RESIDUES OF CHLORFENVINPHOS AND 2,4-DICHLOROACETOPHENONE IN COW MILK IN WESTERN KENYA

BY

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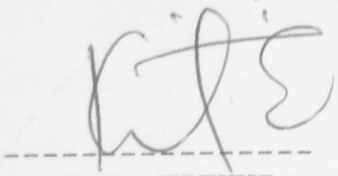
A thesis submitted in partial fulfilment for the degree of Master of Science of the University of Nairobi.

1995

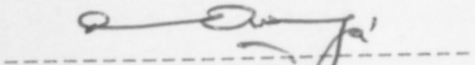
DECLARATION

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

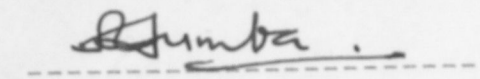


E.N. KITUYI

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



PROF. S.O. WANDIGA



DR I.O. JUMBA

Dedication

To my late father, Richard Mukhisa Kituyi

ACKNOWLEDGEMENT

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DEFINITIONS

ADI-Acceptable Daily Intake

a.i.- active ingredient

ChE - cholinesterase enzyme

ECF - East Coast Fever

FAO - Food and Agricultural Organization

GC-MS - Gas chromatography-mass spectrometry

GDP - Gross domestic product

HPLC - High performance liquid chromatography

ICIPE - International Centre for Insect Physiology and Ecology

ILCA - International Livestock Centre for Africa

ILRAD - International Laboratory for Research on Animal Diseases

ILO - International Labour Organization

KCC - Kenya Co-operative Creameries

KEMRI - Kenya Medical Research Institute

KARI - Kenya Agricultural Research Institute

LBDA - Lake Basin Development Authority

MRL - Maximum Residue Limit

NADPH -Reduced nicotinamide adenine dinucleotide phosphate

2-PAM - 2-pyridinealdoxime methiodide

UNEP - United Nations Environment Programme

WHO - World Health Organization

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ABSTRACT

A survey of the literature revealed that livestock production ranks second to cereals, provides employment and helps in alleviating poverty especially in the rural areas of Western Kenya; that a number of acaricides are available of which Steladone 3ØØEC is the most commonly used for the control of tick-borne diseases such as East Coast Fever in grazing cattle in the area; that chronic or acute exposure to these acaricides can have serious health effects on man; and that no comprehensive research had been carried out determine the fate and potential accumulation of these chemicals in cows' milk.

A study was therefore conducted in which one hundred samples of cows' milk were collected from individual farms and several delivery centres in the districts of Bungoma and Trans Nzoia in Western Kenya at different periods of the year. The objective was to determine the fate and residual effect of chlorfenvinphos, the active ingradient in Steladone 3ØØEC, as affected by season, butterfat content and method of acaricide application (whether by dipping or handspraying).

Samples were subjected to chlorfenvinphos analysis by high pressure liquid chromatography and a combined gas chromatography - mass spectometric technique following the mandatory liquid extraction and clean-up operations. Results were based on butterfat content which was determined gravimetrically after extraction into hexane.

Concentrations of chlorfenvinphos varied between Ø.52 and 3.9Ø µg/kg in the dry season; and from 1.58 to 1Ø.96

g/kg in the samples collected during the wet season. Dipped cows gave milk which was significantly higher ($P<0.05$) in the acaricide content than hand-sprayed animals. The chlorfenvinphos degradation product, 2,4-dichloroacetophenone was detected in only 12 % of the samples, at concentrations ranging from 3 - 276 µg/kg. Concentrations of both the acaricide and it's metabolite were positively correlated with the content of butterfat in the milk.

Reference to the 1993 Codex Alimentarius showed that the acaricide residue concentrations all fell below the recommended critical level of 8 µg/kg, suggesting that health risks arising from dietary exposure by the adult residents in the study area may not be serious. However, for infants, the danger may be significant since the accepted daily intake was exceeded by between 7 - 15 times.

It is suggested that monitoring programes be initiated to generate data on pesticide residues on a regular basis. Educational programmes to sensitize the farm-workers and dip manangers on the hazards posed by poor acaricide handling and careless spillages will be an added advantage. Nursing mothers should be encouraged whenever possible to breastfeed their young to reduce exposure to acaricides through intake of cow's milk.

CHAPTER ONE

INTRODUCTION

1.1 Livestock Production in Kenya

Livestock are of particular importance in the farming economy of Kenya.Almost all farmers own livestock of the various species, and about 73% of the total land area of the country (412,ØØØ km^2)can only be exploited by some form of livestock husbandry and tourism. The livestock industry constitutes 7.7% of the Gross Domestic Product (GDP), and livestock products provide more than one third of the total foods available in the country.

There are a total of 9,671,ØØØ head of cattle in Kenya comprising crossbreeds, grade animals and the indigenous East African Zebu. Approximately 8Ø% (1,2ØØ,ØØØ) of the grade milking cows are raised on small holdings of less than 2Ø hectares(ha),while the rest , about 27Ø,ØØØ are found on large scale farms (> 2Ø ha),(ISNAR,1985). The current breed composition of the grade dairy herd is approximately 27, 13, 16 and 8% pure breed Ayrshire, Fresian, Guernsey, and Jersey, respectively.The remainder (36%) are crossbreeds and the East African Zebu. Other forms of livestock production in the country include beef cattle, poultry, sheep, goats and to a lesser extent pigs.

The role of livestock production in the economy of Kenya cannot therefore be compromised, but should instead be supported. In line with this the Government has set up governing policies that aim at encouraging increased

production among the farmers.Such include the production of sufficient animal foodstuffs to satisfy the nutritional needs of the growing population, and animal products other than foods to support resource-based agro-industries; to assist in the alleviation of poverty by improving employment opportunities throughout the livestock industry; and to export surplus animal products (in so far as this objective does not conflict with that of domestic self sufficiency) in order to improve foreign exchange resources.

1.2 The Dairy Industry

Cow milk is an important dietary commodity in Kenya and it is therefore imperative that cattle are protected from parasites and other pests, for maximum production. Milk production is more profitable than beef production.In 1982, 53% of all grade animals, and 27% (4,162,ØØØ) of the indigenous cows in Kenya were able to produce 1,566 million litres of milk, (ISNAR,1985) of which 7Ø, 8 and 22% were produced by small holders, large farms and pastoralists, respectively. This production however dropped by over 3Ø% by 1992 in some regions especially parts of Central and Rift Valley Provinces. This drop was attributed to increasing production costs, especially for drugs,feeds and replacement of breeding stock, poor market prices, lack of credit facilities and poor management skills(KNDP,1994).

Milk sales were estimated at 6ØØ million litres in 1992 or 38% of the total production. The major proportion of the milk produced was consumed by producers' families.Small

holders sold their milk, partly through the Kenya Co-operative Creameries (KCC), partly through local co-operatives and the remainder directly to consumers. In some regions, especially the Western and parts of the Eastern provinces, there has been a dramatic increase in milk production over the last 6-8 years. This was probably due to the rapid increase in the number of grade cattle for milking and donor assistance through dairy development programs.For instance, the objective of the Rural Milk Collection and Cooling Centres Program, partially funded by the Finnish Government, has been to improve the collection and processing of milk produced by small holders.Ongoing projects are currently centred in Bungoma and Meru districts.

One major constraint affecting the livestock industry, however, is the prevalence of numerous tick-borne diseases, some of which are fatal to the animals.Typical examples are East Coast Fever (ECF), anaplasmosis, babesiosis, redwater and heartwater, theileriasis and cowdriosis (Tatchel,1983).

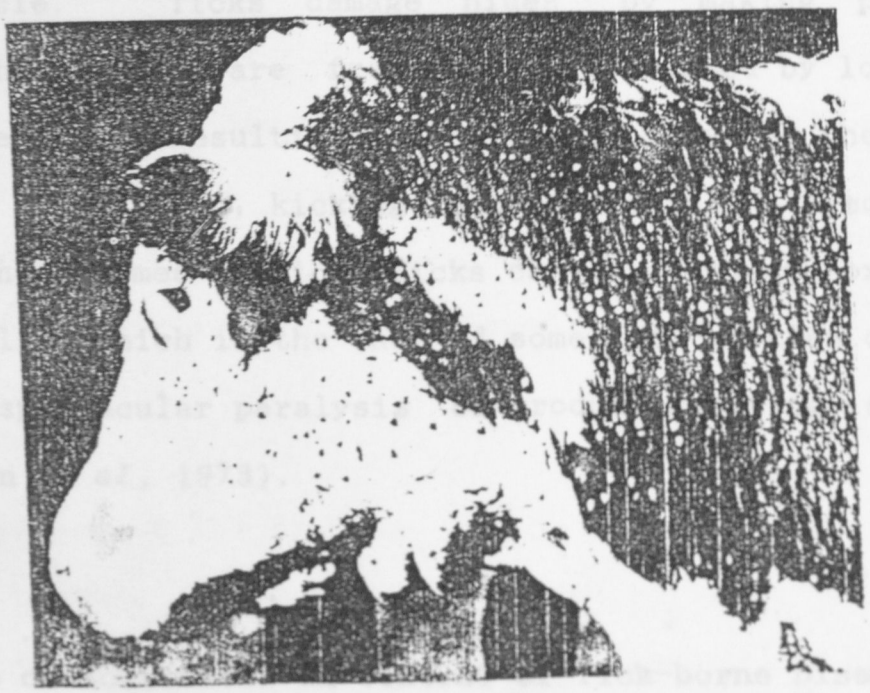
1.3 Ticks and Tick-borne Diseases

Ticks are eight-legged arachnid parasites that may use several different hosts during their life cycle as well as spending several months of each year on the ground. They themselves are frequently parasitized by one or more of a long list of bacterial, rickettsial, viral and protozoal organisms. Many of these organisms can produce disease when

when transmitted to livestock.

The most common tick species in Kenya are *Boophilus annulatus*, *B. decoloratus*, *Rhipicephalus appendiculatus*, *Amblyomma variegatum*, *R. evertsi evertsi*, and *B. microphus*. These are mainly found in Western Kenya and most parts of the Rift Valley. Plate (a) shows an animal heavily infested with *B. microphus* in one of the study farms. The ticks are mainly concentrated around the neck of the animal where neither the tongue nor tail can reach to interfere with the blood sucking.

Plate (a)



Heavy infestation of the tick *Boophilus microplus*, on a Hereford

The protozoan parasite *Theileria parva* causes severe and often fatal diseases in cattle in eleven African countries, namely Burundi, Kenya, Malawi, Mozambique, Rwanda, Sudan, Tanzania, Uganda, Zaire, Zambia and Zimbabwe. The

brown ear tick, *R. appendiculatus* transmits *T. parva* parasites to cattle which then develop East Coast Fever(ECF), and if left untreated die within a few weeks. The chief habitat of the brown ear tick is the Savannah grasslands, whose climate and vegetation give conditions optimum for the survival of the parasite (ILRAD, 1992).

Apart from disease transmission, ticks can cause damage to their hosts in several other ways. They are voracious blood suckers:it is estimated that 1-3 ml of host blood is required to enable a female tick to complete its parasitic life cycle. Ticks damage hides by making puncture wounds.These wounds are frequently exacerbated by localized tissue reactions resulting in irritation to which the animal responds by licking, kicking and scratching. Frequently, the wound becomes septic. Ticks can also inject toxins via their saliva which in the case of some tick species can give rise to spectacular paralysis or produce sweating sickness (Harrison *et al*, 1973).

1.4 Use of Acaricides in Control of Tick-borne Diseases

Acaricides are chemicals used for the control of mites, the highly diversified tiny arthropods of the class Arachnida, order Acarina,to which all common ticks belong.The practice of regularly dipping or spraying cattle with acaricides to prevent tick infestation generally has proved effective in preventing the transmission of tick-borne diseases in Africa. The main disadvantages of this control method are its dependency on water which is often

scarce, the rising costs of acaricides, the difficulty in maintaining dip and spray infra-structures, the development of tick resistance to the acaricide, contamination of the environment by acaricides, occurence of acaricide residues in food, and the adverse effects of intensive tick control on endemic stability for tick-borne diseases.

These problems have stimulated research into new and innovative disease control methods. Increased knowledge of tick biology in some instances has allowed more effective timing of acaricide applications and also allowed a desirable reduction in their frequency and menace(ILRAD, 1992). Organochlorine (OC) acaricides are largely outmoded because of residue occurence and persistence problems and the high resistance factors. The organophosphate (OP) acaricides are still effective though resistance to the compounds in some areas has developed recently.

1.5 Statement of the problem

The biggest drawback encountered in milk production that mostly goes unnoticed is the issue of pesticide residues in milk. These chemicals are used in various forms in crop and animal production and when ingested through pretreated feed, contaminated water or dermally after spraying, they pose serious health risks to both the animal and man through the milk we take. Saxena and Siddiqui (1983) found high levels of some pesticides in maternal blood, placenta and umbilical-cord blood of still-born.They reported that lindane together with aldrin, dieldrin and

p,p'DDT were the cause of still-birth,in both man and animals.

Carcinogenicity in test mammals has been reported in various studies though similar work in man has yet to be reported.Kanja (1988),reported high levels of p,p'DDT and aldrin (17 and 18 times respectively,higher than the recommended limit) in mothers milk sampled from various provinces in Kenya.In a related study,high levels of the OC insecticide, lindane (upto 1ppm) have been detected in samples of human milk procured from mothers attending clinics in Nairobi (Wandiga and Mutere,1988).

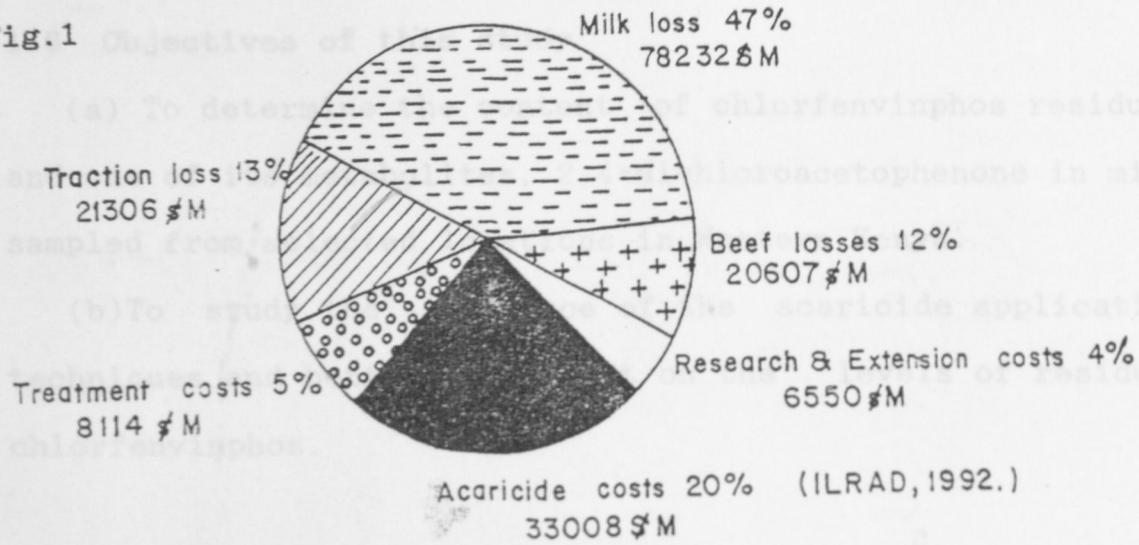
The prolonged ingestion of even small amounts of some of these persistent chemicals through cow milk or other sources can unquestionably cause harm (Gunther, 196Ø). Infants and young children tend to receive a greater portion of their total diet from a smaller number of foods (mainly milk) than do adults. This dietary restriction can aggravate pesticide exposure to the subjects,particularly where such products originate from a treated source.Since children are known to be uniquely sensitive to some forms of toxicity, it is important to have accurate intake data for this age group to assess the impact of pesticide residues in their diet.

Acute pesticide poisoning cases continue to increase every year. WHO reports 3 million cases of poisoning and 22Ø,ØØØ deaths annually worldwide. Also, 25 million symptomatic poisonings occur among agricultural workers in developing countries (Mbugguss,1994). In 1988, the Kenya Medical Research Institute (KEMRI) reported 35Ø,ØØØ cases of

acute poisoning, and a death toll of 7ØØ in Kenya (KEMRI,1988).At least two cases of acute pesticide poisoning are reported daily at the Kenyatta National Hospital,Nairobi (Waiyaki et al,1988). Despite all these, FAO calls for an annual increase of 3% in pesticide imports and use upto the year 2ØØØ towards achievement of "food for all" policy.

A KARI/ILRAD/ILCA collaborative study in the endemic region of eastern, central and southern Africa estimated total losses due to East Coast Fever (ECF) in 1989 to be US$ 168 million. Fig.1 includes an estimated mortality of 1.1 million cattle (ILRAD,1992)

Fig.1



(ILRAD,1992.)

Despite the continued annual use of about 2.5 million tonnes of pesticides in world agriculture (Helsel,1987), pests are destroying about 35% of all potential crops before harvest (Pimentel and Pimentel,1979) primarily to insects, plant pathogens and weeds. After harvest, an additional 2Ø% of the crops are destroyed by insects , micro-organisms, rodents and birds. Thus , nearly half of all potential food in the world is being destroyed annually by pests, despite

all effort to control them.

Due to these reasons, cow milk and agricultural food crops, which are important sources of pesticide intake by humans require programmes that would constantly monitor upward deviations from the limits recommended by the Codex Alimentarius Commission of the Food and Agricultural Organization (FAO) and WHO, hence the purpose of this project.

1.6 Objectives of this study

(a) To determine the content of chlorfenvinphos residues and one of its metabolites, 2,4-dichloroacetophenone in milk sampled from selected locations in Western Kenya.

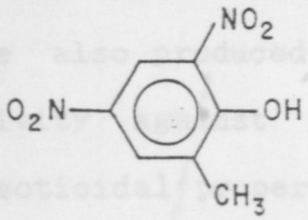
(b)To study the influence of the acaricide application techniques and butterfat content on the levels of residual chlorfenvinphos.

CHAPTER TWO

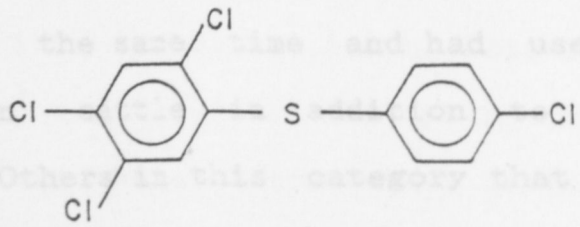
LITERATURE REVIEW

2.1 Historical Development and Use of Acaricides in Kenya

Acaricide use in Kenya for livestock production dates back to the early 192Øs when arsenicals such as arsenic trioxide were used for parasite control on sheep and cattle upto the late 194Øs. Azobenzenes and other hydrazine compounds such as 2-methyl-4,6-dinitrophenol [1] and 1,2,4-trichloro-5-[4-(chlorophenylthio] benzene [2],so were various sulfides,



[1]

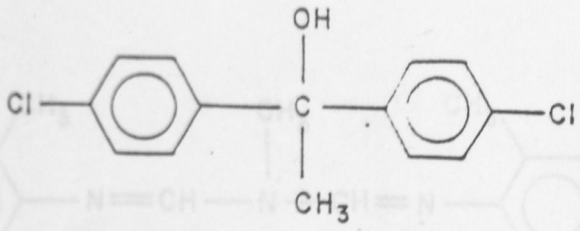


[2]

sulfones and sulfonates which were introduced as purely contact acaricides.This had the advantages of long residual action, low phytotoxicity and low mammalian toxicity.They were mainly effective at the egg and larval stages. From 1948, sulphites were developed as non-systemic acaricides that were effective against all stages of numerous spider mite species.They were well tolerated by plants and had low acute mammalian toxicity.

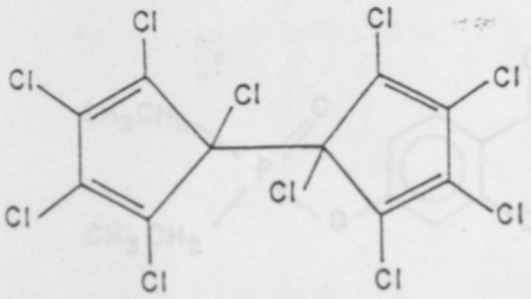
Diphenylcarbinols were developed from 1955 as contact acaricides. Dichlofol [3], was the first diphenylcarbinol

acaricide manufactured. However, due to development of resistance by the parasites in many parts of the world, more products were released into markets.

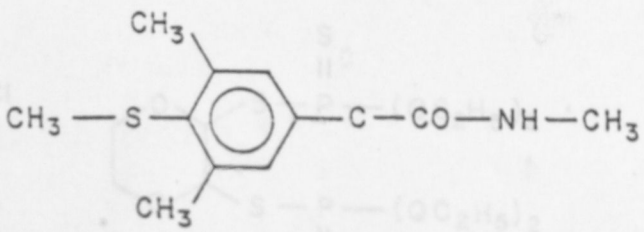


[3]

Chlorohydrocarbons like Dienochlor [4] were produced as early as 1960, followed later by fluorinated compounds like Lambrol and Nissol in 1965. Carbamates like Mexacarbate were also produced around the same time and had useful activity against ticks on cattle in addition to its insecticidal properties. Others in this category that may still be found on the market include Methiocarb [5].



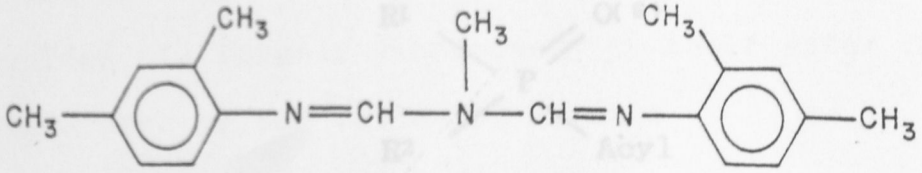
[4]



[5]

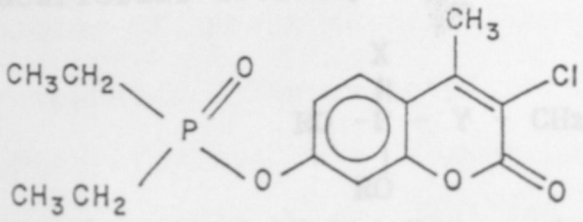
Organotin compounds like the tricylcohexylstannane derivatives (Cyhexatin and Azocyclotine) were found to have acaricidal properties and became common between 1968 and 1976. However, they were soon replaced by heterocyclics and formanidines which had superior acaricidal and ovicidal

activity, even against OP-resistant strains. An example currently in use in Kenya is Amitraz [6] (commonly called Triatix^{R}), first developed in 1973.

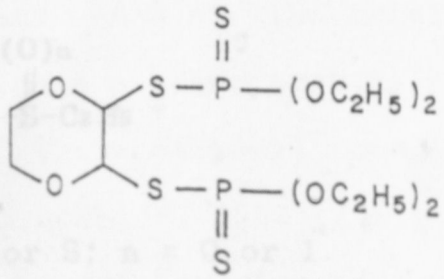


[6]

This is effective against all stages of numerous phytophagus mites as well as against ticks and some insects. Organochlorines which came into the market in the late 196Øs were replaced by pyrethrins and later by the synthetics. In the mid 197Øs, group I OPs were introduced, including compounds like Coumaphos [7] and Dioxathion [8].



[7]

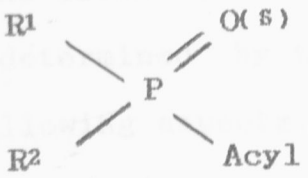


[8]

In 1981, the Kenya Veterinary Laboratories at Kabete reported further cases of resistance that now warranted the marketing and use of group II OP compounds. Chlorfenvinphos was therefore recommended for the affected areas in Kenya, and is the acaricide of the moment in most areas.

2.2 The Organophosphate Acaricides: Structure and Activity Relationships

Organophosphates having insecticidal activity may be traced back to a basic structure that is defined by the general formula:



which predicts that a biologically active phosphate will be obtained when either sulphur or oxygen is directly bound to the pentavalent phosphorus. In the skeleton structure, R^1 and R^2 may be alkoxy, alkyl or amino residues, while 'acyl' represents the anion of an organic or inorganic acid (e.g. fluorine, cyanate, thiocyanate) or of other acidic residues such as enol and mercapto groups. Systemic phosphates of the following general structure have acaricidal activity:

where R = C_2H_5; X = S or O; Y = O or S; n = O or 1.

The following general structure however describes the group of OPs to which development of resistance towards it is rather slow.

where R = CH_3 or C_2H_5; X = O or S; and Z may be any other

group.

Figure 2 gives the structures of common OP acaricides used in both veterinary and crop production in Kenya.

The ecological and biochemical behaviours of the OP pesticides is largely determined by their ester character, distinguished by the following aspects.

(a)Ease of chemical hydrolysis

(b)Trans-esterification and/or phosphorylation

(c)Dealkylation of triesters to diesters

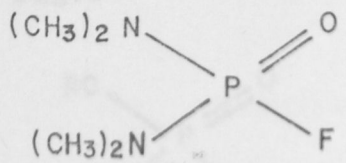
(d)Structural and toxic properties

(e)Resistance phenomena

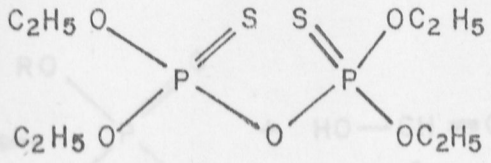
Acaricidal phosphates must possess an electron withdrawing substituent bonded to the phosphorus. Such compounds are susceptible to nucleophilic attack at the phosphorus atom. As one would expect, the enol phosphates are hydrolysed relatively rapidly in alkali but have a half-life in acidic media, of about a tenth longer than that of the trialkyl phosphates. The cause of this is again a prior protonation, which favours the formation of the carbonyl compound corresponding to the enol (Fest and Schmidt, 1983) (Fig.3).

The factors above have been useful in accounting for reduction in acute toxicity either by a replacement of O with S at the P-atom and vice versa,or a switch from diethyl phosphates to dimethyl analogues. In the case of O-phenylphosphates,steric effects have been used to explain the alteration of enzyme affinity and detoxification caused by the substituent in the meta position.

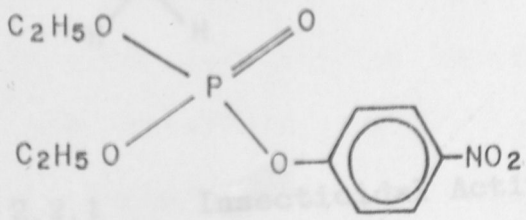
Fig. 2 Structures of common OP acaricides



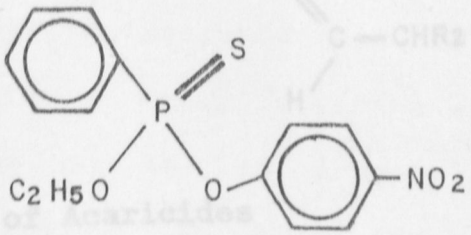
dimefox



sulfotepp



paraoxon



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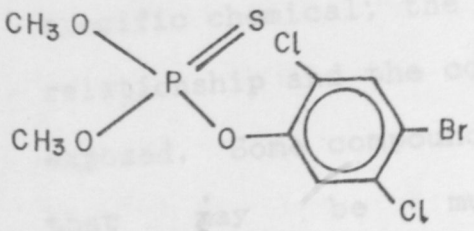
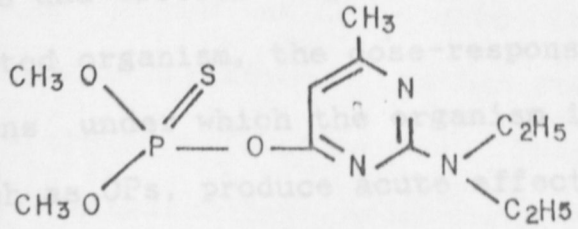
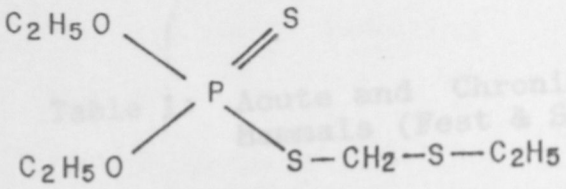


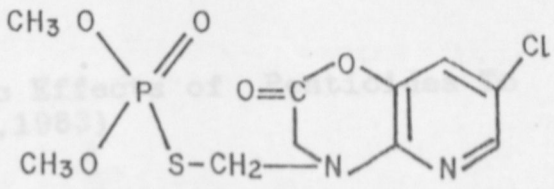
bromophos



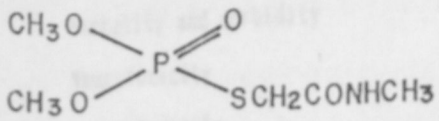
pirimiphos — methyl



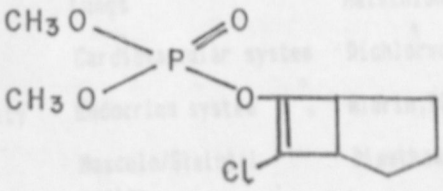
phorate



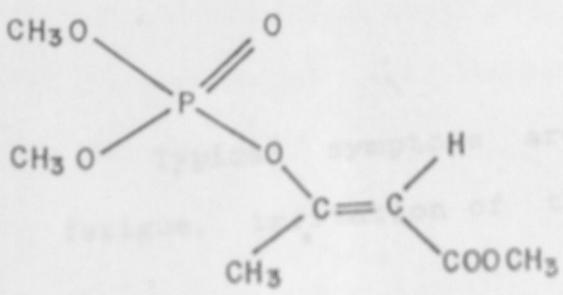
Azamethiphos



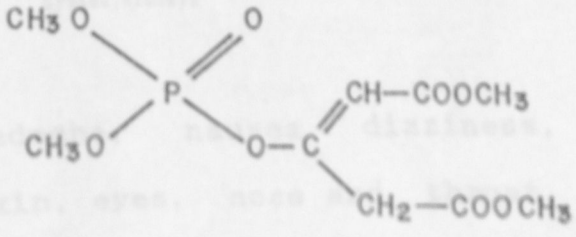
Omethoate



heptenophos

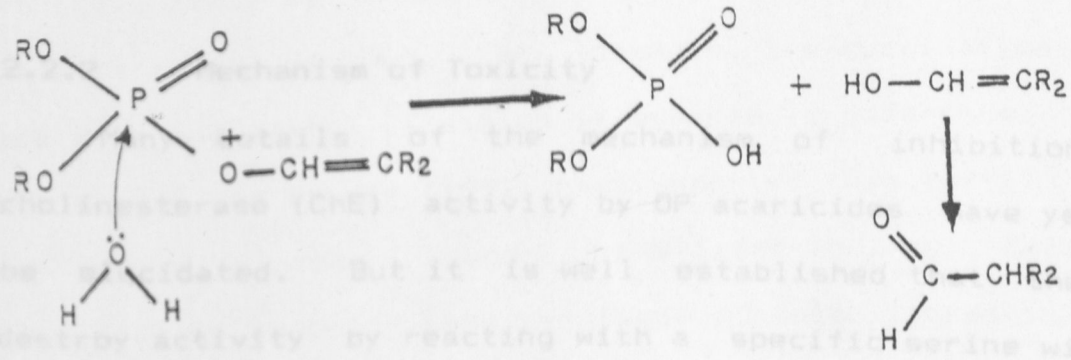


merinphos



bomyl

Fig.3



2.2.1 Insecticidal Activity of Acaricides

Pesticides are biologically active compounds that can produce a myriad of symptoms and effects depending on the specific chemical, the affected organism, the dose-response relationship and the conditions under which the organism is exposed. Some compounds, such as OPs, produce acute effects that may be mutagenic, teratogenic or even carcinogenic(Table 1).

Table 1: Acute and Chronic Toxic Effects of Pesticides To Mammals (Fest & Schmidt,1983)

Acute toxicity	Chronic toxicity	Target organ toxicity	Example of pesticides
Mortality and morbidity	Carcinogenicity	Lungs	Malathion,DDT
Neurotoxicity	Mutagenicity	Cardiovascular system	Dichlorvos,DDT
Immunotoxicity	Development toxicity	Endocrine system	Aldrin,DDT
Dermal toxicity		Musculo/Skeletal system	Dimethoate
Reproductive toxicity		Liver	Aldrin/Dieldrin
		Haematopoietic system,Kidneys	Aldrin

Typical symptoms are headache, nausea, dizziness, fatigue, irritation of the skin, eyes, nose and throat,

a,b).

2.2.2 Mechanism of Toxicity

Many details of the mechanism of inhibition of cholinesterase (ChE) activity by OP acaricides have yet to be elucidated. But it is well established that the OPs destroy activity by reacting with a specific serine within the catalytic centre of the enzyme to produce O,O-dialkylphosphoserine (Kennedy,1991). In this form, the enzyme is unable to hydrolyse choline esters. There is a direct correlation between the degree of OP-induced ChE inhibition and the degree of phosphorylation. Within ChE, only the active site serine is phosphorylated by OPs. These studies have been done using radiolabelled compounds (^{32}P and ^{3}H) (Kennedy,1991).

Consider the oxon metabolite of the common OP pesticide fenitrothion, reacting with active centre serine [9] (Fig.4).

The phosphorylated ChE [10] is inactive, but it can spontaneously dephosphorylate to its active form. The rate of regeneration of activity is directly proportional to the rate of dephosphorylation. In some situations, 'aging' of the enzyme occurs by the spontaneous dealkylation of one of the alkyl groups of O, O-dialkylphosphoserine (Kennedy, 1991). Aged ChE [11] is permanently inhibited. Unlike the non-aged form, an aged enzyme cannot be reactivated by 2-pyridinealdoxime methiodide (2-PAM) and other chemical reactivators.

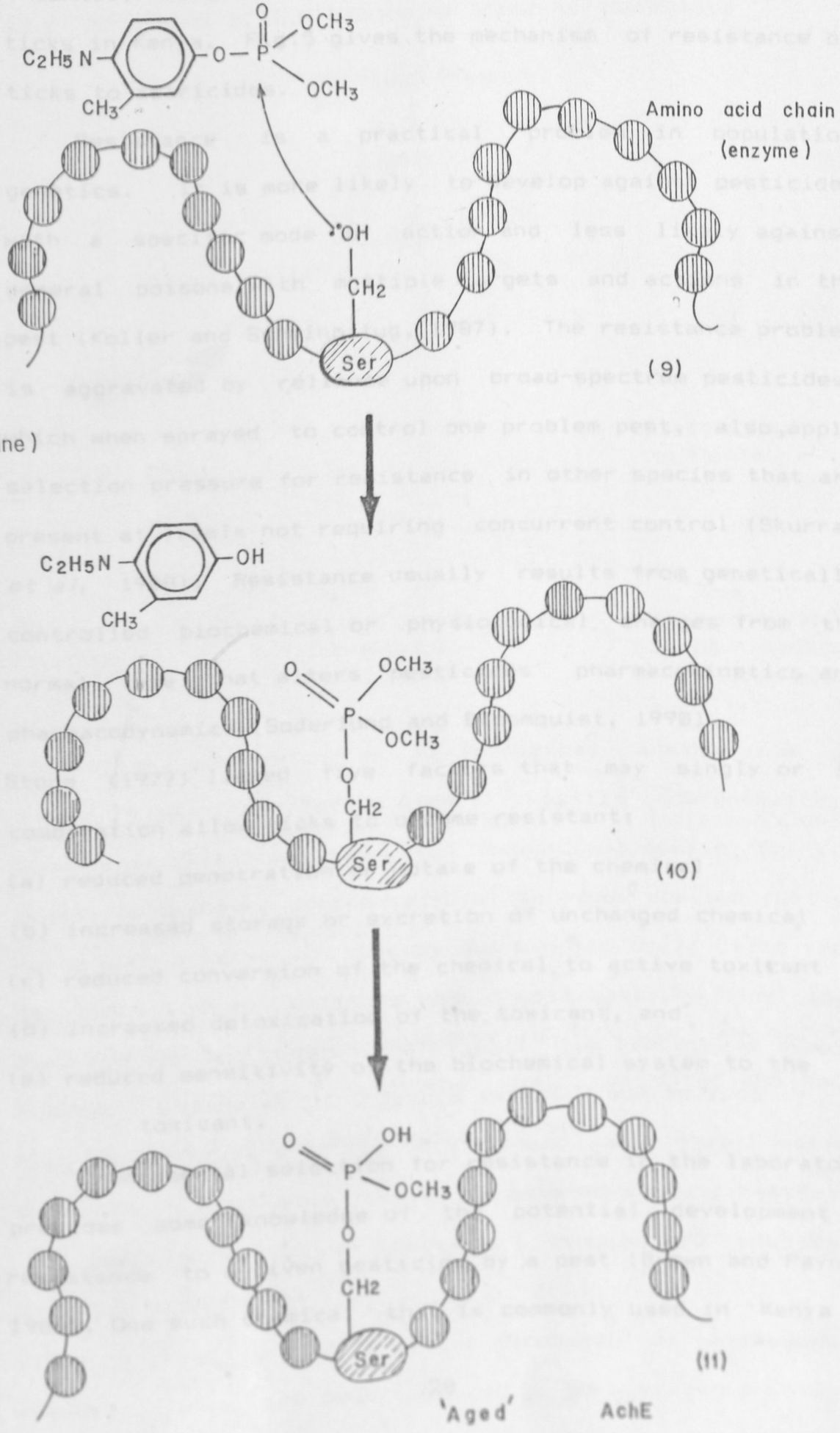
It is possible that several pesticides applied together can cause more damage than expected from their cumulative effects. This is synergism, and is a condition typical and characteristic of OPs. For instance, the combination of parathion and methyl parathion is especially hazardous.

Rengam and Snyder (1991) have reported that certain OPs such as dichlorvos cause a pesticide-induced delayed neurotoxicity (PIDN). Long and large diameter fibres in the spinal cord and peripheral nervous system are particularly susceptible. Prolonged conditions result in muscle weakness that may progress to paralysis. The feet and legs are usually more severely affected than the hands and arms. Onset is usually 2-4 weeks after the acute exposure. Thus, although OPs are the most widely used group of insecticides, the greatest health danger paused to field workers is acute poisoning. OPs are responsible for the largest number of cases of acute systemic illness every year in California among field workers exposed to pesticide residues (Kennedy, 1991). Poisoning from these compounds is attributed to the interference with the enzyme cholinesterase. Early indications of poisoning include headache, dizziness and flu-like symptoms;and victims usually have a lower-than-normal blood cholinesterase(ChE) activity.

2.3 Resistance

Resistance to acaricides by various tick species has been recorded, dating back to 1973 when *B. microphus* resisted arsenicals.Even today various OP group I compounds

Fig. 4 Mechanism of ChE inhibition by an OP compound.



like dioxathion (commonly called Delnav) and coumaphos (Asuntol) have also been reported to be resisted by various ticks in Kenya. Fig.5 gives the mechanism of resistance by ticks to acaricides.

Resistance is a practical problem in population genetics. It is more likely to develop against pesticides with a specific mode of action and less likely against general poisons with multiple targets and actions in the pest (Koller and Scheinpflug, 1987). The resistance problem is aggravated by reliance upon broad-spectrum pesticides, which when sprayed to control one problem pest, also apply selection pressure for resistance in other species that are present at levels not requiring concurrent control (Skurray *et al*, 1988). Resistance usually results from genetically controlled biochemical or physiological changes from the normal type that alters pesticides' pharmacokinetics and pharmacodynamics (Soderlund and Bloomquist, 1990).

Stone (1977) listed five factors that may singly or in combination allow ticks to become resistant:

(a) reduced penetration or uptake of the chemical

(b) increased storage or excretion of unchanged chemical

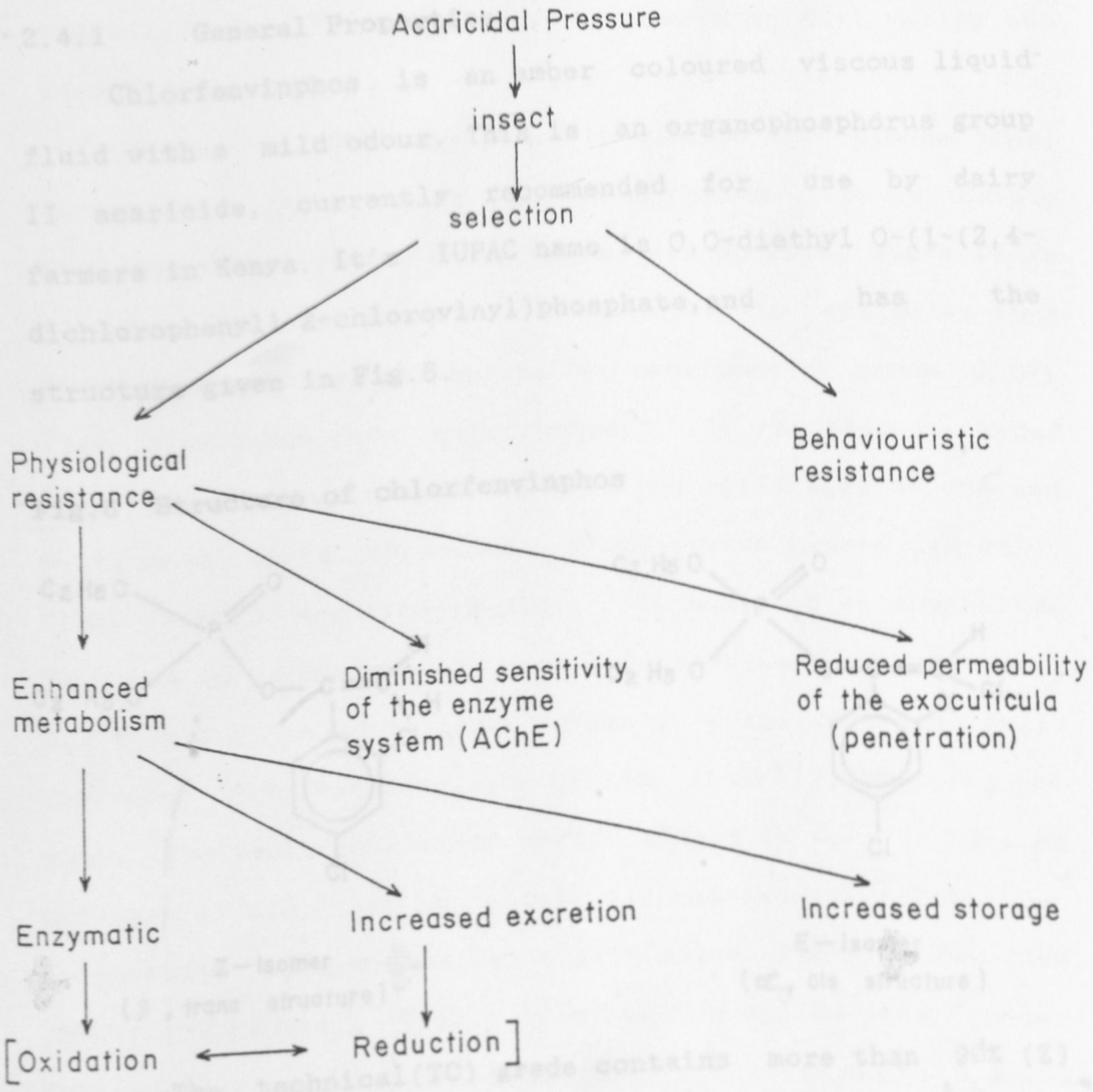
(c) reduced conversion of the chemical to active toxicant

(d) increased detoxication of the toxicant, and

(e) reduced sensitivity of the biochemical system to the toxicant.

Experimental selection for resistance in the laboratory provides some knowledge of the potential development of resistance to a given pesticide by a pest (Brown and Payne, 1988). One such chemical that is commonly used in Kenya as

Fig.5 Mechanism of resistance by ticks to acaricides



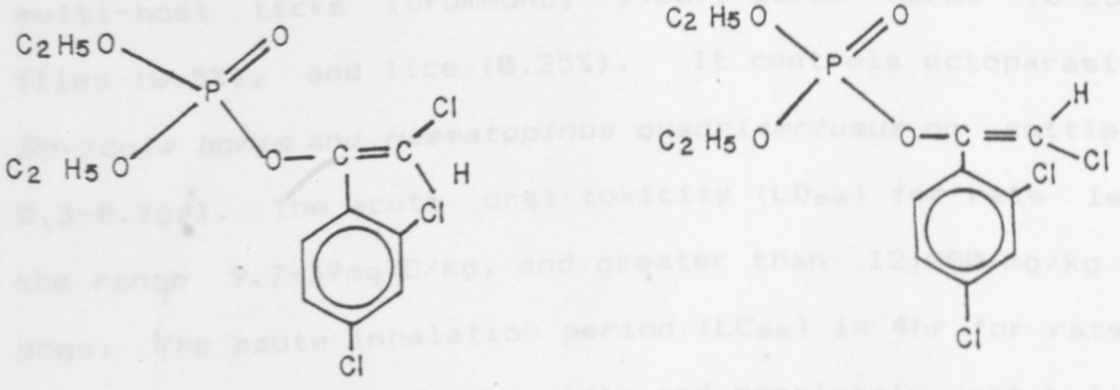
an acaricide and whose residues in food is the current subject of discussion around the world, and forms the basis of this study is chlorfenvinphos.

2.4 CHLORFENVINPHOS

2.4.1 General Properties

Chlorfenvinphos is an amber coloured viscous liquid fluid with a mild odour. This is an organophosphorus group II acaricide, currently recommended for use by dairy farmers in Kenya. It's IUPAC name is O,O-diethyl O-(1-(2,4-dichlorophenyl)-2-chlorovinyl)phosphate, and has the structure given in Fig.6.

Fig.6 Structure of chlorfenvinphos



Z – isomer
(β, trans structure)

E – isomer
(α, cis structure)

The technical(TC) grade contains more than 90% (Z) and (E) isomers, in a typical ratio of 8.4:1 respectively. It is miscible with acetone, dichloromethane, ethanol, hexane, kerosene, propylene glycol and xylene. Several formulations bearing different commercial names for the compound are *Birlane* (for crop protection) and *Supona* (veterinary), both from Shell, *Sapecron* and *Steladone* (Ciba-Geigy), *Apachlor* (Rhone-Poulenc) and *Supadip* (Welcome), most of which are available on the Kenyan market. *Steladone* is

sold as an emulsifiable concentrate, *STELADONE 300EC*, containing 300g/l of chlorfenvinphos active ingradient. Over 98% of the dairy farmers in Rift Valley use it for tick control.

Steladone 300EC has been formulated for plunge dips, spray-races, handdressing and handspraying. It is safely used on sheep, goats, dogs, horses and donkeys, where it is strictly applied dermally. It is lethal to all ticks that have developed resistance to OC compounds or group I OPs like dioxathion and quintiophos. It is also a broad spectrum ectoparasiticide, controlling all stages of one and multi-host ticks (Drummond, 1960), screw worms (0-25%), flies (0.5%), and lice (0.25%). It controls ectoparasites *Bovicola boris* and *Haematopinus quadripertusus* on cattle at 0.3-0.7g/l. The acute oral toxicity (LD_{50}) for rats is in the range 9.7-39mgTC/kg, and greater than 12,000 mg/kg for dogs. The acute inhalation period (LC_{50}) is 4hr for rats at 0.05mg/l of air. It is rapidly and completely metabolized in mammals; metabolites being eliminated within a few days (Pesticide Manual, 1991). In other countries like France, the material is used as a residual spray (0.05-0.1% a.i.) for the control of flies in dairy barns (FAO/WHO, 1990).

2.4.2 Formulation

The active ingredient chlorfenvinphos is insoluble in water and cannot be pulverized because of its physical properties. It can only be dissolved in organic solvents for application, but due to the limitations of using these solvents, like the high volatility, cost of formulation and

damage to tissues (Ghosh, 1989), methods have been developed to spread the active ingredient dissolved in a small volume of solvent over a large area. The medium of dispersion of this preparation is water, but due to its immiscibility with the organic solvents used, emulsifying agents are added to this solution. The agents are surface active, characterized by a molecule with both lipophilic and hydrophilic moieties on either end. Their function is to modify the properties of the interface between the dispersed and continuous phase.

Steladone 300EC is an emulsifiable concentrate which when diluted with water produces a very stale but well dispersed milky emulsion. The emulsion maintains the correct concentration of acaricide in the dip wash over a long period of time. Animals whose coats are thoroughly wetted will have all ectoparasites killed. The available oily solvents facilitate adherence of the active ingredient onto the animal's coat hence giving it protection for a period of 3-5 days. Some of these solvents act as stickers to improve spray retention on surface. Some are penetrants to facilitate the penetration activity of the active ingredient into the target organism. The oily nature of the solvents enables penetration through the waxy cuticle of ticks. Humectants are added to delay evaporation of the water carrier. Stabilizing agents are incorporated to reduce the possible speedy degradation of the acaricide during storage.

2.4.3. Methods of Application

(a) Plunge dips: Initial filling is done by diluting *Steladone 300EC* at a rate of 1 litre of acaricide to 600 litres of water. Replenishing, is done by diluting 3 litres of Steladone 300EC into 1000 litres of water (1:333) to give a dip wash concentration of 0.05%. Plate (b) shows cattle fully immersed in a plunge dip.

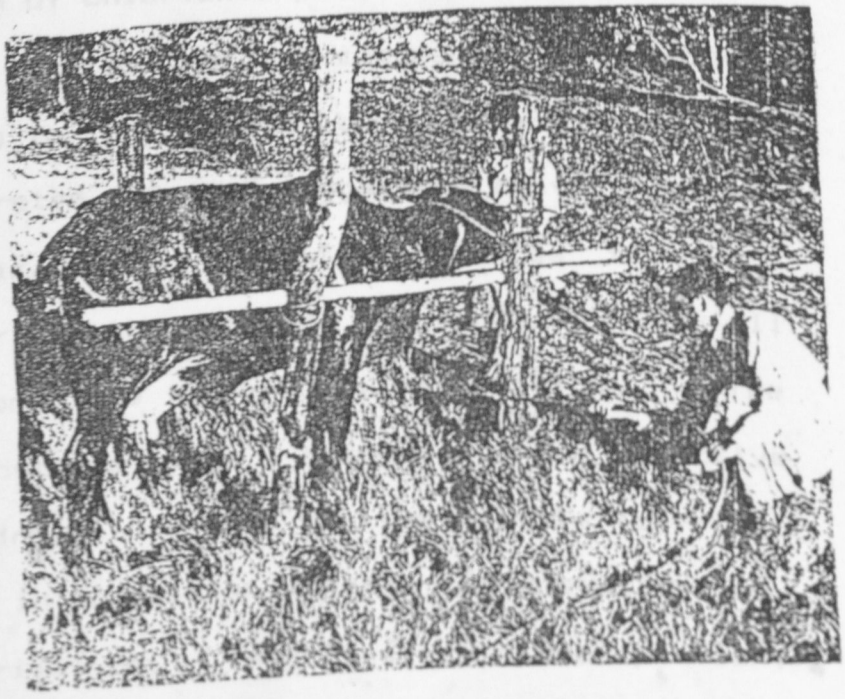
Plate (b)

(b)Spray race: Initial filling contains chlorfenvinphos at a concentration of Ø.Ø5% but is boosted to retain strength after every 1ØØ head of cattle are sprayed. The boosting is done by adding 1Øml into the tank (1 ml chlorfenvinphos per head). Plate (c) is an example of the spray race mode.

Plate (c)

(c) Handspraying: Steladone 300EC is diluted at 1:600 (25 ml in 15 litres of water) in a knapsack handsprayer. This conveniently sprays 3-4 mature cattle allowing enough spraywash to ensure thorough wetting of the coat. Both brown ear and red legged ticks transmit ECF and are eliminated by spraying the ears and tail base where they reside, respectively.

Plate (d) local farmers handspraying an animal in a village



(d) Handdressing: 2 ml of Steladone 300EC are added to 1 litre of water and applied with swab or brush around the ears and horn base as well as under the tail. The exercise therefore involves application of the emulsion to localized parts of the animal's sites for ticks.

The application frequency depends on the weather conditions and the parasite being controlled. On the animal, all organophosphate compounds are broken down by UV light of the sun and the residual effect is a maximum 6 days. For ticks, the animals are therefore treated at least once a week. In cases where there is an outbreak of high ECF,or during rainy seasons, treatment is twice weekly on a 3/4/3 day interval.

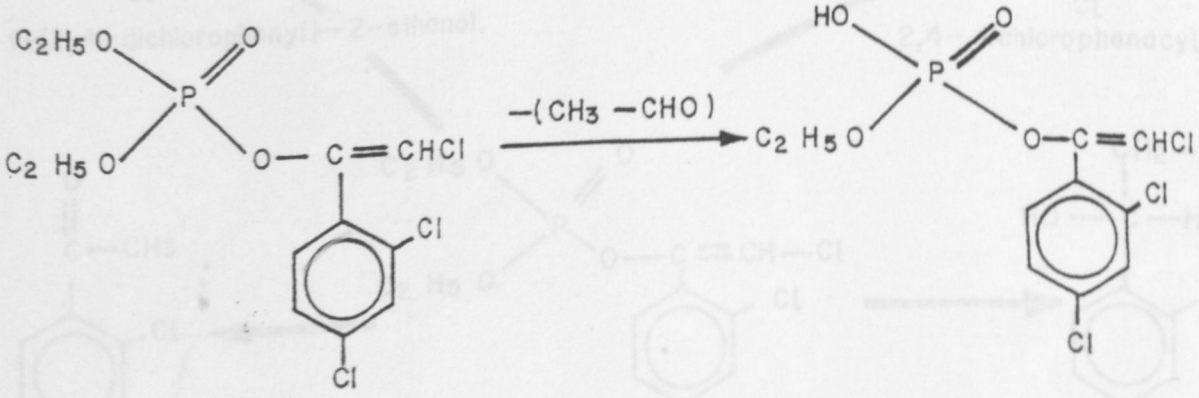
2.4.4 Metabolism of Chlorfenvinphos

When organisms take up foreign compounds, they protect themselves by trying to convert them to innocuous substances which are then excreted. Animal organisms convert foreign compounds into polar, water soluble substances that are excreted as such or in the form of conjugates. Not all metabolic conversions are indeed detoxifications. The phosphates for instance are detoxified to inactive metabolites by hydrolysis. Some of the simple chemical reactions involved in these conversions include oxidation, reduction, isomerization, hydrolysis, dealkylation, degradation at the carboxyl group and conjugation.

As a rule, insecticides are not degraded by just one of the above mechanisms but several reactions occur simultaneously. In mammals, the detoxification of the insecticides is mainly carried out in the liver owing to its high esterase content, the toxic oxygen compounds being hydrolysed. The O-dealkylation of triesters to give water soluble diester acids is the third most important mechanism

of detoxification *in vivo*. This mechanism plays an important role in selectivity and resistance both in plant and animal organisms. Of the various biochemical mechanisms studied, the oxidative O-dealkylation has been proven in the case of chlorfenvinphos (Fest and Schmidt, 1983). Reduced nicotinamide adenine dinucleotide phosphate (NADPH) and oxygen are required for the reaction, the end products being O-desethyl-chlorfenvinphos and acetaldehyde (Fig.7).

Fig.7.

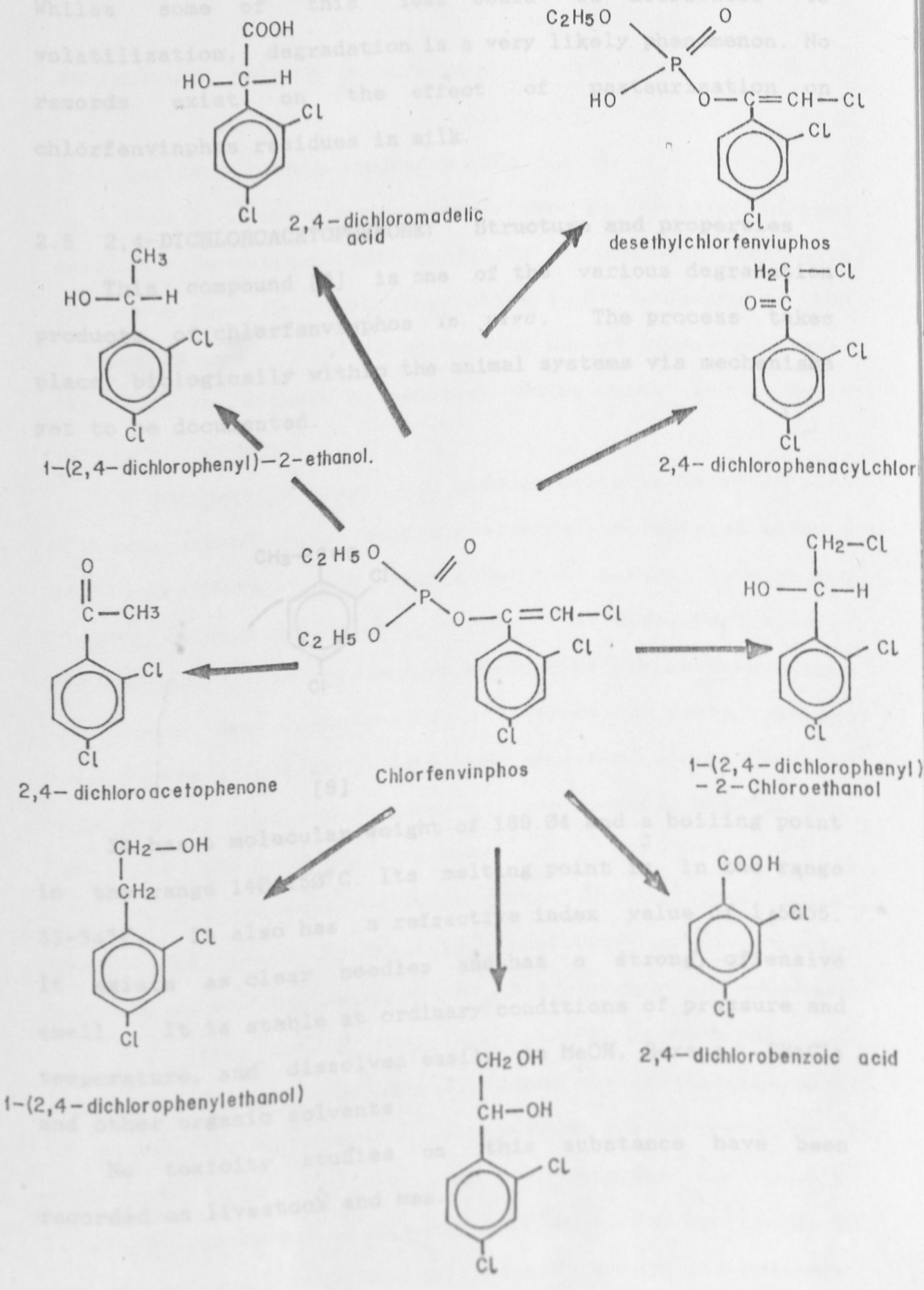


In dairy animals, chlorfenvinphos undergoes metabolic degradation to yield various products(Fig.8) all detected in milk fat (Moye, 1986).

2.4.5 Thermal Stability

The rate of thermal decomposition of solid chlorfenvinphos is negligible below 90°C and is slow even at 120°C-130°C (Beynon *et al*, 1973). However, in solution the degradation rate is higher and is also likely to be so in heated crops.

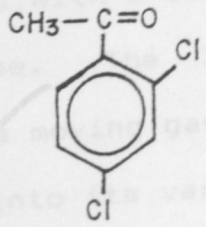
Fig. 8 Metabolites of chlorfenvinphos detected in milk



Whilst some of this loss could be attributed to volatilization, degradation is a very likely phenomenon. No records exist on the effect of pasteurization on chlorfenvinphos residues in milk.

2.5 2,4-DICHLOROACETOPHENONE: Structure and properties

This compound [9] is one of the various degradation products of chlorfenvinphos *in vivo*. The process takes place biologically within the animal systems via mechanisms yet to be documented.



[9]

It has a molecular weight of 189.Ø4 and a boiling point in the range 14Ø-15Ø°C. Its melting point is in the range 33-34°C. It also has a refractive index value of 1.5635. It exists as clear needles and has a strong, offensive smell. It is stable at ordinary conditions of pressure and temperature, and dissolves easily in MeOH, Hexane, CH_2Cl_2 and other organic solvents.

No toxicity studies on this substance have been recorded on livestock and man.

CHAPTER THREE

INSTRUMENTAL METHODS OF ANALYSIS

3.1 Gas liquid chromatography (GC)

This is a technique commonly used in the determination of pesticide residues both qualitatively and quantitatively. The technique involves the process of separating the components of a mixture by making use of their partition coefficients between a gaseous moving phase and a solid stationary phase.

A compound in liquid or gaseous state is injected into a column packed with a suitable material impregnated with a stationary phase. The liquid is then pushed through the column using a moving gas such as nitrogen. Partition of the compound into its various components takes place along the column. Each component is ionized by the flame (in the case of flame ionization detection) and the current produced subsequently lowers the standing flame current. This is then amplified and detected as a peak.

The FID was employed because of its high sensitivity, wide range, great reliability, simplicity of construction and operation. The response of this detector is dependent upon the formation of ionic species when carbon compounds are burnt in a hydrogen flame. In GC, the identification of the constituent compounds is based on the correlation of retention times of the peak of interest to those of reference standards. Similarly, peak area is proportional to the amount of compound that has passed through the detector.

3.2 High Pressure Liquid Chromatography (HPLC)

The main components of a HPLC are a high pressure pump, a column/injector system, and a detector. In addition, components such as solvent reservoirs, in-line filters, pressure gauges, recorders, integrators and minor components may be required. In HPLC the eluent from the solvent reservoir is filtered, pressurized and pumped through the column packed with relevant organic material. A mixture of solutes injected at the top of the column is separated into components on travelling down the column and the individual solutes are monitored by the detector and recorded as peaks on a chart recorder.

The two common detectors known are the Refractive Index (RI) detector and the ultra violet (UV) detector. The UV detector is preferred as it simply measures the change in UV absorption as the solute passes through a flow cell at a fixed wavelength. The column, usually a metal or glass tube is packed with high molecular weight organic material like the C-18s, to give high-efficiency separating columns. Columns may be run isocratically (with fixed eluent composition) or in the gradient elution mode in which the mobile phase composition varies during the run. Like for the GC, quantitative analysis is carried out by measuring the peak areas or peak heights in the chromatogram, whereas qualitative analysis is achieved by comparing the retention times of peaks of interest with those of reference standards.

The **Reverse Phase** HPLC mode is commonly adopted. Molecules that are highly polar give long retention times

and peak tailing problems in adsorption (normal phase) chromatography. The remedy here lies in resolving the compounds using a non-polar stationary phase in conjunction with a polar mobile phase, like methanol/water mixture (Moye,1986). The polar molecules now have little affinity for the hydrophobic support and are eluted relatively quickly by the aqueous mobile phase, hence most polar molecules are eluted first. Examples of column packings of this type include Permaphase ODS, Spherisorb ODS and μBondapak C_{18} (Pryde and Gilbert,1985).

3.3 Mass Spectrometry

Liquid samples are volatilized under vacuum in a heated reservoir (about 1μg), and the vapour is leaked into the ionization chamber. To facilitate volatilization, especially of samples with high boiling points, heating of the reservoir is necessary. Solid samples are introduced into the ionization chamber on the tip of an insertion probe.

The sample is bombarded with a high energy (70 eV) stream of electrons from the ion source. The energy absorbed by the molecules promotes ionization by loss of electrons from bonding and non-bonding orbitals. The ions formed by the removal of one electron from the original molecule are called molecular or parent ions. Some of the molecular ions split into smaller (daughter) ions and neutral fragments. Both positive and negative ions are formed, but only the positively charged species are of concern. A controlled positive potential is used to repel the positive ions out of

the ionization chamber. An acceleration plate with a positive potential of 2000 V is used to accelerate the positive ions down the tube into the magnetic field. The ions are deflected by the magnetic field depending on their mass/charge (m/e) ratio. The ion beam is thus split into component ion beams of different m/e ratios. Each ion beam in turn is made to pass through a collector slit and impinges on a collector plate. Each ion acquires an electron from the plate. A flow of current is produced in the collector circuit, amplified and recorded as a function of the m/e ratio. The size of each peak is a measure of the relative number of ions in each beam.

Combined gas chromatography/mass spectrometry (GC-MS), is a method of choice for analysis of organic mixtures. The ability to separate a complex mixture of organic compounds and identify the individual components, or to look for a specific compound in a complex mixture are the two primary applications of GC-MS. The requirements for the use of GC-MS are that the mixture be amenable for analysis by GC, and that the compounds or their derivatives must be volatile but not thermally labile.

Mass spectrometry has been employed for the detection and confirmation of various classes of fumigants and for analysis of pesticide residues in food and water by both single and multiple ion monitoring. Although a weak molecular ion cluster may be evident at various m/e ratios, the most intense peak in the spectrum of the standard is normally employed for analytical purposes.

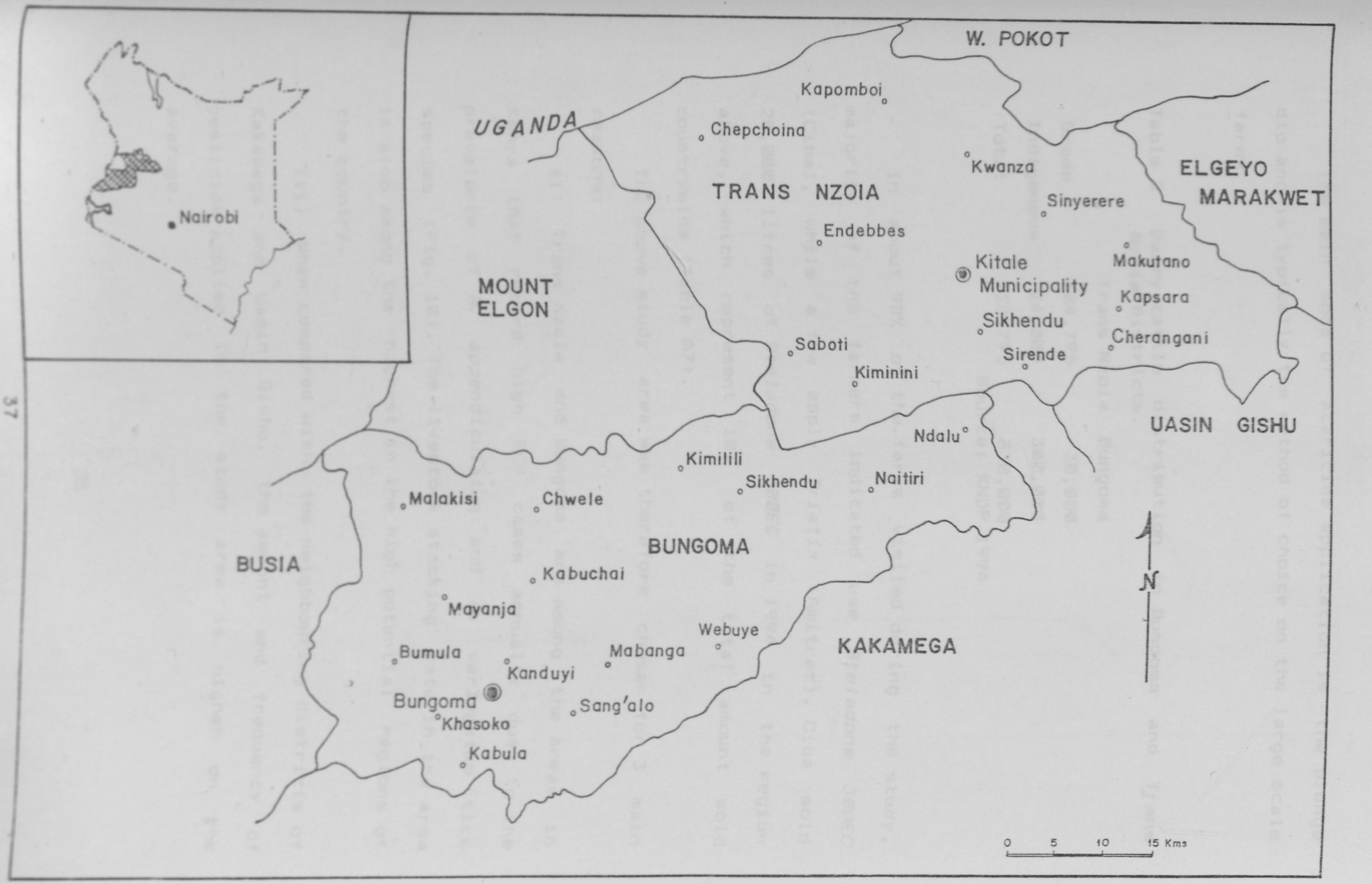
CHAPTER FOUR

MATERIALS AND METHODS

4.1 STUDY AREA

The sampling sites covered an area which lies between 5900-6300ft above sea level, comprising two districts: Bungoma and Trans-Nzoia in Western Kenya (Fig.9). Annual temperatures vary between 9.5°C-30.6°C and mean annual rainfall values fall in the range 900 to 1200 mm. The only KCC factory in the area is located at Kitale.There are various other small coolers and processing units operative in a number of Co-operative society premises and large scale farms.Trans Nzoia has 407 cattle dips, 154 of which are privately owned. The rest are communal, half of which are found in Saboti division. Bungoma has 186 cattle dips while Kanduyi division has the highest number. 60% of the dipping facilities are underutilized due to poor maintanance of the infra-structure and lack of extra income by the farmers to pay for dipping fees. In Bungoma, the majority Zebu cattle kept by farmers are resistant to most tick-borne diseases hence see no need to dip them, and in most cases the animals kept are of low economic value in terms of milk production (KNDP,1994). Table 2 gives the dairy cattle distribution in the area. The region is characterized by a large number of commercial dairy farms owned by individual farmers, government institutes and Agricultural Developement Corporation.

Fig. 9 STUDY AREA



The main mode of acaricide application is the plunge dip and is typically the method of choice on the large scale farms.

Table 2 Dairy cattle distribution in Bungoma and Trans Nzoia Districts.

	Trans Nzoia	Bungoma
Grade	104,795	30,000
Indigenous	24,000	300,000
Total	128,795	330,000

Source: KNDP,1994

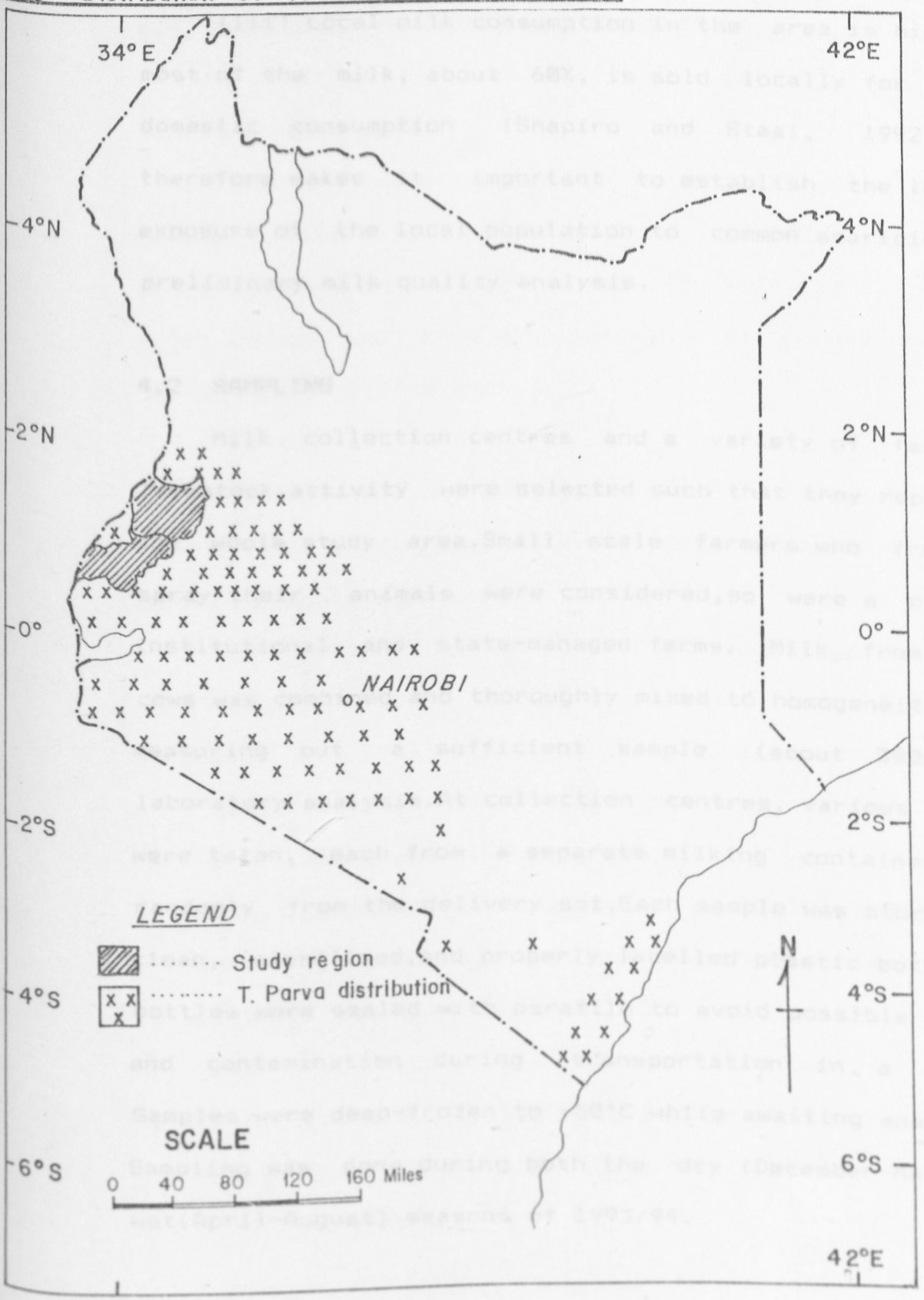
In about 98% of the farms visited during the study, majority of the farmers indicated use *Steladone 300EC* (Ciba), while a few apply *Triatix* (Amitraz). Ciba sold 20,000 litres of *Steladone 300EC* in 1994 in the region above, which represent 10% of the total amount sold countrywide (Table A7).

The above study area was therefore chosen for 3 main reasons:

(i) Trans Nzoia and Bungoma are among the areas in Kenya that record high ECF cases annually due to the prevalence of *R. appendiculatus* and *A. variegatum* tick species (Fig. 10). The livestock stocking rate in the area is also among the highest in the high potential regions of the country.

(ii) When compared with the neighbouring districts of Kakamega and Uasin Gishu, the amount and frequency of pesticide applied in the study area is higher on the average.

Fig.10 Distribution of Theileria parva in Kenya



(iii) Local milk consumption in the area is high since most of the milk, about 60%, is sold locally for direct, domestic consumption (Shapiro and Staal, 1992). This therefore makes it important to establish the level of exposure of the local population to common acaricides from preliminary milk quality analysis.

4.2 SAMPLING

Milk collection centres and a variety of farms with livestock activity were selected such that they represented the whole study area.Small scale farmers who frequently spray their animals were considered,so were a number of institutional and state-managed farms. Milk from various cows was combined and thoroughly mixed to homogeneity before measuring out a sufficient sample (about 300ml) for laboratory analysis.At collection centres, various samples were taken, each from a separate milking container chosen randomly from the delivery set.Each sample was stored in a clean, sterilized,and properly labelled plastic bottle. The bottles were sealed with parafilm to avoid possible spillage and contamination during transportation in a coolbox. Samples were deep-frozen to -50°C while awaiting analysis. Sampling was done during both the dry (December-March) and wet(April-August) seasons of 1993/94.

4.3. LABORATORY MATERIALS AND REAGENTS

4.3.1. Glassware

All glassware (including the borosilicate vials and syringes) was soaked in detergent, washed thoroughly and dried at 100°C in the oven. They were all rinsed first with pure acetone and then with n-hexane to remove traces of contamination before use.

4.3.2. Solvents and Reagents

The following reagents were used in the extraction and chromatographic screening.

(i) **n-Hexane, acetone** and **dichloromethane** were doubly distilled on a glass column and purity checked using gas chromatography (GC) with flame ionization detection (FID).

(ii) **Acetonitrile** and **methanol** were of high pressure liquid chromatography (HPLC) grade.

(iii) **Acetonitrile saturated with n-hexane(ASH)** was prepared by adding equal volumes of hexane and acetonitrile to a 500ml separatory funnel and shaking for about 2 minutes. The mixture was then let to stand for the two layers to separate. The lower layer was retained for use.

(iv) Where applicable,**distilled deionised water(DIW)** was used throughout.

(v) **Sodium sulphate** and **Florisil gel** were heated overnight at about 300°C in an oven, then cooled and stored moisture-free before use.

(vi) **Saturated sodium sulphate solution:**200ml of DIW

was placed in a beaker and pre-treated sodium sulphate added to saturation. Fresh solutions were prepared each working day and stored in sealed bottles before use.

(vii) Ø.1M Phosphate Buffer (pH=6.Ø): This was made by dissolving 1.361g of KH_2PO_4 in 1ØØml of water to make Ø.1M solution. Aliquots of it were added to about 2Ø ml of Ø.1M NaOH with stirring in a sterile beaker, until a pH of 6.Ø was reached. The solution was kept in a dark place under refrigeration, for a week's use.

(viii) Glass wool (fibre glass): A reasonable size of glass wool was stuffed in a Soxhlet extractor and enough n-hexane added to the reservoir. The reservoir was heated under reflux for 4 hr after which the glasswool was removed, dried and stored in a dessicator before use.

4.3.3 Standards

Chlorfenvinphos:

An aliquot(7.4µl) of a chlorfenvinphos standard of 99.5% purity and density 1.361g/l was added into a 1ØØml volumetric flask using a Hamilton syringe and made to the mark with dichloromethane. This provided a stock solution of 1ØØppm.

2,4-dichloroacetophenone:

Ø.Ø1g of the crystalline compound was dissolved in dichloromethane in a 1ØØml volumetric flask then made to the mark to provide 1ØØppm stock solution. Both stock solutions

were kept in the dark under refrigeration as they are susceptible to degradation under UV light.

4.3.4 Analytical Instruments

(i) Gas Chromatography: A Perkin-Elmer Model 8500 fitted with FID was used for chromatographic measurements.

(ii) A VWR Scientific pH meter was used for determination of pH.

(iii) A Beckmann Model 334 Gradient Liquid Chromatograph, equipped with a Hitachi 100-40 UV Detector, and Spherisorb ODS and µBondapak C_{18} columns was used for all HPLC measurements.

(iv) A GC-Mass Spectrometer Model VG 12-250 fitted with a capillary Ultra 1 column was used.

4.4. SAMPLE TREATMENT

4.4.1 Butter Oil Extraction

The procedure reported by McLeod and Ritcey (1973), was used,with some modification in certain steps.

Milk (100g) was transferred quantitatively into a Waring Commercial blender. 90ml of acetone and 50ml of pure n-hexane were then added and the mixture blended for three minutes to give a slurry which was then transferred immediately into six 40ml centrifuge tubes.The blender was rinsed with 30ml hexane followed by 14ml of water and the rinsings added to the tubes. The mixed slurry was then

centrifuged for 5 minutes at 2000 r.p.m.(IEC Clinical Centrifuge, CAT 801). The yellow hexane phase containing butterfat was drained into a 250ml beaker using a teat-pipette.5g of sodium sulphate was added and the mixture shaken and left to stand for ten minutes. The contents were filtered (Whatman No.1) and the solvent evaporated using a rotary evaporator(Achelis & Sons, Germany). The oil was then transferred qualitatively to a pre-weighed vial using a minimum (1ml portions) of dichloromethane. The dichloromethane was then evaporated on a waterbath set at 40°C. The weight of the dry butter-oil residue was recorded.

4.4.2 Partitioning

The butter-oil obtained above was dissolved in 10ml of hexane and contents transferred quantitatively to a 125ml separatory funnel using two 10ml- portions of hexane. The solution was then extracted by vigorously shaking with four 5ml-portions of ASH. To the acetonitrile phase was added 5ml of dichloromethane and 5ml of phosphate buffer followed by 50ml of hexane. The mixture was shaken vigorously for 1 minute,before adding 100ml of water and 15ml of saturated sodium sulphate. The mixture was shaken again for 2 minutes and the layers allowed to separate. The (lower) acetonitrile/water phase was discarded,and the hexane phases washed twice with 5ml portions of water. The organic phase was filtered through Whatman No.1 filter paper and 25g of sodium sulphate. The funnel was rinsed with 25ml hexane, and the washings combined. The solution was finally concentrated

to about 10ml using a rotary evaporator.

4.4.3 Extract Clean-up

A clamped glass column measuring 30cm long by 22mm internal diameter, previously rinse with acetone and n-hexane was set and a bud of glasswool was stuffed at the constriction. A slurry of Florisil was prepared by stirring a mixture of 9g of Florisil and 40ml of hexane in a beaker. The slurry was then added to the column to a height of about 10cm. To this Florisil bed was added sodium sulphate to a height of 1cm.

The butter oil extract (section 4.4.2) dissolved in hexane was transferred to the column quantitatively with two 5ml portions of hexane,taking precautions to allow the hexane layer to traverse the sodium sulphate bed before addition of the next hexane portion. The column was then eluted with 25ml of hexane followed by another 50% hexane in dichloromethane. The final eluant was 25ml of pure dichloromethane. The eluent portions were combined and solvents evaporated using a rotary evaporator. The residue was finally transferred to a storage vial using 5ml of dichloromethane. All extracts were stored in a refrigerator awaiting chromatographic analysis.

4.5.0 HPLC ANALYSIS

4.5.1. The Mobile Phase

Preliminary tests with various concentrations of methanol and acetonitrile in water established that 70% aqueous methanol gave effective separation of chlorfenvinphos peaks from those of the metabolite 2,4-dichloroacetophenone within a short time (15 min). A further advantage of this solvent system was the elimination of interferences due to non-polar substances.Prior degassing of the mobile phase with O_2-free N_2 improved column efficiency.

4.5.2. Recovery Tests

One ml aliquots of standard chlorfenvinphos and the metabolite 2,4-dichloroacetophenone in the concentration range 0.003-0.05ppm and 7.5-100ppm, respectively,were added to weighed amounts of a milk sample taken from the same source. The spiked samples were blended and extracted in accordance with the sample procedure described previously. The clean butter oil extracts obtained were analysed by HPLC and the amounts of each added standard calculated to check the effect of sample matrix, clean up procedure and the extraction steps on the recovery.

4.5.3 Sample Batch Analysis

The butter oil extracts were each redissolved in 2ml of methanol and aliquots analysed in triplicate by HPLC at a fixed attenuation setting.Measurable peak heights for both compounds were recorded and the concentrations of the two

compounds calculated.

4.5.4. Confirmatory Analysis

A few of the milk samples that showed residues of chlorfenvinphos and 2,4-dichloroacetophenone were further subjected to comparative analysis using GC-MS detection so as to confirm their identity and presence by the various characteristic ion species. This is the most convincing confirmatory procedure (Stan,1992) in pesticide residue analysis in food and environment. The approach adopted here was that reported by Gilbert *et al*,(1987).

4.6 OPERATING CONDITIONS

4.6.1 HPLC

The following conditions were established for sample analysis by high pressure liquid chromatography.

Mobile phase:	70% methanol: 30% water
Column (RP):	µBondapak C_{18}
Wavelength:	UV 254 nm
Flow rate:	2 ml/min.
Chart speed:	1 cm/min.
Temperature:	ambient
Loop:	fixed at 20 µl

4.6.2 GC-MS (VG-12-250)

Column: Capillary Ultra1 (50m)
Carrier Gas: Helium
Run Time: 52 minutes
Programme:

Run Stage :	1	2	3
Temperature (°C):	50	200	280
Iso time (min) :	0	0	2
Ramp rate(°C/min):	5	4	

4.6.3 GC (Perkin Elmer 8500)

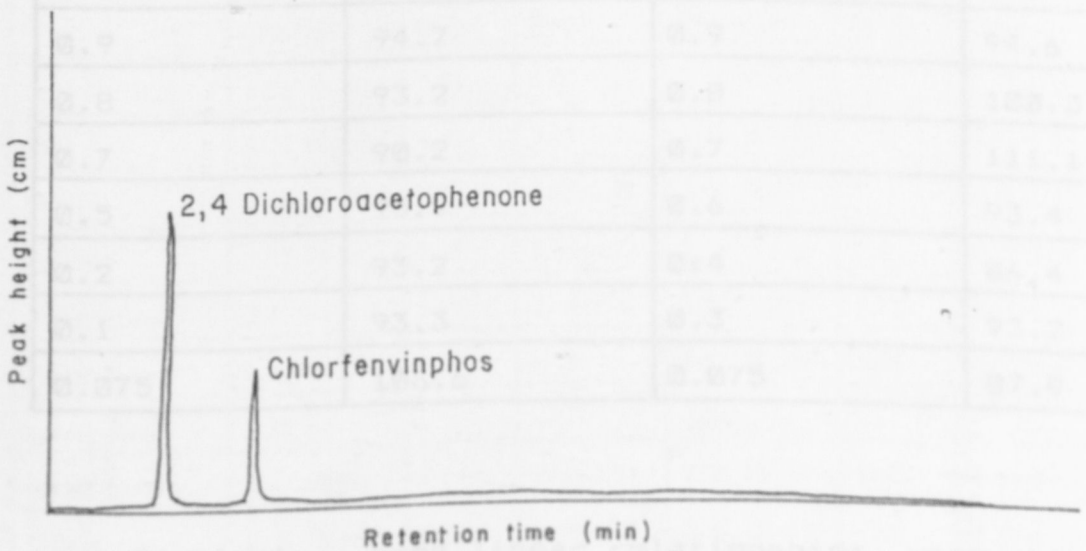
Column: Cappilary, SE-45
Carrier Gas: Nitrogen
Run Type: Isothermal 180°C

CHAPTER FIVE

RESULTS

The optimum conditions (section 4.6) gave satisfactory resolution when recovery and calibration data were assessed both for the acaricide and its metabolite. Good linear relationships were obtained between peak heights and concentrations or amounts of compound added; slope, intercept and r^2 were Ø.444, Ø.ØØØ39 and Ø.99Ø8 for chlorfenvinphos and 18.544, 9.598 and Ø.9928 for 2,4-dichloroacetophenone (Figs.A.1 and A.3). A typical chromatogram is shown in Fig. 11.

Fig. 11 A typical chromatogram showing separation between the peaks of chlorfenvinphos and 2,4-dichloroacetophenone



5.1 Recovery Tests

These were determined using the standard addition techniques. Working standards in the range Ø.Ø75 - 1.Ø ng for chlorfenvinphos and (Ø.Ø75 - 1.Ø µg) for 2,4-

dichloroacetophenone were added to selected milk sample aliquots (100g) which had previously been analysed and the content of residue and metabolite recorded. Extraction and analysis were carried out through the entire procedure under similar condition. Percent recovery was calculated from the difference between the original sample concentration and the net spiked (sample + standard) concentration. The mean percentage recovery data are shown in Table 3.

Table 3 Recoveries of chlorfenvinphos and 2,4-dichloroacetophenone from spiked cow milk.

CHLORFENVINPHOS		2,4-DICHLOROACETOPHENONE	
Amount Added (ng)	% Recovered	Amount Added (µg)	% Recovered
1.0	89.0	1.0	92.4
0.9	94.7	0.9	94.6
0.8	93.2	0.8	100.3
0.7	90.2	0.7	111.1
0.5	95.9	0.6	93.4
0.2	93.2	0.4	86.4
0.1	93.3	0.3	93.2
0.075	106.6	0.075	87.9

Similarly, good linear relationships were obtained in both cases; slope, intercept and r^2 were 3.755, -0.059 and 0.936 for chlorfenvinphos and 65.208, 1.638 and 0.9817 respectively for 2,4-dichloroacetophenone (Fig. A.2 and A.4).

5.2 Batch Analysis Results

Tables A.1-A4 gives the analysis results for all the 1ØØ samples tested for chlorfenvinphos and 2,4-dichloroacetophenone. It can be observed from these data that in the dry season, no metabolite was detected at all, while in the rainy season only five cases tested positive to it.

5.3 INFLUENCE OF WEATHER-CHANGE ON FAT CONTENT

Analysis results for samples collected from selected centres in both seasons are presented in Table 4.

Table 4 Variation of milkfat content (%) with seasonal change

CENTRE	DRY SEASONS			WET SEASON		
	Mean(±SD)	Median	Range	Mean(±SD)	Median	Range
Sang'alo Inst.	3.83 (Ø.26)	3.86	3.59-4.31	3.47 (Ø.61)	3.5	2.83-4.Ø6
LBDA	4.28 (Ø.86)	4.92	2.69-5.35	3.95 (Ø.97)	4.34	2.51-4.6
FTC	6.16 (1.27)	6.71	3.Ø8-6.79	5.32 (Ø.87)	5.46	4.2Ø-6.17
Kitinda	5.93 (Ø.84)	5.79	5.42-6.72	3.31 (Ø.52)	2.93	2.72-3.97
Nzoia Scheme	3.7 (1.17)	4.19	2.44-4.74	3.68 (1.3)	3.68	2.76-4.61
Okwomi Farm	4.2Ø -	-	-	3.89 (Ø.87)	3.89	3.37-4.41
Barasa Farm	3.99 -	-	-	3.24 -	-	-
Kituyi farm	2.61 (Ø.9)	-	1.98-3.64	2.15 (Ø.3)	2.15	2.13-2.17
TOTALS	4.34 (Ø.18)	4.Ø9	1.98-6.79	3.63 (Ø.67)	3.Ø8	2.13-6.17

(SD= Standard Deviation)

The butterfat content in the milk was generally higher ($P<Ø.Ø5$) in samples collected during the dry season than in those taken during the wet season in both districts.

5.4 EFFECT OF SEASONAL CHANGE ON CHLORFENVINPHOS RESIDUES IN MILK

Tables 5 and 6 give data on the residues of chlorfenvinphos in milk sampled during the two seasons.

Table 5 Chlorfenvinphos residues (µg/kg) in milk from Bungoma district.

CENTRE	WET SEASON		DRY SEASON	
	Mean (±SD)	Median	Mean (±D)	Median
Sang'alo Inst.	2.88 (0.33)	2.7	1.46 (0.95)	1.17
LBDA	4.53 -	-	1.28 (1.07)	1.28
FTC	2.79 (0.14)	2.79	1.18 (0.12)	1.16
Kitinda Centre	3.62 (0.74)	3.92	1.84 (0.66)	1.84
Ndalu Centre	6.75 (0.76)	7.00	2.18 (1.44)	2.18
Khakula Farm	6.03 (4.58)	6.03	1.44 -	-
Okwomi Farm	5.14 (3.49)	5.14	N.D. -	-
Barasa Farm	N.D.	-	N.D.	-
Kituyi Farm	2.04 -	-	3.9 -	-
Okonya Farm	2.45 (1.13)	2.45	-	-

N.D.= Not detected

Table 6 Chlorfenvinphos residues (µg/kg) in milk from Trans Nzoia district.

CENTRE	WET SEASON		DRY SEASON	
	Mean (±SD)	Median	Mean (±D)	Median
Cherangani Centre	3.86 (0.84)	3.86	1.97 (1.21)	1.32
Nzoia Scheme	3.00 (0.3)	3.00	2.20 -	-
Wambwa Farm	1.85 -	-	2.26 (0.61)	2.26
Kihara Farm	2.79 -	-	1.30 (0.21)	1.30
ADC Farm	3.27 -	-	-	
Soy Sambu Centre	3.67 (1.33)	3.9	-	
Bikeke Centre	4.61 (3.01)	3.09	-	
Kapsara Centre	3.05 (2.05)	2.19	-	
Sikhendu Centre	10.40 (0.83)	10.40	-	

Milk samples collected in the wet season had far higher values of chlorfenvinphos residues than those taken during the dry season (P <0.05). Only at one sampling point (Sikhendu) did the residue content exceed the Codex MRL of 8

µg/kg milkfat for 1993. Otherwise the quantities remained slightly below the recommended limit (on average) during the rainy season and extremely low during the dry season.

5.5 VARIATION OF CHLORFENVINPHOS RESIDUES WITH MILKFAT CONTENT

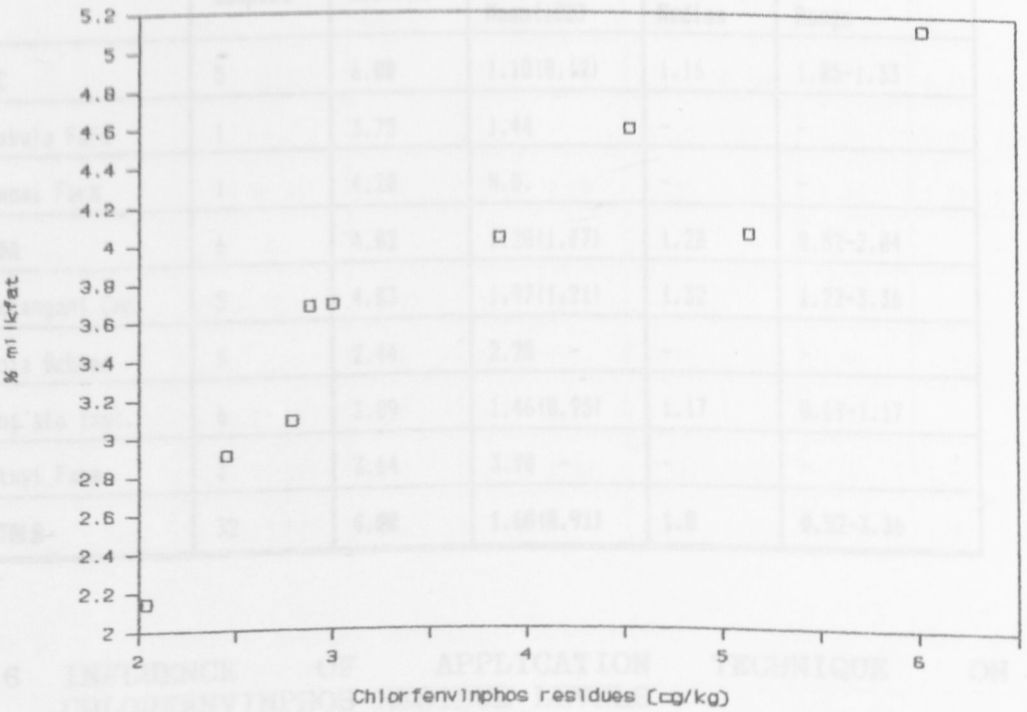
Table 7 gives the data on chlorfenvinphos residues and milkfat content for the wet season.

Table 7 % milkfat and mean levels of chlorfenvinphos (µg/kg) residues in wet season samples.

CENTRE	No. of Samples	Mean % milkfat	Chlorfenvinphos Residues		
			Mean(±SD)	Median	Range
Khakula Farm	2	5.13	6.03(4.58)	6.03	2.79-9.27
Okwomi Farm	2	4.06	5.14(3.49)	5.14	2.67-7.60
LBDA	4	4.60	4.53 -	-	-
Cherangani Cen.	3	4.03	3.86(0.84)	3.86	3.32-4.41
Nzoia Scheme	2	3.69	3.00(0.3)	3.00	2.79-3.21
Sang'alo Inst.	4	3.68	2.88(0.33)	2.70	2.66-3.29
FTC	4	3.09	2.79(0.14)	2.79	2.69-2.89
Okonya Farm	2	2.91	2.45(1.13)	2.45	1.65-3.25
Kituyi Farm	2	2.15	2.04 -	-	-
TOTALS	**25**	**3.70**	**3.64(0.83)**	**4.01**	**1.65-9.27**

A plot of the milkfat content vs concentration of chlorfenvinphos residues gave Pearson's correlation coefficient of 91% (for 14 d.f.) suggesting a linear relationship between the two at P <0.05, especially for samples collected in the wet season (Fig.12).

Fig.12 Relationship between % milkfat and chlorfenvinphos concentration in wet season.



(Regression constants: r^2=Ø.822, slope=Ø.6Ø7, y intercept=1.5)

By contrast, there was absolutely no relationship between the acaricide residues and milkfat content for samples collected during the dry season (r=-9.98). Table 8 gives the data obtained.

Table 8 % milkfat and mean levels of chlorfenvinphos (µg/kg) residues in dry season samples.

CENTRE	No. of Samples	Mean % milkfat	Chlorfenvinphos Residues		
			Mean(±SD)	Median	Range
FTC	5	6.00	1.18(0.12)	1.16	1.05-1.33
Khakula Farm	1	3.75	1.44	-	-
Okwomi Farm	1	4.20	N.D.	-	-
LBDA	6	4.02	1.28(1.07)	1.28	0.52-2.04
Cherangani Cen.	5	4.03	1.97(1.21)	1.32	1.22-3.36
Nzoia Scheme	5	2.44	2.20 -	-	-
Sang'alo Inst.	6	3.89	1.46(0.95)	1.17	0.69-1.17
Kituyi Farm	3	3.64	3.90 -	-	-
TOTALS	**32**	**4.00**	**1.68(0.91)**	**1.8**	**0.52-3.36**

5.6 INFLUENCE OF APPLICATION TECHNIQUE ON THE CHLORFENVINPHOS RESIDUE LEVELS

Samples were analysed from centres which observed strict maintenance of dipping infrastructure, concentration of active ingredient (a.i.) in the dip solution and frequency of treatment in both districts. Tables 9 and 10 depict the results obtained.

Table 9 Mean (± Standard Deviation) chlorfenvinphos residues in samples obtained from dipped cows.

CENTRE	CHLORFENVINPHOS RESIDUES (µ g/kg)					
	WET SEASON			DRY SEASON		
	Mean	Median	Range	Mean	Median	Range
Sang'alo Inst.	2.88 (0.33)	2.7	2.66- 3.29	1.46 (0.95)	1.17	0.69- 1.17
LBDA	4.53 -	-	-	1.28 (1.07)	1.28	0.52- 2.04
FTC	2.79 (0.14)	2.79	2.69- 2.89	1.18 (0.12)	1.16	1.05- 1.33
ADC Farm	3.27 -	-	-	-	-	-
Sikhendu Centre	10.40 (0.83)	10.37	9.78- 10.96	-	-	-
Ndalu Centre	6.75 (0.76)	7.08	5.86- 7.3	2.14 (1.44)	2.96	1.12- 3.16
Bikeke Centre	4.61 (3.01)	3.09	2.67- 8.07	-	-	-
Kitinda Centre	3.57 (0.74)	3.92	2.97- 3.98	1.84 (0.66)	1.84	1.37- 2.31
Cherangani Cen.	3.86 (0.84)	3.86	3.22- 4.41	1.97 (1.21)	1.32	1.22- 3.36

Table 10 Mean (± Standard Deviation) chlorfenvinphos residues in samples obtained from handsprayed cows

CENTRE	CHLORFENVINPHOS RESIDUES (µ g/kg)					
	WET SEASON			DRY SEASON		
	Mean	Median	Range	Mean	Median	Range
Wambwa Farm	1.85 -	-	-	2.26 (0.61)	2.26	1.83- 2.69
Khakula Farm	6.03 (4.58)	6.03	2.79- 9.27	1.44 -	-	-
Okwomi Farm	5.14 (3.49)	5.14	2.69- 7.60	N.D.	-	-
Kituyi Farm	2.04 -	-	-	3.90 -	-	-
Kihara Farm	2.79 -	-	-	1.3 (0.21)	1.3	1.15- 1.45
Okonya Farm	2.45 (1.13)	2.45	1.68- 3.25	-	-	-
Barasa Farm	N.D.			N.D.		

N.D.= Not detected

It is observed that the residues are generally higher in milk sampled from animals that are dipped and lower in those from the handsprayed. A t-test analysis showed that

levels were significant (t=3.695) at both 5% and 1% levels for the wet and dry seasons, respectively. Generally, no notable trend was observed between residues due to both dipping and handspraying during the dry season (t=Ø.114; P<Ø.Ø5).

5.7 RESIDUES OF 2,4-DICHLOROACETOPHENONE IN MILK

Analysis of 2,4-dichloroacetophenone residues, the breakdown product of chlorfenvinphos, showed no detectable levels in samples collected during the dry season (Table A1-A4). By contrast, the wet season samples were found to have detectable residues of 2,4-dichloroacetophenone, but there was no relationship between residues of 2,4-dichloroacetophenone and the milkfat content (Table 11).

Table 11 Residues of 2,4-dichloroacetophenone compared with those of chlorfenvinphos in some wet season samples.

CENTRE	% Milkfat	2,4-dichloroacetophenone (µg/kg)	Chlorfenvinphos (µg/kg)
Khakula Farm	5.13	118.4	6.03
Ndalu Centre	4.27	21.8	7.08
Bikeke Centre	6.62	71.8	3.09
Sikhendu Centre	3.88	276.8	1.18
Kihara Farm	5.59	3.3	2.79

5.9 CONFIRMATORY ANALYSIS

The GC-MS reference spectra for both chlorfenvinphos and 2,4-dichloroacetophenone and suitable ions chosen for monitoring are presented in Figs. 13-16. Monitoring ions were chosen such that they were of optimum mass and in high abundance for achievement of good analytical sensitivity and specificity.

For chlorfenvinphos, the ions selected for monitoring were m/e 81, 109, 276, 295 and 323 while for 2,4-dichloroacetophenone, the ions selected for analytical monitoring were m/e 43, 145 and 173. The results indicated that only technical grade chlorfenvinphos was present and not any of the isomeric forms. The base peak for this grade is typically 267 while that for the cis- and trans- isomers is 29 (Fig.A.10).

The capillary GC retention times of the two compounds (35.46 for chlorfenvinphos and 24.35 for 2,4-dichloroacetophenone) agreed with those of their standards to within 0.2-1%, on the basis of the proven performance of the equipment. At least three individual selected ions in either compound had coincidental maxima (Figs. A.7- 9).The relative abundances of chosen ions fluctuated to within 10% of the values obtained for the standards which satisfies the criteria for confirmation of a compound of interest. The signal-to-noise ratio, established according to IUPAC rules (Gilbert et al, 1987) at the least intense of the masses being monitored always exceeded three.

Fig. 13 GC Chromatogram for chlorfenvinphos standard

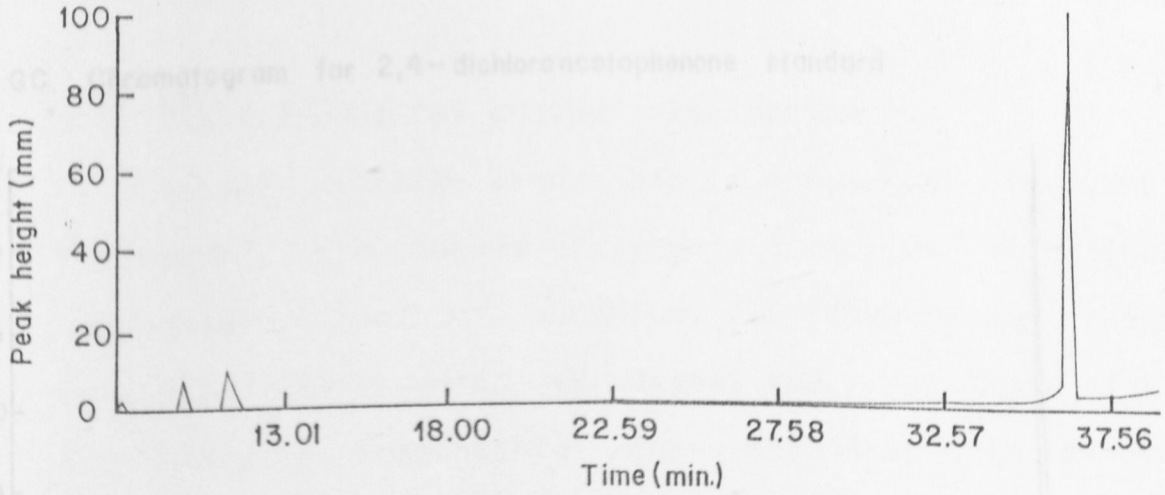


Fig. 14 Electronic ionization spectrum for chlorfenvinphos

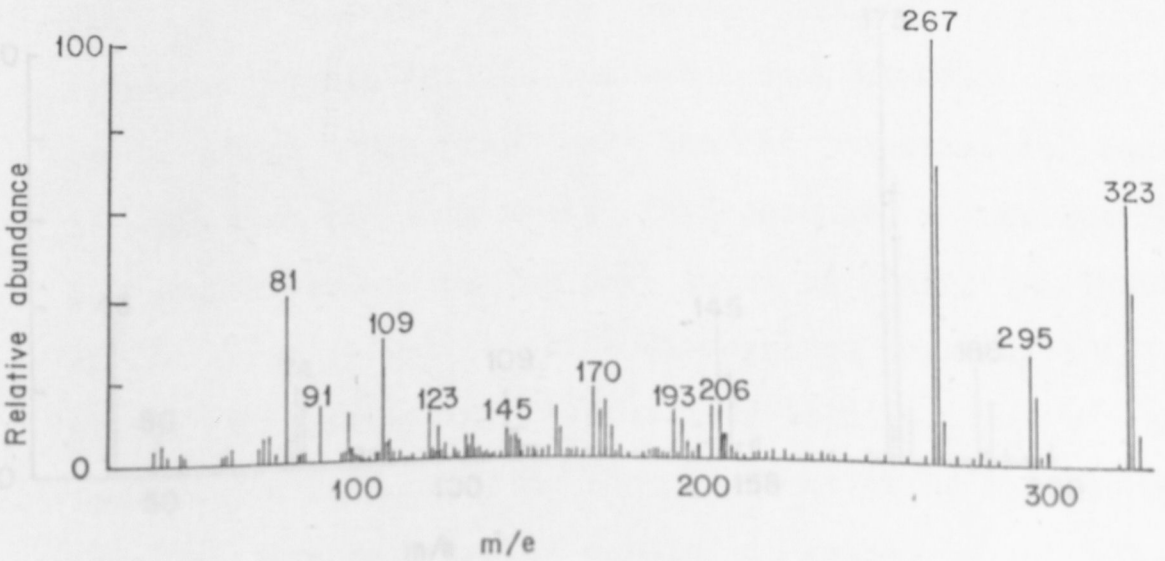


Fig. 15 GC Chromatogram for 2,4-dichloroacetophenone standard

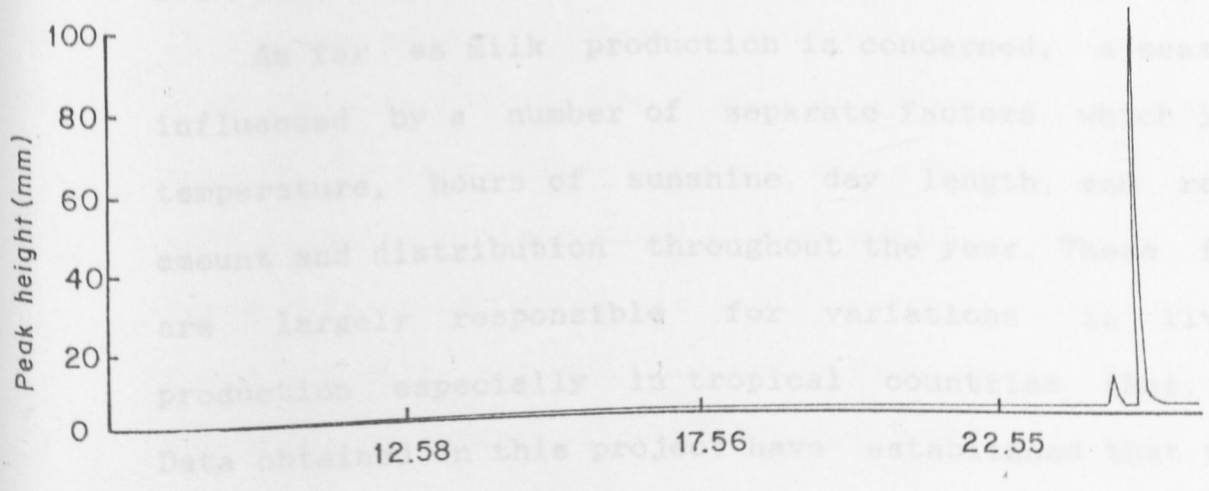
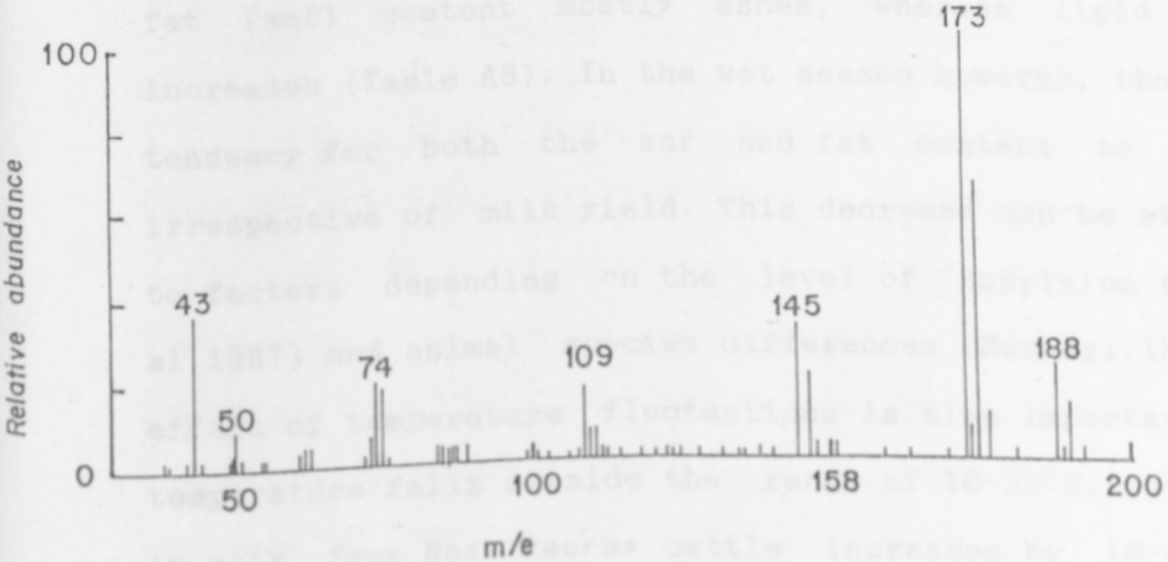


Fig. 16 Electronic ionization spectrum for 2,4-dichloroacetophenone



CHAPTER SIX

DISCUSSION

6.1 Variation of fat content with season

As far as milk production is concerned, a season is influenced by a number of separate factors which include temperature, hours of sunshine, day length, and rainfall amount and distribution throughout the year. These factors are largely responsible for variations in livestock production especially in tropical countries (Rai, 1985). Data obtained in this project have established that the fat content of milk was higher in samples collected during the dry season than during the wet period (Table.7 and 8).

In the dry season, the total yield of milk tends to decrease, accompanied by a parallel decrease in solids-not-fat (snf) content mostly ashes, whereas lipid content increases (Table A8). In the wet season however, there was a tendency for both the snf and fat content to decrease irrespective of milk yield. This decrease can be attributed to factors depending on the level of nutrition (Webb et al,1987) and animal species differences (Musangi,1969). The effect of temperature fluctuations is also important: when temperature falls outside the range of 10-32°C, fat content in milk from *Bos taurus* cattle increases by 10-40% while snf, lactose and protein contents decrease. Fat content generally changes by 0.1 to 0.3% for each 10° change in temperature (Musangi,1969).

The contents of both butterfat and snf reflect two

main effects: stage of lactation and seasonal variation in availability (Musangi,1969). During drought there is a tendency by the lactating animals to be heavily dehydrated, leading to a high concentration of total solids in the milk. In the wet season, however, water-loss is minimal and the milk yield is higher. The high quality feeding maintained on some of the farms, like the Lake Basin Development Authority (LBDA), Kenya-Finland Rural Dairy Project and the Bungoma Farmers Training Centre, is depicted by the high fat content found in milk samples collected from these centres. The thick-yellow butterfat obtained during the extraction stage of these particular samples signified high levels of carotenoids attributed to roughage-rich feeds.

6.2 Influence of fat content and the effect of acaricide application method on chlorfenvinphos residue levels

Farmers treat their animals with acaricide, usually minutes after the milking process in the mornings of the designated days. This is done in plunge dips or by handspraying in the homes. Animals treated by either technique have been reported to produce milk that contains acaricide to varying degrees of contamination (Chamberlain and Hopkins,1962).

Fats as compounds of triglycerides, phospholipids and cholesterol esters (Table A8), are carried by the blood circulatory system to the mammary glands in the form of lipoprotein complexes.After dipping or spraying the animal,

some acaricide may be ingested or absorbed directly through the dermal pores thereby ending up in the circulatory system. Studies using radiolabelled ^{32}P-chlorfenvinphos have indicated that the amount of radioactive material in the blood reaches a maximum build-up 2 hours after treatment, which indicates rapid absorption of the acaricide and its products through the skin of cattle. Chlorfenvinphos like other lipophilic drugs and pesticides, binds itself very strongly to complex plasma proteins. This binding facilitates migration to other parts of the body (Beynon *et al*, 1973). Like most organophosphates,the characteristic low polarity of chlorfenvinphos enables it to traverse into the lipid phase of the milk system. This phase movement is facilitated by the physiological pH(5.8-6.4) which is conducive for *in vivo* metabolic processes of the pesticides within the udder environment.

The Codex Alimentarius Commission of the FAO/WHO has set limits as to what quantities are allowable. According to the 1993 pesticide report (Table A9) the acceptable daily intake (ADI) for chlorfenvinphos is 2 µg/kg of body weight, while its recommended Maximum Residue Limit (MRL) is 8 µg/kg of milkfat in milk and milk products from cattle, sheep and goats. Only the Sikhendu area in Trans Nzoia district recorded levels higher than the MRL value in all the samples collected, probably due to large dung and mud quantities in the dips or high application frequencies. In this area there are about 8 public plunge dips.

Codex MRLs for fat soluble pesticide residues in milk

and milk products are expressed on a **whole product** basis.For a milk product with a fat content less than 2%, the MRL applied should be half those specified for milk while those with a fat content more than 2% should be 25 times the MRL value for milk when expressed on a fat basis (Codex Alimentarius,1993). On the basis of these guidelines, the milk consumed in the study area at that time was generally safe for human consumption except in the few centres where levels were higher than the stipulated MRL values.

During the hot dry period there's good evidence that some of the volatile organic solvents present as formulation additives evaporate easily, rendering the active ingradient (a.i.) unstable and exposed to air and ultra violet (UV) light.The UV light is known to initiate a photochemical reaction that isomerizes some of the trans-isomer to the unstable and less reactive cis-chlorfenvinphos (Beynon et al,1973). Some a.i. will be lost via wind-assisted vapourization. Despite the rapid dermal absorption and elimination potential of this compound by cattle (Chamberlain and Hopkins,1962) quite little will be available for dermal intake in this case. A higher percentage of the detected acaricide residues may be due to other absorption routes mainly via the gastro-intestinal ingestion, inhalation and direct drip-off from the animal especially in rainy weather. Due to this inconsistency in the absorption routes,coupled with factors such as vapourization,the amount and pattern of residues reaching the milkfat will definitely be affected, consequently

leading to the low levels found in the dry season. As far as the amount of residues of chlorfenvinphos in milk is concerned, the application technique has no significant effect during this season.

Animals on almost all farms visited graze and stay in the open fields at all times including the rainy weather, which is usually characterized by dull, cloudy days. These conditions promote prolonged retension of the acaricide on the animal's skin.The retension is also favoured by the properties possessed by the stickers, penetrants,humectants and stabilizers which are formulation additives. Rain water is known to add to the wetting of the animal coat. Due to its high boiling point and lipophorbic nature (relative to the a.i. and formulation additives) it tends to provide an extra layer over the acaricide, hence maximum dermal absorption is expected. However, elimination of the acaricide or its metabolites has been shown to be rapid from the low concentrations found in the urine and feaces one week after treatment (Chamberlain and Hopkins, 1962). These observations possibly explain why the levels obtained in this project were generally higher in the wet than the dry season, irrespective of the butterfat content. The graph in Fig.12 indicates a linear relationship between the fat content and chlorfenvinphos residue concentrations, suggesting a stable and consistent pattern of absorption of chlorfenvinphos into the fat portion of milk.

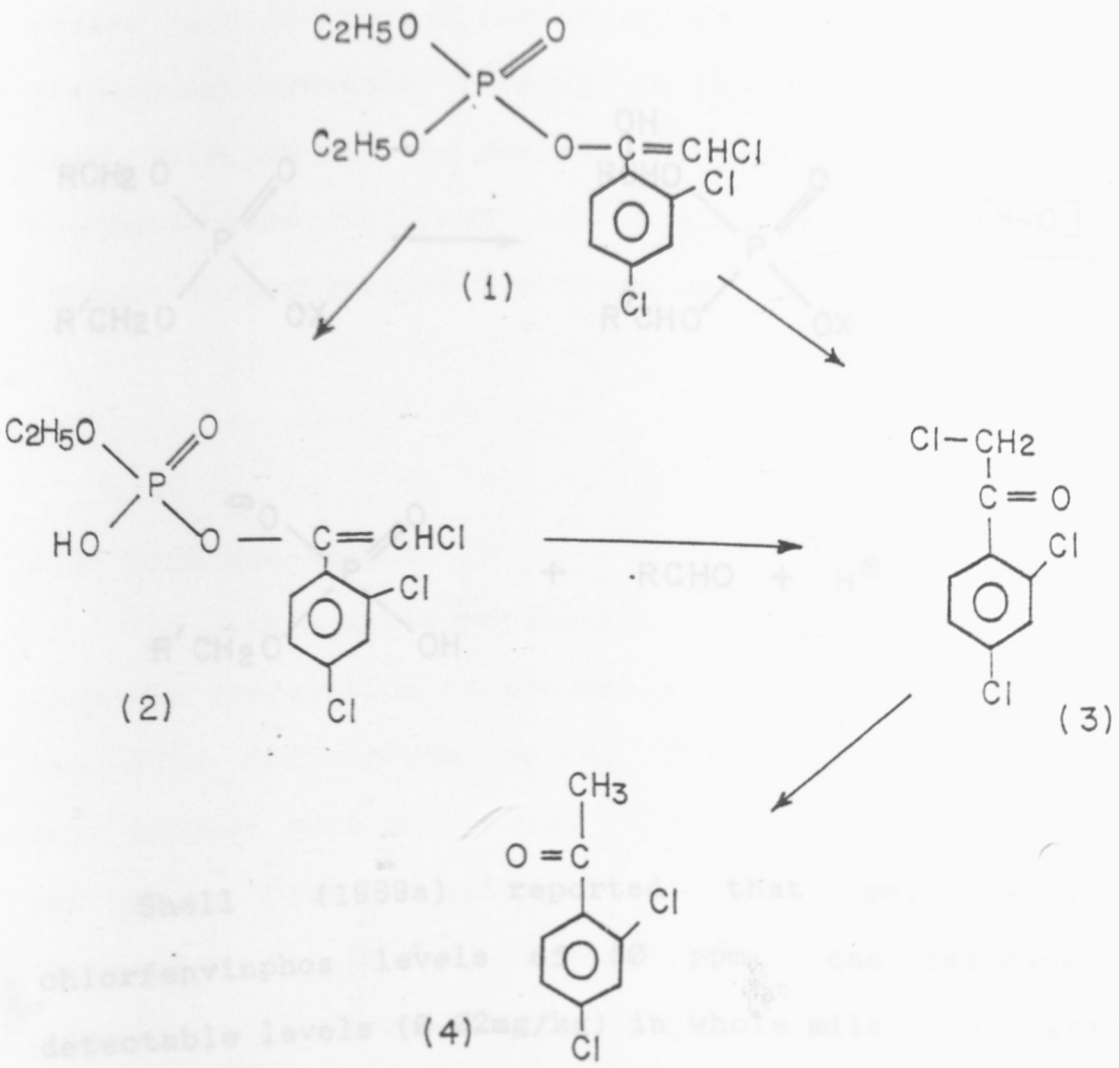
The higher degree of ingestion of the acaricide by animals during dipping and the definite maximum coverage in

the process are not the only possible factors leading to higher residues. The cowdung and mud noted in most of the public plunge dips especially in Bungoma, remain on the animal's coat and promotes dermal entry of the acaricide retained within it. Direct drip-off onto the udder then teats during the milking process is yet another possible route through which contamination occurs during the wet season. Spraying is carefully done in most of the cases leading to negligible ingestion via possible inhalation of the vapours. Dipped animals therefore tend to maintain higher residue quantities than the hand-sprayed ones in this season.

6.3 Residue patterns of 2,4-dichloroacetophenone and its relationship with that of chlorfenvinphos

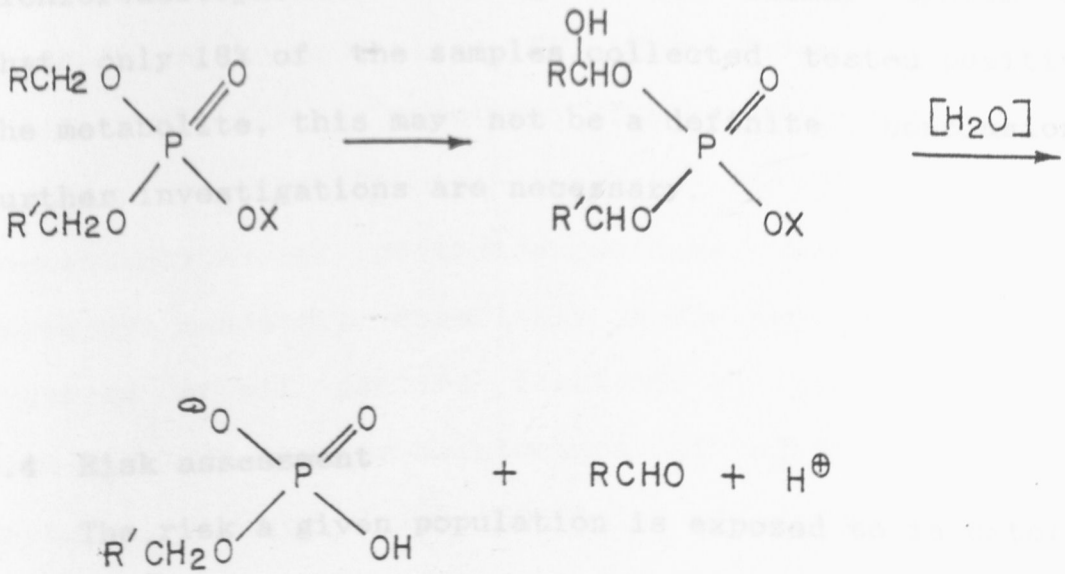
The metabolic fate of chlorfenvinphos in mammals has been extensively investigated by various workers who all suggest that the most probable detoxification reaction in mammals is the desethylation of chlorfenvinphos [1] to the phosphate diester, desethyl chlorfenvinphos [2] (Fig.17). This compound is not only excreted rapidly but is also further metabolised to the intermediate, 2,4-dichlorophenacyl chloride [3]. The latter may also arise directly from chlorfenvinphos by enzyme-catalysed hydrolysis subsequently leading to the formation of 2,4-dichloroacetophenone [4] under normal physiological conditions (Beynon et al,1973).

Fig.17 Formation of 2,4-dichloroacetophenone in mammals



Studies on the desethylation of chlorfenvinphos have shown it to be an oxidative process. The enzyme (a mono-oxygenase) catalysing this process is located in the microsomal fraction of the mammalian liver and requires molecular oxygen and reduced nicotinamide adenine dinucleotide phosphate ($NADPH_2$) for activity. The products of its action are desethylchlorfenvinphos and acetaldehyde (Fig. 18).

Fig. 18 Mechanism of the enzyme-catalysed oxidation process



Shell (1969a) reported that only with feed chlorfenvinphos levels of 5Ø ppm can residues reach detectable levels (Ø.Ø2mg/kg) in whole milk. The degradation products of chlorfenvinphos (Fig.18) were also detected in milk.

In a lactating cow injected intramuscularly with Ø.58 mg C^{14}-chlorfenvinphos per kg body weight, Ø.2% radioactivity was found in the milk, which contained 75% unchanged chlorfenvinphos, together with small amounts of2,4-dichloroacetophenone, 1-(2,4-dichlorophenyl)ethanol and 2,4-dichloromandelic acid.

The conclusion made from Table 11 suggests that levels of 2,4-dichloroacetophenone increase as residual

chlorfenvinphos increases. The various favourable conditions mentioned in the previous section in the rainy season lead to more chlorfenvinphos and consequently 2,4-dichloroacetophenone forming in the animal system. Given that only 18% of the samples collected tested positive to the metabolite, this may not be a definite conclusion and further investigations are necessary.

6.4 Risk assessment

The risk a given population is exposed to is determined from the correlation of the experimental values to the MRLs and ADIs, for a given period. The residue levels found in this project were on average lower than the residual limits set by the Codex Alimentarius (1993). The ADI for infants (under 4 months of age) is worked out on the basis that they consume an average 120g (Ø.12 kg)of milk per day (WHO/UNEP/ILO, 1987). The residual limit is then determined from the expression:

$$ADI(\mu g/kg) \times Ø.12$$

whose value for chlorfenvinphos is Ø.24µg/kg. The levels found in the milk samples from both seasons exceeded this limit by between 7-15 times considering the combined mean chlorfenvinphos residue levels (1.68 µg/kg in dry season and 3.64 µg/kg for wet season) (Table 7).

6.5 Current and future investigations

A new threat to Kenya in the field of pesticide use comes in the wake of the recent European Union Council directives on Maximum Residue Limits (MRLs) of pesticides in agricultural commodities (EEC,1993). These directives put great emphasis on scrutiny of any foodstuff that is exported to the European market, for any unacceptable concentrations of pesticide residues. New stategies are therefore mandatory especially in the area of education and training of all parties involved, on pesticide handling, safe-use and proper maintenance of equipment. Kenya for instance, exports large quantities of butter to parts of Europe and once such residues are detected, the product may be rejected leading to loss of foreign exchange as well as markets.

The Kenya Agricultural Research Institute (KARI) has recently launched a pesticide residue level testing facility for the horticultural export market. A similar one is yet to be launched but highly recommended for the dairy products, which are only tested at the point of destination by the importing country. This facility will ensure regular monitoring of pesticides in potential milk producing zones to ensure not only high quality, residue-free dairy products but also enlighten the farmers on the proper understanding and practice of safe pesticide handling and application techniques.

Field trials in Kenya using Boran heifers immunized against ECF have shown that animals dipped weekly gain an

average of 78 g per day more than undipped animals over a 30 week period (De Castro *et al*, 1985). It has also been demonstrated that high tick numbers cause considerably greater liveweight losses in tick-susceptible Boran cattle than in tick-resistant animals of the same breed (De Castro,1987). The International Laboratory for Research on Animal Diseases(ILRAD) is currently conducting research aimed at developing safe and efficient vaccines that will protect cattle against ECF. In the Uasin Gishu district of Rift Valley Province, and the Kaloleni division at the Coast, preliminary results indicate that immunization is the most cost-effective tick-borne disease control method and has an additional advantage of reducing present acaricide application by up to 75%. In both areas, greater economic returns will accrue from immunizing grade rather than Zebu cattle(ILRAD,1993).

Biotechnology has recently promised new biological controls to replace pesticides, with the hope that a method that uses microorganisms will give satisfactory results that will lead to its recommendation to farmers. Such a technique will be cost-effective and easy to use as predicted by various authorities (NRC, 1987; Pimentel, 1987). However, although the introduction of such techniques will help reduce the use of acaricides, solutions to some serious environmental problems need to be addressed (Pimentel *et al*, 1989).

6.6 Conclusion

(i) Residues of chlorfenvinphos have been found in milk at levels generally below the recommended limit.

(ii) Little or negligible levels of 2,4-dichloroacetophenone were detected in the milk during the entire study period, suggesting a fast excretion possibility for this metabolite from the animals' physiological system.

(iii) The wet season has been found to be quite significant in the study of the various parameters affecting chlorfenvinphos residues in milkfat. The concentrations of these residues are generally higher than those for the dry season regardless of all other factors.

(iv) Chlorfenvinphos residue levels increase with an increase in butterfat content in milk during the wet season.

(v) Dipping animals during the wet season results in milk with higher chlorfenvinphos residue levels. By contrast, milk from handsprayed cows contained lower levels of the acaricide.

(vi) Butterfat content is higher in the dry season than in the wet period.

(vii) Cow milk produced during this period was safe for consumption to the adult population, but risky for the infants.

6.7 Recommendations

In view of the findings in this study, it is imperative that relevant government organs, non-governmental organizations and the pesticide industry at large endeavour to rationalize the situation through some of the following steps.

(a) Monitoring of all tick-infested areas in Kenya (Fig.1Ø) for residues of chlorfenvinphos and all its other metabolites should be initiated.

(b) The Veterinary Department should investigate why there was persistently higher levels than the recommended limit in all samples collected in the wet season in the Sikhendu area.

(c) The management of the public plunge dips, especially those in Bungoma district should be improved to reduce the mud and dung content.

(d) The identification of the health effects of long term exposure of chlorfenvinphos in the general population is difficult but extremely important. Every effort should be made to perform epidemiological studies to elucidate this problem, including identification of milk consumers from areas found to have high concentrations of chlorfenvinphos residues.

(e) Relevant training and information on pesticide safety should be given to various classes of society.

(f) Nursing mothers should be encouraged to strictly breastfeed their babies and avoid cow milk especially from the study area, since even boiled milk would not be safe for

infants. Thermal decomposition of chlorfenvinphos has been found to be negligible at temperatures upto 130°C.

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APPENDIX 1

Fig. A1 Calibration curve for chlorfenvinphos

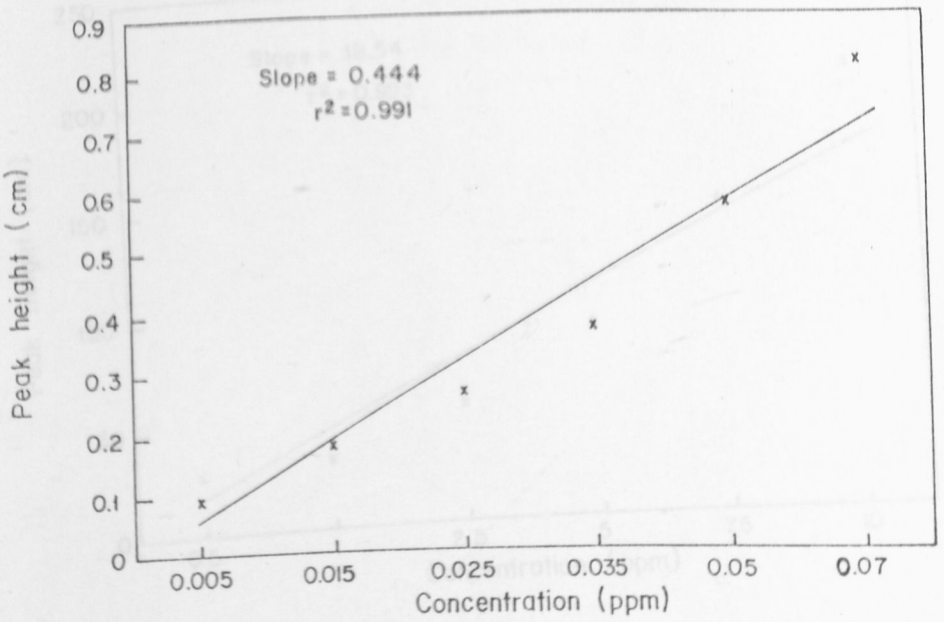


Fig. A2 Recovery graph for chlorfenvinphos from spiked milk samples.

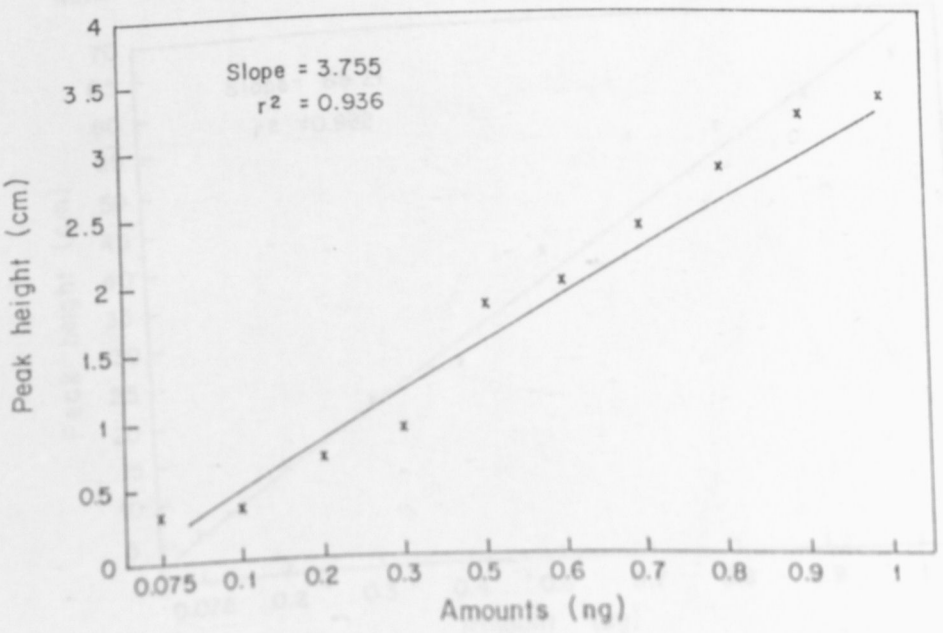


Fig. A3 Calibration curve for 2,4 – Dichloroacetophenone

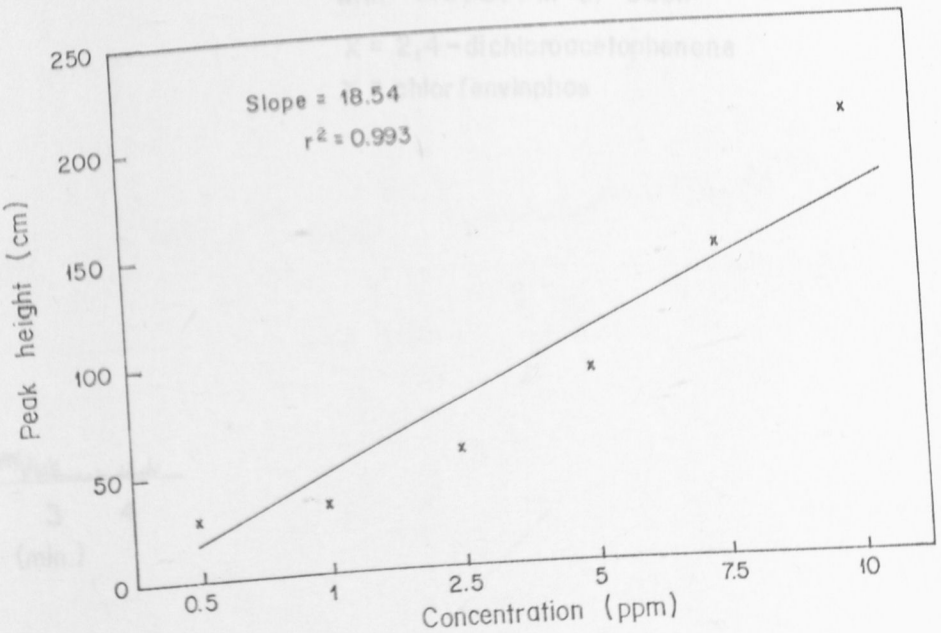


Fig. A4 Recovery graph for 2,4 – Dichloroacetophenone from spiked milk samples

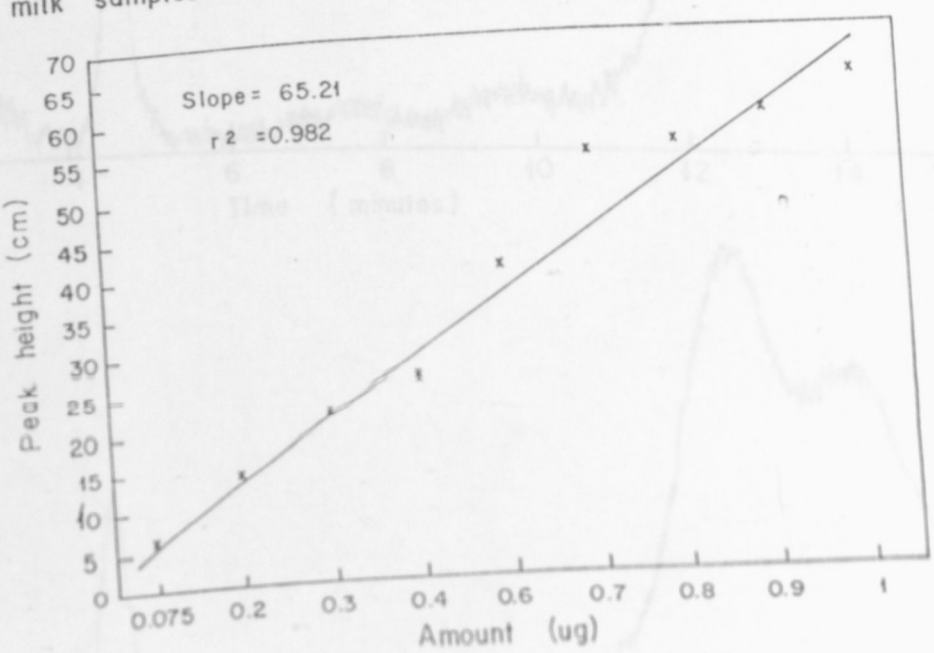


Fig. A5 HPLC chromatogram showing; (a) Separation between peaks of chlorfenvinphos and 2,4-dichloroacetophenone standards.

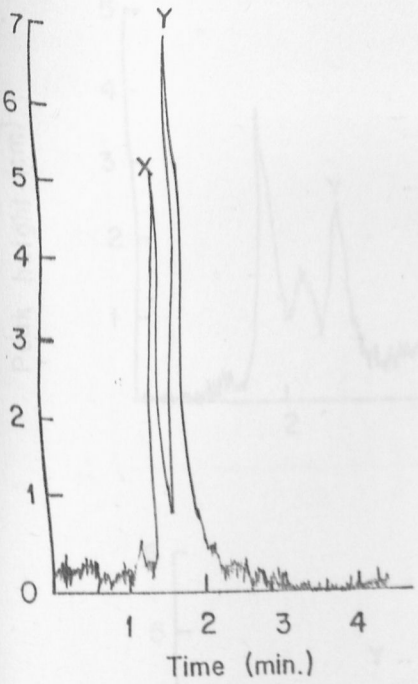
(b) Peaks from a pure milk extract.

(c) Peaks for both compounds due to milk fortified with 0.005 PPM of each

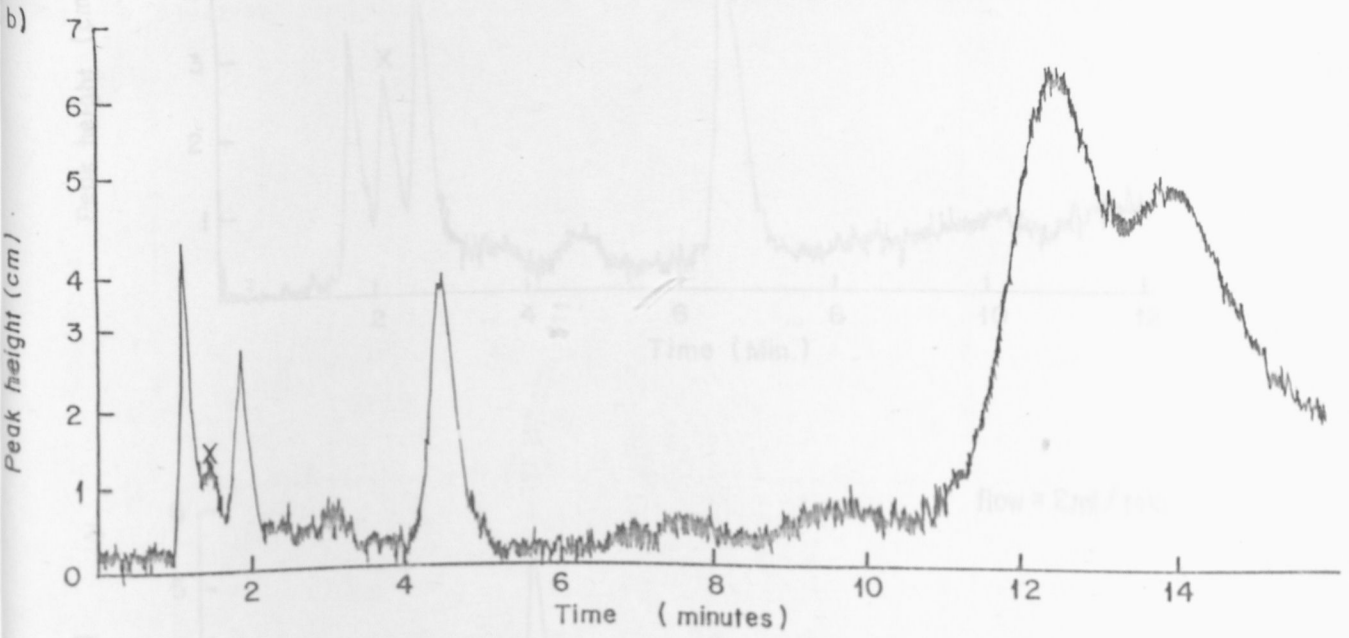
X = 2,4-dichloroacetophenone

Y = chlorfenvinphos

a)



b)



c)

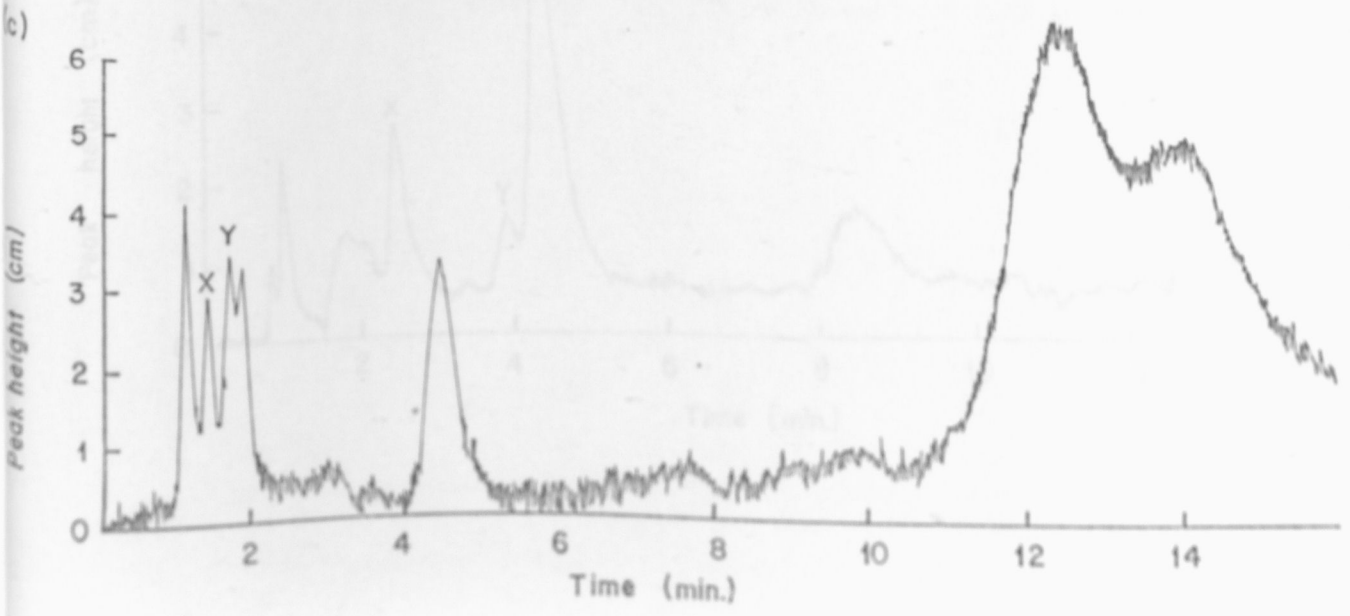
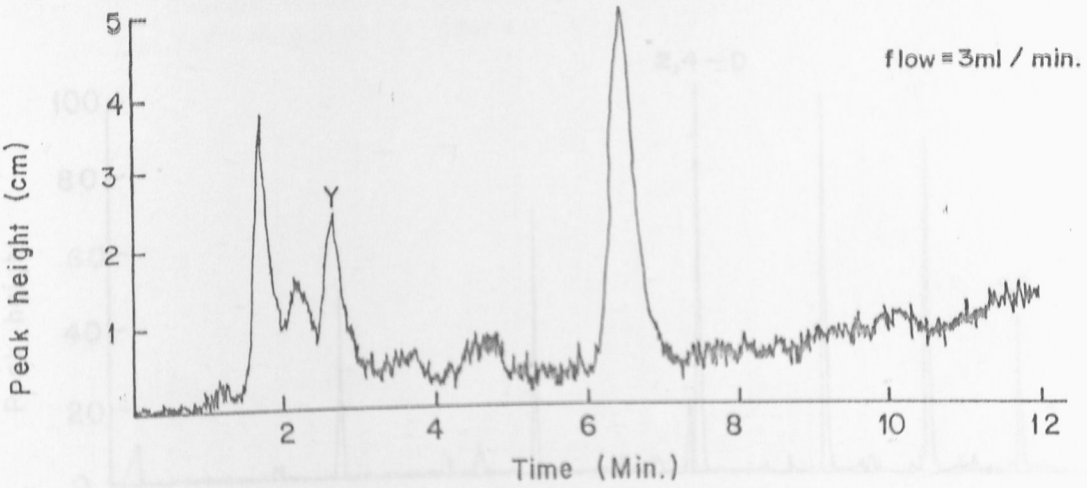
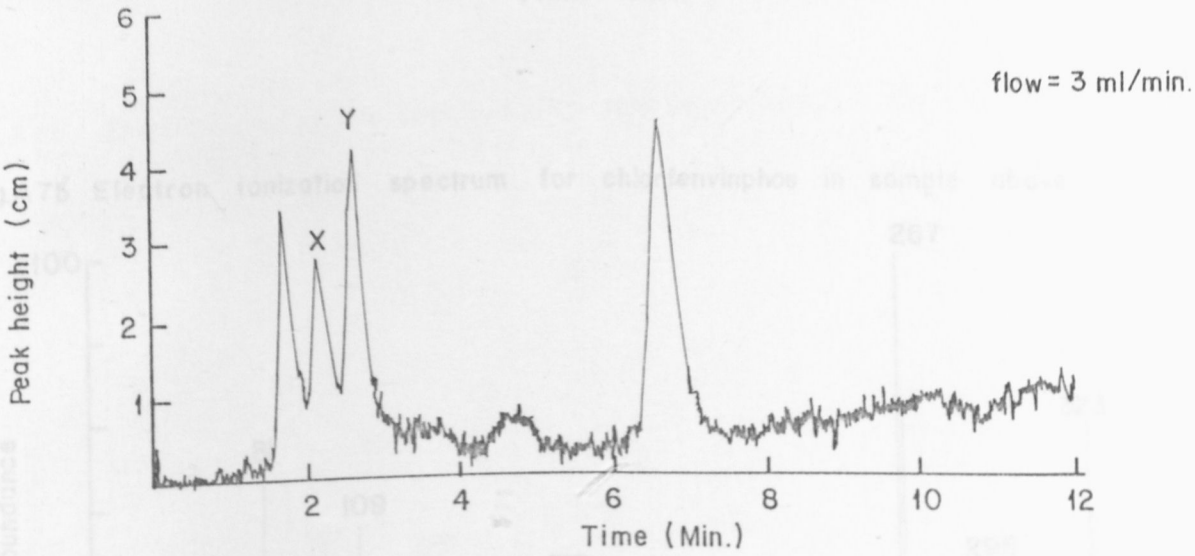
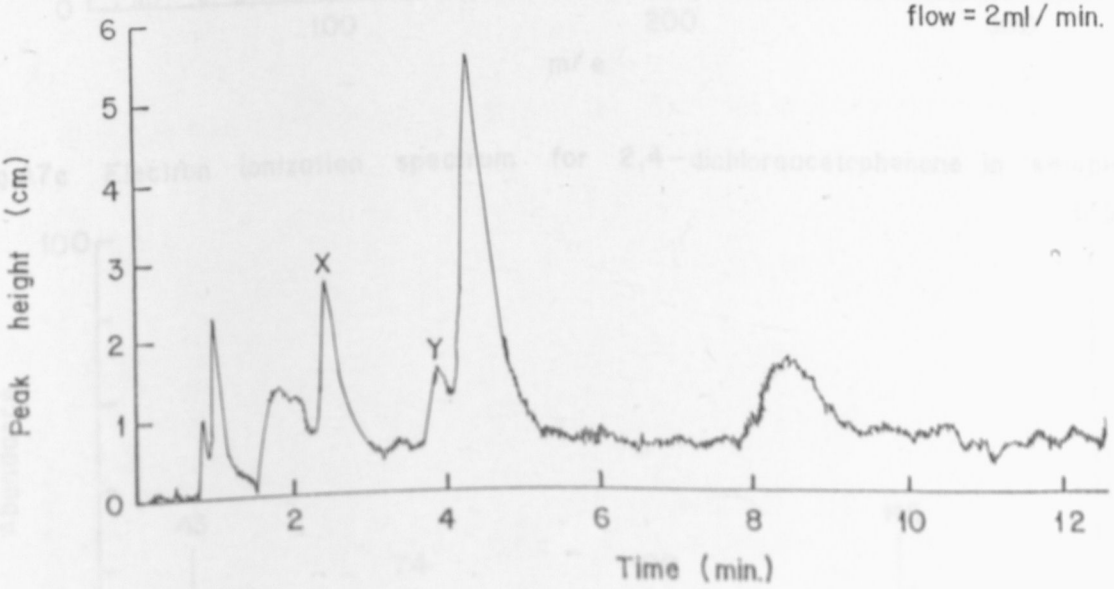


Fig. A6 HPLC chromatogra for three arbitrary sample extracts.







APPENDIX 3

Fig.A7a GC chromatogram for milk extract fortified with 0.005 ppm of both chlorfenvinphos (SD) and 2,4-dichloroacetophenone (2,4-D)

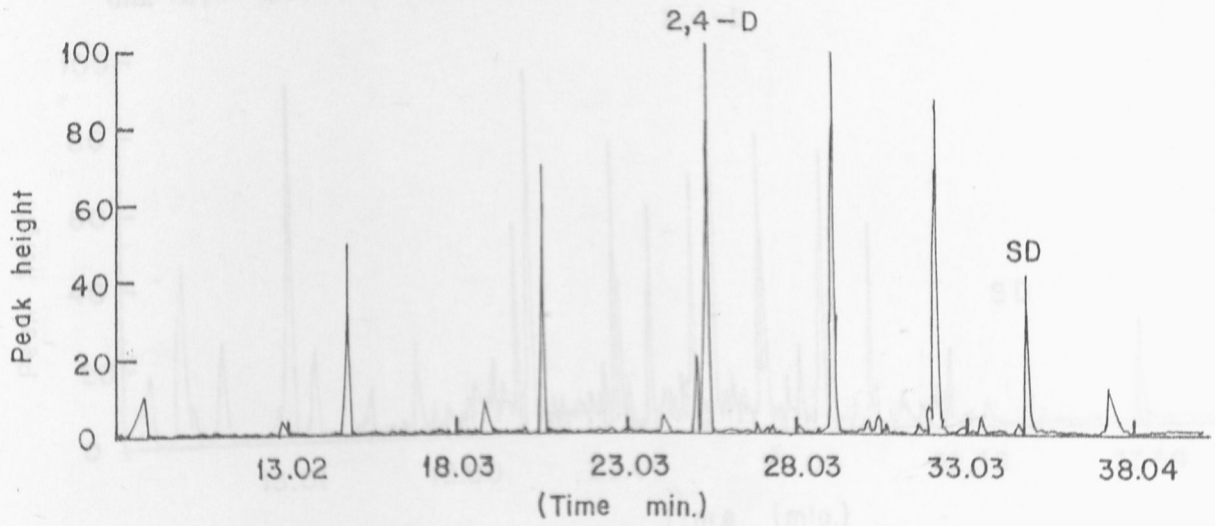


Fig.A7b Electron ionization spectrum for chlorfenvinphos in sample above

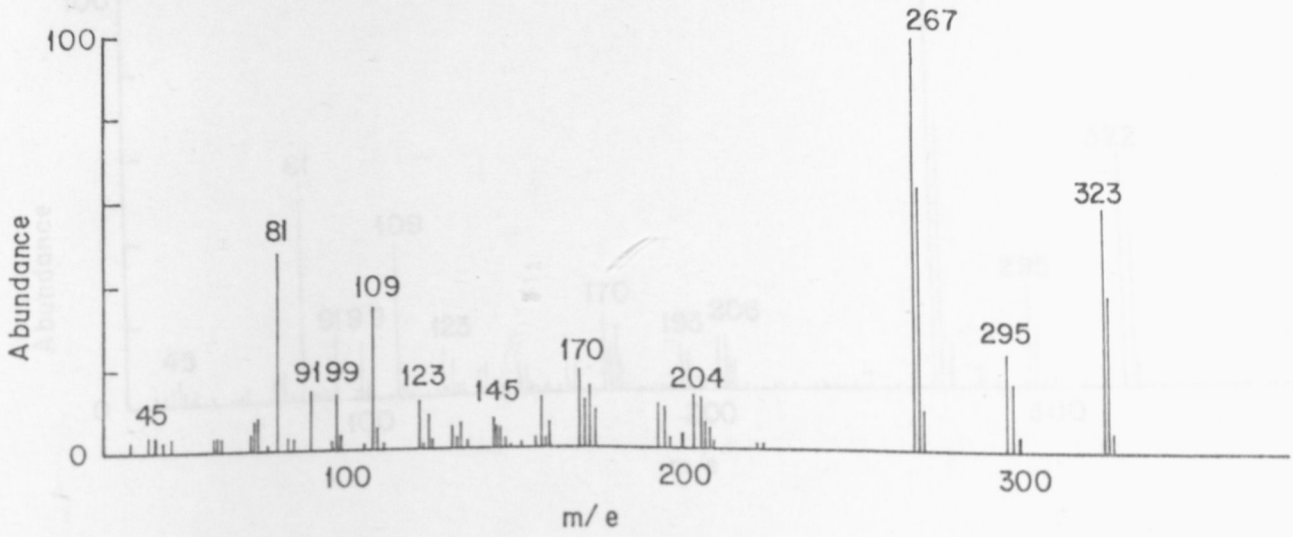


Fig.A7c Electron ionization spectrum for 2,4-dichloroacetophenone in sample above.

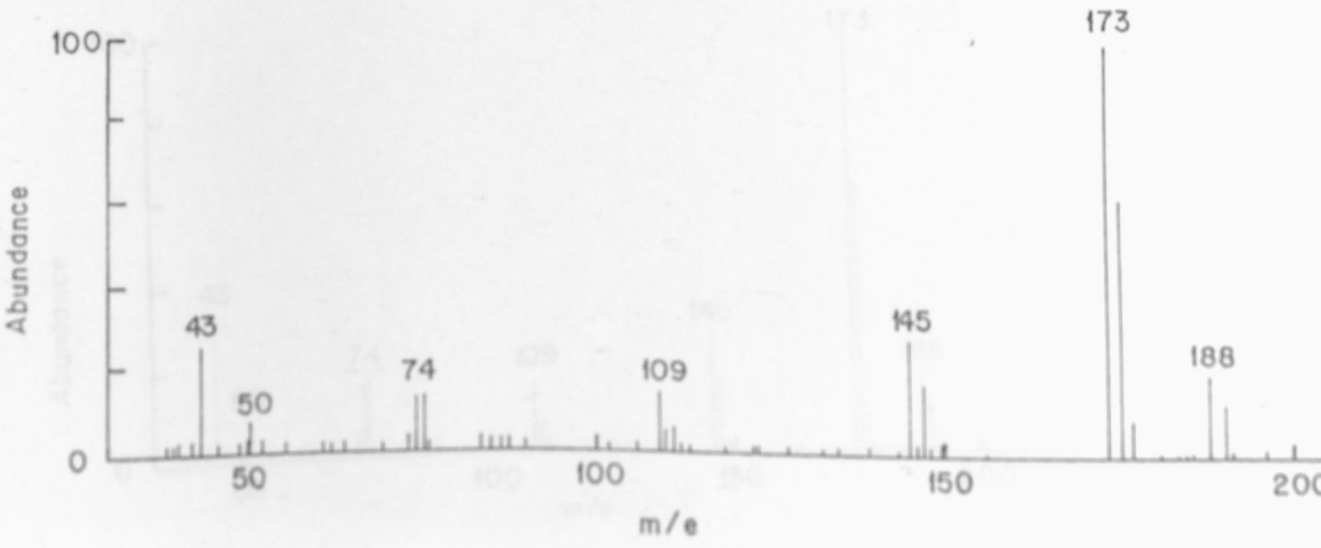


Fig. A 8a GC chromatogram for milk extract showing peaks of chlorfenvinphos (SD) and 2,4-dichloroacetophenone (2,4-D)

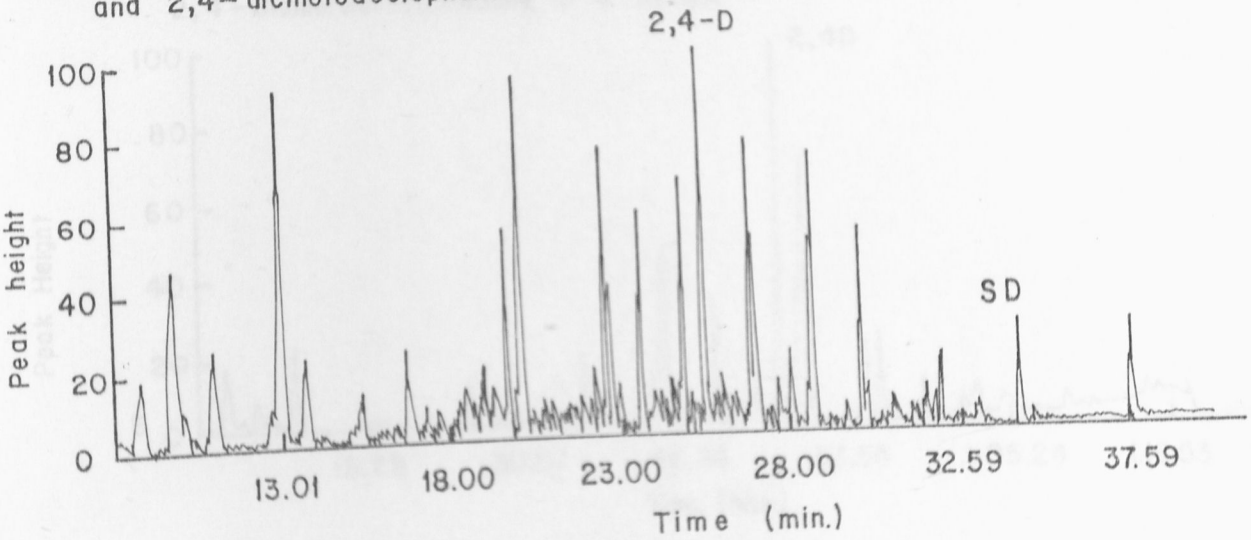


Fig. A 8b Electron ionization spectrum for chlorfenvinphos in sample above

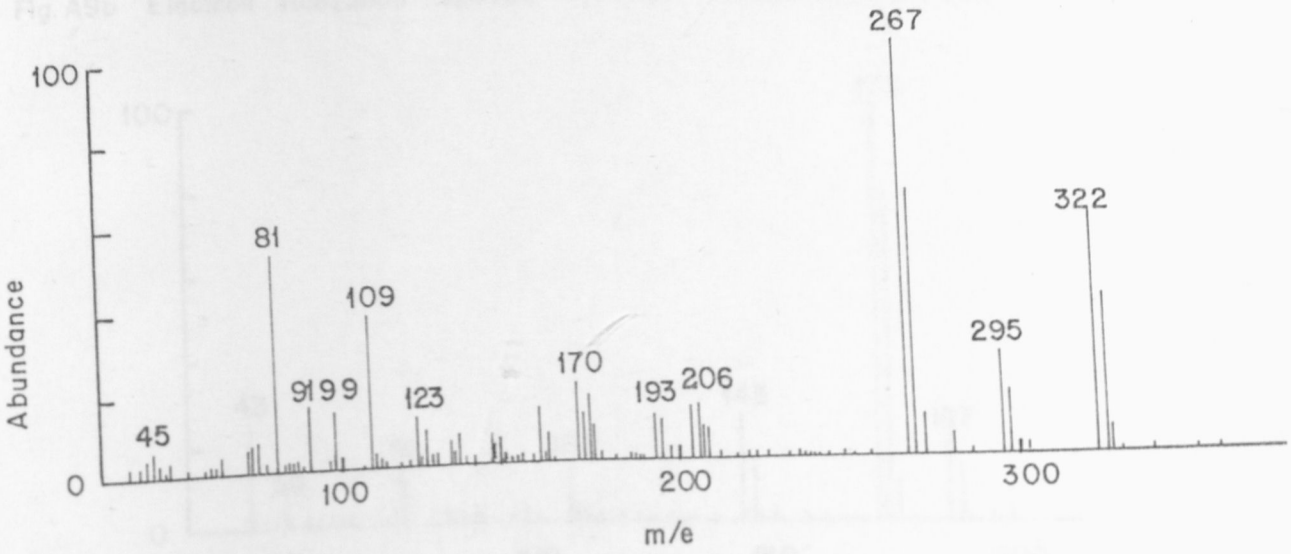


Fig. A 8c Electron ionization spectrum for 2,4-dichloroacetophenone in sample above

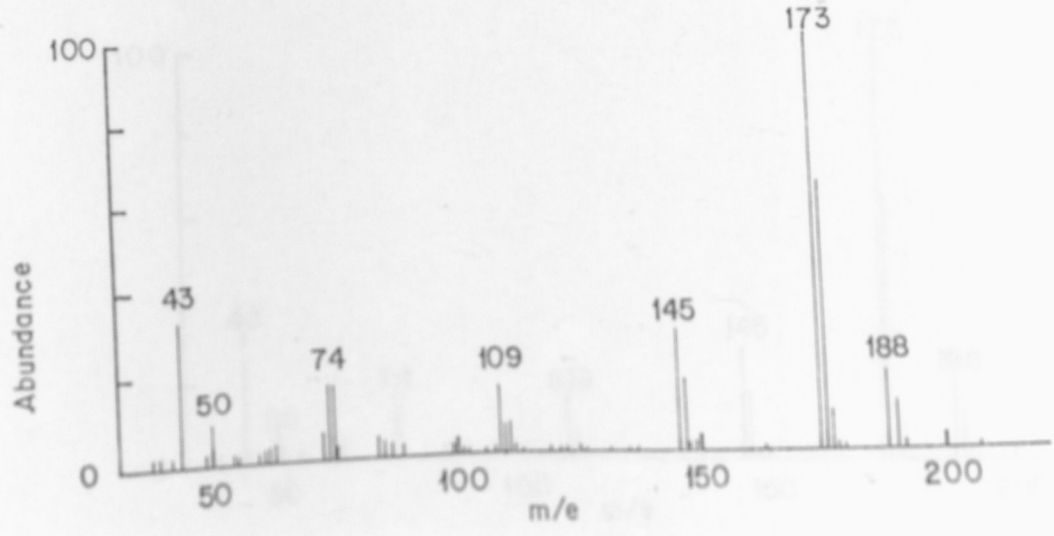


Fig. A9a Actual GC Chromatogram for milk extract showing the peak of 2,4 – dichloroacetophenone in a sample

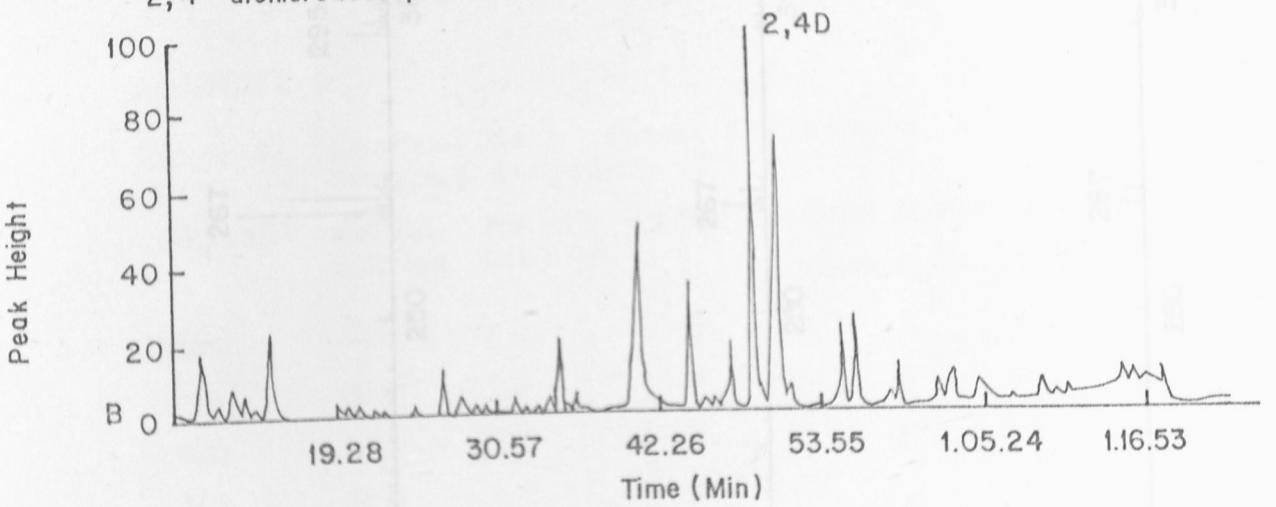
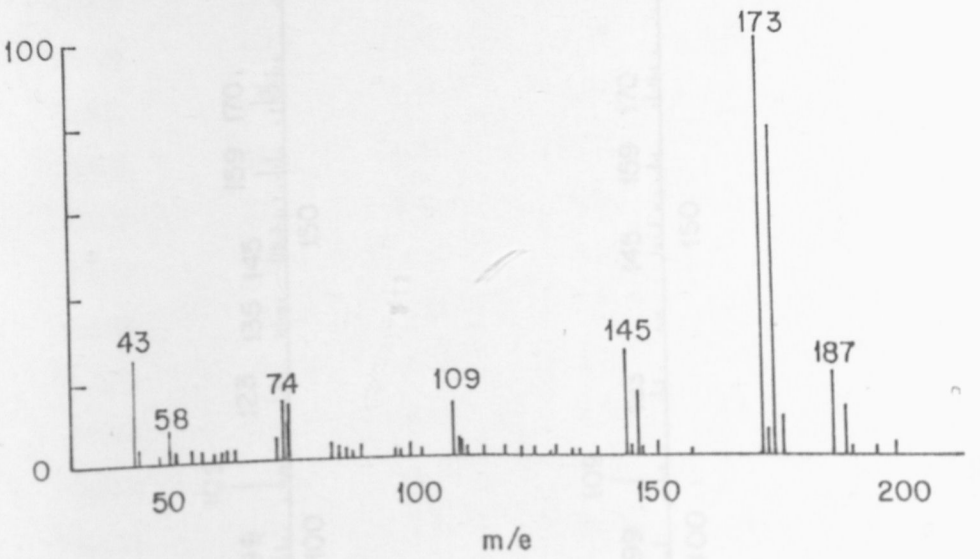


Fig. A9b Electron ionization spectra for 2,4 – Dichloroacetophenone in sample above



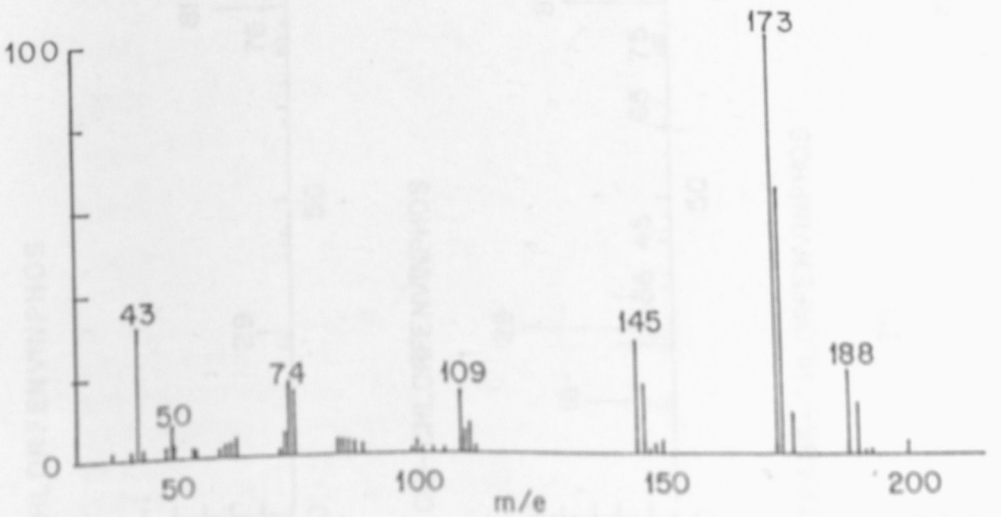
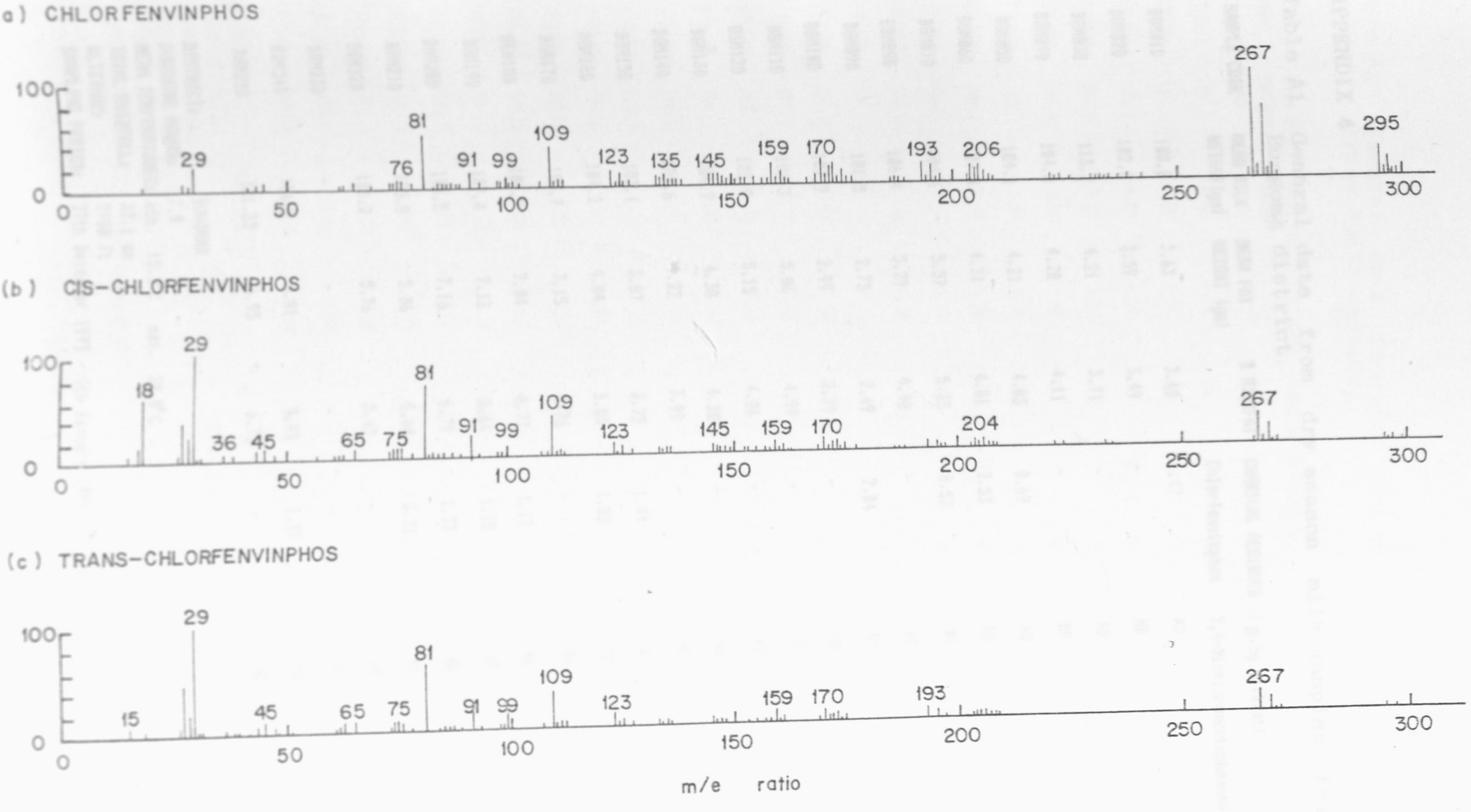


Fig. A10 Electron ionization spectra for chlorfenvinphos standards



APPENDIX 4

Table A1 General data from dry season milk samples from Bungoma district.

SAMPLE CODE	MEAN MILK WEIGHT(gm)	MEAN FAT WEIGHT (gm)	% MILKFAT	CHEMICAL RESIDUES (µg/kg milkfat)	
				Chlorfenvinphos	2,4-Dichloroacetophenone.
BGM01D	100.8	3.63	3.60	1.17	ND
BGM02D	102.8	3.59	3.49	-	ND
BGM03D	113.7	4.21	3.71	-	ND
BGM04D	104.0	4.28	4.11	-	ND
BGM05D	104.1	4.21	4.05	0.69	ND
BGM06D	107.4	4.31	4.01	2.53	ND
BGM07D	104.1	5.57	5.35	0.52	ND
BGM08D	106.4	5.29	4.98	-	ND
BGM09D	101.5	2.73	2.69	2.04	ND
BGM10D	107.2	2.99	2.79	-	ND
BGM11D	101.3	5.06	4.99	-	ND
BGM12D	105.9	5.15	4.86	-	ND
BGM13D	104.2	4.38	4.20	-	ND
BGM14D	105.6	4.22	3.99	-	ND
BGM15D	103.1	3.87	3.75	1.44	ND
BGM16D	104.2	4.04	3.88	1.05	ND
BGM17D	105.7	7.15	6.76	-	ND
BGM18D	104.9	7.04	6.71	1.17	ND
BGM19D	107.4	7.13	6.64	1.15	ND
BGM20D	104.5	7.16	6.79	1.33	ND
BGM21D	103.8	5.86	5.64	2.31	ND
BGM22D	106.2	5.76	5.42	-	ND
BGM23D	-	-	-	-	ND
BGM24D	106.2	6.01	5.93	1.37	ND
BGM25D	101.33	6.95	6.72	-	ND

DISTRICT: BUNGOMA
SUNSHINE HOURS: 7.8
MEAN TEMPERATURES: min. 15.3°C max. 29.8°C
TOTAL RAINFALL: 32.1 mm
ALTITUDE: 5980 ft
SAMPLING PERIOD: 27th December 1993 - 4th January 1994

Table A2 General data from dry season milk samples from Trans Nzoia district.

SAMPLE CODE	MEAN MILK WEIGHT (gm)	MEAN FAT WEIGHT (gm)	% MILKFAT	CHEMICAL RESIDUES (µg/kg milkfat)	
				Chlorfenvinphos	2,4-Dichloroacetophenone
KTL01D	104.2	4.36	4.19	ND	ND
KTL02D	106.2	5.03	4.74	ND	ND
KTL03D	103.9	4.87	4.69	ND	ND
KTL04D	103.8	2.53	2.44	2.2	ND
KTL05D	104.0	2.54	2.44	ND	ND
KTL06D	105.7	3.59	3.40	ND	ND
KTL07D	106.4	3.78	3.55	1.12	ND
KTL08D	98.3	2.24	2.28	ND	ND
KTL09D	106.0	1.34	1.26	3.16	ND
KTL10D	-	-	-	-	ND
KTL11D	106.8	3.61	3.38	ND	ND
KTL12D	108.3	3.79	3.49	ND	ND
KTL13D	102.2	3.70	3.62	ND	ND
KTL14D	104.1	3.55	3.41	2.69	ND
KTL15D	105.2	3.04	2.89	1.83	ND
KTL16D	112.5	4.21	3.73	1.32	ND
KTL17D	111.6	4.57	4.09	1.22	ND
KTL18D	103.8	4.43	4.27	3.36	ND
KTL19D	102.3	3.76	3.68	ND	ND
KTL20D	102.8	3.37	3.28	ND	ND
KTL21D	101.8	4.74	4.66	1.45	ND
KTL22D	103.1	5.97	5.79	1.15	ND
KTL23D	103.8	2.28	2.20	ND	ND
KTL24D	104.1	2.06	1.98	ND	ND
KTL25D	104.5	3.81	3.64	3.9	ND

DISTRICT: TRANS NZOIA
SUNSHINE HOURS: 7.1
MEAN TEMPERATURES: min. 10.6°C max. 27.2°C
TOTAL RAINFALL: 30 mm
ALTITUDE: 6220 ft
SAMPLING PERIOD: 27th - 28th December 1993

Table A3 General data from wet season milk samples from Bungoma district

SAMPLE CODE	MEAN MILK WEIGHT(gm)	MEAN FAT WEIGHT(gm)	% MILKFAT	CHEMICAL RESIDUES (µg/kg milkfat)	
				chlorfenvinphos	2,4-Dichloroacetophenone
BGM01W	100.8	4.00	3.97	ND	ND
BGM02W	99.6	3.40	3.41	3.98	ND
BGM03W	99.7	3.12	3.13	3.92	ND
BGM04W	101.8	2.77	2.72	2.97	ND
BGM05W	102.4	2.12	2.07	3.25	ND
BGM06W	96.5	2.07	2.13	2.04	ND
BGM07W	100.6	0.015	2.17	ND	ND
BGM08W	101.1	3.78	3.74	1.65	ND
BGM09W	96.6	3.91	4.06	3.29	ND
BGM10W	103.6	2.93	2.83	ND	ND
BGM11W	102.8	4.03	3.92	2.7	ND
BGM12W	100.6	3.09	3.07	2.66	ND
BGM13W	100.8	2.53	2.51	ND	ND
BGM14W	99.02	4.22	4.26	ND	ND
BGM15W	99.7	4.39	4.41	ND	ND
BGM16W	96.9	4.46	4.60	4.53	ND
BGM17W	104.8	3.54	3.37	2.67	ND
BGM18W	107.9	4.76	4.41	7.6	ND
BGM19W	103.4	6.02	5.82	2.69	ND
BGM20W	100.9	5.14	5.10	2.89	ND
BGM21W	102.4	4.30	4.20	ND	ND
BGM22W	104.8	4.33	4.13	9.27	234
BGM23W	109.6	6.71	6.12	2.79	2.74
BGM24W	100.4	6.19	6.17	ND	ND
BGM25W	100.2	3.50	3.24	ND	ND

DISTRICT: BUNGOMA
SUNSHINE HOURS: 6.9
MEAN TEMPERATURES: Min 14.9°C Max 29.1°C
TOTAL RAINFALL: 662.7 mm
ALTITUDE: 5900 ft
SAMPLING PERIOD: 28th MAR - 2nd APR 1994

Table A4 General data from wet season milk samples from Trans Nzoia district.

SAMPLE CODE	MEAN MILK WEIGHT (gm)	MEAN FAT WEIGHT (gm)	% MILKFAT	CHEMICAL RESIDUES (µg/kg milkfat)	
				chlorfenvinphos	2,4-Dichloroacetophenone
KTL01W	97.8	3.56	3.64	5.86	ND
KTL02W	103.4	5.22	5.05	7.3	ND
KTL03W	102.0	4.35	4.27	7.08	21.8
KTL04W	102.3	5.47	5.35	3.32	ND
KTL05W	102.1	3.68	3.61	ND	ND
KTL06W	102.4	2.77	2.71	4.41	ND
KTL07W	103.6	4.84	4.67	3.9	ND
KTL08W	107.9	5.64	5.22	4.88	ND
KTL09W	99.6	4.57	4.59	2.24	ND
KTL10W	101.8	6.74	6.62	3.09	71.0
KTL11W	97.7	1.18	1.21	8.07	ND
KTL12W	94.7	2.83	2.99	2.67	ND
KTL13W	107.3	3.12	2.91	3.27	ND
KTL14W	97.2	5.01	5.16	ND	ND
KTL15W	100.8	3.13	3.10	5.39	ND
KTL16W	103.4	6.91	6.68	1.58	ND
KTL17W	104.1	4.96	4.76	2.19	ND
KTL18W	102.1	3.96	3.88	10.96	27.6
KTL19W	102.0	4.64	4.55	9.78	ND
KTL20W	103.1	3.93	3.81	ND	ND
KTL21W	99.4	5.56	5.59	2.79	3.31
KTL22W	109.5	5.04	4.60	ND	ND
KTL23W	105.4	4.73	4.49	1.85	ND
KTL24W	97.8	2.70	2.76	2.79	ND
KTL25W	95.9	4.43	4.61	3.21	ND

DISTRICT: TRANS NZOIA
SUNSHINE HOURS: 7.0
MEAN TEMPERATURES: Min 12.4°C Max 27.2°C
TOTAL RAINFALL: 622 mm
ABLTITUDE: 6220 ft
SAMPLING PERIOD: 29th - 30th MAR 1994

Table A5

SAMPLING GUIDE

SAMPLING STATION	DRY SEASON	WET SEASON
Sang'alo Institute	BGM01D BGM02D BGM03D BGM04D BGM05D BGM06D	BGM09W BGM10W BGM11W BGM12W
Lake Basin Dev. Authority	BGM07D BGM08D BGM09D BGM10D BGM11D BGM12D	BGM13W BGM14W BGM15W BGM16W
Kitinda Dairy Co-op. Society	BGM21D BGM22D BGM23D BGM24D BGM25D	BGM01W BGM02W BGM03W BGM04W
Farmers Training Centre	BGM16D BGM17D BGM18D BGM19D BGM20D	BGM19W BGM20W BGM21W BGM24W
Khakula Farm	BGM15D	BGM22W BGM23W
Okwomi Farm	BGM13D	BGM17W BGM18W
Okonya Farm	-	BGM05W BGM08W
Barasa Farm	BGM14D	BGM25W
Kituyi Farm	KTL23D KTL24D KTL25D	BGM06W BGM07W
Ndalu Farm	KTL06D KTL07D KTL08D KTL09D KTL10D	KTL01W KTL02W KTL03W
Cherangani Centre	KTL16D KTL17D KTL18D KTL19D KTL20D	KTL04W KTL05W KTL06W
Soy Sambu Centre	-	KTL07W KTL08W KTL09W
Bikeke Centre	-	KTL10W KTL11W KTL12W
ADC Farm Endebbes	-	KTL13W KTL14W
Kapsara Centre	-	KTL15W KTL16W KTL17W
Sikhendu Centre	-	KTL18W KTL19W
Nzoia Scheme Centre	KTL01D KTL02D KTL03D KTL04D KTL05D	KTL24W KTL25W
Wambwa Farm	KTL11D KTL12D KTL13D KTL14D KTL15D	KTL22W KTL23W
Kihara Farm	KTL21D KTL22D	KTL20W KTL21W

Table A6 SOLVENT PROPERTIES OF CHROMATOGRAPHIC INTEREST

SOLVENT	ϵ^{R1203}	Viscosity (mPa 20°C)	Density	R.I.	B.p.(°C)	UV cut off (nm)
Hexane	0.01	0.33	0.659	1.375	69.0	200
Toluene	0.29	0.59	-	1.496	110.6	285
Diethyl ether	0.38	0.23	0.713	1.353	34.6	220
Chloroform	0.48	0.57	1.5	1.443	61.2	245
Dichloromethane	0.42	0.44	1.366	1.424	40.1	235
Acetone	0.56	0.32	0.818	1.359	56.5	330
Ethyl Acetate	0.58	0.45	0.901	1.370	77.15	260
Acetophenone	0.65	0.37	0.782	1.344	82.0	190
Ethanol	0.88	1.20	0.789	1.361	78.5	210
Methanol	0.95	0.60	0.796	1.329	64.7	205
Acetic Acid	Large	1.26	1.049	1.372	117.9	-
Water	Large	1.00	1.000	1.330	100.0	-

Table A7 SALES OF STELADONE 300EC IN KENYA BY CIBA.

YEAR	AMOUNT IN LITRES
1990	377,000
1991	256,000
1992	250,000
1993	190,000
1994	200,000

Table A8 COMPOSITION OF BOVINE MILK LIPIDS

Class of Lipids	% Total Milk Lipids
Tryglycerides of fatty acid	95-96
Diglycerides	1.26-1.59
Monoglycerides	0.016 - 0.038
Ketoacid glycerides	0.35-1.28
Ketonogenic glycerides	0.03-0.13
Hydroxyacid glycerides	0.6-0.78
Lactonogenic glycerides	0.06
Neutral glyceryl ethers	0.016-0.020
Neutral plasmalogens	0.04
Free fatty acids	0.10-0.44
Phospholipids (total)	0.08-1.00
Sphingolipids	0.06
Sterols	0.22-0.41
Squalene	0.007
Carotenoids	0.0007-0.0009
Vitamin A (based on free alcohol)	0.0006-0.0009
Vitamin D	0.5×10^{-7}-2.1×10^{-6}
Vitamin K	0.0001

Table A9 CODEX REPORT 1993

CHLORPHENVINPHOS

JMPR: 71, 84 (Year of JMPR evaluation or subsequent review)
ADI: 0.002mg/kg body weight (1971-year of confirmation)
Residue: Chlorfenvinphos, sum of E- and Z- isomers fatsoluble.

COMMODITY

CODE NO.	NAME	MRL (mg/kg).
VB0041	Cabbage, Head	0.05
VR0577	Carrot	0.40
FC0001	Citrus fruits	1.00
VO0440	Egg plant	0.05
GC0645	Maize	0.05
MM0095	Meat	0.20 (fat)V
MM0187	Milk of Cattle, Goats and Sheep	0.008 FV
VO0450	Mushrooms	0.05
VA0385	Onion, bulb	0.05
SO0697	Peanut	0.05
VR0589	Potato	0.05
GC0649	Rice	0.05
CM1205	Rice, polished	0.05
VR0508	Sweet potato	0.05
VO0448	Tomato	0.10
VR0506	Turnip, garden	0.05
GC0654	Wheat	0.05

F (following MRLs for milk) - Residue is fat soluble
V (following MRLs for products of animal origin) - The MRL accomodates veterinary uses.