there are several factors on the farmer's side that have made EBDCs the chemical of choice. Compared to other fungicides, used in tomatoes, EBDCs or generally the DCs, are not only affordable (Kingsland and Sitter, 1986), they are also readily available and the farmer enjoys a variety of formulations from different companies. In addition, they fit well within the farming systems as well as market requirements.

Hassal *et al.* (1990) explained that on one side, EBDCs are very unstable in nature with the implication that the pre-harvest interval is as short as three days, while on the other side, tomatoes have to be harvested at the right stage to meet market requirements, on. While, EBDCs have low acute toxicity to humans, they are broad-spectrum and have been known to be protective against leaf spot and potato scab, among others. Thus, the worldwide consumption of dithiocarbamates of between 25,000 and 35,000 metric tonnes per year (Kingsland and Sitter, 1986) could be attributed to the above factors.

The ethylene-bis-dithiocarbamates, however, share a common manufacturing process contaminant, ethylenethiourea (ETU) (Hylin, 1973; Marshall, 1977; FAO, 1980; Dearfield, 1994; Vettolazzi *et al*., 1995). Ethylenethiourea (ETU; imidazolidine-2-thione), and isopropylene (i-PTU; 4-methylimidazolidine-2-thione) are, respectively, degradation products of the ethylenebisdithiocarbamate (EBDC) and propylenebisdithiocarbamate (PBDC) fungicides, collectively called alkylenebisdithiocarbamates (ABDCs), which includes some of the most widely used fungicides in agriculture and horticulture (Startin *et al.* 2005). The level of ETU in EBDCs may increase during storage and upon exposure of EBDC fungicides to air, moisture and high temperature (Blasquez, 1973; Bontoyan and Looker, 1973; Kumar and Agarwal, 1991).

The toxicological concerns raised against EBDCs pesticides are mainly associated with this degradation product and processing contaminant, Ethylenethiourea (ETU). ETU is suspected to have carcinogenic properties and is also maternally teratogenic (Ulland *et al*., 1972; Graham *et al*., 1973; Tsuchikya *et al.* 1992). It has also been shown to be a weak genotoxic compound, capable of inducing chromosomal aberrations and genetic malformations (Tsuchikya *et al*., 1992; Scarabelli *et al*., 1993; Dearfield, 1994; Franekic *et al.*, 1994). Moreover, ETU has thyroid toxicity (WHO, 1988) and has been shown to interfere with the production of the tri-iodothyronine (T_3) and thyroxin (T_4) hormones (Nebbie and Fink-Gremmels, 1996), resulting in thyroid disorders (WHO, 1988).

A special report by the International Union of Pure and Applied Chemistry (IUPAC 1977) covers the most important chemical properties of ETU and the dynamics of its while the 1980 Joint Meeting of FAO panel of experts on pesticide residues in food and the environment and the WHO expert group on pesticide residues.

1.2 Problem Statement

ETU is the main metabolite of EBDCs which can be formed either at EBDC formulation, storage, application, under field conditions and any other stage of the tomato value chain. ETU is a class 2B carcinogen (IARC, 1991). Also causes thyroid dysfunction (WHO 1988).

The situation is exacerbated by extensive and massive use of EBDCs in production of tomatoes in Kenya. There is also an information gap on factors that contribute to ETU occurrence in foods at the time of consumption in Kenya.

1.3 Objectives

1.3.1 Overall Objective

Assess how the fungicide formulation, growing environment, and value addition chain of tomatoes influence dietary exposure to ETU.

1.3.2 Specific Objectives

The following were the specific objectives pursued.

1. To conduct a survey to assess fungicide usage in the Mwea Division.

2. Assess and quantify the amount of ETU contaminant in formulated DC products.

3. Assess and quantify the levels of ETU in tomatoes sprayed with DCs.

4. Assess the effect of washing on the level of ETU in cooked and uncooked tomatoes.

5. Assess the effect of cooking on the formation of ETU in tomatoes sprayed with DCs.

6. Investigate the effect of market chain on ETU levels in tomatoes sourced from Mwea, Wakulima, Githurai and Juja fresh air markets.

7. Assess the occurrence of ETU in processed tomato products.

1.4 Hypothesis

The formation of ETU in formulated dithiocarbamates is a function of the nature of the dithiocarbamate as well as the subsequent treatment the commodity receives. The hypotheses to be tested are:-

1. There are statistically different levels of ETU in different dithiocarbamates formulations.
2. There are statistically different levels of ETU in tomatoes sprayed with different dithiocarbamates.
3. Prior washing of tomatoes exposed to EBDCs before cooking significantly reduces the level of ETU.
4. Different fungicide formulations applied during tomato production in Mwea predispose different levels of ETU in foods.
5. There is occurrence of ETU in processed tomato products sold in Kenyan markets.

1.5 Justification

There is information gap on factors that contribute to ETU occurrence in foods at the time of consumption in Kenya, making food safety regulation incomplete. Holland *et al*. (1994) reported lack of information in the literature about the levels of ETU residues left in prepared food after home-cooking of raw crops containing EBDC residues and recommended gathering such information through investigation.

The current method used by monitoring laboratories to determine dithiocarbamate residues in food involves the analysis of carbon disulfide gas (CS_2) generated after hydrolysis of the compound present in the sample. The method is seriously inadequate, as it does not identify the origin of the CS_2 detected and, therefore, it cannot be relied upon for determination of ETU toxicity in foods. It is also worth to note that it is only recently that High Performance Liquid Chromatography equipment has become available in Kenyan institutions. This development, unlike Thin Layer Chromatography and CS_2 determination, will facilitate specific and sensitive detection of ETU present in concentrations as low as 0.05% in the human diets. Thus determination of ETU will provide a fair assessment of exposure to these EBDCs.

Also, these investigations are of prime public health importance in Africa, where dithiocarbamates are applied in the farming systems and in which continent the prevalence of HIV/AIDS is highest. Moreover, these investigations are timely as they come in the wake of pressurized safety demands by EUREP-GAP for any exporter, who would wish to access the European market.

Chapter 2

LITERATURE REVIEW

2.1 Background Information on ETU Impurities

Humans can be exposed to pesticides and their impurities through direct handling, contact with environmental residues, and dietary intake. Technical pesticides, although by definition being 'pure active ingredient', also may contain complex mixtures of other minor chemical components due to process variables, side reactions, and impurities in starting materials. For some impurities such as ETU, this may lead to the allocation of maximum concentration limits in technical grade products (FAO, 1994).

The toxicological tests carried out with technical products of typical composition for registration purposes include assessment of toxic potency of the impurities present in the test material. However, the composition of the technical product may vary, particularly with respect to impurities and potentially also the toxicity of the product, depending on the manufacturing process and sources of starting materials. This is especially of concern in the case of generic pesticides, which may be produced and formulated by many manufacturers under widely varying conditions, with different materials, and a range of quality control standards.

Because the composition of the technical products may reveal the manufacturing process, information on the composition of technical active ingredient and formulation of pesticides is considered to be commercially confidential. It is usually available only for government registration authorities and relevant international advisory committees on a confidential basis (Ambrus *et al.* 2003).

The identification of unknown impurities in a pesticide product at the mg/kg level is a very difficult analytical task because of the complex nature of the technical products. Prediction of the possible impurities that may occur has to be done. This may be aided by a study of the routes of synthesis and side reactions in the manufacturing process as well as impurities in the starting materials. Additional consideration should be given to the known impurities in pesticides of similar chemical structure (Baron *et al.* 1978).

The relevance of the impurities is assessed by taking into account their relative toxicity compared with active ingredient and storage stability.

2.2 Safety Specification of Plant Protection Products

There may be substantial differences in the toxicological safety of products of the same active ingredients manufactured under different conditions or from different additives such as the carriers, adjuvant and surfactants. Thus, toxicological data based on a particular formulated product cannot simply be extrapolated to other generics as some of the ‘inert ingredients’ may influence toxicity. Extended storage may also lead to formation of degradation products, which pose dietary intake hazards to consumers of the treated foods (FAO, 1999).

2.3 Sources of ETU

Ethylene thiourea (ETU) is a decomposition product of ethylene bis-dithiocarbamate (EBDCs), the most widely used class of fungicides in the world (Kumar *et al.* 1991). Most of the ETU in crops, immediately, after the application of EBDCs arises from the ETU present in EBDC formulations as an impurity produced during their manufacture or formed during their storage (Camoni *et al.* 1988).

According to Lentza-Rizos (1990), ETU may also be taken up by plants from the soil following the breakdown of EBDCs. Kumar and Agarwal (1993) reported that ETU could be translocated from one part of the plant to another; this confirms that ETU is systemic and can easily accumulate in plant tissues, particularly the fruits. The common EBDC chemicals include mancozeb, metiram, zineb, maneb and nabam, whose degradation pathway is illustrated in Figure 1.

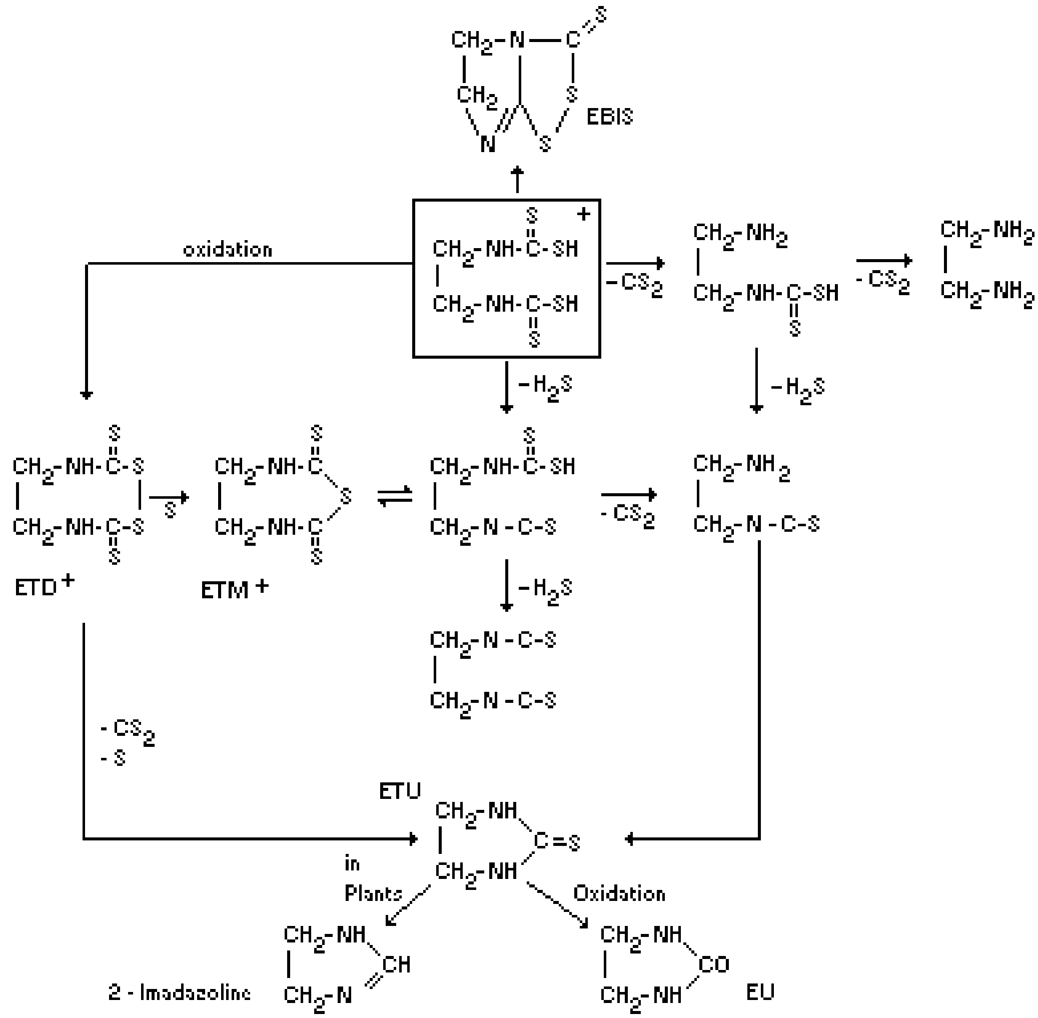

Figure 1. Degradation pathway of ethylene-bis-dithiocarbamates; + = polymeric products.

Source: FAO (1994). Plant Production and Protection

Mancozeb remains unchanged in the soil for only short periods of time. One study recovered only 1.16% of mancozeb seven days after application to silt loam soils, while the half-life was measured three days on fine sand. Soil microorganisms readily break down Mancozeb. One of the break-down products of mancozeb is ETU.

2.4 Health Concerns on ETU

ETU exhibits moderate acute toxicity upon oral administration in rats. The LD_{50} in mice and rats has been reported to be 3000 and 1832 mg/kg, respectively, (U.S. EPA, 1984) and 545 mg/kg in pregnant rats (FAO, 1994).

Rose *et al.* (1980) explained that ETU, being an anti-thyroid, brings about a decrease in the level of circulating thyroid hormones and the decreased level causes stimulatory feedback of the pituitary gland and, consequently, an increased release of thyroid stimulating hormone. ETU acts by blocking the iodination of thyroxine precursors, thus reducing the synthesis of the thyroid hormones. Iodide peroxidase catalyses the iodination of tyrosine and the coupling of the resultant iodotyrosyl residues to produce the active hormones tri-iodothyronine (T_3) and thyroxine (T_4).

Kurttio *et al.* (1986) examined the alterations of function and morphology of the thyroid gland in rats induced by ETU administration. ETU at concentration

10.6-23mg/kg inhibited dose-dependently T4 and (T_3) secretion. It also increased the basal TSH secretion 10-fold, obviously due to lack of negative feedback by T_4 and T_3. No morphological alterations have been induced in thyroids but ultra structural changes have been observed namely an increased number of myelin bodies, dilatation of rough endoplasmic reticulum and increased vacuolization in the epithelial cells of thyroids follicles.

Datson *et al*. (1987), summarizing toxicological properties, stated that the most prominent aspect of ETU toxicity in the adult rat is its action as goitrogen, causing hyperplasia of the thyroid as well as a large decrease in circulating thyroid hormone levels. As neither hypothyroidism nor thyroxine (T_4) supplementation has any effect on teratogenicity of ETU, it seems likely that the development effects of ETU are not mediated by the maternal thyroid state.

In addition to having the potential to cause goiter, a condition in which the thyroid gland is enlarged, this metabolite has produced birth defects and cancer in experimental animals. Ethylene thiourea is believed to induce thyroid tumors through the suppression of thyroxine syntheses, leading to hyperplasia of the thyroid gland (WHO 1988) and other geno-toxic effects (Dearfield, 1994). After proof of its toxicity in 1969, ETU has been placed in a class of hygienically hazardous substances (Chovancova *et al.* 1985). In 1991, the International

Agency for Research on Cancer (IARC) classified ETU as a Type 2B carcinogen and an anti-thyroid compound.

According to Teramoto *et al*. (1980) ETU has been shown to be nitrosable and converted to N-nitroso ETU (NO-ETU), which is strongly mutagenic in acidic conditions. NO-ETU can also be formed from nitrates during their metabolic reduction in mammalian species and during micro-biological conversion in vegetables stored at room temperature for prolonged periods.

Kenya, like many other economies in Africa, is now contending with the HIV/AIDS scourge. The viral load in those living with HIV/AIDS is associated with increased energy requirements to maintain the basal metabolic rate (BMR), which is under the influence of growth hormones, T_4 and T_3. Thus, ethylene thiourea, with its anti-thyroid properties, retards energy production (Saieva, *et al.* 2004). Energy is, particularly, required for absorption of all nutrients and to maintain life. Through lack of required energy, the life span of those infected with the virus is greatly reduced. Needless to say, the nutritive value of any food component is useless if it harbors such potentially toxic substances. The Codex Committee for Pesticides Residues (CCPR) has set 0.05mg/kg for tomatoes (FAO/WHO 1989a). The 1993 JMPR (FAO, 1994) did set the MRL for ETU in humans as 0.004 mg/kg body weight.

2.5 Previous Work on ETU

Residues of pesticides in food are influenced by the storage, handling and processing that occur between harvesting of raw agricultural commodities and consumption of prepared foodstuffs. It is also worth to take note that detailed studies on the fate of metabolites during food processing are lacking for most pesticides (IUPAC 1994). Indeed Kontou et al. (2004) acknowledged that the degradation mechanism of the EBDCs is complex, and the exact reaction mechanisms still remain not well understood. It is recommended in the JMPR evaluations of 1989 that further investigations be undertaken on the fate of dithiocarbamate residues during preparation and cooking with particular reference to their conversion to ETU (FAO/WHO 1989b).

According to Kontou *et al*. (2004), thermal treatment of raw agricultural commodities containing EBDC could result in extensive conversion to the more toxic ETU. Snapbeans treated with maneb have been found to contain ETU after commercial canning (US EPA, 1984). When three vegetable crops (Watts *et al*., 1974) or grains (Rosenberg and Siltanen, 1979) were cooked in water at 100° C for 15 min and 30 min, respectively, conversion factors as high as 30% (w/w) have been observed. The fate of residues in homogenates in pears (Ripley and Simpson 1977) or grapes (Ripley *et al*. 1978) heated at 100° C, were also reported.

As far as the effect of tomato processing on EBDC residues is concerned, the results reported are somewhat variable. Newsome (1976), observed conversions in the range 37.7-48.8% on molar basis for tomatoes with several EBDC formulations and heated at 100° C for 10 min, while Ripley and Cox (1978) reported that the respective factors for mancozeb containing tomato samples processed under same conditions were less than 21% (mol mol^{-1}).

Kontou *et al.* (2004), after a thorough study on kinetics of maneb degradation during thermal processing, reported a positive relationship between the formation of ETU from dithiocarbamates and temperature. The study failed to indicate how the type of dithiocarbamate affected dietary exposure to ETU, as only one type of dithiocarbamate, maneb, was assessed in the study.

Ripley *et al.* (2000) criticized the conventional residue level criteria of determining the safety status of natural foods initially exposed to dithiocabamates. He lamented of how most monitoring laboratories relied on the detection of carbon disulfide (CS_2) generated after acid digestion of any dithiocarbamates present in the crop to authenticate the food material as being safe. Ripley and his co-authors in their arguments quoted EPA and NIOSH 1984 reports, which document formation of ETU during cooking, adding that the use of MRL criteria in raw produce was misleading as far as dithiocarbamates were concerned. However, all these studies are not exhaustive in themselves as they were conducted in temperate regions, particularly in Italy and Brazil. Thus, this ETU residue study is a step ahead in providing relevant and up to date information on formation of ETU in a tropical environment.

Hajslova *et al.* (1986), studied the fate of ethylene-bis(dithiocarbamate) fungicides during processing of contaminated apples. Along with confirming that ETU is formed during heat processing of dithiocarbamate-treated produce, the investigation revealed that ETU could persist for as long as nine months of storage. However, the study failed to address the level of ETU that is bound to occur during formulation of dithiocarbamates.

In the United states, the U.S. EPA cancelled the use of EBDCs fungicides on 11 crops due to evidence of carcinogenicity in animals (U.S. EPA, 1996). Newsome and Laver (1973) reported deregistration of Zineb, a compound similar to mancozeb, for use as an antifungal in all countries. Similar safety concerns led to deregistration of maneb a dithiocarbamate fungicide that was common in Kenya in the early seventies. All these legal steps have been taken due to the devastating effects of ETU whose predisposing factors were addressed in the study.

Saieva, *et al.* (2004, on studying the urinary excretion of ETU after exposure to EBDCs found ETU excretion in laboratory animals to be relatively slow; it begins 6h after oral administration in rats and peaks after 24h. This could imply that there is adequate exposure for ETU to impact negatively on the body physiology via the dietary pathway, whose information on occurrence is currently inadequate.

Aprea *et al.* (1998) used reverse phase HPLC with spectrophotometric detection at 232nm to analyse the levels of ETU in urine after extraction with dichloromethane. Saieva *et al.* (2004) used the same analytical criteria to determine the level of urinary ETU among exposed Italians. Debbarh and Moore (2002) developed a simple and convenient HPLC method for determination of ETU in biological samples using Lichrosorb column. It is this validated analytical criteria that this study will adopt to analyse ETU in tomatoes.

In a monitoring study of 5888 food items conducted by the US EPA samples, residues of ETU, and in 19 per cent, residues of EBDCs were detected (US EPA 1996*)*. Thus there is an obvious need to acquire more information on ETU formation for purposes of control of the levels of this metabolite on agricultural crops of which the maximum residue level (MRL) was set as low as 0.05 ppm by the European Union (Kumar *et al.* 1997)

2.6 Review of Methodology

Many papers have described methods for the determination of ETU and the earlier literature has been reviewed by Bottomley *et al.* (1985) and Lentza-Rizos (1990). However, other workers have often commented on the unsatisfactory performance of many of the reported methods (Nitz *et al.* 1982; Krause 1989; Ahmad *et al.* 1995). Oxidation of ETU during extraction has been a cause of poor recovery (Otto *et al.* 1977). For instance, oxidation of ETU during extraction has been noted to be a cause of poor recovery with the AOAC Official Method (AOAC, 1995) which involves extraction by blending sample and diatomaceous earth with methanol-water after the addition of sodium chloride.

Several workers have reported methods based on UV detection (Lehotay *et al.* 1992; Ahmad *et al.* 1995; Kontou *et al.* 2001; Garcinuno *et al.* 2004). Startin *et al.* (2005) criticized the clean–up step owing to its tedious nature and resulting in underestimation and recommends a simple rapid procedure in which ETU is directly extracted into an appropriate solvent such as methanol or water.

Smith *et al.* (1988) monitored ETU in lettuce directly by reversed phase HPLC on an ODS silica column and UV detection. They examined a range of solvents (methanol, ethanol, water, dichloromethane and chloroform) for use in the extraction of spiked samples. Water and methanol were reported to have the highest recovery levels attributing this to high solubility of ETU in these solvents. ETU is readily soluble in water seconded by methanol but it is less soluble in chloroform and dichloromethane. Thus extraction with water or a mixture of water and methanol was recommended. Chovancova *et al.* (1985) indeed used the same solvents for determination of ethylenethiourea in grapes and wine and recommended the same for ETU determination in other plant samples such as tomatoes.

Kobayashi *et al.* (1986) developed a method for ETU determination, in onions, tomatoes, watermelons, and lettuce, involving extraction with aqueous methanol and quantification with UV detection. The detection limit conversion reported is 0.01 mg/kg and recoveries ranged from 76% to 90% at the 0.4 mg/kg fortification level. Kocourek and Zemanova (1987) detected ETU at concentration levels of 0.05 mg/kg in fresh and processed products (apple juice, apple puree, onions strawberries, tomato paste, wine and apple cider) by HPLC using a UV detector at 240nm.

Application of UV detector in the determination of ETU has been recommended by Lehotay *et al.* (1992), Ahmad *et a.,* (1995), Kontou *et al.* (2001), and lately by Garcinuno (2004). This procedure has found wide acceptance as they are more accurate, quick and convenient.

One aspect of methodology of ETU determination that should be noted is the possibility of conversion of EBDC residues to ETU during the analytical procedure. Van der poll *et al.* (1993) discredited the derivatisation as part of analytical procedure and any heat treatment to EBDCs such as concentration through rotary evaporation as these resulted in exaggerated levels of ETU from EBDC degradation. Use of derivatisation procedure in preparation process could result in even higher levels due to EBDC decomposition. Pease and Holt (1977) examined this problem in detail for maneb. They found that 9.1% (by wt) of maneb in spiked samples may be changed to ETU during the analytical procedure of several methods tested, namely those of Onley and Yip (1971); Onley and Storherr (1975); Newsome (1972) and Haines and Adler (1973) which was later published by Otto *et al.* (1977). Nash (1974) did report that 1-2% of Maneb and Zineb may degrade to ETU during benzylation process.

Chovancova (1985) also reported the potential increase of ETU content by conversion of Mancozeb to ETU during the derivatisation procedure, and estimated this to be about 2%. Bolzoni *et al.* (1993) and Matuyama *et.al.* (1994) documented that the use of derivatization as an addition preparation step, increased the time taken for analysis and possibility of errors as well as low recoveries while Startin *et al.* (2005) reported that with HPLC, derivatization was quite unnecessary. Smith *et al.* (1988) found it necessary to remove suspended particles of residual EBDCs co-extracted from lettuce leaves by filtering the methanolic extracts, because if these were carried into subsequent analysis steps, their degradation could have caused an apparent increase in ETU content.

Hoagland and Frear (1976) pointed out that the facile degradation of ETU could cause problems of innacurate determination using TLC and advised that special precautions should be taken during analysis. Smith *et al.* (1988) noted that ETU was unstable in methanolic extracts thus recommending that extraction process of ETU to be simple and short, and analysis immediate to minimize losses. All these precautions were taken into account during this study in order to obtain meaningful results.

Chapter 3

MATERIALS AND METHODS

3.1 Study Area

Mwea is one of the three divisions in Kirinyaga district. It is a semi-arid area falling in agro-ecological zone III (UL 4), but a recognized horticultural zone in Kenya. A great proportion of tomatoes sold in Nairobi city and its suburbs, originates from this region. This withstanding, analysis for ETU residues would provide the basic information about the level of ETU exposure in raw tomatoes in Kenya.

3.2 Research Design

The set-up involved a case study survey, field experimentation, stratified random sampling, laboratory determination of ETU, statistical data analysis by ANOVA and independent t-test.

Cross-sectional survey on EBDC usage in Mwea. The study area was divided into seven strata. Each location formed a stratum. The data obtained from the survey was cross tabulated for descriptive statistics.

Systematic sampling was adopted for each stratum in which for every five farms one respondent was interviewed on farming practices using a semi-structured questionnaire.

3.2.1 Field Survey on Usage of Dithiocarbamates in Mwea Division

A survey to assess fungicide usage in Mwea Horticultural zone in Kirinyaga District was conducted. The study area was divided into four segments namely; Ngurubani, Kagio, Kutus and Kimbimbi according to where the farmers source the agricultural inputs. The demarcation of the administrative locations of Mwea division that were covered in the study is shown in plate 1 in the appendices section.

A questionnaire was prepared to gather information on crop protection practices, usage of fungicides, availability of extension services, post-harvest treatment and marketing channels from a random sample of these farmers. Local suppliers of agrochemicals were also identified in an interview with the farmers in the four blocks. Information was gathered on their career background, education level, experience, EUREP GAP regulations, as well as peak season of fungicides.

3.2.2 Laboratory Reagents and Materials

Methanol analytical grade, labels, filter papers, aluminium foil, parafilm were obtained from Westford Laboratory Supplies Limited., Kampala, Uganda. Methanol HPLC grade was obtained from Kobian Chemicals Limited., Kenya, while Ethylenethiourea standard 99.8% purity was ordered from Fluka, France, through Kobian Chemicals Limited.

3.2.3 ETU Analysis in Dithiocarbamate Fungicides

Fungicide products consisting of mancozeb (Sancozeb^{R}, Pennicozeb^{R}, Milthane Super^{R}, Dithane-M-45^{R}, Oshothane^{R}), blue copper (Cuprocaffarro^{R}), propineb (Antracol^{R}), and mancozeb/metalaxyl (Ridomil^{R}) samples were obtained from agro-chemical shops in Mwea Division, Kirinyaga district, the area of study.

From each formulation, three samples were analysed for ETU, which could have resulted from processing or storage conditions. All the batches analyzed were within the statutory shelf life specification of two years. The dithiocarbamate samples are represented in the Plate 3 in the appendices section.

3.2.4 Standard Preparation

Stock standards were prepared by weighing 10-20 mg of ethylene thiourea in 10-ml volumetric flasks (Debbarh, et al. 2002) and diluting to volume with diluting solvent; methanol-water (1:4). A sufficient number of analytical standards were prepared to generate a calibration curve. Spiking the standard with 0.002g of ETU was applied to confirm peak identity.

3.2.5 ETU Determination by HPLC

The HPLC system consisted of a model 600E pump, ODS Packed Lichrosorb^{R} 100RP8 (5µl) column and R/P UV detector set at wavelength of 243nm which was maintained at a temperature of 40°C; the eluate was thus monitored at 243nm. Other conditions that prevailed include pump pressure of 83kgf/cm^{2}, a flow rate of 0.4mlmin^{-1} with a mobile phase of water/methanol ratio of 90:10 (v/v) (isocratic). The mobile phase was first degassed with ultrasonic bath for one hour at a frequency of 1000. Preliminary experiments were conducted to validate the analytical methods used.

3.2.6 Assessment of ETU Levels in Tomatoes Sprayed with DCs.

This step involved investigations of ETU formation from different dithiocarbamate formulations under field conditions. A flat piece of land measuring 40 m by 7 m was identified meters away from Thiririka river in Juja Location, Ruiru, Division Thika District. After a field survey, five fungicides that had been found to be the most popular among tomato farmers, were selected for field experimentation. A 10g sachet of certified tomato seeds (Cal J. variety) was obtained from Regina^{R} Seeds Limited, from a seed stockist and the seedlings were raised on a 2.5M by 1M nursery.

The seedbed plots were laid in Randomised Complete Block Design (RCBD) as shown in Plate 2 in the appendix. This design, despite the block effects, was necessary so as to control local variation. Five treatments consisting of the various formulations plus a control were allocated to the plots in triplicates. This involved spraying the tomatoes with the fungicides on weekly or fortnight basis (depending on weather conditions). The control plot was sprayed with plain water. The tomato plots were exposed to similar field conditions and cultural practices. Spray barriers in form of polythene sheets were erected around every plot to avoid spray drift during fungicide application. Then application rate was 5g in two litres of water. No chemical control method was adopted against insect pest infestation so as to avoid interferences during laboratory analysis.

Tomato fruits were harvested on the eighth day after pesticide application for ETU analysis in the laboratory. Tomato samples picked were preserved at -20°C until analysis. These samples are shown in Plate 4 in the appendices.

3.2.7 Tomato Sample Preparation

A 100 g chopped tomatoes was blended in a high-speed blender for two minutes. The homogenate was transferred into a 50ml plastic beaker and the pH determined. A 2 g sample of the tomato homogenate was weighed and transferred into a 50 ml conical flask. Then 10 ml of methanol, analytical grade, was added and topped to the mark with 38ml distilled water. All this was done in triplicates for each sample and then put on a mechanical shaker set at 300 revolutions per minute for 15 minutes as described by Kontou *et al.* (2004).

The resulting aqueous solution was then filtered with 12, 5 gauge filter papers three times. The filtrate was further filtered with suction in Buchner funnel through a 1 cm layer of hyflo super cel and occasionally with cellite. All this is as per the OSHA method 95 for ETU extraction, as modified by Knio *et al.*, 2000 and Kontou 2004. The resulting filtrate was then transferred into sample vials and 10µg/ml was injected into the HPLC column.

Preliminary analyses were conducted to confirm peak identity. This was by spiking the analytical sample with 0.002g of ETU and determining the change in peak area. Samples awaiting HPLC analysis were kept at 5°C in a refrigerator (Kontou *et al* 2004). The experiment was replicated with a different batch of samples using the same procedure.

3.2.8 The Effect of Washing on ETU levels in Cooked and Uncooked Tomatoes

This experiment followed a dichotomous key in which there was a set of washed tomatoes and a set of unwashed tomatoes. Washing was meant to remove the surface deposits of fungicides and this was in distilled water to avoid introduction of new contaminants. The ETU levels in these tomatoes were either analysed raw or after cooking. The extraction procedure was as described in section 3.2.7 above.

3.2.9 Effect of Market Chain on ETU Levels in Tomatoes

The experiment was intended to complement objective 4. Mwea market was used to assess the level of ETU in tomatoes sold in the Kenyan Market. Stratified random sampling of tomatoes (Variety *California. J*.) from various market segments was adopted. Deliberate effort was made to ascertain value addition regimes practiced in by both retail and wholesale markets. The collected samples were assessed for ETU residues/contaminants as described in Section 3.2. The data obtained from laboratory analysis was expressed in percentages or parts per million through computations. Duncan Multiple Range Test (DMRT) was adopted to test for statistical differences between means. Genstat package was used to perform the statistical analyses.

3.2.10 Experiment on Formation of ETU during Cooking

To investigate the effect of cooking on EBDCs residues, 50 g samples of tomato homogonates (washed and unwashed) from both controlled field experimentation and fresh markets were placed in a 100 ml round bottom glass flasks connected with a vertical condenser. The samples were heated to a temperature of over 90°C for 15 minutes as a simulation of normal cooking. The samples were immediately cooled under running water and analyzed for ETU formation. All analysis was done in triplicates.

This step was of importance, since the residues of the active ingredients of the dithiocarbamates are known for their instability, particularly when subjected to heat and that ETU is the primary metabolite. Analysis of tomato stew was thus necessary as far as dietary exposure to ETU is concerned.

3.2.11 Assessment of ETU in Marketed Tomato Products

Non-stratified random sampling of various types of processed tomato products in the market was carried out to assess the effect of processing, and particularly heat treatment with reference to ETU formation. The products sampled were tomato purees, tomato ketch-up and tomato-sauce from Kenyan supermarkets. All these samples were kept at a temperatures of -5°C until analysis for thermal stability. The samples are shown in Plate 5 in the appendix.

To conduct ETU assessment, two gram from either of tomato product was weighed and put into a 50 ml conical flask. Then 10 ml of methanol (analytical grade) was added and topped to the mark with 38 ml distilled water. Further details of analysis are as described in section 3.2.7.

Chapter 4

RESULTS AND DISCUSSION

4.1 Survey of Fungicide Usage in Tomatoes in Mwea Division.

A survey on usage of agro-chemicals in horticultural farming in Mwea division indicated that 68% of the respondents (tomato growers) relied mainly on EBDCs to control fungal diseases in tomatoes. This is shown in figure 2. The products were found to be in stock in 96% of the agrochemical shops in Mwea region.

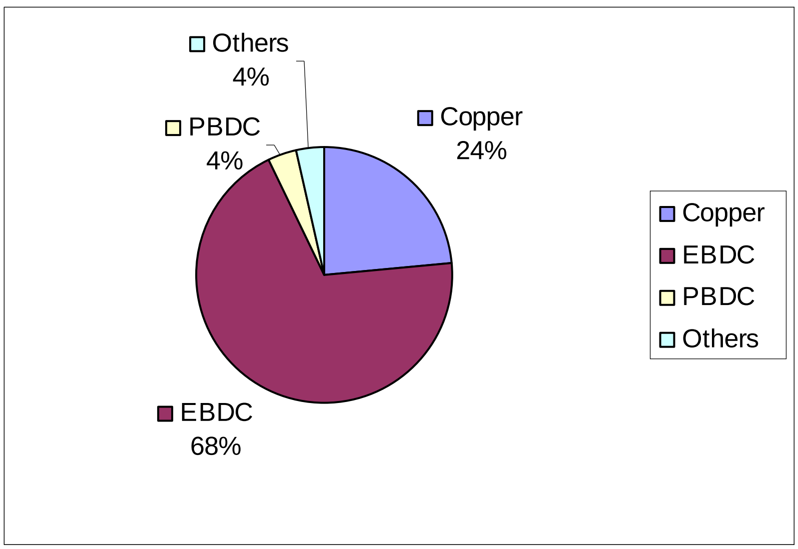

Figure 2: Usage of different fungicide classes in tomatoes in Mwea Division

The survey also showed that Sancozeb[R] was the most popular and composed more than 40% of all fungicides applied in tomatoes. This is depicted in figure 3.

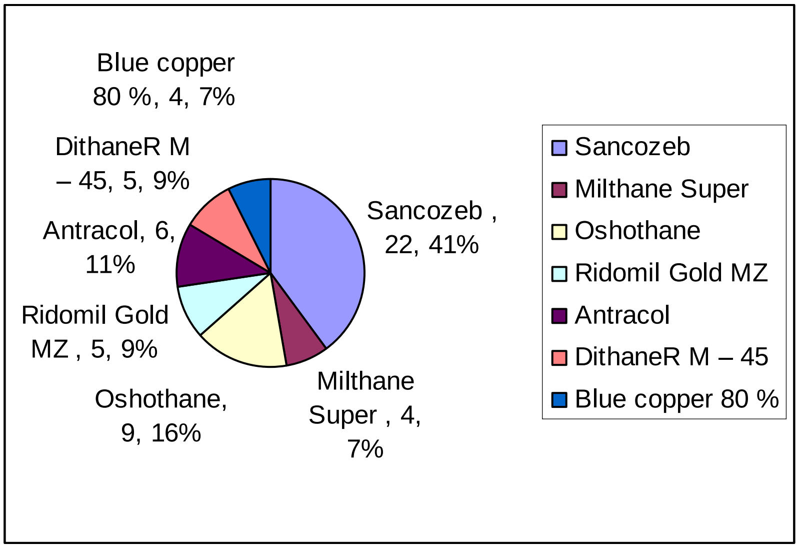

Figure 3: Usage of individual fungicide in tomatoes in Mwea Division

Twenty six out of thirty four (76%) agrochemical stockiest in Mwea reported that Sancozeb[R] was the best selling agrochemical in Mwea seconded by Pennicozeb[R] as they were able to turn volumes of the same all the year round with peaks in November and December. Sixty five per cent of the farmers interviewed reported that, following the widespread recommendation of Cuppro-caffaro[R] (Copper hydroxyxanide) in french beans under EUREP GAP regulations; they were adopting the same in tomatoes.

As figure 4 shows, out of the fifty five farmers interviewed during the survey only two farmers (4%) have had the government extension worker visit their farm for inspection and advisory purposes. Twenty nine out of fifty five farmers (53%) interviewed in various locations of Mwea division complained of conflicting messages among private service providers (agro-vet salespersons included) while 62% reported that they could not translate the message conveyed into practice in their farming systems.

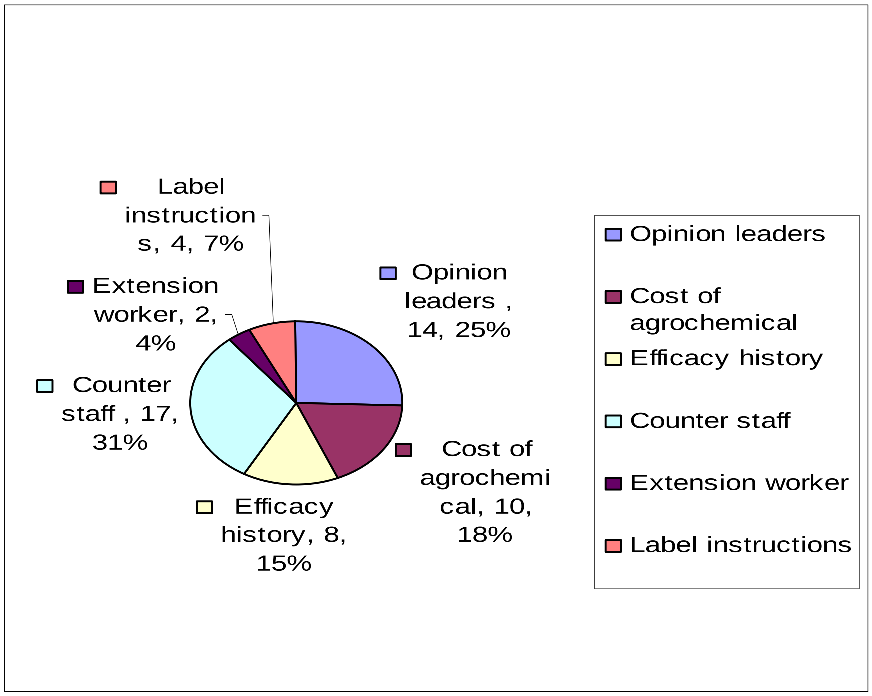

Figure 4: The main factor in the choice of fungicide for use in tomatoes in Mwea Division.

An informal survey of counters of the agro-chemical shops revealed that over eighty per cent of the attendants did not have tangible training in agriculture or on agro-chemicals yet ninety two per cent of farmers solely depended on them regarding how to administer the pesticide bought from their premises. Over 40 farmers (80%) admitted failure to read the pest control product label partly due to the tiny writings and partly due to the assumption that the information contained is too complicated and therefore not meant for them. Forty nine of the fifty five respondents (89%) reported that they produced tomatoes for sale in fresh air markets.

4.2 ETU Standard Curve

The retention time of ETU in the HPLC column was found to 15 minutes as demonstrated by the chromatograph in appendix 1. Calibration curve was constructed by plotting detector response versus concentration (μg/ml) of thiourea under excel spreadsheet. When the peak area was plotted against the respective standard concentration, a high level regression coefficient (0.9902) of the standard curve was observed and this is shown in Figure 5. The resultant equation, y=396502x, was then used to compute the sample concentrations for the various analysis.

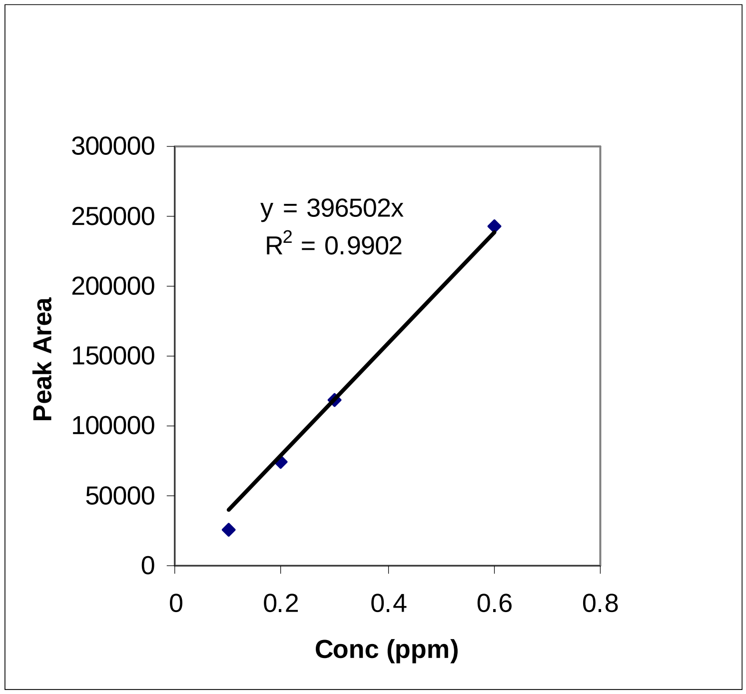

Figure 5: ETU Standard Curve

4.3 ETU Contaminant in EBDCs

According to the results of the HPLC analysis, all EBDC were found to have ETU contaminants which ranged from 0.17% in Ridomil^{R} to 4.73 % in Sancozeb^{R} as shown in Table 1. The levels, except for Sancozeb^{R} and Oshothane^{R}, are within the acceptable limits of 0.5% permissible ETU level in EBDCs given by the Italian Health Authority (Camoni *et al.* l988). No ETU was detected in Antracol (Propineb), which is a PBDC. Levels of ETU for the different fungicides were found to be significantly different ($P<0.05$). These results suggest that ETU contamination begins at the formulation stage and this is in agreement with a report by Bontoyan and Looker (1973) and Dearfield (1994). The levels may increase during storage and upon exposure of EBDC fungicides to air, moisture and high temperature (Blasquez 1973, Kumar and Agarwal 1991).

Table 1: ETU Contaminants in Formulated Fungicides

Agro-chemical Fungicide	Mean n=3 ETU Conc. (%)
Dithane^R M – 45	$0.39^a \pm 0.03$
Milthane super^R 80 WP	$0.37^a \pm 0.02$
Oshothane^R 80 WP	$1.54^b \pm 0.26$
Ridomil Gold MZ^R	0.I7^c ± 0.01
Antracol^R 70 WP	n.d
Sancozeb^R 80 WP	$4.73^b \pm 0.47$
Electis^R	$0.18^c \pm 0.28$
Blue copper 80 %	n.d

Legend:-

n – sample size

n.d - not detected

R – Registered trade name

S.E – Standard error of the mean

Superscripts a, b, c… – indicates difference between means

- means with similar letters are significantly different

Trotter and Pardue (1982) demonstrated that the the amount of ETU in formulation of EBDCs increases on storage if the product is kept at 49° C and at 80 per cent relative humidity. Kontou (2004) found that ETU increased, during storage for 39 days at 49 °C and 80% relative humidity, from an initial content of 0.02 - 2% to a final level of 0.13 - 14.5%. The degradation dynamics of formulations from different manufacturers varied. In addition Kontou (2004) proposed that ETU could persist in the environment for a period of 10 weeks. In 1998, the Plant Protection Department of Vietnam tested commercial formulations of EBDCs, for ETU content, and found that nearly one-half (46.3%) exceeded the Italian specification level of 0.5% (Camoni *et al* 1988). Zineb formulations were found to be the most frequently (77.9% of samples) contaminated. Bontoyan et al (1972) tested the ETU content of 28 different EBDC formulations of 5 manufacturers. The samples of pesticides less than two years old contained ETU in the range of 0.04-2.02 % whereas; ETU was present at a level of 2.73% in a product more than five years old.

4.4 ETU Levels in Tomatoes under Field Experiment

There were significant differences in ETU levels in different fungicides ($p<0.05$). The amount of ETU detected could be attributed to ETU contaminants in EBDCs. Oshothane-treated tomatoes, on average, were found to have the highest level of ETU while Antracol (propineb) had the least. Tomatoes sprayed with Ridomil Gold MZ [R] which is formulation of 640g mancozeb and 40 metalaxyl had the same levels of ETU as those that were under Milthane Super [R] (80% Mancozeb) shown in table 2.

Table 2: ETU Levels in Tomatoes Sprayed with Dithiocarbamates

Agrochemical sprayed	ETU level (mg/kg) Mean ± SE
Milthane Super[R] 80WP	3.8 ± 0.7[a]
Ridomil Gold MZ [R]	3.8 ± 1.2[a]
Dithane[R] M- 45	7.1 ± 1.6[b]
Oshothane [R] 80 WP	10.2 ± 1.9[c]
Antracol[R] 70WP	0.6 ± 0.4[d]
Control (sprayed with water)	0.0[d]

Legend:-

R – Registered Trade name

S.E – Standard error of the mean

Superscripts a, b, c… – indicates difference between means

- means with similar letters are significantly different

All these levels are statistically different ($p<0.05$) and exceed the 0.05 ppm permitted residue limit for ETU in food materials set by the European Union (Kumar et al 1997). The levels of ETU in tomatoes sprayed with DCs and that found in EBDCs fungicides were found to be highly correlated with a Pearson correlation coefficient of 0.893. Further analysis indicated that the level of ETU in tomatoes was dependent on the level of ETU in the EBDC product itself. The regression coefficient was found to be 0.80 indicating that the level of ETU in tomatoes depended greatly on the amount of ETU in fungicide used. This is illustrated in figure 6.

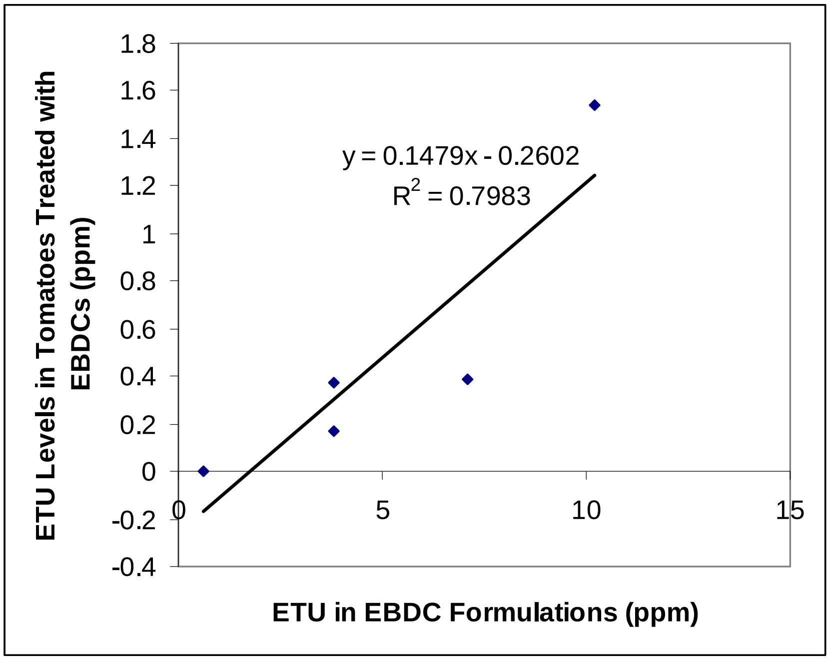

Figure 6: Linear regression model of ETU levels in tomatoes treated with EBDCs and ETU in EBDC formulation used.

These findings are consistent with those of Cassanova and Dachaud (1976) who found a good correlation between the concentration of ETU expected on tomatoes sprayed with mancozeb, maneb or zineb containing known concentration of ETU, and the actual amount of ETU determined.

Smith *et al.* (1988) sprayed lettuce with aqueous suspensions containing high levels of maneb, zineb, or mancozeb, avoiding any pesticide run-off onto the soil. The level of ETU present in each of the pesticide formulation was previously determined. The initial levels of ETU detected on the leaves corresponded to the amounts of ETU present in the applied fungicide formulation as tabulated below.

Table 3: ETU Deposits on Crops Immediately after Treatment with EBDC Fungicides

Crop	Fungicide	ETU detected (mg/kg)	Reference
Pears	Zineb	0.02	Ripley and Simpson (1977)
Grapes	Mancozeb	0.03	Ripley *et al*. (1978)
Tomatoes,	Maneb	0.74	Ripley and cox (1978)
	Mancozeb	0.96	

Thus the importance of the 'quality' of the formulation applied to plants led many countries such as Italy to set limits to the percentage of ETU allowed in EBDC formulations (FAO 1979).

In some instances, the use of EBDCs has not been reported to give rise to measurable residues of ETU in raw agricultural commodities at harvest (JMPR 1980, FAO 1988). Kakaricova *et al.* (1988) showed that the amount of ETU varies in relation to the amount EBDC present in harvested grapes: must and wine produced from grapes treated with mancozeb 14 or 28 d before harvest contained detectable ETU residues, whereas those made from grapes harvested 42 d after treatment did not. However, post harvest handling may greatly influence the ETU level at the point of consumption.

4.5 Effect of Washing and Cooking on ETU Levels

The analysis of the results indicated that cooked-washed and cooked-unwashed had significant differences ($P<0.05$). ETU levels shot from a mean of 10.2 mg/kg to 23.9 mg/kg after cooking. As far as tomatoes exposed to EBDCs are concerned, uncooked-washed tomatoes had the lowest levels (9.4 ppm), followed by those tomatoes that were washed before cooking which had a mean of 10.2 ppm. As Table 4 shows, tomatoes that were cooked-unwashed gave the highest ETU levels (23.9 ppm), followed by those that were neither washed nor cooked (12.2 ppm).

Table 4: ETU Levels in Tomatoes from Different Markets and Treatments

Market	Treatment ETU (mg/kg)			
	W/N.C	W/C	N.W/C	N.C/N.W
Wakulima	11.2 ± 0.5[a]	13.6 ± 0.1[c]	39.9 ± 0.5[e]	17.5 ± 0.1[d]
Githurai	8.3 ± 0.7[b]	7.6 ± 0.1[b]	12.9 ± 0.5[c]	10.3 ± 0.6[a]
Juja	7.9 ± 0.7[b]	9.8 ± 0.8[a]	24.6 ± 0.6[d]	10.6 ± 0.8[a]
Mwea	10.1 ± 0.5[a]	9.9 ± 0.3[a]	18.4 ± 0.7[d]	11.5 ± 0.9[a]
Mean	9.4 ± 0.7[a]	10.2 ± 0.2[a]	23.9 ± 0.8[d]	12.2 ± 0.4[c]

Legend:

W/N.C - Washed /Not Cooked

W/C - Washed/ Cooked

N.W/C - Not Washed/ Cooked

N.W/N.C - Not Washed /Not Cooked

n = number of tomato batches picked per analysis ↔5

a, b, c & d- imply means followed by the same letter are not significant.

Independent t-test gave no significant differences in ETU levels between washed-raw and those that were washed and cooked ($p<0.05$). However, the levels in all the samples analysed exceeded 0.05 ppm residue limit set by European Union in 1997 for ETU in foods. Thus washing of tomato produce to remove the surface residues, which have a potential of being converted to ETU in the subsequent treatments such as cooking, appears critical in minimizing dietary exposure to ETU.

The observation of higher ETU levels in cooked tomatoes has been reported in other plant materials initially treated with EBDCs upon cooking (Blasquez, 1973; Ripley and Cox, 1978; Kumar and Agarwal 1991, Zhang *et al*. 1991). Kumar and Agarwal (1991) observed 34.2% increase in radioactivity in form of ETU after cooking tomatoes initially treated with Dithane M-45[R], making a conclusion that there was degradation of the parent fungicide at high temperature of cooking in water.

Watts *et al.* (1974) reported that about 20% ETU was formed during cooking of tomatoes fortified with Mancozeb. Newsome and Laver (1973) and Marshall (1977) have reported similar observation regarding ETU levels after cooking. Ross *et al.* (1978) reported about 34% conversion of mancozeb to ETU as a result of boiling in water. Kontou (2004) reported total elimination of the residues of the parent compound through sterilization as they are converted to ETU.

The results of this study are consistent with the initial findings of Philips et al. (1977) that 33-87% of the EBDCs and the majority of the ETU residues are removed following a simple washing procedure of the raw agricultural produce. Casanova and Guichon (1988) studied the percentage of EBDC residues removed by washing from spinach treated with mancozeb, maneb, or zineb. The reduction in pesticide concentration was greater when initial deposit was higher. Marshall and Jarvis (1979) found that tomato juice from tomatoes washed in running water for ten minutes contained 52% less EBDC and 50% less ETU than that made from unwashed tomatoes.

Trotter and Pardue (1982), working with raw, unwashed spinach, detected up to 2.3 ppm ETU on dithiocarbamate-treated leaves and between 5 and 80 ppm (µg/g wet weight basis) of the dithiocarbamate upon washing the leaves.

Kumar and Agarwal (1993) in a controlled experiment, found that there was a decrease in Mancozeb from 47.5%, which were reduced to 22.3% of the original (5.53µg/gm fresh weight) after washing. Marshall (1982) observed that 30-55% of EBDC residues were removed by washing tomatoes for 10 min in water. Cheah (1985) reported that in tomatoes the mancozeb residues were reduced to

0.58 mg/kg CS_2 from 3.8 mg/kg CS_2 after washing. The author, thus, concluded that prior to washing of the surface of vegetables even for a short time removes a large proportion of the EBDC fungicide residues. However, most of the ETU metabolite remained.

The importance of washing is any agricultural produce initially exposed to EBDCs is strongly supported by the study conducted by Gonzalez *et al.* (1989) who observed that canned spinach sprayed three times in the field with maneb at weekly intervals at the recommended rate and harvested one week after the last treatment contained 63 ppm, if canned without any washing treatment.

Hassal (1990) reported that the high residue levels of EBDCs occasionally observed in vegetables in the late 1980s sold in Canadian market had led to their restricted use as a strategy to curb chronic exposure to ETU.

4.6 Effect of Market Chain on ETU Levels

As regards marketing chain, the main destination market, particularly Wakulima (wholesale) market in Nairobi, had the highest levels of ETU (39.9 mg/kg) for tomatoes that were cooked unwashed. Overall, 10.2 mg/kg of ETU was observed in tomatoes that were cooked washed which could be contaminants in the EBDC used to control fungal diseases, an average increase of 13.7 mg/kg ETU was from conversions of EBDC residues during cooking of unwashed tomatoes. The increase in ETU residues was computed to be 134.4%.

The explanation to such a state of affairs lies in the survey findings that wholesale merchandise did not allow washing of fungicide deposits on the surface of the produce. Indeed, from observation, the unwashed tomatoes from Wakulima market had visible surface deposits of fungicide residues. Alongside reduction of surface EBDC residues, washing helped to reduce the ETU metabolites which could be in EBDC products as contaminants during formulation. The difference in ETU levels is depicted in the figure 7.

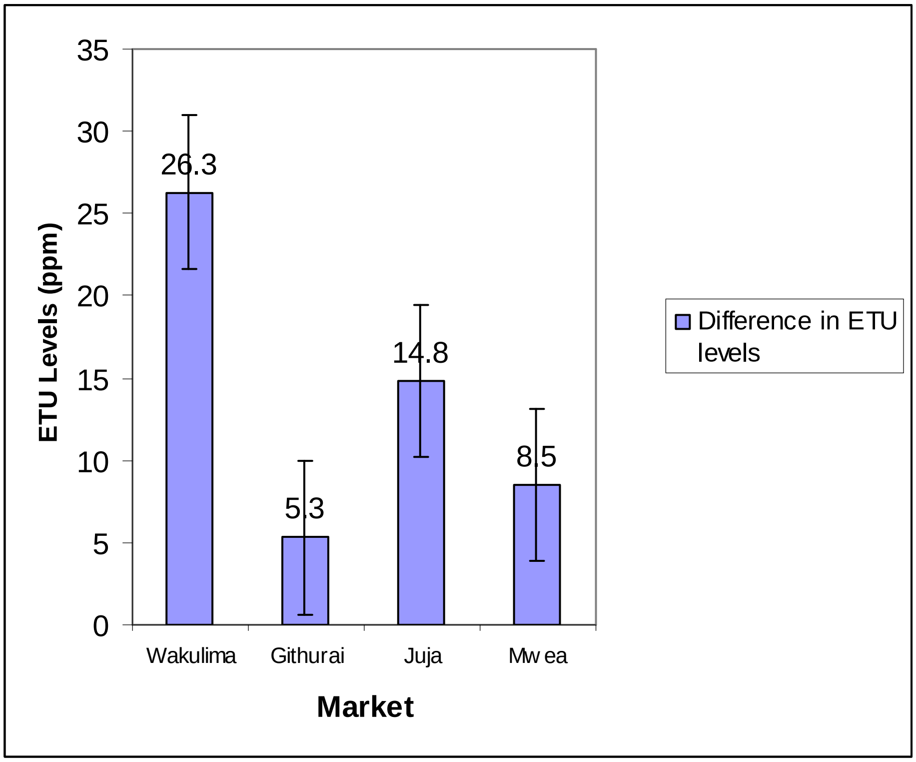

Figure 7: Difference in ETU levels between washed and unwashed tomatoes after cooking.

According to the European Union, processing studies have to be carried out when there is a reasonable expectation that processing may convert the residues into species of greater toxicological concern (Commission of the European Communities 1997, Kontou, *et al.*, 2004). The higher levels of ETU detected in the samples that were cooked unwashed could be attributed to surface deposits of EBDCs that degraded into ETU during the cooking process. This observation supports the findings of Watts *et al.* (1974) who reported that about 20% ETU was formed as a result of boiling fortified samples with mancozeb. Rose *et al*. (1978) reported about 34% conversion of Mancozeb to ETU in apples as a result of boiling in water. It was also reported by Cheah (1985) that 12-79 % of Mancozeb residues were removed from tomato on boiling for 10 minutes as they get degraded into ETU. In addition, Kontou *et al.* (2004), reported a total conversion of maneb residues during sterilization of pre-treated samples.

As far as the rate of degradation is concerned, Kontou *et al.* (2004) noted that conversion of Maneb to ETU at 90°C was dominant at the first initial steps of the reaction. This finding explains the occurrence of high ETU levels observed in the unwashed tomatoes cooked for 10 minutes during the study.

4.7 ETU in Canned Tomato Products

Tomato paste exhibited the lowest levels of ETU, which were even below the detectable limit in all the samples analyzed. Tomato sauce, on the other hand, had the highest levels (2.79 ppm) of ETU. ETU levels in tomato ketch-up and tomato puree averaged 0.71ppm and 0.85 ppm, respectively. This observation is in line with the findings of Knio *et al.* (2000) who reported that ETU being water soluble, it was bound to dominate in tomato products derived from juicy content. The results of the analysis are presented in table 5 b.

Table 5: ETU Levels in Processed Tomato Products Sampled from Kenyan Supermarket

Canned Tomato Product	Mean[b] ETU (ppm)		S.E[a].
Tomato ketch-up	0.71[a]	±	0.11
Tomato paste	n.d[e]		
Tomato sauce	2.79[b]	±	0.04
Tomato puree	0.80[a]	±	0.07

Legend:

a-standard error.

e-not detected

b - Mean value of six readings from three samples; (means followed by the same letter are not significant according to the Duncan's multiple range test at 95 % confidence level.)

ETU detected in these products could primarily be attributed to degradation of EBDC residues during heating processes.

According to Ankumah and Marshall 1984, the levels of ETU in the processed products vary greatly according to the origin and history of the raw materials specifically the tomatoes and the disease management practices adopted during production. Kontou *et al.* (2004) on thermal stability studies of ETU observed no decline in ETU recovery even at temperatures of 90°C for 80 min. and thus concluded that ETU is a stable terminal product of EBDC degradation. Other previous work report that ETU could persist upto six months and was extremely stable toward hydrolysis and heating (Mak *et al.* 1984).

Chapter 5

CONCLUSION AND RECOMMENDATIONS

Initial findings that ETU is formed during the heat processing of EBDC-treated foods (IUPAC 1977) are confirmed in this study and information on facile formation of ETU from EBDCs has been provided. Due to variation in ETU levels among products of the same active ingredient, the regulatory authorities should evaluate the pesticide products made by manufacturers individually. The manufacturing site(s) and approved suppliers of active ingredients used for formulation of pesticides should be specified before the product is registered. Essentially there should be a limit on acceptable levels of ETU in technical EBDC products.

It can be concluded from the study that the conventional efficacy evaluation for registration of a pesticide product in Kenya is not solely sufficient to assure safety of produce. The high conversion factors determined for the thermal processes underlie the necessity of controlling the level of EBDC residues in raw agricultural commodities, as recommended by Kontou *et al.* (2004). The study concludes that if the washing of the tomatoes is not thorough before cooking, the amount of toxicant that may form constitutes a potential health hazard, in terms of ETU formation.

ETU formation from produce initially exposed to EBDC has been observed to be promoted by cooking processes and is thus of great significance in processed foods.

The survey findings conducted in Mwea Division on agro-chemical usage imply extension and regulation bottlenecks. Most farmers who depend on agro-vet counter staff for advisory are ill advised on the choice of fungicides and the rates. Consequently, there is urgent need for the government regulation on extension to avoid conflicting extension messages which may predispose economic losses and health hazards.

Agricultural extension services needs to be extended to post-harvest handling for enhanced food safety. There should be training programmes for counter-staff on administration of fungicides in general.

Cooking tomatoes sprayed with EBDCs without prior washing posed the highest dietary exposure to ETU. The level of ETU in harvested tomatoes was found to be highly correlated to the level of ETU contaminant in EBDC. With the exemption of processed tomato products, all the raw and cooked tomatoes had ETU levels that were significantly higher ($P<0.05$) than the guideline levels for ETU residues in food (0.1-0.01 mg/kg) as recommended by FAO/WHO organizations (FAO, 1978).

A study should be conducted on how conversion factors relate to temperature and EBDCs conversion to EBDCs. Lastly, the nature of processing environment for tomato products in various companies in Kenya merits further investigations.

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APPENDICES

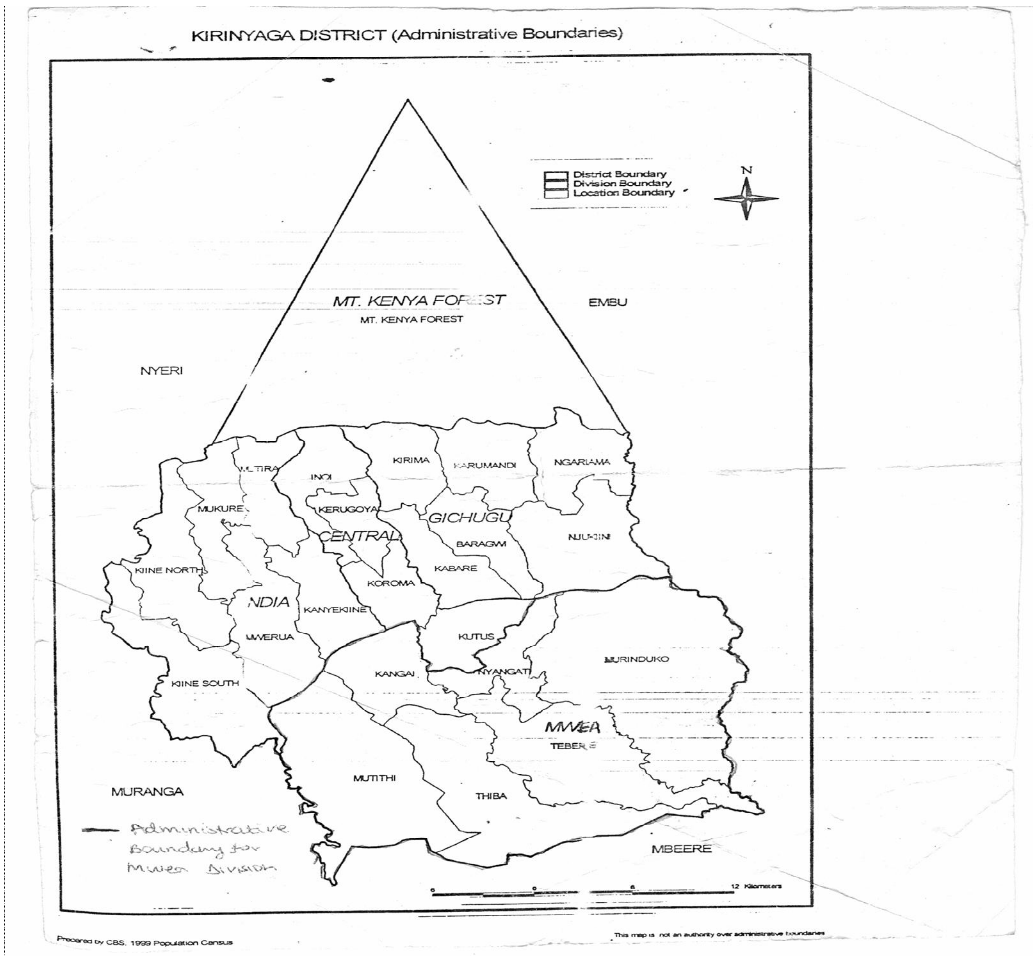
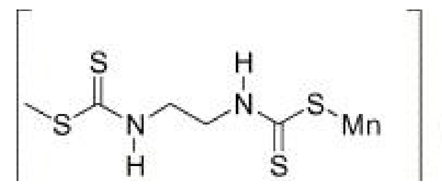
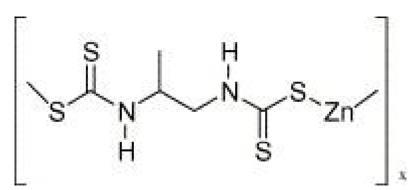


Plate 1:
Kirinyaga District Map

Mancozeb
(monomer)



Propineb MW = 289.8
(monomer)



B11	B14
B13	B15
B12	B16

B22	B24
B25	B26
B23	B21

B36	B35
B33	B31
B34	B32

Plate 2: Illustration of field experimentation set-up under RCBD: three blocks, five treatments and a control. The 1st & 2nd digit refer to the block number and treatment applied respectively.

Field Layout

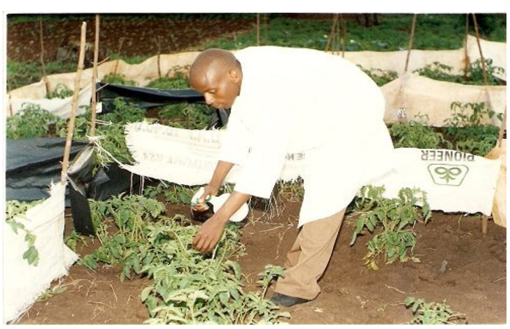
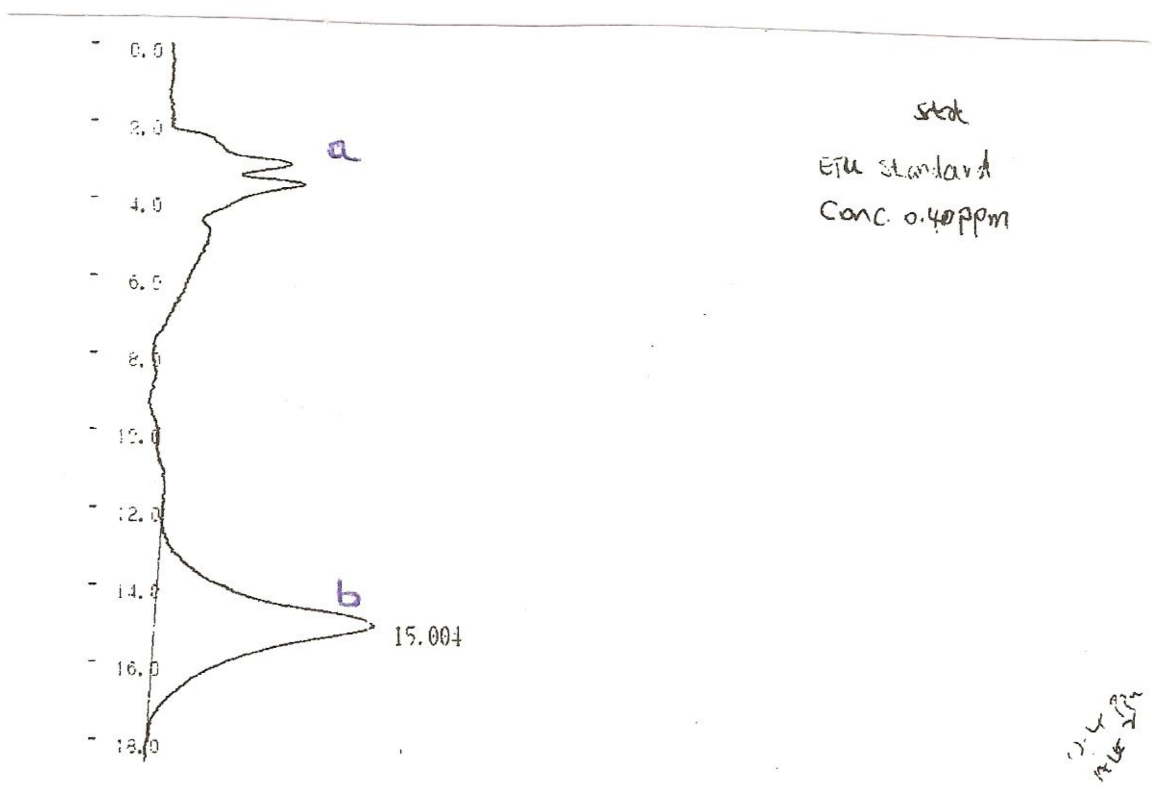


Plate 3: Some DCs Investigated for ETU Contaminants from Mwea Division.

Plate 4: Samples from field experimentation ready for ETU analysis.

Plate 5: Processed Tomato Products for ETU Analysis

Appendix 1: Chromatographic Presentation of 0.40 ppm ETU Standard from HPLC Analysis.



Legend:

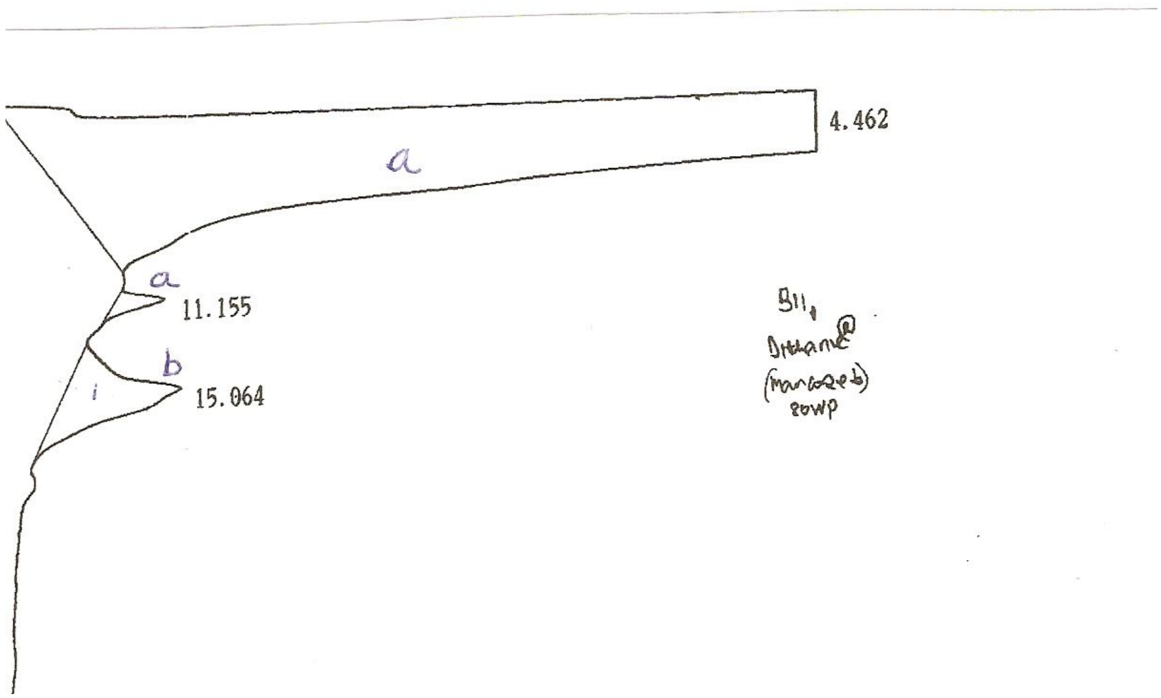
a – peak due to solvent system inter-phase

b – peak from ETU standard

Note:

- Purity of the standard was 99.8%.

Appendix 2: Chromatographic Presentation of ETU level in tomatoes sprayed with Dithane M45, one of the EBDC investigated.



Legend:

a – peaks for inert substances in the fungicide formulation

b – peak for the ETU in the fungicide formulation

Appendix 3: Questionnaire used in the Survey

A Field Survey on Dithiocarbamates Usage by Tomato Growers in Mwea, Division, Kirinyaga District.

Questionaire No. …….

Name of Interviewer ……….. Date of Data Collection ……………………….

Location ……………

Dear Farmer/Respondent,

I am a student at Jomo Kenyatta University of Agriculture. I am doing research on food safety as part of my study. I require information on fungicide usage in tomatoes for this study. I would appreciate if you provided me with this information. I assure you that the information that you provide will remain confidential and will only be used for the current study. Kindly take some time to answer the following questions.

Welcome.

1. Name of the farmer ---

2. Location of the farm: District---------------- Division-------------- Location-----------   Sub-location--------- -   Village-----------------

3. What is the size of your farm? A. Less than a quarter of an acre B. Between a half and two acres C. More than two acres. (Choose one)
4. Do you grow your tomatoes for A. Sale, B. For home consumption, or C. both? If for sale, what is your target market? A. Canning, B. Export, C. Freshmarket.
5. For how long have you been in tomato farming? A. Less than one year. B. Between two and ten years. Over ten years.
6. Which variety of tomato are you currently growing? A. Money maker B. Cal. JVF C. Riograde D. Hybrids such as Monyala F1, Caltana F1, Eden e.t.c.
7. What is the reason behind the choice of the variety? A. Yields B. Resistance to Pests & Diseases C. Availability D. Input and management costs
   E. Any other (specify)---.
8. How old is the current tomato crop on your farm ? A. Less than one month B. Two to three months C. More than three months.
9. Which crop was previously grown where you are growing tomatoes? A. Tomatoes

B. Potatoes C. Any other (specify)---
----

10. Which diseases are of economic concern in your tomato growing? A. early and late blight B. any other (specify)---------------------------- ----------------
----

11. Which chemicals do you depend upon to control the above fungal diseases in your tomatoes?

Disease	**Control**

12. What guides you in determining what type of fungicide to use? A. Cost of Fungicide. B. Disease severity C. Extension services D. Influence of opinion leaders E. Any other (specify)--
----

13. After how many days of transplanting your tomatoes to the seedbed, do you apply the first fungicide spray? A. One week or Less, B. Two weeks, C. Three weeks or more.

14. What guides you in determining how much fungicide to apply? A. Stage of disease development B. Cost of the fungicide C. Instructions on the product label D. Any other (specify).---

15. What other fungicide do you use, when and how often? Fill in space provided in the table below.

Fungicide	Disease	Timing details	Frequency

16. On average how many times do you spray with one fungicide before changing to another within one cropping season? A. Once B. Twice C. More than twice. D. Uses only one type of fungicide.

17. (Optional) On what grounds do you switch from one fungicide to another? A. Efficacy B. Cost C. Pesticide Residues D. Any other (specify)-----------------------

18. How many times, on average, do you spray with fungicides within a cropping season? A. Once B. Twice C. Thrice or more.

19. After spraying with fungicide, how long do you wait before harvesting your tomatoes?

Fungicide sprayed by the farmer	**Days he/she waits before harvesting.**

20. Have you ever heard of EurepGAP or KenyaGAP Regulations? Yes/No.
21. Have you attended any training on EurepGAP or KenyaGAP? Yes/No.

    If yes when and by who?---
    ----
22. Have you made any arrangement to meet EurepGAP/KenyaGAP requirements? Yes/ No.
23. From your own point of view, how do you rate the effort/task required to comply with EurepGAP regulations? A. Easy B. Normal C. Difficult D. Impossible.

24. Do you have access to agricultural extension services? A. Yes B. No. .If yes how often? A. Once a week. B. Once a month C. Once a year. Do you find the services to be of any value? A. Yes B. No.

Part II

Appendix: Agro-chemical stockist Questionnaire

1. Business name of the ago-chemical shop------------------------------
2. Location of the business --
3. Highest level of formal education, or professional qualification of the counter staff attendant .A Primary B. Secondary C. Post-Secondary.
4. Number of years in agro-chemical business. A. Less than five years B. Between five and ten years C. Over ten years. (tick one)
5. List of dithiocarbamate formulation in stock. Fill in accordingly in the table below.

Trade name	**Manufacturer**	**Distributor**

6. Best selling dithiocarbamate in the area --

   ----

7. Peak season of fungicides in general. A. March to July B. August to September

   C. October to December D. January to February.

8. Proportion of dithiocarbamates to the total fungicide in the shop A. Less than 10%

   B. 10 -50% C. 50-95% D. Over 95%.

Any other comment---

Thank you,

Kariuki, George M.

(Student JKUAT)