"FATE OF CHLOROTHALONIL ON SNOWPEAS AND ASSESSMENT OF ITS RESIDUES IN FRENCH BEANS AND PASSION FRUITS IN NAIROBI"

BY

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I56/8048/2006

THESIS SUBMITTED TO THE BOARD OF POST GRADUATE STUDIES IN PARTIAL FULFILLMENT OF THE DEGREE OF MASTER OF SCIENCE IN ENVIRONMENTAL CHEMISTRY OF THE UNIVERSITY OF NAIROBI

NOVEMBER 2010

DECLARATION

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

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This thesis has been submitted for examination with our approval as University Supervisors.

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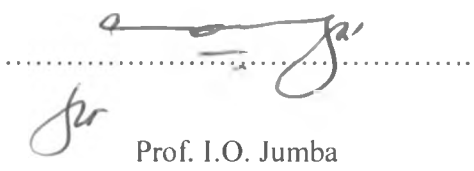

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DEDICATION

This work is dedicated to my dear parents and family who have been a source of inspiration, love and encouragement.

ACKNOWLEDGEMENT

I wish to express my sincere gratitude to my supervisors; Professor Shem O. Wandiga and Professor Isaac O. Jumba for their contribution, professional advice and technical assistance. Their interest, comments and suggestions, despite their busy schedule are highly appreciated. Their advice and support during the experimental stage, data analysis and interpretation, and writing up have been enormous. I further acknowledge KEPHIS for giving me time and resources to undertake this work.

I would like to acknowledge the valuable contribution of Mr. Vincent O. Madadi for his technical support during entire period of the project. Special thanks to the entire research team of the Pesticide Research Laboratory of Department of Chemistry, University of Nairobi. I further thank the school of biological sciences, University of Nairobi for providing working space in the green house.

My acknowledgement goes to the Soil Physics Laboratory of the National Agricultural Research Laboratories of the Kenya Agricultural Research Institute Nairobi, for their technical advice on soil sampling and analysis during the study. I further acknowledge the Pest Control Products Board for the valuable information on chlorothalonil that they provided. I further thank Mr. Samuel Kimani Muhia a farmer in Magumu Location of South Kinagop Division for providing the soil used in this study. Special thanks to the almighty God, our Heavenly Father for his love and grace through which I was able to accomplish this work.

ABSTRACT

Chlorothalonil (2, 4, 5, 6-tetrachlorophthalonitrile) is a broad spectrum contact fungicide. It has both agricultural and household uses. Residues of chlorothalonil are regularly found on farm produce of plants that are grown using the fungicide. The maximum residue limit (MRL) for chlorothalonil ranges from 0.01mg/Kg in bananas to 70 mg/Kg in chili dry pepper; while the Acceptable Daily Intake (ADI) is 0.03 mg/Kg.

In this study, the fate of applied chlorothalonil in soil and snow peas and its residue levels on French beans, passion fruits and snow peas sold in Nairobi were established. Controlled and treated experiments were conducted in a green house on soil obtained from Magumu location of south Kinangop division, Nyandarua District, Central province. Chlorothalonil residues were soxhlet extracted from samples using organic solvents, detected and quantified by Gas Liquid Chromatograph with an Electron Capture Detector and confirmed by Electron Impact Ionisation Gas Chromatograph mass spectrometer (EI-GCMS).

Results obtained indicate that chlorothalonil has a dissipation half-life of 2 days in snow peas and 10.2 days in the soil. At a Pre-harvest interval of 21 days chlorothalonil residues measured 0.0126 mg/Kg for snow peas grown on untreated soil and 0.0245 mg/Kg for those grown on treated soil. Both levels were below both EU and Codex MRLs of 5 and 2 mg/Kg respectively and the ADI of 0.03mg/Kg for snow peas.

Chlorothalonil residue levels in assessed fruits and vegetables ranged from non-detectable (ND) levels to 0.012 mg/kg in snow peas, ND to 0.021mg/kg in French beans and ND to 0.009 mg/Kg in passion fruits. The experimental Limit of Detection for the method was established to be 0.004 mg/Kg while the Limit of Quantitation was 0.013 mg/Kg. An average enrichment factor of 3.86 was applied during analysis to enable detection and quantitation of residues at

low levels. The observed residues did not violate both the EU and Codex MRLs. However, presence of chlorothalonil residues on passion fruit indicates unauthorized use of the fungicide, indicating potential misuse of pesticides.

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ABBREVIATION

ADI	Acceptable Daily intake
AEZ	Agro-ecological zones
a.i	Active Ingredient
AOAC	International Association of Official Analytical Chemists
ASALs	Arid and semi arid lands
BCPC	British Crop Production Council
BW/day	Body weight per day in Kg
CA	Chemical Abstracts
CAS	Chemical Abstract Service
EPZA	Export Processing Zones Authority
FAO	Food and Agriculture Organization
GAP	Good Agricultural Practice
GC-ECD	Gas Chromatography with Electron Capture Detection
GC-MS	Gas chromatography - Mass Spectrometry
GDP	Gross Domestic Product
GSH	Glutathione
HPLC	High Performance Liquid Chromatography
IAEA	International Atomic Energy Agency
ILO	International Labour Organization
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
JKIA	Jomo Kenyatta International Airport
JMPR	Joint FAO/WHO meeting on Pesticide Residues
KARI	Kenya Agriculture Research Institute
KEPHIS	Kenya Plant Health Inspectorate Service

mg/Kg	Milligrams per Kilogram
MRL	Maximum Residue Limit
NARL	National Agriculture Research Laboratories
NIST	National Institute of Standards and Technology
PCPB	Pest Control Products Board
PHI	Pre - Harvest Interval
RASFF	Rapid Alert System for Food and Feed
SDA- 47525	2, 4, 5 trichloro-6-hydroxy-3-cyanobenzamide
SDS- 46851	3 Carbomoyl-2, 4, 5- trichlorobenzoic acid
SDS - 47623/4	2, 4, 5 – trichloro- 3cyanobenzamide
SDS -19221	2,3,4,6 Tetrachloro-5-cyanobenzamide
SDS-3701	4 hydroxy 2, 5, 6, trichloroisophthalonitrile
SDS-46042	4,6,7 trichloro-3-oxo-2,3-dihydrobenzo[*d*] isothiazole-5-carbonitrile
STMR	Supervised Trial Median Residue.
TMDI	Theoretical Maximum Daily Intake
UNEP	United Nations Environment Programme
UNIDO	United Nations Industrial Development Organisation
USDA	United States Department of Agriculture
US-EPA	United States Environment Protection Agency
WHO	World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

1.1 General Background

Agriculture is the leading sector in the Kenyan economy in terms of its contribution to the Gross Domestic Product (GDP). Kenya relies on rain-fed agriculture. The country has four major Agro Ecological Zones namely the highlands, Savanna, coastal and arid and semi arid lands (ASALs). The zones have distinct humidity ranges, mean annual temperature and rainfall patterns and altitudes that largely dictate their respective ecological potentials.

Agricultural activities are concentrated in the highlands (high potential), savannah, and coastal (medium potential) AEZs (NEAP, 1994). The coast, eastern plateau and the lake basin experience two rainy seasons; long rains extend from March to May and the short rains last from October to December. The Highlands of western Kenya (Kericho, Kisumu, Kakamega, Kisii, and Eldoret) have a single rainy season lasting from March to September. The hottest time period falls in the months of February and March and the coldest is July and August [Kenya Meteorological Department, 2009].

The agricultural sector provides food to the population. It also provides employment either directly or indirectly to about 70% of the total work force in Kenya [Ouma and Majanja, 2006]. The sector also accounts for over 65% of Kenya's total export earnings while providing the basis for the development of the other areas of the economy especially the manufacturing sector through provision of raw materials. It also contributes immensely to poverty eradication in the country.

Kenya produces approximately 3,000,000 tons of vegetables, fruits and cut flowers annually, of which approximately 100,000 tons (about 3.3%) are exported. The European Union imports about 90% of Kenya's horticultural exports [Ouma and Majanja, 2006]. Kenya exports 5% of the fresh produce (fruits and vegetables) it produces annually and the remaining 95% is consumed locally. The rapid growth of this sector has had positive effects on the communities in the form of employment and the national economy in the form of valuable foreign exchange.

Pesticides are a useful tool in production of fresh produce. However, unlike other agricultural inputs such as fertilizers, manure, seeds, etc, pesticides pose a potential risk to both human beings and the environment. Inappropriate use of pesticides may lead to presence of harmful residues in both the produce and the environment. As far as the horticultural industry is concerned, adherence to Maximum Residue Limit (MRL) requirements is the main concern.

The UK and France are the primary markets for fresh vegetable produce from the country, with a share of over 30% by volume [EPZA, 2005]. Sales of Kenya based pre-packed high quality vegetables have been increasing annually – particularly snow peas, sugar snaps, baby vegetables, runner beans and French beans. Large volumes of airfreight to the UK are available on both scheduled and charter flights [EPZA, 2005].

1.2 Regulation, Use and Distribution of Pesticides in Kenya

1.2.1 Pesticide Regulation

The earliest recorded legislation dates from 6th September 1921 when the public health Act, Cap 242 was passed by the colonial government. A second act of parliament dealing with cattle cleansing, Cap 358, was passed on 27th April 1937. This Act prescribed various preparations for

destroying ticks. These preparations are still retained in law though several amendments have modified the original prescriptions [Wandiga *et al.*, 2003].

A voluntary precautionary scheme for the agricultural industry was adopted when the colonial government declared emergency rules (at the height of the struggle for independence). This scheme led to the Poisonous Substances Ordinance of 1954. The Ordinance was based on the UK Act of 1952, which provided for the protection of employees against risk of poisoning by certain substances used in Agriculture, incidental and connected matters [Wandiga *et al.*, 2003].

The Pharmacy and Poisons Act was passed by Westminster on 1 May 1957. Included in this Act were the control veterinary drugs and poisons with additional rules on the selling and labeling of poisons including pesticides. Upon independence, the Kenyan parliament passed an Act on 11 May 1965 for prevention of adulteration of food, drugs, chemical substances, and incidental and connected matters.

In the Food, Drug and Chemical Substances' Act Cap 254, pesticides were given particular attention and the term 'chemical substance' was defined to refer to any substance or mixture of substances prepared, sold or presented for use as a germicide, insecticide, rodenticide, disinfectant, antiseptic, vermicide and detergent. For the first time, it also set tolerance levels (in ppm) for pesticides in foodstuffs. [Wandiga *et al.*, 2003; KLR 2010].

Other legislative laws passed by parliament that have a bearing on pesticides use, distribution and control include the Agriculture Act Cap 318 of 1955, the Fertilizer and Animal Foodstuffs Act Cap 345, The Plant Protection Act Cap 324 of 1979 and the Pest Control Products Act which came into law in 1983. The latter was established to regulate the importation, exportation, manufacture and distribution of products used for the control of pests. It also addresses the

organic function of pesticides on plants and animals and the Agricultural produce (export) Act cap 319 of 1923 that provided for the grading and inspection of agricultural produce to be exported, and generally for the better regulation of their preparation and manufacture.

1.2.2 Pesticide use and Distribution in Kenya

The use of pesticides in Kenya has steadily increased since 1986 from Kshs. 580.2 million to kshs. 4.5 billion, in 2005/06 [PCPB, 2006]. The major active substances involved were Glyphosate, 1, 3-Dicholoropropene, Amitraz, Mancozeb, D'allethrin, Chlorothalonil, Copper hydroxide, Cuprous oxide, Dimethoate, Metolachlor + Atrazin; Sulphur, Diazinon, Methyl bromide, Deltamethrin, 2, 4-Dichlorophenoxyacetic acid and Cobox in order of decreasing volume.

Out of the 7708 metric tonnes, imported only 82 metric tonnes were exported to neighbouring countries and involved the active ingredients pirimiphos methyl, permethrin and chlorfenviphos [PCPB, 2006]. Table 1.1 below shows a summary of quantity of pesticides imported in the 2003/2004 financial year through to 2008/2009 while Table 1.2 shows the value of imports in the same period [PCPB, 2006, and unpublished report from PCPB]

Table 1.1: Quantity of Pesticides Imports (in Tonnes)

Category	2003/2004	2004/2005	2005/2006	2006/2007	2007/2008	2008/2009
Insecticide	2465	2881	2844	2475	2887	2995
Fungicide	1657	2031	2361	3190	2651	2340
Herbicide	1396	1538	1311	1859	2289	2933
Others	723	597	1192	1225	1330	1413
Total	**6241**	**7047**	**7708**	**8749**	**9157**	**9681**

Table 1.2: Value of Pesticides Imports (in '000,000' Kshs)

Category	2003/2004	2004/2005	2005/2006	2006/2007	2007/2008	2008/2009
Insecticide	2411	2077	2031	1181	3909	2079
Fungicide	925	1113	1506	1251	602	3153
Herbicide	571	650	620	324	206	944
Others	142	133	337	362	191	1167
Total	**4049**	**3973**	**4494**	**3443**	**4908**	**7343**

1.3 Pesticide Residues in Food

Incidences of produce having high levels of pesticide residues have been recorded. Some of these pesticides are known carcinogens and may have long-term effects like nervous system disorders and immune suppression. Un published report from KEPHIS on a market basket and farm gate survey on tomatoes conducted in 1994 indicated levels above maximum residue limits (MRLs) for dithiocarbamates (specifically Mancozeb).

In 2005, Kenyan passion fruit and French beans were intercepted in Sweden and Belgium during routine monitoring. The passion fruits were found to have dithiocarbamates and Chlorothalonil residues, while the French beans were found to have chlorothalonil, Dimethoate, Tebuconazole and Omethoate residues [KEPHIS, 2006].

A study on dissipation and degradation of Malathion and dimethoate from soil and garden pea plant was carried out by Getanga [Getanga, 1999]. He reported the dissipation rate of dimethoate from the foliar surface of the garden pea to be 29 days ($t_{1/2}$ = 29 days), while the foliar

dissipation rate of malathion from the pea plant showed that rate of decline is fast during the initial stages and relatively slow afterwards.

1.4 Pesticide Residues in the Environment

Previous studies indicate presence of pesticides in environmental samples. Organochlorine pesticides residues comprising of 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (p, p'-DDT), 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane (p, p' –DDD), 1,1-dichloro-2,2-bis(p-chlorophnyl)ethylene (o, p'-DDE), Aldrin, Dieldrin, α-endosulphan, β-endosulphan, endosulphan sulphate, 1,2,3,4,5,6-Hexachlorocyclohexane (α-HCH, β-HCH, γ-HCH), heptachlor, heptachlor epoxide, methoxychlor, and endrin, and organophosphate residues of diazinon, dimethoate, malathion, fenitrothion, and ethyl parathion were detected at varying frequencies and concentrations in water, soil, weeds and fish samples [Madadi, 2005]. He reported that the total residues of DDT, HCH, methoxychlor and endrin were below the WHO limit guidelines for drinking water, where as aldrin, Dieldrin, heptachlor, heptachlor epoxide and endosulphan were above the recommended values.

In another study, [Getanga *et al.,* 2004] found α-BHC, β-BHC, γ-BHC (lindane), endosulphan, heptachlor, aldrin, heptachlor epoxide, dieldrin, endrin and methoxychlor in soil from sugar belt zone of Lake Victoria basin. They reported higher levels of α-BHC, β-BHC and lindane.

1.5 Problem Statement

Horticulture is among the leading foreign exchange earner and employer either directly or indirectly with about 70% of the producers being smallholder farmers. Kenya imports most pesticides from the developed countries. Though pesticides residue trials to provide data for registration of pesticides and the establishment of maximum residue limits are carried out,

instances of farm produce having pesticide residues above the MRL continue to occur. Furthermore these trials are carried out at the country of origin of the pesticides most of which have climatic conditions that are different from the Kenyan one. The continued occurrence of pesticide residues above MRLs brings into question the level of application of good agricultural practice (GAP) by farmers. Chlorothalonil is commonly used in Kenya on a variety of crops; and with current challenges, there is need to carry out a study on its fate, this study focused on snow peas grown under local climatic conditions and also involved assessment of its residues in other fresh produce in Nairobi markets, in particular French beans and passion fruits.

1.6 Objectives

The main objective was to generate data on the fate of Chlorothalonil applied to snow peas under local conditions,

The specific objectives were;

1. To study the fate of chlorothalonil applied to snow peas under local climatic conditions.
2. To quantify Chlorothalonil residues from treated snow peas.
3. To assess chlorothalonil residues on snow peas, French beans and passion fruits from selected markets in Nairobi.

1.7 Study Hypothesis

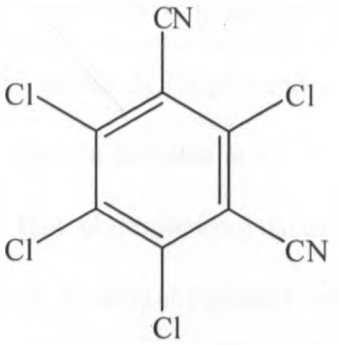
The study hypothesis was that; Pesticide residues above MRL are found on produce if a crop is treated with a pesticide without observing Good Agricultural Practice, which includes observing the set pre-harvest interval for that specific pesticide.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Physical and Chemical Properties of Chlorothalonil

Chlorothalonil is a broad spectrum non-systemic fungicide [IPCS, 1996]. Its chemical names are 2,4,5,6-tetrachloro-3-cyanobenzonitrile (ISO), tetrachloroisophthalonitrile (IUPAC) and 2, 4, 5, 6- tetrachloro-1, 3-benzenedicarbonitrile (CAS). Its structure is given in figure 1.1 below.



2,4,5,6-tetrachloroisophthalonitrile
Chemical Formula: $C_8Cl_4N_2$

Figure 1.1: Structure of Chlorothalonil

The CAS registration Number for chlorothalonil is 1897- 45-6. Chlorothalonil has an octanol water partition coefficient (K_{ow} log P) of 2.88 – 3.86 [IPCS, 1995]. At 25°C, the K_{ow} log P is 2.91 and it is not fat soluble. The technical grade has purity greater than 97% and contains the impurities; tetrachlorophthalonitrile (less than 0.1%); tetrachloroterephthalonitrile (0.1-1.6%); pentachlorobenzonitrile (0.5-2.5%); partially chlorinated dicyanobenzenes (0.2 -1.0%); unchlorinated dicyanobenzenes (0.1-1.6%); hexachlorobenzene (HCB) (0.03%) and xylene insolubles (0.35%).

Chlorothalonil is a colourless, odourless crystalline solid with a melting point of 250 – 251 ^{0}C and a boiling point of 350 $^{\circ}$C at 730 mmHg pressure [IPCS, 1995]. It is thermally stable under normal storage conditions and is stable to UV radiation. It is chemically stable in neutral or acidic aqueous solutions. However it decomposes at pH 9, with the rate of decomposition following first order kinetics at 1.8% per day at 25 ^{0}C [IPCS, 1995]. It has a half-life of 38.1 days in aqueous media at pH 9 [Farm chemicals, 1994].

Chlorothalonil is non-corrosive in pure form, and non-volatile under normal field conditions. It has a vapour pressure of 5.72 x 10 $^{-7}$ torr at 25 $^{\circ}$C [Farm chemicals, 1994] and is slightly soluble in xylene (80g/l); acetone, dimethyl sulfoxide (20g/l) and cyclohexanone, dimethlyformamide (30g/l) all at 25 $^{\circ}$C. It is readily soluble in benzene at 25 $^{\circ}$C but insoluble in water (0.81 mg/L) [IPCS, 1995]. It has been shown that chlorothalonil is unstable to light when dissolved in benzene and that 2, 3, 5-trichloro-4, 6-dicyanobiphenyl is the condensation product [IPCS, 1995].

Chlorothalonil has been produced commercially since 1969 by the chlorination of isophthalonitrile or by treatment of tetrachloroisophthalonyl amide with phosphorus oxychloride [IPCS, 1995]. As a fungicide, it is not only used in agricultural farming but is also applied on turf, lawns and ornamental plants. It was first registered for use in the U.S in 1966 on turf grass. The first food crop registration was in 1970 for potatoes [EPA, 1999]. It is the second most widely used agricultural fungicide in the U.S. [Caroline, 1997]. It is used in the control of many fungal diseases in a wide range of crops, including pomes and stone fruit, citrus, berries, bananas, green vegetables, coffee, tea, peanuts, potatoes, onions, almonds, bush and cane fruits, cranberries, strawberries, pawpaw, mangoes, coconut palms, oil palms, cucurbits, tobacco, rice,

soya beans, sugar beet, mushrooms, cotton, maize, rubber, vines, hops, vegetables, pepper and cereals. It is also used in wood and paint preservation [FAO, 1996; BCPC, 2006; IPCS, 1996].

Chlorothalonil has three main formulations: suspension concentrates (SC), wettable powder (WP) and water dispersible granules. It contains two main recognized contaminants: hexachlorobenzene (HCB) maximum up to 0.004% and decachlorobiphenyl up to 0.003% [FAO, 2005]. The formulations are readily diluted with water and applied by ground or aerial spray system.

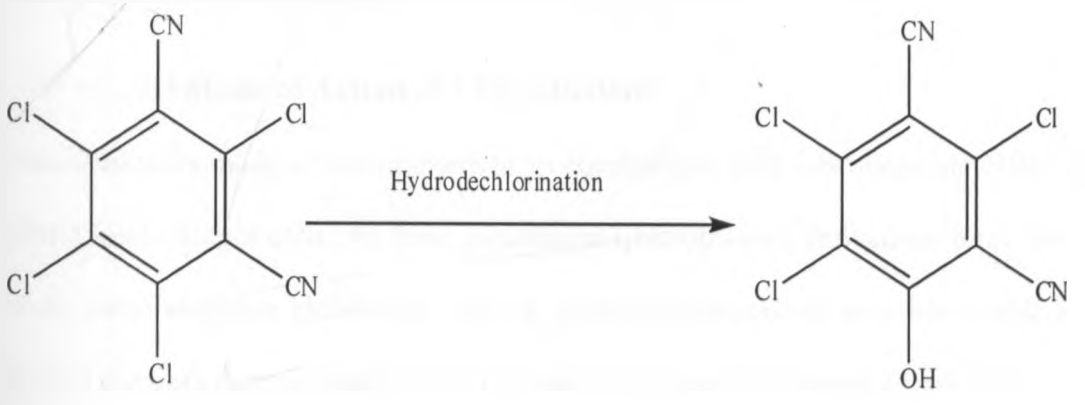
In Kenya, chlorothalonil is commonly sold under the trade names Daconil 720 SC, Bravo 500g/L SC, Clortocaffaro 75% WP, Koban 750g/L, Ranko 75WP (750g/L), Rankonil 500 SC, Clortocaffaro 500g/L, Rova 500 F LOW , Dakota 50 FW 50% SC, Twigathalonil 720SC, Rova 75 WP 75%, and Folio Gold 537.5g/Kg. Folio contains a combination of Chlorothalonil 500g/L and Metalaxyl 37.5g/L [PCPB, 2007]. In other countries, it is sold under the trade names Faber, Repulse, Exotherm, Nopcocide (a preservative in paint and adhesives), Fourtuf, Exotherm termil, Termil, and Sweet [FAO, 1996].

Chlorothalonil is registered in Kenya for use in the control of leaf and ear disease in wheat, coffee berry disease in coffee, bean rust, angular leaf spot, anthracnose and botrytis on French beans and aschochytes, downy mildew and botrytis on snow peas, early and late blight in tomatoes, downy mildew and leaf spot on cucumber, downy mildew, and botrytis and black spots on Roses [PCPB, 2007]. Unpublished reports from PCPB indicate an upward trend in imports of chlorothalonil rising from 84970 kilograms in 2003 to 277430 kg in 2008. All imported chlorothalonil is consumed locally. Ranconil 500 SC and Daconil 720 SC are registered

for use on snow peas in Kenya. Typical active ingredients application rates vary from 1.2 to 2.5Kg/ha for crops such as beans, celery and onions [IPCS, 1996].

2.2 Metabolism of Pesticides in Plants

When pesticides are sprayed on plants, material not adhering tenaciously to the plant surface slough off immediately and the remaining portion of the original deposit becomes an effective deposit which then begins to be absorbed into the plant [Getenga, 1999]. The disappearance of these pesticides from the leaf surface after interception is exponential and often follows first order kinetics [Willis and Dowell, 1987]. The primary metabolite (breakdown product) of chlorothalonil is 4-hydroxy-2, 5, 6-trichloroisophthalonitrile and has been detected in soils, plants, and animals following application of the pesticide. It is about 30 times more toxic than chlorothalonil itself and is more persistent and mobile in soil [Caroline, 1997]. Figure 2.2 represents chlorothalonil and its primary metabolite.



Chlorothalonil

4-hydroxy-2,5,6-trichloroisophthalonitrile

Figure 1.2: Chlorothalonil and its Primary Metabolite

A study conducted in California, on bent grass gave a dissipation half-life ($t_{1/2}$) of 4.9 days [Wu *et al.*, 2001].The residue levels and degradation rate of chlorothalonil in cucumbers, peppers and cranberry tomatoes grown under green house conditions indicated $t_{1/2}$ periods of 5.3 days for cucumbers, 7.3 days for peppers and 11.5 days for cherry tomatoes [Varlverde *et al.*, 1993].

In Massachusetts, dislodgeable foliar and whole fruit residues of chlorothalonil and degradation products were assessed in cranberry bog over a growing season. The dissipation of dislodgeable foliar chlorothalonil residues followed first order kinetics with estimated $t_{1/2}$ of 12.7 days [Putnam *et al.*, 2002]. All residues of harvested fruit were well below the US EPA tolerance for fresh cranberries. However in spring cabbage grown in an open field, the $t_{1/2}$ was 1.8 days. If the cabbage were treated once at normal dosage of pesticide, the MRL was not exceeded. But, if the vegetable was treated four times at the maximum dosage with a five day's interval the $t_{1/2}$ of chlorothalonil in the vegetable was 4.1days and the final residual amount exceeded its MRL in cabbage [Zhi-Yong *et al.*, 2007].

2.3 Mode of Action of Chlorothalonil

Chlorothalonil's mode of action involves its combination with a biomolecule called glutathione (GSH) inside fungus cells. As these glutathione-Chlorothalonil derivatives form, they tie up all of the cells' available glutathione, leaving glutathione-dependent enzymes unable to function. Several enzymes that are important in cellular respiration (the process by which large molecules are broken down and provide the cell with energy) are glutathione dependent. Their inhibition leads to Chlorothalonil's toxic effects [Caroline, 1997].

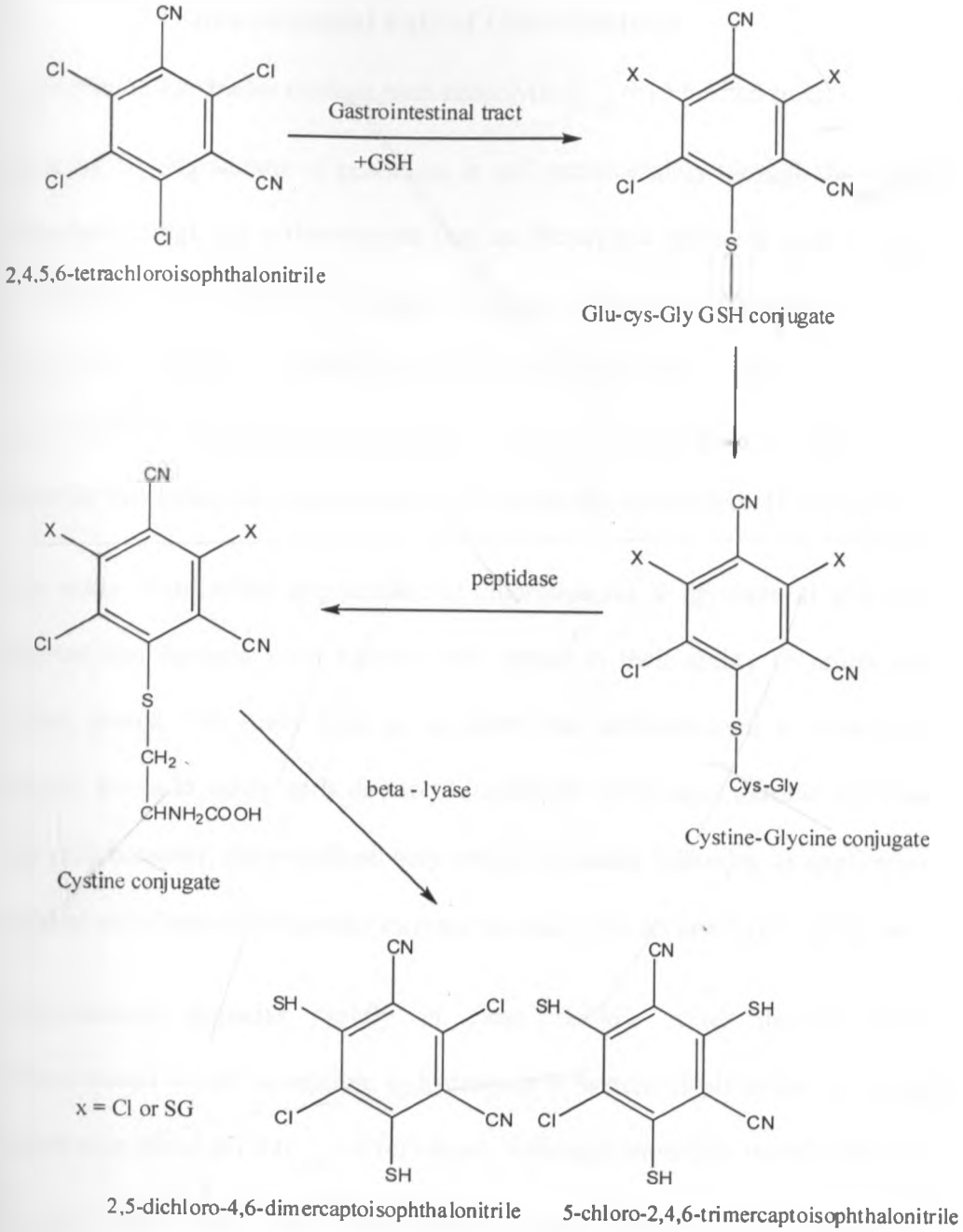
Human exposure to chlorothalonil may occur during production and application of the product as a fungicide, bactericide and nematocide and from ingestion of crop residues in food stuff or

animal residues in food of animal origin. It has been detected in some foods; however, chlorothalonil is classified as an active ingredient pesticide unlikely to present acute hazard in normal use [WHO, 2005]. The residues of chlorothalonil in plants remain as parent compound while in ruminants, the major identified metabolite is the 4 – hydroxyl derivatives and no parent material is found [BCPC, 2006].

The toxicokinetics of chlorothalonil in rats was reviewed by [Wilkinson and Killeene, 1996]. After oral administration, the amount of chlorothalonil absorbed is dose related. Thus, while approximately 30% of an administered dose of up to 50mg/kg body weight was absorbed, absorption at higher dose decreased, being only 15% at 200mg/kg body weight. Chlorothalonil reacts readily with glutathione and glutathione conjugation is the primary route of metabolism. The liver is the major organ for the conjugation of chlorothalonil with glutathione.

The major urinary metabolites are trithiomonochloroisophthalonitrile and dithiomonochlorophthalonitrile and the corresponding methylthio derivatives. The di- and tri glutathione conjugates formed in the liver may be secreted into the bile, undergo enterohepatic circulation as intact glutathione conjugates or cysteine conjugates, return to the liver for further processing and be transported directly to the kidney as demonstrated by their presence in the blood. The chlorothalonil metabolites arriving in the kidney consist of a mixture of di- and tri-glutathione conjugates, cysteine s-conjugates and possibly some mercapturic acids. The glutathione conjugates are completely cleaved in the proximal tubes by γ-glutamly transpeptidase and dipeptidase to the cysteine s- conjugates, which are subsequently cleaved by β-lyase to the corresponding thiol derivatives. Since mercapturic acids have not been identified in the urine, these compounds are probably deacetylated to corresponding cysteine conjugates, which may undergo bioactivation to a reactive thiol by β-lyase [Wilkinson and Killeene, 1996].

Figure 2.3 illustrates a proposed pathway for chlorothalonil transformation to thiol derivative in the rat kidney. Dogs and monkeys excrete little or none of these derivatives in the urine. Chlorothalonil has low acute oral and dermal toxicity in rats and rabbits, with LD_{50} above 10,000mg/ kg body weight. It irritates the skin and eye. The main effects of chlorothalonil are observed in the stomach and kidney and lesions in these tissues were observed in two-year studies in rats, mice, and dogs. In rats hyperplasia of the kidney and fore stomach was observed at doses of 3.8mg/kg body weight per day.



2,4,5,6-tetrachloroisophthalonitrile

Glu-cys-Gly GSH conjugate

Cystine conjugate

Cystine-Glycine conjugate

2,5-dichloro-4,6-dimercaptoisophthalonitrile

5-chloro-2,4,6-trimercaptoisophthalonitrile

Cys-Cystine; Gly-Glysine; Glu-Glutamine

Figure 2.3: Proposed Pathways for Chlorothalonil Transformation to Thiol Derivative in Rat Kidney.

2.4 Environmental Fate of Chlorothalonil

Chlorothalonil degrades through both photolytic ($t_{1/2}$ = 10 hr) and microbial processes ($t_{1/2}$ = 7 – 68 days). Biodegradation of pesticides in soil occurs mainly through the biochemical processes of bacteria, fungi, and actinomycetes that use the organic molecule as an energy source [Eeden *et al.*, 2000].The rate of decomposition of pesticides through biodegradation is however influenced by the soil temperature affecting enzyme activity, the water, clay and humic content of the soil that determine the degree of adsorption that will occur and the molecular structure of the pesticide that influences its accessibility for microbial metabolism [Eeden *et al.*, 2000].

In a study of microbial degradation of chlorothalonil in agricultural soil Eeden *et al.* (2000) reported that bacteria from various soils varied in their ability to utilize chlorothalonil as a carbon source. The study findings indicated that chlorothalonil is unlikely to accumulate to harmful levels in sandy soils due to utilization by indigenous aerobic soil bacteria. In red and clay soils however, chlorothalonil may persist for weeks following its application because certain physical and chemical properties increase the risk of its accumulation [Eeden *et al.*, 2000].

Chlorothalonil degrades rapidly in clear, shallow water through aqueous photolysis. Chlorothalonil is not susceptible to hydrolysis in waters of pH below 9, but does hydrolyze in waters at or above pH 9 ($t_{1/2}$ = 40-60 days). Although photolytic transformation of chlorothalonil is more rapid than biotic metabolism, aqueous photolysis is limited to environmental compartments where clear, shallow waters are exposed to direct sunlight. Therefore, the main route of dissipation for chlorothalonil in the environment is expected to be through aqueous, biotic degradation ($t_{1/2}$ = 7-29 days).

Chlorothalonil degrades under both aerobic aquatic conditions ($t_{1/2}$ = 7-16 days) and aerobic terrestrial conditions ($t_{1/2}$ = 22-68 days), and through anaerobic degradation ($t_{1/2}$ = 21-29 days). Chlorothalonil has low water solubility 0.9mg/litre and low mobility in soil. Moderately persistent in soil, chlorothalonil is metabolized to its' more toxic and persistent 4-hydroxy metabolite [Caroline, 1997; Eeden *et al.*, 2000]. The half- lives for dissipation of the 4-hydroxy metabolite in soils range between 6 and 43 days [IPCS, 1996].

In the environment, chlorothalonil is highly toxic to birds, fish and aquatic invertebrates [Eeden *et al.*, 2000]. Although studies have indicated that chlorothalonil is not expected to affect human reproduction due to its relatively low bioaccumulation factor [Eeden *et al.*, 2000], it has been characterized by the US EPA as likely to be a human carcinogen by all routes of exposure'' [Eeden *et al.*, 2000].

Chlorothalonil is degraded by substitution of chlorine with hydroxyl group (dechlorination) and hydrolysis of the nitrile group. Figure 2.4 presents some of the known transformations which occur with chlorothalonil under various environmental conditions. Another breakdown product formed in soil is m-phthalodinitrile. While m-phthalodinitrile can cause headaches, nausea, confusion, and loss of consciousness, in general its toxicological properties have not been investigated [Caroline, 1997].

In a study on the environmental fate of chlorothalonil and its metabolite under field – variable conditions simulating a tropical environment in Costa Rica, the dissipation of chlorothalonil was rapid with a $t_{1/2}$ of 2.2 and 3.9 days in soil and banana leaves, respectively [Alicia *et al.*, 2007]

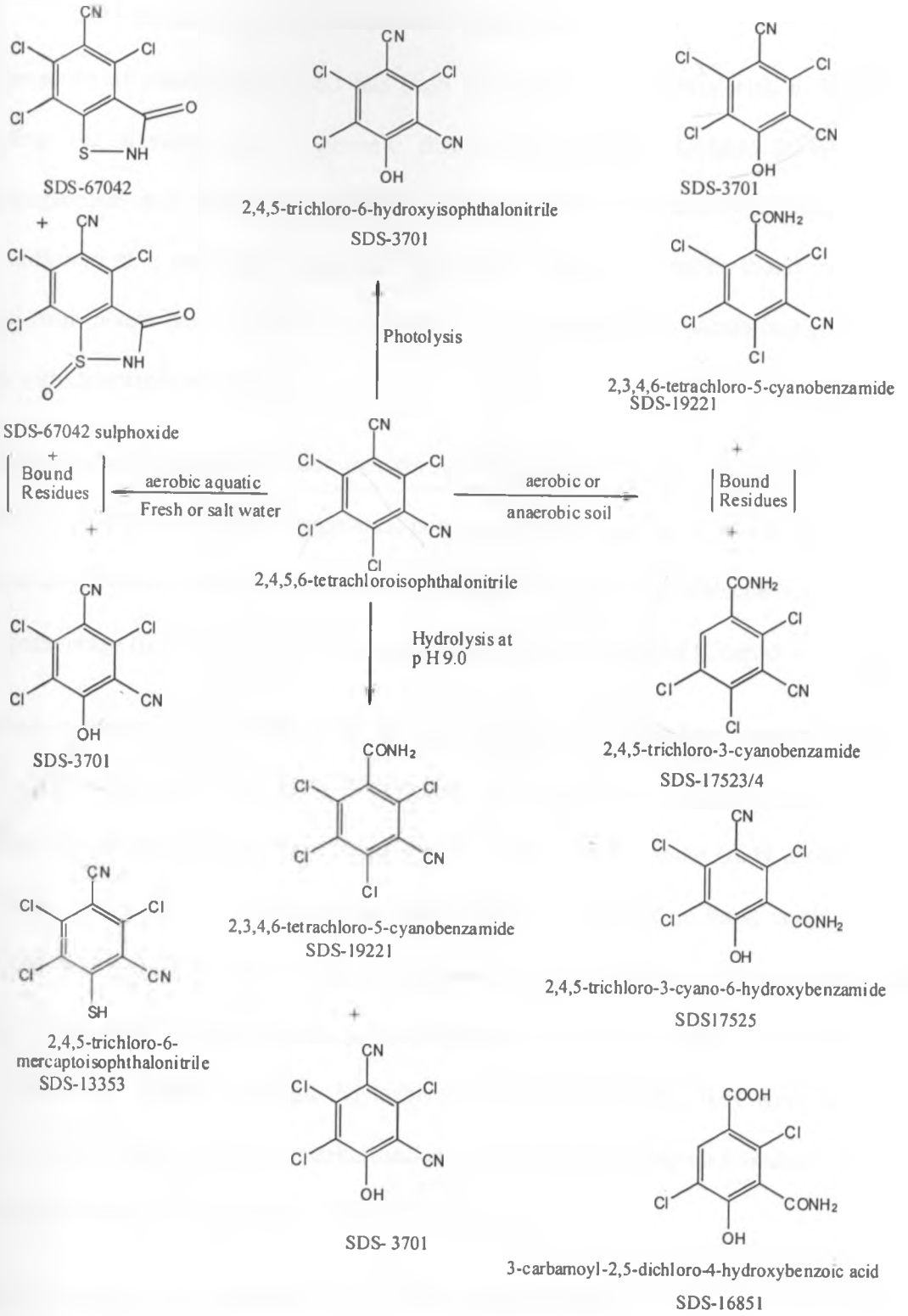


Figure 2.4: Chlorothalonil Metabolism under various Environmental Conditions

2.5 Contamination of Food with Pesticides

The presence of chemicals in food has been described as a potential risk to human health, including the development of chronic diseases [Souza and Caldas, 2006]. In Brazil, dithiocarbamates and ten organophosphates were analysed and found in samples of prepared food collected at a university restaurant. Residues of dithiocarbamates found in the samples ranged from levels below 0.10 to 0.24 mg/kg CS_2, with vegetable salads being the dominant group with detectable residues.

In the Philippines a pesticide residue monitoring study of commonly consumed fruits, vegetables and cereals including tomatoes, eggplants, potatoes, bananas, cabbages, carrots, mangoes, okra, rice and string beans found that 18.5% of the samples collected had detectable residues, with a small percentage (0.4%) having concentrations above the Japan MRLs [Chen *et al.*, 2006].

In Kenya, a survey of deltamethrin and lamda cyhalothrin in vegetables reported mean residue levels ranging between 0.0130 and 0.3400 mg/kg during the dry seasons and between non-detectable level and 0.1100 mg/kg during the wet season. In the same study it was found that vegetables consumed in urban areas had higher residues compared to those consumed in rural areas with mean residue levels reaching a maximum of 0.3400 mg/kg in the samples analysed from the urban areas. In the same study, samples analysed form the rural area had levels between 0.0012 and 0.11 mg/kg. Although the levels were below the MRL, they were however well above the ADI [Njagi, 2005]. A market basket and farm gate survey on tomatoes conducted in 1994 indicated mancozeb levels above MRL at farm gate.

In 2005, Kenyan passion fruit and French beans were intercepted in Sweden and Belgium. The passion fruits were found to have unacceptable residues of dithiocarbamates and Chlorothalonil,

while the French beans were found to have Chlorothalonil, Dimethoate, Tebuconazole and Omethoate [KEPHIS, 2006]. The residues are based on the whole fruit and vegetable presented for sale.

In Ireland a monitoring programme was conducted in 2004 for three different food groups for which MRLs have been established thus, food of plant origin (including fruit and vegetables), cereals and food of animal origin (meat, milk, and dairy produce) by the department of agriculture and food [DAFPCS, 2006]. Of the samples taken, 27 % were of domestic origin, 44% were imports from other EU countries and 29% were imports from countries outside the EU . Samples were analysed for residues of up to 118 pesticides and metabolites. Fourty eight percent of samples analysed contained no detectable pesticide residues, while the remaining 52% contained one or more detectable residues of which 3.4% contained residues in excess of the MRLs. In all, residues of 53 different pesticides were detected.

In the monitoring programme Kenyan produce was found to have residues. In particular, Avocado was found to contain fludioxinil at 0.02 mg/Kg, while passion fruit samples were found to have propargite residues at 0.08mg/kg and chlorothalonil at 0.15 mg/kg. While there are no MRLs for propargite the levels exeeded the EU MRL for chlorothalonil (0.01mg/kg). Four samples of Peas with pods had detectable residues. One conatined dimethoate at 0.13mg/kg which was above the EU MRL 0.02mg/kg. The second one was found to contain dimethoate and tebuconazole at 0.12 and 0.05 mg/kg, respectiveky and although there is no MRL for tebuconazole, the dimethoate MRL (0.02mg/kg) was exceeded. The third sample contained dimethoate and omethoate at 0.22 and 0.1 mg/kg, respectively but both were below the codex and EU MRL 1mg/kg for mangetout/sugar peas.The fourth sample contained residues of kresoxim-methyl at 0.02mg/kg which fell below the MRL 0.05 mg/kg.

Of the 64 cereals samples analysed, 13 contained pesticide residues but none of which exceed the MRLs.Kenya however, does not export cereals to the EU. Foods of animal origin including bovine, ovine, porcine, dairy products, venison, poultry, eggs and honey samples were also in the 2004 PRM programme. 127 samples of bovine meat were analysed. Six (all originating from Ireland) of these contained detectable pesticide residues. Four of the samples contained DDT as the metabilite p, p'- DDE, one contained HCB and the sixth contained diazinon. No MRLs were exceeded for p, p'- DDE (1.0 mg/kg fat), HCB (0.2 mg/kg fat) and diazinon no MRL [DAFPCS, 2006].

Of the 65 samples of ovine meat analysed, five (all originating from Ireland) contained detectable residues in kidney fat. Four of these contained DDT as the p, p'- DDE metabilite and one contained HCB. The residues levels were however very low (less than 0.005mg/kg fat) was considered to result from former use of pesticides. In 59 porcine, 67 dairy, 5 venison, 10 eggs and 10 honey samples analysed, no pesticide residues were detected. However, in poultry, out of the 28 samples analysed, two contained detectable levels: one of dieldrin and the other of p, p'-DDE both at levels below 0.01mg/kg fat [DAFPCS, 2006].

2.6 Contamination of Food with Chlorothalonil

Residues of chlorothalonil are regularly found in produce that was grown using the herbicide. For example, the U.S. Dept. of Agriculture found chlorothalonil residues on 32 percent of their celery samples and 7 percent of their green bean samples in 1992; 50 percent of the celery samples and 12 percent of the green bean samples in 1993; and 14 percent of the green bean samples in 1995 [Caroline, 1997]. The upward trend could probably be attributed to increased use of the fungicide.

The use of chlorothalonil on bananas is registered in Australia with multiple treatments and Pre harvest intervals of 1 to 0 days at an application rate of 1.1-2.16 kg ai/ha and in Latin America for aerial application at a rate 0.88-1.63 kg ai/ha. Two Australian trials on unbagged bananas reported to the 1993 JMPR which were according to the Australian GAP of 10 applications at the rate 1.1 or 2.2 kg ai/ha with 1 day PHI had resulted in residues of 0.6 and 2.0 mg/kg [FAO, 1997], respectively. In three of four Latin America banana trials evaluated by the 1993 JMPR the residues were below 0.01mg/kg. In the fourth trial on unbagged fruit carried out in Costa Rica in 1985 with 10 aerial applications at 1.75 kg ai/ha, the maximum residues in six field samples was 0.12mg/kg six days after treatment [FAO, 1997].

The Codex MRL for chlorothalonil ranges from 0.01 mg/kg in bananas (bagged) and sweet corn (corn – on – the - cob) to 70 mg/Kg in dry, chilly. The Codex MRL for common bean (pod and/ or immature seeds) is 5mg/kg [Codex, 2006]. There are no Codex MRLs for snow peas and passion fruits. However, the EU/UK has MRLs for these commodities set at 0.01mg/Kg for Passion fruit, 2mg/Kg for peas with pods (Legume vegetables fresh) and 5mg/Kg for beans (with pod) legumes vegetables fresh, and these MRLs came into force in October 2008 [Pesticide Residues Committee, 2008]. Table 2.1 represents national and international MRLs for selected commodities.

The allowable daily intake (ADI) for chlorothalonil is 0.03mg/Kg bw/day [FAO, 2006], while the Theoretical Maximum Daily Intake (TMDI) is 20% of the Allowable Daily Intake giving a TMDI of 0.006 mg/Kg. Crop residues are composed mainly of the parent compound. This has raised concern to the horticulture stake holders especially with the new regulations on pesticide application requiring that there be no trace of pesticide residue in fruits, vegetables and cut flowers intended for the EU markets.

Table 2.1: Chlorothalonil MRLs for Selected Commodities in mg/kg

Commodity	Codex MRLs	EU MRLs	US MRLs	Japan
Banana	0.01[1]	0.2	0.05[2]	0.2
Barley	0.1	0.1	-	-
Beans dry	0.2	0.01	0.1	0.2
Bean, snap	5	5	5	-
Peas, edible podded	-	2	5	2
Passion fruit	-	0.01	3	3
Tomatoes	5	2	5	5

[1] Based on bagged bananas; [2] This MRL refers to Banana, edible pulp.

Source; USDA Foreign Agriculture Service web site [USDA FAS, 2010].

Chlorothalonil does not have the chemical properties of a molecule that is likely to leach through soil and contaminate water. However, it has been found in groundwater in some states in the U.S. In addition, it binds strongly to organic acids in water, which can result in elevated concentrations. Chlorothalonil ability to contaminate water long distances from where it is used has been demonstrated in the U.S. [Caroline, 1997].

2.7 Snow Peas Growing

The snow Peas *(Pisum sativum var.macrocarpon)* is a distinct botanical cultivar or subspecies of *Pisum sativum.* The pod is slab-sided and is eaten before the string develops and the peas start to swell.

Peas require a cool, relatively humid climate and are grown at higher altitudes in the tropics with temperatures from 7 to 30°C. Production is concentrated between the Tropics of Cancer and 50° N. As a winter annual, peas tolerate frost to -2°C at the seedling stage, although top growth may be affected at -6°C. Winter hardy peas can withstand -10°C, and with snow cover protection, tolerance can be increased to -40°C [Slinkard *et al.*, 1994]. As described in Slinkard *et al.*, (1994), the optimum temperature levels for the vegetative and reproductive periods of peas were reported to be 21 and 16°C, and 16 and 10°C (day and night), respectively. "Temperatures above 27°C shorten the growing period and adversely affect pollination.

A hot spell is more damaging to peas than a light frost. Peas can be grown successfully during midsummer and early fall in those areas having relatively low temperatures and a good rainfall, or where irrigation is practiced. For very early crops, a sandy loam is preferred, however, for large yields where earliness is not a factor, a well-drained clay loam or silt loam is preferred [Duke, 1981].

The harvesting of snow peas is the most critical and costly part of farming period. The consumer requires the pods to be of maximum size but without any development of the seeds. The pods must be completely flat, as at this stage they contain no cross-fibres and the whole pod can be eaten. Under most conditions this will necessitate harvesting every 3 days.

In Kenya, snow peas production is a relatively recent introduction which is growing in importance due to its demand and value in the export market mainly in Europe. Snow peas do well in the upper and lower highland zones at altitude between 1,500 and 2,600m. Snow peas prefer cool temperature between 12 and 20°C for cultivation and humid areas with well distributed rainfall of 1500 to 2100mm per annum. Well drained soils rich in organic matter are

suitable for snow peas production. Silty clay loam soils are ideal and optimum soil pH range is 5-7. In Kenya the suitable areas for snow peas production includes Kiambu, Meru (Timau area), upper Murang'a, Nyeri, Taita Taveta, Mt. Elgon, Bomet, Machakos, Koibatek (Timboroa) Kericho, and upper Kisii districts.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the Study Area

The fate of chlorothalonil in snowpeas was conducted in Nairobi, Kenya under green house conditions in 2008. Nairobis' geographical coordinates are 1° 17′ 0" south, 36° 49′ 0" east. Its 1,661m above sea level and has four main seasons in a year namely; Warm, sunny, dry season (mid-December to Mid-March), main rainy season (mid- March to May), cool rather cloudy, dry season (June to mid-October) and secondary rainy season (mid-October to Mid-December). In this study, snow peas, french beans and passion fruits were bought from two municipal markets (Githurai and Kangemi) located in the outskirts of the city and two supermarkets located within the Central Business District (CBD).

The green house was located at the College of Physical and Biological Sciences within the school of Biological Sciences, Chiromo Campus. The mean temperature of the green house during the study period was 24.9°C.

3.2 Materials

3.2.1 Chemicals Used

Chlorothalonil analytical standard used for identification and quantification of residues ; PCB 155 and PCB 18 analytical standards used as internal standard and syringe standards respectively, were of high purity (above 98%), and were obatained from Dr. Ehrenstorfer. HPLC and Pesticide residue grade dichloromethane and hexane, and anhydrous sodium sulphate analar were obtained from Pyrex East Africa. While the acetone, isoocatane, neutral alumina

(Aluminium oxide) and general purpose reagent grade acetone were obtained from Fabrics and supplies. Activated charcoal and mercuric chloride were obtained from Merck.

3.2.2 Equipments and Apparatus Used

Sampling of soil core was done using stainless steel core samplers and rings of diameter 5cm and a depth of 5cm provided by NARL, while soil samples for fertility evaluation was sampled using a sampling auger provided by KEPHIS. Separating funnels, beakers and volumetric flasks made of pyrex glass, one litre polyvinyl pots and watering trays, self sealing polythene and khaki sampling bags, gloves, 2ml agilent sample vials and caps, and spatulas were provided by KEPHIS. Analytical balances type A-160 from Fisher, Mettlor toledo model AG245 and Adam model AFP-2100LC were used for weight measurements. Gallenkamp oven model OV-160 was used for moisture content determinations and glassware drying. Horbart food processor and stainless steel blender used in chopping and homogenising samples were also provided by KEPHIS. Soxhlet extraction apparatus were used for the extraction whereas Buchii RE 111 rotary evaporator was used for concentrating sample extracts.

Agilent 6890N and Varian chromapack-3800 gas chromatographs equiped with a mass selective and Electron capture detectors respectively were used for identification and quantification of chlorothalonil residues.

3.2.3 Test Soil and Water

Soil for the study was obtained from a farm located in Magumu location of South Kinangop division, Nyandarua district of Central province, a high potential agricultural zone while bore hole water available in the green house was used to irrigate plants.

3.3 Methodology

3.3.1 Green House Experiments

Determination of Moisture Content

Approximately 1g portion of the test soil and every soil sample analyzed was weighed into a pre-weighed crucible and contents placed in the oven at 110 ^{O}C for 24 hours. The crucible was then placed in a desiccator to cool to room temperature. The crucible and its contents were re-weighed and the loss in weight attributed to moisture content of the sample.

Fate of Chlorothalonil in Soil

Based on the moisture content of the test soil, the mass of soil used to set up the experiment was determined from the equation: MT1= MSØW + MS (where, MT1 is the total mass of the soil at field capacity, MS is mass of oven dry soil, ØW is the percentage of water content). Using the same relationship the mass of the soil at field capacity was established using the equation: MT2= MSØFC + MS where, ØFC is the water content at field capacity. The difference between MT2 and MT1 is the amount of water added to the soil to have the water content at field capacity.

Approximately 700g of test soil at field condition was weighed into eleven one litre pots to form eleven pots for chlorothalonil dissipation study in soil (equivalent to 661.29 g of oven dry soil). Three replicated pots for dissipation on soil with snow peas plants, the tenth for soil without plants and the final one on sterile soil. The soil was sterilised using mercuric chloride. 243.46 ml of borehole water was added to the soil to adjust its water content to field capacity. The soils were maintained at field capacity during growth of snow peas. The pots for chlorothalonil dissipation in soil were treated with chlorothalonil (Daconil 720g/l) at the rate indicated on the

instruction label, 2 litres per hectare equivalent to 1.44 kg a.i /ha. The soil treatment spray mixture was analysed to give the initial dose applied to the soil.

Fate of Chlorothalonil in Snow peas

Additional three replicate one litre pots of soil were prepared and moisture content of soil adjusted to field capacity as for the fate study on soil above, to form nine pots for chlorothalonil dissipation study on snow peas growing on untreated soil. The three replicated pots for chlorothalonil dissipation on soil with snow peas plants were used to study the fate of chlorothalonil on snow peas growing on treated soil.

Diammonium phosphate (DAP) fertilizer was applied at the recommended rate of 50kg per acre which translated to 0.1901 g DAP. Six seeds were sown in each pot. The plants started germinating on the sixth day and germination was complete by the ninth day. Thinning of weak plants was carried out on the twentieth day leaving only three plants per pot. When the plants were 49 days old they were treated with chlorothalonil (Daconil 720g/l) at the label application rate by use of a hand sprayer. The plants growing on treated soil were sampled before spraying. The temperature of the green house was monitored throughout the experiment.

3.3.2 Assessment of Chlorothalonil Residues

Residues of chlorothalonil in snow peas, French beans and passion fruits marketed in Kangemi and Githurai municipal markets and two selected leading supermarkets in Nairobi were assessed. The choice of Githurai and Kangemi markets was to be able to capture produce origination from the central and upper eastern regions and the rift valley and kinangop regions respectively. Sampling from supermarkets was to capture produce that does not meet grading requirements for export market. These commodities were considered for the survey due to their importance to

Kenya as export commodities and increasing popularity among the Kenyan population as production grows to meet export volumes.

3.3.3 Sampling and Sample Preparation

Test Soil

Soil samples for physical analysis were sampled using core samplers and ring to obtain undisturbed soil. A sample consisted of two cores of undisturbed soil rings taken at 0-15 cm for the top soil and 15-30 cm for the sub soil. Upon collection the core rings were covered and transported to the laboratory for analysis.

Samples for fertility evaluation and fertilizer recommendation were taken with the aid of a sampling auger up to a depth of 30 cm. The sample was then placed in a khaki bag and transported to the laboratory for analysis.

Green House Sampling

Sampling of the soil was done an hour before treatment with chlorothalonil and an hour after treatment. Soil sampling was done on the day of spraying representing day zero and there after seven, fourteen and twenty one days following spraying giving a total of sixteen samples. The samples were wrapped in aluminum foil and placed in a self sealing polythene bag and transported to the laboratory.

The initial sampling for the plants was carried out an hour before treatment and an hour after spraying. Sampling of the plants was done by cutting the leaves at the petiole with a pair of scissors. This was done for day zero, seven, and day fourteen after spraying. Day twenty one, twenty eight and thirty five incorporated picking the green peas since they were ready for harvesting. Samples were wrapped in aluminum foil and placed in a self sealing polythene bag

and transported to the laboratory. A total of eighteen samples in replicates were taken giving a total of fifty four samples.

Once in the laboratory, sample preparation started immediately where possible. In the event that sample preparation could not start immediately, samples were stored in a deep freezer at -20 ^{0}C to maintain their integrity.

Market Sampling

Sampling of French beans, passion fruits and snow peas from the open-air markets and super markets in Nairobi was by buying the commodity just like a customer would. Samples comprised of one kilogramme of the commodity. Sampling was carried out in January, March and July corresponding to the different seasons described in section 3.1. A total of thirty two samples were collected: ten snow peas, twelve French beans and ten passion fruits. Some of the commodities were however missing in the markets and super markets at the time of sampling particularly snow peas and passion fruit during the warm and dry, and cool and dry seasons.

Sample Processing

The laboratory samples of fruits and vegetables were homogenized using Hobart food processor. The homogenized wet analytical sample (10g) was placed in a mortar. For every gram of wet sample, 3g of baked out anhydrous Na_2SO_4 was added. This mixture was ground using a pestle to a homogeneous powder. This was covered with aluminum foil and left overnight to dry further.

10g of each soil sample was weighed into a beaker and baked out Na_2SO_4 added in the ratio of 1g of sample to 2 g of Na_2SO_4. The contents were stirred to obtain dry mixture, covered with aluminum foil and left overnight to dry further.

3.3.4 Glassware Cleaning

All glassware for sampling, extraction and clean up was thoroughly cleaned by soaking in soapy water for at least an hour followed by cleaning then rinsed with clean tap water followed by distilled water then with redistilled acetone. The glassware was then oven dried at 105^0C while calibrated glassware like pipettes were air dried to minimize loss of calibration that may arise due to expansion and contraction during oven drying and subsequent cooling.

3.3.5 Preparation of Reagents and Analytical Standards

Preparation of Drying Agent

The drying agent Na_2SO_4 was prepared by baking approx. 3g of Na_2SO_4 per 1g of sample for 16 hours at 400 ^{0}C to remove contaminants like phthalates.

Drying and Activating Column Materials

Aluminum oxide, Al_2O_3 (15g per sample) was dried overnight at 200 ^{0}C to make it 100% active (remove all water). It was then deactivated with water (8% w/w) by adding 16 ml of HPLC grade water to 184 g of activated Al_2O_3 in a 250 ml round bottomed flask and shaking by hand until all lumps were gone. The round bottomed flask was put on a shaking table for half an hour then left overnight to condition.

Purification of Analytical Reagents

All reagents that were not HPLC or Pesticides residue grade were purified by triple glass distillation. The resultant distillate was tested for purity by concentrating the reagent ten times then reconstituting in 1 ml pesticide residue grade isooctane. The reconstituted isooctane extract was subjected to GC analysis to detect any pesticide contaminant

Preparation of Analytical Standards

Stock solutions of analytical standards were prepared from high purity analytical standard reference materials. The amount of analytical standard required to prepare stock solutions was calculated using the formula: $C = (M \times P) / V$ where C is the concentration of the stock solution being prepared, M is the mass to be taken, P is the purity of the analytical standard as a fraction and V is the final volume of the stock solution. The stock solutions were prepared in isooctane.

Calibration curve series of standards was prepared from the stock solution. The series consisted of eight calibration levels, which were obtained by diluting the stock solution. The dilutions were based on the formula: $C_1V_1 = C_2V_2$ where, C_1 is the concentration of the stock solution, V_1 is the volume of stock solution to be taken; C_2 is the concentration of the standard to be prepared and V_2 is the final volume of standard to be prepared. The concentrations are calculated by exactly weighing the stock solution and isooctane additions. This was achieved by weighing the empty flask together with its cork, transferring the required volume of standard the flask then weighing flask containing the standard followed by topping up with the appropriate solvent and a final weighing to obtain the weight of solvent. The weight of the entire contents was also recorded for monitoring evaporation of the standards.

3.3.6 Analytical Methods

Physicochemical Parameters of Soil

The test soil was analysed for chlorothalonil and both physical and chemical parameters to obtain baseline information. Soil analyses were carried out using methods described in the physical and chemical methods of soil analysis [MoANAL, 1980]. Soil analysis involved physical and chemical analyses. The physical analyses included texture analysis also called mechanical or

particle size analysis by pipette method, complete moisture retention (pF) where undisturbed soil core samples were subjected to negative pressure suction (kaolin box apparatus) and positive external gas pressure (pressure cooker) to remove water, and Bulk density determined according to the core method where the double cylinder core samplers were used, porosity and particle specific density. The double cylinder core samplers used were 5cm diameter by 5cm height.

The chemical analysis included pH determined in a 1:1(w/v) soil-water suspension with a pH meter, total organic carbon using the calorimetric method, where all organic carbon in the soil sample was oxidised by a 15 ml mixture of concentrated sulphuric acid and 5% potassium dichromate in 1:2 ratio v/v at 150°C for thirty minutes to ensure complete oxidation. Barium chloride is added to the cool digest, mixed thoroughly and allowed to stand overnight. The amount of organic carbon in sample is determined by concentration of chromic ions (Cr^{3+}) produced after oxidation spectrophotometrically at 600nm.

Total Nitrogen was also analysed using the Kjeldhal method where organic nitrogen compounds in soil are digested with concentrated sulphuric acid and selenium mixture as a catalyst to convert Nitrogen to ammonium sulphate. The digest was made alkaline with sodium hydroxide and the released ammonia distilled off and collected in boric acid indicator solution and titrated against standard acid.

Other available nutrients like phosphorous, magnesium, manganese, calcium potassium and sodium were analysed using Mehlic 1 (double acid) method. Oven dry soil was extracted in 1:5 ratios (w/v) with a mixture of 0.1M hydrochloric acid and 0.5M sulphuric acid; where the acid replaces the bulk exchangeable metal cations and the sulphate anion is exchanged for phosphate.

The P, Mg and Mn were determined calorimetrically while Ca, K and Na were determined by flame photometry.

Soluble salts were determined by electrical conductivity while the available trace elements (Iron, Zinc and copper) were analysed by extracting oven dry soil with 0.1 M HCl in a 1:10 ratio (w/v) then elements determined with atomic absorption spectrophotometer (AAS)

Physicochemical Parameters of Water

The water used for irrigating the plants was analysed for irrigation suitability. Analysis involved testing for its pH and electrical conductivity (EC), soluble salts of sodium, potassium, calcium, magnesium, carbonates, bicarbonates chlorides and sulphates; as a guide to its suitability for irrigation.

The pH and EC are determined by direct measurement using a pH and electrical conductivity meter respectively. Sodium and potassium contents were determined directly by use of the flame emission spectroscopy while chlorides were determined by use of a chloride meter. Sulphates concentration was determined by the barium chloride method where sulphates are precipitated as barium sulphate. Carbonates and bicarbonates were determined titrimetically by use of 0.02M sulphuric acid with phenolphthalein and methyl red indicators respectively. Calcium and Magnesium were analysed by titrating their salts with EDTA using Eriochrome black T as the indicator; then determined by Atomic absorption spectroscopy.

Determination of Chlorothalonil

The methodology used in analysis of chlorothalonil involved: homogenization of samples, extraction of samples with a suitable organic solvent, clean up of the solvent extract using column chromatographic techniques and analysis of the cleaned up extract with capillary GC

with ECD detector. Confirmatory analysis was performed using GC-MS.

Quantitative determination was made by comparison with external standards and use of an eight-point calibration curve of standards. The results for the soil samples were based on dry matter weight while the fruits and vegetable samples were based of fresh weight. Internal standard PCB 155, syringe standard PCB 18 and spiked samples were included for quality control of the analysis.

3.3.7 Sample Extraction

The dry sample obtained after processing section 3.3.3 was transferred to a soxhlet thimble and 100µl of 1ppm PCB 155 solution added as an internal standard. 130 ml of hexane: acetone (3:1v/v) was added to a 500 ml round bottomed flask. Anti-bumping chips were added to allow smooth boiling. The Soxhlet apparatus were set up and extraction allowed to proceed for 16 hours.

To the extract, 2ml of isooctane was added as a keeper and then the extract was rotary evaporated to 3ml. The extract was quantitatively transferred to a tube and then evaporated with a gentle stream of nitrogen to 1 ml.

3.3.8 Extract Clean - up

Anhydrous Na_2SO_4 was packed in appropriate clean up column (with frit) to 1cm height (equivalent to 2g) and 15 g of Al_2O_3 followed by 0.05g of activated charcoal and another 1 cm of Na_2SO_4. After every addition, the column was tapped to allow the particles to settle uniformly. The column was then conditioned by eluting with 15 ml of hexane.

The Soxhlet extract of the sample was transferred quantitatively to the column. The tube was further rinsed with five 1 ml portions of hexane and the rinsing transferred to the column as soon

as the sample extract had eluted without leaving the column to dry completely. The column was then eluted with 100ml of hexane: dichloromethane (1:1) mixture. The eluate was collected in a 250 ml round bottomed flask. 2ml of isooctane was added to the resulting eluate to act as a keeper and the combined eluants rotary - evaporated to 3 ml. The extract was transferred to a GC sample vial and further concentrated to 1 ml using a gentle stream of nitrogen. 100 µl of 1 ppm PCB 18 was added as an injection check standard. The extract was now ready for analysis by GC- MS

3.3.9 Gas Chromatograph-Mass Spectrometer (GC-MS) Analysis

An Agilent GC-system 6890N, Inert Selective Mass Detector (MS) –system 5975, and an Agilent split/splitless injector 7683 B series were used for qualification and quantification of chlorothalonil in the sample extracts. The GC-MS was run using Helium as the carrier gas while injections were made on the splitless mode.

3.3.10 GC System Conditions

The GC column used was a Dimethyl phenyl silicone DB 1701, with a flow of 1.2ml/minute equivalent to a velocity of 42 cm/sec and a pressure of 17.7 psi on the constant flow mode. The inlet (injector) temperature was set at 270 ^{0}C while the oven was set at an initial temperature of 90 ^{0}C for 2 minutes followed by a temperature rise at the rate of 15^{0}C per minute up to 165 ^{0}C for the first ramp. A second ramp of 2 ^{0}C per minute to a final temperature of 250 ^{0}C was set. After the second ramp the temperature was held for 1 minute bringing the run time to 50.50 minutes.

3.3.11 MS System Conditions

The MS Quadruple temperature was set at 150 ^{0}C while the MS ionic source (electron impact) at 230 ^{0}C the auxiliary temperature was set at 295 ^{0}C. The samples were run on a Single Ion Mode (SIM) where the following Ions were targeted; Chlorothalonil target ion mass 266 and qualifier ion 268. For PCB 155 the target ion mass was 360 and qualifier 362,while for PCB 18 the target ion mass was 256 and qualifier 258,

Upon ensuring that the correct method was loaded on the GC, a test injection was performed by injecting level one standard (0.12 mg/kg). The response of all the compounds was compared to the response of the same level injected in the multi-level calibration of the last sequence. If both were comparable then a new sequence would be started for the current analysis.

3.3.12 Injection

The order of injection was as follows: first a solvent blank run (isooctane) followed by calibrating standards starting with most diluted standard to the most concentrated and then blank (isooctane). The isooctane run was followed by the sample extracts starting with the blank samples, then reference and the real samples and finally the calibration standards. The last injection was the mid-calibration curve standard.

3.4 Quality Assurance and Quality Control

Control pots for both fate of chlorothalonil in soil and snow peas were set alongside the experimental pots. The control pots were sufficiently separated to exclude any contamination from the treated pots.

Analysis of controls was carried out alongside the samples to ascertain that no artefact in the crop derived from local conditions gave rise to interference in the analysis and to establish the

transport and storage stability of any residue. Controls were fortified with chlorothalonil at known concentrations (1.0, 2.5 and 5.0mg/kg) to establish the recovery level of the pesticide from the crop by the analytical method.

3.5 Statistical Data Analysis

The results were analysed using excel programme. Calibration standard series used were evaluated for within laboratory reproducibility acceptability using the Horwitz equation CV. Results are presented as mean of triplicate analyses with standard deviation.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Physicochemical Characteristics of Test Soil and Water

The soil used in this study was ranked to be 75 % clay, 8.1 % sand, and 16.9 % silt. The soil had a bulk density of 0.72g/cc. It was slightly acidic with a pH of 6.23. The percent total nitrogen was 0.42 while the organic carbon was 3.57 %. All macro elements potassium, calcium, magnesium and sodium were adequate except phosphorous which was low at 28ppm based on the Mehlich double acid method (Mehlich1) whose critical level for phosphorous is 30 ppm. The micro-elements copper, iron and manganese were adequate while zinc was low at 4.00ppm. The saturation point of the soil at PF 0 was 84.48% while the field capacity point of the soil at PF 2.0 and 2.3 were 62.17% and 42.67%, respectively. The permanent wilting point of the soil at PF 4.2 was 20.45%. The average moisture content of the soil sample was 2.38 %. Table A1 in the appendix resents physicochemical characteristic of the test soil.

Borehole water was used to irrigate the plants. The water had a p H of 8.79, sodium content of 4.15 m.e per litre, chlorides content of 5.738 m.e per litre, conductivity of 336 µs/cm (micro Siemens per centimeter) and a sodium adsorption ratio (SAR) of 15.63 making it a medium salinity-medium sodium water. Contents of other elements were: potassium 0.12m.e per litre, calcium and magnesium 0.001 m.e per litre, bicarbonates 0.098 m.e per litre and sulphates 0.023 m.e per litre. A summary of the chemical characteristics of the water are presented on Table A2 in the appendix.

4.2 Chlorothalonil Analytical Method Performance

4.2.1 Method Accuracy

Recovery data for the method were generated by fortifying blank samples with 100 µl of 1 mg/kg chlorothalonil stock solution. These were then analysed to establish the recovery of the analyte. The cleanup stage was optimised to obtain recovery of 96.38% for an elution with 200mls Hexane, 103.22% for the elution with 100ml of 1:1 mixture of dichloromethane: hexane and 115.50 % for the elution with 200ml of 1:1 mixture of dichloromethane: hexane. 100mls of dichloromethane: hexane mixture was therefore used as the clean up mixture.

Samples of passion fruit, French beans and snow peas were spiked with chlorothalonil at different concentrations and the percentage recoveries calculated. Figure 4.1 presents TIC chromatogram of a spiked sample extract. The recoveries of chlorothalonil in the three matrices ranged between 70% and 120% Table 4.1

Table 4.1: Method Recovery data in % for Chlorothalonil Residues

Commodity	1.0mg/Kg	2.5mg/Kg	5.0 mg/Kg
Snow peas	115.06	81.39	88.98
French beans	101.97	107.97	64.57
Passion fruit	108.02	98.86	96.45

The data on reveries was tested for outliers using Dixon Q test. The Q calculated was less than Q critical value at 95% confidence level. Thus none of the reported recoveries is an outlier. Table A6 in the appendix presents Dixon Q-test for chlorothalonil recovery data in the three matrices.

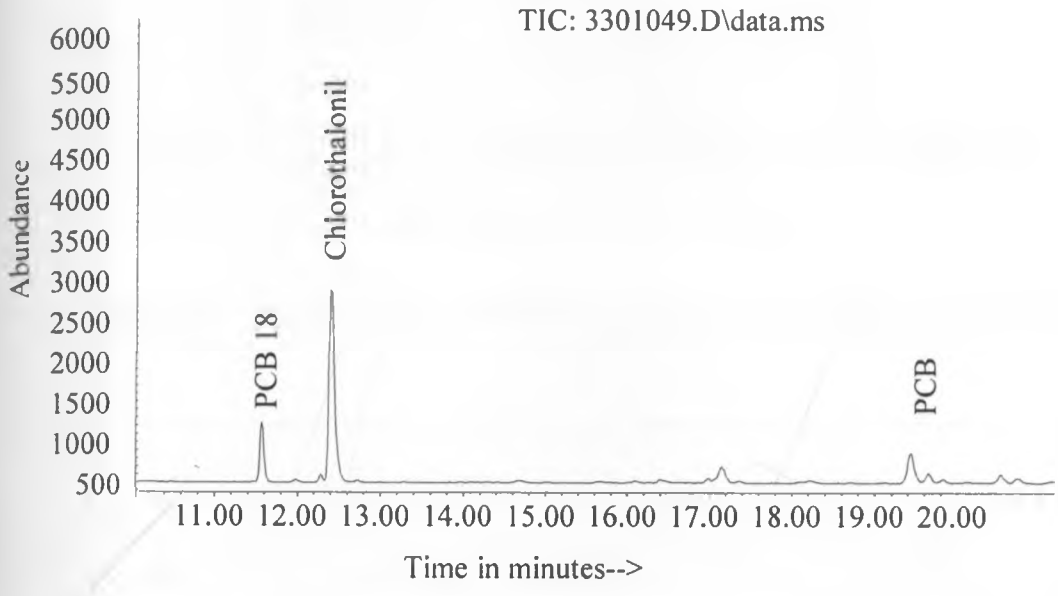


Figure 4.1: Chromatogram of a Spiked Sample Extract

4.2.2 Method Precision

Relative standard deviations and reproducibility CVs for the calibration standards used to qualify and quantify chlorothalonil are as shown in Table 4.2. Figures 4.2, 4.3 and 4.4 present chromatogram of lowest calibration standard (0.005mg/kg), calibration curve for chlorothalonil and ionic mass spectra for chlorothalonil respectively.

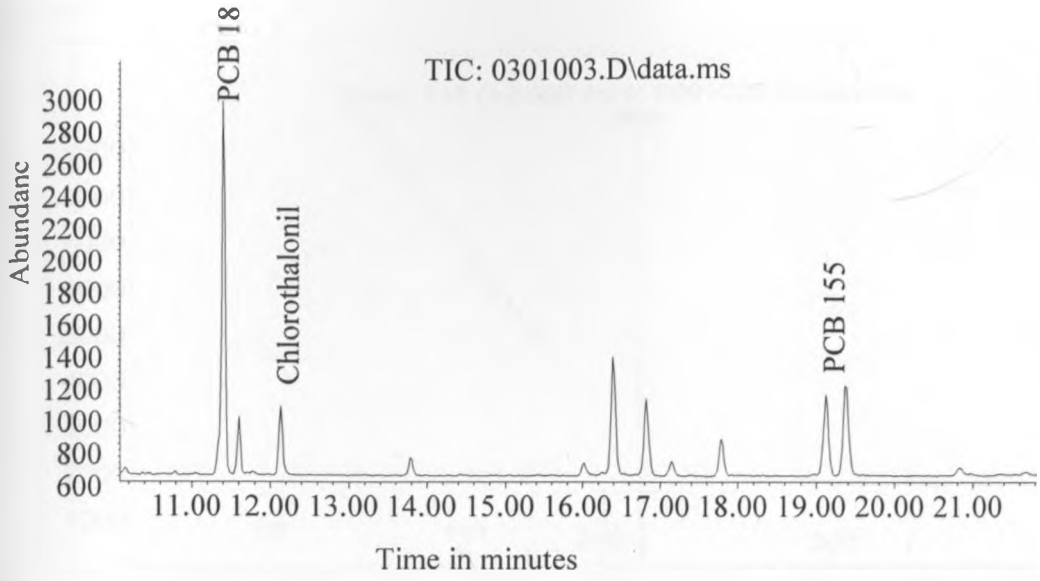


Figure 4.2: Chromatogram of Chlorothalonil Lowest Calibration Standard

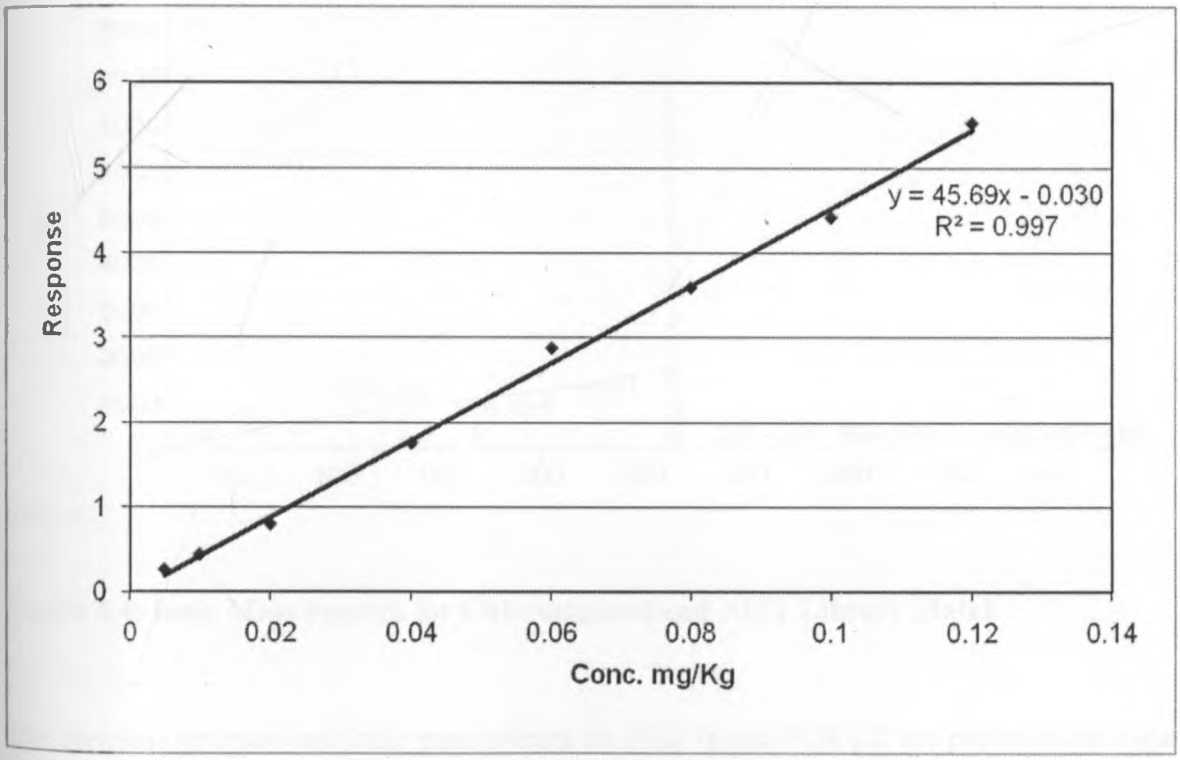


Figure 4.3: Calibration Curve for Chlorothalonil

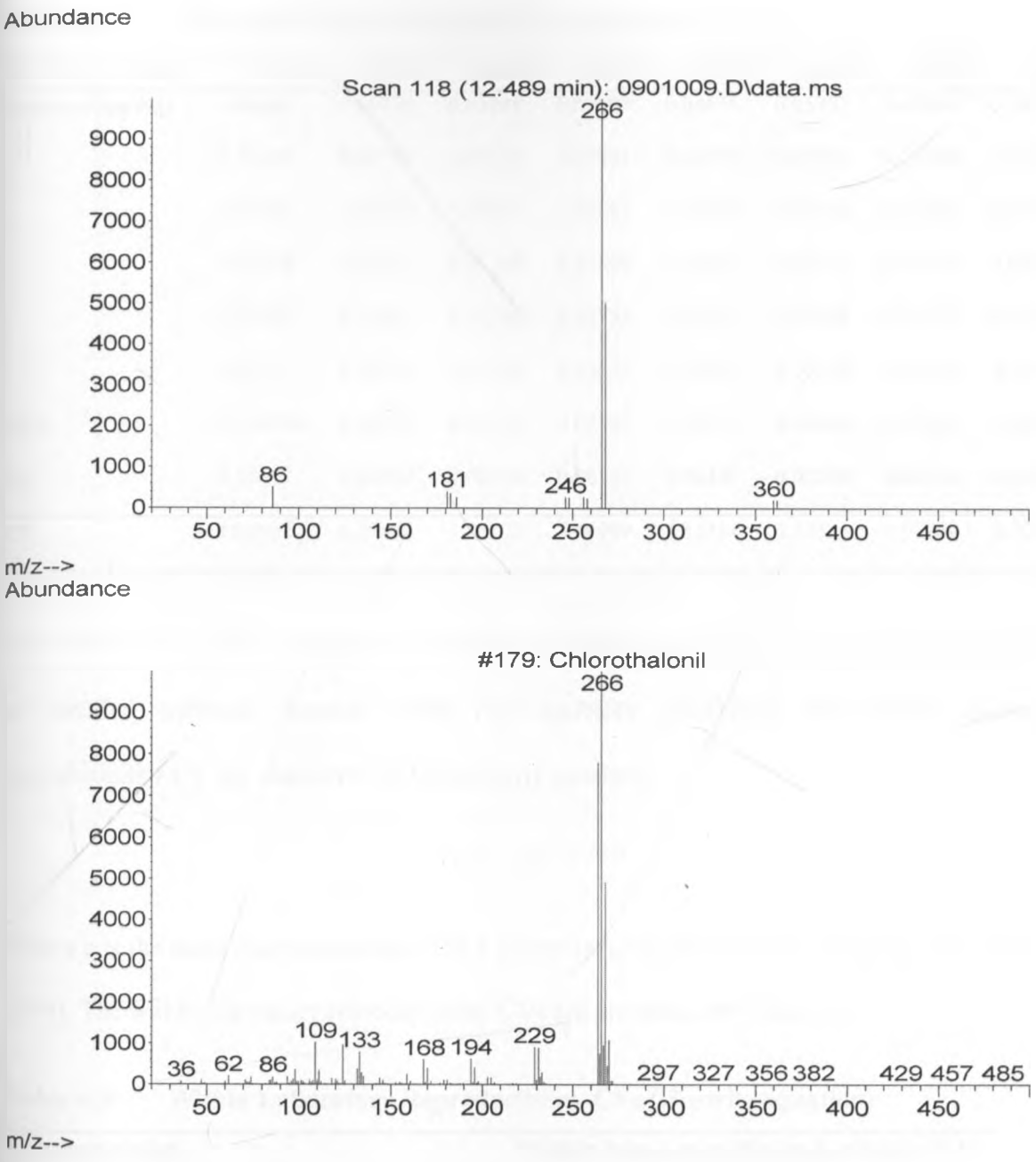


Figure 4.4: Ionic Mass Spectra for Chlorothalonil and NIST Library Match

The chemical structure and ionic mass spectra for PCB 18 and PCB 155 are presented on Figures A1 and A2 respectively of the Appendix.

Table 4.2: Calibration Curve Standards Mean, SD and CV.

Std Conc. (mg/Kg)	0.004	0.007	0.015	0.031	0.049	0.068	0.090	0.112
Response(mg/Kg)	0.00486	0.00776	0.01288	0.02697	0.04656	0.05707	0.07009	0.08829
	0.00486	0.00771	0.01327	0.02901	0.04719	0.05936	0.07239	0.09064
	0.00495	0.00744	0.01291	0.02687	0.04678	0.05726	0.07042	0.08842
	0.00428	0.00761	0.01328	0.02889	0.04663	0.06262	0.07194	0.08917
	0.00582	0.0084	0.01349	0.02753	0.04561	0.05899	0.07324	0.09133
	0.0035	0.00678	0.01229	0.02652	0.04204	0.05758	0.07349	0.09418
Mean	0.004954	0.00778	0.01316	0.02785	0.04655	0.05906	0.07161	0.08957
SD	0.00077	0.00052	0.00042	0.00107	0.00191	0.00208	0.00141	0.00223
CV	15.59320	6.73483	3.25023	3.84909	4.11311	3.535	1.97739	2.50011

The obtained CV for the calibration standards are within the acceptable range for repeat analyses of certified reference material under reproducibility conditions. The Within laboratory reproducibility CV are calculated by the Horwrtz equation:

$$CV = 2^{(1-0.5\text{Log } c)}$$

Where c is the mass fraction expressed as a power (exponent) of 10 (e.g. 1mg/kg $=10^{-3}$) [Nollet, 2004]. The within laboratory reproducibility CVs are presented on Table 4.3.

Table 4.3: Within Laboratory Reproducibility CVs (Horwitz equation)

Content(mg/kg)	Within laboratory Reproducibility C.V
0.001	45*
0.010	32*
0.100	23
1.000	16

***As low as possible: In practice <22%**

The standard deviation for the sample triplicates was calculated and ranged between ±0.0012 and ±14.5393 µg/Kg for the degradation samples, ±0.0098 and ±2.1637 µg/Kg for the market survey samples, and ±0.428 and ±2.2393 µg/Kg for the calibration curve standards.

4.2.3 Limit of Detection and Limit of Quantitation

Limit of detection (LOD) and Limit of Quantitation (LOQ) were established by injecting standards with known low concentrations. A comparison of the signals from known low concentration standard with those arising from baseline noise was done. The LOD was determined to be the region where the signal to noise ratio S/N ratio was 3:1 while the LOQ was determined to be the region where the S/N ratio 10:1. The experimental LOD was established to be 3.77µg/kg while the LOQ was established to be 12.55µg/kg equivalent to 0.004 and 0.013mg/Kg respectively. The results for the residues assessment are presented in µg/kg since they were detected at very low concentrations.

4.3 Fate of Chlorothalonil in soil

The soil spraying mixture contained 454.60 mg/Kg Chlorothalonil. While the results based on dry weight for the dissipation of chlorothalonil in soil are presented on Table 4.4. Figure 4.5 represents chromatogram of chlorothalonil residues in soil. Table A3 in the appendix presents moisture content of analysed samples.

Table 4.4: Results of Chlorothalonil Dissipation in Soil in mg/kg

Sample name	Day 0	Day 7	Day 14	Day 21
Control	<LOD	<LOD	<LOD	<LOD
Sterile soil without plants	15.478	7.783	6.009	8.142
Treated soil with plants	21.561	11.627	7.935	9.129
Treated soil without plants	12.061	0.756	2.393	0.583

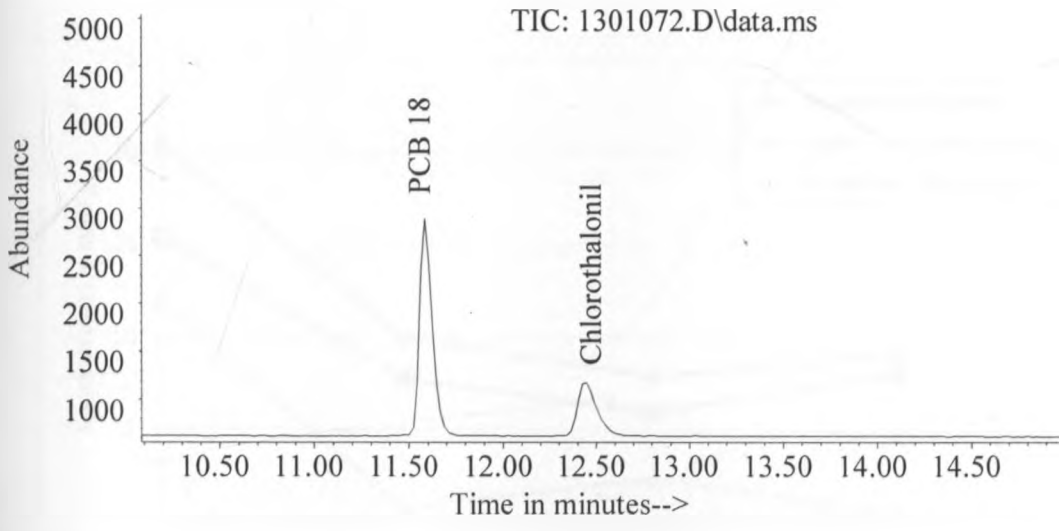


Figure 4.5: Chromatogram of Chlorothalonil Residues in Treated Soil on Day 21

The trend of dissipation (Figure 4.6) was obtained by plotting determined concentration of Chlorothalonil versus time in days for sterile soil without plants, non sterile soil without plants and non sterile soil with plants. This study was conducted to establish whether application of chlorothalonil on crops growing on chlorothalonil treated soil result in elevated residue. The

trend shows a relatively similar degradation pattern of Chlorothalonil in sterile soil without plant compared to non sterile soil with plants implying microbial degradation is minimal. Eeden *et al* [2000] reported that chlorothalonil will probably not accumulate to harmful levels in sandy soil, due to utilization of chlorothalonil by indigenous aerobic soil bacteria. In red and clay soils, however, chlorothalonil may remain present in the soil environment for weeks following its application, because certain physical and chemical properties increase the risk for its accumulation. The concentration of Chlorothalonil was slightly higher in the soil with plants compared to soil without plants and this could be attributed to high moisture content in the soil without plants since the pots were given same amount of water.

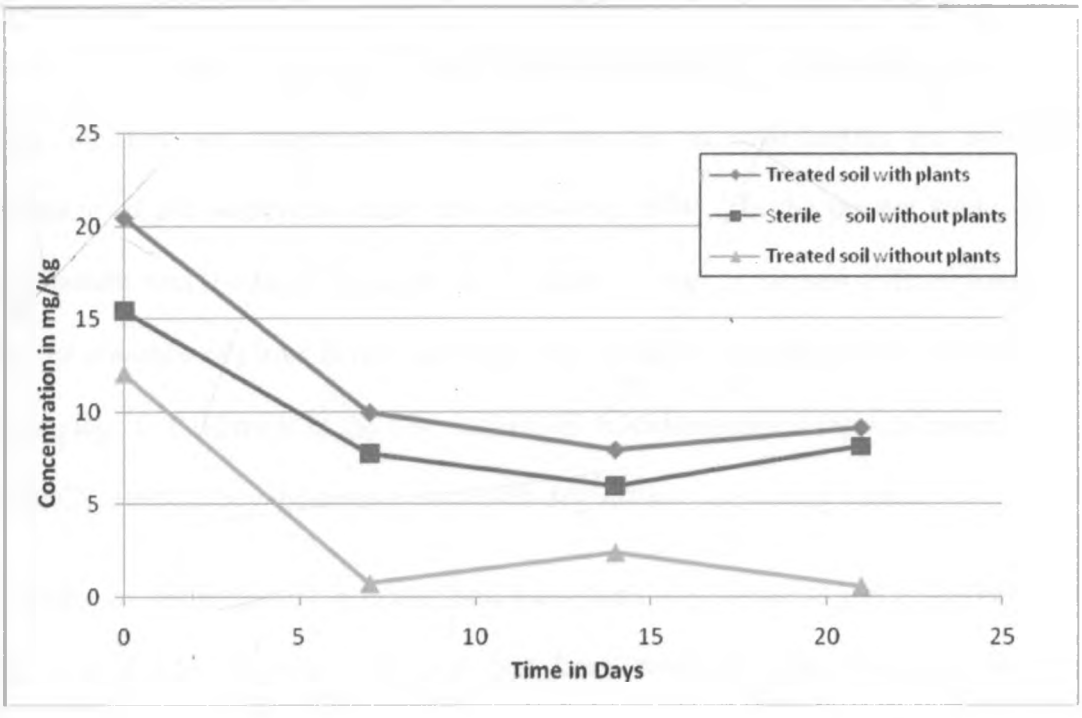


Figure 4.6: Trends of Chlorothalonil Dissipation in Clay Soil

Using the data obtain (Table 4.4), the half-life ($t_{1/2}$) of chlorothalonil was calculated through regression analysis assuming the loss of chlorothalonil follows a first order kinetic thus;

$$\ln (C/C_o) = -kt \ldots\ldots \text{(Equation 1)}$$

Where C is concentration of chlorothalonil at a time t, C_o is concentration of chlorothalonil at time t = 0 and k is the first order rate constant, which depends on water activity.

$$\text{From equation 1 above, } t_{1/2} = -(1/k) \ln 0.5 \ldots\ldots \text{(Equation 2)}$$

The half- life of chlorothalonil in soil with plants, sterile soil without plants and soil without plants was found to be 10.34, 10.19 and 6.03 days respectively. This is within reported ranges from previous studies in which laboratory experiments, reported a half- life ranging from 4 to 40 days in various types of soil. The degradation rate increased with increasing organic matter content, moisture and temperature with little loss due to volatilization but appeared to be independent of pH within the range of 6-8 [JMPR, 1974]. In the present study, the organic matter content was the same however; the moisture content of the soil differed from pot to pot due to the presence of plants in one pot. Table 5 in the appendix presents the moisture content of soil samples. In field trials in the US, the half-life of chlorothalonil residues ranged from 26-45 days [IPCS, 1996]. They indicated a decreased degradation rate during winter.

The decline of chlorothalonil in treated soil with plants was similar to that of sterile soil without plants (Table 4.5). Although the soil had been sterilised using mercuric chloride at a concentration of 500mg/kg, the irrigation water was not sterilised. This led to reintroduction of microorganisms into the soil, explaining the similarity in trend of decline to the soil with plants (Figure 4.7).

Table 4.5: Decline of Chlorothalonil Residues in Soil mg/kg

Day	Treated soil with plants (ST)	Sterile soil without plants (SST)	Treated soil without plants (SCA)
0	20.47	15.48	12.06
7	9.96	7.78	0.76
14	7.93	6.01	2.39
21	9.13	8.14	0.58

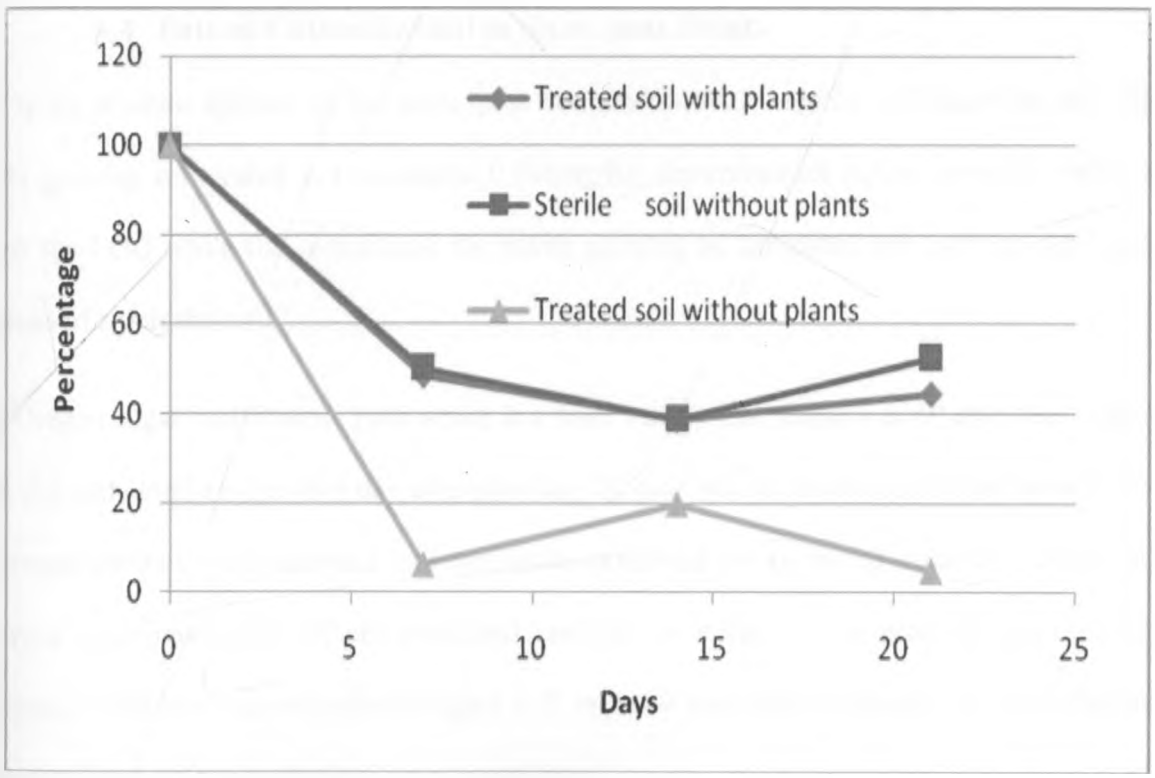


Figure 4.7: Decline of Chlorothalonil in Clay Soil

The decline of chlorothalonil in soil without plants was more rapid compared to the other two.

This could be attributed to the high moisture content of the soil since all pots received equal amounts of water, and for this pot there were no plants to utilise the water.

Laboratory experiments reported by IPCS for five soil types' representatives demonstrated that the half-life values for the hydroxyl metabolite ranged from 36 days in sandy loam soil type to 220 days in clay type soil. It has been shown that bacteria isolated from soil are capable of metabolizing chlorothalonil in culture media. It can be deduced that soil microorganisms play a role in the rapid degradation of chlorothalonil in soil [Duane, 1970].

4.4 Fate of Chlorothalonil in Snow peas Plants

The spray mixture applied to the snow peas contained 454.60 mg/Kg of Chlorothalonil. The plants growing on treated soil contained 0.006mg/Kg chlorothalonil before spraying which is below the LOQ while the control and the plants growing on untreated soil had non detectable residues of chlorothalonil.

The Oregon sugar pod II snow peas which is a bush variety that matures in 60 days was treated with chlorothalonil on the 49th day after planting, 21 days before the expected first harvest day. Collected samples were analysed and the results presented on Table 4.6 obtained. Figure 4.8 presents a chromatogram of chlorothalonil residues in treated snow peas on day 21. The dissipation trends of chlorothalonil (Figure 4.9) in snow peas plants growing on chlorothalonil treated soil and on non treated soil were established.

Table 4.6: Decline of Chlorothalonil Residues in Snow peas mg/kg

Day	Both Soil and Plants Treated	Treated plants	Untreated plants (control)
0	29.651	26.363	<LOD
7	19.197	7.238	<LOD
14	3.060	0.939	0.009
21	0.022	0.003	<LOD
28	0.009	<LOD	<LOD

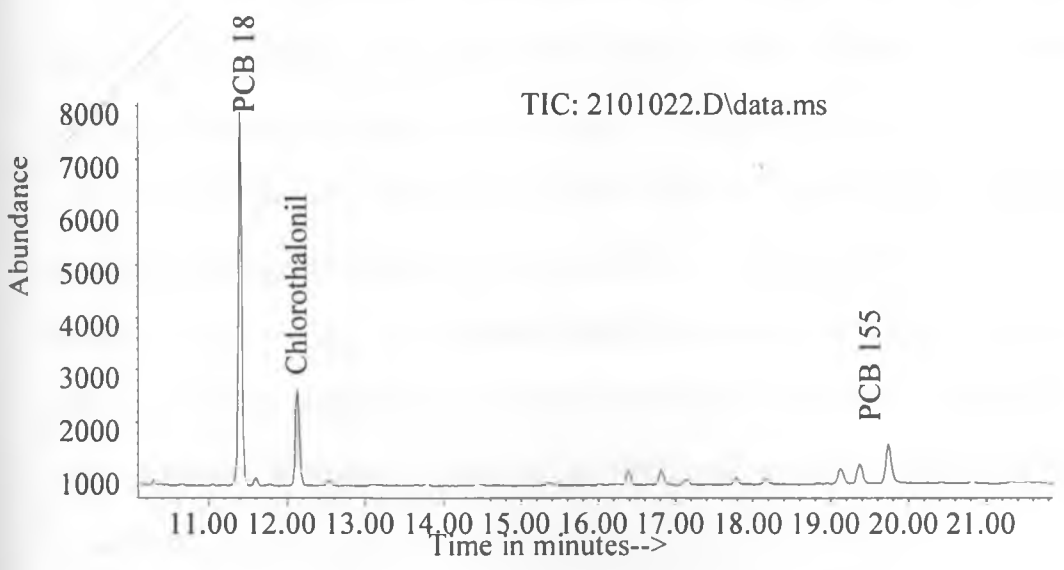


Figure 4.8: Chromatogram of Chlorothalonil Residues in Treated Snow peas on Day 21

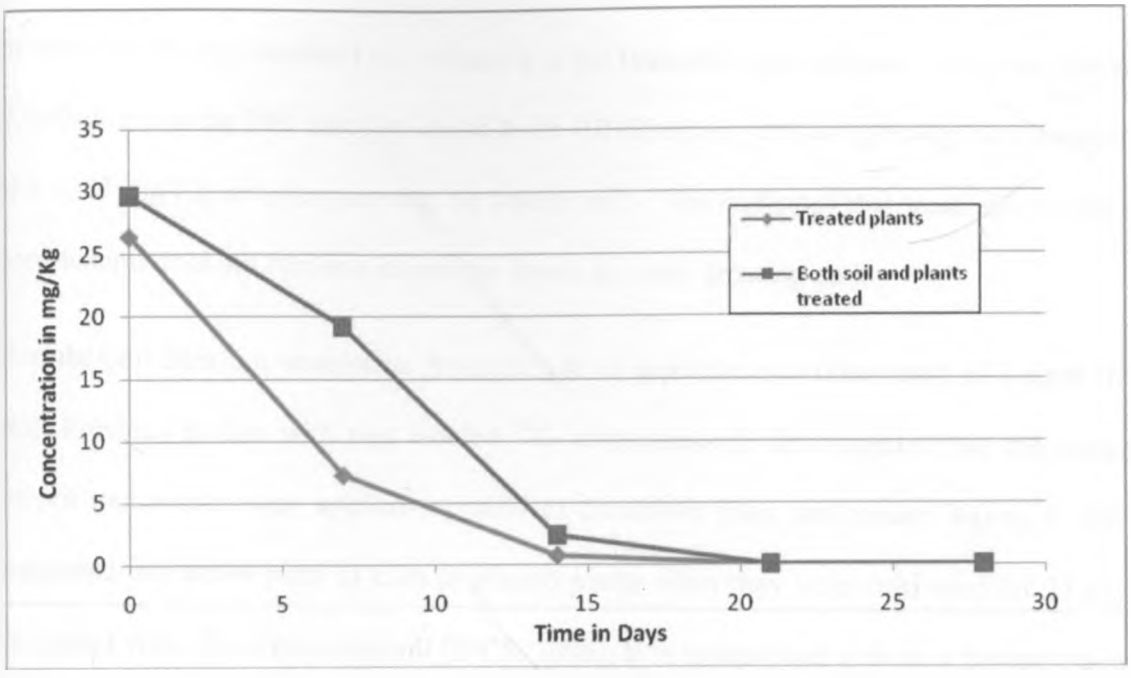


Figure 4.9: Trends of Chlorothalonil Dissipation in Snow peas Plants

The dissipation of chlorothalonil in both plants is rapid within the first 14 days then gradual after thereafter. The half-life of chlorothalonil in snow peas was calculated using equation 2 after k was determined and was found to be 1.90 days for chlorothalonil growing on untreated soil and 2.05 days for snow peas growing on soil treated with chlorothalonil. This is comparable with its half- life of 1.8 days in cabbage (*Brassica oleracea* L. variety capitata) [Zhi-Yong *et al.*, 2007]. Half-lives of 4.9, 3.9, 5.3,7.3,11.5 and 12.7 days were reported in bent grass studied in California [Wu *et al.*, 2002], bananas leaves in Costa Rica [Alicia *et al.*, 2007], cucumbers, peppers and cherry tomatoes in Spain [Valverde et *al.*,1993] and cranberry bog [Putnam *et al.*, 2003], respectively.

The concentration of chlorothalonil on the pre-harvest interval (PHI) day, (21) was calculated using the equation $C_t = C_o e^{-kt}$ Where, C_t is concentration of chlorothalonil at t = 21(PHI), C_o is

concentration of chlorothalonil at t = 0 and k is the first order rate constant. The concentration of chlorothalonil on the PHI day was found to be 0.0126mg/Kg on peas growing on untreated soil and 0.0245 mg/Kg on peas growing on treated soil. This indicates that treatment of soil with chlorothalonil does not increase its residue levels in plants growing on it.

Chlorothalonil does not translocate from the site of application to other parts of a plant [IPCS, 1996]. Previous studies with ring labeled ^{14}C- chlorothalonil, demonstrated that chlorothalonil does not translocate when applied topically to cucumber, bean and tomato leaves. It was not translocated into aerial parts of corn or tomato plants when they were cultivated for 23 days in soils treated with ^{14}C- Chlorothalonil [IPCS, 1996]. It is metabolized only to a limited extent on plants and the 4-hydroxy metabolite is usually <5% of the residue [IPCS, 1995]

The amount of residue at harvest depends upon factors such as the application rate, time interval between last application and harvesting, and the type of crop. Residues are composed mainly of chlorothalonil and only negligible amounts of the hydroxy - metabolite. Table 4.7 presents decline of chlorothalonil residues in some food crops [IPCS, 1996].

Table 4.7: Decline of Chlorothalonil Residues

Days after treatment	Pears mg/kg	Apples mg/kg
0	3.85	2.35
7	2.48	1.73
14	2.00	0.92
28	1.35	0.98

4.5 Assessment of Chlorothalonil Residues

4.5.1 Snow peas

Ten snow peas samples were analysed for chlorothalonil residues. The survey targeted twelve samples but Githurai market and supermarket U did not have the commodity at the time of sampling. The results of the tested samples are presented in table 4.10

Table 4.8: Results of Chlorothalonil Residues (µg/Kg ±SD) in Snow peas

Seasons	Kangemi Market	Githurai Market	Supermarket T	Supermarket U
Warm and dry (January)	2.34±0.42	*	<LOD	12.41±1.22
Long rains (March)	<LOD	1.06±0.009	<LOD	7.81±0.21
Cool and dry (July)	<LOD	<LOD	<LOD	*

* Commodity not available in the market at the time of sampling.

Chromatogram of snow peas extract with non-detectable residues is presented on figure 4. 10. The results show that chlorothalonil residues were detected in snow peas from three out of the four market outlets surveyed. The trends Figure 4.11 show that High residues were observed during the warm and dry season at levels ranging from 2.34 to 12.41 µg/kg while the long rains season had residues ranging from 1.06 to 7.81 µg/kg. During the cool and dry season residues were all below the detection limit. Out of the ten samples tested, only four had detectable

residues representing forty percent. All detectable residues were below the MRL (5 mg/Kg) and the ADI (0.03 mg/Kg).

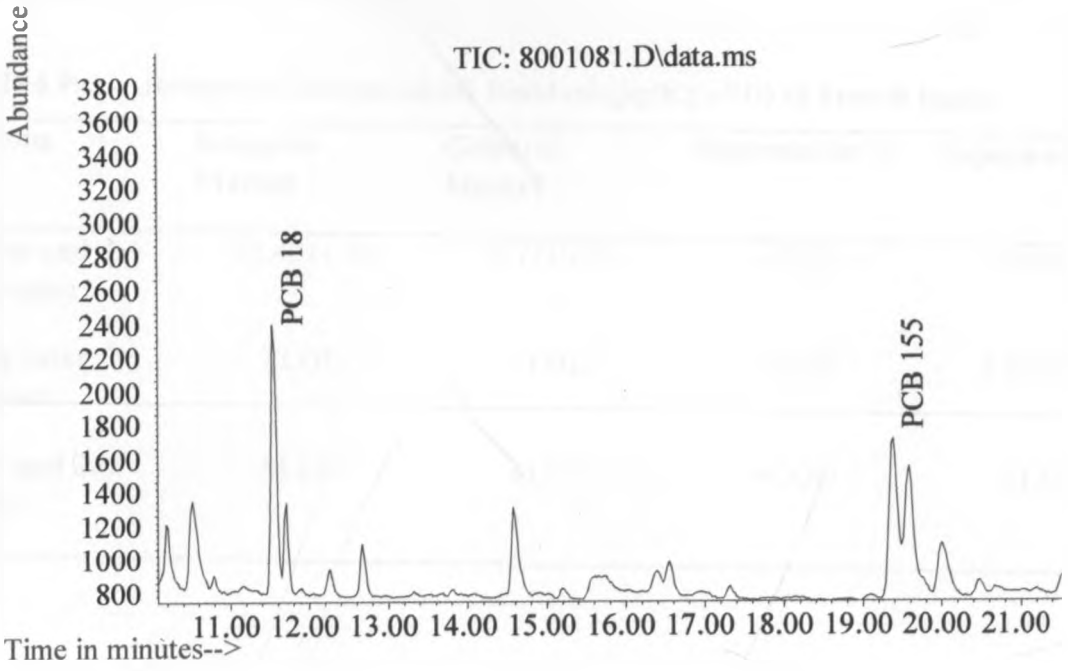


Figure 4.10: Chromatogram of Snow peas Extract with Non Detectable Residues

Figure 4.11: Trends of Chlorothalonil Residues in Snow peas at Market Outlets

4.5.2 French beans

A total of twelve French beans samples were collected and analysed for chlorothalonil residues. The results are presented in table 4.9

Table 4.9: Results of Chlorothalonil Residues (µg/Kg ±SD) in French beans

Seasons	Kangemi Market	Githurai Market	Supermarket T	Supermarket U
Warm and dry (January)	21.40±1.29	1.77±0.07	<LOD	1.60±0.46
Long rains (March)	<LOD	<LOD	<LOD	0.81±0.01
Cool and dry (July)	<LOD	<LOD	<LOD	<LOD

Detectable residues were found in four of the twelve samples collected. Chromatogram of French beans extracts with non-detectable residues of chlorothalonil are presented on figures 4.12. Three of these were collected during the dry and warm season while the fourth one was collected during the long rains season (Figure 4.13). Higher residues were observed during the warm and dry season ranging from 1.60 to 21.40 µg/Kg, compared to the long rains season that reported 0.81µg/Kg. No detectable residues were reported during the cool and dry season.

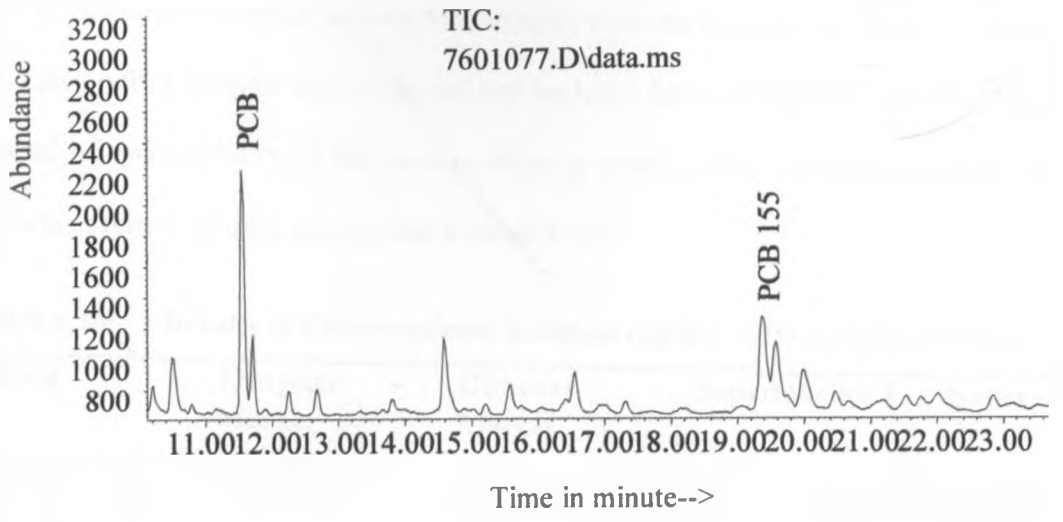


Figure 4.12: Chromatogram of French beans Extract with Non Detectable Residues

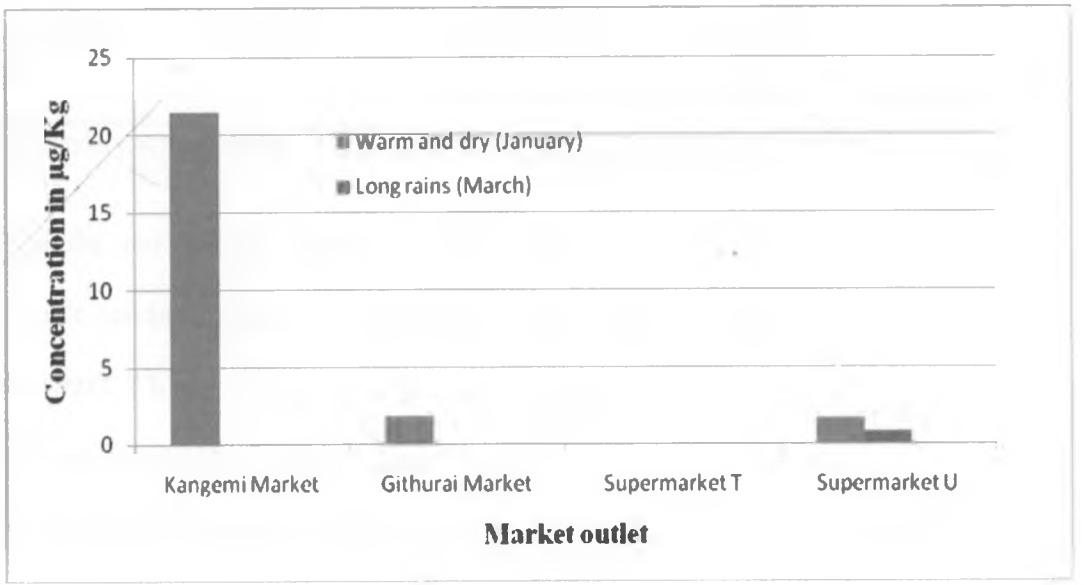


Figure 4.13: Trends of Chlorothalonil Residues in French beans at Market Outlets

4.5.3 Passion Fruit

The survey targeted twelve passion fruit samples from the four market outlets; however a total of ten passion fruit samples were collected and analysed for chlorothalonil residues. The deficit was caused by unavailability of the passion fruits at some market outlets at the time of sampling. Results of tested samples are presented on table 4.10

Table 4.10: Results of Chlorothalonil Residues (µg/Kg ±SD) in Passion Fruits

Season	Kangemi Market	Githurai Market	Supermarket T	Supermarket U
Warm and dry (January)	<LOD	3.22±0.34	*	1.92±0.11
Long rains (March)	2.58±2.16	<LOD	3.04±1.74	<LOD
Cool and dry (July)	<LOD	8.66±1.80	<LOD	*

*Commodity not available in the market at the time of sampling.

Out of the ten samples tested, five had detectable residues while the remaining had non-detectable residues. Figure 4.14 presents a chromatogram of chlorothalonil residues in a passion fruit extract. Chlorothalonil residues were detected in tested samples during the three seasons. Higher residues were reported during the cool and dry season at 8.66 µg/Kg compared to the warm and dry and rainy seasons which reported residues ranging from 1.92 to 3.22 µg/Kg and 2.58 to 3.04 µg/Kg respectively.

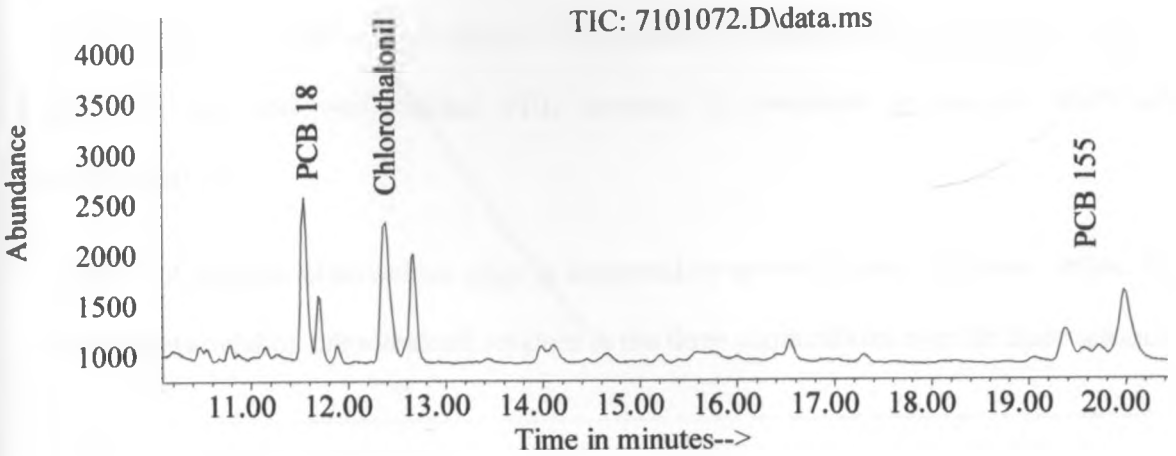


Figure 4.14: Chromatogram of Chlorothalonil Residues in a Passion Fruit Sample

Figure 4.15 presents trends of chlorothalonil residues in passion fruits in the three seasons of the survey.

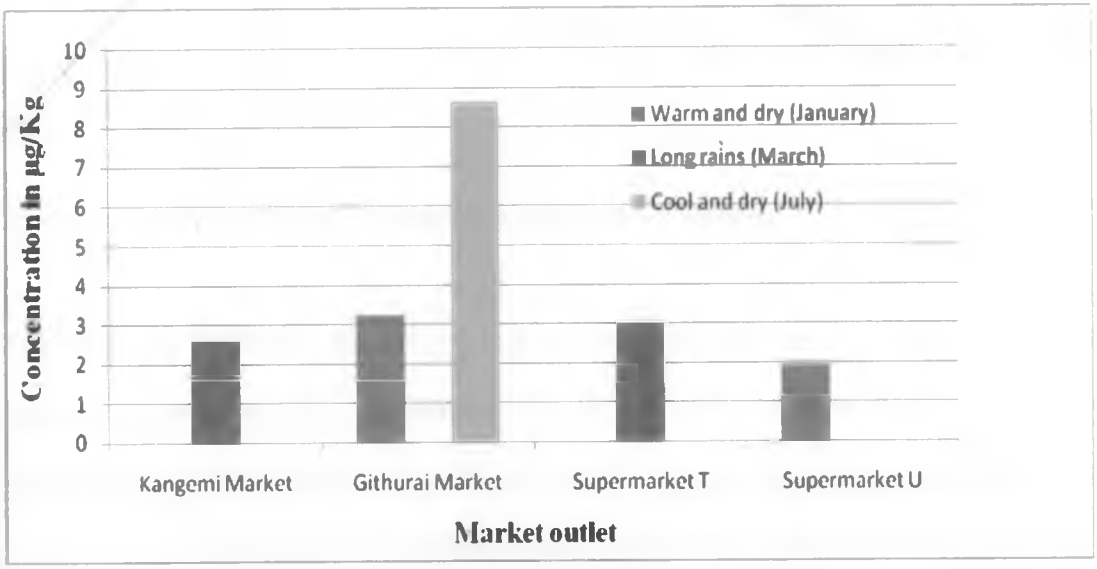


Figure 4.15: Trends of Chlorothalonil Residues in Passion Fruits from Market Outlets

Presence of chlorothalonil residues on both snow peas and French beans below their MRLs is expected with observance GAP since it is registered for use on the commodities with clear application rate and well defined PHI; however its presences in passion fruit indicate unauthorized use.

The level of residues observed on crops is depended on several factors discussed earlier. Figure 4.16 presents trend of chlorothalonil residues in the three commodities over the three seasons.

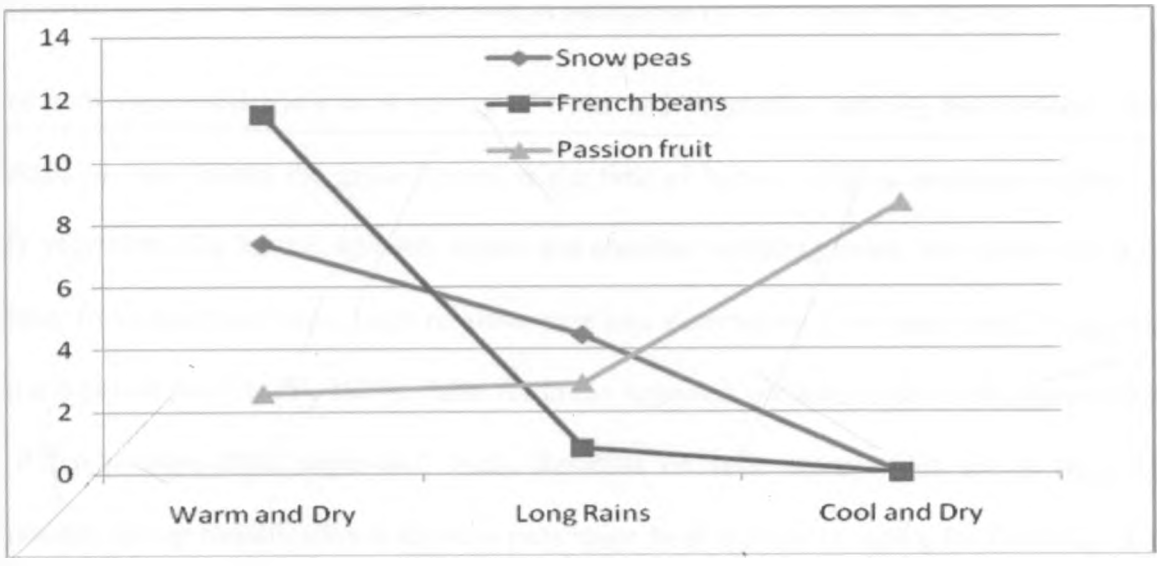


Figure 4.16: Trend of Chlorothalonil Residues over Seasons

In addition to application rate, Pre-harvest Interval and crop type, environmental factors such as temperature, precipitation and humidity and air movement (wind) influence the persistence of pesticides on plants. The total quantity and form of a pesticide reaching plants and soil depends on the site and method of application, type of equipment together with the formulation type. These consequently influence pesticide persistence on both the plants and soil [Edwards, 1975]. Plant factors that affect persistence include plant species, nature of harvested crop, structure of

cuticle and metabolic activities like rate of uptake, growth, translocation storage and excretion [Edwards, 1975]. In this study the observed trend may be attributed to environmental factors since a general decrease of residue levels in snow peas and French beans is observed as precipitation increases and temperature decrease. Being non-systemic chlorothalonil may be washed off during the rainy season leading to lower residues. In passion fruits, no specific trend was established. Since chlorothalonil is not registered for use on passion fruit, application rate and pre-harvest interval observed play a role in addition to the environmental factors.

Data from supervised trials on a variety of fruits and vegetables indicate that residues were detected on most above the ground crops at the time of harvest. Higher residues occurred on leafy vegetables like lettuce, spinach, celery and crucifers including kales, with lower levels on melons, fruits and root crops. High residues were also detected on lima bean plants, sugar beet tops and peanut hay [JMPR, 1974]. Table A4 in the Appendix presents residues of chlorothalonil on different crops from supervised trials. Residues on snap beans which are in the same commodity group classification with snow peas range from 0.04 to 14 mg/kg for five trials at an application rate of 1.7 a.i. kg/ha with a pre-harvest interval ranging from 0 to 7 days for five to eight applications. When the application rate was raised to 2.5 a.i. kg/ha, the observed residues ranged from 0.8 to 10 mg/kg for three trials with a PHI of 0-7 days for eight applications.

The lower residues ranging from 0.003 to 0.022 mg/kg in snow peas considered under this study could be attributed to the longer pre-harvest interval of 21 days with only one application. Other factors could be the climatic conditions. The resulting residue levels do not violate the codex and EU MRLs (5 and 2 mg/kg respectively).

Samples with detectable residues represent 40.6% of the surveyed samples. Of the positive samples, 15.6% were passion fruit while remaining 25 % were snow peas and French beans with 12.5% each this percentage is high compared to findings of other surveys and monitoring programmes. This can be attributed to the enrichment factor employed in quantifying chlorothalonil leading to low LOD 0.004mg/kg. This was necessitated by the low MRL for chlorothalonil in passion fruits. Although the residues were below the MRLs, for both snow peas and French beans, there is no codex MRL for chlorothalonil in passion fruit. However, the UK MRL is 0.01mg/kg set at the limit of determination. Where MRLs are established at the limit of determination it signifies that there is either no legal use of the pesticide on that crop or that legal use will not result in detectable residues being present in the harvested food product [DAFPCS, 2006]. The detected levels were below the MRL and Allowable daily intake; this was achieved by employing an enrichment factor. The mean enrichment factor for the analysed samples was 3.86.

A pesticide residues monitoring programme conducted in Ireland in 2004, found Kenyan produce to contain detectable pesticides residues. Out of the 855 routine samples analysed, almost half 48% had detectable residues. 29% of the surveyed samples were imports from countries outside of the EU. Out of the positive samples 1.9% contained chlorothalonil residues. Chlorothalonil was detected on Pome fruits from Brazil at 0.02mg/kg a level below the MRL (1mg/kg), Blue berry from Spain at 0.35 mg/kg which is above the MRL 0.01 mg/kg, cranberry from the US at 0.07 mg/kg which is below the MRL 0.2 mg/kg, redcurrants from Ireland at 0.04 which is also below the MRL of 10 mg/kg. Tomatoes from Spain were found to have residues of chlorothalonil at 0.03 mg/kg while the MRL is 0.05 mg/kg where as head cabbage from the same country had 0.25 mg/kg while he MRL is 0.3 mg/kg. Other commodities that had chlorothalonil

residues are beans with pod from Spain with 0.1 mg/kg, celery from South Africa (0.41 mg/kg), Spain (0.32 and1.38 mg/kg), and Ireland (0.05mg/kg). All were below the MRL of 10 mg/kg. Passion fruits from Kenya were found to have chlorothalonil residues at 0.15 mg/kg a level that is above the EU MRL (0.01 mg/kg) [DAFPCS, 2006].

In Kenya Pest control products are evaluated and registered for use by the Pest Control Products Board (PCPB). Chlorothalonil is registered for various uses on different crops; however it is not registered for any use on passion fruit. According to PCPB, any other uses outside the registered uses are not authorized. Therefore the use of pest control products in a manner that is inconsistent with directions on approved labels is prohibited [PCPB, 2007]. Detection of chlorothalonil residues on passion fruit confirms that farmers are using the fungicide in a manner that is inconsistent with the directions on approved label.

The unauthorised use of chlorothalonil on passion fruit could mean that the fungicide is working well to control pests and diseases on this crop. The danger of the practice is that farmers could be using the same application rate and PHI on passion fruit as on the other crops, whereas pesticides behave differently on different crops. This could result in presence of unacceptable residue levels on the fruits leading to an interception like the one received from Ireland. An interception of the produce is very expensive for the farmer, exporter and Kenya at large because it would be branded a bad source.

Another survey conducted by the Pesticide residue committee (PRC) of the UK in 2008 where 4129 samples were tested reported that around half the samples tested did not have detectable pesticides residues while the percentage of food tested which contained residues above trading standards and the MRLs remained at 1.2%. The monitoring programme aimed at foods where residues were expected to be found. In this programme, a total of 2309 samples of fruits and

vegetables were tested for up to 212 pesticides. Residues were found in 1484 samples (64.3%) with 49 samples (2.1%) containing residues above the MRL. Residues above MRL were reported in apples, beans in pods chilli peppers, Chinese cabbage, cucumber, grapes, melons, oranges, pears, peas in pods, potatoes, spinach, tomatoes and yams. Chlorothalonil residues were not detected in any of the samples; however, four samples of beans in pods originating from Kenya contained dimethoate residues above MRL, one contained omethoate residues above the MRL while the other three had detectable residues of the same pesticide. One of the four samples contained residues of methomyl above MRL and detectable residues of dicofol in addition to the dimethoate and omethoate. Produce from other countries including India, China, Egypt, Ghana and Morocco Contained pesticide residues. These countries compete for the EU market with Kenya, and Kenya must strive to maintain the market by meeting the trade requirements for this market.

The assessment considered a total of thirty two sample thirteen of which contained detectable residues of chlorothalonil representing approximately forty one percent (40.6%) of surveyed fruits and vegetable. This percentage is lower compared to the Ireland's pesticide residue monitoring programme in 2004 that reported fifty two (52%) percent of the analysed samples to contain detectable residues. This could be attributed to the number of samples considered and pesticide commodity combination. In the Ireland monitoring programme, seventy seven different fruits and vegetables were analysed for one hundred and eighteen pesticides compared to thirty two in the this study. In 2008 the pesticide residue committee of the UK reported that sixty four (64.3%) of tested samples had detectable residues. While the present study was a baseline assessment targeting a single pesticide in three commodities, the Ireland was a monitoring programme and the UK Surveys found most residues in speciality commodities like beans and

yams who's MRLs are set at detection limit because they are not grown in many parts of Europe thus no scientific information to base MRLs on. This explains why in the present study chlorothalonil residues are high in passion fruit compared to French beans and snow peas.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

Chlorothalonil was found to persist more in soil than in the snow peas with a half-life of 10 days in soil compared to 2 days in snow peas plants. There was no significant difference between residues of chlorothalonil in snow peas growing on treated and untreated soil; therefore it is not absorbed by snow peas from soil. Chlorothalonil residues on the pre-harvest day were below both the EU and Codex MRLs. Thus, appropriate use in accordance with instructions on the label, on commodities for which it is registered and with observance of good agricultural practice, resultant produce is compliant with market and safety requirements.

Chlorothalonil residues were detected in the three commodities assessed indicating its use. The results obtained shows that all commodities analyzed were compliant since the levels of chlorothalonil residues observed were below both the Codex and EU/UK MRLs and the ADI. However, chlorothalonil was detected in passion fruit; a commodity on which it's used is not registered. This constitutes an unauthorized use. Detection of chlorothalonil on the passion fruits at levels above 0.01mg/Kg in the EU results in interception; and indeed Kenyan passion fruits have been intercepted previously. These interceptions are very expensive for the farmer, exporter and Kenya at large as a fresh produce source since such interceptions lead to notifications being circulated to EU member states and the European Economic Area (EEA) through the Rapid Alert System for Food and Feed (RASFF). This can lead to loss of this market and consequently foreigh exchange earnings.

5.2 Recommendations

Although residues of chlorothalonil in French beans and snow peas were below the set MRLs, they were detected in passion fruit, a crop that has no Codex MRLs and who's EU MRL are set at 0.01mg/Kg, the LOD. The codex has no MRL for this pesticide because no trials have been conducted so far for generation of the necessary data for setting MRLs, neither is there data on a crop that the MRLs for chlorothalonil on passion fruit could be extrapolated from. Passion fruit is an important crop for Kenya and therefore, there is need for trials to be set up to generate data for the establishment of its MRLs. The National Taskforce on Horticulture in Kenya needs to request the manufacturers to support setting up of supervised trials to generate the required data for setting the MRLs. This would also generate information to establish the application rate and PHI for chlorothalonil in passion fruits.

National Pesticide Residues Monitoring and Surveillance is recommended on produce sold in Kenyan markets and those destined for export to protect consumers, assess implementation of GAP, detect unauthorized or misuse of pesticides and support trade.

Further study on the dissipation trends of chlorothalonil in tropical soils cores to establish the dissipation rate is recommended. This could be coupled with a leaching ability of the fungicide in tropical soils.

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APPENDIX

Table A1: Test soil Fertility evaluation Parameters and Physicochemical properties

Parameters	Test Soil	Critical level	Comment in relation snow peas growing
pH	6.23	5.5	Slightly Acidic
Total Nitrogen %	0.42	0.2	Adequate
Organic carbon %	3.57	0.5	Adequate
Phosphorous (ppm)	28	30 ppm (Mehlich 1)	Low
Potassium (me %)	1.28	0.2	Adequate
Calcium (me %)	5.0	2.0	Adequate
Magnesium me %	2.48	1.0	Adequate
Manganese me%	0.49	0.11	Adequate
Copper (ppm)	1.94	1.0	Adequate
Iron (ppm)	73.0	10.0	Adequate
Zinc (ppm)	4.00	5.0	Low
Sodium me %	0.10	2	Adequate
Clay %	75	40	Clay soil, texture grade C
Silt %	16.9	-	
Sand %	8.1	-	
Bulk Density	0.7	-	
PF 0	84.48	-	Saturation point
PF 2	62.17	-	Field capacity
PF 2.3	42.67	-	Field capacity
PF 4.2	20.45	-	Permanent wilting point

Table A2: Irrigation water Suitability Parameters

Parameters	Irrigation water	Critical level	Comment
pH	8.79	N/A	
Conductivity(μs/cm)	336	1	Very High
Sodium (m.e/L)	4.15	N/A	
potassium (m.e/L)	0.12	N/A	
Calcium (m.e/L)	0.14	N/A	
Magnesium (m.e/L)	0.001	N/A	
Carbonates (m.e/L)	ND	N/A	
Bicarbonates (m.e/L)	0.098	N/A	
Chlorides (m.e/L)	5.738	N/A	
Sulphates (m.e/L)	0.023	N/A	
Sodium Adsorption Ratio (SAR)	15.63	5.0	Very High

Note

Note Applicable is used because the quality of irrigation water is depended on SAR and EC. The SAR is a ratio of Na ions to Ca and Mg ions expressed as;

$$SAR = \frac{NA^{+}}{\sqrt{(Ca^{2+} + Mg^{2+})}}$$

Table A3: Moisture Content (%) for Soil Samples

Day	Sterile soil without plants (SST)	Treated soil with plants (STA)	Treated soil with plants (STB)	Treated soil with plants (STC)	Treated soil without plants (SCA)	Control (SCT)
0	1.77	2.39	1.66	1.79	0.10	2.16
7	2.56	1.57	1.63	1.61	3.40	3.55
14	2.73	1.58	1.90	1.63	3.39	3.27
21	1.72	3.77	7.20	2.92	1.63	1.37

Table A4: Summary of Chlorothalonil Residues from Supervised Trials

crop	Applications		PHI (days)	No of trials	Residues in mg/Kg	
	Rate (a.i.kg/ha)	Number			Total range	Range mean per trail
carrots	1.3	13	12	1	0-0.7	0.15
Broccoli	1.3	9	1-15	3	0.01-9.0	0.01-6.0
Cabbage	2.5	7-9	0-7	5	0-0.2	<0.01-0.4
Cucumber	0.8	4-9	0-6	5	0-1.1	0.05-0.9
Watermelon	1.7	8-10	0-14	4	0.03-1.0	0.08-0.5
Potatoes	1.3	3-13	12-23	16	0-0.07	0-0.02
Sweet corn	2.5	11	0-7	3	0.01-0.1	0.04
Snap beans	1.7	5-8	0-7	5	0.04-14	0.1-11
	2.5	8	0-7	3	0.8-10	2.3-5.4
Lima beans	1.7	13	0	1	10-13	12
Oranges	1.3	1-14	0-14	3	1.8-5.1	2.7-4.3
Kales	2.5	3	0-14	4	1.5	2.8-62
Spinach	1.3	5	3-8	2	4-48	14-29
Lettuce head	1.7	4-7	0-14	5	0.1-100	1.3-86

Table A5: Summary of Market Survey Results

Season	Market out let	commodity	GC-MS Results (µg/Kg)	Reported µg/Kg	Enrichment Factor
Warn and dry	Kangemi	Snow peas	6.95	2.34	2.98
	Kangemi	French beans	66.02	21.40	3.08
	Kangemi	Passion fruit	0.00	0.00	2.28
	Githurai	French beans	5.49	1.77	3.09
	Githurai	Passion fruit	8.85	3.22	2.75
	Supermarket U	Snowpeas	25.83	12.41	2.08
	Supermarket U	French beans	5.24	1.60	3.27
	Supermarket U	Passion fruit	4.30	1.92	2.24
	Supermarket T	Snowpeas	0.00	0.00	2.18
	Supermarket T	French beans	0.00	0.00	3.77
Rainy season	Kangemi	Snow peas	2.14	0.37	5.79
	Kangemi	French beans	2.53	0.48	5.32
	Kangemi	Passion fruit	4.98	2.58	1.93
	Githurai	Snowpeas	5.22	1.06	4.94
	Githurai	French beans	2.26	0.54	4.23
	Githurai	Passion fruit	0.00	0.00	2.10
	Supermarket U	Snowpeas	25.50	7.81	3.26
	Supermarket U	French beans	5.27	0.81	6.49
	Supermarket U	Passion fruit	0.00	0.00	199
	Supermarket T	Snowpeas	0.00	0.00	4.04
	Supermarket T	French beans	1.09	0.33	3.31
	Supermarket T	Passion fruit	5.22	3.04	1.72
Cool and dry	Kangemi	Snow peas	3.17	0.83	3.83
	Kangemi	French beans	0.00	0.00	3.78
	Kangemi	Passion fruit	0.00	0.00	9.39
	Githurai	Snowpeas	0.00	0.00	3.75
	Githurai	French beans	0.00	0.00	2.68
	Githurai	Passion fruit	28.93	8.66	3.34
	Supermarket U	French beans	0.00	0.00	2.18
	Supermarket T	Snowpeas	0.00	0.00	3.08
	Supermarket T	French beans	0.00	0.00	2.86
	Supermarket T	Passion fruit	2.06	0.58	3.53

Table A6: Dixon Q-Test for Chlorothalonil Recovery Data.

Spike concentration	Snow peas	French beans	Passion fruit
1.0mg/kg	81.38	64.57	96.45
2.5mg/kg	88.98	101.97	98.86
5.0mg/kg	115.06	107.97	108.02
Dixon Calc. Row 1	-0.225653207	-0.861751152	-0.208297321
Dixon Calc. Row 3	0.774346793	0.138248848	0.791702679
Dixon critical CL=95%	**0.97**		

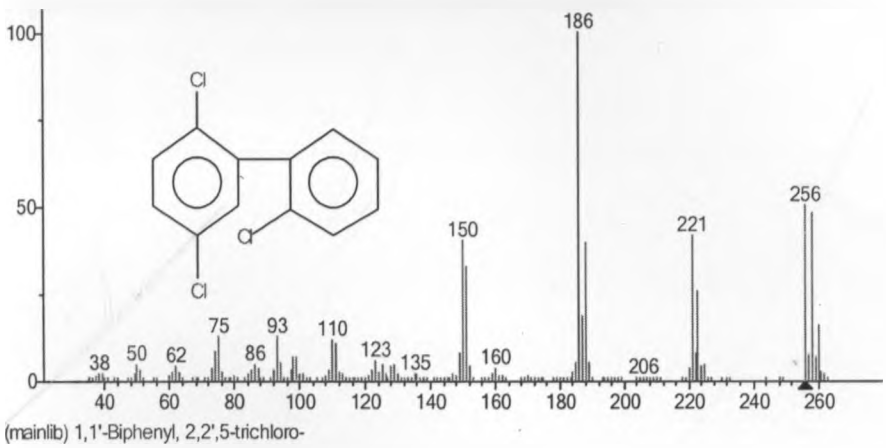

Figure A3: Chemical structure and ionic Mass Spectra of PCB 18

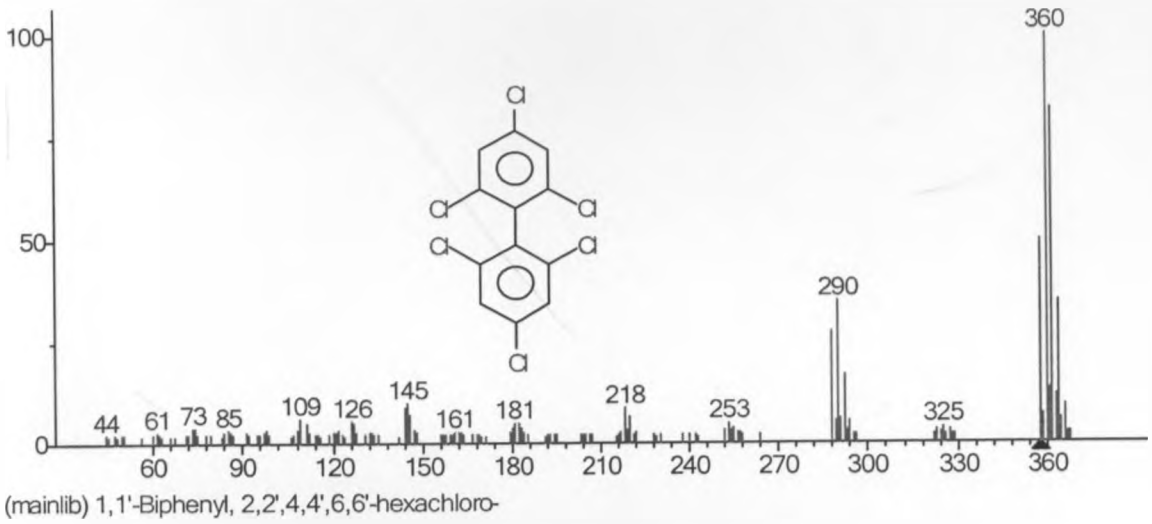

Figure A2: Chemical structure and ionic Mass Spectra of PCB 155