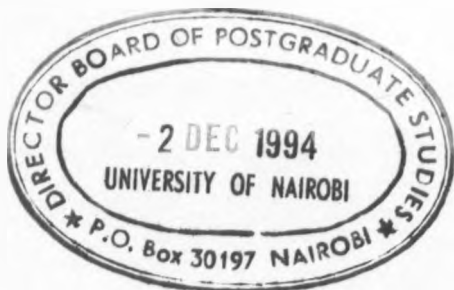


**STUDIES ON THE DISSIPATION AND METABOLISM OF SOME  
INSECTICIDES UNDER KENYAN ENVIRONMENTAL CONDITIONS.**

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A thesis submitted for the fulfilment of the degree of Doctor of Philosophy,  
University of Nairobi.

1993



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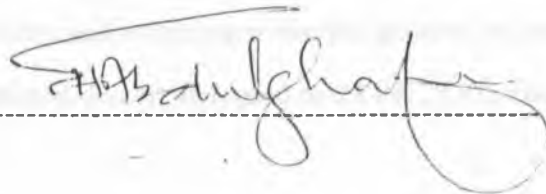
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## ABSTRACT

The dissipation and degradation of  $^{14}\text{C}$ -p,p'-DDT (over 172 days),  $^{14}\text{C}$ -p,p'-DDE (189 days) and  $^{14}\text{C}$ -parathion (72 days) were studied in tropical soils of Chiromo, Nairobi under field conditions. The three chemicals were found to dissipate under field conditions much more rapidly from tropical soils than from soils in the temperate regions. The dissipation curves were found to be biphasic with a rapid rate of disappearance from 0-37 days after application for DDT, 0-56 days for DDE and 0-7 days for parathion and a slower phase thereafter. The bound residues initially constituted a very small percentage of the applied pesticide, 1.3% for DDT, 10.4% for DDE and 2.4% for parathion but gradually increased to 5.9% after 64 days with DDT and 20% after 189 days with DDE. Bound residue levels of parathion in soil increased to about 25% after 72 days. Based on first order kinetics an overall half-life of disappearance for DDT, DDE and parathion in soil were calculated as 65, 145, and 48.4 days, respectively. In extracts of DDT treated soil, the main degradation products identified were DDE and a small amount of DDD. While the amount of p,p'-DDT decreased with time, there was a slow increase in the amount of extractable p,p'-DDE from the soil. However, in DDE treated soils p,p'-DDE remained unchanged throughout the experimental period. Ethyl parathion and its metabolites, paraoxon, p-nitrophenol and p-aminophenol were found in extracts of parathion treated soil during the first 21 days of the experiment. The amount of ethyl parathion in the extracts decreased with time while the amount of paraoxon, p-nitrophenol and p-aminophenol slowly increased to 23.9%, 6.7% and 3%, respectively during this period.

The effects of solar radiation on the dissipation of  $^{14}\text{C}$ -p,p'-DDT from non-sterile soil were also studied under laboratory conditions. The volatilization and mineralization

of  $^{14}\text{C}$ -p,p'-DDT was quantified. It was found that volatilization was the major dissipation process and the dissipation rate was significantly increased by solar radiation.

The dissipation and distribution of  $^{14}\text{C}$ -carbofuran in flooded and non-flooded soils under field conditions in the Kano plains at Ahero were studied for a period of 111 days. Carbofuran was found to be non-persistent and the dissipation trend was similar to studies from other tropical and sub-tropical countries. The disappearance was more rapid under flooded conditions with over 85% of the pesticide lost in flooded soil and over 60% lost in non-flooded soils after 111 days. A high percentage of carbofuran residues was found bound to the soil, although bound residue formation was higher in non-flooded soils. The vertical distribution of carbofuran residues remained in the top 10 cm layer of flooded soil after 111 days whereas there was a more uniform distribution of the residues vertically after 54 days and 111 days in non-flooded soils.

The adsorption/desorption and mobility of carbofuran in samples of agricultural soils collected from Ahero and Chiromo were also investigated. The amount of carbofuran adsorbed to Ahero soil was slightly higher than that of Chiromo soil i.e. 32.3% and 29% at 5 ppm, respectively. The results show that adsorption depended on both the content of clay and organic matter and the adsorption of carbofuran to Ahero soil to attain equilibrium was more rapid. The Freundlich constants of carbofuran in the two soils were:  $K_F = 15.49 \mu\text{g/g}$  and  $1/n = 1 \mu\text{g/ml}$  for Ahero soil and  $K_F = 15.14 \mu\text{g/g}$  and  $1/n = 1 \mu\text{g/ml}$  for Chiromo soil. Clay content seemed to influence adsorption to Ahero soil more than organic matter content while organic matter content influenced adsorption to Chiromo soil more. At equilibrium, there was stronger adsorption of carbofuran to Chiromo soil and only 39% was desorbed from the soil after 6 hours while 70% of the adsorbed pesticide was desorbed from Ahero soil. After 48 hours of leaching, 33% of the total residues were found in the leachate from Ahero soil and 29% in the leachate from

the Chiromo soil. More types of metabolites were identified from the Chiromo soil column confirming greater microbial and chemical activity in this soil column.

Two experimental models simulating the traditional storage conditions prevalent in Kenya i.e. the open basket model and the modern wooden box model were used to study the rate of dissipation and fate of malathion residues in maize grains (*Zea mays*) and beans (*Phaseolus vulgaris*). The maize and beans were stored for periods of upto one year at ambient temperatures averaging 23°C. The grain samples were initially treated with radiolabelled  $^{14}\text{C}$ -malathion dust prior to storage and portions analysed at regular time intervals for malathion, malaoxon, isomalathion and the hydrolytic metabolites, malathion  $\alpha$ -monocarboxylic acid and malathion  $\beta$ -monocarboxylic acid using a combination of chromatographic, radioisotopic and mass-spectrometric techniques.

The findings showed a gradual penetration of malathion into the grains in amounts which were slightly higher in maize than in beans irrespective of the method of storage. After 51 weeks of storage, 34-60% of the initial residues was persistent in all grains. The total residual levels were slightly higher in beans than in maize irrespective of the storage method though the persistence was a little higher in the wooden box than in the open basket. The rates of dissipation of the insecticide decreased with storage time, following a biphasic pattern. Following first order reaction kinetics, the following half-lives were obtained: maize grains stored in open basket- 194 days, maize grains stored in closed wooden box- 261 days, beans stored in open basket- 259 days and beans stored in closed wooden box- 405 days. The beans stored in the wooden box had higher levels of bound residues than those sampled from the open basket. This trend was similar in maize grains although the concentrations were lower. The analysis of malathion metabolites by GC confirmed the degradation trend of the residues.

ALUMNI

DEDICATION

To my late father, Jakobo Lala Wajwang'a

## ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to the German Academic Exchange Service (DAAD) for the assistance given to me both financially and in the form of a fellowship during my studies. I am also very grateful to the International Atomic Energy Agency (IAEA) for a training fellowship and the radiolabelled chemicals provided to me under Research Contract Nos. KEN/5/016 and 315RC/5566/R1/RB, respectively. I wish to thank the Director of Kenya Industrial Research and Development Institute for allowing me to take a study leave to pursue this course.

I sincerely thank my supervisor, Prof. Shem O. Wandiga for his guidance and encouragement throughout my studies. I also wish to thank Dr. F. V. Acholla for encouraging me to take up this topic and Prof. A.H.S. El-Busaidy for going through the thesis draft.

I am very grateful to Prof. W.C. Dauterman of the Department of Toxicology, North Carolina State University, USA for his tireless efforts to make me understand the application of radioisotope techniques in pesticide metabolism studies and for accepting to read and correct all the manuscripts. His assistance contributed a great deal towards the completion of this thesis. I also wish to thank Prof. A. Kettrup of the Institute of Ecological Chemistry, GSF, Munich for accepting me in his laboratory where I did most of the analytical work.

To my wonderful colleagues, Juma Makopa and Peter Ng'ang'a for being such a lively team to work with.

Finally to my wife, Anne and children, Lalah, Obara, Wasonga and Mboss for their loving support.

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## GENERAL INTRODUCTION

### PESTICIDES: BENEFITS AND RISKS

A pesticide is any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any insect, rodent, nematode, fungus, weed or any other form of terrestrial, aquatic or animal life or virus, bacteria or other organisms which is declared to be a pest except viruses, bacteria or other microorganisms on or in living man or other animals; or any substance or mixture of substances intended for use as a plant regulator, defoliant or dessicant (Green et al., 1977; Munga, 1985; Murphy, 1986). They are used in agriculture and public health to produce a crop of higher quality, reduce the input of labour and energy in crop production and to control vector borne diseases by combating veterinary and human pest related problems (Brown, 1972; Mathews, 1979).

Pesticides occupy a rather unique position among the many chemicals that man encounters everyday in that they are deliberately added to the environment for the purpose of killing or injuring some form of life (Murphy, 1986) and depending on their intended use, they are classified as insecticides ( used against harmful insects), acaricides ( used against ticks and mites), herbicides ( against weeds), fungicides ( against plant fungal diseases), rodenticides ( against rodents e.g. mice, rats), nematicides (against nematodes), avicides ( against birds), molluscicides ( against molluscs) and synergists of both herbicides and insecticides (Hodgson, 1991).

Insecticides represent one group of pesticides that are used in large quantities and have a well known history of causing toxic effects in non-target organisms (Murphy, 1986). In deed, both the greatest successes and environmental failures associated with pesticides such as rapid agricultural development, disease vector control in public health

and pollution of the environment in the developed world have involved the use of insecticides (Hodgson, 1991). Insecticides can also be classified according to their mode of action and/or according to their chemical nature i.e. the organochlorines, organophosphates, carbamates and pyrethroids. Apart from having in common the capability of destroying life of one form or another, the other important characteristic of pesticides is that just as there is diversity in their chemistry and physicochemical properties, there is also a great diversity in their types of action and their primary targets (Murphy, 1986). In this study, four insecticides i.e. DDT (an organochlorine), malathion and parathion (organophosphates) and carbofuran (a carbamate) were investigated with regard to their metabolism in the environment and their brief history, chemistry and modes of action are discussed below (Corbett, 1974; Matsumura, 1975; Kuhr, 1976; Mghenyi, 1988).

Chemical pesticides have been used for over a thousand years to control pests. The first synthetic insecticides were inorganic in nature and contained substances such as arsenic, copper, mercury and boron as active ingredients (Edwards, 1970). Examples are Paris green, acetate and arsenite of copper crystals used in the USA against the Colorado beetle in potatoes in 1867, lead arsenate against gypsy moth in 1892, sodium fluoride and boric acid against cockroaches in 1930's. Other important chemicals used for pest control were the organic insecticides which were natural plant derivatives e.g. nicotine (from tobacco), pyrethrum (from the chrysanthemum) and rotenone (from the roots of the derris plant (Mathews, 1979; Dix, 1981).

DDT was first synthesized in 1874 by Zeigler and its insecticidal properties discovered in 1939 by Mueller (CRC: organochlorines). It had profound effect on the whole subsequent history of pest control (Hodgson et al., 1991). Its low cost, broad spectrum of activity, selective toxicity, persistence and ease of formulation made it the most widely used insecticide ever introduced. Pure DDT consists of white needles which

melt at 108-109°C, has low water solubility and a strong lipophilic character that favours its accumulation in biolipids (Farm Chemicals, 1991). It is very stable because its molecule is constructed entirely from C-C, C-H and C-Cl bonds all of which tend to be chemically inactive under normal environmental conditions. The only possible reaction it undergoes is loss of hydrogen chloride in the aliphatic centre.

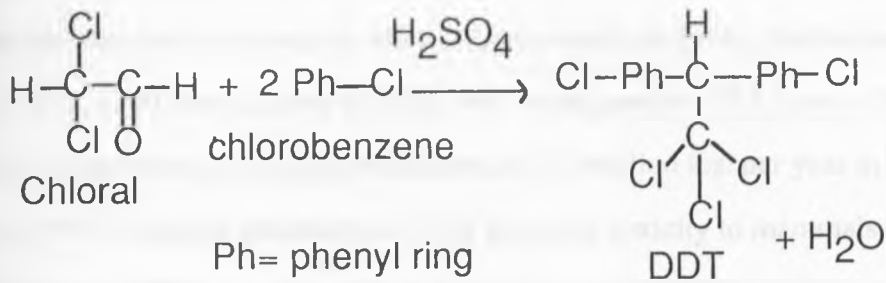


Fig. 1 The synthesis of DDT

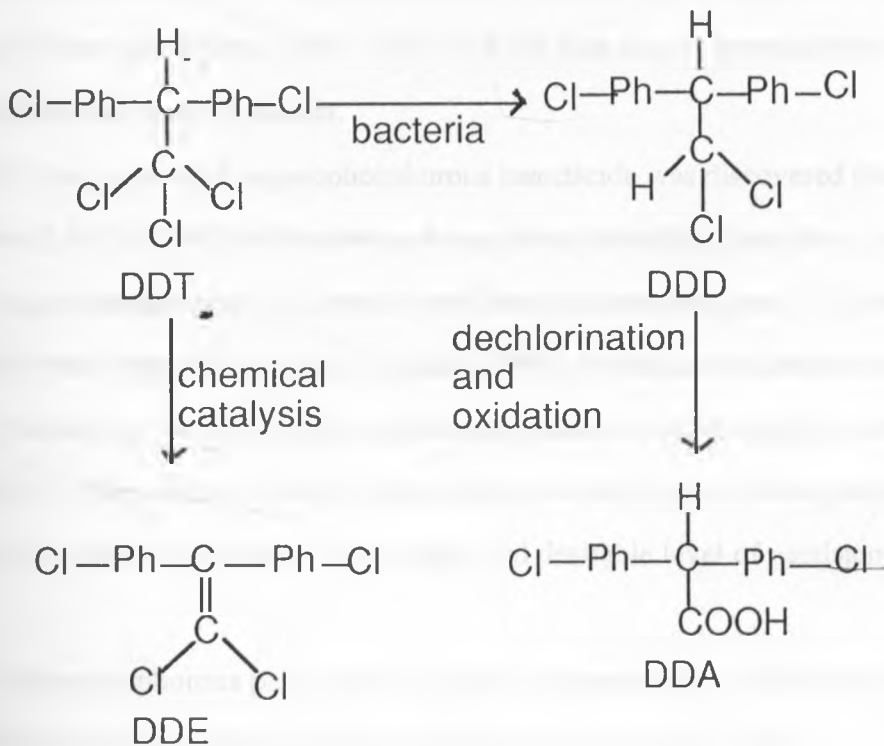
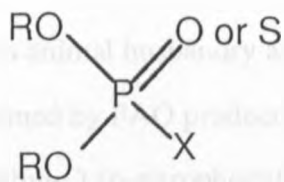


Fig. 2 The metabolic pathway of DDT in soil.

DDT is synthesized by the condensation of chloral with chlorobenzene in the presence of sulfuric acid (Mark et al., 1966). In addition to the desired product p,p'-DDT, 20-30% of o,p'-DDT and trace of o,o'-DDT are formed as impurities. Commercially, DDT is relatively easy to produce and therefore very cheap. It was manufactured in large quantities during the second world war and its use by the US Army arrested a potential epidemic of typhus in 1943 in Naples for the first time in medical history. Its continued use also reduced a large death toll from malaria in India (Hartley and West, 1967). In 1961, 1200 formulations of DDT were being used in USA against 240 different agricultural pests leading to a peak production of 180 million lbs. per year in 1963 (Hodgson, 1991). Another advantage of DDT is its low toxicity to mammals and its lack of skin irritancy. DDT is a neuro-excitant which affects the peripheral sensory fibres and motor units initially and then the peripheral nervous system (Hassall, 1990). Its action on nerves has been attributed to a delayed shut-off of sodium gates during the action potential (O'Brien and Hilton, 1978). This blockage then causes hyperactivity followed by convulsions and death in insects.

The first successful organophosphorous insecticide was discovered during the second world war in 1941 by Schrader and was called schradan. Since then, over 100,000 organophosphorous compounds have been screened and over 100 have been marketed for their insecticidal action (Hassall, 1990). Parathion was discovered in 1944 and this opened way for the development of many others such as malathion, diazinon, dimethoate, etc. The objective of the search into more and more of these pesticides was to find improved selectivity, safety to mammals, and desirable level of persistence (Hassall, 1990).

Organophosphorous pesticides are esters of phosphoric or thiophosphoric acid and most of them have the general structure shown below (Lin et al., 1983).



R= methyl, ethyl  
 X= aliphatic, aromatic or  
 heterocyclic group.

Fig. 3 The general structure of organophosphorous insecticides.

X is the leaving group which is normally joined via an ester or thioester linkage i.e. P-O-X or P-S-X. The structural variability of organophosphorous compounds is reflected in their wide range of physicochemical properties and also in the considerable diversity of mechanisms by which they can be attacked by enzymes which consequently leads to species selectivity and minimal risk of uniform development of tolerance to all the organophosphorous compounds. The varying physicochemical properties include different vapour pressures at room temperature, different solubilities in water, their chemical stability and toxicity to mammals. The wide spectrum of physicochemical and biological properties of these compounds enables them to have a wide range of uses in agriculture and in animal hygiene. They can be classified as contact insecticides e.g. mevinphos, persistent contact e.g. malathion, and systemic compounds e.g. dimethoate depending on their practical uses and modes of action.

Organophosphorous pesticides became popular as a replacement for organochlorines due to their low persistence in the environment even though their cost was sometimes as much as three times higher than that of the organochlorines. This is illustrated by a survey of pesticide usage in 1983 in the UK. The data collected was from a variety of farms and small holdings using pesticides since 1976 and showed that the extent of organophosphorous pesticide useage in agriculture was increasing (Smart,

1983). This was also true in animal husbandry and those used as herbicides and as growth regulators. This was confirmed by FAO production yearbooks 1979-1985.

Parathion, O,O-diethyl-O-(p-nitrophenyl) phosphorothioate, was first synthesized in 1944 as O,O-diethyl phosphorothioic acid ester of p-nitrophenol and recognized as an insecticide in 1945 (Smart, 1983). The great interest in parathion (the second organophosphorous insecticide after TEPP which was used as a substitute for nicotine against aphids) was stimulated in the 1950's due to its outstanding insecticidal properties and very broad spectrum of activity (Sethunathan et al., 1977; Smart, 1983). The cleavage of the P-O-phenyl linkage of parathion is not a hydrolytic reaction, *in vivo*, but is an oxidative process mediated by an NADPH-dependent oxidase (Muirhead-Thomas, 1971; Sethunathan et al., 1977). Parathion can be synthesized by the reaction of thiophosphoryl chloride  $\text{PSCl}_3$  with ethanol in the presence of an acid-binding agent followed by reaction with sodium salt of p-nitrophenol (Fig. 4).

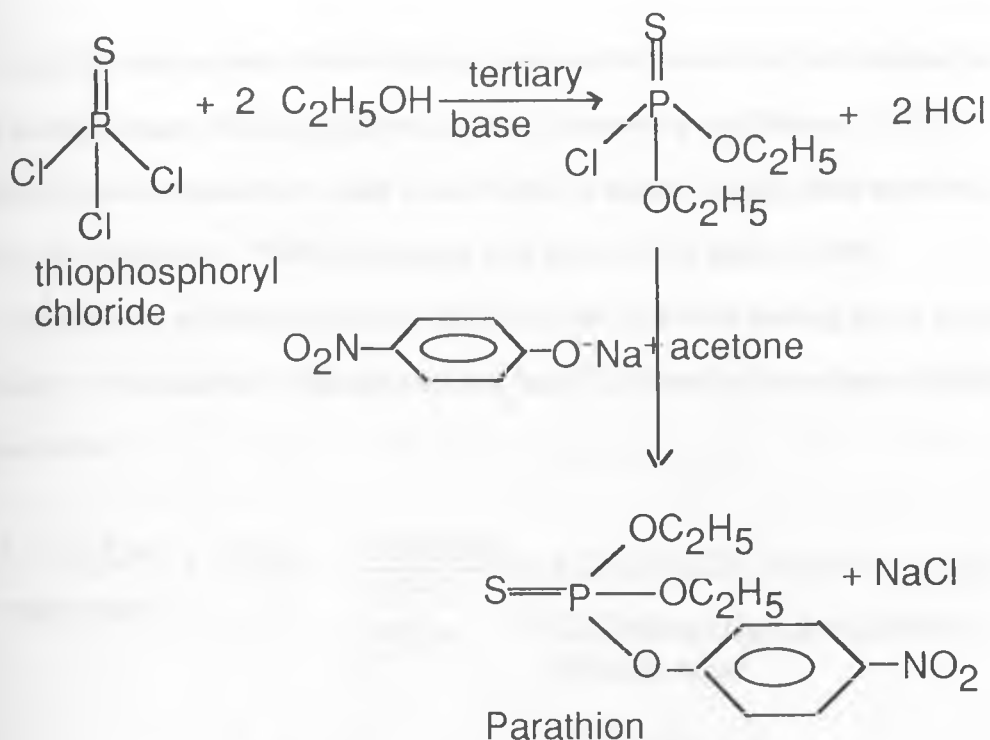


Fig. 4 The synthesis of parathion

Parathion acts by phosphorylation of acetylcholinesterase and affects the neural transmission of impulses through the nervous system of target organisms e.g. arthropods or mammals (Smart, 1983; Hassall, 1990).

Malathion, O,O-dimethyl-S-(1,2-dicarboethoxyethyl) phosphorodithioate, is one of the most generally used organophosphorous pesticides (Hassall, 1990). It was developed in 1950 by the American Cyanamid company. It is a yellow oil with low water solubility (145 mg/L at room temperature), and is soluble in most organic solvents except for petroleum ether. Its lipophilic nature enables it to get into the waxy cuticle of leaves. Malathion has high insecticidal activity, is expensive but safe to use, selective and in its pure form has moderate toxicity (LD<sub>50</sub> of 12,500 mg/Kg in rats) (Lin, 1983). Crude malathion and its formulations which contain impurities are far more toxic to mammals.



These impurities are not only formed during commercial production but can also be formed during storage (Hollingshaus et al., 1981; Armstrong and Fukuto, 1987).

Impurities present in malathion make it more toxic to higher animals than expected (Hassan and Dauterman, 1968; Dauterman and Main, 1984; Miles, 1989).

Malathion is a dithiophosphate with a succinic acid ester leaving group and can be synthesized by the reaction of diethyl maleate with O,O-dimethyl phosphorodithioic acid as shown below.

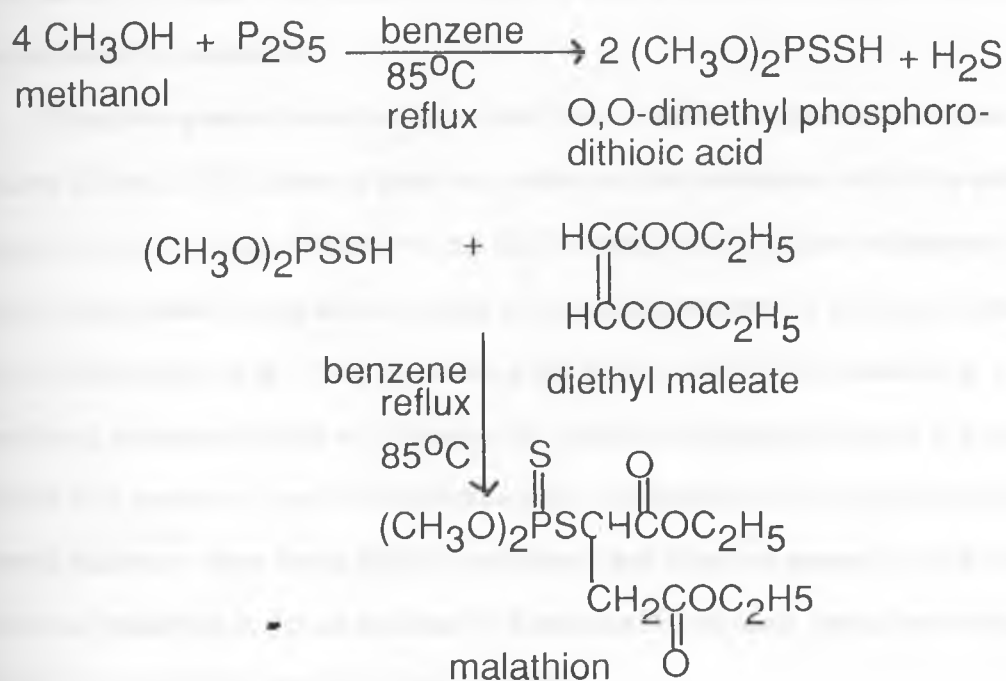


Fig. 5 The synthesis of malathion.

Principally, malathion is used worldwide to control aphids and similar sap sucking insects, weevils, small beetles, scale insects and red spider mites on vegetables, field crops, fruits, nuts, stored grains and on domestic animals (Lin et al., 1984). It undergoes activation before it reaches at the site of action in the nervous system of the

insect. This activation is an oxidative process which renders the product more polar than the original molecule so that it possesses systemic action whereas the original molecule does not. This product is malaoxon and it is formed by replacing the thiono sulfur atom with oxygen. Malaoxon is a much more powerful cholinesterase inhibitor. The formation occurs in insects which possess a very active oxidative enzyme system and activates malathion by attacking P=S linkage so that the insect's own enzyme system actually helps to kill it (see Fig. 8). In organisms in which carboxylesterases are active (e.g. vertebrates), one of the ethyl groups from the succinic ester moiety is removed by hydrolysis leading to detoxication of malathion.

Impurities present in malathion makes it more toxic to higher animals than expected (Miles, 1989). Some of these impurities are low molecular weight by-products and possess high toxicity of their own e.g. O,S-dimethyl-S-(1,2-dicarboethoxyethyl) phosphorodithiolate (isomalathion) which is particularly known for its delayed toxic effect (Hollingshaus et al., 1981; Armstrong and Fukuto, 1987). Isomalathion is a potentiating substance which will increase the toxicity of malathion since it is a powerful inhibitor of  $\beta$ -esterases (one of the constituents of carboxylesterase in higher animals). It prevents malathion from being rapidly hydrolysed and therefore potentiates the action of malathion. Malathion is not an inhibitor of  $\beta$ -esterase. Other toxic impurities include O,O,S-trimethylphosphorothioate (Miles, 1989).

The third major class of modern synthetic pesticides was the carbamates introduced in the 1950's starting with carbaryl. Like the organophosphates, they are acetylcholinesterase inhibitors that are non-persistent, do not bioaccumulate and tend to have lower mammalian toxicity (Hodgson et al., 1991). The other major carbamates include carbofuran and aldicarb. Currently the commercial market is dominated by the organophosphates and the carbamates though other compounds such as synthetic

pyrethroids which are often more selective are growing in importance (Hodgson et al., 1991).

Carbamate insecticides are more closely related in biological action and with regard to resistance development to organophosphorous insecticides (Hartley and West, 1967; Hassall, 1990). They are frequently used against insects that do not readily respond to organophosphorous compounds. They are in most cases more expensive than common organophosphorous compounds but their production is steadily increasing (Kuhr and Dorough, 1976). Their use is appropriate in controlling recalcitrant insects that do not readily respond to organophosphates e.g. whiteflies, leafminers, ants, mealy bugs, scale insects, cockroaches, earwigs and wasps and to control aphids and other pests that have developed resistance to organophosphorous insecticides. Their general structure is given below.

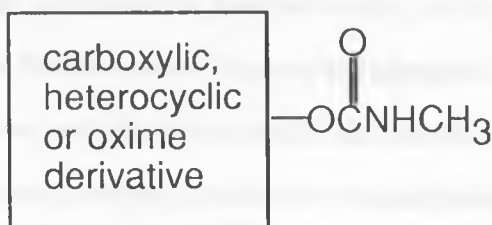


Fig. 6 The general structure of carbamates

The principal mechanism of carbamate toxicity is the inhibition of cholinesterase activity involving carbamylation of the esteric site of acetyl cholinesterase (Hassall, 1990). This effect is more labile and takes a shorter time than in the organophosphorous compounds. Thousands of carbamates have been screened for insecticidal activity but only 20 are currently marketed in significant quantities (Farm Chemicals, 1991).

Carbamates also have a narrower spectrum of toxicity than the organophosphates and their toxicity is influenced by their chemical structure as well as the physicochemical and

biophysical factors (Felsot et al., 1985). Carbamate insecticides are absorbed and metabolised rapidly in living systems and the number of metabolites formed is considerably larger. Strongly hydrophilic carbamates (e.g. carbofuran) are slow to penetrate the cuticle and do not show contact toxicity. When they get inside, knockdown is quick and there is usually no recovery. Strongly lipophilic carbamates (e.g. carbaryl), on the other hand, penetrate easily and give a rapid knock down that is followed by a degree of recovery depending on dose (Quraishi, 1977).

Carbofuran (2,3-dihydro 2,2-dimethyl-benzofuran-7-yl-N-methylcarbamate) is a widely used carbamate insecticide. It is an N-methylcarbamate ester of a heterocyclic phenol i.e. 2,3-dihydro 2,2-dimethylbenzofuranol. It is a systemic and contact insecticide and nematicide which has a broad spectrum of activity against many agricultural pests (Aquino and Pathak, 1976). It has low mammalian toxicity (LD50 11 mg/kg in rats) but is very toxic to invertebrates and should be handled with a lot of care. It has been extensively used worldwide for the control of pests in sugarcane, sugarbeet, maize, rice and coffee. It has rapid action and kills both nymphs and adults within 20 minutes. The metabolic attack on carbofuran involves oxidation or hydroxylation of the ring itself (Suett, 1986).

Over one thousand pesticide chemicals are in use throughout the world today, a quarter of which are mainly used in agriculture (Hodgson et al., 1991). The use of these chemicals is recognized worldwide as effective, simple, quick method of pest control. Without chemical control, crops would be ravaged by disease, insect pests, and weeds and there would be severe loss of food (Mathews, 1979). To illustrate the importance of pesticides, the control of wild oat *Avena spp* in cereals can prevent losses of grain upto 350 kg/ha (Mathews, 1979). Virtual weed-free conditions are now possible using the range of herbicides available to prevent such losses.

Various types of pesticides are used in the control of insects, rodents and other pests that are involved in the life cycle of vector borne diseases such as malaria, filiarisis, yellow fever, typhus etc. Worldwide estimates of the lives saved by using DDT for example to eradicate malaria and other disease vectors are numbered in the millions (Klaassen et al., 1986). It has been estimated that well over 1000 million people would be exposed to malaria if the highly successful use of insecticides were to be interrupted. Despite intensive research into alternative methods of pest control, chemical control is still the most effective method of controlling most insect pests, weeds and diseases. Higher living standards of an ever increasing human population will undoubtedly require increased use of pesticides particularly in the developing countries which were using less than 10% of the world pesticide production in 1977 (Edwards, 1966; Edwards, 1973; Xiang, 1981).

During the development of many of the original pesticides man was so preoccupied with the effectiveness of his pesticides that little consideration was given to what would eventually happen to the steadily increasing volume and number of chemicals that were being released into the environment (Dix, 1981). Though their use is beneficial one important disadvantage is their dispersal and persistence in the environment (Xiang, 1981). Organochlorines, for example, are the most persistent in the environment (Edwards, 1973; White, 1974).

Large amounts of pesticides and their residues are therefore distributed in the environment and enter the drainage canals, rivers, lakes and oceans via transportation by wind, water and through domestic and industrial sewage (Sharon et al., 1980; Sharon and Mark, 1980). Pesticides applied directly to water to control aquatic pests and erosion of contaminated farm soil account for the greatest contribution of pesticides in water bodies (Edwards, 1966). DDT residues have been reported for example in water mainly adsorbed to particulate matter (Giddings and Monroe, 1970; Bierman and Swain, 1982). Because

of its high solubility in fat and low solubility in water, DDT is easily bioconcentrated in bodies of fish and other aquatic organisms (Miles and Harris, 1978; Meier et al., 1983). The storage of organochlorine pesticides in human fatty tissues and blood samples is now well known (Hickey and Anderson, 1968; Menzie, 1972). The effect of pesticides on terrestrial environments due to their persistence in soil have also been reported in many studies (Nasim et al., 1971; Cooke and Stringer, 1982; Buck et al., 1983).

Pesticides also have a negative effect on the ecology of non-target organisms (Stickel, 1974). Their residues and metabolites have been found in wild birds and mammals all over the world and can be passed from one animal generation to another through the placenta, milk or eggs (Stickel, 1974; Hill and Wright, 1978). The impact of DDT on the ecology have been shown to occur in the decline in the number of certain bird species attributed to increased egg-shell thinning due to its residues found in these birds (Hickey and Anderson, 1968; Menzie, 1972). There is a correlation in time between geographical areas of high DDT usage and the effects on local populations living in areas of low useage (Perfect, 1980; UNEP, 1986). In Kenya, monitoring of animal products e.g. meat, milk and organ tissues, have shown that the continuous use of the organochlorine pesticides leaves chemical residues. The amounts of these residues were found to be greatest in districts with high agricultural activities (Kaine, 1976).

Another problem associated with pesticide use is the rapid development of resistance if most of the pest population is exposed to the specific pesticide and if the pest can multiply quickly or if there is some immigration of unexposed individuals. The effects of persistent, broad spectrum chemicals e.g. the organophosphates have been shown by the upsurge of pest populations when the balance between the pests and their natural enemies is destroyed as a result of pesticides killing not only the target but also the non-target predators (Hassall, 1990). Organophosphate and carbamate insecticides have been adopted in many countries as a preferred means of pest control. Intensive use

of these chemicals which target the enzyme acetylcholinesterase may result in insecticide resistance of pest species conferred by an altered form of acetylcholinesterase. The alteration in acetylcholinesterase causes a change in kinetics of inhibition with various insecticides reducing their efficacy in competing for the enzyme with its normal substrate acetylcholine (Hemingway, 1991). The problem of resistance is often magnified by the use of different pesticides against the same pest.

## **METABOLISM OF PESTICIDES IN SOIL**

Synthetic organic pesticides have been in widespread use for more than 50 years during which they have contributed greatly to increased worldwide food production and improved human and animal health (Plimmer, 1988; MacRae, 1989). Despite the great benefits associated with the use of agricultural pesticides, they are metabolized in soils leaving metabolic residues some of which are toxic to non-target species including humans. Some of these metabolites persist in soil posing a great danger to the environment. This has led to a number of studies aimed at understanding the degradation mechanisms of these pesticides in soils.

The environmental fate of a pesticide in soil is dependent upon the physical and chemical properties of the soil (Plimmer, 1988) and the movement or the disappearance of pesticides from the site of application can be caused by run-off, leaching and/or volatilization. The dissipation pathways are also influenced by physical/chemical properties of the pesticide itself i.e. volatility, vapour pressure and the nature of the surface, type of formulation, method of application and agricultural conditions. It is important to understand the degradation of pesticides not only because of degradation products as environmental contaminants but also because degradation reduces the effectiveness of the pesticide (Karns et al., 1986). Due to potential dangers to living organisms some of the more persistent insecticides in the environment have been replaced

by less persistent ones which rapidly disappear from environmental components such as soil, plants, grains etc.

The disappearance of a pesticide from soil may not only reflect its degradability but may also show our inability to detect its residues by conventional procedures i.e. its potential conversion to bound compounds which cannot be extracted by the usual organic solvents (Lichtenstein et al., 1977). Using radiolabelled pesticides it has been observed that a portion of the pesticide residues remains in the soil after solvent extraction with the usual solvents following extraction of incubated pesticide treated soils (Khan and Hamilton, 1980). These residues are determined by combustion of the extracted soil to yield  $^{14}\text{CO}_2$  which is then analysed by liquid scintillation counting. Recently, it has also been analysed by high temperature steam distillation and by supercritical fluid extraction method which is of great advantage because it does not alter the chemical nature of bound residues which can then be identified (Roberts and Standen, 1981; Khan et al., 1990). Bound residues may be the parent chemical or its degradation products. In many cases, persistent soil bound residues result from both the parent and one or more of the degradation products. Typically, bound residues constitute 20-70% of the total residues present in the soil (Klein and Scheunert, 1982). This shows that a significant portion (20-70%) of pesticides applied in agriculture remains in soil as persistent residues bound to the soil colloids. There are two main mechanisms involved in bound residue formation (Calderbank, 1989) which include surface adsorption to soil and covalent bond formation. The covalent bond formation process is predominant in the binding of degradation products. Organic matter is largely responsible in the initial binding process but certain expanding clays which bind some pesticides more firmly than organic matter may be involved later. The clay and organic matter (colloids) in particular have very large surface areas and provide millions of square metres of active site surface in the top centimetres of single hectare. These active surfaces of finely divided soil colloids affect the behaviour of



chemicals in soil with regard to how readily they are retained (adsorbed), leached, or chemically decomposed (MacRae, 1989).

As time progresses, bound residues tend to become more bound, more resistant to degradation and show little evidence of biological activity (Khan, 1982). This may be the slow chemical incorporation of the residues into humic fraction of the soil and/or the result of migration of residues to less accessible binding sites which requires an energy barrier to be overcome (Calderbank, 1989). The bound residue levels therefore increase indefinitely but reach a plateau level when the amount being degraded is equal to the amount being formed each year. Bound residue levels depend on the type of pesticide, soil, climatic factors e.g. organochlorines (7-25% of applied pesticide), organophosphates (18-80%), carbamates (32-70%), fungicides (chlorophenols) (45-90%), and herbicides (ureas) (34-90%).

The significance of bound residues and their bioavailability is well addressed and their physicochemical and biological characteristics in soil is well documented (Lichtenstein and Khan, 1977; Helling and Krivonak, 1978a). Khan (1982) describes two categories of bound residues i.e. bioavailable bound residues in soil which are not taken up by plants/animals in the soil or bound residues in plants which are not absorbed from the gastrointestinal tract when administered orally to animals and are excreted in faeces. The bioavailable bound residues in soils are those that are taken up by plants or animals and those in plants which are absorbed from gastrointestinal tract if taken orally. The biological significance of bound residues in grains have also been demonstrated in wheat grains treated with radiolabelled deltamethrin and stored in the laboratory for 168 days (Khan et al., 1990) and it was found that 11% of total applied pesticide was in bound form. The FAO has recognized the biological significance of bound residues and in 1986 the joint FAO/IAEA division of Isotope and Radiation Application of Atomic Energy for Food and Agricultural Development initiated a programme on the biological activity and

bioavailability of bound pesticide residues using nuclear techniques which emphasized studies on grain bound residues and their bioavailability to experimental animals and toxicological potential (FAO/IAEA, 1986; Stratton and Wheeler, 1986). It has been found that bound residues in stored wheat treated with deltamethrin when fed to rats are highly bioavailable and the residues are detected in urine and faeces (Khan et al., 1990). Recently, Aly and Dauterman (1992) extracted and identified bound fenvalerate and diflubenzuron from wheat using supercritical fluid chromatography and showed their bioavailability in rats. They also demonstrated their biological activity in fathead minnows and housefly larvae, respectively.

Microorganisms have been established as a major means of destroying pesticide chemicals in soil and water through their involvement in the biodegradation and detoxification of these pesticides (Coats and Somasundaran, 1991; Somasundaran et al., 1991). MacRae (1989) has summarized the literature from 1981 to 1987 on the metabolism of pesticides and structurally related compounds in soil. During this period, research into the microbial metabolism of a diverse range of pesticides was extensively conducted and the role of microorganisms in the degradation of the three types of pesticides of interest in this study i.e. organochlorine, organophosphate and carbamate insecticides has been clearly established. However, the metabolism of pesticides in soil cannot be viewed in terms of microbes in isolation i.e. without the consideration of other factors such as the chemical, photochemical and physical factors including temperature, humidity, and volatility which may affect biodegradation (Mulla et al., 1981).

### **Metabolism of parathion**

It was reported sometime ago (Lichtenstein and Schulz, 1964) that microorganisms are associated with the breakdown of parathion and methyl parathion in soil by hydrolysis to give p-nitrophenol. Sterilization of the soil samples by autoclaving

was found to destroy 90% of the degradation activity but was not affected by gamma radiation treatment of the soils (Kishk et al., 1976). The major pathways in the metabolism (degradation) of parathion in soil and water environments are nitro group reduction and hydrolysis both mediated by microorganisms (Sudhakar-Barik et al., 1979). The nitro group is reduced to the amino group making the molecule less active. The formation of paraoxon, aminoparathion, p-nitrophenol, and p-aminophenol from parathion in soil has been reported and they are produced partly by hydrolysis in soil which is largely due to microbial activity (Fuhreman and Lichtenstein, 1980). After repeated applications of parathion or its hydrolysis product p-nitrophenol to an anaerobic flooded soil, the degradation pathway was found to shift from reduction to hydrolysis (Sethunathan and Yoshida, 1973; Sudhakar-Barik et al., 1979). This shift occurred as a result of the proliferation of parathion-hydrolysing microorganisms that utilize p-nitrophenol as an energy source. It has been established that microbial populations build up in soil and water systems following repeated applications of a wide array of pesticides which serve as a source of carbon and energy for growth (Waid, 1972). These studies were however done under laboratory conditions and the shift is known uniquely for parathion.

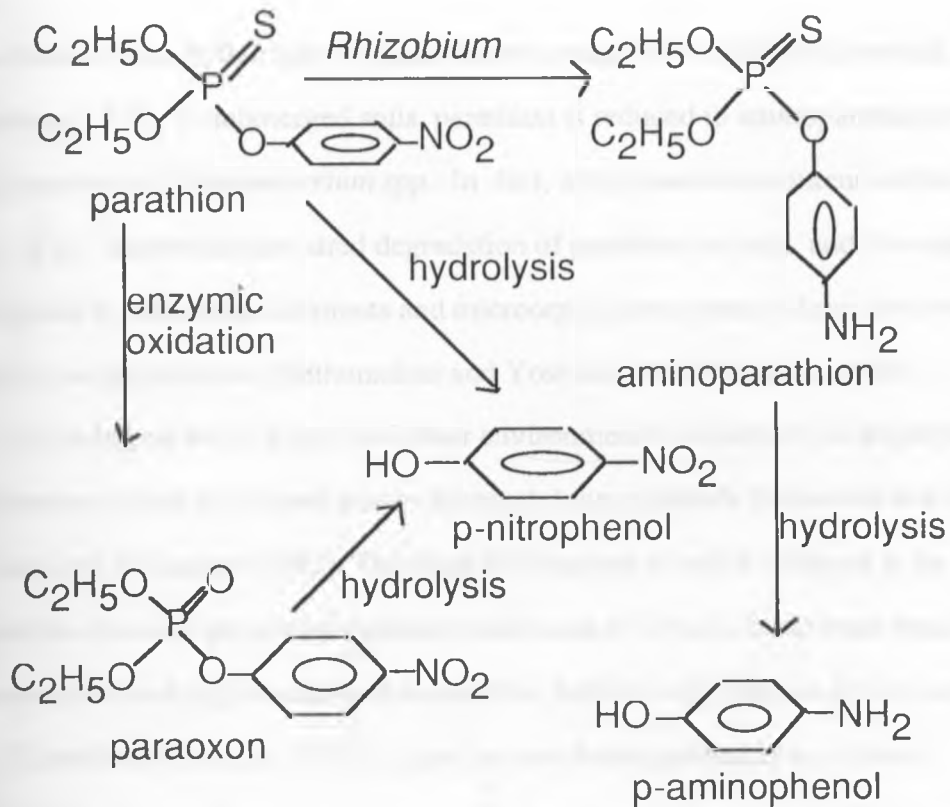


Fig. 7 The metabolic pathway of parathion in soil.

The rate of degradation of parathion in a flooded soil increases progressively after each successive application. Aminoparathion has been recovered as a major product after the first addition of parathion and aminoparathion and p-nitrophenol after the second addition while only p-nitrophenol was found after the third addition. This shows that degradation of parathion shifted from reduction to hydrolysis after repeated application to flooded soil (Sethunathan and Yoshida, 1973).

The degradation of parathion in flooded soil follows first order kinetics but hydrolysis proceeds at a remarkably faster rate than reduction. Sethunathan and Yoshida (1973) investigated the persistence of parathion in five soils under flooded and non-flooded conditions and found that it degraded faster in near neutral soils under flooded conditions than under non-flooded conditions. Parathion has also been reported to

degrade faster in soils with higher organic matter content due to higher microbial activity (Sethunathan, 1973). In submerged soils, parathion is reduced to aminoparathion by microbial activity of *Flavobacterium spp.* In fact, a high moisture content and the presence of soil microflora favoured degradation of parathion in soils and the major route of metabolism in soils, lake sediments and microorganisms in pure culture involved nitro reduction to aminoparathion (Sethunathan and Yoshida, 1973; MacRae, 1989).

Depending on the soil type and other environmental conditions, large proportions of parathion may bind to soil and persist for much longer periods (Reynolds and Metcalf, 1962; Klein and Scheunert, 1982). This high binding rate to soil is believed to be due to the formation of amino groups by metabolic reactions in the soil. It has been found that high temperature and high humidity decreased the half-life of parathion in soil and its toxicity (Ronald and Baetcke, 1973). Light has also been implicated as a factor responsible for the breakdown of parathion. The exposure of parathion to light resulted in the formation of cholinesterase inhibitors which were chromatographically different from parathion (Ronald and Baetcke, 1973; Katan et al., 1976; Sethunathan et al., 1977).

### Metabolism of DDT

Organochlorine pesticides are well known for their persistence in the environment particularly in soil. In temperate regions, most organochlorines persist for several months and even years (Hassall, 1990). This is especially so when high concentrations of the pesticides are applied to soils with high clay and organic matter content. The three major pathways by which DDT is degraded in soil are shown in Fig. 2. The first pathway involves dehydrochlorination to DDE which is the major metabolite identified in soil and in animal tissues treated with p,p'-DDT. The enzyme that catalyses this reaction is DDT dehydrochlorinase (DDTase) and the reaction is dependent on the presence of glutathione. The other metabolite p,p'-DDD is formed by reductive dechlorination, a

process which occurs widely in nature. This reduction is brought about by a catalytic but non-enzymatic reaction involving the participation of a haeme compound, riboflavin and a reduced nucleotide. For example the anaerobic conditions at the bottom of a pond can lead to DDD production by similar non-enzymic means. Dicofol is formed by oxidation of DDT. The dissipation and degradation of p,p'-DDT has been found to be more rapid in tropical soils (half-lives ranging from 64-110 days) than in temperate soils and the metabolites DDE and DDD have been identified and quantified in the same soils (Giddings and Monroe, 1972; El-Zorgani, 1976; Wandiga and Natwaluma, 1984; Wandiga and Mhgenyi, 1988).

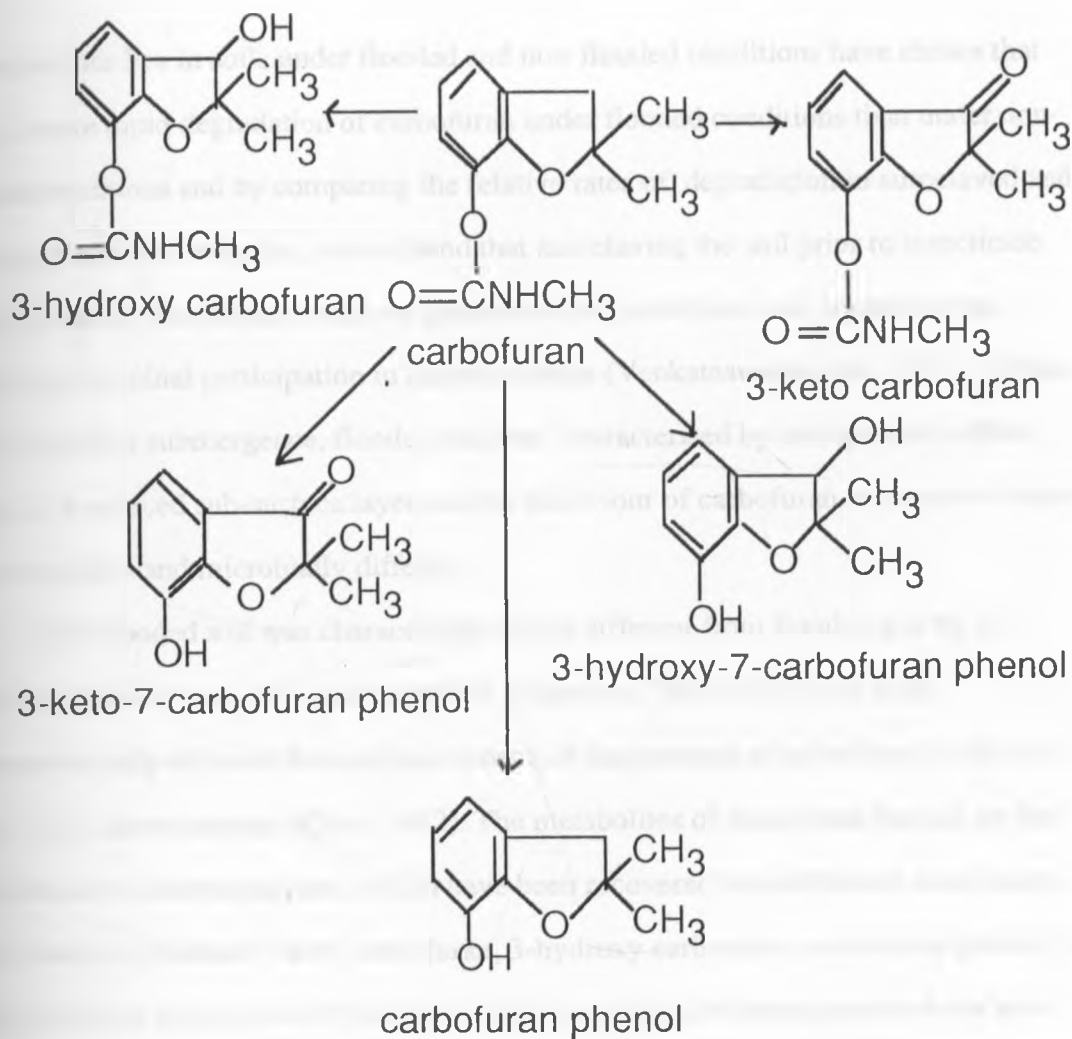


Fig. 9 The metabolic pathway of carbofuran.

#### Metabolism of carbofuran.

Carbamates are used extensively as a replacement for organochlorines because they are less persistent (Caro et al., 1973). Their fate in soil is less understood than the other two types of pesticides. However, microorganisms have been isolated and characterized from soil which are capable of carrying out some form of degradation of carbofuran (Williams et al., 1976; Venkateswarla et al., 1977; Felsot et al., 1981).

Laboratory studies in soils under flooded and non-flooded conditions have shown that there is more rapid degradation of carbofuran under flooded conditions than under non-flooded conditions and by comparing the relative rates of degradation in autoclaved and non-autoclaved soil samples, it was found that autoclaving the soil prior to insecticide incorporation in the soil increased the persistence of carbofuran to a greater extent, indicating microbial participation in its degradation (Venkateswarla et al., 1977). Within a few days after submergence, flooded soil was characterised by an oxidized surface layer and a reduced sub-surface layer and the behaviour of carbofuran in these two zones was chemically and microbially different.

Non-flooded soil was characteristically as different from flooded soil by its physical, chemical (e.g. pH), and microbial properties. This contributed to the characteristically different fate and mechanism of degradation of carbofuran in the two types of soil environments (Chen, 1982). The metabolites of carbofuran formed by the involvement of microorganisms which have been recovered from different soils (loam, sandy, and clay) include 3-keto carbofuran, 3-hydroxy carbofuran, carbofuran phenol, 3-keto carbofuran phenol, and 3-hydroxy carbofuran phenol. Microorganisms have also been found to contribute to increased mineralization of carbofuran in soil (Lichtenstein et al., 1977).

More recent studies involving carbofuran metabolism have been conducted in sub-tropical soils. These include work done by Panda (Panda et al., 1988) which showed that incorporated carbofuran was more stable than surface applied carbofuran. This could be explained by the relatively higher pH of the surface soil and flooded water immediately in contact with the surface soil layer. Highly volatile insecticides have shown increased persistence when incorporated into non-flooded soil and their persistence was found to increase with the depth of incorporation. This increased persistence was attributed to reduced losses by volatilization and photochemical



degradation (Vollner et al., 1980; Panda et al., 1988). A depth of a few mm into the soil is highly oxidized and is dominated by aerobic microorganisms, whereas the sub-surface is in a reduced state and has facultative/obligate anaerobic microorganisms. Carbofuran is therefore hydrolysed both in non-flooded and flooded soils but slightly more rapidly under flooded conditions. In a flooded rice field, the difference in pH between flood water and sub-surface soil is more pronounced and carbofuran undergoes degradation primarily by hydrolysis and the rate of this hydrolysis increases with soil pH (Karns et al., 1986).

## **METABOLISM OF PESTICIDES IN STORED GRAINS**

Grains whether for consumption or export are usually required to meet a variety of quality standards including those governing pesticide residues which must be below the tolerance limits as set up by each country (Holland et al., 1982). With the expanding range of crops being grown and the presence of a diversity of pests and diseases there is a constant need for residue information for new crops and pesticides (Smart, 1987). Dried grains, fruits, nuts and cereal products are subject to infestation by many species of insects and consequently require some insecticidal treatment during storage (Lindgren et al., 1983). Postharvest use of insecticides is therefore becoming more frequent to prevent losses of stored foodstuffs by insect infestation and increased sanitation standards. For example, in Australia malathion had been used as a grain protectant since 1965 in sorghum which led to the development of malathion resistant strains in 1983 (Bengston et al., 1983). To meet the needs of increased sanitation standards required for export grain, the level of control of storage pests in this country is very high.

A number of developed countries support pest control methods to prevent postharvest losses. It is known that insects and mites are harmful to stored products and, GTZ, the German Technical Exchange programme has been involved in training

specialists to protect products from insects and mites infestation in the tropics (Weidner, 1989) and has come up with a review of some stored products insect problems in Africa especially *Postephanus truncatus* on maize and cassava, *Callosobruchus maculatus* on *Vigna* and *Phaseolus* seeds and the main insecticides used against these pests including synthetic pyrethroids, organophosphates and hydrogen phosphide (a fumigant).

The worldwide post harvest losses of grains account for 10% of the total harvest including 35% in India and 46% for sorghum in Nigeria. The United Nations through the FAO, the World Bank, the International Foundation for Science, GTZ, and IDRC set up Post Harvest Food Loss Trust Fund programme aimed at reducing this loss by 50% in 1985. To start with, it mainly set off to determine the post harvest damage with special reference to rural conditions. The amounts and persistence of insecticidal residues in stored products depend upon the physical and chemical properties of both the insecticide and the material being treated as well as the toxicity of the chemical to the insect being controlled (Barbara and Madisen, 1983; Rengasamy and Parmar, 1986). Therefore, there exists a wide area in which to assess and evaluate the amount of residues in stored products. The recent increase in the number of pesticide residue cases has led to increased research during the last 20 years on pesticide metabolism in stored food products. Some of the parameters used in estimating post harvest damage include weight loss, increase in moisture content, total infestation, temperature, CO<sub>2</sub> concentration, bacterial infection, weight of dust and foreign particles. These parameters also influence the degradation of pesticide residues in stored grains .

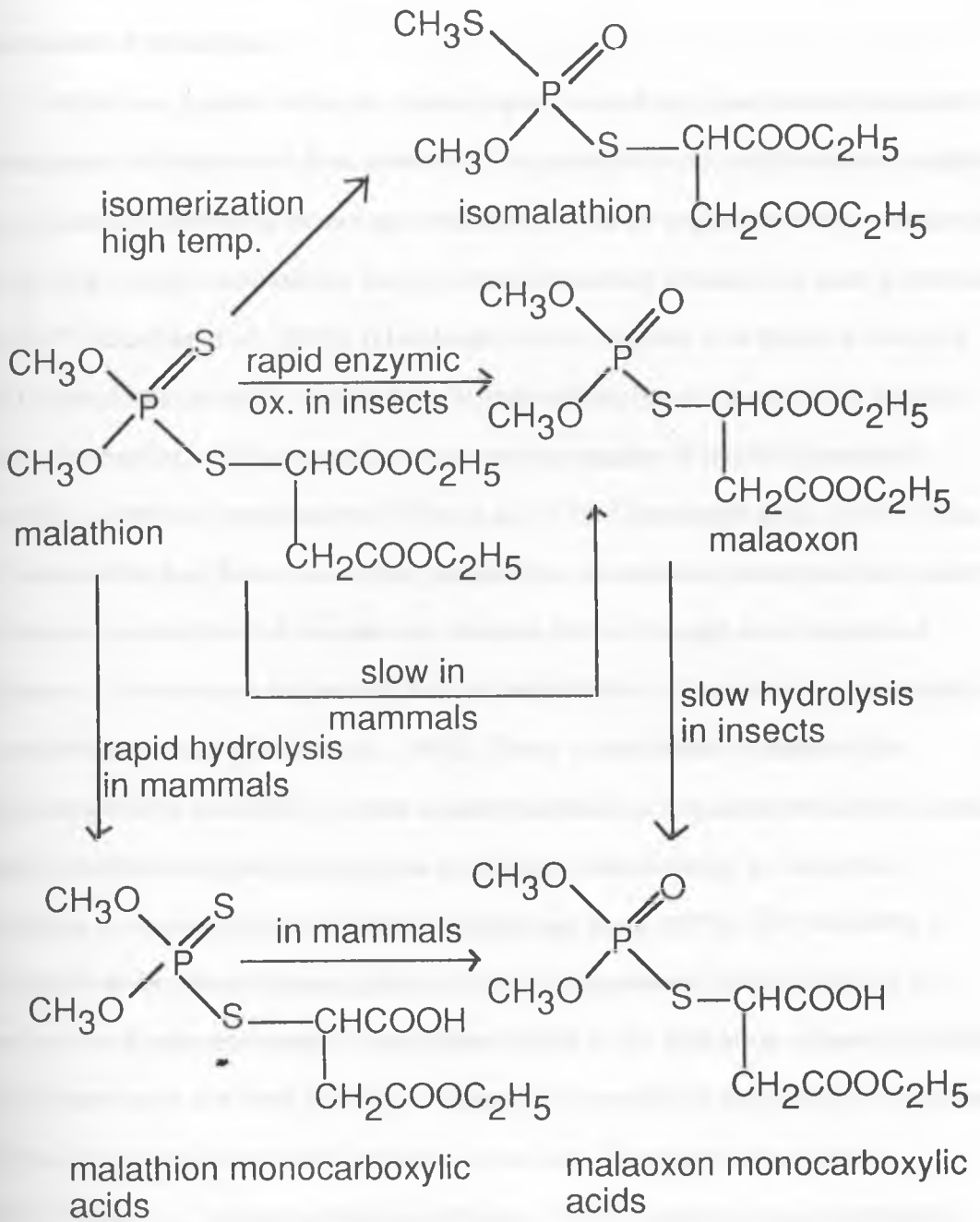


Fig. 8 The metabolic pathway of malathion

## Metabolism of malathion

Malathion, applied either as a dust or spray is used as a post harvest treatment to prevent insect infestations of food products. The protection may last for several months to an entire season depending on storage conditions. It can be applied in empty storage bins or over bulk storage commodities and has been extensively studied as a grain protectant since 1958 (Gunther et al., 1958). It has been known for some time that low levels of toxic contaminants occur in technical grade organophosphorous insecticides formed either as by-products during manufacture or on degradation of technical products especially at elevated temperatures (Miles et al., 1979; Greenhalgh et al., 1983). It has also been known that numerous organophosphorous compounds potentiate the toxicity of malathion to warm-blooded animals and resistant insects through a mechanism of inhibition of the esterases responsible for the detoxication of malathion by hydrolysis of the carboethoxy group (Casida et al., 1963). There is additional evidence of the potentiating effects exhibited by some organophosphorous impurities which are normally present in technical organophosphorous pesticides on the toxicity of the active ingredients to warm blooded animals (Pellegrini and Santi, 1972). The similarity of substitution at the phosphorous moiety and the use of common intermediates in the manufacture of organophosphate insecticides results in the formation of several common toxic contaminants and their presence changes the properties of the insecticide and may enhance hazards associated with its handling and use. These impurities include isomalathion, O,O,O-trimethylphosphorothioate, O,O,S-trimethylphosphorothioate, O,O,S-trimethylphosphorodithioate, O,S,S-trimethylphosphorothioate (Greenhalgh and Marshall, 1976). The existence and development of impurities in malathion formulations is an important factor since these impurities alter the toxicity of malathion to animals. Samples of malathion-dispersable powders stored in tropical countries at high temperatures were analysed and the content of the S-methyl isomer was found to be as

high as 3.3% and rat oral LD<sub>50</sub> was 500 mg/kg compared to those of fresh powders <0.3% S-methyl isomer content and LD<sub>50</sub> >2500 mg/kg (Miles et al., 1979). The rate of formation of the S-methyl isomer was found to be dependent upon the type of formulation.

Owing to the importance of these metabolites, several studies have been conducted to follow the behaviour of malathion in different types of grains under different storage conditions. Rapid and sensitive methods of analysis involving GC, HPLC, GC-MS and NMR have been developed for these residues (Krzeminski and Landman, 1963; Greenhalgh et al., 1983; Mensah, 1987; Wilkins and Mason, 1987; Fenske and Leffingwell, 1989; Wilkins, 1990). The studies showed that the formation of the residues was influenced by the type of storage, type of surface e.g. concrete which catalysed a very rapid breakdown of malathion. It was found that there was a loss of malathion during milling and it found its way into the endosperm by penetration through the bran over long periods of storage time (Mensah, 1987). The greatest loss of malathion from the grains occurred during the first month of storage but slower degradation occurred thereafter (Rowlands and Horter, 1967; Gozek and Artiran, 1980). The degradation of malathion in grains was also accelerated by the presence of dockage (e.g. small particles of broken grains, dust etc.) which reduced its effectiveness (Anderegg and Madisen, 1983). When the grains were sterilised, the degradation was much slower showing that enzyme activity contributed to the degradation of malathion. This degradation was dependent on the type of grain, for example, there was a faster rate of degradation in wheat than in maize (Rowlands and Horter, 1967). Bound residue formation also occurred in stored grains and their toxicological significance has been studied (Khan et al., 1990). Studies on isomalathion which is a toxic metabolite of malathion started in 1976 as a result of malathion poisoning in Pakistan which was found to have been caused by high levels of isomalathion in the formulation used. It has been

found that isomalathion just like malathion undergoes degradation on solid pesticide formulation carriers and container surfaces to give products such as O,S,S-trimethylphosphorodithioate, O,O,S-trimethylphosphorothioate, etc. (Rengasamy and Parmar, 1986; Rengasamy and Parmar, 1987).

## OBJECTIVES

In 1967, Crammer estimated that, in global terms, losses in plant production upto harvest as the result of pests, diseases and weeds constituted one third of the potential yield. In Kenya a further 30-70% loss occurred during post harvest storage (Personal Communications, 1989). Pest management approaches have global dimensions but the emphasis and method of implementation of pest control varies from country to country. Pesticide regulations have been in effect for several decades in developed countries (Von Moltke, 1986) and they have concurred with obtaining data for registration and then subsequent enforcement of the laws and regulations. The procedure followed in reaching a regulatory decision on whether a given pesticide should be registered and under what conditions it should be used involves a series of decisions based on scientific evaluation of all available data on the efficacy and potential adverse effects in order to ensure overall needs and interests of the society (Wilkinson, 1986) i.e. taking care of potential risks rather than expected economic benefits. These regulations only apply to the individual country involved in the regulatory decision and not developing countries which do not have the necessary technology and where pesticide misuse is indeed the most worrisome aspect of pesticide usage. The Food and Agriculture Organization of the United Nations (FAO) foresees a steady annual increase in the use of pesticides of 4.5% in global terms to prevent agricultural food losses (Adam, 1986) and most of this increase will occur in the developing countries. They will need to intensify food production by use of fertilizers and pesticides in order to feed their ever increasing population. Recognizing the risks that these chemicals have on both man and the environment and that the quality of pesticides available in many developing countries often do not meet minimal standards of quality and field performance, FAO's member countries adopted an international code of conduct

on the distribution and use of pesticides which covered specifications and testing of physical and chemical properties.

The persistence and fate of pesticides in the environment depends upon the climatic conditions. The loss of pesticides through volatilization, photodecomposition and microbial degradation which are influenced by soil type, water, rainfall, temperature, humidity and method of application has been found to be more rapid under tropical and sub-tropical conditions (Kraus, 1980). It would therefore be necessary to assess the environmental fate of pesticides under actual tropical conditions before registering them for use in the tropics. Since 1980's this fact has contributed to a number of research projects financed by FAO/IAEA in a number of developing countries to understand the fate of pesticides in tropical regions (IAEA, 1988).

Requirements by EPA emphasize metabolism studies of pesticides in soil (Laskowski et al., 1983) with the intention to identify breakdown products that may accumulate in soil. The use of radiolabelled  $^{14}\text{C}$ -compounds in soil cylinder tests under field conditions has been widely accepted as a rapid and precise method for the determination of mobility, rate of formation of metabolites and disappearance of the parent compound (Harvey, 1983). The results of these tests will then determine the formulations to be used and the methods of application in order to reduce the environmental and human risks associated with pesticide use.

In Kenya a variety of pesticides for use in both agriculture and public health (Appendices, Tables 1, 2 and 3) are imported in large quantities. Normally these pesticides are used following specifications set in the countries of manufacture. These specifications do not conform to local climatic conditions and coupled with lack of trained personnel and adequate tools to screen these pesticides, they are often applied in excessive amounts to compensate for their rapid environmental dissipation and to ensure expected results. There is growing public awareness on the benefits and risks in pesticide



use but no supporting scientific studies have been conducted to generate data that show the disappearance and fate of these pesticides under the local climatic conditions.

Only few studies have been carried out to show the the disappearance of DDT from local soils under field conditions (Wandiga and Natwaluma, 1984; Mghenyi, 1988).

The objectives of the present study were to apply radioisotope techniques to follow the dissipation and metabolism of DDT, DDE and parathion in Chiromo soil, the dissipation, distribution and metabolism of carbofuran in flooded and non-flooded soils of the Kano plains at Ahero under field and laboratory conditions and to follow the dissipation and metabolism of malathion in stored maize (*Zea mays*) and beans (*Phaseolus vulgaris*) under two different storage conditions widely used in Kenya. Chromatographic and mass-spectrometric methods of analysis were used to identify the metabolites.

## CHAPTER I

### FATE OF $^{14}\text{C}$ -p,p'-DDT IN KENYAN TROPICAL SOILS

Key words: p,p'-DDT, soil, solar radiation

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## INTRODUCTION

The use of DDT has been banned or greatly restricted in many countries because of its persistence under temperate climates (Giddings and Monroe, 1972). DDT residues have been detected in soil, water, air, food, wildlife and man after long periods.

Detectable amounts were found in soil even after 50 years after application (White, 1974; DeCock and Rand, 1984). However, DDT and other organochlorine pesticides are still extensively used in developing countries including Kenya, though mainly for the control of disease vectors. Studies have shown that DDT does not persist for long periods under certain tropical and sub-tropical conditions (El-Zorgani, 1976; Perfect, 1980; Wandiga and Natwaluma, 1984). The long residual activity and economic viability of DDT in control of agricultural pests and its efficacy in public health have led to more intensive investigations into the behaviour of DDT under tropical conditions to provide the necessary data to evaluate its safety (IAEA, 1988).

The distribution and persistence of DDT in soil is influenced by several factors including solar (UV) radiation, temperature, rainfall, pH and the presence of microorganisms. The loss of the pesticide via volatilization, photodecomposition and microbial activity can therefore be considerably high under tropical conditions where intense solar radiation, high temperatures and humidity prevail (Sethunathan et al., 1977; IAEA, 1988).

This paper reports the results of a study to follow the dissipation and degradation of  $^{14}\text{C}$ -p,p'-DDT in soil under field and laboratory conditions using radiotracer techniques.

## MATERIALS AND METHODS

### Chemicals

Uniformly labelled  $^{14}\text{C}$ -p,p'-DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane); specific activity: 15.3 mCi/mmol and radiochemical purity of 97% by TLC and HPLC was obtained from New England Nuclear GmbH, Vienna, Austria. Non-labelled p,p'-DDT, p,p'-DDE and p,p'-DDD (all 99% pure by TLC) were obtained from

Greyhound Chromatography and Allied Chemicals, UK. Distilled methanol, n-hexane, toluene and ethanolamine were obtained from suppliers in Nairobi. Crystalline 2,5-diphenyl oxazole (PPO) for use in scintillation cocktail was purchased from Sigma Chemical Company, UK.

### **Apparatus**

A mortar and pestle were used for soil sample homogenization and all samples awaiting analysis were kept in a refrigerator in the laboratory. A paper lined standard glass tank, precoated silica gel 60 F 254 plates and a Spectroline UV lamp were used for TLC analysis. An oven was used for soil moisture content determination while a Soxhlet apparatus and a rotary evaporator were used for extraction and concentration of soil extracts, respectively. A Perkin Elmer Gas Chromatograph Model 8500 fitted with a Perkin Elmer GP-100 graphics printer, a packed OV210 column and an EC detector was used for analysis of DDT and its derivatives. A Beckman LS6800 liquid scintillation spectrometer was used for counting. Combustion was made in an OX-600 Harvey biological oxidizer. Special quartz glass tubes (60 cm long and 7 cm diameter) were used for the solar radiation experiments.

### **Field Application and Sampling**

A soil sample collected from the experimental site had the following properties: pH 6.3; sand 22%; clay 46%; silt 32%; nitrogen 0.15%; organic carbon 1.96%; texture: clay (Ref. Kenya National Agriculture Laboratories, Kabete).

The field study began in January, 1990 and was completed in July, 1990. A plot measuring 7 metres by 7 metres was chosen in a location near the Department of Chemistry, University of Nairobi. The plot was prepared by removing all weeds and stones. Hard PVC cylinders (length: 15 cm; diameter: 10.4 cm) were driven into the soil

with 2 cm left protruding above the soil surface to prevent flow of run-off water and left undisturbed for one week before applying the pesticide. To each cylinder 8  $\mu\text{Ci}$  of  $^{14}\text{C}$ -p,p'-DDT and 10 mg of non-labelled p,p'-DDT in 10 mL of n-hexane were added. The first three cylinders were dug out at random soon after pesticide application to determine the initial residues. The other cylinders were sampled in triplicate, at various intervals up to 172 days. The upper 10 cm of soil from each cylinder was removed, air-dried overnight, ground in mortar and pestle and thoroughly mixed.

### Analytical Methods

Approximately 50 grams of soil sample (in triplicate) was Soxhlet extracted using 150 mL of methanol for 4 hours. An aliquot of the extract was added to the cocktail and counted to determine the extractable residues. Non-extractable (bound) residues were determined by combustion of the extracted soil and counting. The methanol extracts (9 mL) were treated with distilled water (10 mL) in a separatory funnel and mixed thoroughly by shaking. This was then partitioned three times with 15 mL portions of n-hexane; the extracts were combined and then reduced for GC and TLC analysis. The following GC conditions were used: detector temperature  $350^{\circ}\text{C}$ ; oven temperature  $200^{\circ}\text{C}$  (Isothermal for 50 minutes); carrier gas :white spot nitrogen at 75 mL/min; injection volume 1  $\mu\text{L}$ . The identification was made by co-chromatography with authentic p,p'-DDT, p,p'-DDE and p,p'-DDD while quantitation was achieved by comparing peak areas of sample injections with those of the standards.

### Weather Data

The daily readings of the weather data in Chiromo (altitude 1661 m) were taken by the Department of Meteorology in Nairobi. These included the daily maximum and

minimum temperatures, the daily sunshine hours, the daily amount of rainfall and the hourly relative humidity. These are summarized in Appendices, Fig. 1.2.

### Effect of Solar Radiation under Laboratory Conditions.

The soil sample was collected from the same location in the field and air-dried for two days in the laboratory. Some 1320 g of this soil sample was treated with 50 mL of n-hexane solution containing 40  $\mu\text{Ci}$  of  $^{14}\text{C}$ -p,p'-DDT and 50 mg of non-labelled p,p'-DDT. Approximately 50 g of this treated sample was Soxhlet extracted with 150 mL of methanol and the extractable and bound residues determined as described above.

Approximately 635 g of the treated soil was then transferred to a quartz tube and tightly corked from both ends. Another sample of 635 g soil was placed in a similar quartz tube covered with black polythene paper. The exposed quartz tube allowed efficient penetration of sunlight while the covered quartz tube was taken as the control. The tubes were set up and placed on top of a two storey building from 8.00 to 17.00 hours every day for six weeks. Using a low vacuum pump, the tubes were flushed into 20 mL 0.1N NaOH solution through preextracted polyurethane plug for 30 minutes every morning and evening. The polyurethane plug was used to trap all the organic volatiles produced inside the tube while the NaOH solution absorbed the  $^{14}\text{CO}_2$  formed as a result of mineralization. Sampling was done weekly for six weeks and the analysis included counting of the Soxhlet extracted polyurethane plug, and the NaOH solution.

## RESULTS

The following Table 1.1 shows the results of the dissipation of  $^{14}\text{C}$ -p,p'-DDT from soil over a period of 172 days. Total residues dissipated rapidly to 11% in less than 6 months. Bound residue formation progressed to a peak of 5.9% after 64 days and then

declined to 2.3% at the end of the experimentation period. Overall half-life of dissipation was calculated to be 64.6 days (See Appendices, Fig. 1.3).

**Table 1.1**

The Dissipation of DDT in Kenyan soil.

| Days after treatment | Extractable residues |      | Bound residues   |     | Total residues % * |
|----------------------|----------------------|------|------------------|-----|--------------------|
|                      | $\mu\text{g/g}$      | % *  | $\mu\text{g/g}$  | % * |                    |
| 0                    | 13.30 $\pm$ 0.41     | 76.2 | 0.19 $\pm$ 0.02  | 1.3 | 77.5               |
| 9                    | 12.92 $\pm$ 0.19     | 74.0 | 0.19 $\pm$ 0.013 | 1.2 | 75.5               |
| 16                   | 10.19 $\pm$ 0.21     | 58.3 | 0.38 $\pm$ 0.014 | 2.1 | 60.4               |
| 23                   | 8.21 $\pm$ 0.22      | 47.1 | 0.19 $\pm$ 0.07  | 1.2 | 48.4               |
| 37                   | 2.45 $\pm$ 0.04      | 14.1 | 0.38 $\pm$ 0.01  | 1.5 | 15.6               |
| 51                   | 2.55 $\pm$ 0.13      | 14.5 | 0.94 $\pm$ 0.18  | 5.4 | 19.9               |
| 64                   | 2.17 $\pm$ 0.07      | 12.4 | 1.04 $\pm$ 0.019 | 5.9 | 17.3               |
| 112                  | 3.02 $\pm$ 0.23      | 17.3 | 0.38 $\pm$ 0.23  | 2.1 | 19.4               |
| 172                  | 1.41 $\pm$ 0.04      | 8.3  | 0.44 $\pm$ 0.25  | 2.3 | 10.6               |

\*100% = Radioactivity in 8  $\mu\text{Ci}$ .

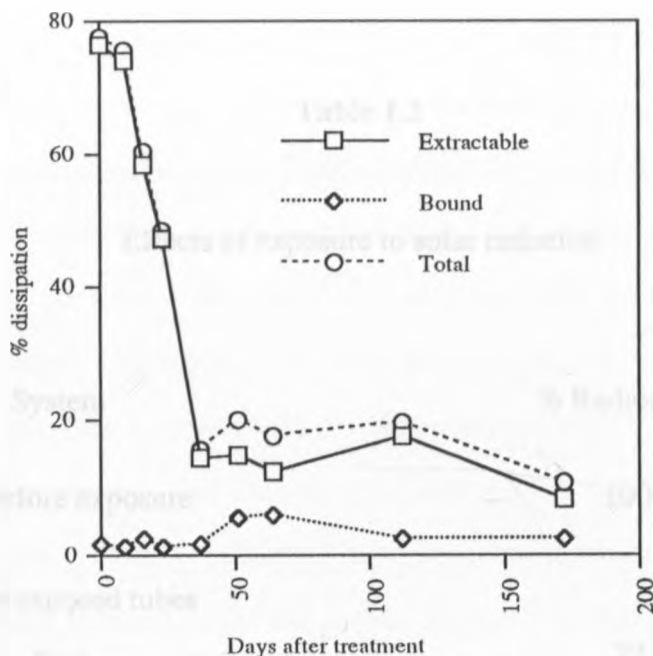


Fig. 1.1 The percent dissipation of DDT in Kenyan soil.

The GC analysis of the hexane extracts showed the presence of DDT, DDE and DDD revealing a gradual slow decline in the relative concentration of p,p'-DDT to 79% after 172 days. In parallel p,p'-DDE increased gradually to 21% after the same period. Trace amounts of p,p'-DDD could be detected during the experimentation period, but these did not show a pattern.

Table 1.2 shows the amount of  $^{14}\text{C}$ -residues remaining in the soil before and after exposure to solar radiation and of volatilization and mineralization of  $^{14}\text{C}$ -p,p'-DDT. The analysis of  $^{14}\text{C}$ -residues in light-exposed and non-exposed quartz tubes indicated 27.1% and 10.9% losses in radioactivity respectively. Of these losses 18.6% was due to volatilization and 8.5% due to mineralization in the exposed quartz tube. In the dark tube 6.0% was lost by volatilization and 4.9% by mineralization. These observations show that solar radiation enhances the volatilization and mineralization of DDT in non-sterile soil.



Table 1.2

## Effects of exposure to solar radiation

| System                           | % Radioactivity |
|----------------------------------|-----------------|
| Soil before exposure             | 100 *           |
| <sup>14</sup> C in exposed tubes |                 |
| Soil                             | 72.9            |
| Organic volatiles                | 18.6            |
| <sup>14</sup> CO <sub>2</sub>    | 8.5             |
| <sup>14</sup> C in dark tubes    |                 |
| Soil                             | 89.1            |
| Organic volatiles                | 6.0             |
| <sup>14</sup> CO <sub>2</sub>    | 4.9             |

\* Equivalent to 37.88 µg/g.

## DISCUSSION

The organochlorine, p,p'-DDT has been found to be one of the most persistent pesticides in temperate soils where half-lives of upto 50 years have been reported (Guenzi and Beard, 1970). In our studies, a half-life of 65 days was found which is comparable to those reported from other tropical countries (El-Zorgani, 1976). The degradation of this chemical in soil is therefore influenced by soil type, organic matter

content, soil pH, soil microorganisms and climate (eg. rainfall, solar radiation, soil and air temperatures). For example, high organic matter contents have been shown to be inversely related to the rate of loss of DDT residues. This has been found to be the case in studies conducted in forest soils with high organic matter contents where DDT has been shown to be highly persistent. Since DDT has very low water solubility (maximum 1% by weight) and therefore little influenced by leaching in the soil, volatilization and photodecomposition play a major role in its disappearance from the this soil. Laboratory and field studies have shown that the rate of volatilization is directly influenced by temperature, presence of water in the soil and the vapour pressure of the pesticide. Volatilization is also affected by the soil texture and has been found to be inversely related to the surface area of the soil. Solar radiation intensity (which acts on the soil surface) influences the change of DDT into DDE and is an important parameter to consider in the initial days when the pesticide is still on top of the soil surface. In our laboratory studies, it was apparent that solar radiation (UV range) increases the rate of both the volatilization and mineralization of DDT in this soil. Microorganisms also play an active role in the degradation of DDT in the soil and the conversion of DDT to DDD has been shown to be microbial and highly sensitive to O<sub>2</sub> (Burge, 1971). However, DDD constituted a very small percentage of the metabolites recovered from the soil extracts in our field experiment.

A lot of rainfall was recorded on the first day after application of DDT to the soil which resulted in the incorporation of the pesticide into the inner soil layer, partially preventing a very rapid loss by volatilization. There was, as a result, more persistence during the first nine days. DDT disappeared most rapidly during the first one and a half months particularly between day 23 and day 37 after pesticide application concurring with the high temperatures experienced between Jan 23 and March 4. During the 37 days after treatment, there was a great deal of rainfall and the dissipation rate was slower

showing that degradation due to chemical and microbial activities in the soil i.e. the formation of DDE and DDD metabolites, greatly influenced the rate of disappearance of DDT from the soil during this period. At this time, the rate of formation of bound residues also increased and this lowered the total residue (extractable+bound) dissipation rate.

## CONCLUSIONS

DDT disappeared rapidly from soil under field conditions with a half-life of 65 days. The highest losses through volatilization paralleled the prevailing high temperatures in the period between 23 to 37 days after application. In soil, the main degradation mechanism converted DDT to DDE and a small amount of DDD. Conversion to CO<sub>2</sub> was also demonstrated in the laboratory. The results indicate that DDT is not likely to accumulate in the field and its dissipation occurs at rates higher than those reported from temperate regions and comparable to those of non-persistent pesticides. Its degradation is influenced by both the soil characteristics and climatic factors. The major dissipation process from the Chiromo field soil is volatilization which is influenced mainly by high temperatures and intense solar radiation existing in this environment. Studies on the behaviour of this pesticide in water, plant uptake and on solid surfaces representing its various applications are recommended in order to understand its overall persistence in the Kenyan environment.

## ACKNOWLEDGEMENT.

The authors wish to thank the International Atomic Energy Agency for the financial assistance, under research contract No. 5566/RB.

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## CHAPTER 2

### THE PERSISTENCE OF $^{14}\text{C}$ -p,p'-DDE IN KENYAN TROPICAL SOILS

Key words: DDE, soil, half-life

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## INTRODUCTION

Studies conducted in temperate regions have shown that the organochlorine pesticide p,p'-DDT and its main metabolite, p,p'-DDE have a high persistence and bioaccumulate in these environments. They are therefore considered environmental contaminants (Giddings and Monroe, 1972; White, 1974; Bierman and Swain, 1982; De Cock and Rand, 1984). However, DDT is cheap, safe to use, easily available and is very effective in disease vector control (Cooke and Stringer, 1982; IAEA, 1988). It is therefore still widely used in the third world. A number of studies conducted in these countries suggest that DDT and DDE are less persistent in the tropics than in temperate zones (El-Zorgani, 1976; Perfect, 1980; Wandiga and Natwaluma, 1984). High temperatures, high humidity, intense solar radiation and different soil characteristics degrade DDT faster as

shown by shorter half-lives and different degradation pathways (Weber, 1980).

Additional studies are required to evaluate the safety of these chemicals in a tropical environment. This paper reports the results of a study conducted on the dissipation and degradation of  $^{14}\text{C}$ -p,p'-DDE in Chiromo soil under field conditions.

## MATERIALS AND METHODS

### Chemicals

Uniformly labelled  $^{14}\text{C}$ -p,p'-DDE (1,1-dichloro-2,2-bis(4-chloro- $^{14}\text{C}$ -phenyl)ethene); specific activity : 1138.6 MBq/mmol and radiochemical purity of 97% by TLC was obtained from IAEA, Vienna. Non-labelled p,p'-DDT, p,p'-DDE and p,p'-DDD standards (all 99% pure by TLC) were obtained from Greyhound Chromatography and Allied Chemicals, UK. Distilled methanol, n-hexane and toluene were obtained from suppliers in Nairobi. Crystalline 2,5-diphenyl oxazole (PPO) and 2,2'-p-phenylene-bis(4-methyl-5-phenyloxazole), (dimethyl POPOP), for use in liquid scintillation spectrometry were purchased from Sigma Chemical Company, UK. A Harvey C- $^{14}$  cocktail was obtained from R. J. Harvey Instrument Corporation, USA.

### Apparatus

A mortar and pestle was used for soil sample homogenization and all samples awaiting analysis were stored at 4 °C in the laboratory. A paper lined standard glass tank, precoated silica gel 60 F 254 plates and a Spectroline UV lamp were used for TLC analysis. An oven was used for soil moisture content determination while a Soxhlet apparatus and a rotary evaporator were used for extraction and concentration of soil extracts, respectively. A Packard Tri-Carb 1000 TR Liquid Scintillation Spectrometer

was used for quantitation of the radioactivity. Soil samples were combusted in an OX-600 Harvey Biological Oxidizer.

### Field Application and Sampling

A soil sample collected from the experimental site had the following properties : pH 6.3; sand 22%; clay 46%; silt 32%; nitrogen 0.15%; organic carbon 1.96%; texture clay. (Ref: Kenya National Agricultural Laboratories, Kabete).

The experiment began in August, 1992 and was completed in March, 1993. A plot measuring 7 metres by 12 metres was selected in a location near the department. The plot was prepared by digging deep and removing all weeds and stones. Hard PVC cylinders with lengths of 15 cm and diametres of 10.4 cm, were driven into the prepared soil leaving 2 cm protruding above the soil surface so as to prevent losses due to surface run-off. The cylinders were left undisturbed for one week. To each cylinder was then added 5  $\mu\text{Ci}$  of  $^{14}\text{C}$ -p,p'-DDE and 8.6 mg non-labelled p,p'-DDE in 10 mL n-hexane resulting in a specific activity of 183 MBq/mmol. Two cylinders, chosen at random, were immediately dug out to determine the initial residues. The other cylinders were sampled in duplicate at various time intervals upto 189 days. The upper 10 cm of soil from each harvested PVC cylinder was removed, air-dried overnight in the laboratory, uniformly ground and then mixed thoroughly. The moisture content of the soil was then determined.

### Analytical Methods

Extractable residues were obtained by taking approximately 50 grams of the soil sample (in triplicate) and extracting with 150 mL of methanol for 4 hours in a Soxhlet apparatus. The methanol extract was then reduced to 10 mL by rotary evaporation. One mL was then added to 8 mL of cocktail and counted. The remaining methanol extract was

diluted with distilled water (10 mL) in a separatory funnel and mixed thoroughly by shaking. This was partitioned three times with 15 mL portions of n-hexane, the extracts combined, dried over anhydrous sodium sulphate and then reduced for TLC analysis. The identification was made by co-chromatography with p,p'-DDT, p,p'-DDE and p,p'-DDD standards. Non-extractable (bound) residues were determined by combusting 1.5 grams of the extracted soil in a Harvey Biological Oxidizer. The  $^{14}\text{CO}_2$  produced was trapped in 7.5 mL of the Harvey cocktail and counted. This procedure was repeated for non-extracted soil samples to verify the total residues recovered from the soil. Total residues which are tabulated below (Table 2.1) were obtained by adding together extractable and bound residues.

#### Weather Data

The daily weather readings at Chiromo (altitude 1661 m) were taken by the Department of Meteorology in Nairobi and are summarised in the following figure (Fig. 2.4 and 2.5). The data taken included the daily maximum and minimum temperatures, the daily sunshine hours, the daily amount of rainfall and the hourly relative humidity.



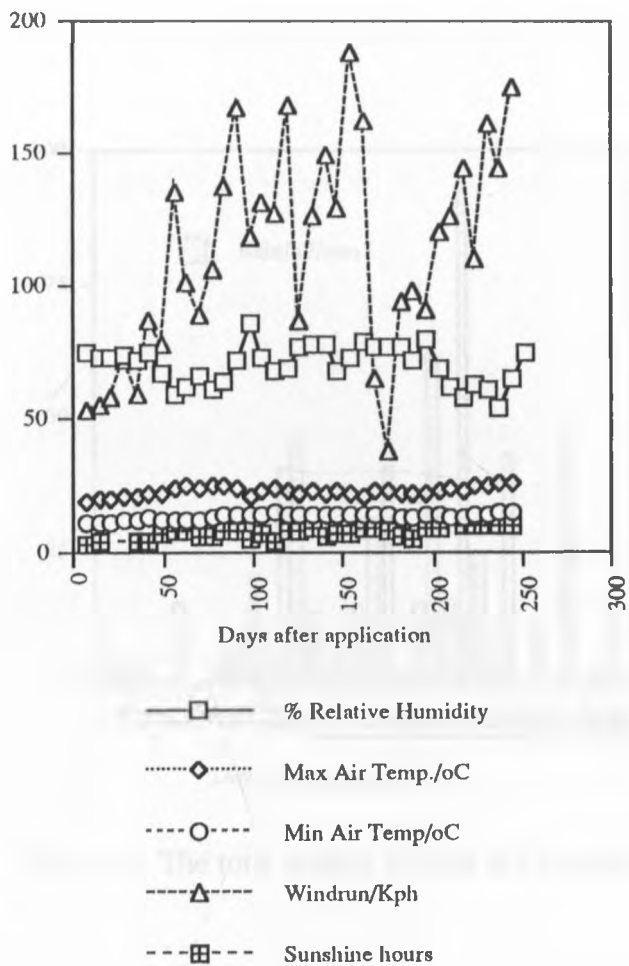


Fig.2.4. The average weekly weather data at Chiromo.

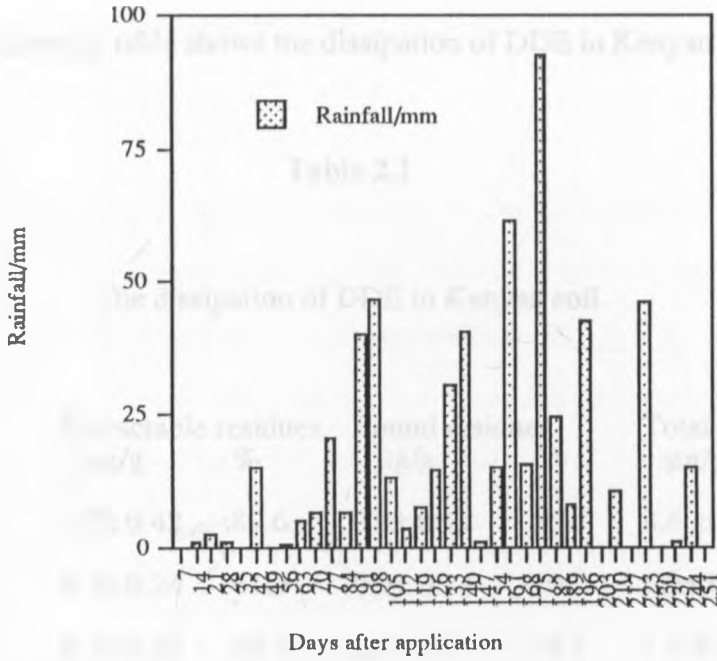


Fig.2.5 The total weekly rainfall at Chiromo.

## RESULTS

The following table shows the dissipation of DDE in Kenyan soil.

Table 2.1

The dissipation of DDE in Kenyan soil.

| Days after application | Extractable residues |      | Bound residues  |      | Total residues  |      |
|------------------------|----------------------|------|-----------------|------|-----------------|------|
|                        | $\mu\text{g/g}$      | %    | $\mu\text{g/g}$ | %    | $\mu\text{g/g}$ | %    |
| 0                      | 7.7 $\pm$ 0.42       | 89.6 | 0.9 $\pm$ 0.12  | 10.4 | 8.6 $\pm$ 0.27  | 100  |
| 7                      | 5.3 $\pm$ 0.24       | 61.9 | 0.9 $\pm$ 0.14  | 10.7 | 6.2 $\pm$ 0.19  | 72.6 |
| 14                     | 5.3 $\pm$ 0.94       | 61.6 | 2.1 $\pm$ 0.04  | 24.5 | 7.4 $\pm$ 0.49  | 86.1 |
| 21                     | 5.0 $\pm$ 0.96       | 58.7 | 1.3 $\pm$ 0.36  | 15.0 | 6.3 $\pm$ 0.66  | 73.7 |
| 28                     | 4.5 $\pm$ 1.63       | 52.0 | 2.2 $\pm$ 0.29  | 25.7 | 6.7 $\pm$ 0.96  | 77.7 |
| 42                     | 4.2 $\pm$ 0.81       | 48.4 | 1.4 $\pm$ 0.60  | 16.8 | 5.6 $\pm$ 0.74  | 65.2 |
| 56                     | 3.8 $\pm$ 1.33       | 43.9 | 1.8 $\pm$ 0.27  | 20.4 | 5.6 $\pm$ 0.80  | 64.2 |
| 70                     | 3.4 $\pm$ 0.59       | 39.5 | 1.1 $\pm$ 0.32  | 13.2 | 4.5 $\pm$ 0.46  | 52.7 |
| 84                     | 3.7 $\pm$ 0.28       | 43.6 | 1.4 $\pm$ 0.17  | 16.2 | 5.1 $\pm$ 0.22  | 59.8 |
| 98                     | 3.1 $\pm$ 0.40       | 36.4 | 1.1 $\pm$ 0.05  | 13.3 | 4.2 $\pm$ 0.22  | 49.7 |
| 112                    | 2.1 $\pm$ 0.08       | 24.9 | 1.7 $\pm$ 0.38  | 19.5 | 3.8 $\pm$ 0.23  | 44.4 |
| 140                    | 1.7 $\pm$ 0.41       | 19.8 | 1.7 $\pm$ 0.24  | 19.9 | 3.4 $\pm$ 0.33  | 39.6 |
| 189                    | 1.8 $\pm$ 0.24       | 20.4 | 1.7 $\pm$ 0.15  | 19.9 | 3.5 $\pm$ 0.20  | 40.3 |

Note: 100% represents total (extractable+ bound) residues determined on zero day. The total amount of DDE applied per cylinder was 8.65 mg which was equivalent to 10.06  $\mu\text{g/g}$  of soil.

DDE dissipated to 40% of the total initially recovered residues in about four and a half months. While the extractable residue levels declined with time, the amount of bound residues increased rapidly during the first month, rising to 25.7% and then gradually decreasing to 20% of the total residue recovered at the end of the experiment. The dissipation pattern is shown in Figure 2.1. The overall half-life of dissipation was calculated by regression analysis to be 145 days (Appendices, Fig. 2.3). The TLC analysis of the n-hexane extracts revealed only the presence of p,p'-DDE. No p,p'-DDT nor p,p'-DDD were detected in the extracts during the experiment.

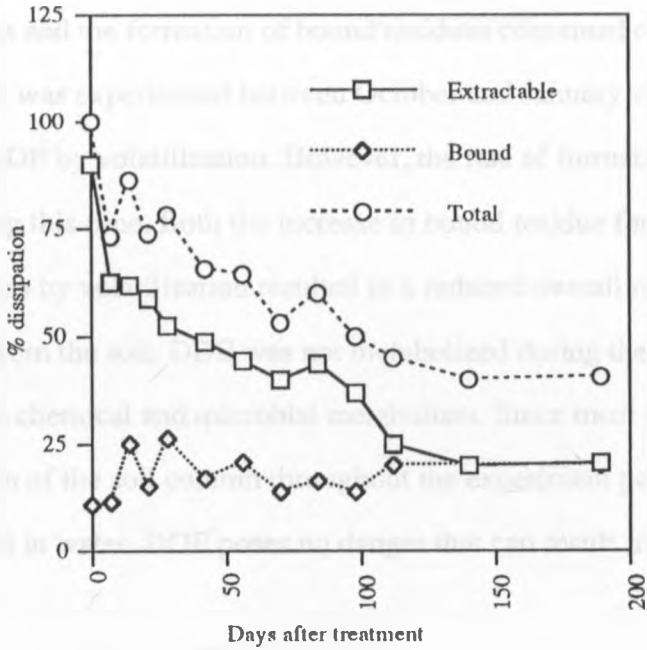


Fig. 2.1 The percent dissipation of DDE in Chiromo soil.

## DISCUSSION

DDE disappeared fairly rapidly from the Chiromo soil under field conditions with a half-life of 145 days. During the first month, there was no rainfall and both the air and soil temperatures and relative humidity were high. These factors accelerated the rapid loss of DDE from the soil by volatilization. The formation of bound residues was higher than that of DDT slowing down the overall dissipation of DDE from the soil and resulting in increased persistence. DDE was also found to be more stable to chemical and microbial attack in the soil. This seems to suggest that the formation of bound residues in soils treated with DDT first goes through the formation of DDE by chemical catalysis followed by the binding of DDE to the soil matrix. In the DDE treated soil, the initial bound residues on zero day constituted 10.4% of the total recovered radioactivity. This is rather high due to the fact that the soil samples were air-dried for two days in the laboratory

prior to analysis and the formation of bound residues continued during this drying period. A lot of rainfall was experienced between October and January which led to a reduction in the loss of DDE by volatilization. However, the rate of formation of bound residues increased during this time. Both the increase in bound residue formation and the decrease in the rate of loss by volatilization resulted in a reduced overall rate of disappearance of this chemical from the soil. DDE was not metabolized during the experiments, showing its resistance to chemical and microbial metabolism. Since most of the DDE was located in the top 13 cm of the soil column throughout the experiment period and because it is not easily dissolved in water, DDE poses no danger that can result from underground leaching.

### CONCLUSIONS

DDE disappeared fairly rapidly from Chiromo soil under field conditions with a half-life of 145 days. In the soil, p,p'-DDE was not metabolized. These findings indicate that DDE is not persistent in Kenyan soils but dissipates at higher rates than those reported for temperate regions. However, DDE appeared to dissipate less rapidly than DDT though bound more to this soil than DDT.

### ACKNOWLEDGEMENT

The authors wish to thank the International Atomic Energy Agency (IAEA) for the financial assistance given under research contract Nos. 5566/RB and KEN/5/016. We are also grateful to Dr. W.C. Dauterman for technical and laboratory assistance.

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## CHAPTER 3

### THE DEGRADATION OF PARATHION IN TROPICAL SOILS

Key words: Parathion, field conditions, half-life

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#### INTRODUCTION

Parathion, O,O-diethyl O-p-nitrophenyl phosphorothioate, is one of the oldest and most widely used agricultural pesticides (Muirhead-Thomson, 1971). It is resistant to chemical hydrolysis under acidic and neutral conditions but is easily hydrolysed under alkaline conditions (Sethunathan et al., 1977). Despite this resistance to chemical attack, parathion is relatively short-lived in most environments (Klein and Scheunert, 1982). It has been found to be less persistent than the organochlorine pesticides following studies conducted in temperate regions (Katan et al., 1976). Depending on the soil type and other environmental conditions, large proportions of parathion may bind to soil and can persist for much longer periods (Reynolds and Metcalf, 1962; Klein and Scheunert, 1982). This



high binding rate to soil is believed to be due to the formation of amino groups by metabolic reactions in the soil. The behaviour of parathion however may be different under tropical conditions because of higher temperatures and humidity, more intense UV radiation and different soil microorganisms (Sethunathan et al., 1977). Despite the increased use of this agricultural pesticide in Kenya, its behaviour and fate have not been investigated. In this paper we report the results of a study conducted to follow the dissipation and degradation of parathion in Nairobi soil under field conditions using radiotracer techniques.

## MATERIALS AND METHODS

### Chemicals

Uniformly ring labelled  $^{14}\text{C}$ -parathion (specific activity: 10 mCi/mmol) and non-labelled parathion (purity: 98% by TLC) were obtained from IAEA, Vienna. Non-labelled standards ethyl parathion, paraoxon, p-aminophenol and p-nitrophenol were obtained from the Institute of Ecological Chemistry GSF, Munich. Distilled methanol, n-hexane and toluene were purchased from suppliers in Nairobi. Crystalline 2,5-diphenyl oxazole (PPO) and 2,2'-p-phenylene-bis-(4-methyl-5-phenyl oxazole) (dimethyl POPOP) for use in liquid scintillation spectrometry were obtained from Sigma Chemical Company, UK. Harvey carbon-14 cocktail was obtained from R.J. Harvey Instrument Corporation, USA.

### Apparatus

Soil samples awaiting analysis were stored at 4°C in the laboratory. A mortar and pestle were used for soil sample homogenization while a paper-lined standard glass tank, precoated polygram sil 'G/UV 254 TLC plates and a Spectroline UV lamp were used for

TLC analysis. An oven was used for the determination of moisture content while a Soxhlet apparatus and a rotary evaporator were used for extraction and concentration of the soil extracts, respectively. A Packard Tri-Carb 1000 TR Liquid Scintillation Spectrometer was used for counting. Sample combustion was made in an OX-600 Harvey Biological Oxidizer.

### Field Application and Sampling

A soil sample collected from the experimental site had the following characteristics : pH 6.3; sand 22%; clay 46%; silt 32%; nitrogen 0.15%; organic carbon 1.96%; texture clay (Ref: Kenya National Agricultural Laboratories, Kabete).

The field experiment began on March 16, 1993 and lasted two and a half months. A plot measuring 6 m by 6 m was chosen in a location near the Department of Chemistry, University of Nairobi. The plot was prepared by digging deep and removing all weeds and stones. Twenty hard PVC cylinders, measuring 55 cm in length and 6 cm in diameter were driven into the prepared soil leaving 3 cm protruding above the soil surface so as to prevent loss of soil due to surface run-off. After one week 2.5  $\mu\text{Ci}$  of  $^{14}\text{C}$ -parathion ( specific activity 281  $\mu\text{Ci}/\mu\text{mol}$ ) and 2 mg non-labelled parathion were added to the soil surface in each cylinder. Two cylinders, chosen at random, were immediately dug out to determine the initial residues. The other cylinders were sampled in duplicate at various time intervals upto 72 days. The soil from each harvested PVC cylinder was removed, air-dried overnight in the laboratory, uniformly ground and mixed thoroughly. The moisture content of the soil was then determined.

### Analytical Methods

Approximately 50 grams (in triplicate) of soil sample was extracted in a Soxhlet apparatus with 150 mL of methanol for 4 hours. The extract was reduced by rotary

evaporation to 10 mL. The extracts (1 mL) were analysed by liquid scintillation counting. The remaining 9 mL were concentrated to about 2 mL and placed on a Silica gel column (20 cm long and 1.5 cm diameter) with a 1 cm layer of anhydrous sodium sulphate on top. Elution was with 100 mL of distilled n-hexane. The eluate was reduced to 5 mL and dried with anhydrous sodium sulphate. This was then analysed by TLC and GC. The solvent system for TLC was  $\text{CHCl}_3$ : hexane: acetic acid (8:2:1, v/v/v) and the plates were viewed at 254 nm. The GC conditions were as follows: Hewlett Packard Model 437 with FID detector and a Hewlett Packard 3385 automatic integrator; column: HP-5 cross-linked with 5% phenyl methyl silicone (25 m \* 0.2 mm \* 0.5  $\mu\text{L}$  film thickness); temperature: 80°C for 1 min then 20°C/min upto 260°C and held for 20 min; carrier gas: nitrogen at 1.5 mL/min; detector temperature 250°C; injector temperature 250°C.

Non-extractable (bound) residues were determined by combustion of the extracted soil. Approximately 1.5 grams of extracted soil were combusted in a Harvey Biological Oxidizer and  $^{14}\text{CO}_2$  trapped in 7.5 mL Harvey cocktail and counted. This procedure was repeated for non-extracted soil. Total residues were determined by adding together the radioactivity associated with extractable and bound residues and comparing them with the residues determined by combustion of the unextracted soil sample.

### Weather Data

The daily weather readings at Chiromo (Altitude 1661 m) were taken by the Department of Meteorology in Nairobi. These included the maximum and minimum temperatures, sunshine hours, rainfall and the hourly relative humidity (see Figures 3.4 and 3.5 below).

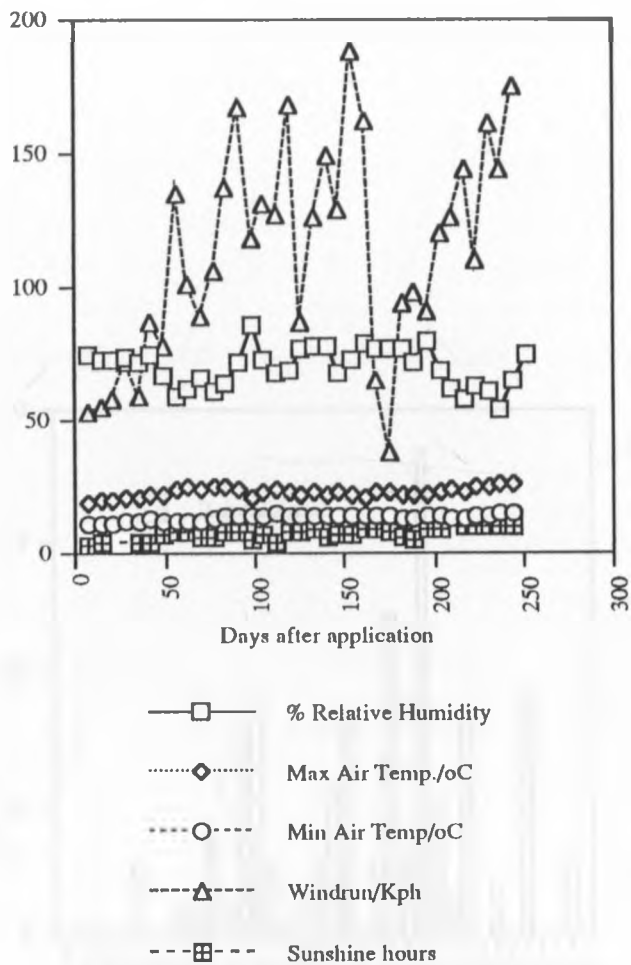


Fig. 3.4 The average weekly weather data at Chiromo.

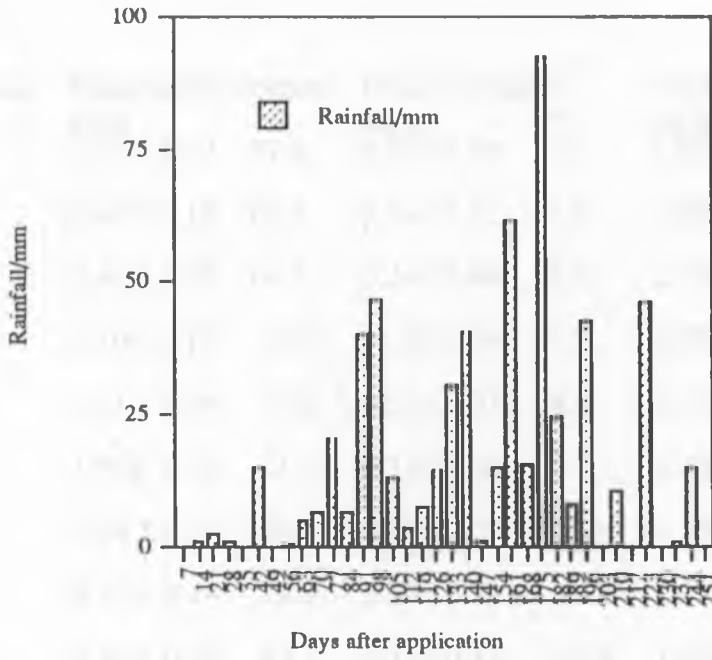


Fig. 3.5 The total weekly rainfall at Chiromo.

## RESULTS

Table 3.1

The dissipation of parathion in Kenyan soil.

| days after treatment | Extractable residues |      | Bound residues  |      | Total residues  |      |
|----------------------|----------------------|------|-----------------|------|-----------------|------|
|                      | $\mu\text{g/g}$      | %    | $\mu\text{g/g}$ | %    | $\mu\text{g/g}$ | %    |
| 0                    | $2.73 \pm 0.27$      | 97.6 | $0.09 \pm 0.02$ | 2.4  | $3.60 \pm 0.14$ | 100  |
| 3                    | $2.45 \pm 0.18$      | 68.0 | $0.14 \pm 0.01$ | 3.8  | $2.59 \pm 0.10$ | 71.9 |
| 7                    | $2.48 \pm 0.37$      | 68.8 | $0.24 \pm 0.06$ | 6.7  | $2.72 \pm 0.22$ | 75.5 |
| 14                   | $2.10 \pm 0.17$      | 58.5 | $0.26 \pm 0.04$ | 7.3  | $2.37 \pm 0.11$ | 65.8 |
| 21                   | $1.91 \pm 0.19$      | 53.2 | $0.31 \pm 0.07$ | 8.6  | $2.22 \pm 0.13$ | 61.7 |
| 28                   | $1.85 \pm 0.14$      | 51.5 | $0.35 \pm 0.08$ | 9.7  | $2.20 \pm 0.11$ | 61.2 |
| 42                   | $1.60 \pm 0.19$      | 44.5 | $0.57 \pm 0.09$ | 15.9 | $2.18 \pm 0.14$ | 60.5 |
| 56                   | $0.71 \pm 0.14$      | 19.8 | $0.90 \pm 0.23$ | 24.9 | $1.61 \pm 0.18$ | 44.7 |
| 72                   | $0.54 \pm 0.06$      | 15.1 | $0.71 \pm 0.11$ | 19.6 | $1.29 \pm 0.17$ | 35.7 |

The total residue levels fell rapidly from 3.6 ppm on zero day to about 1.29 ppm at the end of the experiment period ( Table 3.1 and Fig. 3.1). An overall half-life of disappearance within this period was found to be 48.4 days (see Appendices, Fig. 3.3). The first week showed the highest rate of dissipation followed by a slower rate between 7 and 42 days and then a sudden rise in the dissipation rate between 42 and 72 days. This is probably a result of increased rainfall causing a sudden rise in degradation by microorganisms giving metabolites which went into bound residue formation during

these later days. The dissipation curve shows a biphasic pattern. By regression analysis, the dissipation data fitted into a first order kinetics model and a half-life of dissipation of 48.4 days was calculated. This is lower than the half-values obtained for DDT (64.6 adys) and DDE (145 days) in the same soil.

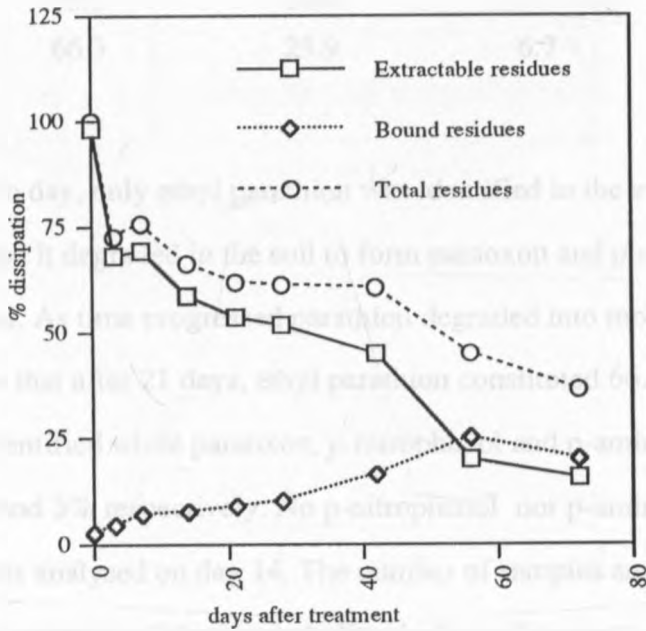


Fig. 3.1 The percent dissipation of parathion in Chiromo soil.

By TLC and GC the following metabolites were identified in the methanol extracts of soil samples (Table 3.2).

Table 3.2.

Parathion metabolites in the soil extracts.

| Days after treatment | ethyl parathion | paraoxon | p-nitrophenol | p-aminophenol |
|----------------------|-----------------|----------|---------------|---------------|
| 0                    | 100             | 0        | 0             | 0             |
| 7                    | 83.2            | 11.6     | 3.6           | 1.6           |
| 14                   | 83.5            | 16.4     | -             | -             |
| 21                   | 66.3            | 23.9     | 6.7           | 3.0           |

On zero day, only ethyl parathion was identified in the soil extracts. However after three days it degraded in the soil to form paraoxon and p-nitrophenol and p-aminophenol. As time progressed parathion degraded into more and more of these metabolites so that after 21 days, ethyl parathion constituted 66.3% of the total metabolites identified while paraoxon, p-nitrophenol and p-aminophenol constituted 23.9%, 6.7% and 3% respectively. No p-nitrophenol nor p-aminophenol was detected in the soil extracts analysed on day 14. The number of samples analysed were not adequate to rule out the presence of these metabolites in the soil extracts.



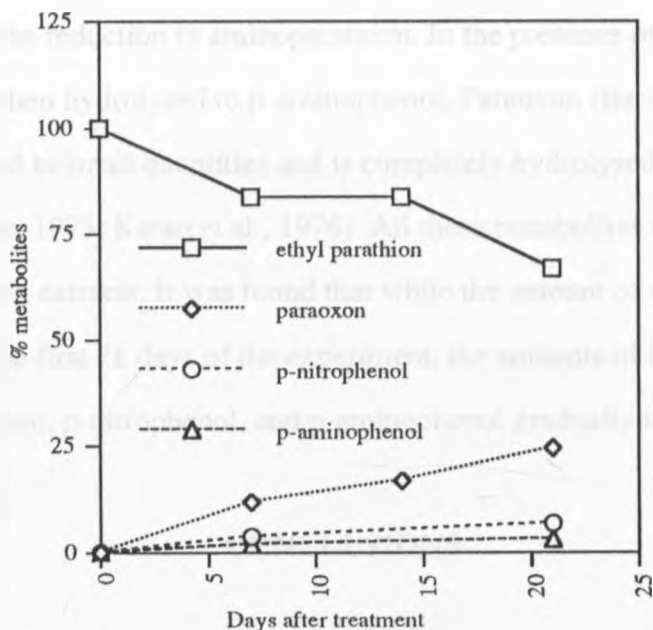


Fig. 3.2 The percent metabolites in the soil extracts.

## DISCUSSION

The disappearance of parathion from the Chiromo soil was very rapid. In the first three days when the pesticide was still on the top of the soil column, the dissipation was mainly due to volatilization. As the pesticide moved into the soil as a result of rainfall, microbial activity and the formation of bound residues increased and these then played a major role in the rate of disappearance of the pesticide. This mainly occurred between 3 and 42 days after application of the pesticide to the soil. After 56 days, the air and soil temperatures and the hours of sunshine increased. This accelerated the rate of dissipation by volatilization while the bound residue levels began to decline. Two pathways have been postulated for microbial degradation of parathion in soil i.e. the hydrolysis to

p-nitrophenol and the reduction to aminoparathion. In the presence of oxygen, the aminoparathion is then hydrolysed to p-aminophenol. Paraoxon (the oxygen analogue of parathion) is formed in small quantities and is completely hydrolysed to p-nitrophenol (Joiner and Baetcke, 1973; Katan et al., 1976). All these metabolites were identified and quantified in our soil extracts. It was found that while the amount of ethyl parathion decreased during the first 21 days of the experiment, the amounts of its major metabolites, paraoxon, p-nitrophenol, and p-aminophenol gradually increased.

## CONCLUSIONS

Parathion was less persistent than has been reported for DDT and DDE degradation in this tropical soil. It dissipated following a biphasic pattern which is also found to be the case with the other pesticides, the half-life of dissipation being 48.4 days (compare DDT: 64 days and DDE: 145 days). However, parathion accumulated more in the bound form than DDT. Despite its high toxicity to mammals (acute oral LD<sub>50</sub> of 3.3 mg/kg in female rats), parathion does not persist in this tropical soil environment.

## ACKNOWLEDGEMENT

The authors wish to thank the International Atomic Energy Agency (IAEA) for the financial assistance given for this study under research contract Nos. 5566/RB and KEN/5/016. We are also grateful to Dr. W.C. Dauterman for technical and laboratory assistance.

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## CHAPTER 4

### THE ADSORPTION/DESORPTION AND MOBILITY OF CARBOFURAN IN SOIL SAMPLES FROM KENYA.

Key words: Carbofuran, soil type, Freundlich Adsorption/Desorption constants, leaching profile, metabolites

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### INTRODUCTION

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl-N-methylcarbamate) is a very effective systemic and contact insecticide and nematicide used against a wide range of agricultural pests (Aquino and Pathak, 1976; Caro et al., 1973). Though less persistent than most organochlorine pesticides, carbofuran is more toxic to animals, very water soluble and tends to degrade faster under flooded soil conditions (Caro et al., 1973; Venkateswarlu et al., 1977; El-Zorgani et al., 1980). The adsorption and desorption

characteristics which determine the movement of pesticides through the soil profile, their bioavailability, microbial degradability and persistence depend upon the soil properties such as organic matter content, clay content and the physical/chemical properties of the pesticide i.e. size, shape, solubility in water, pK values and polarity (Wondimagegnehu and Foy, 1986; Calderbank, 1989).

In Kenya, carbofuran is used extensively against rice pests in paddy fields and because of its acute toxicity the fate of its residues in terms of their persistence and mobility is of great concern (Anonymous, 1985; Plimmer, 1980). The aim of this study was to investigate the adsorption/desorption and mobility of carbofuran in samples of soil collected from the Ahero and Chiromo areas of Kenya.

## MATERIALS AND METHODS.

### Soils.

The two soils used in this study were collected from two different agricultural regions of Kenya i.e. the lowland heavy, sandy soils of the Kano Plains at Ahero and the red, silt loam soils from Chiromo in Nairobi. A third type of soil was a standard sandy soil obtained from the Institute of Ecological Chemistry, GSF, Munich. The composition and physical characteristics of the three soils are presented in Table 4.1. The soil samples were air-dried in the laboratory, ground with mortar and pestle and sieved through a 2 mm mesh.

### Chemicals.

Labelled  $^{14}\text{C}$ -carbofuran (2,3- $^{14}\text{C}$ -dimethyl-2,3-dihydro/3- $^{14}\text{C}$ /benzofuran-7-yl-methylcarbamate); specific activity: 728.3 MBq/mmol and radiochemical purity of 97% by TLC was obtained from the Institute of Isotopes, Budapest, Hungary. Non-labelled

standards of carbofuran, 3-hydroxycarbofuran, 3-ketocarbofuran and 3-ketocarbofuran phenol were kindly supplied by the Institute of Ecological Chemistry, GSF, Munich. Anhydrous calcium chloride, residue grade dichloromethane, acetone, n-hexane and methanol and HPLC methanol, acetonitrile, and water were used including Hydroluma cocktail for scintillation counting.

### Apparatus.

Some 100 mL glass centrifuge tubes, overhead shaker, 1200 rpm centrifuge were used in the adsorption/desorption studies. A low pressure rotating pump and glass columns (size: diameter 4.6 cm; height 27 cm) were used in the leaching experiments. Other apparatus used include rotary evaporator, Soxhlet apparatus and nitrogen evaporator for concentrating the sample extracts. Equipment used were a Berthold BF 8000 Liquid Scintillation Spectrometer, a Packard 306 Automatic Sample Oxidizer, a Berthold UV-lamp at 254 nm, a Berthold Automatic Linear Analyzer Scanner and a Hewlett Packard HPLC with ODS column fitted to a Berthold Two Channel UV-radioactive detector LB510 and an automatic fraction collector.

### Experimental Methods.

#### Adsorption/Desorption.

The procedure followed was that developed by the EEC for a ring test exercise (Protocol, 1988). Five grams of dry soil were weighed into 100 mL glass centrifuge tube and 25 mL of 0.01 M  $\text{CaCl}_2$  solution containing a mixture of cold carbofuran and  $^{14}\text{C}$ -carbofuran was added. A blank centrifuge tube containing only 5 grams of soil in 25 mL 0.01 M  $\text{CaCl}_2$  solution and two control tubes containing only 0.01 M  $\text{CaCl}_2$  solution and radiolabelled carbofuran were also prepared. For the other tubes tests were conducted in

duplicate. The experiments were done at 5 ppm (0.025 mg cold carbofuran and  $0.0045\mu\text{Ci }^{14}\text{C}$ -carbofuran), 1 ppm (0.005 mg cold carbofuran and  $0.0009\mu\text{Ci }^{14}\text{C}$ -carbofuran) and 0.2 ppm (0.001 mg cold carbofuran and  $0.00018\mu\text{Ci }^{14}\text{C}$ -carbofuran) each in 25 mL of 0.01 M  $\text{CaCl}_2$  solution. The samples were shaken on an overhead shaker at room temperature (about  $25^\circ\text{C}$ ) for 22 hours, a period of time which was found to be sufficient to attain equilibrium. Samples were centrifuged at 0, 2, 4, and 22 hours after shaking at 1200 rpm for 10 minutes. Then a 2 mL aliquot of supernatant was removed and added to 15 mL Hydroluma cocktail for counting. The difference between the amounts of  $^{14}\text{C}$ -residues found in the standard solution and the supernatant was taken to be the amount adsorbed by the soil.

Desorption was determined on the same samples used for adsorption by removing a known volume of the supernatant (75%) from the tube after 22 hours and then replacing it with pesticide free 0.01 M  $\text{CaCl}_2$  solution. Two successive desorption extractions were made. Each time the tubes and the contents were shaken and centrifuged as described above and 2 mL aliquot removed and counted after 0, 2, 4, 6, 22 hours of shaking.

### Soil Column Leaching.

Soil samples were packed in glass columns (diameter: 4.6 cm) to a height of 27 cm with a glass wool plug at the bottom (Wondimagegnehu and Foy, 1986). Each soil column was saturated with 0.01 M  $\text{CaCl}_2$  by passing the solution through it upward by capillarity for 12 hours. The column was then allowed to drain free overnight with 0.01 M  $\text{CaCl}_2$  solution passing through it at a predetermined rate. The dry mass of soil in each column was 550 grams. Approximately 5.01 mg of  $^{14}\text{C}$ -carbofuran (244,200 dpm) was placed on the soil column and washed with 0.01 M  $\text{CaCl}_2$  solution at the rate of 7 mL per hour for 48 hours. After 48 hours the volume of the leachate was measured and analysed

for radioactivity. The soil column was carefully removed and divided into 0-5 cm, 5-10 cm, 10-15 cm, 15-20 cm, 20-25 cm and 25-27 cm sections for analysis of  $^{14}\text{C}$ -residues.

#### Analysis of Residues in Water and Soil.

The volume of the leachate was analysed by adding 2 mL aliquots to a cocktail for LSC. The metabolites in the leachate were analysed by taking 100 mL, acidifying with a few drops of 0.25 M HCl and extracting 3 times with 50 mL  $\text{CH}_2\text{Cl}_2$ . The extracts were combined and reduced to dryness in a rotary evaporator at about 20°C and then redissolved in 10 mL of methanol for TLC and HPLC. An aliquot of the extract was spotted on precoated silica gel plates and developed in n-hexane:acetone (60:40 v/v). The plates were visualized under the UV lamp at 254 nm and the presence of  $^{14}\text{C}$ -carbofuran in the extract was further confirmed in a TLC scanner. The metabolites were also analysed by a Hewlett Packard HPLC with column : ODS; Solvent: acetonitrile/water (7:3 v/v); Flow rate: 1.2 mL/min; Injection volume: 10 $\mu\text{L}$ .

To determine the carbofuran residues in soil, the soil samples from each section of the column were air-dried in the laboratory and 25 grams weighed and Soxhlet extracted with 150 mL of dichloromethane and an aliquot taken for counting. The extracted soil was air-dried and combusted to determine the bound residues. The dichloromethane extract was reduced to dryness, redissolved in methanol and analysed by TLC and HPLC as outlined above.

## RESULTS

Table 4.1 shows the results of the physical characteristics of the soil samples used for the adsorption/desorption and mobility tests of carbofuran in soil. Before the



experiments were run, several preliminary tests were performed on standard sandy soil samples obtained from the Institute of Ecological Chemistry, GSF, Munich.

**Table 4.1**  
The physical characteristics of the soils used.

| Soil      | pH  | Sand<br>% | Clay<br>% | Silt<br>% | OC<br>% | Moisture<br>% |
|-----------|-----|-----------|-----------|-----------|---------|---------------|
| Ahero     | 7.0 | 38        | 37        | 25        | 1.08    | 5.3           |
| Chiromo   | 6.1 | 30        | 42        | 28        | 1.22    | 6.5           |
| GSF Sandy | 6.0 | 97        | 5.1       | 3.2       | 0.7     | 0.69          |

Note: OC denotes organic carbon.

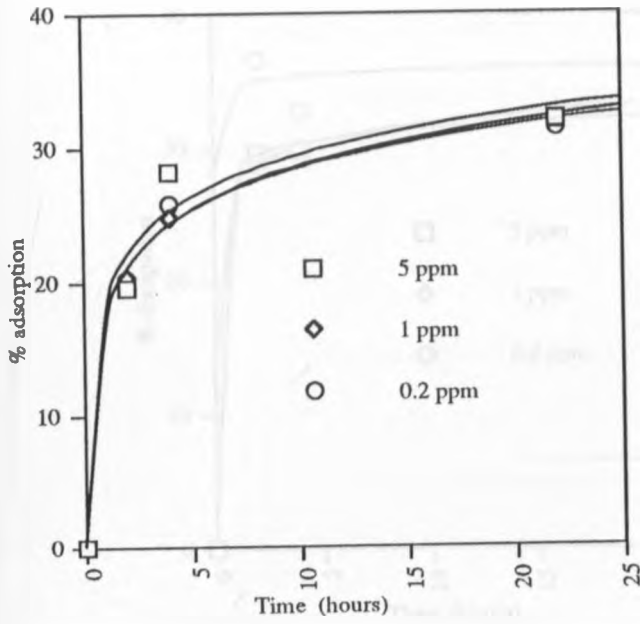
Tables 4.2 and 4.3 show the results of adsorption and desorption of carbofuran in Chiromo and Ahero soils. The amount of carbofuran adsorbed to Ahero soil was slightly higher than the amount adsorbed to Chiromo soils. In Ahero soil samples the amount of pesticide adsorbed (as % of applied pesticide) also increased when the concentration of pesticide applied was lowered i.e. 32.3% with 5 ppm, 33.2% with 1ppm and 36.8% with 0.2 ppm of carbofuran applied to the soil. The results show that adsorption depends on both clay content and organic matter content of the soil. As evidently shown, more carbofuran is adsorbed onto Ahero soil than Chiromo soil, although it was more difficult to remove the adsorbed pesticide from Chiromo soil than from Ahero soil. There was stronger adsorption of carbofuran onto Chiromo soil and, of the total amount of pesticide adsorbed onto Ahero soil, upto 70% was desorbed while only about 39% of the total pesticide adsorbed onto Chiromo soil was removed within the 6 hours of the desorption

test. It was also observed that the adsorption of carbofuran onto Ahero soil to attain equilibrium was more rapid than onto Chiromo soil.

**Table 4.2**

Adsorption (as % of applied pesticide)

| Time<br>(hours) | Ahero soil |      |       |      |       |      | Chiromo soil |      |       |      |       |      |
|-----------------|------------|------|-------|------|-------|------|--------------|------|-------|------|-------|------|
|                 | 0.2 ppm    |      | 1 ppm |      | 5 ppm |      | 0.2 ppm      |      | 1 ppm |      | 5 ppm |      |
|                 | µg         | %    | µg    | %    | µg    | %    | µg           | %    | µg    | %    | µg    | %    |
| 0               | 0          | 0    | 0     | 0    | 0     | 0    | 0            | 0    | 0     | 0    | 0     | 0    |
| 2               | 0.37       | 36.6 | 1.43  | 28.5 | 7.38  | 29.5 | 0.20         | 19.6 | 1.02  | 20.3 | 4.9   | 19.6 |
| 4               | 0.21       | 32.8 | 1.07  | 29.9 | 5.25  | 29.8 | 0.21         | 25.7 | 0.98  | 24.7 | 5.65  | 28.1 |
| 22              | 0.15       | 36.8 | 0.83  | 33.2 | 4.00  | 32.3 | 0.18         | 31.3 | 0.96  | 31.9 | 4.61  | 31.9 |

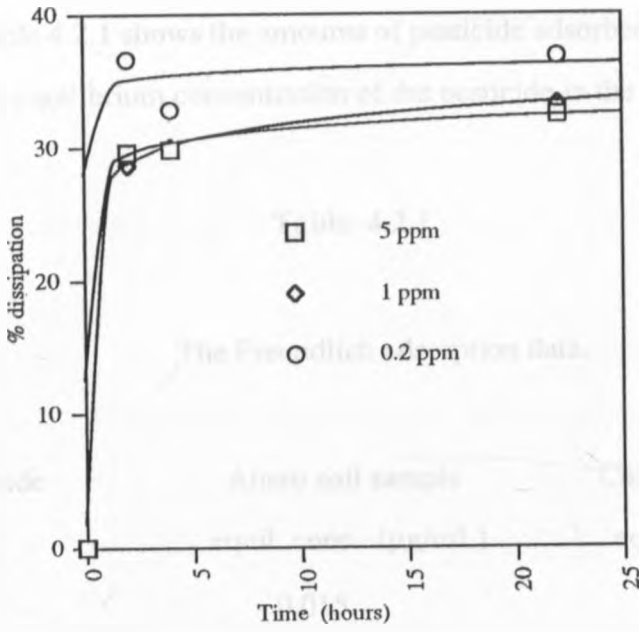


$$y = 10.551\text{LOG}(x) + 18.636 \quad r^2 = 0.806$$

$$y = 10.872\text{LOG}(x) + 17.496 \quad r^2 = 0.990$$

$$y = 10.542\text{LOG}(x) + 17.643 \quad r^2 = 0.932$$

Fig. 4.1 The percent adsorption of carbofuran in Chiromo soil.



$$y = 2.819\text{LOG}(x) + 28.424 \quad r^2 = 0.965$$

$$y = 4.503\text{LOG}(x) + 27.163 \quad r^2 = 1.000$$

$$y = 1.176\text{LOG}(x) + 34.520 \quad r^2 = 0.078$$

Fig. 4.2 The percent adsorption of carbofuran in the Ahero soil.

The following table 4.2.1 shows the amounts of pesticide adsorbed per gram of soil ( $\mu\text{g/g}$ ) versus the equilibrium concentration of the pesticide in the test solution ( $\mu\text{g/mL}$ ).

**Table 4.2.1**

The Freundlich adsorption data.

| amount of pesticide adsorbed ( $\mu\text{g/g}$ ) | Ahero soil sample<br>equil. conc. ( $\mu\text{g/mL}$ ) | Chiromo soil sample<br>equil. conc. ( $\mu\text{g/ml}$ ) |
|--|--|--|
| 0.2  | 0.015  | 0.013  |
| 1.0  | 0.066  | 0.064  |
| 5.0  | 0.324  | 0.320  |

The data from the adsorption of carbofuran to both the Ahero and Chiromo soils given in table 4.2.1 above fitted well into the Freundlich adsorption isotherm equation which relates the amount of test substance adsorbed to the concentration of the test substance in solution at equilibrium i.e.  $X = K_F C^{1/n}$ , where  $X$  = amount of pesticide adsorbed per gram of soil ( $\mu\text{g/g}$ ), and  $C$  = equilibrium concentration of the pesticide per mL of 0.01 M  $\text{CaCl}_2$  solution.  $K_F$  and  $1/n$  are the Freundlich constants. From the plot of  $\text{Log } X$  versus  $\text{Log } C$ , the gradient =  $1/n$  and the  $\text{Log } X$  intercept =  $\text{Log } K_F$ . These constants were found to be  $K_F = 15.49 \mu\text{g/g}$  and  $1/n = 1.0$  for the Ahero soil and  $K_F = 15.14 \mu\text{g/g}$  and  $1/n = 1.0$  for the Chiromo soil.

Table 4.3

Desorption (as % of adsorbed pesticide) at 5 ppm .

| Time<br>(hrs)    | Ahero soil   |              | Chiromo soil |              |
|------------------|--------------|--------------|--------------|--------------|
|                  | % desorption | µg pesticide | % desorption | µg pesticide |
| 0                | 30.8         | 2.49         | 13.3         | 1.06         |
| 2                | 29.1         | 1.55         | 13.3         | 0.91         |
| 4                | 22.9         | 0.87         | 10.2         | 0.61         |
| 22               | 20.3         | 0.58         | 9.8          | 0.56         |
| Total desorbed   |              | 5.49         |              | 3.14         |
| Total adsorbed   |              | 8.08         |              | 7.98         |
| % total adsorbed |              | 70%          |              | 39%          |

Table 4.4 shows the soil column leaching profile of carbofuran in the three soils. The distribution of carbofuran in sections of the soil columns is shown as the total radioactivity (extractable plus bound residues) in the section as a percentage of the total radioactivity applied to the column at the beginning of the experiment. The experiment lasted 48 hours. These percentages are expressed as µg of carbofuran equivalents based on total recovered activity in each section of the column and the specific activity of  $^{14}\text{C}$ -carbofuran used. The results show very rapid vertical movement of  $^{14}\text{C}$ -carbofuran and metabolites down the soil columns. After 48 hours, 33% of total radioactivity was recovered in the leachate from the Ahero soil, 29% in the Chiromo soil and 60% in the

sandy soil leachate. This again suggests a stronger adsorption of carbofuran (due to higher clay and organic matter contents) in the Chiromo soil than the other soils. Sandy soil had very little clay and organic matter compared to the other soils and as expected there was more extensive leaching of carbofuran in this soil. The following table 4.4 shows the leaching profile of carbofuran in the three soils after 48 hours.

**Table 4.4**

Leaching profile of carbofuran in the soils.

| Section of Column | Ahero soil               |         | Chiromo soil             |        | Sandy soil               |        |
|-------------------|--------------------------|---------|--------------------------|--------|--------------------------|--------|
|                   | mg carbofuran equivalent | % total | mg carbofuran equivalent | %total | mg carbofuran equivalent | %total |
| 0-5 cm            | 0.2                      | 3.95    | 0.28                     | 5.6    | 0.026                    | 0.52   |
| 5-10cm            | 0.14                     | 2.78    | 0.56                     | 10.5   | 0.017                    | 0.34   |
| 10-15cm           | 0.30                     | 6.01    | 0.53                     | 10.5   | 0.019                    | 0.38   |
| 15-20cm           | 0.63                     | 12.51   | 0.62                     | 12.2   | 0.033                    | 0.65   |
| 20-25cm           | 0.79                     | 15.83   | 0.67                     | 13.3   | 0.112                    | 2.24   |
| 25-27cm           | 0.98                     | 19.54   | 0.23                     | 4.38   | 0.201                    | 4.02   |
| Leachate          | 1.65                     | 33      | 1.62                     | 29     | 3.007                    | 60.0   |

Note: 550 gm of soil sample in the column was treated with 5.01 mg  $^{14}\text{C}$ -carbofuran.

The same amount of soil and chemical was used for the three types of soil.

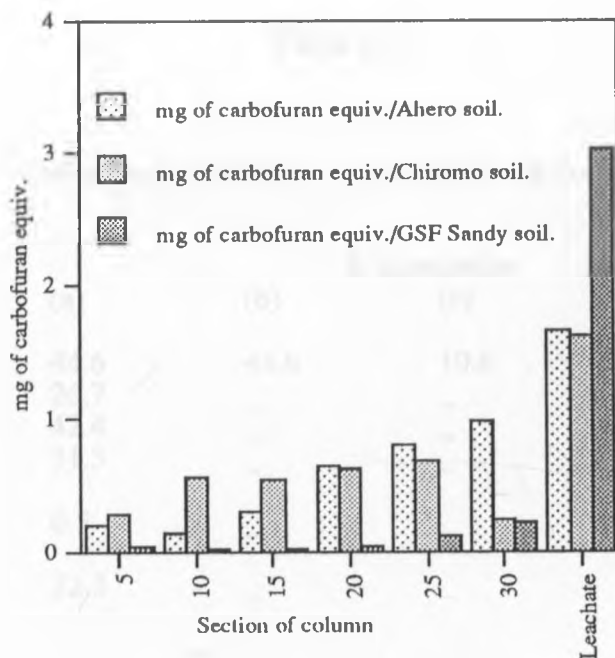


Fig. 4.3 The leaching profile of carbofuran in the three soils.

Tables 4.5, 4.6 and 4.7 show some of the metabolites that were identified by HPLC. The identification was based on cochromatography with authentic standards and the presence of  $^{14}\text{C}$ -carbofuran equivalents was also confirmed by TLC scanning after developing sample extracts in *n*-hexane:acetone (60:40 v/v). The HPLC was with an ODS column, solvent system: acetonitrile/water (70/30 v/v); flow rate 1.2 mL/min; injection volume 10  $\mu\text{L}$ . The absorbance was at 205.4, 215.4, and 280.6 nm. The ratios of each metabolite against the total number of metabolites identified in each section of the column are given below.



Table 4.5

Carbofuran metabolites in the Ahero soil column.

| Section of column | % Metabolite |      |      |      |      |
|-------------------|--------------|------|------|------|------|
|                   | (a)          | (b)  | (c)  | (d)  | (e)  |
| 0-5 cm            | 44.6         | 44.6 | 10.8 | —    | —    |
| 5-10 cm           | 26.7         | —    | —    | 52.2 | 21.1 |
| 10-15 cm          | 42.4         | —    | —    | —    | 57.6 |
| 15-20 cm          | 31.3         | —    | —    | 68.7 | —    |
| 20-25 cm          | —            | —    | —    | —    | —    |
| 25-27 cm          | 6.5          | —    | —    | 63.5 | 30.0 |
| Leachate          | 32.8         | —    | —    | 55.6 | 11.6 |

Note: (a) represents carbofuran; (b) represents 3-hydroxycarbofuran; (c) represents 3-ketocarbofuran; (d) represents 3-hydroxycarbofuranphenol (e) represents 3-ketocarbofuranphenol. A blank space denotes that the metabolite was not detected.

Table 4.6

Carbofuran metabolites in the Chiromo soil column.

| Section of column | % metabolite |      |      |     |      |
|-------------------|--------------|------|------|-----|------|
|                   | (a)          | (b)  | (c)  | (d) | (e)  |
| 0-5 cm            | 27.6         | —    | 36.3 | —   | 36.1 |
| 5-10 cm           | 29.4         | 6.2  | 17.3 | —   | 47.1 |
| 10-15 cm          | 43.9         | 28.9 | —    | —   | 27.1 |
| 15-20 cm          | 69.5         | —    | —    | —   | 30.5 |
| 20-25 cm          | 52.3         | 20.6 | —    | —   | 27.2 |
| 25-27 cm          | 43.1         | —    | 41.1 | 5.9 | 15.7 |
| Leachate          | 92.2         | 4.03 | 0.67 | —   | 3.16 |

Table 4.7

## Carbofuran metabolites in GSF Sandy soil column

| Section of column | % metabolite |      |     |     |      |
|-------------------|--------------|------|-----|-----|------|
|                   | (a)          | (b)  | (c) | (d) | (e)  |
| 0-5 cm            | 6.8          | 88.8 | —   | —   | 4.4  |
| 5-10 cm           |              |      |     |     |      |
| 10-15 cm          |              |      |     |     |      |
| 15-20 cm          | 37.8         | 62.2 | —   | —   | —    |
| 20-25 cm          | 52.3         | —    | —   | —   | —    |
| 25-27 cm          |              |      |     |     |      |
| Leachate          | 52.3         | —    | 3.0 | 3.3 | 41.4 |

## DISCUSSION

The results show very rapid vertical movement of carbofuran residues down the soil columns. Some 33% of total radioactivity was recovered in the leachate from the Ahero soil while 29% and 60% were recovered from the Chiromo and GSF sandy soil columns, respectively. The effect of microorganisms on the degradation of carbofuran in the soil columns is the result of the formation of more metabolites and these seem to bind more to the Chiromo soil sample. Also many of these metabolites were present in the leachate in trace amounts indicating that most of them had eluted from the soil columns.

In the Ahero soil column, the main compounds along the column were carbofuran, 3-hydroxycarbofuran phenol and 3-ketocarbofuran phenol. 3-Hydroxycarbofuran and 3-ketocarbofuran were found only in the top 5 cm of the soil column. In the Chiromo soil column all the metabolites, carbofuran, 3-

hydroxycarbofuran, 3-ketocarbofuran, 3-hydroxycarbofuran phenol, and 3-ketocarbofuran phenol were found spread out along the entire column. However, in the leachate mainly carbofuran was found. In the GSF Sandy soil column, the main metabolites were carbofuran, 3-hydroxy carbofuran and 3-ketocarbofuran phenol.

The adsorption of carbofuran to both the Ahero and Chiromo soils was high (32.3% and 31.9% at 5 ppm, respectively). However there was less adsorption in the sandy soil. These results suggest that the adsorption of carbofuran in the Kenyan soils was mainly influenced by the amount of clay and organic matter in the two soil samples. However, 70% of the adsorbed pesticide was removed by desorption after 22 hours in the Ahero soil and 39% desorbed from the Chiromo soil. The data from the adsorption of carbofuran to both the Ahero and Chiromo soils fitted well into the Freundlich adsorption isotherm equation which relates the amount of pesticide adsorbed to its concentration in solution at equilibrium. The Freundlich constants of carbofuran in the two soils were found to be  $K_F = 15.49 \mu\text{g/g}$  and  $1/n = 1.0 \mu\text{g/mL}$  for the Ahero soil and,  $K_F = 15.14 \mu\text{g/g}$  and  $1/n = 1.0 \mu\text{g/mL}$  for the Chiromo soil.

## CONCLUSION

These results confirm that the physical/biochemical characteristics of the soil greatly influence the rate of adsorption/desorption and leaching of carbofuran and therefore, apart from the climatic factors, affect its disappearance and degradation in the soil environment.

## ACKNOWLEDGEMENTS

The authors wish to thank the German Academic Exchange Service (DAAD) for the financial assistance.

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## CHAPTER 5

### THE DISTRIBUTION AND DISSIPATION OF CARBOFURAN IN A PADDY FIELD IN THE KANO PLAINS OF KENYA.

Key words: Carbofuran, flooded and non-flooded paddy field conditions, dissipation, metabolism, vertical distribution.

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## INTRODUCTION

Carbofuran (2,3-dihydro-dimethyl-7-benzofuranyl methylcarbamate) is a systemic and contact insecticide and nematicide which has a broad spectrum against many agricultural pests (Aquino and Pathak, 1976). It is very effective in the control of major rice pests i.e. the green leafhoppers, brown planthoppers, stemborers and whorl maggots (Aquino and Pathak, 1972; Venkateswarlu et al., 1977). Although less persistent, carbofuran is more toxic to animals than most organochlorines (LD<sub>50</sub> 11 mg/kg in rats,

dogs and chickens) (Caro et al., 1973). It has been studied in a number of countries with regard to its fate in different environments. Studies conducted in India and China (Jinhe et al., 1989; Panda et al., 1988) have shown that carbofuran degraded non-enzymatically and by microorganisms under sub-tropical conditions. The degradation occurs by hydroxylation at the benzylic carbon to give 3-hydroxycarbofuran and 3-ketocarbofuran under upland conditions and via hydrolysis to form phenols i.e. carbofuran phenol, 3-hydroxycarbofuran phenol, and 3-ketocarbofuran phenol under flooded conditions (Jinhe et al., 1980; Jinhe et al., 1989). A significant part of this pesticide remained in the form of bound residues in the soil, rice plants and fish tissues (El-Zorgani et al., 1980; Jinhe et al., 1980; Vollner et al., 1980; Jinhe et al., 1989). It is hydrolysed both in non-flooded and flooded soils but slightly more rapidly under flooded conditions and it shows increased persistence when incorporated into non-flooded soils as a result of reduced losses by volatilization and photochemical degradation (Vollner et al., 1980; Panda et al., 1988). The persistence increased with the depth of soil incorporation of the insecticide.

In Kenya, carbofuran ( in the form of 5% technical Furadan® granules) is applied at the rate of 0.5-4 kg a.i./ha for the control of soil dwelling, foliar feeding insects and mites at the seed furrow in rice paddy fields. Approximately 97% of all the rice in Kenya is produced by irrigation schemes covering 9000 hectares (Anonymous, 1985).

With the increasing population, efforts are being made to intensify rice production and to increase this acreage. This will result in an increase in the use of carbofuran at least in the near future. Carbofuran residues and its toxic carbamate metabolites are therefore of great concern in terms of its persistence, mobility and toxicity to fish and mammals (Plimmer, 1988; MacRae, 1989). This study follows the dissipation, metabolism and distribution of carbofuran in flooded and non-flooded soils of the Kano plains of Kenya under field conditions.

## MATERIALS AND METHODS.

### Chemicals.

Ring labelled  $^{14}\text{C}$ -carbofuran (carbofuran/ benzofuranyl-ring 2C-14); specific activity: 11.9  $\mu\text{Ci}/\text{mmol}$  and radiochemical purity of 99% by TLC was purchased from International Isotopes, Munich. Non-labelled standards: carbofuran, 3-hydroxy carbofuran, 3-ketocarbofuran, carbofuran phenol, 3-ketocarbofuran phenol and 3-hydroxycarbofuran phenol were kindly supplied by the Institute of Ecological Chemistry, GSF, Munich while 5% technical Furadan® granules were purchased from Rhone Poulenc, Nairobi. Distilled methanol, dichloromethane, acetone and toluene were obtained from suppliers in Nairobi. HPLC grade methanol, benzene, ethyl acetate, and water were used. Crystalline 2,5-diphenyl oxazole (PPO) and POPOP both for use in scintillation cocktail were purchased from Sigma Chemical Company, UK. A Harvey cocktail for  $^{14}\text{CO}_2$  absorption was purchased from J.H. Harvey Inst. Corp., US.

### Apparatus.

PVC pipes (diameter: 3.7 cm and length : 20 m ) were purchased and cut into 55 cm long cylindrical tubes to be inserted into the soil of the paddy field. A mortar and pestle for soil sample homogenization was used and all samples of soil and pesticide residue extracts awaiting analysis were stored at 4°C. Paper lined standard glass tanks, precoated silica gel SIL G/UV 254 plates and a Spectroline UV lamp was used for TLC analysis. An oven was used for the determination of moisture content while a Soxhlet apparatus and a rotary evaporator were used for extraction and concentration of the soil extracts. A Berthold LB2832 Automatic TLC Linear Analyzer was used to locate the radioactive spots. A Tricarb 1000TR Perkin Elmer Liquid Scintillation Spectrometer was



used for radioassay and the bound residues were combusted in an OX-600 Harvey Biological Oxidizer.

### Field Application and Sampling.

The experimental site was located at Ahero, 350 km west of Nairobi (Lat. 0°-12'S; Long. 24°-50'E; Alt. 3854 ft). The field work was planned in collaboration with Ahero Rice Research Station, National Irrigation Board. The weather conditions which include the relative humidity, air temperatures, rainfall, windspeed of this location were recorded by the Department of Meteorology, Nairobi during the experimental period. A soil sample collected from the experimental site gave the following properties: pH 7.0; sand 38%; clay 37%; silt 25%; organic carbon 1.08% (Ref. Bayerische Hauptversuchsanstalt für Landwirtschaft der Technische Universität, Munich, Germany).

The field study began in January, 1993 and lasted 111 days. A small plot measuring 18 metres by 6 metres was chosen within the irrigation scheme where rice had not been previously cultivated. The plot was prepared by tractor and then by hand to remove all weeds and stones as is usually done for rice cultivation. The plot was then subdivided into three equal parts (each 6 by 6 metres). Two parts were completely drained to give non-flooded conditions while one part was flooded with water to the level usually used in rice cultivation. The plots were then left undisturbed for one week after which 36 hard PVC cylinders as described previously were driven into the soil with three centimetres left protruding above the soil surface to prevent run-off water. The tubes were left undisturbed for one week before applying the pesticide.

The plots were divided into A, B, and C. In plot A (flooded paddy soil) and plot B (non-flooded soil), each cylinder received 1.7  $\mu\text{Ci}$   $^{14}\text{C}$ -carbofuran and 11.7 mg of cold standard carbofuran equivalent to 20 ppm. In plot C (non-flooded soil), each cylinder received 1.7  $\mu\text{Ci}$  of  $^{14}\text{C}$ -carbofuran and 0.4 grams of 5% technical furadan granules

equivalent to 20 ppm. The labelled and non-labelled chemicals were mixed in 8.5 mL of methanol in a vial prior to application. The chemicals were applied to the surface of the soil and then covered with a thin layer of soil. The first two cylinders from the three plots were carefully removed and wrapped in plastic bags soon after pesticide application for determination of the initial residues. The other cylinders were sampled in duplicate at various time intervals upto 111 days.

### Analytical Methods.

The soil samples were carefully removed from the PVC cylinders by cutting each pipe laterally into sections of 0-10 cm, 10-20 cm, 20-30 cm, 30-40 cm, 40-50 cm. The soils from each section was separated, mixed and approximately 10 grams taken for analysis to determine the distribution of carbofuran along the soil column. The sample was placed in a 250 ml conical flask and 50 mL acetone was added, stirred and left to settle and then the acetone decanted. This was done twice and the extracts were combined and filtered to remove any remaining soil particles. From the acetone extract, 2 mL were taken and counted. The extracted soil samples were left in the flasks to air-dry, reweighed and 1.5 grams taken for combustion in an OX-600 Harvey Biological Oxidizer for 4 minutes. The  $^{14}\text{CO}_2$  produced was absorbed in a Harvey cocktail and counted. All analyses were done in duplicate.

The remaining soil samples from each section of the pipe were combined, air-dried in the laboratory and then ground in a mortar and pestle and thoroughly mixed. Approximately 50 grams of the soil sample (in duplicate) were extracted in a Soxhlet apparatus using 150 mL dichloromethane for 4 hours and the extractable residues were determined by counting 1 mL of the extract. Non-extractable (bound) residues were determined by combusting 1.5 grams of extracted soil and counting the  $^{14}\text{CO}_2$  trapped in the Harvey cocktail.

## RESULTS

Tables 5.1, 5.2 and 5.3 show the results of the dissipation  $^{14}\text{C}$ -carbofuran from flooded and non-flooded soils over a period of 111 days. These are also summarized in the following figures.

Table 5.1

The dissipation of non-formulated carbofuran in flooded soil.

| Days after treatment | Extractable     |      | Bound           |      | Total           |      |
|----------------------|-----------------|------|-----------------|------|-----------------|------|
|                      | $\mu\text{g/g}$ | %    | $\mu\text{g/g}$ | %    | $\mu\text{g/g}$ | %    |
| 0                    | 11.2            | 57.0 | 1.8             | 9.0  | 13.0            | 66.0 |
| 18                   | 4.6             | 23.3 | 5.8             | 29.3 | 10.4            | 52.6 |
| 25                   | 4.3             | 21.9 | 3.4             | 17.4 | 7.7             | 39.3 |
| 40                   | 1.8             | 9.4  | 8.1             | 40.9 | 9.9             | 50.3 |
| 54                   | 1.4             | 10.4 | 2.3             | 17.8 | 3.7             | 27.8 |
| 111                  | 1.2             | 7.4  | 2.2             | 12.8 | 3.4             | 20.2 |

Table 5.2

The dissipation of non-formulated carbofuran in non-flooded soil.

| Days after treatment | Extractable     |      | Bound           |      | Total           |      |
|----------------------|-----------------|------|-----------------|------|-----------------|------|
|                      | $\mu\text{g/g}$ | %    | $\mu\text{g/g}$ | %    | $\mu\text{g/g}$ | %    |
| 0                    | 14.21           | 84.5 | 1.75            | 10.4 | 15.96           | 94.9 |
| 18                   | 9.98            | 59.4 | 8.86            | 52.7 | 9.41            | 55.9 |
| 25                   | 3.55            | 22.2 | 5.89            | 36.9 | 9.44            | 59.1 |
| 40                   | -               | -    | 7.38            | 46.2 | -               | -    |
| 54                   | -               | -    | 7.28            | 45.6 | -               | -    |
| 111                  | 2.94            | 18.4 | 2.56            | 17.8 | 12.25           | 36.2 |

Table 5.3

The dissipation of 5% technical furadan in non-flooded soil.

| Days after treatment | Extractable |      | Bound |      | Total |      |
|----------------------|-------------|------|-------|------|-------|------|
|                      | µg/g        | %    | µg/g  | %    | µg/g  | %    |
| 0                    | 11.76       | 56.5 | 2.01  | 9.6  | 13.77 | 66.3 |
| 18                   | 5.05        | 24.2 | 6.52  | 31.3 | 11.57 | 55.5 |
| 25                   | 3.28        | 15.7 | 10.03 | 48.1 | 13.31 | 63.8 |
| 40                   | 0.83        | 4.0  | 6.52  | 31.3 | 7.35  | 35.3 |
| 111                  | 1.41        | 6.7  | 7.24  | 34.8 | 8.65  | 41.5 |

Mass of dry soil in cylinder Plot A: 1017 grams.

Plot B: 1191 grams.

Plot C: 961 grams.

Table 5.4

The vertical distribution of carbofuran residues in the PVC cylinders (as % of the total recovered residues) under field conditions.

| Days after treatment plot | 40   |      |      | 54   |      |      | 111  |      |      |
|---------------------------|------|------|------|------|------|------|------|------|------|
|                           | A    | B    | C    | A    | B    | C    | A    | B    | C    |
| 0-10 cm                   | 79.2 | 51.3 | 50.8 | 76.3 | 72.0 | 70.0 | 28.3 | 52.2 | 49.6 |
| 10-20 cm                  | 6.3  | 31.4 | 22.2 | 9.2  | 14.2 |      | 24.8 | 11.6 | 19.2 |
| 20-30 cm                  | 5.7  | 6.8  | 11.4 | 11.3 | 10.1 | 15.4 | 17.0 | 28.8 | 17.4 |
| 30-40 cm                  | 5.2  | 5.1  | 8.5  | 1.8  | 2.6  | 7.0  | 15.2 | 5.0  | 8.7  |
| 40-50 cm                  | 3.6  | 5.4  | 7.0  | 1.4  | 1.0  | 6.7  | 14.7 | 2.4  | 5.0  |

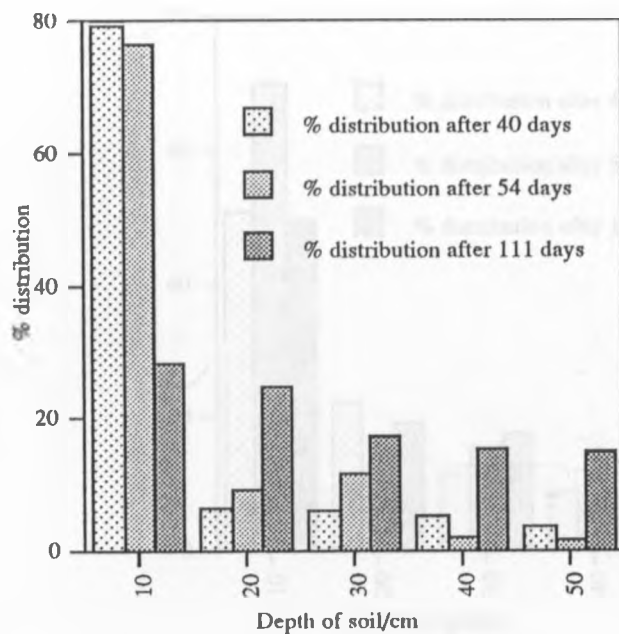


Fig. 5.1 Plot A: The distribution of carbofuran with soil depth.

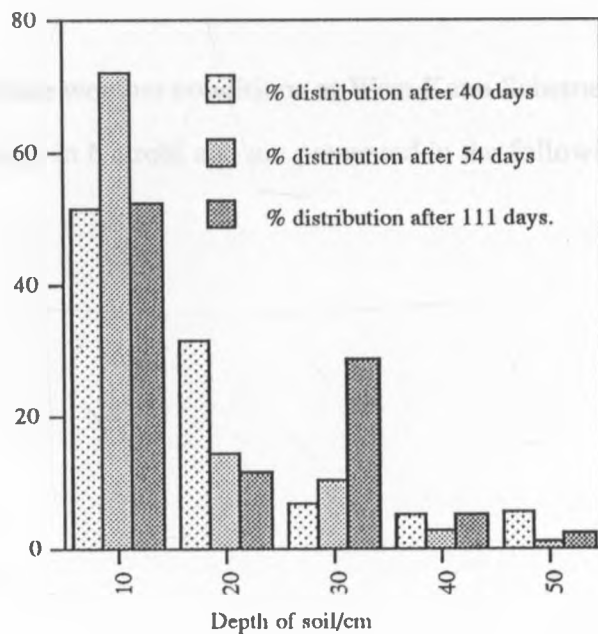


Fig. 5.2 Plot B: The distribution of carbofuran with soil depth.

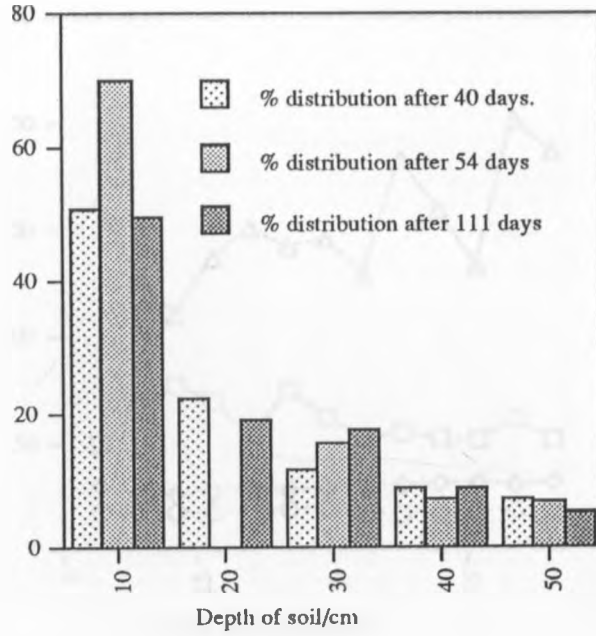


Fig. 5.3 Plot C: The distribution of carbofuran with soil depth.

#### Weather Data.

The daily readings of the weather conditions at West Kano Scheme were taken by the Department of Meteorology in Nairobi and are presented in the following figures.

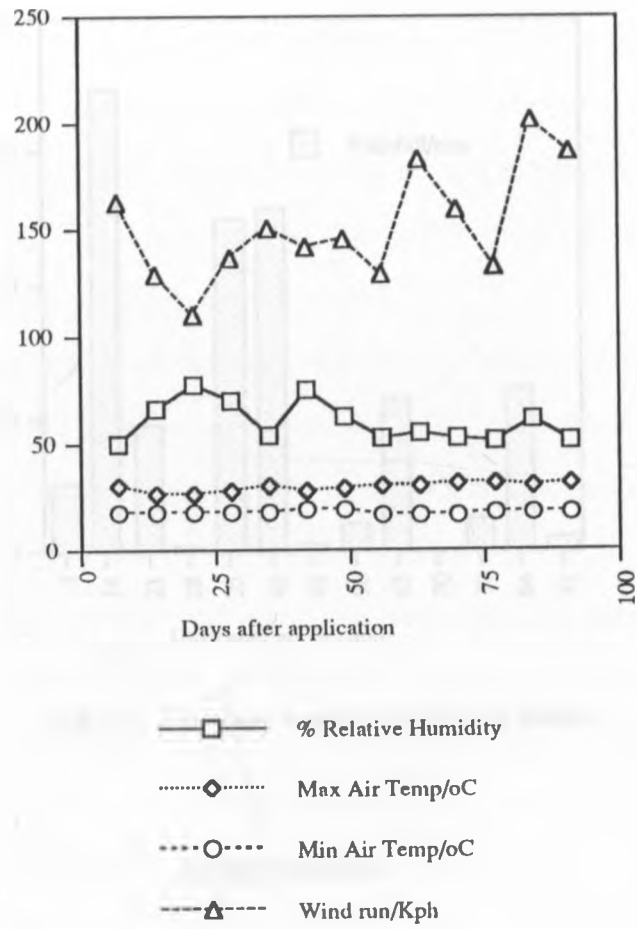


Fig. 5.4 The average weekly weather data at Ahero.

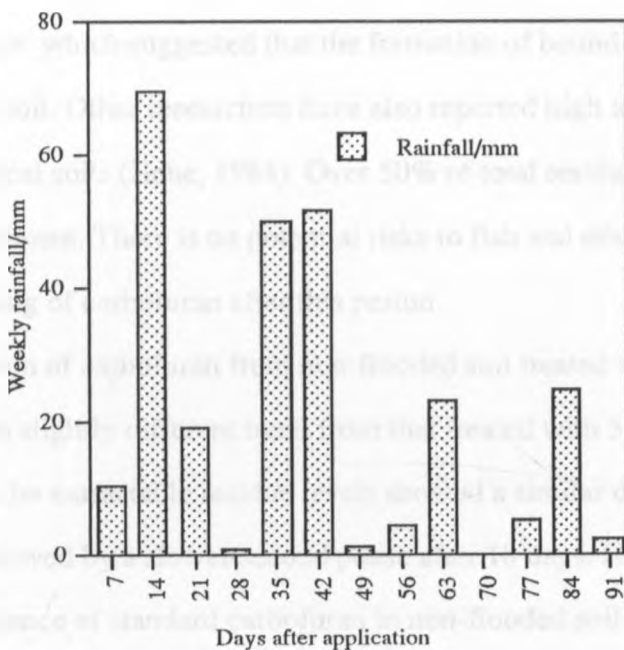


Fig. 5.5 The total weekly rainfall at Ahero.

## DISCUSSION

The recovery of carbofuran residues from the soil samples was low because the amount of pesticide lost in the water as a result of co-evaporation during the air drying process was not taken into account. This was particularly true in the case of the flooded soil samples. In terms of extractable residues there was rapid dissipation of carbofuran from 11.21 ppm to 4.58 ppm after 18 days in flooded soil. The rate of dissipation slows down from this time to the end of the experiment. The trend shows a biphasic pattern with fast disappearance upto 18 days followed by a slower rate of dissipation. The bound residue level was high at 9 % of the total residue recovered on zero day because the samples had to be carried from Ahero to Nairobi overnight and had to be air-dried before the analysis. In flooded soil the bound residue went upto about 29.3 % of the recovered pesticide after 18 days and was just over 40 % after 40 days. This agrees with the findings



of the adsorption tests which suggested that the formation of bound residues was very rapid in this type of soil. Other researchers have also reported high levels of bound residues in sub-tropical soils (Jinhe, 1984). Over 50% of total residues disappeared within 25 days of the experiment. There is no potential risks to fish and other organisms in soil due to direct poisoning of carbofuran after this period.

The dissipation of carbofuran from non-flooded soil treated with standard carbofuran showed a slightly different trend from that treated with 5% technical Furadan® although the extractable residue levels showed a similar dissipation pattern- a rapid first phase followed by a slower second phase after 18 days. However, there was slightly more persistence of standard carbofuran in non-flooded soil but still a loss of over 50% of the total residues occurred after 25 days. The bound residues were also higher upto 59% after 54 days. The dissipation of carbofuran from flooded soil was more rapid than that in non-flooded soils with levels approaching 40% in less than 25 days and falling below 20% after 111 days in terms of total residues.

The maximum and minimum air temperature at Ahero was fairly constant in the range 26-30°C (max) and 14-18 °C (min). This is typical of this region. However, there was a distinct difference in the % relative humidity and the amount of rainfall recorded, being higher between 0-18 days. These climatic factors coupled with soil microbial characteristics played a major role in the rapid dissipation and degradation of carbofuran from the soil and also in the formation of bound residues.

In flooded soil most of the carbofuran residues were found in the top 10 cm of the soil even after 111 days. This is a potential danger to fish due to surface water contamination. In fact, most of the pesticide tended to stay in the water above the soil surface, since carbofuran is very water soluble. About 18% of the residues were in the 40-50 cm section of the soil column after 111 days. However, in non-flooded soils there was definite movement of the pesticide (most in the 20-30 cm section) vertically

downwards. These results show that there is very little risk of ground water contamination with carbofuran in this type of soil.

## CONCLUSION

Carbofuran can be considered to be non-persistent in this tropical soil which concurs with the findings of previous studies conducted in other tropical countries. High temperature, high % relative humidity, rainfall and soil microbial properties stimulated the disappearance of this pesticide from the soil. The dissipation was more rapid under flooded conditions than under non-flooded conditions and carbofuran existed in the soil mostly in a bound form 2-3 weeks after application. In flooded soil, most of the residues were found in the top 10 cm layer of soil presenting great danger to fish and other organisms. Contaminated water may find its way into the nearby canals and the River Nyando which passes through this area during the first 3 weeks of pesticide application.

## ACKNOWLEDGEMENTS

The authors wish to thank the German Academic Exchange Service (DAAD) and the International Atomic Energy Agency (IAEA) for the financial support under Research Contract No. KEN/5/016. We are also grateful to Dr. W.C. Dauterman for the technical and laboratory assistance.

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## CHAPTER 6

**THE METABOLISM OF MALATHION RESIDUES IN STORED  
BEANS (*Phaseolus vulgaris*) AND MAIZE (*Zea mays*).**

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**1. INTRODUCTION**

Malathion (O,O-dimethyl S-(1,2-dicarboethoxyethyl) phosphorodithioate) is a widely used organophosphorous pesticide due to its low persistence in the environment and high insecticidal activity (Lin et al., 1983). It is used worldwide for the control of pests in vegetables, field crops, fruits, nuts, stored grains and on domestic animals (Lin et al., 1984). In Kenya, malathion is mainly used to control insect pests in stored grains. It is commercially available as 2% Malathion dust and applied at the rate of 500 grams of the dust per tonne of dry grains (Annual Report, 1988). It controls pests by both contact and vapour activity.

However, malathion has been the subject of many biochemical studies relating to its metabolism and toxicity (Chiu et al., 1968; Hassan and Dauterman, 1968; Dauterman

and Main, 1968; Lin et al., 1983; Lin et al., 1984). Some studies have also been conducted to follow its persistence and fate in soil, plants and grains (Mensah, 1987; Nigel, 1987). Pure malathion has moderate toxicity (LD<sub>50</sub> of 12,500 mg/kg for rats) but crude malathion and its formulations contain impurities which are far more toxic to mammals. These impurities are not only formed during commercial production but can also develop in the grains during storage (Lin et al., 1984; Miles, 1989). The most toxic of these products is the oxidation product malaoxon which is responsible for the insecticidal activity of malathion. Additional metabolites can arise from hydrolysis, isomerization of the P-S and S-C linkages and demethylation giving malathion  $\alpha$ - and  $\beta$ -monocarboxylic acids, O,O-dimethyl phosphorodithioate and isomalathion etc. Several methods for the extraction, purification and quantification of malathion metabolites in grains have been reported and include extraction with acetonitrile/water, filtration, liquid-liquid partitioning followed by TLC, HPLC, GC and GC-MS (Anderegg and Madisen, 1983; Berit, 1986; Rengasamy, 1989; Wilkins, 1990).

This paper reports our studies on the behaviour and persistence of malathion applied to dry maize grains and beans stored in two different storage models simulating the traditional open basket and the closed modern box. The aim was to follow the dissipation of malathion and to identify some of its metabolites.

## 2. MATERIALS AND METHODS

### 2.1 Chemicals

<sup>14</sup>C-malathion (O,O-dimethyl S-(1,2-dicarboethoxy-(1,2-<sup>14</sup>C)-ethyl phosphorodithioate); specific activity: 24.5 mCi/mmol and radiochemical purity: 98% (by TLC) was obtained from International Isotopes, Munchen, Germany and 2% (a.i.) technical malathion powder was purchased from Rhone Poulenc in Nairobi. Non-labelled

standards, malathion, malaoxon, malathion monocarboxylic acids and isomalathion were obtained from the Institute of Ecological Chemistry, GSF, Munchen, Germany. The organic solvents used viz: isooctane, ethylacetate, n-hexane, methanol were all of residue analysis grade. Methanol, water and acetonitrile of HPLC grade were used for HPLC analysis and Permaflour® and Hydroluma® cocktails were used for bound and aqueous residues, respectively. All these solvents were obtained from the Institute of Ecological Chemistry, GSF, Munich. Crystalline PPO and POPOP used in liquid scintillation spectrometry were purchased from Sigma Chemical Company, UK.

## 2.2 Apparatus

A Waring blender was used for sample homogenization and all samples awaiting analysis were stored at -4 °C in the laboratory. Precoated silica gel 60 F 254 TLC plates, paper-lined standard glass tanks saturated with solvents: n-hexane+ ethylacetate (60+ 40 by volume) and a Berthold UV lamp were used in TLC analysis. A Berthold Automatic Linear Analyzer TLC Scanner was also used to scan the TLC plates.

A glass column (length: 30 cm; diameter: 1 cm) plugged with glass wool and filled upto 20 cm with Florisil (60-100 mesh ASTM) was used for clean-up of internal extracts while a Hewlett Packard GC model 437 fitted with a Hewlett Packard 3385 Automatic System Integrator and a capillary column HP-5 crosslinked with 5% phenyl methyl silicone (25 m \* 0.2 mm \* 0.5 uL film thickness) available at the Institute of Ecological Chemistry, GSF, Munich, Germany was used. Other instrumentation used were a Hewlett Packard HPLC with ODS column, a Finnigan ITS 40 GC-MS and a Packard 306 Automatic sample oxidizer, a Berthold BF 8000 liquid scintillation spectrometer, and a Beckman LS6800 Scintillation Spectrometer (available at ILRAD , Nairobi).

### 2.3 Sample Treatment and Sampling Procedure

Two types of storage models were used i.e. the traditional open basket (cylindrical basket: height-11 cm, and diameter- 25 cm) made of straw was bought from a local market and the modern wooden box (24.5 cm \* 24.5 cm \* 24.5 cm) was made in the Science Workshop of the University of Nairobi. Fresh beans and maize grains were obtained from a rural farm in Ahero where chemicals had not been applied. They were air-dried in the laboratory for 5 days. 750 grams of each dry material were treated with a mixture of 12.5  $\mu\text{Ci}$  of  $^{14}\text{C}$ -malathion and 0.38 grams of 2% Malathion dust equivalent to 10.36 ppm of the pesticide. The grains were thoroughly mixed in a polythene bag, transferred to the appropriate storage model in the laboratory and stored at 20-24  $^{\circ}\text{C}$  for 12 months.

Sampling and analysis started 6 hours after application then continued after 2 weeks, 1,2,3,4,6,9 and 12 months. Each time, grain samples (25 g) were taken in triplicate from each model, washed with distilled water (3 X 30ml) and the washings retained. The water extracts were analysed for surface residues by mixing 1 ml with 8 ml of Hydroluma cocktail and counting. The washed samples were air-dried overnight, homogenized in a Waring blender and Soxhlet extracted with methanol (150 ml) for 4 hours. The extract was concentrated to 10 ml in a rotary evaporator before adding 1 ml to 8 ml of scintillator for counting. The water phases were partitioned with n-hexane (3 X 30 ml) and the combined organic layer concentrated to 10 ml before determination of metabolites by TLC and GC.

### 2.4 Analytical Methods

One gram samples of the extracted grain were combusted in a Packard 306 oxidizer and the  $^{14}\text{CO}_2$  produced trapped in Carbosorb®, mixed with Permaflour® solvent and counted for determination of bound residues.



Precoated silica gel plates were used to identify some the metabolites in both the water extracts (surface residues) and the methanol extracts. Development was done in paper lined standard glass tanks and the solvent system was n-hexane+ ethyl acetate (60+ 40 by volume) and the metabolites were viewed at 254 nm. TLC scanning was used to confirm the presence of  $^{14}\text{C}$ -malathion residues.

The analytical method followed for LC, GC, HPLC and GC-MS was a modification of that reported by Cotham and co-workers (Rengasamy, 1989). The methanol extract was evaporated to dryness and redissolved in ethylacetate (5 ml) and cleaned up on a Florisil column by elution with 15% ethylacetate in isooctane (75 ml). The eluate was concentrated and then blown down to 2 ml with nitrogen. A sample of eluate (1  $\mu\text{l}$ ) was injected into the GC for analysis with the following conditions: temp- 80°C for 1 min, then a 20°C/min upto 260°C, hold for 20 min; detector- FID at 300°C; injector temp- 250°C; carrier gas- nitrogen at 1.5 ml/min. For recovery, 10 ml of 200 mg/l of standard malathion and malaoxon in isooctane were added to the top of the Florisil column and eluted with 15% ethylacetate in isooctane (75 ml) and concentrated as described above before injection into the GC. By comparing their peak areas with those of known standard peaks obtained by direct injection, a recovery of about 77% was achieved. All the methanol extracts were cleaned up prior to GC analysis while the n-hexane extracts were injected directly into the GC. Identification was by co-chromatography with known standards and quantitation by comparing peak areas with those of known calibrated standard peak areas using an automatic integrator.

The metabolite identification was further achieved by a Hewlett Packard GC-MS with EI analysis, Helium gas carrier at a flow rate of 1.5 ml/min at 70 eV and similar GC conditions, and by a Hewlett Packard HPLC with an ODS column and the following conditions: 70% acetonitrile/water; flow rate 1.2 ml/min; injection volume 10  $\mu\text{l}$  with all samples redissolved in methanol and co-chromatographed with malathion, malaoxon,

isomalathion and malathion monocarboxylic acids at 220-215 nm and 220-280 nm respectively.

### 3. RESULTS

Tables 6.1, 6.2, 6.3 and 6.4 show the dissipation of malathion residues from stored grains. By combining all these data, plots of % dissipation against storage period were obtained as shown by Figs. 6.1, 6.2, 6.3, and 6.4. The dissipation curves approximate first order kinetics with a rapid fast phase of disappearance followed by a slower second phase. By linear regression analysis (Appendices Figs. 6.5, 6.6, 6.7 and 6.8), the first order log plots ( $2.303 \log[\% \text{ dissipation}]$  against time) gave the following dissipation constants and half-lives given in Table 7.

Table 6.1

Malathion residues recovered in maize stored in the open basket

| Storage Period<br>(weeks) | Ext. (surface)<br>residues |      | Int.(extractable)<br>residues |      | Bound residues     |     | Total residues     |      |
|---------------------------|----------------------------|------|-------------------------------|------|--------------------|-----|--------------------|------|
|                           | mgkg <sup>-1</sup>         | %    | mgkg <sup>-1</sup>            | %    | mgkg <sup>-1</sup> | %   | mgkg <sup>-1</sup> | %    |
| 0                         | 4.92±0.14                  | 64.5 | 2.61±0.04                     | 34.2 | 0.10±0.004         | 1.3 | 7.62               | 100  |
| 2                         | 2.61±0.07                  | 34.2 | 2.00±0.06                     | 26.3 | 0.11±0.002         | 1.4 | 4.72               | 61.9 |
| 6                         | 2.43±0.11                  | 33.8 | 1.76±0.02                     | 23.1 | 0.19±0.014         | 2.5 | 4.39               | 57.5 |
| 10                        | 1.64±0.03                  | 21.5 | 1.88±0.08                     | 24.7 | 0.19±0.015         | 2.5 | 3.72               | 48.7 |
| 15                        | 1.58±0.03                  | 20.7 | 2.98±0.12                     | 39.0 | 0.20±0.005         | 2.6 | 4.76               | 62.3 |
| 19                        | 1.46±0.07                  | 19.1 | 1.58±0.04                     | 20.7 | 0.33±0.008         | 4.3 | 3.37               | 44.1 |
| 27                        | 1.15±0.06                  | 15.1 | 1.94±0.07                     | 25.5 | 0.43±0.017         | 5.6 | 3.52               | 46.2 |
| 39                        | 0.67±0.03                  | 8.8  | 1.52±0.10                     | 19.9 | 0.62±0.030         | 8.1 | 2.81               | 36.9 |

Table 6.2

Malathion residues recovered in maize stored in closed wooden box.

| Storage period<br>(weeks) | Ext. (surface)<br>residues |      | Int.(extractable)<br>residues |      | Bound residues     |     | Total residues     |      |
|---------------------------|----------------------------|------|-------------------------------|------|--------------------|-----|--------------------|------|
|                           | mgkg <sup>-1</sup>         | %    | mgkg <sup>-1</sup>            | %    | mgkg <sup>-1</sup> | %   | mgkg <sup>-1</sup> | %    |
| 0                         | 4.62±0.39                  | 61.9 | 2.79±0.39                     | 37.5 | 0.01±0.002         | 0.7 | 7.46               | 100  |
| 2                         | 2.43±0.02                  | 32.6 | 2.07±0.33                     | 27.7 | 0.02±0.003         | 1.8 | 4.63               | 62.1 |
| 6                         | 2.25±0.02                  | 30.1 | 1.58±0.08                     | 21.2 | 0.29±0.004         | 3.8 | 4.11               | 55.1 |
| 10                        | 1.64±0.02                  | 22.0 | 2.19±0.02                     | 29.3 | 0.21±0.003         | 2.9 | 4.04               | 54.2 |
| 15                        | 1.94±0.02                  | 26.1 | 3.04±0.35                     | 40.7 | 0.16±0.007         | 2.1 | 5.14               | 68.9 |
| 19                        | 1.40±0.01                  | 18.7 | 1.64±0.05                     | 22.0 | 0.18±0.009         | 2.4 | 3.21               | 43.1 |
| 27                        | 1.21±0.03                  | 16.3 | 1.70±0.10                     | 22.8 | 0.50±0.052         | 6.7 | 3.41               | 45.8 |
| 39                        | 1.09±0.04                  | 14.7 | 2.31±0.13                     | 30.9 | 0.48±0.051         | 6.4 | 3.88               | 52.0 |

Table 6.3

Malathion residues recovered in beans stored in the open basket

| Storage period<br>(weeks) | Ext.(surface)<br>residues |      | Int.(extractable)<br>residues |      | Bound residues     |      | Total residues     |      |
|---------------------------|---------------------------|------|-------------------------------|------|--------------------|------|--------------------|------|
|                           | mgkg <sup>-1</sup>        | %    | mgkg <sup>-1</sup>            | %    | mgkg <sup>-1</sup> | %    | mgkg <sup>-1</sup> | %    |
| 0                         | 6.64±0.07                 | 89.9 | 0.69±0.037                    | 9.0  | 0.09±0.004         | 1.2  | 7.64               | 100  |
| 2                         | 4.87±0.04                 | 63.6 | 1.05±0.045                    | 13.9 | 0.18±0.009         | 2.3  | 6.11               | 80.0 |
| 6                         | 4.40±0.07                 | 58.0 | 0.80±0.04                     | 10.7 | 0.27±0.034         | 3.5  | 5.49               | 72.2 |
| 10                        | 3.53±0.07                 | 46.4 | 1.85±0.025                    | 24.3 | 0.27±0.015         | 3.5  | 5.67               | 74.2 |
| 15                        | 3.53±0.03                 | 46.4 | 0.91±0.073                    | 11.9 | 0.53±0.036         | 7.0  | 4.98               | 65.2 |
| 19                        | 3.09±0.10                 | 40.6 | 0.80±0.031                    | 10.4 | 0.53±0.091         | 7.0  | 4.40               | 58.0 |
| 27                        | 2.00±0.01                 | 26.1 | 1.20±0.02                     | 15.9 | 1.17±0.01          | 15.4 | 4.36               | 57.4 |
| 39                        | 1.31±0.02                 | 17.4 | 0.76±0.015                    | 9.9  | 1.13±0.01          | 14.8 | 3.2                | 42.0 |
| 51                        |                           |      |                               |      | 1.97±0.10          | 25.5 |                    |      |

Table 6.4

Malathion residues recovered in beans stored in closed wooden box.

| Storage period<br>(weeks) | Ext. (surface)<br>residues |      | Int.(extractable)<br>residues |      | Bound residues     |      | Total residues     |      |
|---------------------------|----------------------------|------|-------------------------------|------|--------------------|------|--------------------|------|
|                           | mgkg <sup>-1</sup>         | %    | mgkg <sup>-1</sup>            | %    | mgkg <sup>-1</sup> | %    | mgkg <sup>-1</sup> | %    |
| 0                         | 5.09±0.21                  | 82.1 | 0.98±0.11                     | 16.1 | 0.11±0.06          | 1.8  | 6.18               | 100  |
| 2                         | 3.31±0.06                  | 53.6 | 0.91±0.08                     | 14.6 | 0.25±0.01          | 3.9  | 4.47               | 72.1 |
| 6                         | 3.09±0.08                  | 50.0 | 0.98±0.04                     | 16.1 | 0.36±0.01          | 5.7  | 4.44               | 71.8 |
| 10                        | 2.44±0.09                  | 39.2 | 0.55±0.03                     | 8.6  | 0.36±0.01          | 5.7  | 3.31               | 53.6 |
| 15                        | 2.44±0.02                  | 39.2 | 0.80±0.07                     | 11.4 | 1.13±0.09          | 18.2 | 4.25               | 68.9 |
| 19                        | 2.00±0.07                  | 32.1 | 0.55±0.04                     | 8.6  | 1.31±0.04          | 21.4 | 3.85               | 62.5 |
| 27                        | 1.78±0.01                  | 28.6 | 0.84±0.05                     | 13.6 | 0.62±0.01          | 10.0 | 3.24               | 52.1 |
| 39                        | 1.31±0.02                  | 21.0 | 0.65±0.07                     | 10.7 | 0.95±0.07          | 15.0 | 1.71               | 46.7 |

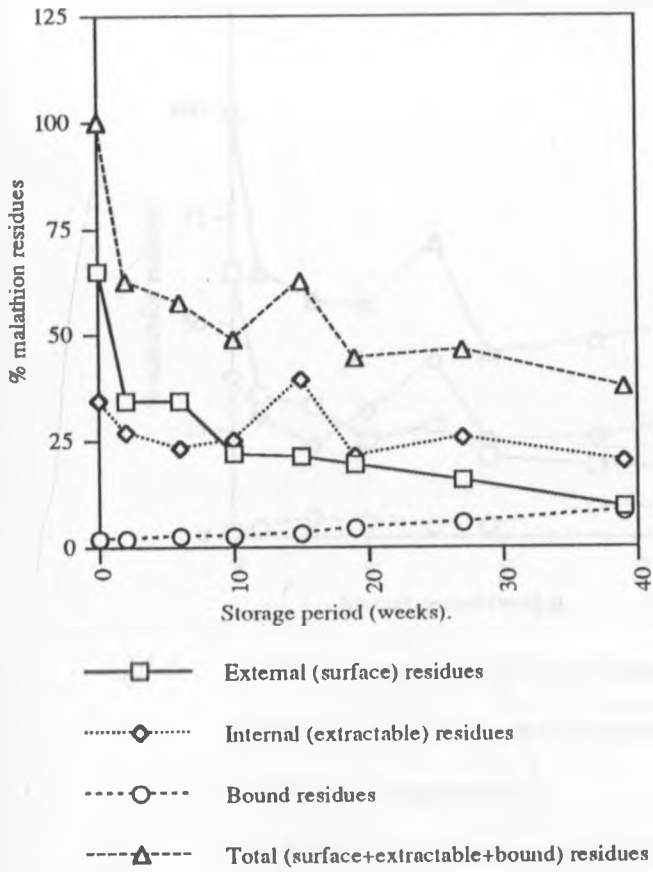


Fig. 6.1 The persistence of malathion residues (as % of total) in maize stored in the open basket.

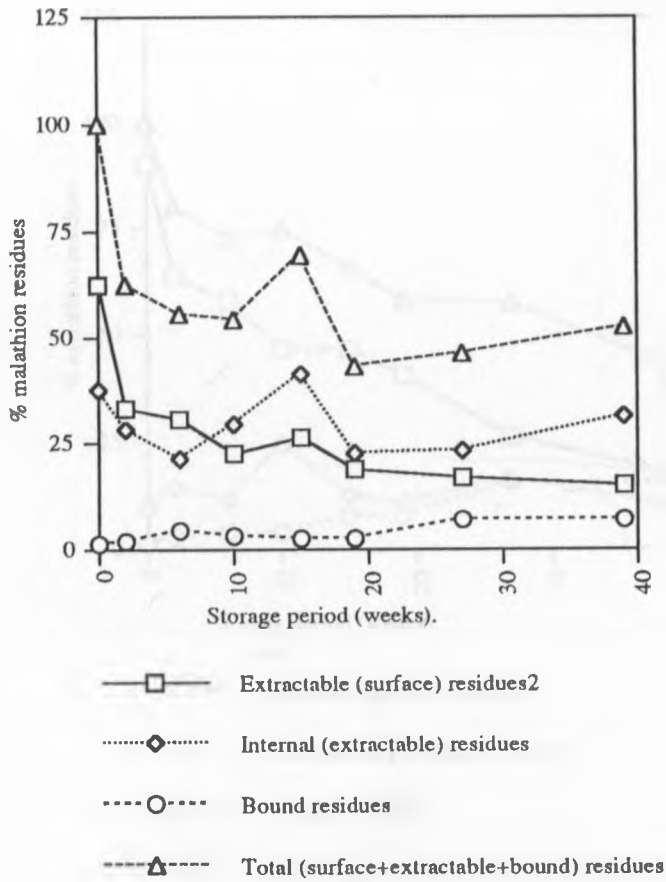


Fig. 6.2 The persistence of malathion residues (as % of total) in maize grains stored in the closed wooden box.



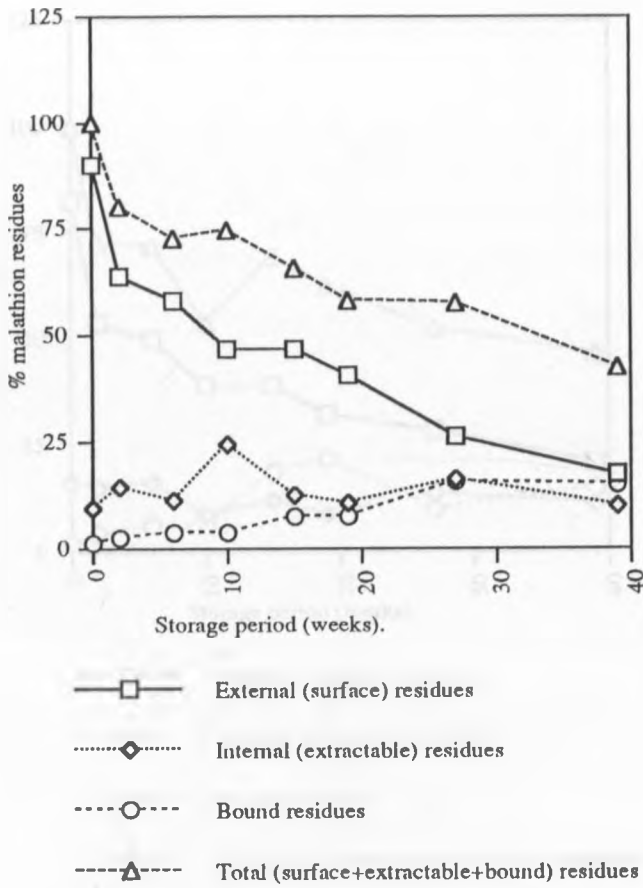


Fig. 6.3. The persistence of malathion residues (as % of total) in beans stored in the open basket.

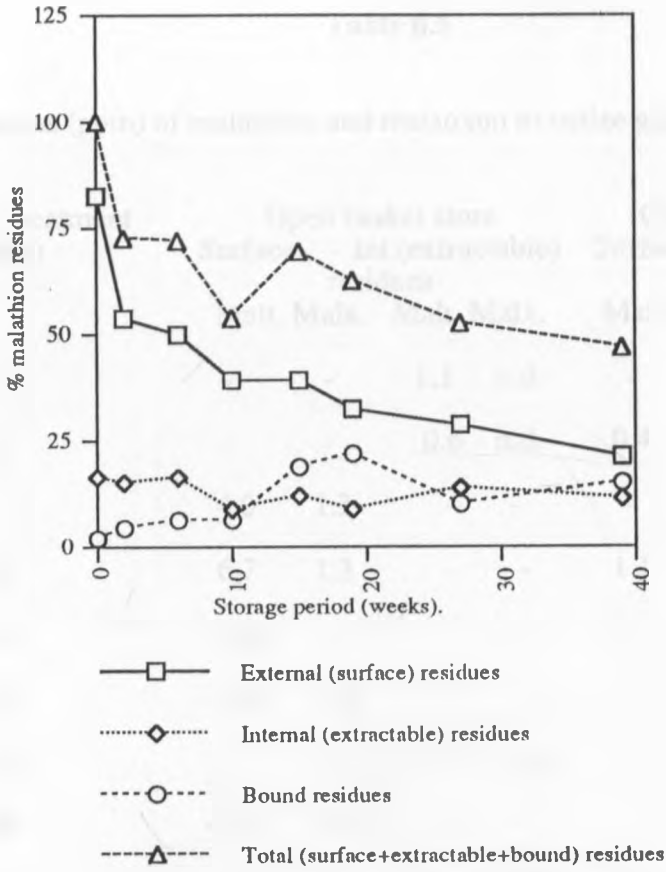


Fig. 6.4 The persistence of malathion residues (as % of total) in beans stored in the wooden box.

Table 6.5

Concentrations (ppm) of malathion and malaoxon in maize grains by GC analysis

| Period after treatment<br>(weeks) | Open basket store |       |                               |       | Closed wooden box |       |                               |       |
|-----------------------------------|-------------------|-------|-------------------------------|-------|-------------------|-------|-------------------------------|-------|
|                                   | Surface           |       | Int.(extractable)<br>residues |       | Surface           |       | Int.(extractable)<br>residues |       |
|                                   | Malt.             | Malx. | Malt.                         | Malx. | Malt.             | Malx. | Malt.                         | Malx. |
| 0                                 | -                 | -     | 1.1                           | n.d.  | -                 | -     | -                             | -     |
| 2                                 | -                 | -     | 0.6                           | n.d.  | 0.4               | n.d.  | 0.6                           | 1.1   |
| 6                                 | 4.0               | 1.3   | -                             | -     | -                 | -     | -                             | -     |
| 10                                | 6.7               | 1.3   | -                             | -     | 1.1               | n.d.  | 0.1                           | 0.9   |
| 15                                | 4.6               | -     | -                             | -     | -                 | -     | -                             | -     |
| 19                                | 3.4               | 1.2   | -                             | -     | -                 | -     | -                             | -     |
| 27                                | -                 | -     | 0.9                           | 0.11  | -                 | -     | 0.1                           | 0.9   |
| 39                                | <0.01             | <0.01 | -                             | -     | -                 | -     | <0.01                         | <0.01 |
| 51                                | -                 | -     | n.d.                          | 0.02  | 0.6               | 2.5   | <0.01                         | <0.01 |

Note: Malt.= malathion

Malx= malaoxon

n.d = not detected.

Table 6.6

Concentrations (ppm) of malathion and malaoxon in beans by GC analysis

| Period after treatment<br>(weeks) | Open basket store |       |                               |       | Closed wooden box |       |                               |       |
|-----------------------------------|-------------------|-------|-------------------------------|-------|-------------------|-------|-------------------------------|-------|
|                                   | Surface           |       | Int.(extractable)<br>residues |       | Surface           |       | Int.(extractable)<br>residues |       |
|                                   | Malt.             | Malx. | Malt.                         | Malx. | Malt.             | Malx. | Malt.                         | Malx. |
| 0                                 | 4.3               | n.d.  | -                             | -     | -                 | -     | -                             | -     |
| 2                                 | 1.2               | n.d.  | -                             | -     | -                 | -     | -                             | -     |
| 6                                 | 2.4               | n.d.  | -                             | -     | 1.5               | n.d.  | -                             | -     |
| 10                                | -                 | -     | -                             | -     | -                 | -     | 0.6                           | 0.1   |
| 15                                | -                 | -     | -                             | -     | -                 | -     | -                             | -     |
| 19                                | 1.1               | 0.5   | 3.9                           | 0.5   | -                 | -     | 1.6                           | 0.3   |
| 27                                | 0.6               | 0.5   | 1.9                           | 0.6   | <0.01             | 0.6   | 0.1                           | 0.2   |
| 39                                | -                 | -     | 0.3                           | <0.01 | -                 | -     | <0.01                         | 0.3   |
| 51                                | -                 | -     | 0.03                          | <0.01 | -                 | -     | -                             | -     |

Table 6.7

The dissipation constants and the half-life values of malathion in the grains.

| Material stored            | Dissipation constant<br>k (mg/kg/day) | Half-life<br>days |
|----------------------------|---------------------------------------|-------------------|
| Maize grains (open basket) | -0.025                                | 194               |
| Maize grains (wooden box)  | -0.019                                | 261               |
| Beans (open basket)        | -0.019                                | 259               |
| Beans (wooden box)         | -0.012                                | 405               |

#### 4. DISCUSSION

The dissipation results showed that high percentage levels of initial residues were found in the methanol extracts after only six hours of storage. This was about 34% in maize stored in the open basket, 37% in maize in the closed wooden box, 9% in beans in the open basket and 16% in beans stored in the closed wooden box. This implies that washing the treated seeds with water did not remove all the pesticide residues from the surface. More residues adhered onto the surface of maize than onto beans. These results re-emphasize the importance of this study with regard to the danger of toxic metabolites that may be consumed with the grains/beans even after washing.

The percentage of residues on the surface decreased gradually with storage time. This decrease was faster in maize and beans stored in the open basket. This could be the result of more exposure to air and subsequent volatilization/evaporation from the stored material. The decrease was from 64% after six hours to 8.8% after 39 weeks for maize grains stored in the open basket, 61% to 14.6% for maize in closed wooden box, 87% to 17% for beans stored in the open basket and 82% to 20.9% for beans in the closed

wooden box. The decrease was also found to be slightly higher in maize than beans irrespective of the method of storage, possibly as a result of higher penetration and less binding of the residues into maize grains with storage time. The dissipation trend from the methanol extracts showed some penetration of malathion residues into both maize grains and beans particularly during the first 15 weeks and the first 10 weeks, respectively. The rate of penetration was found to be a little higher in maize than in beans suggesting that the penetration of this pesticide was dependent on the type of matrix.

The percentage bound residue levels increased gradually with storage time in all stored materials irrespective of the storage method. There appeared to be more biochemical binding of the pesticide in beans. The bound residue levels were higher in maize/beans in the closed wooden box compared to those in open basket. After 39 weeks, the bound residue levels in beans were significantly high i.e. 14.8% in beans stored in open basket and 15% in beans stored in the closed wooden box model.

The total residues (surface+internal+bound) showed dissipation following a biphasic pattern with rapid dissipation during the first 10 weeks and a more gradual one from the tenth to the fifty-first week. In terms of total residues, the dissipation was faster from maize than from beans irrespective of the method of storage although dissipation was a little faster in the open basket. The dissipation curves obtained for both maize grains and beans stored under the two different storage models all approximate first order kinetics with the dissipation constants ( $k$ ) and half-lives summarised in Table 6.7 above.

Comparing the  $R_f$  values of samples spotted with those of the standards, malathion and malaoxon were identified in both the hexane (surface) and methanol extracts. TLC scanning confirmed the presence of  $^{14}\text{C}$ -malathion in the same samples. Due to low concentrations, malaoxon and other possible metabolites could not be identified by TLC scanning. Malathion and malaoxon metabolites were easily identified

by GC in both the n-hexane and the methanol extracts showing that these residues penetrated into the grains during storage (see Tables 6.5 and 6.6).

The HPLC results indicated the presence of malathion and malaoxon in the methanol extracts of both maize and beans samples. This was achieved by filtering the methanol extracts and then directly injecting 10  $\mu$ l of the filtrate into the HPLC. Malathion portion eluting from the column during analysis was collected with the help of an HPLC radiochromatogram fitted with an automatic fraction collector and its identity confirmed by GC-MS. Malathion and malaoxon were detected using the 210-215 nm UV range. The samples were concentrated and resolved in methanol after Florisil clean-up. Isomalathion and malathion monocarboxylic acid metabolites were detected at 222.4 nm and 280.6 nm, respectively in both beans and maize grains sampled after 19 weeks of storage. However no separation of the  $\alpha$ - and the  $\beta$ -monoacids was achieved. The GC-MS results confirmed malathion and malaoxon in the n-hexane (surface) and methanol (internal) extracts samples of both beans and maize grains.

## 5. CONCLUSIONS

Malathion persists longer in beans than in maize irrespective of the method of storage. The dissipation is dependent on the type of storage and is more rapid in the open basket than in the closed wooden box. Over 50% of the pesticide had dissipated from both maize grains and beans after one year of storage. There was penetration of malathion into the seeds during storage and the formation of bound residues also increased during storage. The results show that there is still a considerable level of malathion residues in all the grains even after one year of storage. Malathion and malaoxon metabolites were identified by GC and HPLC, and confirmed by GC-MS in the surface and internal extracts of both maize grains and beans. Isomalathion and malathion monocarboxylic

acid metabolites were also detected by HPLC in the internal extracts of both grain species sampled after 19 weeks of storage.

### ACKNOWLEDGEMENT

The authors wish to thank the German Academic Exchange Service (DAAD) and the International Atomic Energy Agency (IAEA) for the financial support.

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## GENERAL DISCUSSION

The long term effects of pesticide residues in the environment will depend upon the persistence of the parent compound and its metabolites. Persistence refers to the residence time period in which the pesticide and its residues remain in the environment e.g. soil, water, plants, grains etc. The concept of half-life is widely used to express the persistence of pesticides in the environment and refers to the time required for 50% of the pesticide and its residues to disappear from a particular medium. It should be distinguished from the half-life expression used in physical reaction kinetics to show the extent of a reaction involving one or more reactants. The loss of most pesticides in soil follows first order kinetics where the  $\log[\text{residue concentration}]$  or  $\log[\% \text{ dissipation}]$  is directly proportional to the time of residence in the soil.

In general, the most persistent pesticides are the chlorinated hydrocarbon insecticides such as DDT and the cyclodienes e.g. dieldrin. Most organophosphate insecticides rarely persist for a long period of time in soil. Parathion, for example has been shown to have a short residual time in the soil and to lose its insecticidal activity within 2-4 weeks. Carbamate insecticides, however, are slightly to moderately persistent in soils and their residues have been found to disappear completely in 1-4 months. Systemic methyl carbamates such as carbofuran have longer half-lives in soil, ranging between 18-378 days in some countries. In the present study the same order of decreasing persistence was found i.e. DDE > DDT > carbofuran in non-flooded soil > carbofuran in flooded soil > parathion.

The persistence of a pesticide in soil is dependent upon a number of conditions e.g. soil type (clay, loam, silt), organic matter content, pH, microorganisms, liquid flow through the soil, climatic factors (wind, solar radiation, rainfall, soil and air temperatures), application methods and the chemical nature of the pesticide. The loss may

be a result of a combination of mechanisms such as chemical alteration at clay and organic surfaces, volatilization, photodecomposition, leaching and/or microbial degradation. These degradative pathways influenced the dissipation of the pesticides in this study and are briefly discussed in the following paragraphs.

The rate of loss of pesticides by volatilization from the soil is related to the vapour pressure of the pesticide within the soil and its rate of movement to the evaporating surface. The vapour pressure of a pesticide depends upon the air and soil temperature and its adsorption to the soil. Certain pesticides may be distributed throughout the soil profile by vapour phase movement and eventually lost via surface evaporation. The temperature has a direct influence on volatilization by affecting the vapour pressure of the pesticide and the physical/chemical properties of the soil. As the temperature rises, the rate of volatilization also increases. Water also plays a role in pesticide loss by volatilization. Pesticides volatilize much more rapidly from wet soils than from dry soils. As the water evaporates from the soil surface, water-pesticide solution moves upwards towards the evaporating surface by capillary action thereby enhancing pesticide loss by water-evaporation. There is an inverse relation of pesticide volatilization and soil organic matter content. The loss of DDT residues in soil, for example, has been found to be very low when the organic matter content of the soil is high e.g. forest soils. It has also been found that volatilization is inversely related to the surface area of the soil i.e. its texture (Guenzi and Beard, 1970).

Photodecomposition is another mechanism related to volatilization which is responsible for the loss of pesticides and contributed to the dissipation of the insecticides in this study. Solar radiation, particularly in the UV 290-450 nm range with sufficient energy to bring about chemical changes, is responsible for many chemical transformations of pesticides in the environment. However, this mechanism is restricted to the surface of the soil and depends on the intensity, the duration of exposure, the type

of pesticide and the presence of air. In this study this factor may have played a role in the degradation of DDT to DDE in the initial days of the experiment when the pesticide was still on top of the soil surface.

Microbial degradation is another important mechanism affecting the fate and behaviour of many pesticides in soil. Microbial activity is influenced by soil pH, residence time in the soil, soil temperature, moisture content and soil type. Studies showed that DDT incubated with activated sludge under anaerobic conditions was rapidly metabolized to DDD and DDMU. However, under aerobic conditions the incubation of DDT in the same soil for six months resulted only in 25% alteration of DDT to DDE and DDD (Guenzi and Beard, 1968). DDT conversion to DDD has been shown to be microbial and highly sensitive to O<sub>2</sub> (2% O<sub>2</sub> can inhibit this reaction) (Burge, 1971). This anaerobic breakdown of DDT is accelerated by organic matter and high soil pH.

Microorganisms are also involved in the degradation of organophosphates through hydrolysis and oxidation. Two pathways have been postulated for microbial degradation of parathion in soil i.e. the hydrolysis to p-nitrophenol and the reduction to aminoparathion. In the presence of oxygen, the aminoparathion is then hydrolysed to p-aminophenol. Paraoxon (the oxygen analogue of parathion) is formed in small quantities and is completely hydrolysed to p-nitrophenol. These degradation products were all found in the soil extracts in the present study.

Carbofuran metabolism in soil is a complex process involving several microorganisms. The main route of degradation is through hydrolysis at the carbamate linkage where the carbamoyl moiety is degraded to CO<sub>2</sub> and monomethyl amine leaving carbofuran phenol which then binds to the soil (Lichtenstein and Liang, 1987).

*Actinomycetes* have been found to be the most active bacteria in this process. In non-flooded non-sterile soils, there is more rapid mineralization of <sup>14</sup>C-carbonyl carbofuran to <sup>14</sup>CO<sub>2</sub> which can be quantified. However, in flooded soils (less O<sub>2</sub> and a lot of water

available for hydrolysis) hydrolysis of carbofuran is more rapid. This may explain the different rates of dissipation of carbofuran in flooded and non-flooded soils in this experiment.

On the first day after the application of DDT to the soil surface, a lot of rainfall was recorded. This helped in the incorporation of the pesticide into the inner soil layer and so partially prevented a very rapid loss by volatilization. DDT therefore unexpectedly persisted more in the soil during the first nine days. However, in the entire period of the experiment, DDT disappeared most rapidly during the first one and a half months particularly between day 23 and day 37 after pesticide application. This concurs with the prevailing high temperatures experienced between January 23 and March 4. During the 37 days after treatment, there was a great deal of rainfall and the dissipation rate was slower. This shows that degradation due to chemical and microbial activities in the soil i.e. the formation of DDE and DDD metabolites greatly influenced the rate of disappearance of DDT from the soil during this period. The rate of formation of bound residues also increased at this time. The increase in the amount of bound residues therefore lowered the total (extractable+bound) dissipation rate.

The rate of disappearance of DDE from the same soil was comparatively slower than for DDT. During the first month, there was no rainfall and both the air and soil temperatures and the relative humidity were high. These factors accelerated the rapid loss of DDE from the soil by volatilization. The formation of bound residues was higher than that of DDT and this slowed down the overall dissipation of the parent DDE from the soil, resulting in its increased persistence. DDE in the soil extracts was not changed chemically during the experiments. These findings suggest that the formation of bound residues in soils treated with DDT first goes through the formation of DDE by chemical catalysis followed by the binding of DDE to the soil matrix. In the DDE treated soil, the initial bound residues on zero day constituted 10.4% of the total recovered radioactivity.

This is rather high due to the fact that the soil samples were air-dried for two days in the laboratory prior to analysis. A lot of rainfall was experienced between October and January which led to a reduction in the loss of DDE by volatilization. However, the rate of formation of bound residues increased. Both of these factors resulted in a reduced overall rate of disappearance of this chemical in the soil. DDE was not metabolized during the experiments, showing its resistance to chemical and microbial metabolism. Since most of the DDE was located in the top 13 cm of the soil column throughout the experiment period and because it is not easily dissolved in water, DDE poses no danger as a result of underground leaching.

The disappearance of parathion from the same soil was the most rapid of all the chemicals studied. In the first 3 days when the pesticide was still on the top of the soil column, the dissipation was very rapid and was mainly due to volatilization. However, as the pesticide moved into the soil as a result of rainfall, microbial activity and the formation of bound residues increased and these then played a major role in the rate of disappearance of the pesticide. This mainly occurred between 3 and 42 days after application of the pesticide to the soil. During this period the number of metabolites in the soil extracts also increased. After 56 days, the air and soil temperatures and the hours of sunshine increased, accelerating the rate of dissipation by volatilization while the bound residue levels began to decline.

The dissipation of carbofuran from soil was a more complex process than those discussed above. The recovery of carbofuran from the soil was low because the amount of residues lost in the water during the air-drying process was not taken into account. The samples had to be transported to Nairobi overnight and then air-dried in the laboratory for 3 days prior to analysis. This was particularly true for the flooded soil samples. Another source of loss was during Soxhlet extraction in which the volatile dichloromethane was used as the extraction solvent. However, the overall dissipation was more rapid in flooded

soils than in non-flooded soils. In non-flooded soils, the accumulation of bound residues seemed to be a major factor contributing to the relatively slower rate of overall disappearance of carbofuran. The amount of rainfall and the % relative humidity was high between 0-18 days after pesticide application to the soils. During this period, the rate of metabolism by microorganisms was high. Unlike the organochlorines, the disappearance and degradation of carbofuran in soil is complex and is influenced by several factors including adsorption/desorption, leaching (carbofuran is relatively water soluble), formation of metabolites and bound residues which are in turn affected by climatic conditions and soil properties. The data from the adsorption of carbofuran to both the Ahero and Chiromo soils fitted well into the Freundlich adsorption isotherm equation which relates the amount of test substance adsorbed to the concentration of the test substance in solution at equilibrium:

$$\text{i.e. } X = KC^{1/n}$$

where X=amount of pesticide adsorbed per gram of soil ( $\mu\text{g/g}$ )

C=the equilibrium concentration of the pesticide per mL of 0.01 M  $\text{CaCl}_2$  solution ( $\mu\text{g/mL}$ ).

and  $1/n$  and  $K_F$  are the Freundlich constants.

The results showed that carbofuran adsorbed quite rapidly to Ahero soil, reaching 29.5% (at 5 ppm) after six hours. However, due to its high water solubility the bound carbofuran was easily washed down the soil column. These laboratory findings were consistent with the results obtained from the field experiment.

Though malathion is considered non-toxic to humans and is recommended for use in stored grains, it forms toxic metabolites which penetrate into the grains during storage and therefore cannot be removed by washing the grains with water. Some of the toxic metabolites also formed at elevated temperatures e.g. the isomerization of malathion to give isomalathion. The persistence of malathion and the formation of these metabolites in

stored grains depend upon the type of grains, storage conditions (temperature, humidity), type of storage structure and the type and purity of the formulation used. The use of malathion should also be evaluated in terms of its residual lifetime on the treated grains because this determines its effectiveness against pests. In the present experiments, some insect larvae attacked the treated grains stored in the open basket ten weeks after pesticide application.



## CONCLUSIONS AND RECOMMENDATIONS

In addition to the conclusions already made from individual experiments described in the previous sections, it can be generally stated that:

Radioisotope techniques were successfully used in pesticide metabolism studies in soil and stored grains . These techniques were found to be fast, sensitive and accurate.

The fate and behaviour of the insecticides that were studied were found to be influenced by climatic conditions. The insecticides were found to be less persistent in the Kenyan environment than that reported in the temperate regions. The half-lives and fate of these chemicals in the Kenyan environment were similar to those reported in studies conducted in other tropical and sub-tropical countries. Though these pesticides appeared to be less persistent, they formed metabolites some of which are more persistent as well as being more toxic and should be further investigated.

For future studies, laboratory investigations should be carried out to obtain more information on the individual parameters that are responsible for the rapid degradation of these pesticides under tropical conditions. These include the effects of temperature, moisture, pH, and solar (UV) radiation on the metabolism of these pesticides. The contribution of each factor should be investigated in terms of their influence on the rate of volatilization, mineralization and chemical changes under specific conditions. The analytical procedures followed in the clean-up and identification of some of the toxic metabolites whose identification was not conclusive in this study should be modified.

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## APPENDICES

Table 1.

## PESTICIDE IMPORTS IN KENYA ( in tonnes): 1986-1990.

| Year | Insecticides<br>& Acaricides | Herbicides | Fungicides | Others | Total |
|------|------------------------------|------------|------------|--------|-------|
| 1986 | 1076                         | 1129       | 6584       | 808    | 9597  |
| 1987 | 1206                         | 1311       | 7157       | 697    | 10371 |
| 1988 | 1089                         | 2108       | 4259       | 801    | 8257  |
| 1989 | 1571                         | 1148       | 4329       | 665    | 7711  |
| 1990 | 1572                         | 1134       | 1330       | 857    | 4893  |

Source: Pest Control Products Board of Kenya.

Table 2

## Importation of specific insecticides in Kenya: 1986-1992.

| Year | Malathion<br>(technical)<br>(tonnes) | Carbofuran<br>(technical)<br>(tonnes) | Furadan<br>350ST<br>(litres) | Carbosulfan<br>Marshal 250 EC<br>(litres) |
|------|--------------------------------------|---------------------------------------|------------------------------|---|
| 1986 | 20.0                                 | 8.0                                   | -                            | -   |
| 1987 | 15.0                                 | 30.0                                  | 2,000                        | -   |
| 1988 | 9.0                                  | 14.0                                  | -                            | -   |
| 1989 | 16.0                                 | 7.0                                   | 10,000                       | 20,000                                    |
| 1990 | 18.5                                 | 12.0                                  | 16,000                       | -   |
| 1991 | 13.0                                 | 10.0                                  | 21,000                       | -   |
| 1992 | 10.0                                 | 23                                    | 15,000                       | 20,000                                    |

Note: (a). These data are based on applications approved by Pest Control Products Board of Kenya.

- (b). According to the Pest Control Products Board of Kenya, there has been no importation of DDT since 1985.
- (c). Malathion (technical) is used locally for the formulation of 2% Malathion dust, Malathion 50% EC products etc.
- (d). Carbofuran (Furadan) (technical) is used for the preparation of 3G, 5G, and 10G granular formulations.
- (e). Furadan 350ST is used for dressing barley seeds.
- (f). Marshal 250 EC (carbosulfan) is used on cotton, coffee, maize, citrus etc.



Table 3.

The major uses of some of the insecticides in Kenya.

| Insecticide/ trade name                       | Mode of action                     | Major uses   |
|---|------------------------------------|--|
| 1) Carbosulfan<br>Marshal 250 EC              | Insecticide/nematicide             | control of maize stalk borer, coffee berry borer, cotton pests, control of aphids, thrips and lister scale.  |
| Marshal suscon<br>CRG granular                | Systemic<br>Insecticide/nematicide | control of soil pests e.g. termites, grubs, nematodes in coffee nurseries, citrus and macademia forest trees.  |
| Marshal 350 STD                               | Systemic<br>Insecticide/nematicide | seed dressing used in planting/sowing of beans, maize for the control of soil borne pests and early foliar pests e.g. maize leaf hopper (vector of maize streak virus)                   |
| 2). Carbofuran (furan)<br>Furadan 10G         | Systemic<br>Insecticide/nematicide | control of soil insects and nematodes as well as early foliar feeding insects on coffee, bananas, pineapples, pyrethrum nurseries and maize. Applied by mechanical granular applicators. |
| Furadan 5G                                    | Insecticide/nematicide             | used in rice, bananas, beans (as seed dressing), pyrethrum, vegetables, pineapples, maize, coffee. Applied manually.   |
| Furadan 3G                                    | Insecticide/nematicide             | treatment of wheat and barley seeds using seed treatment equipment. Restricted use.  |
| Malathion<br>2% technical<br>malathion powder | Insecticide                        | used mainly against storage pests and in horticultural crops.  |
| DDT   | Insecticide                        | public health control of mosquito larvae.  |

Table 4.

The physical/chemical properties of the insecticides studied.

| Parameter                       | DDT   | DDE  | parathion   | malathion   | carbofuran  |
|---------------------------------|---|--|---|---|---|
| MW (g/mol)                      | 354.51  | 318.03                                     | 291.26  | 330.36  | 221.26  |
| water solubility (mg/l) @20°C   | very low<br>1.7*10 <sup>-7</sup>                              | very low<br>-                              | 24<br>7.4   | 140<br>1.6*10 <sup>-4</sup>                                   | 700<br>2.6*10 <sup>-5</sup>                         |
| vapour pressure mbar @20°C      | -   | -  | 1.3681  | 1.4989  | -   |
| refractive index n <sub>D</sub> | 107-109°C   | 88-90°C                                    | 6.0-6.2°C   | 2.8-3.7°C   | 146-148°C   |
| mp range type/mode              | insecticide   | metabolite of DDT                          | insecticide   | insecticide/ acaricide  | insecticide/ acaricide/ nematocide                  |
| assay                           | HPLC C18-RP. acetone/nitrile/ water. (80/20) UV-230 GC/EC/FID | GC/EC/FID                                  | HPLC C18-RP. acetone/nitrile/ water. (67/33) UV 270 GC/EC/FID | HPLC C18-RP. acetone/nitrile/ water. (70/30) UV 230 GC/FID/NP | HPLC C18-RP. acetone/nitrile/ water. (33/67) UV 272 |
| solubility in organic solvents  | acetone/ hexane/ dichloromethane.                             | acetone/ hexane/ ethanol/ dichloromethane. | acetone/ chloroform/ diethyl ether                            | acetone/ methanol/ chloroform/ ethyl acetate.                 | acetone/ acetonitrile/ dichloromethane.             |

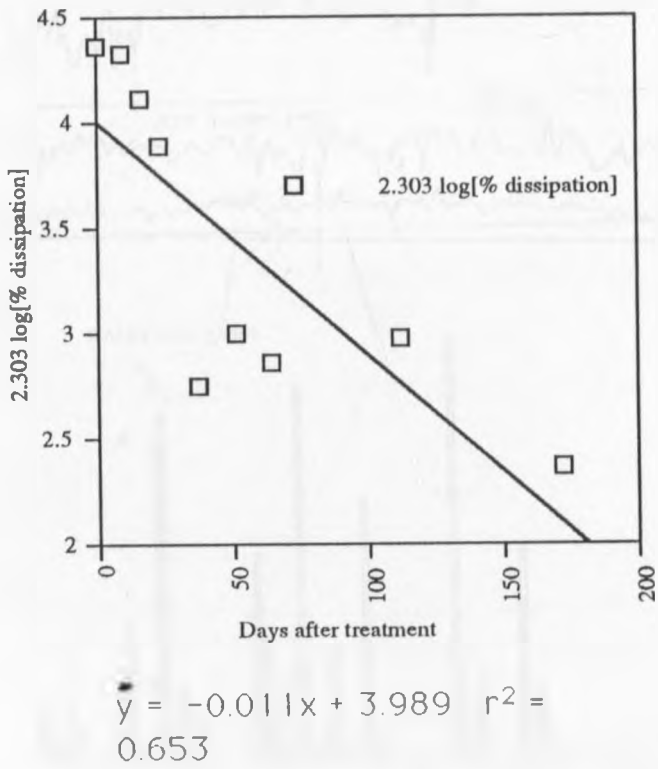
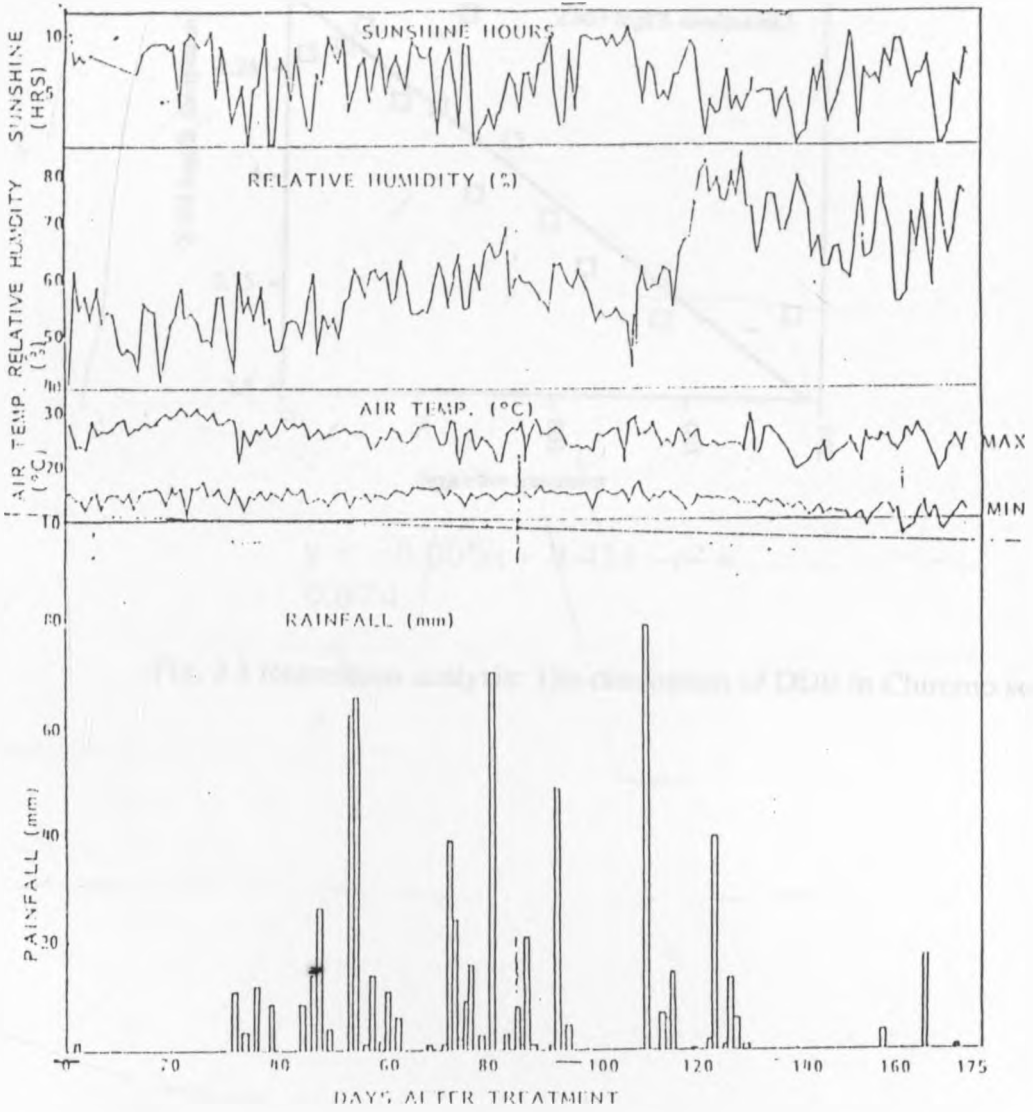


Fig. 1.3 Regression analysis: The dissipation of DDT in Kenyan soil.

Fig. 2 CHIROMO WEATHER DATA



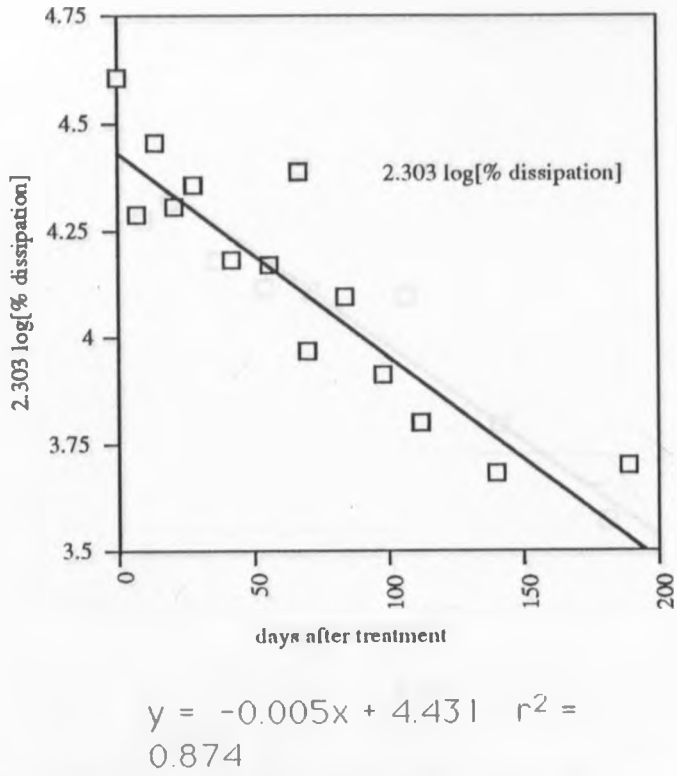


Fig. 2.3 Regression analysis: The dissipation of DDE in Chiromo soil.

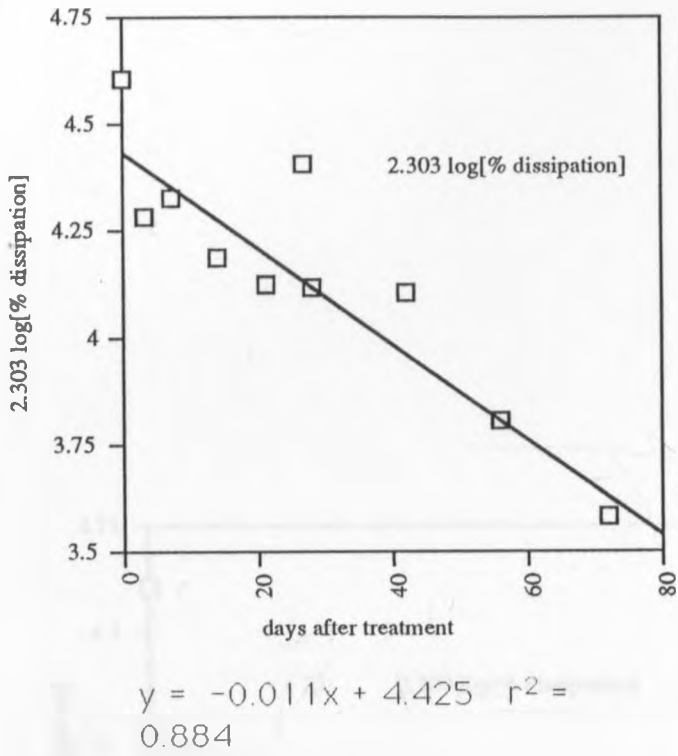
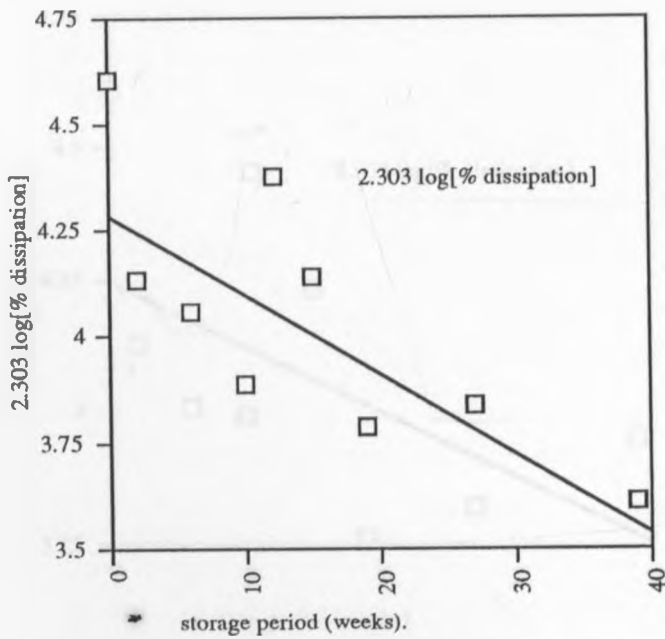
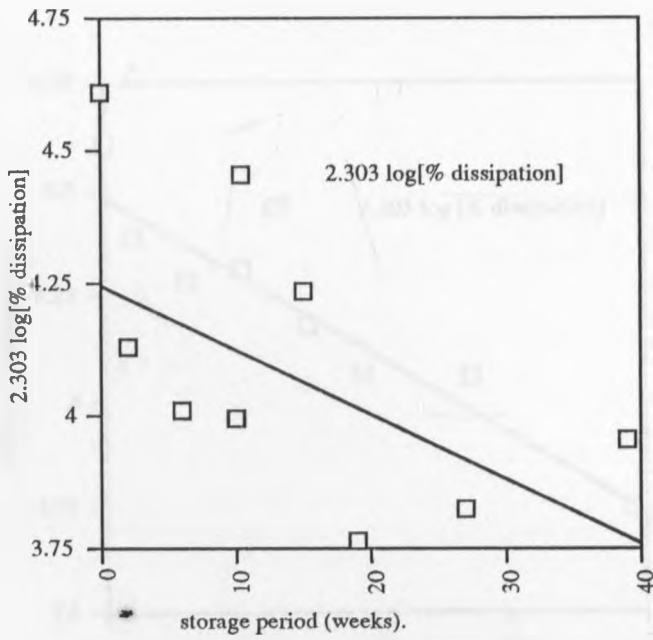


Fig. 3.3 Regression analysis: The dissipation of parathion in Chiromo soil.



$$y = -0.018x + 4.277 \quad r^2 = 0.658$$

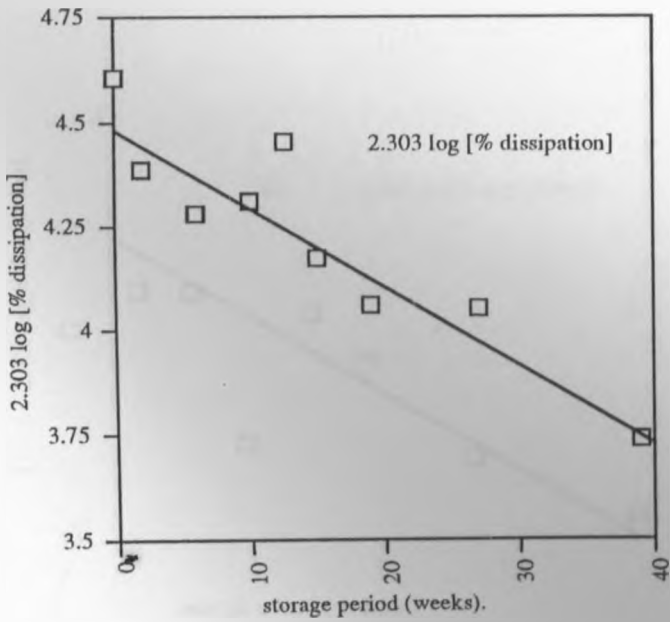
Fig. 6.5 Regression analysis: Malathion residues in maize (open basket).



$$y = -0.012x + 4.242 \quad r^2 = 0.363$$

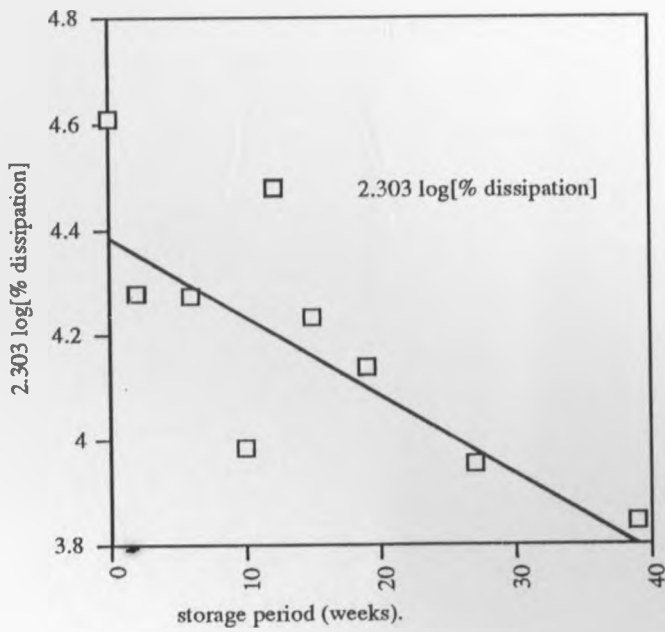
Fig. 6.6 Regression analysis: Malathion residues in maize (wooden box).





$$y = -0.019x + 4.477 \quad r^2 = 0.920$$

Fig. 6.7 Regression analysis: malathion residues in beans (open basket).



$$y = -0.015x + 4.385 \quad r^2 = 0.684$$

Fig. 6.8 Regression analysis: malathion residues in beans (wooden box).