EVALUATING FARMERS KNOWLEDGE IN PESTICIDE HANDLING AND DETERMINING PESTICIDE LEVELS IN MAIZE AFTER VARIOUS PROCESSING METHODS AND DIFFERENT STORAGE CONDITIONS.

BY

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DECLARATION

I declare that this thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

To all those in the world of science and otherwise who devote their energy, intellect and resources towards establishing better and safer crop production systems and healthier food for the society devoid of toxic chemical biocides.

ACKNOWLEDGEMENTS

Working on this thesis was such a remarkable and fulfilling experience for me. But before I was able to finish it, I had to endure all the hardships it entailed; hardships that I would not have endured without the help of others. I believe that in my own humble ways of thinking and acknowledging them in these pages would not be sufficient enough for all the sacrifices they have unselfishly done for me. Yet this is the way I can express my heartfelt gratitude to these generous individuals. I extend my deepest appreciation to: Prof. J. Murungi for her guidance and support in the making of this thesis from beginning until the end, and for accompanying me in the search for research-stations where to carry out the sample analysis. Dr. H. Nyambaka, my thesis and research advisor for his unending patience, considerations and guidance for the successful completion of this thesis as well as for his help in the laboratory. Kenyatta University laboratory technicians especially to Mr. Isaac Mwangi for his patience and unselfish help in handling and setting the GLC instrument. Special thanks also go to Mr. Maina the chief technician for providing me the chemicals and laboratory materials needed to make this study possible.

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LIST OF ABBREVIATIONS

ADI	Acceptable Daily Intake – This is the amount of chemical which can be consumed for a lifetime in the practical certainty that no harm will result.
AOAC	Association of Official Analytical Chemists (USA)
EPA	Environmental Protection Agency (USA)
ECD	Electron Capture Detector
GLC	Gas Liquid Chromatography
GAP	Good Agricultural Practice
HPLC	High Performance Liquid Chromatography
JMPR	Joint FAO/WHO Meeting on Pesticide Residue
LD 50	The Lethal Dose Required to Kill half of a Population of Laboratory test animals.
MRL	Maximum Residue Limit
OP	Organophosphates
PPM	Parts Per Million
P.E.	Petroleum Ether
RH	Relative Humidity
SPSS	Statistical Package for Social Studies.
UV	Ultraviolet
FID	Flame Ionization Detector

EXPLANATION OF TERMS

Grains	Maize seeds
White flour	Milled maize seeds without outer cover (testa) of the seeds
Whole meal	Milled maize seeds with outer cover (testa) not removed
Residue	The fraction (part) of pesticide that is left behind after the pesticide undergoes breakdown process

ABSTRACT

Post-harvest pesticides applied to consumable foodstuffs usually pose a serious but silent threat to human and livestock health. Some pesticides accumulate slowly in various strata of the ecosystem causing irreparable damage to the environment and wildlife. In Kenya huge quantities of these pesticides are used to preserve maize grains during storage. The amount of pesticide residues consumed through maize grains and its products depend on ambient storage conditions of grains, the nature of the active compound of particular pesticide and the processing technique of the grains before consuming. The purpose of this study was to investigate the effects of ambient storage conditions on the concentrations of pesticide residues in post-harvest maize grains and to evaluate the extent to which the consumer is exposed to these pesticides. The research involved a field survey that assessed the knowledge, attitudes and practices of the community regarding the safe use of pesticides (occupational exposure). The study also analyzed the residual concentrations of pesticides in stored maize grains and some of its processed products (milling fractions) such as white flour and whole meal flour. Gas chromatographic method using flame ionization detector (FID) was used for quantization of pesticide residues of two organophosphates (malathion, pirimiphosmethyl) and one synthetic pyrethroid (permethrin) in maize grains and its milling fractions. Three parameters were measured: level of pesticides in two maize milling fractions (whole meal and white flour) and level of pesticide residue that remained on maize grains after various cleaning processes (hulling, waterwash, detergent wash), extent of health risks associated with consuming maize grains and flour treated with pesticides (dietary exposures) and persistence of pesticide residues in maize grains stored under simulated storage conditions (temperature, light and relative humidity). The maize grains samples for the simulated storage were initially treated with 0.3mgkg^{-1} permethrin, 3.9mgkg^{-1}, pirimiphosmethyl and 3.92mgkg^{-1} malathion prior to storage and portions analysed at regular intervals for the pesticide residues. Data was analysed by SPSS and Genstat version 6.1 computer software. Field survey information indicated that more than 95% of farmers in Chilchila do not practice safety precautions during pesticide formulation and application. After six months of simulated storage, at ambient temperatures averaging 22^{0}c and 55% RH the level of the initial residues that persisted on the maize grains was 63.3% permethrin, 64% pirimiphosmethyl and 56% malathion. The rates of dissipation of the pesticide from the maize grains decreased with storage time and followed a biphasic pattern for all storage conditions. Applying first order reaction kinetics, the following halflifes were obtained at the ambient storage conditions of the study area: maize grains treated with permethrin had 130 days; maize grains treated with pirimiphosmethyl had 77 days and the maize grains treated with malathion had 30 days. This trend was repeated for all the other storage conditions, giving a persistence order of permethrin followed by pirimiphosmethyl then malathion. Mean residue concentrations in whole meal were 1.7 mgkg^{-1}, 1.3mgkg^{-1} and 0.16mgkg^{-1} for pirimiphosmethyl, malathion and permethrin respectively. All these residue levels were above the ADI level for each pesticide. Results for white flour indicated mean residue levels of 0.54mgkg^{-1}, for pirimiphosmethyl, 0.53 mgkg^{-1}, for malathion and 0.06 mgkg^{-1} for permethrin, while hazard index analysis for maize grains, whole meal and white flour gave values greater than one for malathion and pirimiphosmethyl. The hazard index analysis was less than one for permethrin. These two observations meant that, the organophosphate pesticides (malathion and pirimiphosmethyl) occurred at levels risky to human consumption in all food products analysed while the pyrethroid pesticide (permethrin) occurred at a safe level for human consumption.

CHAPTER I

1.0 INTRODUCTION

1.1. Background of the study

Agriculture is a very important sector of the Kenyan economy in that the total land under farming is estimated at 2.4 million hectares and supports a population of 30 million people. It generates about 75% of the gross domestic product (GDP) and accounts for 80% of the total national employment (Kenya Bureau of Statistics, 1999). The sector contributes to the overall food needs of the country, provides the domestic industry with agricultural raw materials and promotes industrial development through expanding the market for industrial goods such as pesticides, fertilizers, equipment and machinery. Moreover agriculture helps to finance economic and social development through the net capital from farming to other sectors of the economy (Udoh, 1998). However one of the greatest challenges to the agricultural industry is the issue of pests. These are insects and disease producing organisms, which use plants as their source of food. They feed on plants, leaves, flowers and even fruits. The attack of the insect leads into plant destruction and makes the plants unsafe and unavailable for human consumption (D*eOng, 1979).* Due to this factor, man has come up with various methods to minimize, if not to prevent these insect attacks. Among these methods is the use of pesticides as a means of controlling insects. This method is most popular with farmers due to the fact that pesticides are readily available in the market, easy to administer and provide rapid action against the pest (Calvert *et al.,* 2001).

Natural plant extracts were the earliest form of pesticides. In this category were the flowers of pyrethrum shrub (plate I) which is still used in limited amounts today.

Kenya is the leading producer of pyrethrum in the World today (Aston, 2003). By the beginning of World War II (1940), pesticide selection had widened to several arsenicals, petroleum oils, nicotine, pyrethrum, rotenone, sulfur, hydrogen cyanide gas and cryolite. Indeed it was the World War II that opened the Chemical Era with the introduction of a totally new concept of insect control chemicals – synthetic organic pesticides which were more potent and reliable in eradicating or killing pests (McEwen, 1979)

PLATE 1

Pyrethrum: A suitable alternative to the environmentally unfriendly synthetic pesticides

Some of these chemicals included organophosphates, carbamates, synthetic pyrethroids and insect growth regulators used for the treatment of cereal grains to prevent insect infestation before processing and consumption (Bengston *et al., 1983*).

Prior to the introduction of these synthetic pesticides, significant residues in food involved only two chemicals, mercury and arsenic. Little attention was given to mercury, for its use was limited primarily to soil and seed treatments while residues in harvested crops were not regarded as significant. It was soon learnt that the use of lead arsenate for insect control, especially on grains and leafy vegetables, resulted in high levels of arsenic in the edible products. Thus the first "tolerances" were established for pesticide residues on food (Driesbach, 1977). The concept "tolerance level" was new and was based on toxicological data indicating a maximum level that could be present in foods without any risk of illness from its consumption.

With the development of chlorinated hydrocarbons and their widespread use in agriculture, it became apparent that residues in food were important (Hill and Comardese, 1986). In this study three pesticides (mathion, permethrin and pirimiphosmethyl) that are commonly applied to maize grains were investigated.. In view of the difficulty of assessing the toxicological significance of the pesticide residues in a food product, the joint meeting for pesticide residues (JMPR) often give guideline levels for residues in food products offered for human consumption. Table 1.1 shows these residue levels as recommended for the three pesticides of permethrin, malathion and pirimiphosmethyl in maize products (FAO/WHO, 1994).

Table 1.1. WHO maximum rsidue levels (MRL) and acceptable daily intakes (ADI) for malathion, permethrin and pirimiphosmethyl in maize products (maize meal and white flour)

Pesticide residue	Malathion	Permethrin	Pirimiphosmethyl
MRL ($mgkg^{-1}$)	8	3	5
ADI ($mgkg^{-1}$)	0.02	0.05	0.01

Source: (FOA/WHO, 1994)

Maximum Residual Limit (MRL) is the recommended maximum concentration of a pesticide residue resulting from the use of pesticide according to good agricultural practice (GAP) for the production or protection of the commodity. The acceptable daily intake (ADI) level is the amount of pesticide residue considered safe for human consumption without causing a health risk (FAO / WHO, 1985). Due to the growing dependence on the use of toxic chemical pesticides by farmers, the various components of the agro-ecosystem are affected in several ways including soil degradation, water pollution, air pollution, pests diminution of livestock and wildlife and health risks to man. (Cheng *et al.*, 1977). Pesticide residues are thus widely spread in the atmosphere and biosphere; where they circulate in characteristic paths between the organism and the environment showing a host of interractions (McEwen, 1979). These biogeochemical cycles or pathways are shown in Figure 1.1 where the numerous interactions ensure that the pesticides move and are degraded both biologically and chemically in processes involving the organisms and the insecticides (Goodman and Edward, 1984). Some pesticides degrade into metabolites that are sometimes more toxic than the mother compound and undergo biogeochemical cycles

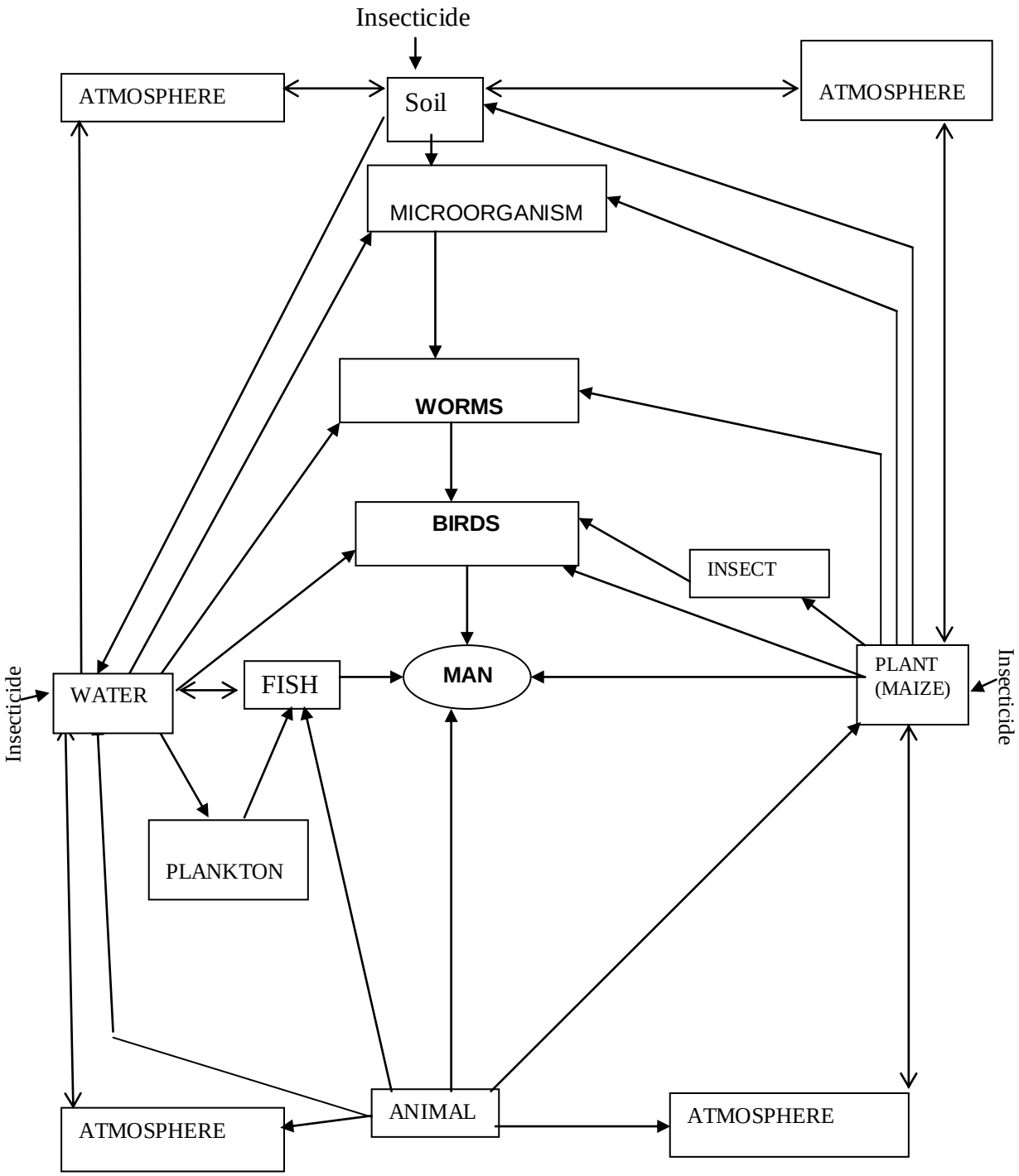


Figure 1.1. Pesticide circulation in Biosphere (biogeochemical cycles)

Source: (Suffet, 1977)

with substantially different residence times in various spheres of the environment (Suffet, 1977). Within this cycle, they will be taken up by man mainly from food and drinking water. In this respect pesticide residues constitute a health risk because most have a tendency of accumulating in vital organs (Golob *et al.*, 1999).

The pesticides interfere detrimentally with metabolic processes of many organisms including man if they are ingested in excessive quantities (Timbrell, 1991). It is therefore important to ensure that the set toxicological limits are not exceeded. Various processes occur that reduce pesticide amounts from the high commodity protection limit (MRL) to the low human consumption limit (ADI). These processes include; loss of pesticide during application on commodity, degradation of pesticide during commodity storage, food processing techniques aimed at reducing pesticide levels and enzyme technology where a pesticide is broken down by an enzyme into non-toxic products. Pesticide degradation is a chemical process that is driven by ambient storage conditions of temperature, humidity and light. Studies on different grains held at a series of controlled storage conditions of temperature and relative humidities show that the loss (degradation) of pesticide from post-harvest application in various grains is predictable (Desmerchelier and Bengston, 1979).

1.2 Statement of the problem

Pesticide residues have been associated with several concerns including risks to human health, death of farm animals and alteration of environment. In Kenya the highest level of such risks occur in rural agricultural areas where the farm worker handles more than 70% of all pesticides used. Chilchila division is one such

agricultural area where pesticides are heavily used on crops including maize, tomatoes, cabbages and coffee. Numerous health problems have been reported from this community which point to the indiscriminate use of pesticides. However inspite of this, little is known on the farmers basic knowledge of the dangers involved in handling, applying and disposing of those pesticide materials. Furthermore, the effectiveness of the cleaning and processing methods used by the farmers in reducing pesticide levels from maize grains is not known. In Kenya, different storage conditions (temperature, light and relative humidity) that should cause optimum breakdown of pesticide on maize grains are not known. In general therefore, farmers in Chilchila division know very little on the levels of pesticide residues carried by common maize foodstuffs (grains, wholemeal flour and white flour) and their health risk incidences to the consumer.

This study, therefore, aimed at answering the following questions;

1. Are the farmers in Chilchila division informed about the dangers of handling pesticides.

2. What is the effectiveness of cleaning methods as practiced in Chilchila division (hulling, water washing, detergent washing, milling) on the reduction of storage pesticides from the maize grains before consumption?

3. Are the concentrations of pesticide residues in maize foodstuffs (grains, white flour and whole meal flour) from Chilchila a health risk to consumers?

4. What is the effect of storage conditions (temperature, light, relative humidity) on the degradation of maize storage pesticide, (malathion, pirimiphosmethyl and permethrin)?

1.3 Null hypotheses

i. Occupational exposure to pesticides through handling, applying and disposing of pesticides pose no danger to the health of farmer in Chilchila Division, Kericho District.

ii. Pesticide amounts found on maize grains, its whole meal flour and white flour from Chilchila Division, Kericho District do not exceed the internationally accepted residue levels set by WHO/FAO.

iii. Different cleaning methods (hulling, waterwash, detergent wash and milling) practiced in Chilchila division are not efficient in removing pesticides from maize grains.

iv. Different storage conditions of temperature, light and relative humidity (RH) cause no significant difference to pesticide residue concentrations on stored maize grains.

1.4. Objectives of the study

General objective: To investigate the health risks to a farmer due to retained (persistent) pesticide residues on maize food stuffs and occupational exposure to pesticides

Specific Objectives

i) To evaluate the farmers' extent of exposure to pesticides while handling, applying and disposing of pesticides and their containers, through a questionnaire.

ii) To determine the levels of malathion, pirimiphosmethyl and permethrin residue in maize foodstuffs (grains, whiteflour and whole meal flour) consumed in Chilchila division, over a period of 120 days.

iii) To investigate the effectiveness of cleaning and processing methods (hulling, waterwash, detergent wash) in removing malathion, pirimiphosmethyl and permethrin residues from maize grains over a period of 90 days.

iv) To determine the effect of different storage conditions of temperature, relative humidity (RH) and light on the degradation of storage pesticides (malathion, pirimiphosmethyl and permethrin) in maize that is stored over a period of 180 days.

1.5 Justification and Output

The study has provided information to the farmers regarding the safety precautions towards pesticide handling, formulation and application. It gave information regarding the levels of pesticide residues in common maize grains, its wholemeal flour and white flour consumed in Chilchila division and therefore create records for the health hazard indices (health risk indicators) of the consumer. This study gave information on how different storage conditions of maize grains usually facilitate a breakdown of pesticide, thus reducing their toxicities. The results from the study can be used by policy makers as a basis of designing and implementing a health education programme aimed at sensitizing farmers on safe ways of using pesticides and grain harvest management. The results can also be used in setting up (MRL) for the purpose of monitoring and controlling pesticide residues on local and imported food grains thus ensuring a safe grain system within the country.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Maize grain production, storage and pest attack

Maize (*Zea mays*) is a valuable cultivated food grain in tropical Africa. It is a tall grass with a large stock, long arching leaves and big seeds (Plate 2.1). Maize grows best in soils of neutral acidity and is easily adaptable to warm, humid climate (Marvin, 1979). In Kenya maize is the most widely grown crop occupying almost half of the acreage used for food crops. In Kericho district the total acreage under maize cultivation is approximately 40,000 Ha with its major storage facilities at NCPB centers of Kericho Town, Kendowa and Kipkelion. It is used for feeding the districts population of 468,500 people (Ministry of Finance and Planning, Kenya, 2002).

Maize is usually stored in traditional stores made of Wickerwork walls raised off the ground with a grass thatch and a plaster of mud or cowdung applied to the framework of stakes. In some cases it is stored in hermetically sealed stores in underground pits (Carllindband, 1980). Storage conditions for grains determine the extent of fungal and insect attack. It is estimated that 10% of the world grain production is lost annually through insect attack during storage (FAO, 1996). In Kenya 18% of harvested grains are lost to pest and fungal attack when no storage pesticides are applied (Rundquist, 1984). Such losses, constitute a significant problem due to the importance of grains in human nutrition and the huge investment in grain production (Desmarchelier, 1977). In warm humid climate that is common in Africa, the major storage pest is the grain weevil (*Sitophilos granaries*) (Kenton and Lindband, 1978).

However in Kenya the greatest maize storage challenge today is a recently identified pest called the large grain borer LGB (*Prostephanus truncates*) (Plate 2.2) (Aston, 2003). The pest locally nicknamed as "Osama", thrives well in stored maize, tunneling it and reducing it to dust. Since this pest is also a woodborer, it attacks the wood that constructs the store, damaging the storage facility (Meikle *et al.*, 1998). Novel approaches have been taken to control the spread of this pest including the introduction of a predator (*Teretrius nigrescens)* from Meso American (Mazur, 1997) and the use of pesticides. However these pesticides persist as toxic residues on the treated grains so that their consumption by man and animal poses a potential health risk (Eduando *et al.*, 2003).

PLATE 2.1

A mature maize: *Zea mays*

2.2 Pesticide usage, adulteration and effects on man

Pesticides are an important factor in grain storage chemistry; where application of contact pesticides and fumigants on grains discourage pest infestation (FAO, 1996). Contact pesticides currently used are organophosphates of low acute mammalian toxicity such as malathion, pirimiphosmethyl and fentrothion or pyrethroids such as permethrin, deltamethrin and decamethrin (Snodgrass, 2001). Organophosphates are effective against most of the grain beetles like *Sitophilus granaries* except *Rhyzopertha dominica* and *Prostephanus truncates* for which treatment with pyrethroid is preferred. Treatment with a mixture of pyrethroid and organophosphate provides an effective way of protecting food grains against a complete range of insect pests (Desmarchelier, 1977). These pesticides gradually breakdown leaving residues on the grains that may be harmful to the health of the consumer. The rate at which these pesticides breakdown is independent of grain type but depends on the storage microclimate of temperature, humidity and light (Walker *et al.*, 1992).

Pesticides substances are usually intended for preventing, repelling or mitigating any pest, where pests can be insects, mice and other animals, unwanted plants (weeds), fungi or microorganisms like bacteria and viruses (DeOng, 1979). They can also be substances intended for use as plant regulators, defoliants or desiccants. By their very nature, most pesticides create some risk of harm to human, animals or the environment because they are designed to kill or otherwise adversely affect living organisms (USEPA, 2004).

Storage of maize grains in Kenya relies heavily on the use of such pesticide chemicals. The commercial names of some of them include actellic super, malper dust, super grain dust, martian doom and skana (Karembu 1990). These pesticides are locally formulated by mixing organophosphates and pyrethroid chemicals as active components (Aston, 2003). The organophosphate active compounds kill insects by disrupting their brains and nervous system; specifically inhibiting the function of a key enzyme in the nervous system called *cholinesterase*. However this *cholinesterase* is also a component of human nerve-to-muscle impulses (Gallo, *et al.*, 1991). Once inhibited the normal ability of animals to respond to external stimuli is destroyed and an animal dies of asphyxiation (Calvert et *al.*, 2001).

PLATE 2.2

An Adult grain borer *(Prostephanus Truncates)*

The most common organophosphate compounds used in maize storage are malathion, parathion and pirimiphosmethyl whose chemical structures are shown in fig. 2.1 (Gallo *et al,* 1991)

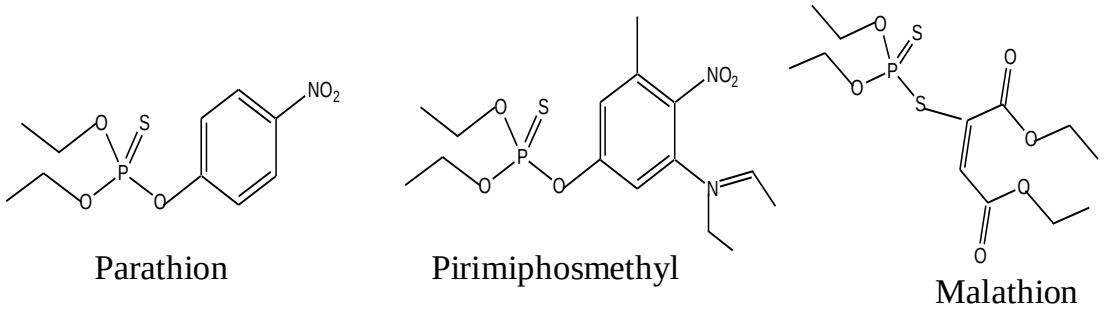


Fig. 2.1 Organophosphate compounds used in grain storage.

Malathion is applied in a variety of industrial and agricultural processes such as grain storage systems and malaria vector control. It is used to increase the strength and toxic insecticidal properties of many pesticide formulations including "actellic super" and "skana" which are analysed in this study. Malathion is "safe" only because of the mammalian liver, an organ with extraordinary protective powers that renders it relatively harmless. The detoxification is accompanied by one of the enzymes of the liver. If however, something destroys this enzyme or interferes with its action, the person exposed to malathion receives the full force of the poison (Barbara, 1993). Thus malathion is of great concern because not only is it acutely toxic, but its toxicity is cumulative. Its acute effects depend on product purity and route of exposure. Other factors which influence the observed toxicity of malathion include the amount of protein in the diet and gender. As protein intake decreases, malathion gets more toxic (Kalayanova *et al.,* 1991).

Malathion has been shown to have different toxicities in male and female rats and humans due to metabolism, storage and excretion differences between the sexes; with females being more susceptible than males (Smith, 1993). Malathion affects the central nervous system, immune system, adrenal glands, liver and blood. Malathion is moderately toxic to birds, with a reported acute oral LD_{50} value for chicken being 525 mg/kg (Wagner, 1990). It is highly toxic to acquatic invertebrates and to acquatic stages of amphibians. However because of the very short half-life, malathion does not bioconcentrate in aquatic organisms (Howard, 1991).

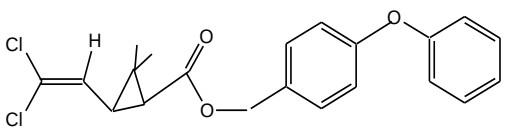
Pirimiphosmethyl is another pesticide belonging to the organophosphate class and is extensively used in grain storage system. This pesticide has been used by man for the last 30years (Bengston *et al.,* 1980). Human exposure to this pesticide mainly occur through air, water and food with typical dietary intake ranging at $0.01mg/kg^{-1}$ day^{-1} for humans (Gallo *et al,* 1991). Pirimiphosmethyl is ingested and retained by living organisms and hence its content in the human body reflects the extent to which it is present as a contaminant in the environment (Hudson *et al.,* 1984).

Major human effects of pirimiphosmethyl are manifested in three organ systems including the central nervous system, the renal system and the haematological system (Rowland, 1991). The toxicity of pirimiphosmethyl like every other organophosphate is based on the fact that it is a potential *cholinesterase* enzyme inhibitor. This affects major components of the nervous system including the Preganglionic neurons of the sympathetic fibres. The toxicity of pirimiphosmethyl to acquatic biota depends on its chemical formulation (Johnson, 1984).

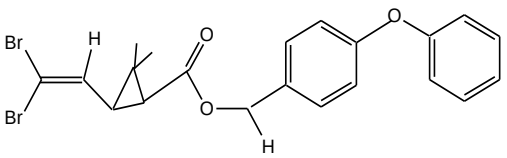
The other group of active chemicals used in grain storage insecticides are Pyrethroids (Fig 2.2.). The Pyrethroids used in the market are synthetic compounds and are more

toxic than natural pyrethrum insecticides since they are more light stable and are resistant to metabolism. They poison the nerve through interfering with transmission of nerve impulses (Sherma, 1998). The most common Pyrethroids used in grain storage are permethrin, decamethrin, deltamethrin, bifenthrin, pyrethrin i and bioresmethrin.

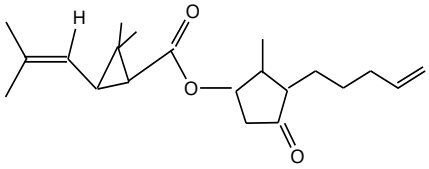
Permethrin is a composite pyrethroid, a broad spectrum cumulative insecticide, and a fast-acting neurotoxin. It is of moderately high toxicity to mammals and readily metabolized with immediate loss of activity. Permethrin is not plant systematic and is readily degraded on soil or plants but has high residual activity on inert surfaces. It is readily absorbed from gastrointestinal tract; may be absorbed by inhalation of dust and fine spray mist but only minimally through the intact skin (Wagner, 1990).



Permethrin



Decamethrin



Pyrethrin I

Fig 2.2. Pyrethroid compounds (Pimentel, 1979)

The health hazard is considerably diminished by the low concentrations of the active ingredient in all formulations. It is important that concentrated formulations be washed immediately from the skin and eyes. Permethrin may be applied to a wide range of grains, fruits, vegetables, tobacco and several non-food crops (Pimentel, 1979). It is a synthetic pyrethroid which acts primarily on the basal ganglia of the central nervous system, causing repetitive nerve action through prolongation of sodium permeability during the recovery phase of the action potential of neurons (Shepard, 1986). In general pyrethroid poisoning is characterized by hyperactivity and hypersensitivity (somatosensory). Permethrin in particular is included among a group of compounds producing the Cs-syndrome in rodents, in sequence; Pawing and burrowing behaviour, salivation, coarse tremors progressing to choreoathetosis and occasionally terminal chronic seizures (Snodgrass, 2001).

Pesticide residues refer to the amount of active pesticide compounds left after being used in killing pests (Hill and Comardese, 1986). Residue level of any pesticide, aside from the physio-chemical properties depends on a number of factors, namely crop and its variety with particular reference to morphological features of leaf, stem and fruit; climatic conditions with induce temperature and rainfall – the degradation being faster under hot/sunny conditions; and doses of pesticides (McEwen, 1979). Most of these pesticides are formulated as to contain residues that further illicit their effects on possible future pest attacks. However, when these are left on the food crops themselves, they may be ingested by man and cause severe poisoning (Smith, 1993). The fact that every meal eaten carries loads of pesticide residues is an inevitable consequence of spraying or dusting of the agricultural crops with pesticides containing these poisons (De Ong, 1979). Pesticides are poisonous in nature not only to the target pests but also to the warm blooded animals including man. Residues left

over the sprayed surface of the crop have become a subject of concern in respect of health hazards to men, animals and environment pollution (Kalayanova *et al.,* 1991). However, if the farmers scrupulously follow the instructions on the labels, the use of agricultural chemicals will produce no residue larger than are permitted by good agricultural practice (GAP). However, most farmers very frequently exceed the prescribed dosages while others mix various pesticides by "cocktailing" in their quest for stronger dose that would eliminate the ever resistant pests (LGB) (Desmarchelier, 1977).

Thus serious problems of pesticide adulteration, dilution and other illegal tampering surrounds pesticide retailing in Africa. For instance, a survey of maize stores in Benin revealed a drop from 13% to 0% of farmers using approved storage pesticides (Meikle *et al.,* 1998). In Ghana most farmers apply a mixture of non-approved products to grains, mainly malathion and DDT, obtained from informal dealers who sell unlabelled products (Kojotsimase, 2003). In Uganda, farmers in 1999 were still buying dieldrin from small traders despite this active ingredient being severely restricted for several years (Pan Africa, 2000). Survey of Tanzania farmers revealed that the 'actellic" "super dust" (ASD) commonly purchased for grain storage is often ineffective due to adulteration by unauthorized dealers (Golob *et al.,* 1999). Over 20% by volume of the pesticides imported into Kenya are extremely or highly hazardous and 20% moderately hazardous, (Ohayo – Mitoko, 1997). Food monitoring surveys carried out in Brazil and Egypt for residues of DDT, lindane, malathion and pirimiphosmethyl showed that 25% and 41% respectively of food samples contained pesticide residues (Doghein *et al.,* 1999).

A study of 50 lactating mothers in India showed average diedrin residue levels of 0.13µg/ml in milk (Prabhu and Pingali, 1993). Perhaps the ultimate answer is to use less toxic chemicals so that the public hazards from their misuse is greatly reduced.

2.3 Summary of toxicological effects of pesticide residue

There are numerous health problems associated with long-term exposure to pesticides. Majority of these problems are caused by lack of knowledge on the safe handling, formulation and application of the pesticide by farmers without protective clothing (Zalom, 2001). A summary of the most common problems that are manifested in different organ systems include:

Eye defects – The eye is very vulnerable to physical and chemical hazards in the agricultural setting. A chronically irritated eye can lead to the formation of apterygium, a vascular that encroaches on the pupil diminishing visual acuity and requiring surgical removal to improve eyesight (Snodgrass, 2001).

Skin effects - Pesticides enter the body mainly through the skin not the respiratory tract contrary to common belief (Wagner, 1990). The quantity varies with working conditions, application techniques, protective equipments and duration of exposure. The arms and the forearm have the highest potential for pesticide contamination. Eczema, a chronic allergic dermatitis characterized by lichenification and fissuring, is a dermatologic health indicator of pesticide exposure. The skin appears thickened with accentuated markings. Other health indicators are the destruction of the distal portions of the toe nails, giving it an “eaten up” appearance (Prabhu and Pingali, 1993).

Respiratory tract effects - some pesticides such as pyrethrum and 2, 4-D severely irritate the lungs and upper respiratory tract (Prabhu and Pingali, 1993). Long term exposure to chemical irritants like pesticides can cause respiratory symptoms such as cough, cold, sputum formation, wheezing, tenderness and decreased chest expansion. Bronchial asthma and other abnormal lung findings are the two respiratory tract indicators of pesticide exposure (McDuffite *et al.,* 2001)

Cardiovascular effects - In acute pesticide poisoning, the heart may be damaged either by direct action on the myocardium or as a result of low tissue oxygenation. Hardening of the blood vessel causes blood pressure elevation. Hence one health indicator is elevated blood pressure (Kalayanova *et al.,* 1991). Organophosphates and 2,4-D have been implicated as causes of myocardial injury or injury to the conducting system as reflected in electrocardiogram changes (Kalayanova *et al.*, 1991).

Gastrointestinal tract effects - Pesticides usually enter the GIT accidentally through the mouth. Carbamate insecticides formulated in methylalcohol and ingested may cause severe gastroenteritic irritation. Organophosphates also irritate the GIT, manifesting as nausea, vomiting and diarrehoea. The health indicator of chronic gastritis is chronically characterized by epigastric tenderness and pain associated with nausea and vomiting (Timbrell, 1991).

Neurologic effects - Organophosphorous compound and 2,4-D are known neurotoxicants. Both have been implicated as causative agents for polyneuropathy, a neurologic disorder that manifest typically as motor weakness in the distal muscles and sensory deficit with a "glove and stocking" distribution (McDuffite *et al.,* 2001). Absence of deep tendon reflexes in the early stages may be the only sign, but neuropathy may be purely motor or purely sensory. Isolated hyporeflexia, another

neurologic index, is a known sensitive indicator of chronic exposure to organophosphorous pesticides (Prabhu and Pingali 1993).

Hematologic affects – A plastic anemia is manifested as pancytopenia exposure to pesticides. Eye, skin, nail, pulmonary neurological and renal problems are significantly associated with long term pesticide exposure (DeOng, 1979).

2.4 Process of pesticide breakdown

2.4.1 Introduction

Degradation process changes most pesticides in the environment to non-toxic or harmless compounds. Within the environment pesticides are altered and this may involve chemical activation or degradation as metabolic reactions mediated by living organisms. Degradation occurs under aerobic or anaerobic conditions. Microbial metabolic degradation pathways for pesticides include among others, β-oxidation, hydroxylation, ether cleavage, dealkylation, dehydrogenation, epoxidation and reduction (McEwen, 1979). Pesticide breakdown when applied to grains occurs under three processes and these are microbial degradation-breakdown of pesticide on the surface of grains caused by microorganism. Photodegradation occurs when pesticide molecules applied to surface of grains acquire radiant energy from sun or from photochemically excited molecules resulting in isomerization, ring substitution, hydrolysis or oxidation. Chemical degradation occurs when storage microclimate of temperature, humidity, aeration, sunlight, pH and adsorption determines the rate of pesticide breakdown (Brown *et al.,* 1997).

A study has reported the use of an enzyme technology for breaking down organophosphate insecticide residues into nontoxic products (Lawrence, 2002). In

the study a rinsate produced from equipment wash down of methyl parathion was treated with an enzyme which rapidly broke it down into 4-nitrophenol, a relatively innocuous and readily biodegradable byproduct. Hydrolysis is probably the major biodegradation process for organo- phosphorus pesticides. One form of microbial oxidation in this group is exemplified by the conversion of thioether moiety of some compounds to their sulphoxide and sulphone analogues. For example, pirimiphosmethyl on grains is degraded and detoxified by hydrolysis of the phosphorous-ester side chain, the rate of hydrolysis increasing with increase in moisture and temperature (FAO/WHO, 1985). In this study the types of degradations examined are both chemical degradation and photodegradation.

2.4.2 Kinetics of pesticide degradation

Reaction kinetics has found a lot of application in analytical chemistry especially in quantitative determinations of trace concentrations of substances due to their low detection limits (Perez-Benditto and Silva, 1988). Studies have shown that pesticide residues from post-harvest application to various grains is a second order process with the rate of degradation being proportional at a fixed temperature to the amount of pesticide and the equilibrium partial pressure in the intergrain space (Desmarchelier, 1977). The kinetics for such second order reactions involving two reactants A and B to form a product C may be expressed as an equation (2.1), though many reactions require more complicated equations.

$$R = -\frac{\partial A}{\partial t} = -\frac{\partial B}{\partial t} = \frac{\partial C}{\partial t} = K[A][B] \text{ ---------- (2.1)}$$

Where the negative signs signifies the decrease in concentration of species A and B, while R is the rate of reaction and K is the rate constant. [A] and [B] represents the

concentration of species A and B respectively at any given time. The term $\partial C/\partial t$ represents the rate of change of concentration with time(Perez-Benditto and Silva, 1988).

For the degradation of pesticide residues from stored grains one of the reactant is assumed to be constant (fixed) throughout the degradation process while the other reactant (pesticide) is varied with time (t). Such reaction is said to follow pseudo-first-order reaction kinetics (Desmachelier and Bengston, 1979).

If in the above example B is in large excess and A is the pesticide studied then equation (2.1) may be expressed as follows:-

$$-\frac{d[A]}{dt} = K[B][A] \text{ ----------(2.2)}$$

$$-\frac{d[A]}{dt} = K^1[A] \text{ where } K^1 = K[B] \text{ ----------(2.3)}$$

On integration of equation (2.2)

$$Log_{10}[A] = \frac{-K[B]_0}{2.303}t + Log_{10}[A]_0 \text{ ----------(2.4)}$$

Equation 2.4 gives us pseudo-first-order equation.

A plot of log [A] versus time (t) gives a straight line whose gradient $-k/2.303$ is equal to the rate of degradation and the y-intercept is equal to $\log[A]_0$. Once the gradient is obtained the concentration of pesticide residue [A] at any given time (t) of a pesticide can be calculated. A study by Bengston *et al.*,(1980) based on post-harvest pesticide application to maize, wheat, oats, paddy rice and sorghum have shown that the rate of degradation of pesticide is proportional at a fixed temperature

to the amount of pesticide and the water activity which was obtained from the equilibrium partial pressure of water vapour in the intergrain space.

Thus the fate of residues of grain protectant insecticides can be predicted from calculations based on several well-defined parameters (Bengston *et al.,* 1980). The recommended (MRL) can therefore be extended to cover all raw grains in the confident expectation that the level of the initial deposit will decline at a predicted rate dependent only on temperature and relative humidity.

Bengston *et al.,* (1980) plotted the mean observed and predicted residue levels of a pesticide at different intervals after application. Fig 2.3 shows the high level of agreement with the mathematical model based on pseudo-first-order Kinetics.

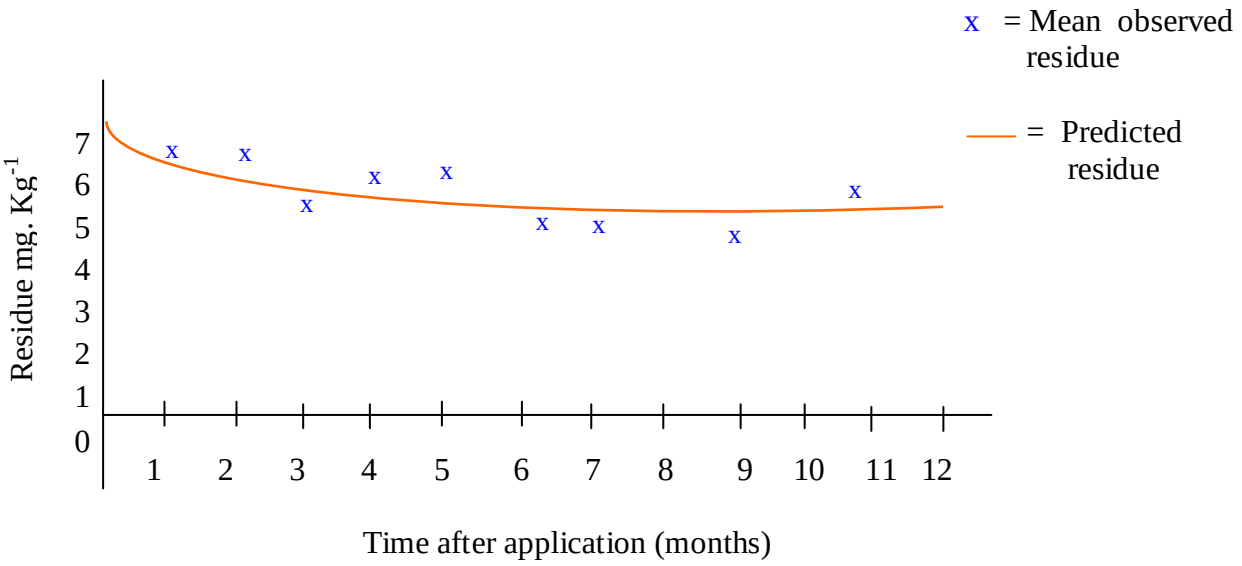


Fig 2.3 A general Pesticide degradation path.

Source: (Desmarchelier, 1977)

Fractional lifetimes are also often used as a means of measuring the rate of degradation for a pesticide. The half-life ($t_{½}$) (time required for initial concentration to get halved) is then calculated using the rate equation shown below

$$R = -\frac{d[A]}{dt} = K[A] \quad \text{----------(2.5)}$$

For a first order reaction, and after one half-life ($t_{½}$) equation (2.5) can be written as below

$$\ln \frac{a}{2} = \ln a - Kt_{½} \quad \text{----------(2.6)}$$

consolidating and rearranging gives

$$t_{½} = \frac{\ln 2}{K} = \frac{0.693}{K} \quad \text{----------(2.7)}$$

2.5 Methods of analysis of pesticides residues on foodstuffs

2.5.1 Classical Methods

Several methods have been used to determine pesticide residue concentrations in foodstuffs. The simplest of these methods is the classical methods. This involves measurement of colour (colorimetric analysis) and volume (volumetric analysis). In the colourimetric technique, the residue is extracted from sample matrix using suitable solvents such as n-hexane/acetone, while colour is by chromophoric groups. The colour is finally measured by matching the test colour with standard colour on a loviband colour scale (Thomas and Chambelain, 1980). This method has been used to study metabolism of pesticides in stored cereals at the pest infestation control laboratory, Slough UK (Rowland, 1991).

Volumetric analysis is a classical method for determining concentration of pesticide residues (WHO, 1985). In this method a pesticide filtrate is acidified with nitric acid

and treated with a few drops of silver nitrate. This mixture is then titrated with 0.1mol/l potassium thiocyanate using phenophthalein indicator (WHO, 1985). Pyrethrin I and Pyrethrin II residues from pyrethrum flowers are determined using this volumetric analysis. However, one of the major limitations of these classical methods is their low sensitivity and are therefore not recommended for trace analysis (FAO/WHO, 1994).

2.5.2 Spectroscopic Methods

Several techniques developed in this category are based on the interaction of molecules with radiation permitting quantitative determination. Some of these are colorimetry, UV spectroscopy and fluorescence absorption spectrophotometry (Suffet, 1977). Colorimetry involves the hydrolysis of the pesticide using potassium hydroxide coupled with a diazo solution at pH 10.3, before absorbance is measured by a spectrophotometer or photoelectric colorimeter at 530 nm (WHO, 1985). A calibration curve is then prepared from which pesticide residues concentrations from samples can be read. The introduction of the *Beakman DV* spectrophotometer with its quartz optics and ultraviolet accessory unit has made it possible to obtain reliable ultra-violet absorption spectra conveniently and within reasonable time (Sherma, 1998). The UV analytical technique for residue analysis is adapted from King (1984). After extraction, absorbance of active compounds is determined with UV spectrometer at optimum quantitative analytical settings. Absorbance is measured at appropriate wavelength of approximately 230 nm.

2.5.3. Enzyme – Immunoassay Technique

Enzyme – immunoassay method for quantitation of residues is a relatively new method published by the American Association of Cereal Chemistry (AACC, 1996). In this method, samples are extracted in methanol, allowed to settle for 1hour then diluted 1:50 in 50mM (Na_3PO_4, 0.9%NaCl, pH_{12}), 0.05% Tween, 1% bovine serum albumin. Microwell plates are then precoated with appropriate antibodies by incubating overnight with 1µg of antibody per 100µl of 50mM Na_2CO_3 buffer pH 9.6 (Skerrit *et al.*, 1992). The diluted extracts (100µl) plus pesticide-peroxidase conjugates (100µl) are added to each microwell plate and incubated for 1hr at 20ºC. The plate is then washed and 160µl peroxidase substrate, 3,3,5,5 – tetramethyl benzidine chromogen added and incubated 30 min at 20ºC. Stopping reagent (40µl of 1.25M H_2SO_4) is added and absorbance measured at 450nm (Hill *et al.,* 1991). Immunoassay results, were calculated from standard response curves, prepared by spiking pesticide standards into methanol or methanol extracts of pesticide free grains.

2.5.4 Chromatographic Methods

Chromatographic analytical techniques for the determination of pesticide residues have been formulated by the American Environmental protection Agency (EPA). These techniques are published in the EPA Manual of Chemical Methods for Chemical and Devices (Zweig and Sherma, 1982). Thin layer chromatography (TLC) is used for the confirmation of residues tentatively identified by gas chromatography. Confirmation is based on comparison of migration distances of the pesticide of interest with authentic pesticide standards run on the same plate. One dimensional development on neutral alumina or silica gel thin layers is most often used for comparison. In addition TLC may be used for semi quantitative analysis of pesticide

residue when a fast estimation of pesticide levels is desired or when the pesticide is unstable during gas chromatography (AACC, 1996). Quantitative analysis is performed by comparing the size or intensity of the pesticide spot, after detection, from the sample with spots from a series of standard solution of same pesticide run on the same plate (King, 1984).

Another technique commonly used for pesticide residue analysis is high performance liquid chromatography (HPLC). In this method the residues are extracted through a solvent system of acetone in petroleum spirit and partitioned between acetone and acetonitrile. The extract is cleaned through Florisil column before HPLC analysis (Tempone, 1979). A column of stainless steel tube 18 cm long and 4.6 mm internal diameter packed with silica and a detector measuring UV absorption at 230 nm is used (Bullock and Derrick, 1976).

The most commonly used technique for pesticide analysis is gas liquid chromatography (GLC) following the recommended methods, (USEPA, 1996). Gas chromatography is an analytical technique for separating volatile substances, based upon the distribution of sample between two phases, a stationary bed of large surface area and a gas which percolates through the stationary bed (Barry and Petzinger, 1981). If the stationary phase is a solid, we speak of a gas solid chromatography (GSC) which depends upon the absorptive properties of the column packing to separate samples. Common packings used are silica gel, molecular sieve and charcoal (Zweig and Sherma, 1982).

The use of gas chromatography in analytical chemistry began in 1905 when it was employed to separate mixtures of gases and vapours (McNair and Bonelli, 1970). These first experiments used adsorption or desorption from solid adsorbents such as active charcoal. However it was Martin and Synge who later introduced the (GLC) technique in 1952. In this method the stationary phase is a liquid spread as a thin film over an inert solid, and the basis of separation is the partitioning of sample between liquid film and gas (McNair and Bonelli, 1970). The GLC method has a very high sensitivity, rapidity and accuracy in its separation, identification and determination of volatile compounds, thus making it the most versatile and selective form of gas chromatography (WHO, 1985). Interest in this analytical method has therefore risen steadily and since 1952, commercial instruments for GLC has appeared in interesting numbers and is estimated that 60,000 gas chromatographs are used in the world today (USEPA, 1997).

2.5.4.1 Theory of gas liquid chromatography

In GLC the components to be separated are carried through the column by an inert gas (carrier gas). The sample mixture is partitioned between the carrier gas and a non-volatile solvent (stationary phase) supported on an inert size-graded solid (solid support). The solvent selectively retards the sample components, according to their distribution coefficients, until they form separate bands in the carrier gas. These component bands leave the column in the gas stream and are recorded as a function of time by a detector.

If the detector response R is plotted against time t, we get a peak that has a Gaussian shape as shown in figure 2.4 below.

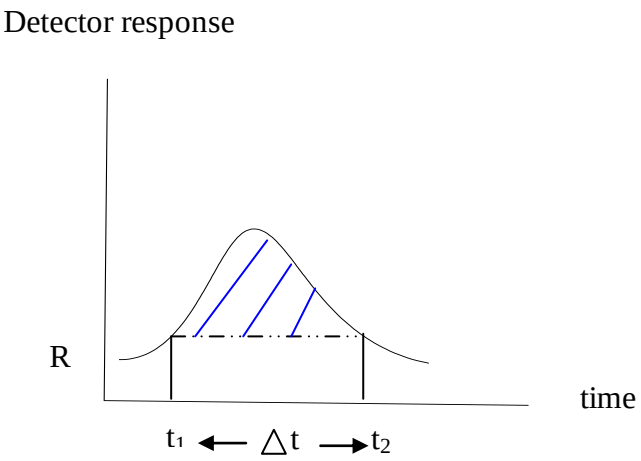


Fig.2.4: GLC Detector response against time.

If the flow rate of carrier gas is kept constant, then the peak area shaded will be directly proportional to the total mass or concentration of a component. During GLC analysis, when a sample is injected, a peak is obtained whose area is a direct measure of the concentration of that sample. In this work, the peak area was used exclusively for the determination of pesticide residue concentrations. A major advantage of this method is that the peak area is less dependent on operating conditions, than the peak height.

2.5.4.2 Instrumentation of GLC

The most important components of GLC are:-

Injection Port

This is a device through which the sample is introduced instantaneously as a "plug" onto the column. The injection port should be hot enough to vapourize the sample so rapidly that no loss in efficiency results from injection technique. On the other hand, the injection-port temperature must be low enough so that thermal decomposition or rearrangement of sample is avoided (Zweig and Sherma, 1982).

A standard technique for the introduction of gases and liquids is to introduce a hypodermic syringe needle through a self-sealing septum lodged in the injection port and inject measured volumes from an attached syringe. To introduce solids, the easiest technique is to dissolve them in a solvent whose response does not interfere with the samples being analysed.

Columns

A column is the part where actual separation of sample components is achieved. In gas liquid chromatography there are both capillary and packed columns. Capillary columns are open tubes of small diameter with a thin liquid film on the wall. Packed columns consist of an inert solid material supporting a thin film of a non volatile liquid. The tube may be either glass, metal or plastic, coiled to fit the chromatograph oven. The solid support, type and amount of liquid phase, method of packing, length and temperature of the column are important factors in obtaining the desired resolution. The dimensions of the column govern the total amount of gas and liquid it will contain (WHO, 1985). Analytical columns are ordinarily1/6″ to1/4″ internal diameter tubing and from 3 to 30 feet in length.

Lengthening a column will increase the separation. Finally the column is conditioned according to recommended methods by the WHO (1985) before use.

In this study a packed megabore chromatographic glass column of 4mm internal diameter and 1.5m long was used.

Detectors

A chromatographic detector is a device, which indicates and measures the amount of separated components in the carrier gas. Detectors may be classified as "integrating" or "differentiating". An integrating detector gives a response proportional to the total mass of component in the eluted zone. The chromatogram produced by an integrating

detector consists of a series of steps in which the distance between consecutive level portion of the curve is proportional to the total mass of component corresponding to that step (McNair and Bonelli, 1970). The titrating burette is an example of an integrating detector. A differentiating detector gives a response proportional to the concentration or mass flow rate of the eluted component. The most familiar example of a detector responding to concentration is a Katharometer. The flame ionization detector F.I.D is an example of detector responding to mass flow rate. The chromatogram produced by a differentiating detector consists of a series of peaks each of which corresponds to a different component. The area under each peak is proportional to the total mass of the component. Differentiating detectors are more commonly used because of their convenience and accuracy. These detectors have high sensitivity, low noise level, a wide linearity of response and are insensitive to flow and temperature changes. In the (F.I.D), hydrogen and air are used to produce a flame (Gilreath, 1964). In this research a differentiating detector (F.I.D.) was used while other common detectors used during pesticide analysis include thermionic detectors.

Recorder

Present practice is to use a strip chart recorder to obtain a permanent record of the results. A 1mv, 1sec full scale response is recommended (McNair and Bonelli, 1970). The potentiometric type recorder used in a GLC is a servo-operated voltage balancing device. The principle of operation is that a balancing motor moves the centre tap of the potentiometer slide wire until the input signal from the chromatograph is balanced by the feedback signal. The amount of motor rotation is proportional to the magnitude of the voltage being measured. The motor rotation is connected to a pen which records on chart paper the voltage variation with time.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study area covered Chilchila Division of Kericho District, Rift Valley Province, Kenya. The choice of the area for study was based on the researcher's first hand experience to the pesticide problem there as he comes from this area. The division is divided into five administrative locations of Kokwet, Kipteris, Kunyak, Kapkoros and Chilchila (Fig 3.1). The area has a population of 37,000 people with 7,000 households (Kenya Central Bureau of Statistics, 1999). Chilchila division has volcanic soils that are rich in minerals and therefore well suited for maize growing. Climatic conditions are hot and humid with one long rainy season between the months of April and September. A maximum rainfall of 1800 mm is experienced while temperatures average at 22^0C (Ministry of Finance and planning Kenya, 2002). The main agricultural activity of the people in this region is the growing of maize for family consumption while coffee is the major cash crop. Other crops such as beans, bananas, sweet potatoes, cassava, tomatoes and cabbages are grown to meet the farmers foodstuffs requirements. Dairy cattle are also kept for milk. Once maize is harvested it is stored for a duration of up to 10 months and this therefore necessitates the application of pesticides.

3.2 Research design

The study was structured in three stages. The first stage involved a field study that aimed at evaluating the farmers extent of exposure to pesticides while handling, applying and disposing of pesticides and their containers. This was conducted through a questionnaire and administered to randomly sampled homesteads (150 homesteads)

of the study area. The second stage involved the collecting of samples of maize grains treated with pesticides by the farmer and the flour milled from the grains. This was collected from 15 randomly sampled homesteads at the end of each month for six consecutive months. Maize grain samples collected from the farmer at the end month were transported to the laboratory and immediately cleaned and processed through various methods (hulling, waterwashing, detergent washing and milling) before being analysed for levels of pesticides. Flour products (wholemeal flour and white flour) were similarly transported to the laboratory after collection at month end.

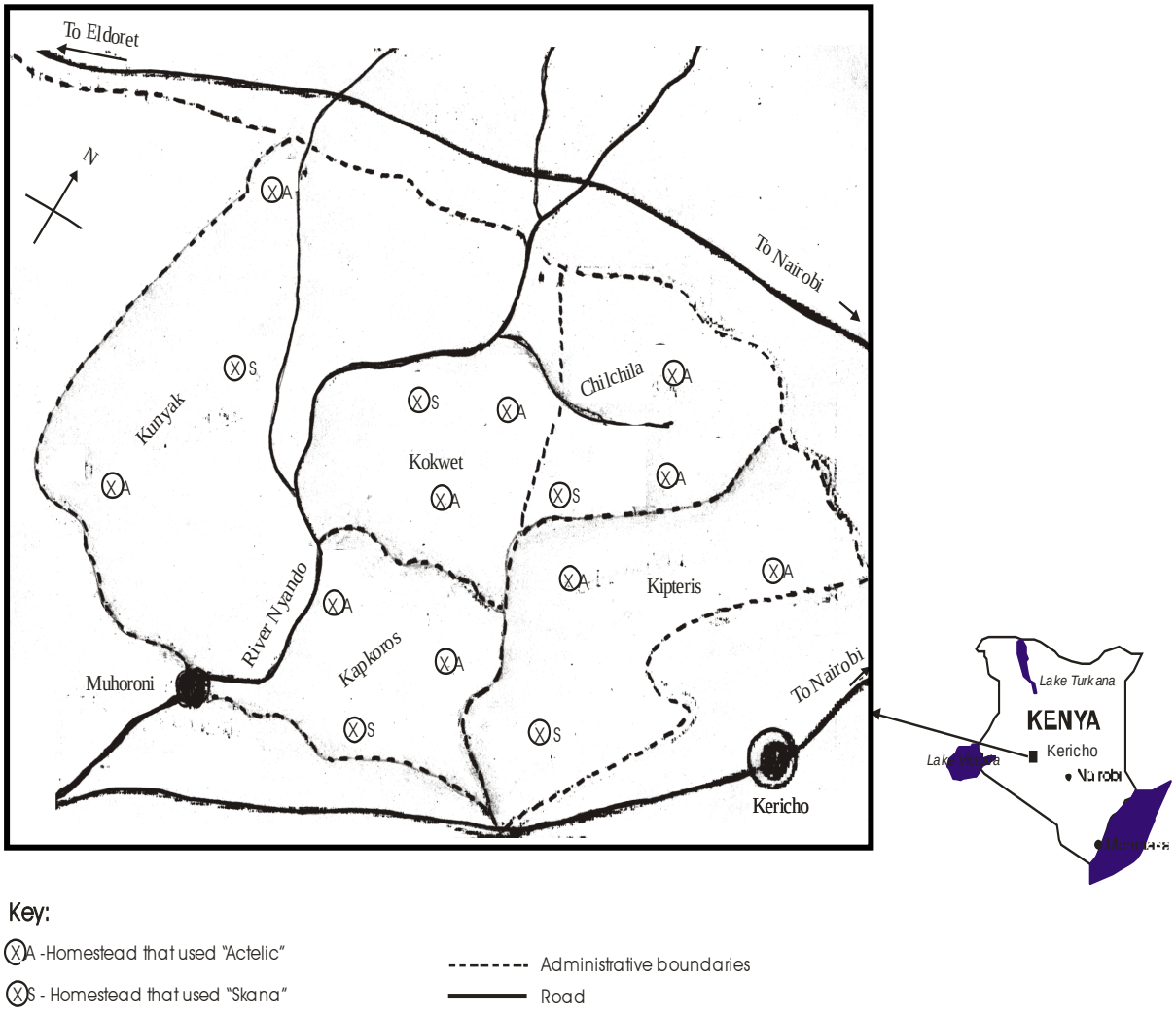


Fig 3.1 Chilchila Division map showing administrative locations and sampling sites

Health risk indices, to the children and adult consumer of the grains and flour commodities were then determined. In the third stage, maize grains were treated at the laboratory with common storage pesticides used in the study area (permethrin, pirimiphosmethyl and malathion). The maize was then stored under simulated different storage conditions (temperature, light, relative humidity) in the laboratory for a period of six months.

3.3 Materials

3.3.1 Sampling sites and sample collection.

A total of 15 homesteads were chosen from the five locations in Chilchila for the monthly collection of the samples. The homesteads were chosen such that two homes applied "actellic super" pesticide while one homestead used "skana" pesticide to treat harvested maize grains. This 2:1 ratio was implied by the number of homestead using each of the commercial pesticide as the questionnaire information indicated. The 15 homesteads chosen for collection of samples are indicated in the pipeline concept, Figure 3.2. Maize grain samples were collected soon after the harvest season in the months of December and January and transported to the laboratory for pesticide treatment, simulated conditions storage and analysis. Maize flour samples were collected at the end of each month from three randomly selected homesteads in each location. In all cases the samples were collected in paper bags that were sealed before transporting to the laboratory. In the laboratory the samples were stored at low temperature ($4^{o}C$) to arrest any pesticides degradation before analysis. The researcher used protective devices such as hand gloves, dust masks and coveralls while collecting these samples.

3.3.2 Preparing samples for simulated storage

The maize grains for simulated storage were treated with a commercial pesticide ("actellic" or "skana") that was applied in the laboratory using a backpack sprayer at constant pressure. A solution containing 60 ml insecticide emulsion (dilution 3:500 in water) was used for each 15 kg grains. For each treatment operation, the grains were spread in a thin layer on a plastic sheet then mixed thoroughly by agitating the plastic sheet with vigorous movements (Eduardo *et al.*, 2003). After treatment the grains were placed in wire gauze boxes and stored under laboratory controlled conditions of temperature, relative humidity (RH) and light. Twenty grams of the grains were then removed and weighed, at intervals of 0, 10, 15, 30, 60, 90, 120, 150, 180 days after treatment and analyzed for levels of pesticide residues using GLC technique.

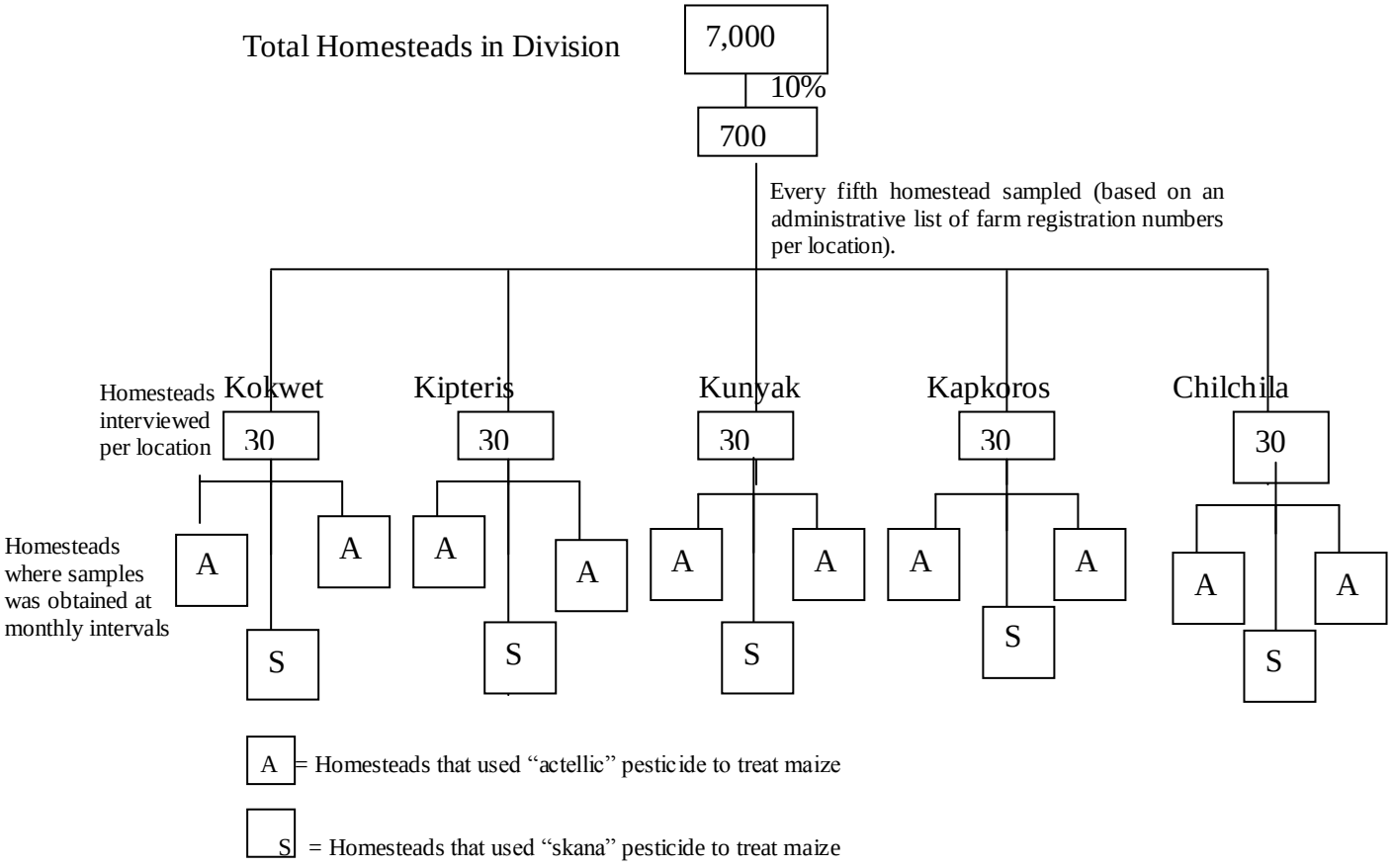


Fig. 3.2 The pipeline concept diagram indicating the random sampling of households for interviews and sample collection.

Source: (Desmmarchelier, 1977)

3.3.3 Storage conditions simulations

The study utilized experimental analysis research design. This was a post test in which the field samples were collected after the application of pesticides, while storage conditions of temperature humidity and light were controlled. The storage conditions of (30^0C, 65% RH) and (26^0C, 60% RH) were set in a designed compartment fitted with a thermostat that regulated temperature inside the box (Appendix C). The compartment had two chambers with the lower chamber set at 26^0C while temperature in upper chamber was 30^0C. The set up was then covered with an opaque polythene material to create a dark chamber. Two trays made of wiregauze were used to hold the maize grains; one treated with "actellic super" while the other was treated with "skana", commercial pesticides. Another set of storage conditions was set by fitting the wire gauze trays into two different dessicators. A petri-dish containing some little water was placed at the bottom of dessicator so that its evaporated vapour helped to create the required humidity. A little silica to help maintain a constant humidity level inside the dessicator was also placed in the dessicator. One dessicator was then placed under direct sunlight during the day and a low voltage electric bulb at night to facilitate evaporation and achieve the higher humidity level of 55%. The other dessicator was placed in a darkroom where there was less evaporation hence lower humidity level of 50%. The storage conditions in the dessicators were therefore controlled to room temperature ranging between 15^0C to 22^0C and relative humidity ranging between 50% to 55%. Relative humidity for both setups were monitored daily through a moisture meter (Model 800 Humidity Sensor) (Appendix C) while the pesticide residue for each set of storage conditions was determined by GLC analysis.

3.3.4 Instrumentational specifications

An analytical electronic balance (mettler Model 1700 MPB) was used for all weight measurements of samples while centrifuging and mixing was achieved through a Centra 787R bench top centrifuge machine. Quantitative determinations of pesticide residue was done through a PYE UNICAM (Model 104) gas chromatographic instrument equipped with a flame ionization detector (FID) using a packed megabore chromatographic glass column of 4mm internal diameter and 1.5 m long. Column packing material (chromosorb W-HP treated with 7.5% OV-210) was used. For precise and reproducible injection of samples into the GLC a 20 µl Perkin Elmer (Model 8310) GC Microliter syringe (701N) was used. All evaporation and concentrations of samples were carried in a standard rotatory evaporator machine.

3.3.5 Reagents and Chemicals

The solvents n-hexane, acetonitrile, acetone, petroleum ether methanol and benzene were analar grade from Kobian Ltd (Nairobi, Kenya). Any commercial grade solvents were distilled to obtain 99.9 % purity. Analar grade pesticide standards of malathion, permethrin and pirimiphosmethyl were obtained from Sigma - Aldrich Ltd (Britain).

3.4 Methods

3.4.1 Questionnaire Administration

Field work study was done through a questionnaire interview guide and observation to obtain information of the farmers risks involved in the handling of pesticides. The questionnaire was administered to randomly selected homesteads in the five locations. Approximately 7,000 households are in this study area and 10% of these households (700) constituted a sample unit(Fig. 3.2). Every fifth household was then randomly

chosen based on administrative list of farms registration numbers per location. This was done until a total of 150 households was obtained for the interview. Thus 30 households per location were visited for the interview and observation exercise. Each location was handled by three research assistants engaged by the main researcher on the basis of understanding the local languages and terrain of the study area. The research assistants were given training on how to conduct the interview by the main researcher. On visiting a selected home, the interview team explained the purpose and importance of the interview and displayed the government research permit allowing for the conduction of the interview. The family head was then issued with the questionnaires and pencil which he / she filled while clarifying for any items he / she didn't understand. For illiterate family heads the research assistant was available to assist in filling the questionnaire. The questionnaires were then collected and packed for later analysis while another member of the team gave a word of thanks to the cooperating interviewee. There was no dropouts on the selected 150 homesteads, since all agreed to answer the questions.

3.4.2 Cleaning and milling the maize grains

Methods of cleaning the maize grains included hulling where the threshed maize grains were placed on a sieving-tray and mechanically agitated and sorted out for foreign solid particles. Water washing method involved placing the maize grains in a water basin half-full of water then scrubbing them thoroughly with bare hands to remove the pesticides from the grain surface. Detergent washing involved putting the grains in a basin and washing them with a liquid mixture of water and a commercial detergent (JIK) before rinsing with clean water. Milling methods were of two types including wholemeal milling where the whole grains were milled into flour while

white flour milling required the removal of outer seed coat (testa) from the maize grains before milling into flour.

3.4.3 Cleaning of glassware and polythene containers

All glassware used in the analysis were cleaned with concentrated chromic acid to remove any stains. They were then washed with a detergent (JIK) free from phosphorus. The containers were first rinsed with distilled de-ionised water before a final rinsing with a solution of Isopropanol. The glassware were then dried in an oven at 100 – 120^{0}C for twenty hours. No rubber or plastic containers were used in this pesticide analysis to minimize contamination of the extracts.

3.4.4 Preparation of standard solutions

The standards used in the study were malathion, permethrin and pirimiphosmethyl. To prepare a standard stock solution for malathion, a 10 mg of the pesticide standard was weighed into a 250 ml volumetric flask and dissolved and made to the mark with acetone to give a solution of 40 µg/ml labeled solution A. One mililitre of solution A was transferred into seven different tubes. Different volumes of acetone were accurately measured and added to make working standard concentrations covering the range of 0.16 µg/ml to 5 µg/ml. These working standard concentrations for malathion were injected into a GLC instrument to obtain a chromatogram of peaks which were used to plot a malathion standard calibration curve. This procedure was repeated for pirimiphosmethly. However, for permethrin a 6.25 mg of the pesticide standard was weighed into a 250 ml flask and dissolved in acetonitrile to give a solution of 25 µg/ml labeled solution B. A serial dilution by addition of acetonitrile to 1ml of solution B yielded working standard concentration covering a range of 0.1 µg/ml to

0.3 µg/ml. These concentration were injected into a GLC to give chromatogram necessary for plotting the permethrin calibration curve.

3.4.5 Sample Extraction

A 10 g sample of grains or the flour was taken and placed in 200 ml flask and 50 ml hexane added then shaken for 15 minutes at 360 cycles per min by an electric shaker. The extract was then centrifuged for 5 minutes at 2,000 rpm. The process was repeated three times to obtain a tripricate of extracts. 5ml aliquots of supernatant from each tripricate extract was transferred to 50ml polypropylene tubes and concentrated under evaporation by means of air flow in water bath at 45^0C. The extract was cleaned by partitioning it between acetonitrile saturated with a 25 ml hexane. After shaking and centrifuging for 5 minutes at 2500 rpm, the hexane layer was drained off and discarded while the acetonitrile layer was transferred to the polypropylene tubes where it was concentrated until almost dry using a water bath at 60^0C and then re-suspended in 5 ml hexane.

The extract was then passed through a column packed with florisil using a mixture of 65% acetone in hexane and eluting at a speed of 2 ml/min. Separation was achieved due to differences in polarity of pesticides. The more polar organophosphates pesticides are eluted first by the more polar eluting solvent of 65% acetone in hexane. After concentration by evaporation in water bath at 45^0C, the organophosphorus residues (malathion or pirimiphosmethyl) were dissolved in 10 ml acetone and transferred to 15 ml graduated tubes as fraction I. Pyrethroid residue (permethrin) which is less polar, was eluted by a mixture of 10% acetone in hexane. This gave

fraction II that was collected in a different polyproplene tube. A 20 µl volume of the extract from fraction I or II was then injected into the GLC instrument for analysis.

3.4.6 TLC analysis

Thin layer chromatography (TLC) was used as the initial step in confirming the presence of the pesticide residue extracted from the sample. A 250 µm thick layer of silica gel slurry was prepared by spreading it on a TLC glass plate. The slurry was prepared by shaking 30 g of adsorbent with a 5 ml hexane for 2 minutes and the layer was activated by heating the prepared chromatoplates in an oven at 100^0C for 2 hours. The layer was divided into tracks by scribbling lines parallel to one edge and 3 cm apart. Two lines were scribed at right angles to the parallel tracks at both ends of the longer sides of the plates. One line represents the spotting line while the other marked the solvent front. The sample extract was spotted as a single application of 2 µl in its own track next to that of the standard. The chromatogram was developed with hexane. The developed TLC plate was irradiated with strong UV light for up to 20 minutes while exposing the adsorbent to water vapour from a steam bath to develop spots and identify the pesticides. The spots in sample extract were identified by comparison of their Rf values with those of the standards.

3.4.7 GLC analysis

A prepared chromatographic column was conditioned by fitting into a GLC oven and heating it overnight (15 hours) at approximately 250^0C with the exit end of the column unconnected to the detector and carrier gas flowing at a rate of 30ml/min. After conditioning, the column was connected to detector and the GLC operation conditions set for analysis, as shown in Table 3.

Table 3.1 Operating conditions of the GLC used in the determinations of pesticide residues

Temperature	
Oven	210^0C
Injection port	210^0C
Flame ionization	250^0C
Gas flow rates	
Hydrogen	30 ml/min
Air	350 ml/min
Carrier gas (nitrogen)	30 ml/min

Source (Youssef *et al.,* 1992)

A 20 µl portion of the standard pesticide solution and sample extract were injected into the GLC column simultaneously. The peak height of the sample injection was used to calculate the peak area by the triangulation method, which was then used to give residue concentration. The concentration was further confirmed through calculations involving the universal equation
(B-1, Appendix B).

3.4.8 Health risk analysis

Health risk estimates were calculated on the basis of pesticide analysis data and exposure assumptions. The following assumptions were made based on the U.S Environmental Protection Agency's (USEPA, 1989) guidelines:

a) Hypothetical body weights of 10 kg for children and 70 kg for adults and

b) Maximum absorption rate of 100% and a bioavailability rate of 100%. Food consumption rates were obtained from questionnaire analysis of the number of members in a family and the amount of maize stored for consumption in six months and included the following:

(i) Ugali / githeri – 1.2 $kgday^{-1}$ for adults (ii) and 0.9 $kgday^{-1}$ for children. Hence for each type of exposure, the estimated lifetime exposure dose $mgkg^{-1}$was obtained by multiplying the residual pesticide concentration ($mgkg^{-1}$) in the food of interest with the food consumption rate ($kgday^{-1}$) and dividing the product by the body weight (kg) (USEPA, 1989). This is shown by equation (B-7, Appendix B). The hazard indices for children and adults were estimated as ratios between exposure dosage value and the reference dosage acceptable daily intake (ADI), which are considered to be safe levels of exposure, over the lifetime (Snodgrass, 2001), shown by equation (B-8, Appendix B).

3.4.9 Data analysis

Mann-Whitney U-test was used to analyse all nonparametric data obtained from the questionnaire and interview guide. Parametric data collected from the GLC analysis was analysed with SPSS and GenStat version 7.1 (Payne *et al.*, 2003) computer software, for various statistical tests to determine whether storage conditions had significant effects on degradation. The impact of storage conditions on pesticide breakdown was analysed using ANOVA tests, t-tests and Tukeys modified tests. The trend relationships for the storage conditions during the pesticide degradation were examined through Pearson Correlation tests while Kruskalwallis tests were used for assessing the compatibility of degradation data from various collection sites.

To explain the rate (speed) of pesticide breakdown a non linear regression analysis was performed with GenStat 7.1 where the decline in pesticide residues with time could be described with Pseudo first order kinetics. The degradation data was characterized using a combination of visual assessment and a λ^2 test (B-9, Appendix B) as recommended by FOCUS (2006). The λ^2 test considers the deviations between measured and predicted values relative to the uncertainty of measurements. Michaelis-Mentons Kinetics were used to explain the various states in the process of pesticide breakdown. Graphical presentations and interpretations of data helped determine the half-lives of various pesticides hence their rates of degradation. Hazard index calculations based on pesticides exposure dosage revealed the level to which a consumer is at risk after consuming maize products treated with pesticides. If the hazard index = (exposure dosage divided by ADI) was greater than 1, ($\geq$ 1) this indicated a health risk to the consumer

(B – 8 Appendix B).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Field survey discussion

The research instruments used in this study for the collection of descriptive data included questionnaires and interviews prepared and administered according to the standard format set by Mugenda and Mugenda (1999) and Gay (1992). Statistical analysis of the response data from the questionnaire (Appendix A) was done through Mann-Whitney U test and One Way Anova tests. The response to each item in the questionnaire was tabulated and its scientific and social relevance discussed.

4.1.1 Family size and farm sizes of Chilchila Division

A household is the sampling unit in this study. It is therefore, necessary to know the number of people residing in each household. Size of the family gives a scientific and social inference to the study. Thus the amount of food (maize) stored and consumed per household is expected to be dependent on size of a family. Table 4.1 shows information on the family and farm size when expressed as frequency and percentage values.

Table 4.1: The frequency and percentage values of family size and farm size

Questionnaire items	Mode of response	No. of respondents (frequency)	Percentage (%)
No. of people per home	1-3	20	13
	4-7	120	80
	8-11	10	7
Size of farm (ha)	0-5	96	64
	5-10	34	23
	10-20	20	13

Most of the families (80%) in the study area are middle-sized with 4 to 7 members, large families with 8 to 11 members accounted for only 7% while small families of 1 to 3 members make 13% of all the families visited. These data indicate that majority of the families consist of 2 to 5 children and hence require enough stored food (maize) for feeding the growing children. Majority of the families are settled in small farms of 0 to 5 ha, which represent 64% of all the farming land in Chilchila division. This means that the families are engaged in small scale peasant farming. Medium sized farms of 6 to 10 ha make for 23% of the land while large farms of 11 to 20 ha accounts for the remaining 13% of the farming land, meaning that large scale farming of any crop is not common in the study area.

4.1.2 Food and cash crop cultivation in Chilchila Division

The types of crops cultivated in the study area were investigated with a view of finding those that require pesticide application. Table 4.2 below shows major crops cultivated number of farmers who cultivate each type of crop and percentage values for each crop cultivated.

Table 4.2: Frequency and percentage values for each crop cultivated

Items	Mode of response	No. of respondents (frequency)	Percentage (%)
Major food crops cultivated	Maize	130	86
	Beans	11	8
	Tubers	3	2
	Bananas	3	2
	Tomatoes	3	2
Major cash crops cultivated	Coffee	105	70
	Sugarcane	45	30

Food and cash crop cultivation data revealed that the community in this area consisted of peasant families engaged in mixed farming with coffee being the major cash crop (70%) while maize (86%) was the major food crop grown. Beans, (8%) and tomatoes (10%) are also grown as minor food crops to supplement the major staple food. The two major cultivated crops (coffee and maize) require pesticide. Other crops cultivated in the area include sugarcane (30%), tubers (2%) and bananas 2% which basically don't need any pesticide application.

4.1.3 Maize harvest and storage facilities in Chilchila Division

The results of this study, where the frequencies and percentage values for maize harvest and its various storage facilities were examined, are shown in table 4.3 below.

Table 4.3: The frequency and percentage values for maize harvest and storage facilities

Item	Mode of response	No. of respondents (frequency)	Percentage (%)
Harvesting months	September	34	23
	October	3	2
	November	113	75
Is maize stored after harvest	Yes	147	98
	No	3	2
Amount of maize stored for consumption (100 kg bags)	1 – 3	22	15
	4 – 7	116	78
	8 – 12	12	7
Storage facility	Traditional granaries	127	85
	Hanging in house & smoking	16	11
	Sealed bins	5	3
	Others	2	1

In Chilchila maize is harvested in two clear harvesting seasons during early September (23%) and the late November (75%) Table 4.3. This reflects two distinct climatic zones of the study area where maize from the warmer lower zone (Chilchila and Kapkaros) locations grow faster and matures early hence harvested in September while maize from the cooler upper zone (Kunyak, Kipteris, Kokwet) locations take longer to grow and slow to ripen hence is harvested later in the year in November. Most of the maize harvested is then stored for family consumption (98%), thus requiring pesticide treatment to avoid insect infestation. The 2% that don't store maize normally sell theirs when it is still in the farm, since they don't have large families to feed. Majority of the families (78%) store 4 to 7 bags of maize for consumption. A few families (15%) store 1 to 3 bags while even fewer families (7%) store 8 to 12 bags of maize for consumption. These storage figures relate closely to the number of members in a family and indicate a consumption rate of approximately one bag per member of the family during the whole storage period.

The most common method of storing the maize was through the traditional granaries (85%). However, this method had a big disadvantage in that the ambient temperature and humidity inside the store were conducive to insect breeding and multiplication. As such, pesticides are an absolute necessity to curtail insect infestation of the grains. Some maize was preserved through smoking and hanging (11%), a method that was popular with the older generation farmers, but which was limited due to its cumbersomeness and lack of capacity to handle bulk storage. Sealed bins were also used by a few farmers (3%) though also limited due to its high cost and lack of bulk storage capacity. There was a rare method of hermetically sealed underground pits which was practiced by an extreme minority of the farmers (1%) who constituted the "others" method of storage.

4.1.4 Maize storage pesticides, their alternatives, sources and storage pests

Maize farmers apply a wide range of pesticides to combat various storage pests (Pimentel, 1979). The source of such chemicals, their effectiveness in controlling pests and the consequent residue health effects are issues of concern to any maize consumer. Therefore Table 4.4 shows the frequencies and percentages of the various storage pesticides available to farmers of Chilchila Division.

Table 4.4: The frequency and percentage values of pesticides, their sources, alternatives and storage pests

Item	Mode of response	No. of respondents (Frequency)	Percentage (%)
Pesticides used to treat maize during storage	Actellic	96	65
	Skana	48	32
	Nova	2	1
	Malathion	2	1
	Blue triangle	2	1
Pesticide alternative	Ashes + sand	55	37
	Smoking then hanging in kitchen	95	63
Sources of pesticides	Retail shops	110	74
	Chemists	5	3
	Open air market	35	23
Changed from one pesticide to another	Yes	104	70
	No	46	30
Maize storage pests	Grain borer *(prostephanus truncaties)*	105	70
	Tropical grain weevils *(sitophila granaries)*	45	30

The greatest challenge to most of the farmers in their maize storage efforts was a grain borer (*prostephanus truncates*), which is also a wood borer and locally nicknamed "Osama". Seventy percent (70%) of the farmers indicated of the havoc that this pest had wrecked on their maize harvest and wooden storage facilities, as well as developing a resistance to available pesticides. Thus sixty five percent (65%) of the farmers in Chilchila opted to use "actellic" pesticide which contained pirimiphosmethyl and permethrin active components, and was more effective on "Osama" and a host of other grain pests. The second most important pesticide was "skana" thirty two percent (32%) and is composed of malathion and permethrin active ingradients. However, it had virtually little effect on "Osama". The other common pest was the traditional tropical grain weevil *(Sitophilus granaries)* at thirty percent (30%) and usually controlled easily by the commercially available pesticides of "actellic" or "skana".

The farmers usually mixed both "actellic" and "skana" pesticides to get a more potent concotion referred to as "gun" which was considered a better formulation to prevent pest infestation although this procedure definitely exposes the consumer to a potential health risk.The other three pesticides; "nova" one percent (1%) "Malathion" one percent (1%) and "Blue triangle" one percent (1%) found a limited use only when the two major pesticides were not available in the market. Analysis by Mann -Whitney U test on the two most common pesticides of "Actellic" and "skana" demonstrated that the "actellic" pesticide had a more significant effect to the grain storage programmes in Chilchila division ($F = 8.2$ $df = 142$ $P<0.05$).

Financial hardships rather than efficiency has driven some farmers to resort to various pesticide alternatives including the age-old methods of smoking and hanging the maize combs sixty three percent (63%) or simply adding a mixture of ashes and sand to the threshed grains thirty seven percent (37%). Smoking kept the maize dry and denied the insects the moisture they needed for existence and multiplication while the ashes and sand mixture scratch the insect cuticle causing them to loose moisture hence die. These two methods had no health risk implications to the consumer but were severely limited by their lack of bulk storage capacity of maize grains.

The most important source of pesticide to the farmers was the retail shops with a percentage of seventy four (74%) at the local shopping centres. Open air market sources contribute twenty three percent (23%) and this constituted the other major pesticide source to the farmers. However, in both these sources, it was noted with concern that the pesticide dealers (sellers) had extremely low or no knowledge on chemical and pesticide handling precautions since many were illiterate. Three percent (3%) of the pesticides came from chemists that were registered with Poisons Control Board (PCB) of Kenya. This meant that most of the pesticides reached the farmers from illegal dealers. Seventy percent (70%) of the farmers indicated that they had changed use of one pesticide to another due to failure of the pesticide to stop infestation. Thirty percent (30%) of the farmers who had not changed use of pesticide were still waiting to see how effective the new pesticide used by their neighbours would act before joining them too.

4.1.5 Farmers knowledge in handling and disposing of pesticides

A farmers level of exposure to pesticides is the most likely cause of the many health ailments that afflict him in his farming activities. The farmer therefore needs to be knowledgeable on the basic safety precautions in relation to pesticide handling so as to minimize obvious occupational exposure hazards. Table 4.5 shows the frequency and percentage results for framers training in pesticides handling, use of protective devices, disposal of pesticide materials and observance of safety interval before consuming grains.

Table 4.5: Farmers training in pesticide handling, use of protective devices, disposal of pesticide materials and safety interval observance.

Item	Nature of response	Frequency	Percentage (%)
Any training in pesticide handling.	Yes No	17 133	11 89
Do you read label on pesticide container.	Yes No	10 140	7 93
Use of protective devices.	Normal clothes Coveralls Goggles Gloves	146 4 Nil Nil	98 2 Nil Nil
Mode of disposing of unused pesticide and empty pesticide containers.	Incineration Inside house Inside store Careless – thrown Away	15 25 30 80	10 17 20 53
Observing safety interval.	Yes No	38 112	25 75

Eleven (11%) percent of all the farmers in the study area had received some training in the safety methods of handling pesticides while eighty nine percent (89%) were

untrained. Application of the Mann-Whitney-U test on the farmer training revealed that a significant health risk is caused by this dominant group of untrained farmers (U_{cal} = 25 df = 149 $P < 0.05$). This is because these untrained farmers determined the amount of pesticide residues reaching the consumer. A few farmers consisting of seven percent (7%), read the instructions on the pesticide labels and adhere to the precautionary advice given. Ninety three percent (93%) of the farmers however, never bother to read the instructions as they were illiterate.

The most common mode of protective dressing used while applying pesticides included the normal domestic clothing and a pair of rubber boots with a percentage of ninety eight (98%). A complete set of protective attire comprising of coveralls, goggles, gloves, headgear and boots was completely non existent in the study area even from the biggest local agro-chemical outlet which deal with agricultural inputs and equipments. Only two percent (2%) of the farmers use coveralls while applying pesticides. This means there is a high level of occupational exposure hazards to the farm worker and the whole household in general.

Disposing of pesticide related materials through incineration (burning) is a scientifically recommended method (Ohayo-Mitoko, 1997). Unfortunately it was the least favoured disposal method accounting for only ten percent (10%) of all disposed pesticide materials in the study area. On the contrary the rather dangerous disposal method of careless-throw-away pesticides was the most common and accounted for fifty three percent (53%) of all disposed pesticide materials. In some families represented by seventeen percent (17%) had the unused pesticide chemicals kept in houses (17%) and this could perhaps be having the consequence of poisoning unsuspecting family members that would mistake them for consumable materials.

Empty pesticide containers were often found in the family's kitchen used to hold edible beverages and spices like tea leaves, salt and sugar in the total ignorance of the dangers of chronic poisoning that could occur. Twenty percent (20%) of the farmers considered their stores as the safest place to keep pesticide materials (Table 4.4)

A Wilcoxon rank-test for disposal methods (incineration and careless-throw-away) showed that there was a significant health risk caused by the latter method ($U_{cal} = 49$ $df = 149$ $P < 0.05$). Seventy five percent (75%) of the families confessed that they never observed any safety interval before consuming the freshly treated grains while only twenty five percent (25%) observed the safety interval. The above analysis shows that in Chilchila, farmers lacked any knowledge relating to the safe handling of pesticides to the detriment of their health and that of other consumers who may use their maize products.

4.1.6 Maize cleaning procedures and cases of illnesses associated with consuming maize treated with pesticides.

Some farmers perform cleaning procedures in an attempt to reduce the amount of pesticide likely to be consumed in a maize diet. While such procedures are effective in lowering pesticide levels on maize, there are other farmers who assume a don't care attitude and never bother to clean their maize grains. Table 4.6 shows such cleaning procedures, symptoms of illnesses and reported cases of illness and death when expressed as frequencies and percentage values.

Table 4.6 Frequency and percentage values for maize cleaning procedure, symptom of illness and reported cases of death of farmers.

Item	Nature of response	Frequency	Percentage
Cleaning procedure Prior to milling and cooking.	Washing (water) Removing outer coat (testa) Hulling	8 6 136	5 4 91
Reported cases of illness after. Consuming pesticide treated maize.	Yes No	140 10	93 7
Most common symptoms of illness.	Diarrhoea and vomiting. Headache and dizziness. Wheezing.	75 45 30	50 30 20
Any death due to pesticide poisoning.	Yes No	2 148	1 99

Hulling, sieving and sorting were the most consistent methods of maize cleaning procedure represented by ninety one percent (91%) prior to milling or cooking. These methods, however, removed very little of the applied chemical pesticide as they were only efficient in removing foreign solids found in the grains. Removing the outer seed coat from the maize grains before milling or cooking is the most efficient methods of cleaning the grains from pesticide residue (Desmarchelier and Bengston, 1979).

Unfortunately no local milling machine (rega rego) in the study area was equipped with the necessary gadget for this milling (sifting) process. Consequently majority families consume whole meal flour containing both seadcoat (testa) and endosperm,

which carries higher pesticide amounts while four percent (4%) of the families can afford to take their maize to Kericho town for the "sift" milling. However, this "sift" flour milling though advantageous in having lower pesticide levels, had a major drawback in that it has low fibre content (USEPA, 2004). Water-washing of the grains before milling was rare and only five percent (5%) of the families washed their grains before cooking. This cleaning method would help to lower the pesticide level if properly adopted.

Approximately ninety three percent (93%) of all the families confirmed that they had noted a health problem associated with pesticides, while seven percent (7%) reported no cases of illness related to pesticides. However reported cases of deaths from pesticide accidents accounted for only one percent (1%) of all the deaths in the area and these were suicidal cases. Chronic pesticide poisoning is the main danger in this study area. Application of Wilcoxon - U test on the reported cases of illnesses after consuming maize treated with pesticides revealed that these chemicals cause a significant health risk in this community ($U_{cal} = 25$ df = 149 $P<0.05$). This danger occurs slowly, and silently but cumulatively through the consumption of trace amounts of pesticides after a number of years (Prabhu and Pingali, 1993). Symptoms exist that show that this chronic poisoning is a serious issue among the maize consumers. Statistical analysis of major symptoms like diarrhoea fifty percent (50%), wheezing twenty percent (20%), headache and dizziness thirty percent (30%) that were commonly diagnosed in the homesteads revealed a significant clinicopathological problem to the consumer of the pesticide treated grains ($U_{cal} = 32$ df = 99 $P<0.05$).

4.1.7 Pesticide use on other agricultural activities

Apart from requiring pesticides for maize storage, the farmer in Chilchila also uses large quantities of pesticides for other agricultural activities around his farm. Such activities include, livestock spraying, weed control, malaria vector control and cash crop (coffee) spraying. Table 4.7 shows some of these activities along with their frequency and percentage values.

Table 4.7: The frequencies and percentage values of other agricultural activities that use pesticides

Item	Nature of response	Frequency	Percentage (%)
Other agricultural activities where pesticides are used	Tomato spraying	60	40
	Coffee spraying	40	27
	Livestock spraying	25	17
	Weed control	20	13
	Malaria control	5	3

Ninety seven percent (97%) of the respondents indicated that they feed their livestock and poultry on pesticide treated grains. These pesticides would be deposited in the adipose tissue of the meat and in the eggs which would then be introduced into the human food chain (Timbrell, 1991). In the category of other agricultural activities, apart from storage activities, tomato spraying took forty percent (40%) of the remaining pesticides. These tomatoes later found their way to the dining table sometime without proper washing to remove the pesticide residues. Coffee and livestock spraying together with weed control took the rest of the pesticide acquired by the farmers at percentages of (27%), seventeen (17%) and thirteen (13%) respectively. Malaria control through indoor spraying and spraying the areas around

the house took three percent 3% of pesticides used. Anova analysis on these various modes of occupational exposure to pesticide residues indicated a significant health risk to the farmer in the division (F_{cal} = 6.8 df = 149 P<0.05).

4.1.8 Farmers recommendations on ways of improving pesticide use around their farms

Farmers had a number of concerns in relation to pesticide availability, use and environmental impact around their farmlands. They recommended the use of natural pesticides, practicing organic farming and a government control in the damping of pesticides as perhaps the best methods of reducing the level of pesticide misuse in their farms. These opinions are shown in Table 4.8 below.

Table 4.8: Frequency and percentage values of the farmers recommendations on ways of improving pesticide use around Chilchila farms

Item	Nature of response	Frequency	Percentage
Recommendation on how to improve pesticide use in farmlands	Use natural products instead of synthetic.	120	80
	Control pesticide dumping.	20	13
	Use of organic farming.	10	7

Majority of the farmers, ninety percent (90%) confirmed that they had observed environmental changes, including death of insects and aquatic life after the use of

pesticides. Anova analysis showed there was a significant change to the environment especially to the insects through spraying of pesticides to crops (F_{cal} = 3.7 df = 149 P<0.05) as observed by farmers. Most farmers represented by eighty percent (80%) felt that people should stop using the imported synthetic pesticides and go back to traditional plants that control pests effectively without threatening the health of the consumer. Some of the plants mentioned were Mibangi (*Mexican marigold*) and Muarobaini (*Neem*). Thirteen percent (13%) of the respondents felt that the government should be more vigilant in controlling pesticide importation and registration so as to avoid the dumping of dangerous chemical pesticides into our country from industrialized nations. Yet still some of the farmers seven percent (7%) suggested a chemical free method of farming, perhaps organic farming which would rid the community of the pesticide health hazards.

4.2 Pesticide Residue in Maize foodstuffs (wholemeal, white flour and grains)

4.2.1 Analytical methods validation

The methods for pesticide residue analysis in this study were validated through various procedures that were carried out on each validation characteristics (parameters) explained below.

Precision

This is the degree of scatter of the analyte concentration for a series of measurements obtained from multiple sampling of a homogenous sample. To test the precision performance of the GLC instrumental procedure a specific of 10 injections of one sample solution was made. For malathion and pirimiphosmethyl pesticides where active components ranged between 0.1 to 1.0% in the sample, the acceptable precision

was 10% RSD, while for permethrin pesticide whose active component ranged below 0.1% in the sample the acceptable precision was 20% RSD.

Accuracy

This is the closeness that a recovered analyte concentration has to the true value. A known concentration of analyte was spiked into a matrix blank to make a spiked-placebo. Such spiked-placebo were prepard in triplicate at three fortification levels within the target concentration range. The analyte concentration in the spiked-placebo was determined using GLC procedure – the quantitative procedure used in final method process. The mean recovered analyte for malathion and pirimiphosmethyl in the triplicate was 80 to 120% (active analyte content 0.1 to 1.0%), while mean recovered analyte for permethrin was 75 to 125% (active analyte content is less than 0.1%).

Selectivity

This is the ability of an instrument to select an analyte concentration from all other components in a sample and give a response that is clearly resolved from the interfering responses. The response of the analyte in test mixture containing the analyte and all potential components, (interferences) was compared with the response of a solution containing only the analyte. The analyte peak was then evaluated for resolution from the nearest eluting peak. The analyte peak was found to have a baseline chromatographic resolution of $\geq$ 1.5 from other sample components (interferences) which was an acceptable resolution meaning the chromatographic parameters chosen for selectivity (column type, mobile phase, flow rate and detection mode) were acceptable.

Linearity

Extent to which the target analyte concentration range has linear relationship with the analyte concentration response from instrument was indicated by value of correlation coefficient (r). A specific of six standards whose concentration span 80 to 120% of the target concentration range (0.2 to 4 $mgkg^{-1}$) were prepared and each analyzed in tripricate. The analyte concentration prepared were plotted against their GLC instrument responses to give straight lines for malathion and pirimiphosmethyl (Fig. 4.1) and permethrin (Fig. 4.2). The correlation coefficients for the three pesticides were malathion (r = 0.9998), pirimiphosmethyl (r = 0.9994) and permethrin (r = 0.9995). Since these r values were all greater than 0.999 analytical methods were judged as acceptable (valid) to measure concentrations within the studied concentration range.

Limit of Quantization (LOQ) and Limit of Detection (LOD)

Limit of quantization (LOQ) was the lowest analyte concentration level and was determined by preparing standard solutions at estimated LOQ concentration (based on preliminary studies). The solution was then injected into GLC and analyzed ten times. The average response and standard deviation (SD) for the 10 times was calculated. The LOQ values were, malathion (0.2 $\pm$ 0.02), pirimiphosmethyl (0.5 $\pm$ 0.01) and permethrin (0.2 $\pm$ 0.01). The limits of detection (LOD) values obtained for the pesticide included malathion (0.03$mgkg^{-1}$), pirimiphosmethyl (0.01 $mgkg^{-1}$) and permethrin (0.01 $mgkg^{-1}$).

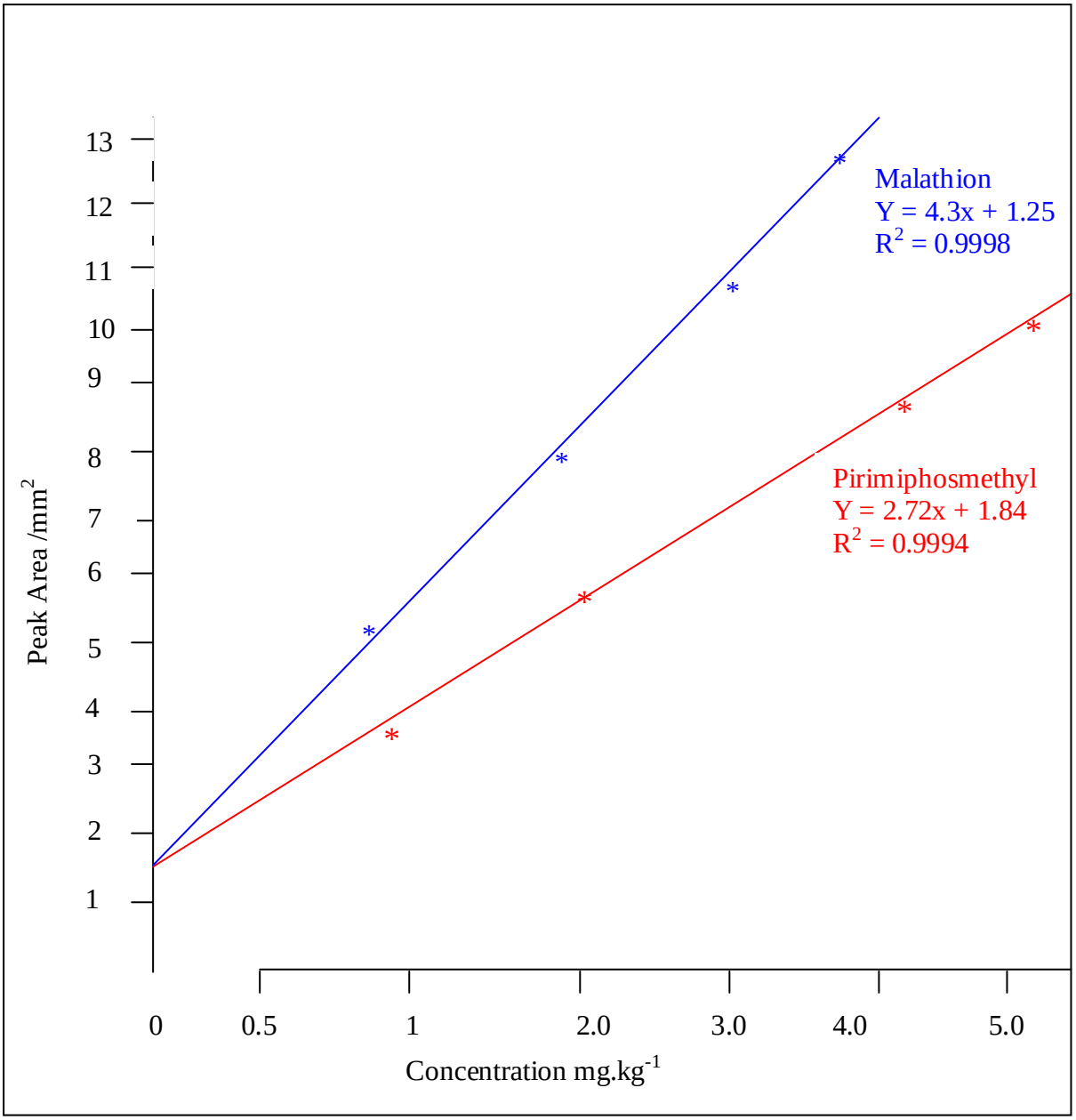


Fig. 4.1: Standard calibration curves for malathion and pirimiphosmethyl

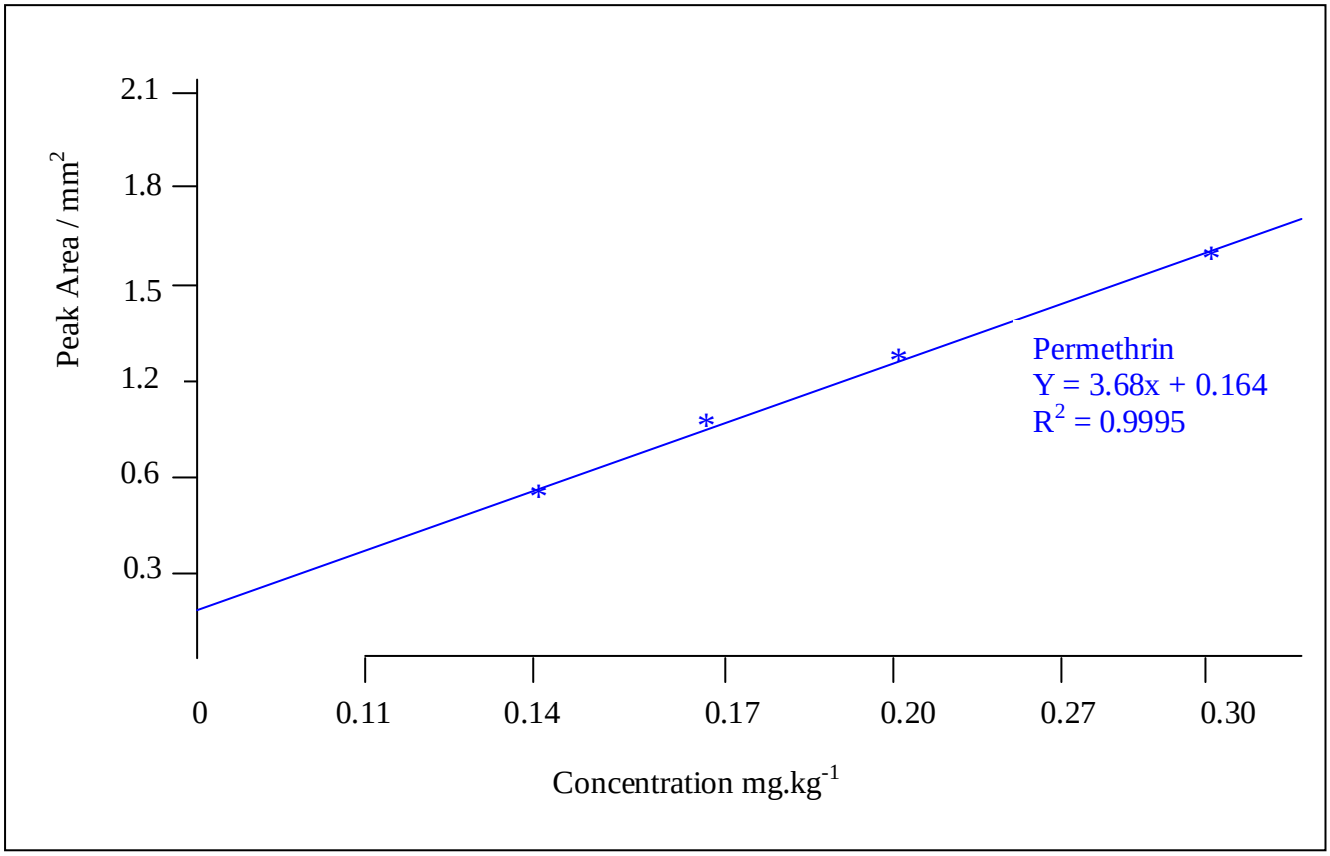


Fig. 4.2 Standard Calibration curve for Permethrin

4.2.2 Malathion residue concentration in maize (grains, white flour and whole meal flour)

The results of the analysis of malathion residue levels in the flour samples (white flour and whole meal) were recorded in Table 4.9. The results indicate that wholemeal flour contained higher malathion residue levels than whiteflour from each collection site. It was observed that residues decreased in both food commodities over the consumption period of 120 days, and that there was no significant correlation in the residue levels for the two flour commodities ($r = 0.616$ $df = 8$ $P = 0.01$). A highly significant difference was observed in the malathion residue found in the two flour commodities with whole meal flour containing higher pesticide levels than white flour ($F = 11.09$ $df = 149$ $P < 0.05$). This difference was attributed to the differences in the milling methods applied. While wholemeal flour carried the testa cover which contained most of the pesticide the white flour carried non of the testa material. White flour obtained from different collection sites did not show significant difference in malathion levels ($F = 0.096$ $df = 74$ $P > 0.05$). The mean residue level for wholemeal flour was 1.40 $mgkg^{-1}$ and for white flour was 0.53 mgkg-^{1} in the whole study area (Table 4.9). In both flour commodities, the mean malathion residue level was above the ADI level (0.02 $mgkgday^{-1}$), for malathion as recommended by the joint meeting for pesticide residues (JMPR) of the World Health Organization (FAO /WHO, 1994.). This means that, a health hazard index analysis needs to be done to establish whether these flour commodities are risky to the health of a consumer. Such a risk will be aggravated by the pesticide retention capacity of a flour commodity.

Table 4.9: Mean levels of malathion residues in maize whole meal and white flour obtained from farmers.

The malathion residue retention behaviour of the whole meal flour was expressed in Fig. 4.3. The profiles show no significant difference in malathion residue for the whole meal collected from various locations in Chilchila division (F = 0.096 df = 149 P > 0.05). This meant that different families from the study area were perhaps exposed to similar levels of malathion residue from wholemeal flour. It also meant that the milling methods for whole meal flour in all the five sites were similar.

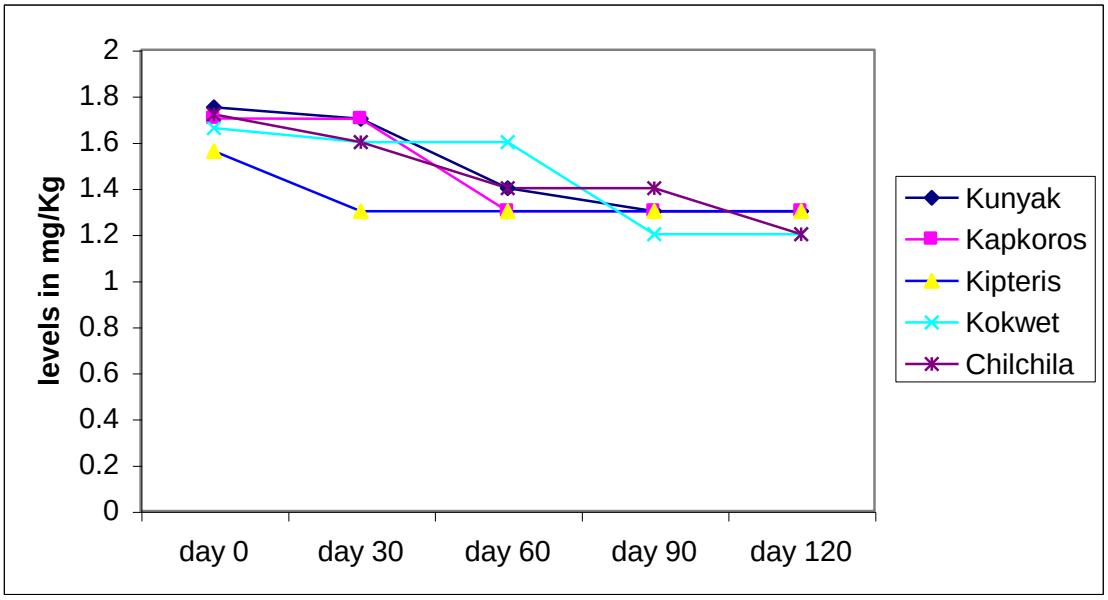


Fig. 4.3: Malathion residue retention profiles for wholemeal from different sites

The residue retention profiles further revealed a decreasing trend for residue levels as the collection time increased. The highest residue level was found in Kunyak collection sites (1.7 $mgkg^{-1}$) while the lowest residue level (1.2 $mgkg^{-1}$) was found in the collection sites of Kokwet and Chilchila. However all the profiles ran above the accepted daily intake (ADI) level of 0.02 $mgkg^{-1}$per day, (FAO / WHO, 1994) implying a possible health risk to the consumer. This longitudinal decrease in residue level over the collection period from each of the collection site was attributed to the breakdown of pesticide through microbial activity on the maize grains milled to

produce the flour. The milling machine also had a running air current mechanism that blew off some of the surface pesticide from the grains before milling into flour thus reducing the pesticide level on the resulting whole meal flour.

Analysis of residue levels in maize products (whiteflour, wholemeal) indicated that milling alone was not completely efficient in reducing pesticide residue levels from grains to acceptable daily intake (ADI) levels as recommend by WHO. Any pesticide residue, above the ADI level exposed the consumer to imminent health risk. The farmers in Chilchila division therefore used a number of other cleaning techniques to reduce the residue levels on the grains. Some of these techniques include, water washing, hulling and detergent washing.

4.2.2.1 Effects of cleaning methods on malathion residues in maize grains

A combination of two of these methods (hulling and water washing) followed by boiling the gains at 100^0C for 90 minutes was the most common cleaning and processing technique used by most households in the study area (Table 4.10). Each cleaning method had different ability of removing pesticide residues, such that thermal processing (boiling at 100^0C for 15 minutes) removed the highest amount of malathion residue (63.2%) while water washing removed the least pesticide amount at (11.07%). Hulling + water washing increased the amount removed to 22.8% and detergent washing (1% neutral soap) removed 35.08% of the pesticide. Hulling plus detergent washing removed even higher amounts of residue at 47.04%. Anova analysis revealed a significant difference in the methods abilities to remove malathion residues ($F = 6.495$ $df = 125$ $P<0.05$). This means that the choice of the cleaning method a consumer used ultimately determined the amount of pesticide consumed.

In Chilchila division the most common cleaning method was the hulling plus water washing. This was followed by thermal processing (boiling at 100^0C) which removed more malathion residue at (71%) and bringing the residues down to a level of 0.65 $mgkg^{-1}$ per day. This reduced the health risks likely to arise from the maize grain consumption. The residue levels decreased with increase in time. Malathion has a less tendency to move into deep waxy layers of grain and therefore remained on the surface of the grains for longer periods where it had maximum contact with degrading microflora (Desmarchelier and Bengston, 1979). It therefore, broke down rapidly within a short storage interval. Anova analysis between storage intervals indicated a significant difference in amount of malathion residues remaining (retained) on maize grains at each interval ($F = 43.47$ $df = 125$ $P< 0.05$). Malathion pesticide has a high solubility in water and therefore any residue remaining on the surface after degradation was easily washed off by water, meaning malathion was not persistent on grains (Focus, 2006).

The malathion levels for maize food commodities of whole meal (1.4 $mgkg^{-1}day^{-1}$) white flour (0.53 $mgkg^{-1}day^{-1}$) and grains cleaned in the most common method of hulling, water wash and boiling (0.65 $mgkg^{-1}day^{-1}$) were combined with the average daily food consumption rates of an adult (1.2 kg day^{-1}) and child (0.9 kg day^{-1}). The resulting product was then divided by the average body weight of the consumer adult (70 kg) or child (10 kg) to give a pesticide exposure dosage value for each of the consumer. The exposure dosage was then divided by the value for the accepted daily intake (ADI) for malathion (0.02 $mgkg^{-1}$) to give a hazard index for each food commodity.

Table 4.10: Malathion residue levels remaining on maize grains after applying various processing methods.

When this index is greater than unit (1) then the consumer is said to be at health risk. Such exposure dosage and hazard index calculations are done using equation (B-7) and (B-8) in appendix B. Results for malathion pesticides are shown in Table 4.11.

Table 4.11 Malathion exposure dosage and hazard index values from maize white flour, whole meal and maize grains (cleaned by hulling, water wash and boiling)

Food commodity	Exposure dosage / $mgkg^{-1}$ x 10^{-2}		Hazard index
White flour	Adult	0.34	0.17
	Children	1.80	0.90
Whole meal	Adult	0.84	0.45
	Children	4.68	2.34
Grains (cleaned by hulling, water wash and boiling)	Adult	1.14	0.57
	Children	6.00	3.00

It was observed that the malathion hazard indices from whiteflour (0.17), whole meal (0.45) and cleaned grains (0.57) revealed no health risk to an adult consumer. This is because their health hazard index values were less than unit (1). However, children in the study area were at a high risk of suffering from pesticide related illnesses through consuming wholemeal (2.34) and maize grains (hazard index = 3.00) treated with malathion pesticide and prepared by the common methods of hulling, water wash and boiling. Hazard index for white flour (0.90) indicated no health risk to children consumers as it is less than unit (1).

4.2.3 Permethrin residue concentrations in maize (grains, white flour and wholemeal)

The results of the analysis of permethrin residues in maize grains, whole meal and white flour are presented on Table 4.1.2. In general it is observed that wholemeal flour contained higher residue levels than white flour from any of the sample collection sites of Kunyak, Kapkoro, Kitperis, Kokwet and Chilchila. The residue levels in each type of flour decreased with increase in collection time. The results also show that all the collection sites had almost the same mean residue levels for wholemeal flour (0.17 $mgkg^{-1}$). Similarly mean residue levels for whiteflour was almost the same for all collection sites (0.06 $mgkg^{-1}$).

Anova analysis indicated a significant difference in the permethrin pesticide levels between wholemeal and white flour ($F=11.85$ $df = 148$ $P < 0.05$). This is attributed to the differences in milling methods where wholemeal floor was milled including the testa that carries most of the pesticide while whiteflour carried none of the testa material. However, Anova analysis for each flour commodity indicated no significant difference in pesticide levels over the duration of storage from 0 to 120 days
($F = 0.961$ $df = 26$ $P > 0.05$). This implies that permethrin undergoes a slow rate of breakdown over the storage time. This is explained by the pyrethroids tendency to move deep into endosperm where it is shielded from microbial degradation effects (Bengston *et al.*, 1983).

Table 4.12: Mean levels of permethrin residues in maize whole meal and white flour obtained from farmers.

The amount of residue retained in white flour at each collection site or analysis date is plotted on graphically (Fig 4.4) to show the permethrin residue retention profile for each collection site. The profile lines show a small decline on residue levels between the initial day (day 0) and the last day (120 days). This implies that milling caused very little difference in permethrin residue level over the whole collection period. The profile lines also run close to each other implying that the milling process in various collection sites yielded similar levels of pesticide though Kunyak collection site had a slightly higher mean residue (0.07 $mgkg^{-1}$) than all the other sites while Kokwet collection site had the lowest mean residue at 0.04 $mgkg^{-1}$. The mean residue level for all the collection sites was 0.06 $mgkg^{-1}$ which was almost equal to the accepted daily intake (ADI) level of 0.05 $mgkg^{-1}$ (FAO/WHO, 1994).

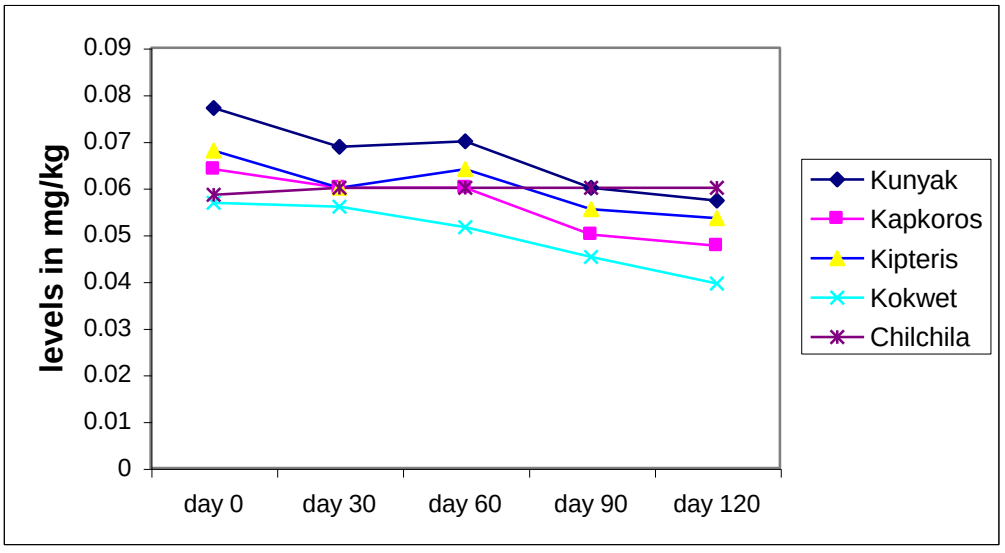


Fig. 4.4: Permethrin residue retention profiles for whiteflour from different sites

Fig 4.5 shows the permethrin residue retention profiles for wholemeal flour from different collection sites in the study area. These profiles revealed that there was no significant difference in the residue levels between all the collection sites, and this observation is confirmed through Anova analysis for the different collection sites ($F = 1.967$ df $= 74$ $P > 0.05$). The mean residue level in wholemeal flour was 0.17 $mgkg^{-1}$ for all collection sites which was above ADI level of 0.05 $mgkg^{-1}$. This high residue level is attributed to the high tendency of permethrin to move deep into endosperm where it was shielded from effects of microbial degradation, photodegradation and hydrolytic degradation that occurs on the grain surface (Bengston *et al.*, 1983).

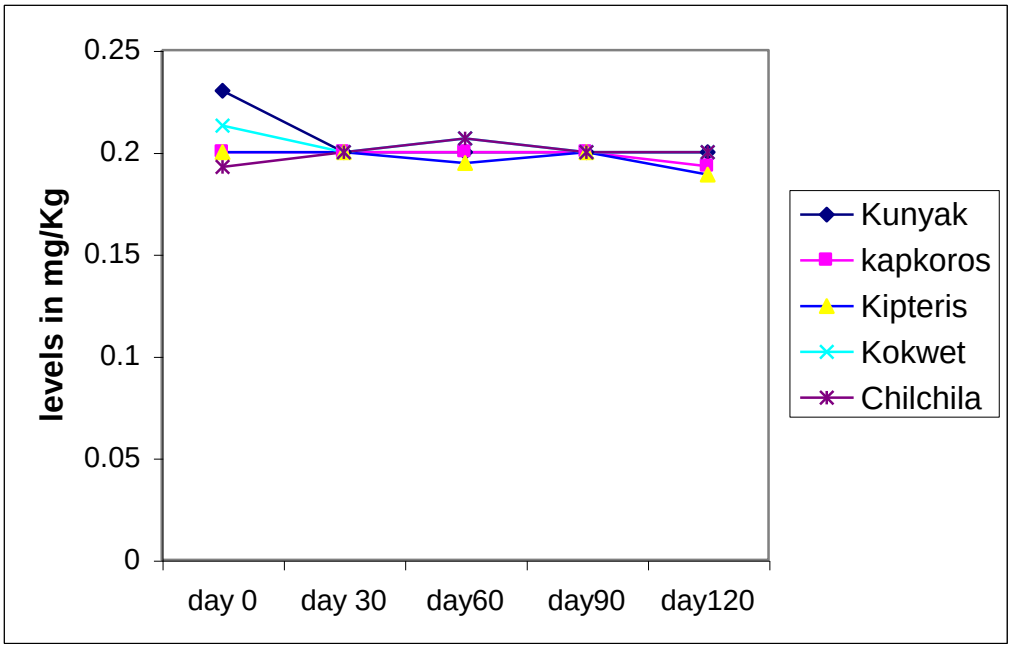


Fig. 4.5: Permethrin residue retention profiles for wholemeal from different sites

4.2.3.1 Effect of cleaning methods on permethrin residues in maize grains.

Permethrin residue retention on grains after performing various cleaning methods was studied through maize grains which were collected from farmers at each month end for four consequtive months. During the first month the grains were collected after every 7 days, then at the end of each month for the next three months. The grains had been treated with a commercial pesticide "skana" which contained permethrin active ingredient at a concentration of 0.3 $mgkg^{-1}$ and stored by the farmer at the common storage facility where temperatures ranged between 15^0C to 22^0C while relative humidity ranged between 50% to 55%.

At the laboratory the grains were then divided into 7 equal samples with each sample being cleaned through simulated cleaning methods similar to those practiced in the study area. Such cleaning methods are shown in Table 4.13. Each sample was then analysed for the residue levels retained after cleaning. The results of the residue retained on maize grains after each cleaning method on the collection analysis date is shown on Table 4.13. Total percentage of permethrin residue removed by various cleaning methods were water wash (10%), hulling plus water wash (20%), detergent wash (30%) and boiling at 100^0C for 90 minutes (50%). The most common cleaning method practiced in the study area was a combination of hulling plus water wash then boiling before consumption. This method removed (63%) of pesticide, leaving an average residue level of 0.09 $mgkg^{-1}$ on the grains. A comparison between permethrin and malathion pesticide amounts removed after the cleaning methods shows that the percentage amount of permethrin removed by the water washing method was less than that of malathion removed by the same cleaning process. Thus water washing removed 10% permethrin, 12% malathion.

Table 4.13: Permethrin residue remaining on maize grains after applying various processing methods.

Less permethrin was removed from grains because it (pyrethroid) is a less polar pesticide than malathion (organophosphates). Permethrin is therefore less effectively washed off from grains surface by polar solvents like water. Also permethrin had a higher tendency to move deeper into the waxy layers (auleron) of the grain where it was inaccessible to the degrading microorganisms. This meant that permethrin could be retained inside the grain for longer periods than malathion. The low permethrin pesticide removal could also be attributed to its low volatility and low hydrolysis like it is for all pyrethroids. The implication of these three observations was that permethrin was more persistent (highly retained) on the grains than malathion. The health risk estimate to the adult and child consumer caused by each of the maize product studied was calculated as shown in Table 4.14.

Table 4.14: Permethrin exposure dosage and hazard index values from maize whiteflour, whole meal and grains (cleaned by hulling, waterwash and boiling)

Food commodity	Exposure dosage $mgkg^{-1} \times 10^{-3}$		Hazard index
White flour	Adult	0.514	0.010
	Children	2.70	0.054
Whole meal	Adult	1.46	0.029
	Children	7.65	0.153
Grains (cleaned by hulling, waterwash and boiling)	Adult	1.54	0.031
	Children	8.10	0.162

The hazard indices for adults from whiteflour (0.010), wholemeal (0.029) and grain (0.031) are each less than unit (1). This indicated no health risk to the adult consumer. Similarly hazard indices for children from white flour (0.054), wholemeal (0.153)

and grains (0.162) are each less than unit (1) thus showing no health risk to children consumers. In general therefore it can be concluded that permethrin residue levels in common maize food stuffs consumed in the study area causes no health risk to either adult or children consumers.

4.2.4 Pirimiphosmethyl residue concentration in maize (grains, whiteflour and wholemeal)

The results of analysis of pirimiphosmethyl residues in maize whole meal and whiteflour are shown in Table 4.15. In general, the wholemeal is found to contain higher mean residue levels (1.76 $mgkg^{-1}$) than whiteflour (0.51 $mgkg^{-1}$) in the whole study area. Pirimiphosmethyl levels for each collection site decreased with increase in collection time from 0 to 120 days for both flour commodities. Pearson correlation analysis for each of the flour commodities indicates no significant linear relationship between residue concentration and collection times ($r = 0.389$ $df = 8$ $P = 0.01$). This suggests that the trend for residue concentration against collection time is perhaps a curvelinear trend.

One way Anova analysis showed that there was a significant difference in the pesticide residue levels of the wholemeal and white flour commodities ($F = 36.586$, $df = 149$ $P < 0.05$). This could be attributed to the milling process that significantly reduced pesticide levels in white flour by removing the outer seed coat (testa) while whole meal flour was milled including the testa that carried high pesticide levels. The white flour contained lower levels of residue which were bound to the endosperm material.

Table 4.15: Mean levels of Pirimiphosmethyl residues in maize whole meal and white flour obtained from farmers.

Trace residues that were bound to lipoprotein material of the grain could not be detected as they could only be extracted through digestion of auleron protein (Rowland, 1991). Majority of the families in Chilichila were exposed to a common average level of pirimiphosmethyl residue from white flour (0.57 $mgkg^{-1}$) so that there was no significant difference in white flour residues collected from various locations ($F = 1.967$ $df = 74$ $P>0.05$). However, since pesticides were concentrated on the outer seed coat (testa) of the grains and the coat was removed while milling, the whiteflour therefore contained low pesticide residue.

The pesticide moved from the testa into the endosperm as the sampling days increased. This raised the pesticide level in endosperm (white flour) as is indicated by the rising profile lines for the first 30 days (Fig. 4.6). After the first 30 days, the pesticide decreased very slightly for the rest of sampling days, because the pesticide in the endosperm was shielded from the various breakdown processes, indicating an average residue of 0.57 $mgkg^{-1}$ for all collection sites. This level was however, greater than the ADI level of 0.01 $mgkg^{-1}$ (FAO / WHO, 1994).

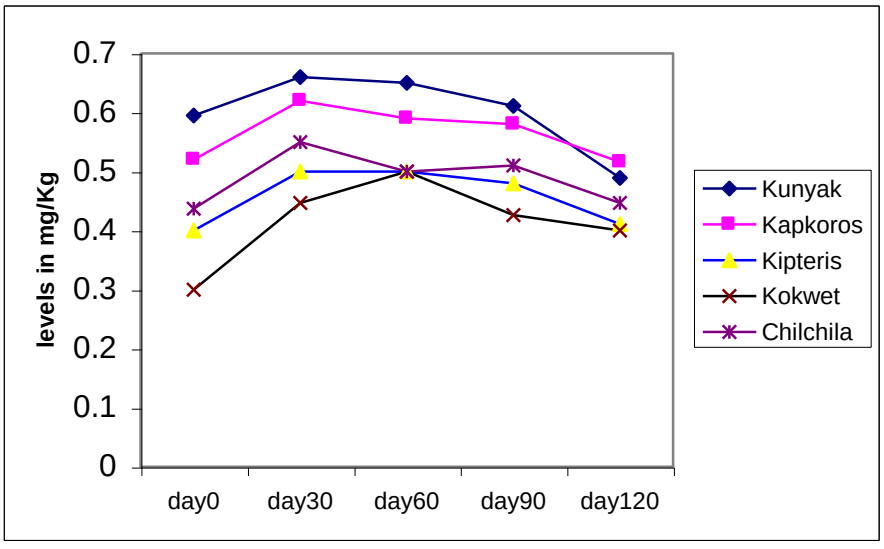


Fig 4.6: Pirimiphosmethyl residue retention profiles for white flour from different sites

The whole meal pesticide residue retention profiles Fig. 4.7 shows that there was significant difference in residues for the wholemeal milling fraction (F= 30.00 df=149 P<0.05) collected from various locations. This means that residue levels in wholemeal flour were not the same between the various locations. This difference could be attributed to the differences on cleaning methods that occurred between the collection sites (locations) which removed different pesticides amounts before milling. Average level of pesticides in the wholemeal flour for the whole study area was 1.76 $mgkg^{-1}$ (Table 4.15) and which was greater than the WHO recommended ADI level of 0.01 $mgkg^{-1}$ day^{-1} (FAO/WHO,1994). The wholemeal residue retention profile lines indicated the decreasing trend from the first day of pesticide application upto the last day of sampling.

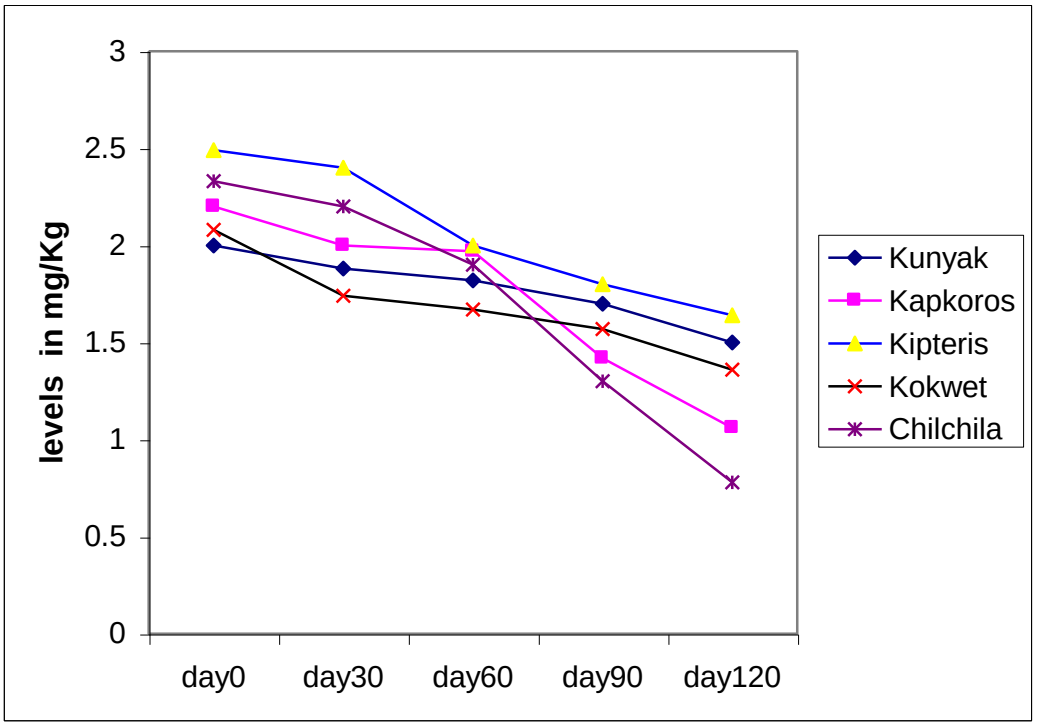


Fig 4.7: Pirimiphosmethyl residue retention profiles in whole meal from different sites

This was due to the reduction of pesticide residue on the seedcoat through various degradation process of microbial, photodegradation and hydrolysis enhanced by storage conditions of light, temperature and humidity in the farmers store. Cleaning methods and milling processes also contributed to the reduction of the pesticide from the grains.

However, milling and degradation processes are not completely efficient in reducing pesticide residue levels from grains. It is therefore necessary to apply extra pesticide removing techniques in order to lower the pesticide levels to acceptable daily intake (ADI) levels recommended by World Health Organization (FAO / WHO, 1994). Some of these techniques included, water washing, hulling and detergent washing. A combination of two of these methods (hulling and waterwashing) followed by boiling the grains at 100^0C was the most common cleaning and processing technique used by most families in the study area.

4.2.4.1. Effect of cleaning methods on pirimiphosmethly levels in maize grains.

Pirimiphosmethyl residue retention on grains after performing various cleaning methods was studied through maize grains which were collected from the farmers at the end of the month and transported to laboratory, where they were divided into 7 equal samples. Each sample was then cleaned through simulated cleaning methods similar to those used in the study area as listed in Table 4.16. After cleaning, the grains were analysed for the pirimiphosmethyl pesticide level that remained (retained) on the grains and the data was recorded in Table 4.16. The percentage of residue removed by each processing method was calculated according to equation (B-10 Appendix B).

Table 4.16: Pirimiphosmethy residues remaining on maize grains after applying various processing methods**.**

It was observed that the residue retention trend for each cleaning method decreased with increase in collection time. One way ANOVA analysis revealed a significant difference in residue retained on maize grains after performing the different cleaning methods ($F = 25.806$ $df = 125$ $P<0.05$). This means that different cleaning methods had different abilities of removing pirmiphosmethyl residue from maize as is indicated by the percentage residue removed by each processing method. Thus the conventional water wash method removed the least pesticide residue (10%). Adding a detergent to water added its power to solubilise the residue thus removing (30%) of pesticide residue.

The hulling process involved the agitating and blowing off a portion of pesticide from the grains before detergent washing thereby making this cleaning combination method more efficient in removing the residue at (45%). Thermal treatment involved boiling the grains for 90 minutes at 100^0C. This method increased volatilization of the pesticide and broke down the residue, thus removing the highest amount of pesticide residue at 60%. The most common cleaning method practiced by the majority families in the study area included hulling plus water wash then boiling. This method removed (67%) of pesticide residue and retained 1.1 $mgkg^{-1}$ pesticide residue; which was a hundred fold above the ADI level (0.01 $mgkg^{-1}$ day^{-1}).

Hazard index analysis for the residue levels in the maize food commodities was necessary in order to reveal the extent of health risk of pirimiphosmethyl to the consumer. Such health risk estimates were calculated through exposure dosage values of whiteflour, wholemeal and maize grains (Snodgrass, 2001) and Table 4.17 shows the values of such hazard indices.

The values for wholemeal flour (1.20) and grains (1.70) indicated a health risk to adult consumers since they are greater than unit (1) while the value for whiteflour (0.39) indicated no health risk to the adult consumer as it is less than unit (1).

All the hazard index values for white flour (2.07), wholemeal flour (6.30) and grains (9.00) indicated a health risk to children consumers since the values are all greater than unit (1).

Table 4.17 Pirimiphosmethyl exposure dosage and hazard index values from maize whiteflour, whole meal flour and grains in (cleaned by hulling, waterwash and boiling)

Food commodity	Exposure dosage ($mgkg^{-1}$) x 10^{-2}		Hazard index
White flour	Adult	0.39	0.39
	Children	2.07	2.07
Whole meal	Adult	1.20	1.20
	Children	6.30	6.30
Grains (cleaned by hulling, waterwash and boiling)	Adult	1.70	1.70
	Children	9.00	9.00

Figure 4.8 shows plots of pirimiphosmethyl residue retained on maize grains after the various cleaning and treatments were carried out. The plots show that the residue amount retained on the grains decreased with time depending on the effectiveness of a particular method in removing the pesticide from the grains. The pirimiphosmethyl, being an organophosphorus pesticide had higher solubility in water and a reduced prospensity to move deep into wax layers of the grains.

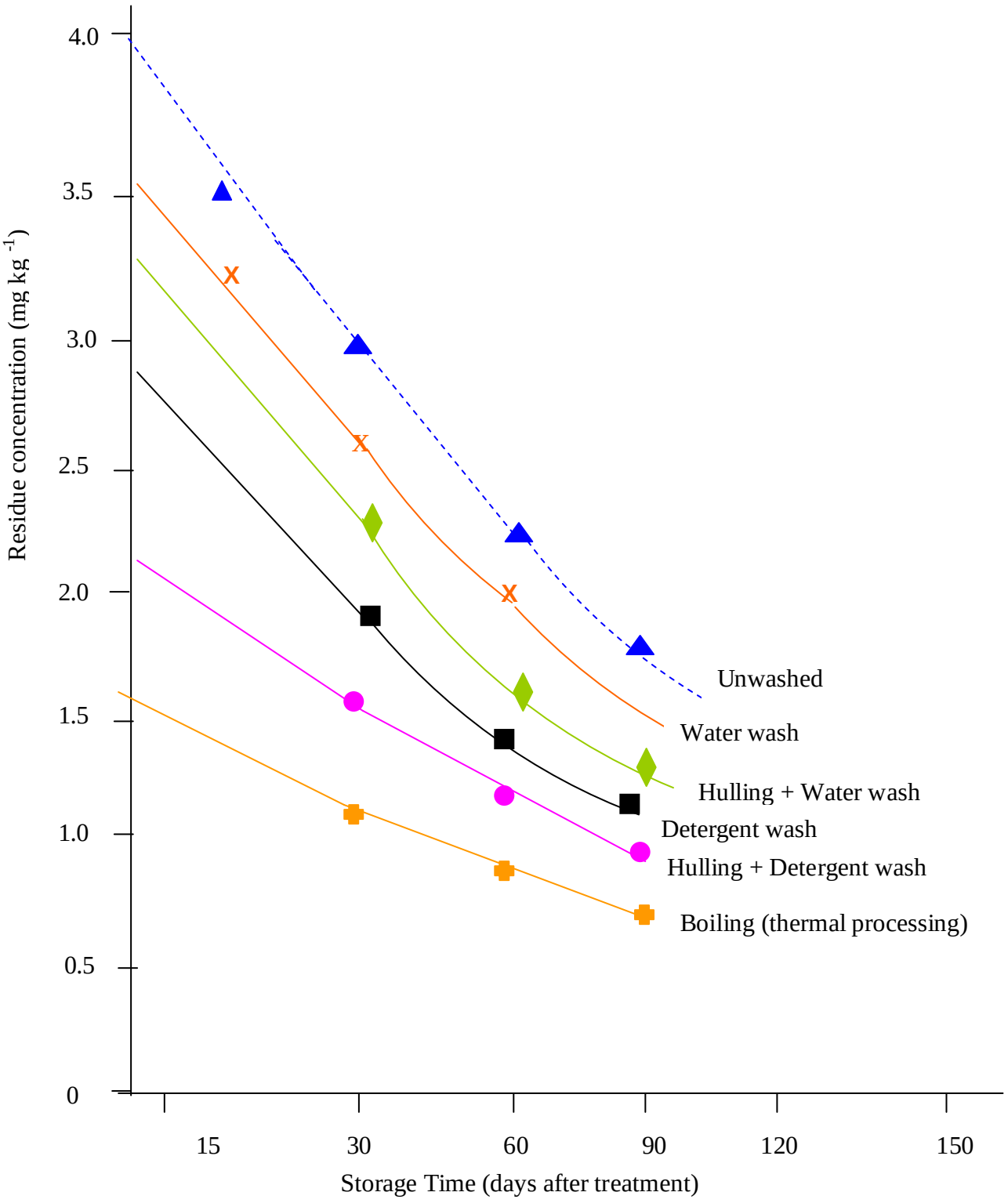


Fig 4.8: Effect of various processing methods on pirimiphosmethyl residues retained on maize grains.

It therefore follows that its residues were confined to the outer surfaces of the grains, and could be removed easily by water washing. Combining a number of cleaning methods like hulling and water washing proved even better in removing the residue, so that the graph line for this cleaning method ran below that of ordinary water wash method.

Adding a detergent to the water made it even a better method for removing the organophosphorous pesticide. This was because detergents usually help to solubilise many organic compounds in water. However, it was the boiling method that removed most of the pesticide from the grains. This was because the heat denatured the pesticide molecule and broke it down. The residue retention profiles (Fig. 4.8) showed that cleaning the grains thoroughly followed by boiling before consuming was an important step towards reducing the levels of pesticides ingested and hence lowering the incidences of health risks in the study area

4.3 Pesticide residue concentrations under different storage conditions

The Result for the analyasis of malathion, pirimiphosmethyl and permethrin residues in maize stored under different conditions of temperature, light and humidity are presented in the following section.

4.3.1 Permethrin residue in maize grain under different storage conditions

The result obtained for the analysis of permethrin residues where recorded in Table 4.18. In general the results indicate that grains stored under high temperature, high humidity and high light conditions contain lower permethrin levels than those stored in low temperature, low humidity and low light.

Table: 4.18: Pesticide residue concentrations in maize grains treated with a commercial formulation (SKANA) at 0.3mgkg^{-1} permethrin and held in different storage conditions for 180 days

The residue on the grain stored under any storage conditions decreased with increase in storage time from 0 to 180 days. For a storage condition of 30^0C, light, 65% RH the residue decreased from 0.25 $mgkg^{-1}$ to 0.18 $mgkg^{-1}$ in a period of ninety (90) days. For a storage condition of 15^0C, light and 50% RH the residue decreased from 0.25 $mgkg^{-1}$ with increase in storage time upto 0.20 $mgkg^{-1}$ and was detectable over the whole period of 180 days. This meant that high temperature and relative humidity (RH) broke down the pesticide at a faster rate than low temperature and relative humidity (RH). At the ambient storage conditions of the study area (22^0C, light, 55% RH) the pesticide levels decreased from 0.25 $mgkg^{-1}$ to 0.19 $mgkg^{-1}$ in the storage period of 150 days. The average pesticide level during this storage period was 0.22 $mgkg^{-1}$ which was above the accepted daily intake (ADI) level of 0.05 $mgkg^{-1}$ day^{-1} (FAO / WHO, 1994) for permethrin. Storage conditions of highlight, temperature and humidity supports degradation microorganisms on the surface of maize grains in the forming of colonies. This therefore enhances the rate at which the microorganisms break the pesticide down. However, low light, low temperature and low humidity are not conducive conditions for the multiplication of microbial organisms and therefore lowers pesticide degradation. Similarly in a farmer's store, where temperature, humidity and light are high, the pesticide breaks down faster than in a storage facility where the temperature, humidity and light are low (Walker *et al*, 1992).

Pearson Correlation analysis for residue concentration and storage time under different storage conditions indicated a nonlinear relationship between the residue concentration and storage time ($r = 0.251$ $df = 8$ $P = 0.01$). This means therefore the pesticide breakdown process has a curvelinear trend as is shown graphically in Fig. 4.9.

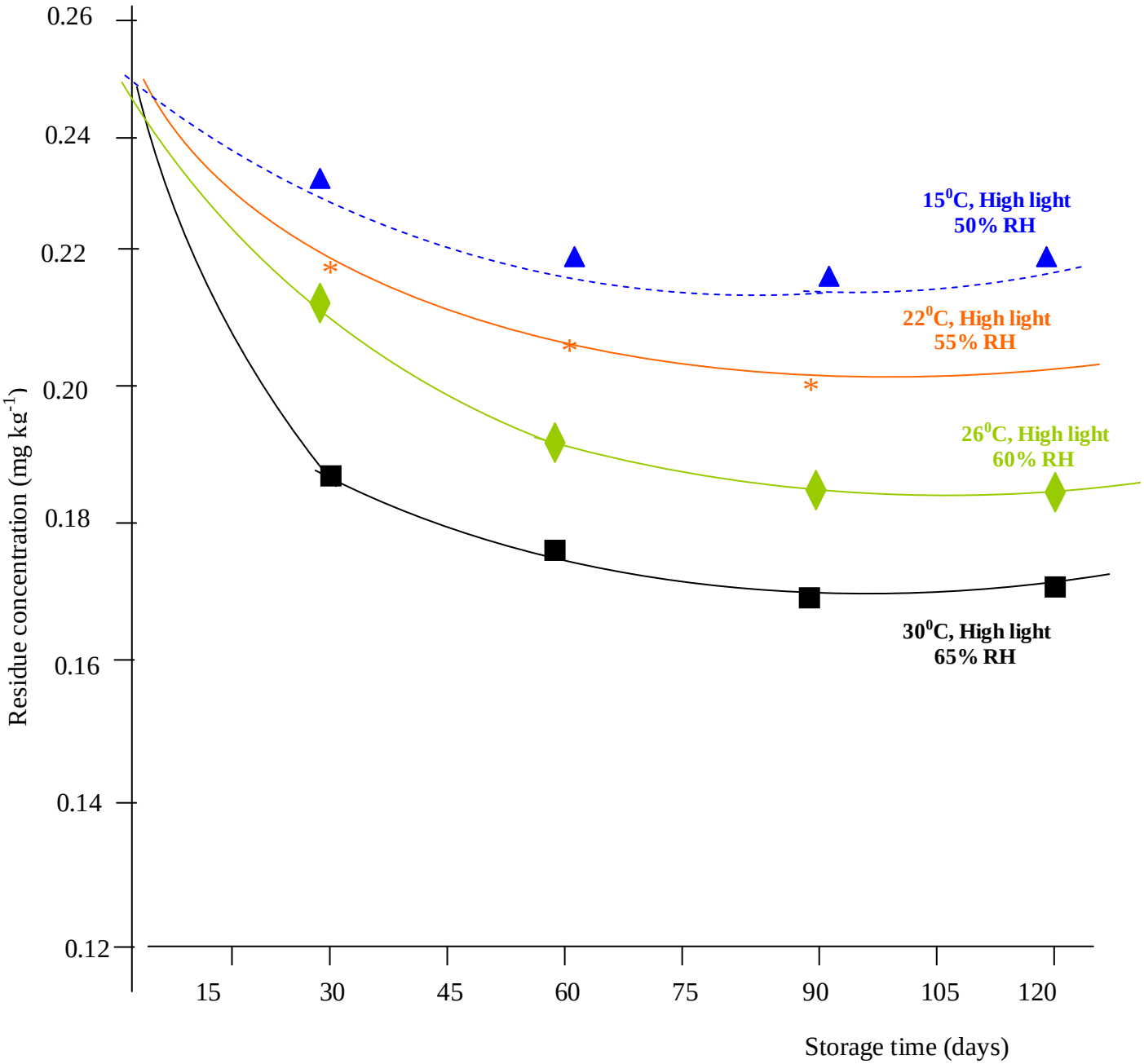


Fig. 4.9: Degradation curves of permethrin pesticide under high light and various storage conditions for temperatures and RH

These trends follow biphasic sigmoidal paths with an initial rapid decrease in concentration during the first 45 days. This is attributed to vigorous extracellular microorganism activity that is controlled by light, temperature and humidity. Pesticides are concentrated at the surface of maize grains shortly after application. The microorganisms are also concentrated at the surface or in the outer regions of maize grains (Priesack and Kisser, 1993). These two relations improve the contact between pesticide and the degrading microbial organisms. The storage conditions of high light, temperature and humidity further supports the thriving microorganisms. These three conditions therefore resulted in a rapid and enhanced initial permethrin degradation. However, there was a slower degradation for the rest of storage period between 45 to 120 days. This was because permethrin moved deeper into cuticular waxes (auleron layers) of the grains after a long storage period. The smaller pores inside the grains were too small to allow significant microorganism colonization. Oxygen and nutrients were also depleted by microorganisms at the surface of the grains leading to insignificant microbial growth inside the grain seeds. As a result the pesticide retained inside the grain was inaccessible to microorganisms thus protected from degradation and therefore led to a low degradation rate.

The degradation mechanism of permethrin involve light as a catalyst. The high light storage conditions use ultra violet light (UV) energy to cause the permethrin pesticide to break down by cleaving the molecule at the C-C bond and C-O bonds at the positions shown in Fig. 4.10.

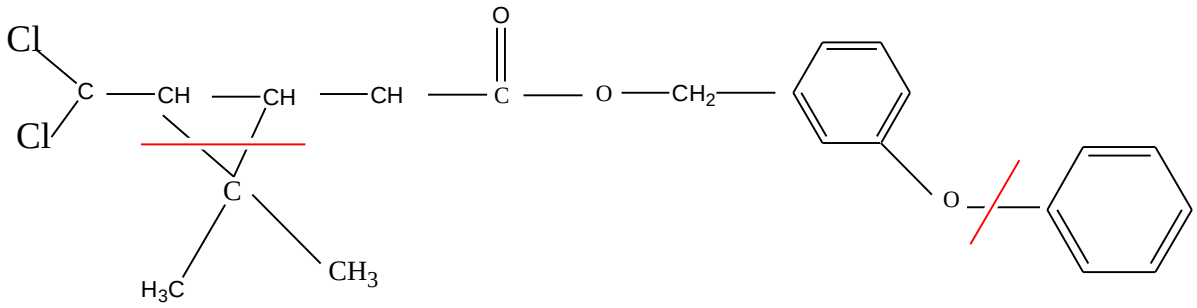


Fig. 4.10 Cleavage of C - C bond and C – O bond by UV in permethrin (Desmarchelier, 1977)

4.3.1.1 Effect of high light on permethrin degradation under different temperature and humidity

This degradation under the highlight, temperature and relative humidity storage conditions approximate first order reaction kinetics. Since the concentration of pesticide in consideration was very small, the first order degradation kinetics could be expressed as (Walker *et al.,* 1992)

$$\frac{dc}{dt} = -kc \quad \text{.....[4.1]}$$

Where (c) is the concentration of permethrin pesticide, (k) is the first-order rate constant and (t) is the storage time. This first-order rate constant is often replaced by half-life ($t_{1/2}$) where

$$t_{1/2} = \frac{\ln 2.}{k} \quad \text{.....[4.2]}$$

The first –order equation can then be written as

$$\frac{dc}{dt} = \frac{-0.693c}{t_{1/2}} \quad \text{.....[4.3]}$$

Since the degradation rate remained constant during the whole degradation process, the residue concentration (C_t) of permethrin at any storage time, (t) during the storage period and an initial concentration (C_0) was given by equations (Rocha and Walker, 1995).

$$c_t = c_0 e^{-kt} = C_0 e^{-0.693t/t_{1/2}} = C_0 [0.5]^{t/t_{1/2}} \quad \text{.....[4.4]}$$

$$c_t = c_0 [0.5]^{t/t_{1/2}} \quad \text{.....[4.5]}$$

When the half-life ($t_{½}$) for a pesticide in a given storage microclimate (temperature, light, humidity) and the initial concentration of pesticide applied (C_0) to maize are known we can calculate the concentration (mg kg^{-1}) of pesticide residue on the maize at any time (t) during its storage. This means that two farmers whose stores have different ambient microclimates and hence different pesticide half-lifes, will have different concentrations of pesticide residue on their maize. The higher the half-life ($t_{1/2}$) the bigger the pesticide residue concentration (C_t) at any time during storage period.

The data for residue concentration [A] in Table 4.18 was given a log transformation so as to yield a linear oriented data for log [A]. A plot of the log concentration log [A] against storage period (days) for the grains gave straight line graphs for each set of storage conditions (Fig 4.11). Reaction kinetic interpretation of the straight lines indicated that the pesticide breakdown was a first order reaction whose mathematical expression was given by equation [1.3] (Perez-Benditto and silva, 1988). The straight line graphs in Fig 4.11 met at a common point on y-axis, Log $[A]_0$, which represented the logarithm of the initial pesticide concentration applied to the grains.

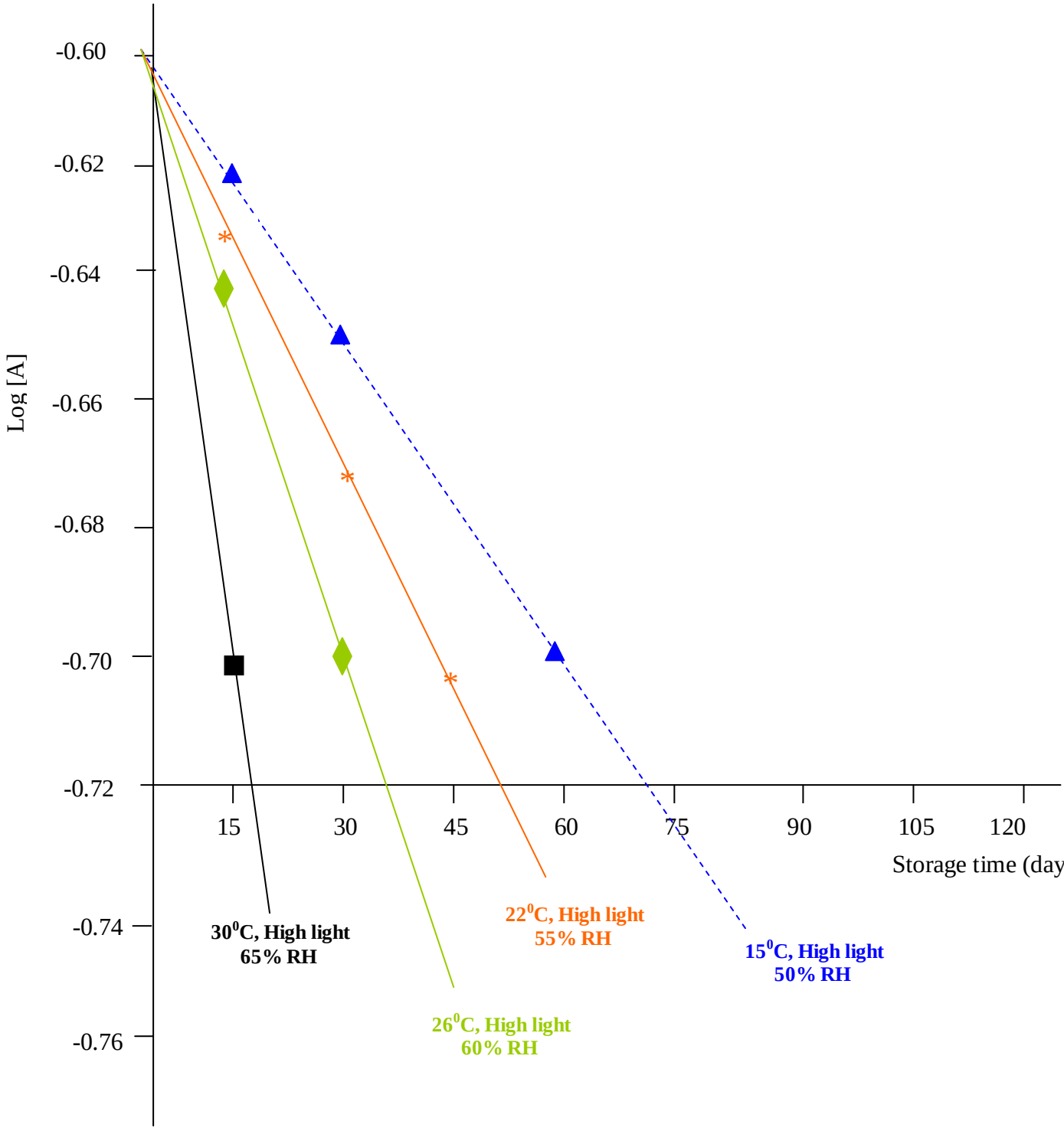


Fig 4.11: Log residue concentration against storage time (days) for different storage conditions of temperature and relative humidity (RH) at high light for permethrin

Each line followed its own degradation path described by a characteristic gradient whose value was equal to the term $k^{-1}/2.303$. It was found that the graph representing the highest temperature and humidity (30^0C, 65% RH) had the highest gradient of 6.60×10^{-3} while the graph representing the lowest temperature and humidity (15^0C, 50% RH) had the smallest gradient of 1.7×10^{-3}. Gradients for the other storage conditions of (22^0C, 55% RH) and (26^0 C, 60% RH) were 2.4×10^{-3} and 3.26×10^{-3} respectively.

Each gradient value of the graph was equated to the gradient term of the equation ($k^{-1}/2.303$) to evaluate the value of k^{-1}, which in turn was used to calculate the pesticide degradation half-life ($t/_{1/2}$) for each storage condition. Table 4.19 shows these half-lifes for the permethrin pesticide. These half-lifes were a direct measure of the pesticide persistence and showed the power of the pesticide (permethrin) to resist degradation. A pesticide with high half-life means it would stay longer in the environment and in the body of a consumer and this translates into a possible heath risk.

Table 4.19: The gradient ($k^{-1}/2.303$), k^{-1} and half-life ($t/_{1/2}$) values for permethrin degradation in maize stored under highlight, varying temperature and relative humidity (RH).

Storage condition	Permethrin		
	$k^{-1}/2.303 \times 10^{-3}$	$k^{-1} \times 10^{-3}$	$t_{1/2}$, days
(15^0C, High light, 50% RH)	1.70	3.75	210
(22^0C, High light, 55% RH)	2.40	5.50	130
(26^0C, High light, 60% RH)	3.26	7.50	93
(30^0C, High light, 65% RH)	6.60	15.00	46

The half-lifes for permethrin (Table 4.19) indicated that its dissipation was dependent on the storage conditions. Grains that were stored at lowest temperature and lowest humidity (15^0C, 50% RH) had the longest half-life (210 days) hence highest residue concentrations. This is because under these conditions the microbial and hydrolytic degradation are low (Rocha and Walker, 1995). Half-life for other storage conditions of (22^0C, 55% RH) was 130 days and (26^0C, 60% RH) was 93 days, respectively. This showed that the higher the storage temperature and relative humidity, the higher the rate of degradation of permethrin pesticide and the lower the concentration of the residue in the environment (Nofziger, 1999).

The high residue concentration (persistence) was attributed to thermal stability, hydrolytic stability and photostability associated with synthetic pyrethroids (permethrin) (Focus, 2006). It was therefore clear that consuming uncleaned maize grains from a store with a low temperature and low humidity (15^0C, highlight, 50% RH) would lead to consuming higher concentrations of pesticide residues than consuming maize stored in higher temperature and humidity (26^0C, highlight, 60% RH). The highest temperature and relative humidity studied (30^0C, 65% RH) had the shortest half life (46 days) and the highest rate of degradation of 6.6×10^{-3} mg kg^{-1}.

A general rate equation for the varying storage temperatures (T_2) relative humidity (RH) and halflife ($t_{1/2}$) is given as (Nofziger, 1999)

$$\log (t_{1/2}) = \log (t_0) - B (T_2 - 30) - \log {}^{RH}/_{65} \quad \text{.....[4.6]}$$

where $t_{1/2}$ is the half-life on grain of temperature (T_2) in degree Celsius and RH is percentage relative humidity while (t_0) is reference half-life on grains stored at 30^0C

and 65% RH. B is a constant related to the storage temperature which is specific for the pesticide applied and was equal to 0.04 degrees^{-1} for permethrin. This rate equation confirmed the half-life values obtained from the graphs in Fig. 4.11.

4.3.1.2 Degradation of permethrin stored under low light and different temperatures and humidity

When permethrin treated grains were stored under lowlight level and varying temperatures and humidity, the residue concentrations were found to follow biphasic sigmoidal trends similar to those of high light. The degradation curves due to lowlight storage however ran above the highlight storage curves thus reflecting the high residue concentration of permethrin when stored at low light conditions. Plots of log residue concentration versus storage time for varying temperatures under low light (dark) storage are illustrated in Fig. 4.12. These low light plots showed smaller (k^{-1}) values compared well with those obtained from the plots of high light. Consequently, the half-life values for the pesticide stored under low light (dark) were higher (Table 4.20) than those for high light. This meant that maize grains treated with permethrin then stored in a dark chamber (low light) would contain higher levels of pesticide residue concentrations compared with those stored in more lighted stores. This is because in the low light storage there was no photo degradation. Only microbial and hydrolytic degradation occurred. This translated into a reduced rate of permethrin degradation.

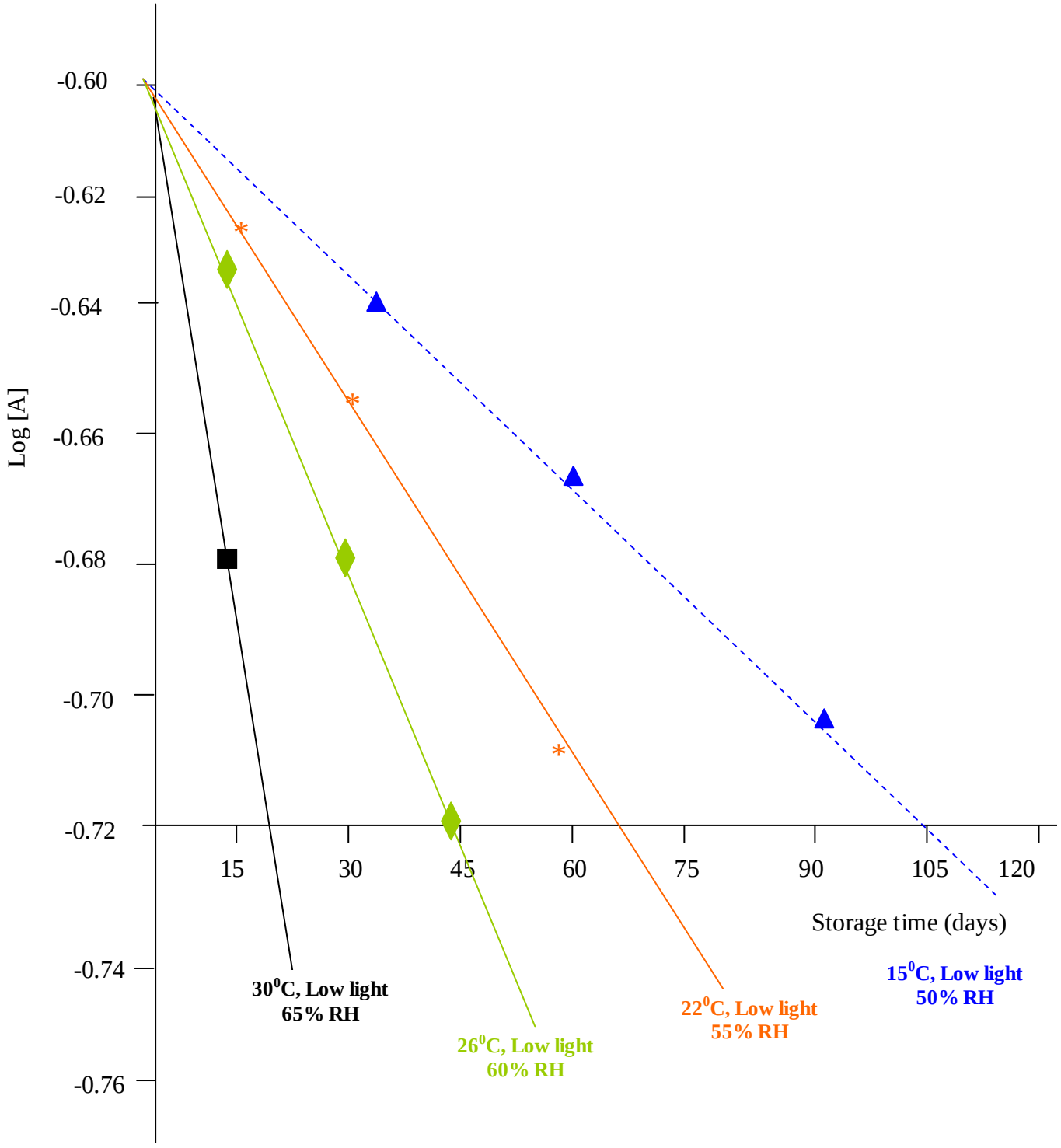


Fig. 4.12 Log residue concentrations against storage time (days) for different storage conditions of temperature and relative humidity (RH) at low light for permethrin

Table 4.20 shows the values of half-lifes ($t_{1/2}$)obtained for grains treated with permethrin and stored under constant low light (dark), but varying temperatures and relative humidity (RH). These values showed that the pesticide breakdown was attributed to temperature and humidity factor. The reaction rates represented by k^{-1} increased with increase in temperature while persistence of the pesticide increased with decrease in temperature.

Table 4.20: The gradient (k^{-1} /2.303), k^{-1} and halflife ($t_{1/2}$) values for permethrin degradation in maize stored under low light, varying temperature and relative humidity (RH) .

Storage condition	Permethrin		
	k^{-1} /2.303	k^{-1}	$t_{1/2,}$ days
(15^0C, Low light, 50% RH)	1.5	2.75	250
(22^0C, Low light, 55% RH)	1.8	4.16	168
(26^0C, Low light, 60% RH)	2.5	5.50	130
(30^0C, Low light, 65% RH)	5.0	12	60

At the low light storage conditions of (15^0C, low light, 50% RH) the degradation rate was smallest 1.5 x 10^{-3} and the half life ($t_{½}$) was longest (250 days). This means permethrin residue concentrations were highest in maize grains stored under such conditions. Low light storage of grains at the ambient storage conditions of the study area (22^0C, 55% RH) carried a mean residue concentration of 0.23 $mgkg^{-1}$ day^{-1} which was higher than that stored under highlight in the same area which carried a mean residue concentration of 0.21 $mgkg^{-1}$ day^{-1} (Table 4.17). At the lowlight storage in the study area the half life was longer (168 days) (Table 4.19) than in highlight storage (130 days) (Table 4.18).

An Anova analysis for low light storage condition revealed no significant impact on the process of permethrin degradation ($F = 0.5$ $df = 71$ $P > 0.05$). Thus low light storage condition did not have a significant effect in breaking down the permethrin pesticide and hence left high levels of active pesticide on the grains for a prolonged period and exposed consumers to health risks for longer periods. Tukeys modified range test found no significant difference between the highest level and lowest level of permethrin residue under low light condition for the storage period of 6 months. This meant that pesticide level in the first day of storage was almost the same as the level after 120 days storage implying that very little breakdown of pesticide occurred under low light storage. Further implication is that a storage facility of low light leads to a low degradation rate of permethrin pesticide applied to maize grains. A two way ANOVA analysis revealed that there was no significant interaction between temperature and low light at constant humidity ($F = 0.12$ $df = 143$ $P>0.05$).

Analysis of permethrin pesticide under low humidity as a degrading factor by Bengston *et al.,* (1983) indicated a comparatively slow decline where its major degradation products were the cis and trans isomers of 3 – (2,2 – dichlorovinyl) – 2,2 – dimethylcyclopropane carboxylic acid (DCVA) plus 3 – phenoxybenzyl alchohol (3 – PBALC) (Fig 4.13). In cereals and grains, residue levels of metabolites DCVA and 3 – phenoxybenzyl alcohol were found to be small compared with corresponding permethrin residue (Desmarchelier and Bengston, 1979). The major permethrin residue was found to lie below the ADI level (0.05 $mgkg^{-1}$) thus posing no serious health risks to the consumer.

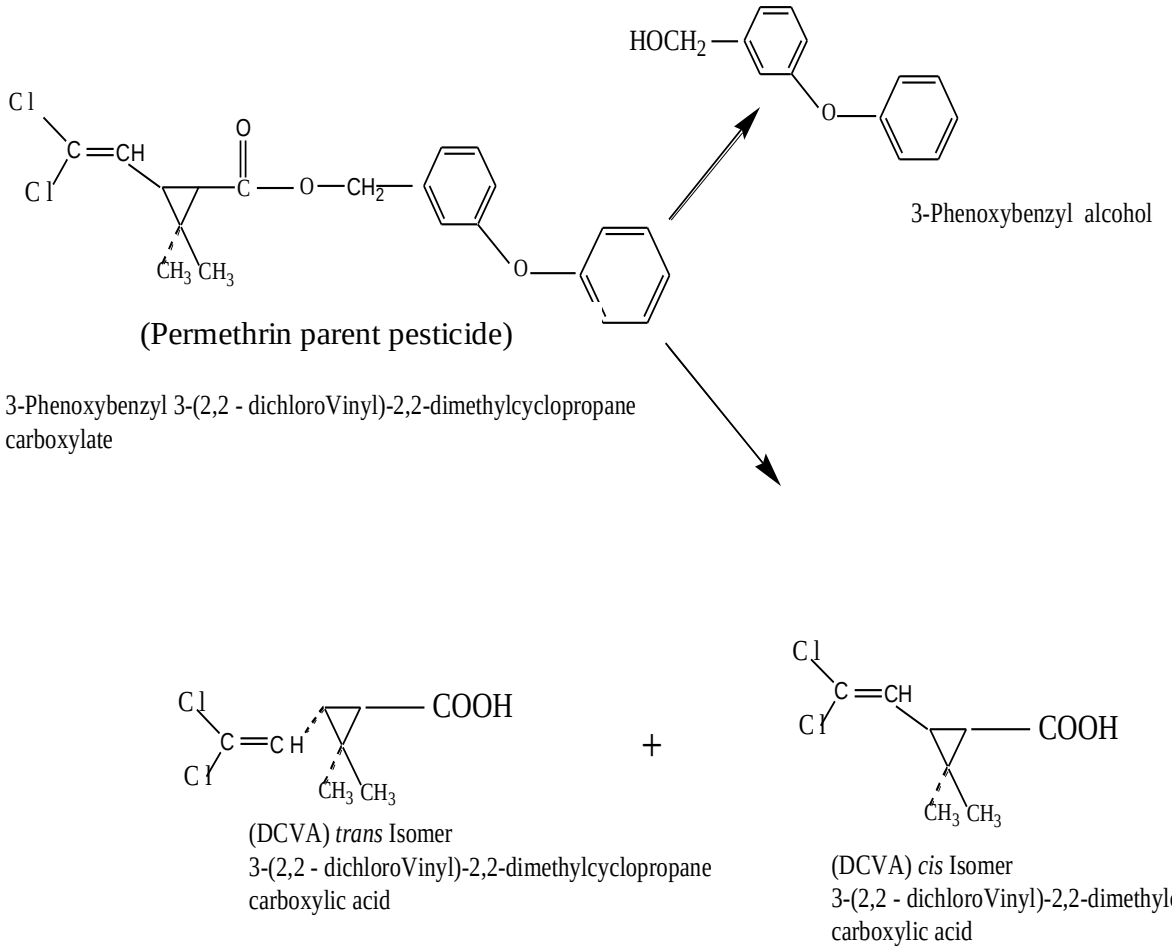


Fig. 4.13: Permethrin and its degradation products (Bengston *et al.*, 1983)

4.3.2 Pirimiphosmethyl residual in maize grains under different storage conditions

The results for the analysis of pirimiphosmethly residue under different storage conditions (temperature, light and humidity) were recorded in Table 4.21. It was found that maize grains stored under high temperature, high humidity and highlight contained lower pirimiphosmethly levels than grains stored in low temperature, low humidity and lowlight. Also the pesticide residue on the grains stored under any of the simulated storage conditions decreased with increase in storage time from 0 to 180 days. At the high temperature, light and humidity storage conditions of (30^0C, light, 65%RH) the residue concentration decreased from 3.9 $mgkg^{-1}$ in a storage period of 180 days. For the lowest storage conditions of (15^0C, dark, 50% RH) the residue concentration decreased from 3.9 $mgkg^{-1}$ to 1.4 $mgkg^{-1}$ in a storage period of 180 days. This meant that the high storage conditions caused the pirimiphosmethyl pesticide to breakdown at a faster rate than the low storage conditions. Ambient storage conditions of the study area (22^0C, light, 55% RH) caused the pesticide to breakdown from a level of 3.9 $mgkg^{-1}$ to 1.1 $mgkg^{-1}$ in a period of 180 days. The mean pesticide residue concentration over this period was 2.4 $mgkg^{-1}$ which was below the maximum residue limit (MRL) value for pirimiphosmethyl (5.0 $mgkg^{-1}$) but above the accepted daily intake (ADI) level of (0.01 $mgkg^{-1}$ day^{-1}) (FAO/WHO, 1994). Storage of grains at (30^0c, light, 65%) gave a lower average pesticide residue concentration (1.98 $mgkg^{-1}$) than storage in lowlight condition (30^0C, dark, 65%) that gave an average residue concentration of (2.23 $mgkg^{-1}$).

Table: 4.21: Pesticide residue concentrations in maize grains treated with a commercial formulation (ACTELLIC) at 4mgkg^{-1} pirimiphosmethyl and held in different storage conditions for 180 days

4.3.2.1 Effect of high light on pirimiphosmethyl degradation undr different temperature and humidity

Plotting the concentration curves for pirimiphosmethyl pesticide under highlight and various storage conditions of temperature and relative humidity (RH) gave the degradation paths in which all curves gave similar shapes and trends but differed in their degradation rates (Fig. 4.15). The trends were characterized by a biphasic degradation pattern. An initial rapid decrease in pesticide concentration occurred during the first 60 days. This was caused by an extracellular enzyme and microbial activity that was controlled by the storage conditions of temperature, light and relative humidity. The high temperatures in these storage conditions favoured a multiplication of microbial cells on the surface of the grains, where most of the pesticide was concentrated during the first 60 days. This enhanced the process of extracellular enzyme and thermal degradation leading to a rapid pesticide breakdown. This was followed by a slower degradation that was attributed to intracellular microbial activity. The role of light in this breakdown process is explained through a cleavage of the phosphorous – oxygen bond in the pirimiphosmethyl molecule at the position shown in Fig. 4.14. A one-way anova analysis confirmed that light had a significant impact on pirimiphosmethyl degradation ($F = 15$ $df = 119$ $P<0.05$)

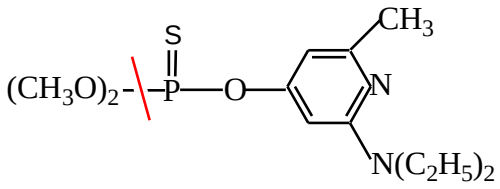


Fig 4.14: Cleavage of phosphorus – oxygen bond in pirimphosmethyl by UV light (Eduardo *et al.*, 2003)

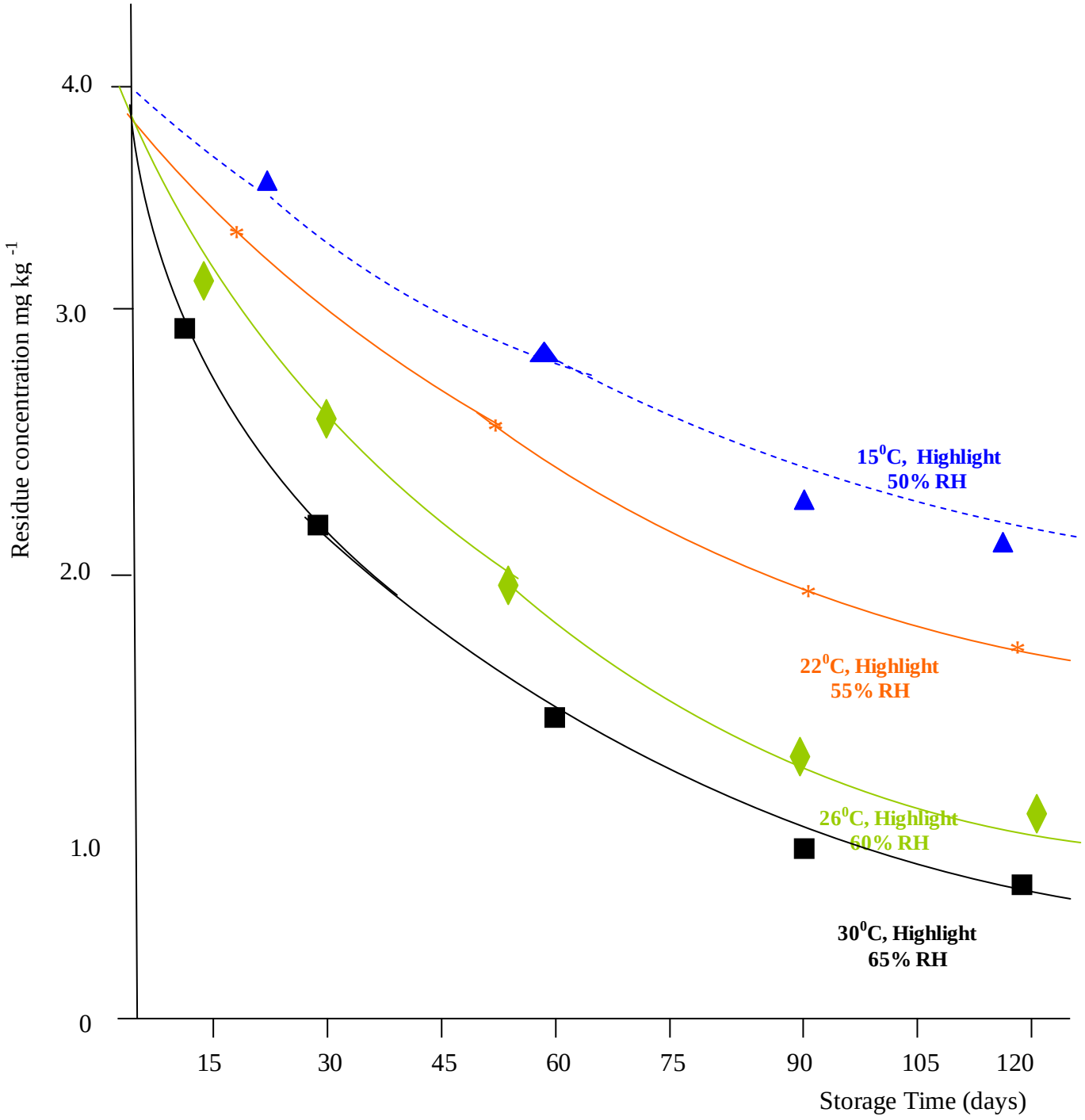


Fig. 4.15: Degradation curves for pirimiphosmethyl under highlight and various storage conditions, for temperature and RH

The biphasic degradation path was explained by the Michaelis-Menton Kinetics (Dykaar and Kitanidis,1996), where the main feature was a constant catalyzing material for a reaction where the microbial cells participating in the degradation were not growing to any significant degree. The order of reaction in Michaelis-Menton kinetics changes with concentration. In this study the pesticide reactant concentration was very low (trace) so that first-order kinetics were fully applicable.

The equation for first – order reaction kinetics [4.5] was applicable in the determination of pesticide concentration at any time during storage. The maximum residue limit (MRL) for pirimiphosmethyl was 5 $mg.kg^{-1}$ while the application rate of "Actellic" pesticide given on label was 5.6 mg kg^{-1}. The ambient storage conditions of the study area (22^0C, highlight, 55%RH) therefore reduced the level of pesticide concentration to 1mg kg^{-1} (Table 4.21) within 180 days of storage. This meant that pirimiphosmethyl was a moderately persistent pesticide and was readily detoxified through breakdown process caused by storage microclimate thus minimizing its health risks.

A logarithm transformation of the data in table 4.21 yielded linear data log[A] for the pirimiphosmethyl pesticide concentration. Plotting the log concentration log[A] data against storage time (days) gave straight line graphs for each storage condition as shown in fig 4.16. These plots were important in the determination of the pesticide half-life under a given storage microclimate of temperature, humidity and light. A straight line equation describing this degradation path was ascribed to each graph and its gradient value determined mathematically. The obtained gradient value was then related to half-life through the reaction – kinetic gradient term of $k^{-1}/_{2.303}$

All the graphs were found to be in agreement with Pseudo first order reaction kinetics equation

$$\log\ [A] = \frac{k^{-1}}{2.303}\ t + \text{constant} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots[4.7]$$

Where the constant term in the equation equals to the y - intercept and its value was $\log [A]_0 = 0.600$ which was equivalent to the initial pesticide concentration applied to grains. The gradient term for reaction kinetics was given by $k^{-1}/_{2.303.}$ A mathematical gradient calculated from the straight line graph was equated to the reaction kinetics gradient term ($k^{-1}/_{2.303}$) in order to obtain the value of k^{-1}. This value of k^{1} was then equated to reaction kinetics term for half-life so that $t_{½} = 0.693/ k^{-1}$. Table 4.22 shows the values for halflife ($t_{½}$) obtained by this calculation for pirimiphosmethyl concentrations under highlight and various storage conditions of temperature and relative humidity.

Table 4.22: The gradient ($k^{-1}/2.303$), k^{-1} and halflife ($t_{1/2}$) values for pirimiphosmethyl degradation in maize stored in highlight and varying temperature and relative humidity (RH).

Storage condition	Pirimiphosmethyl		
	$k^{-1}/2.303 \times 10^{-3}$	$k^{-1} \times 10^{-3}$	$t_{1/2,}$ days
(15^0C, Highlight, 50% RH)	0.27	6.21	115
(22^0C, Highlight, 55% RH)	0.38	8.74	77
(26^0C, Highlight, 60% RH)	0.50	1.15	62
(30^0C, Highlight, 65% RH)	0.70	1.60	42

When treated grains were stored at high light but low temperature and humidity (15^0C, 50% RH) the half-life for pirimiphosmethyl pesticide was 115 days and the degradation rate was 6.21×10^{-3} $mgkg^{-1}day^{-1}$.

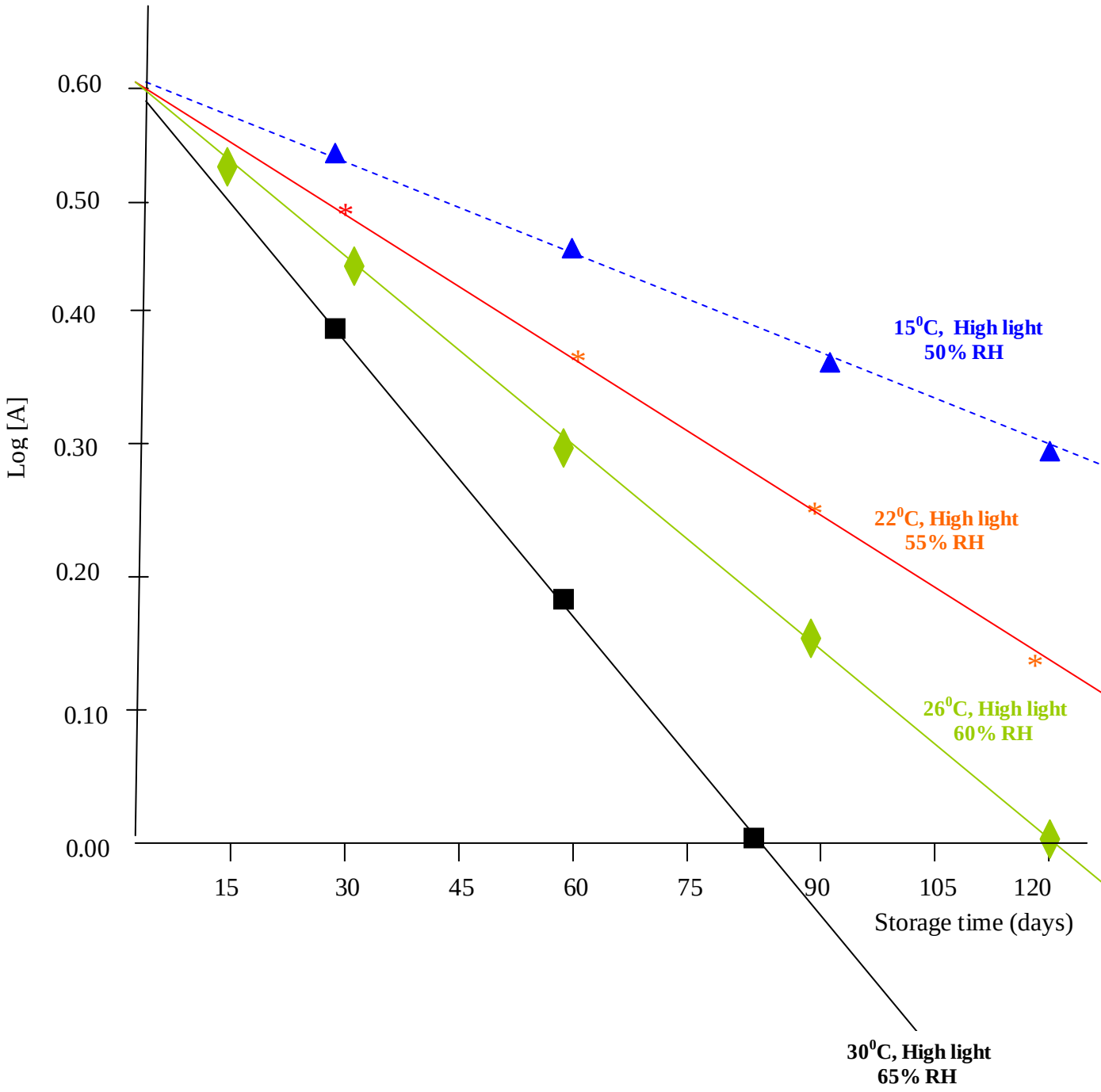


Fig. 4.16: Log residue concentrations against storage time (days) for different storage conditions of temperature and relative humidity at highlight for pirimiphosmethyl

At a higher temperature and relative humidity of (30^0C, 65% RH) a half-life of 42 days and degradation rate of 1.60 x 10^{-3} $mgkg^{-1}day^{-1}$ was observed while in the ambient storage conditions of our study area (22^0C, highlight, 55% RH), pirimiphosmethyl had a 77 days half-life. These half-life results were confirmed by the general rate equation [4.6] where a storage condition (30^0C, 65%RH) and its half-life of (42 days) from Table 4.22 were used as the reference points. Comparisons of half-lifes for permethrin (Table 4.19) and pirimiphosmethyl (Table 4.22) under similar high light storage conditions showed that permethrin had longer half-life than pirimiphosmethyl in all corresponding storage conditions. This meant that permethrin was relatively more climatically stable and persistent than pirimiphosmethyl on maize grains.

4.3.2.2 Degradation of pirimiphosmethyl stored in low light and different temperature and humidity

Maize grains treated with pirimiphosmethyl then stored under low light and varying temperature and relative humidity were analysed and their data plotted. It gave biphasic concentration curves similar to those of storage in high light.The curves for low light storage had smaller gradients than their corresponding curves for high light storage hence their paths lay above those of high light storage when plotted on the same axis.Plotting the log of residue concentration against storage time gave straight line graphs that correspond to each storage condition (Fig. 4.17). The gradient of each of the lines gave a value for the halflife of the storage condition under low light.

The half-life values for the storage conditions are shown in Table 4.23. The storage conditions with highest temperature and humidity (30^0C, 65% RH) gave the steepest line with the highest gradient (0.0056) which represented the biggest rate of degradation of (1.30 x 10^{-2} mgkg^{-1} day^{-1}) (Table 4.23). This degradation rate is smaller compared to the one obtained when storage was in high light (1.61 x 10^{-2} mgkg^{-1}day^{-1}) (Table 4.22). This meant that the pesticide broke down faster under high light due to presence of UV energy than in low light where there was no UV energy. Similarly for all the other storage conditions, it was found that graphs for high light storage had higher gradients (Table 4.22) than those for low light storage (Table 4.23). Therefore at the ambient storage condition of the study area (22^0C, 55%RH) the degradation rate at high light storage was 8.74 x 10^{-3} mgkg^{-1}day^{-1} (Table 4.22) while the rate at low light storage was 7.59 x 10^{-3} mgkg^{-1} day$^{-1.}$ (Table 4.23). This meant that pirimiphosmethyl was more persistent at low light storage facilities ($t_{½}$ = 93 days) than at high light storage facilities

($t_{½}$ = 77 days) in the study area.

Table 4.23: The gradient (k^{-1}/2.303), k^{-1} and halflife ($t_{1/2}$) values for pirimiphosmethyl degradation in maize stored in low light and varying temperature and relative humidity (RH) conditions.

Storage condition	Pirimiphosmethyl		
	k^{-1} /2.303 x 10^{-3}	k^{-1} x 10^{-3}	$t_{1/2,}$ days
(15^0C, Low light, 50% RH)	2.50	5.75	140
(22^0C, Low light, 55% RH)	3.30	7.59	93
(26^0C, Low light, 60% RH)	4.20	9.66	65
(30^0C, Low light, 65% RH)	5.60	13.00	54

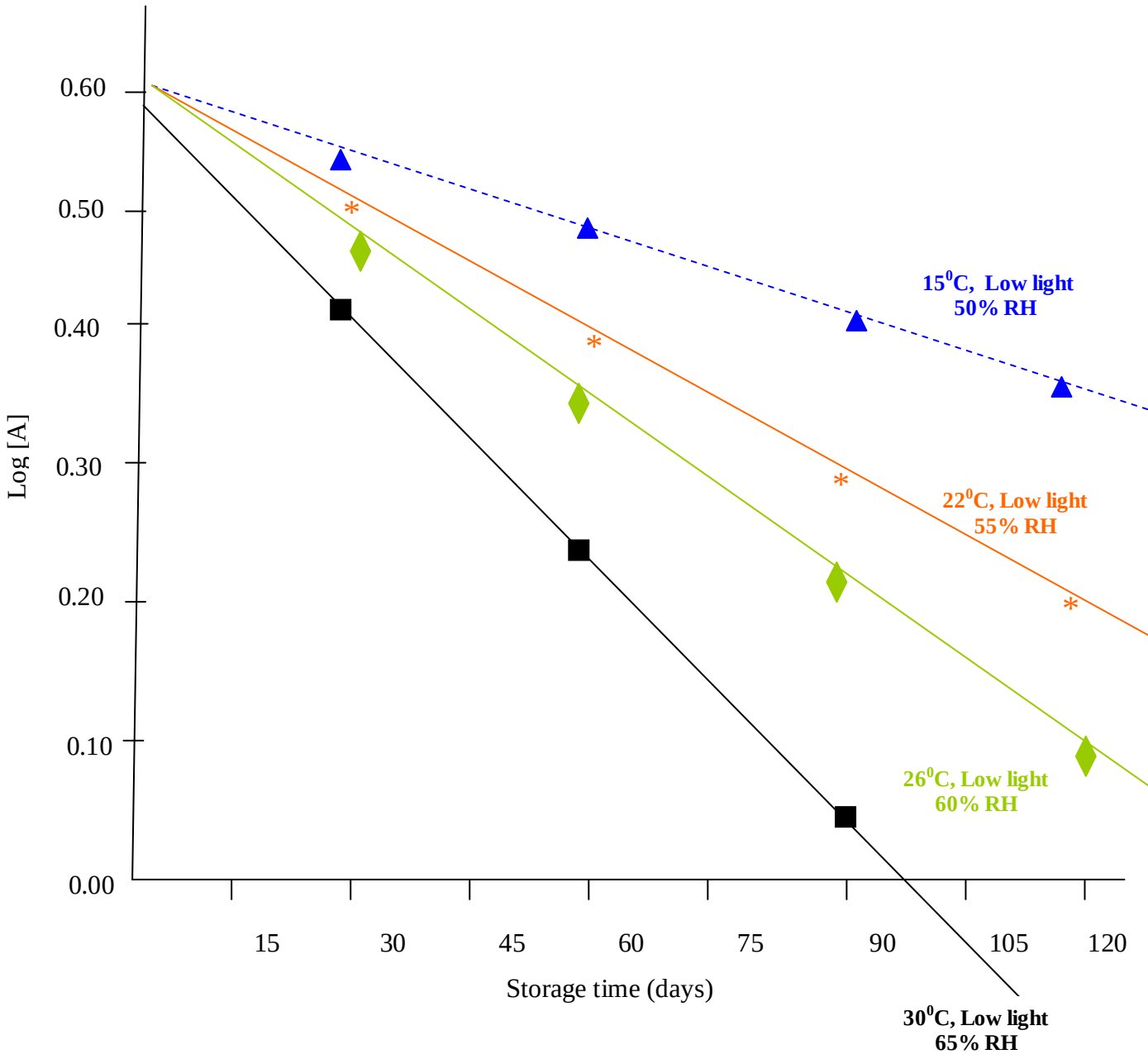


Fig 4.17: Log residue concentrations against storage time (days) at low light for different storage conditions of temperature and relative humidity (RH) for pirimiphosmethyl.

Studies conducted by Rowland (1991) showed that pirimiphosmethyl pesticide on maize grains was degraded and detoxified by hydrolysis of the phosphorus-ester side chain to give hydroxypyrimidine molecule (major product) and N-desethylphosphorus (minor product) as shown in (Fig. 4.18). This hydrolysis process was driven by humidity in the intergrain spaces, so that under storage conditions with high moisture content the hydroxypirimidine was 0.62mg kg^{-1} (Rowland, 1991). The other metabolite of pirimiphosmethyl was N-desethylphosphorus, which was formed in extremely low quantities such as < 0.05 mg kg^{-1} after 6 months of storage (FAO / WHO, 1994).

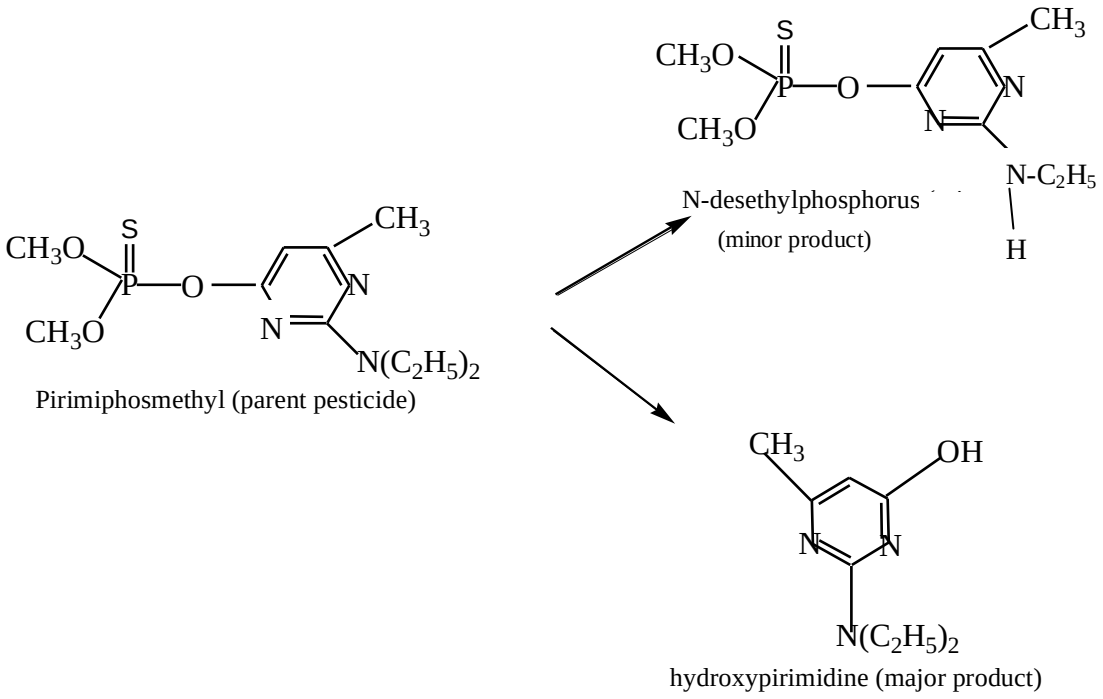


Fig 4.18: Pirimiphosmethyl and its hydrolysis products (Rowland, 1991).

4.3.3: Malathion residue in maize grains under different storage conditions

The results for analysis of malathion residue under different storage conditions of temperature, light and humidity were recorded in Table 4.24. Generally the malathion concentrations for all the storage conditions decreased as storage time increased to 180 days. At the high storage conditions of (30^0C, light, 65% RH), malathion concentration decreased from 3.92 $mgkg^{-1}$ to 0.52 $mgkg^{-1}$ within 60 days. Beyond this, the malathion concentration was not detectable (ND). At low temperature and humidity (15^0C, dark, 50%) the pesticide concentration decreased from 3.90 $mgkg^{-1}$ to 0.22 $mgkg^{-1}$ over 150 days and beyond this, the malathion concentration was not detectable (ND). A comparison of the two storage microclimates revealed that high temperature and humidity caused malathion to degrade faster, taking (60 days) to decrease to ND level than low temperature and humidity which took 150 days to decrease to ND level. The ambient storage conditions prevailing in the study area (22^0C, light, 55% RH) reduced the malathion pesticide concentration from 3.90 $mgkg^{-1}$ to 0.20 $mgkg^{-1}$ within 120 days. The average pesticide residue concentration for the storage period of 120 days was therefore 2.03 $mgkg^{-1}$ day^{-1}which was below the MRL (8 $mgkg^{-1}$) recommended by the World Food Organization FAO but above the acceptable daily intake (ADI) level (0.02 $mgkg^{-1}$ day^{-1}) (FAO/WHO, 1994)

High light storage conditions reduced the pesticide concentration at faster rate than low light storage. Thus a comparison of high light storage at (30^0C, 65% RH) caused breakdown at a faster rate of 2.04 $mgkg^{-1}$ day^{-1} than low light storage in similar temperature and relative humidity (2.0 $mgkg^{-1}$ day^{-1}).

Table: 4.24: Pesticide residue concentration in maize grains treated with a commercial formulation (SKANA) at 4 $mgkg^{-1}$ malathion and held under different storage conditions for 180 days

4.3.3.1 Effect of high light on malathion degradation under different temperature and humidity.

The various paths of decreasing malathion concentration when the maize grains were stored in highlight temperature, and humidity are shown in Fig. 4.20. The decay curves showed that malathion from the grains decreased with storage time and followed a biphasic pattern. The concentration decreased rapidly for the first 30 days before settling into a gradual breakdown for the remaining days. Michaelis-Menton kinetics helps to explain the two phased degradation path of malathion in that the initial rapid breakdown is attributed to extracellular microbial activity that is controlled mainly by the conditions of temperature, light and humidity while the slower degradation was due to intracellular microbial degradation (Dykaar and Kitanidis, 1996). Equation [4.9] for the calculation of changing concentration during pesticide breakdown was applicable in determining the malathion level (quantity) on the stored grains at any time during storage. Table 4.23 showed that malathion residue level was consistently above the accepted daily intake (ADI) level of 0.02 mg kg^{-1} (FAO / WHO, 1994) during the whole of the storage period.

Since high light is a source of UV energy, photodegradation process that breaks the malathion molecule by cleavage of its phosphorus – oxygen bond at the position shown takes place as in Fig. 4.19. One way Anova analysis showed that high light had a significant effect on malathion degradation ($F = 30.2$ $df = 191$ $P < 0.05$).

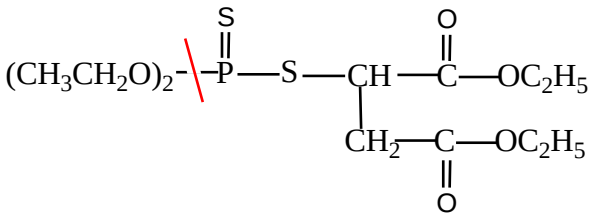


Fig 4.19: Cleavage of phosphorus – oxygen bond of malathion by UV light (Walker *et al*, 1992)

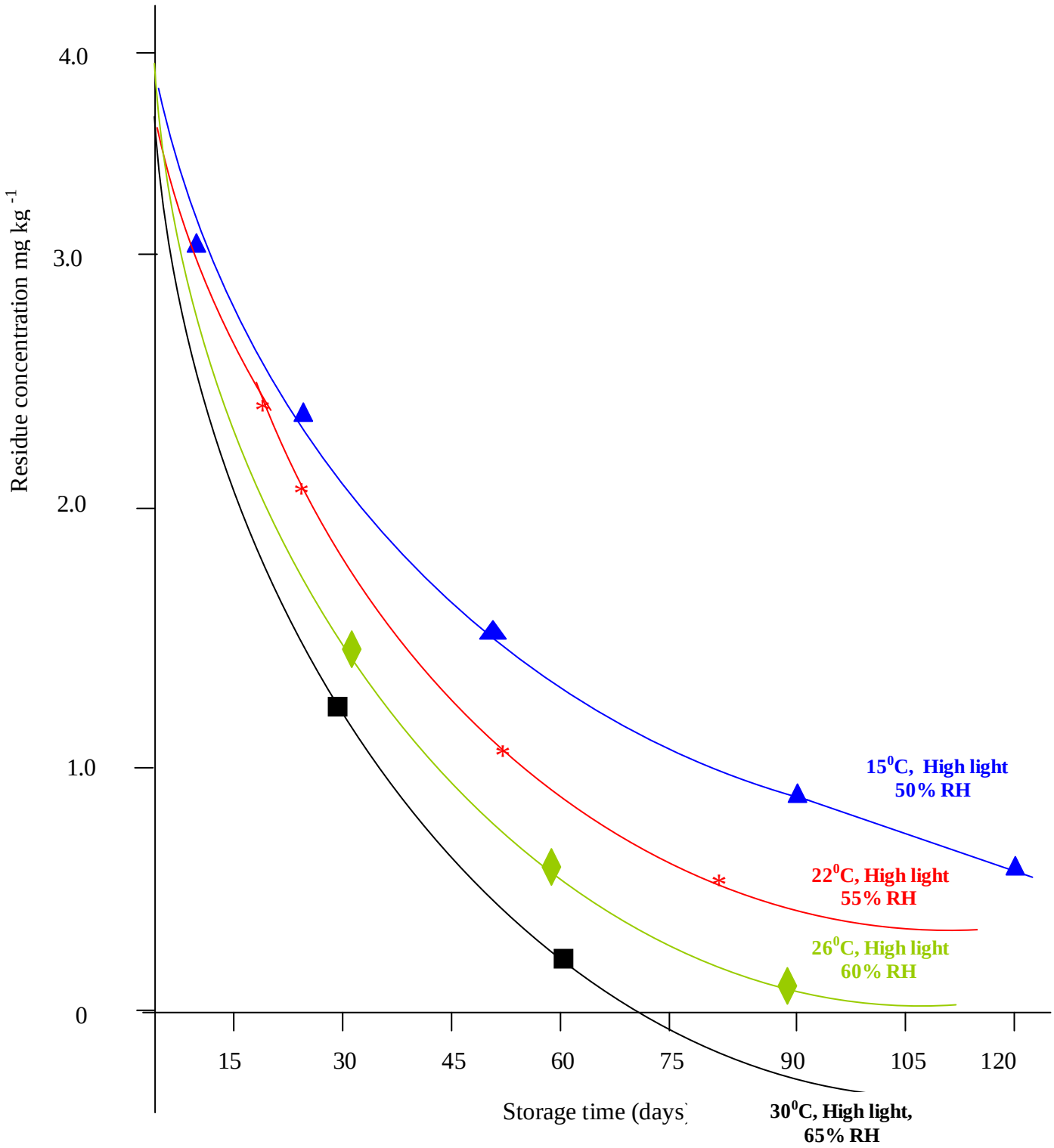


Fig 4.20: Degradation curves of malathion under high light and various storage conditions for temperature and RH.

High temperature storage conditions favour the growth of degradation microorganisms. This leads to a significant degradation rate on pesticide, which decreased as the temperature decreased ($F = 21.50$ df = 191 $P < 0.05$). Malathion has a very low tendency to move deep into the cuticular waxes of the maize grains and it therefore concentrates mainly on the surface of the grain.This exposed the malathion to a high degradation by the microbial cells, which reduced it to a low concentration within a short storage period than pirimiphosmethyl or permethrin.

When the residue concentrations (Table 4.24) were given a log transformation they yielded linear graphs against storage time for all the storage conditions (Fig. 4.21). The plotted lines met at a common y-intercept point and took a negative gradient, showing that the mass of pesticide declined with time. The common y-intercept represents the initial malathion application dosage of 4 mg kg^{-1}. The gradient of each straight line was represented by the term ($k^{-1}/_{2.303}$). When the k^{-1} value was equated to the term that represent half-life in reaction kinetics ($^{0.693}/ k^{-1}$), the value for half-life ($t_{1/2}$) in each storage condition was obtained (Table 4.25).

Table 4.25: The gradient (k^{-1} /2.303), k^{-1} and halflife ($t_{1/2}$) values for malathion degradation in maize stored in high light and varying temperature and relative humidity (RH).

Storage condition	Malathion		
	$k^{-1}/2.303 \times 10^{-3}$	$k^{-1} \times 10^{-2}$	$t_{1/2}$,days
(15^0C, High light, 50% RH)	7.5	1.73	40
(22^0C, High light, 55% RH)	1.0	2.30	30
(26^0C, High light, 60% RH)	1.3	3.07	22
(30^0C, High light, 65% RH)	19.0	4.32	16

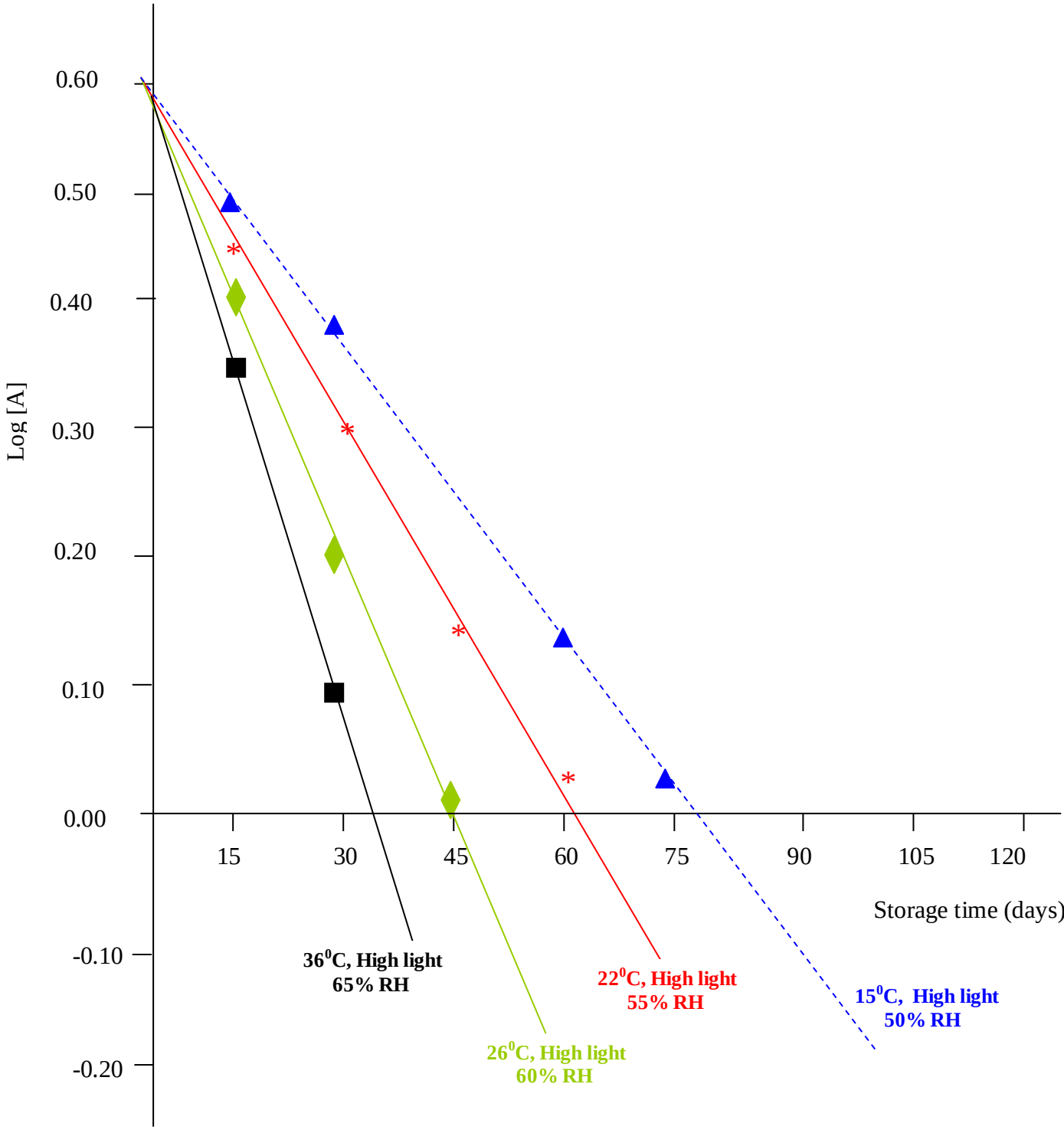


Fig 4.21: Log residue concentration against storage time (days) for different storage conditions of temperature and relative humidity at highlight for malathion

The general rate equation [4.6] for pesticide degradation confirmed the data in Table 4.25 where the reference storage condition was (30^0C, 65% RH) and $t_{1/2}$ was 16 days (Table 4.25). The longest half-life for malathion was 40 days when stored at (15^0C, high light, 50% RH). This compared with 115 days for pirimiphosmethyl and 210 days for permethrin stored under the same storage conditions. This meant that malathion had the highest rate of degradation (1.73×10^{-2} mg kg^{-1}) followed by pirimiphosmethyl (6.21×10^{-3} mg kg^{-1}) and then permethrin (3.75×10^{-3} mgkg^{-1}). Therefore the order of persistence of pesticides on maize grains starting with the most persistence was permethrin, pirimiphosmethyl and malathion respectively.

The shortest half-life for malathion (16 days) occurred at the storage condition (30^0C, high light, 65% RH) with the highest degradation rate of 4.32×10^{-2} mg kg^{-1} day^{-1}. At the ambient storage conditions of the study area (22^0C, high light, 55% RH) the degradation rate for malathion was 2.3×10^{-2} mg kg^{-1} day^{-1} and a half-life of (30 days), which was the shortest period compared with that of pirimiphosmethyl (77 days) with degradation rate of 8.74×10^{-3} mg kg^{-1} day^{-1} and permethrin (130 days) with degradation rate of 5.50×10^{-3} mgkg^{-1} day^{-1}. Malathion therefore was the least persistent pesticide and dissipated most rapidly thus causing a comparatively lower health risks and impact on the environment.

4.3.3.2 Degradation of malathion stored in low light and different temperature and humidity

Table 4.26 show the halflife values ($t_{1/2}$) of malathion degradation in maize stored under low light and varying temperature and relative humidity. It was observed that the storage conditions at low light had lower degradation rates than the corresponding storage conditions at highlight.

Thus at the storage conditions of the study area (22^0C, 55% RH) and highlight the halflife was 30 days while at (22^0C, 55% RH) and low light the halflife was 38 days. At (15^0C, 50% RH) and lowlight the halflife was 45 days. In all the cases malathion was observed to be more persistent on grains stored at low light than those stored in high light. Log concentration of malathion stored at low light and varying temperature and relative humidity was plotted against storage time (days) where straight lines were obtained (Fig. 4.22). The straight line graphs agreed with the first order reaction kinetics model [4.7] whose degradation rates are shown in Table 4.26 as k^{-1} values.

Table 4.26: The gradient (k^{-1} /2.303), k^{-1} and halflife ($t_{1/2}$) values for malathion degradation in maize stored under low light, varying temperature and relative humidity (RH).

Storage condition	Malathion		
	k^{-1} /2.303 x 10^{-3}	k^{-1} x 10^{-2}	$t_{1/2,}$ days
(15^0C, Low light, 50% RH)	6.66	1.53	45
(22^0C, Low light, 55% RH)	8.00	1.84	38
(26^0C, Low light, 60% RH)	12.00	2.76	25
(30^0C, Low light, 65% RH)	15.80	3.64	19

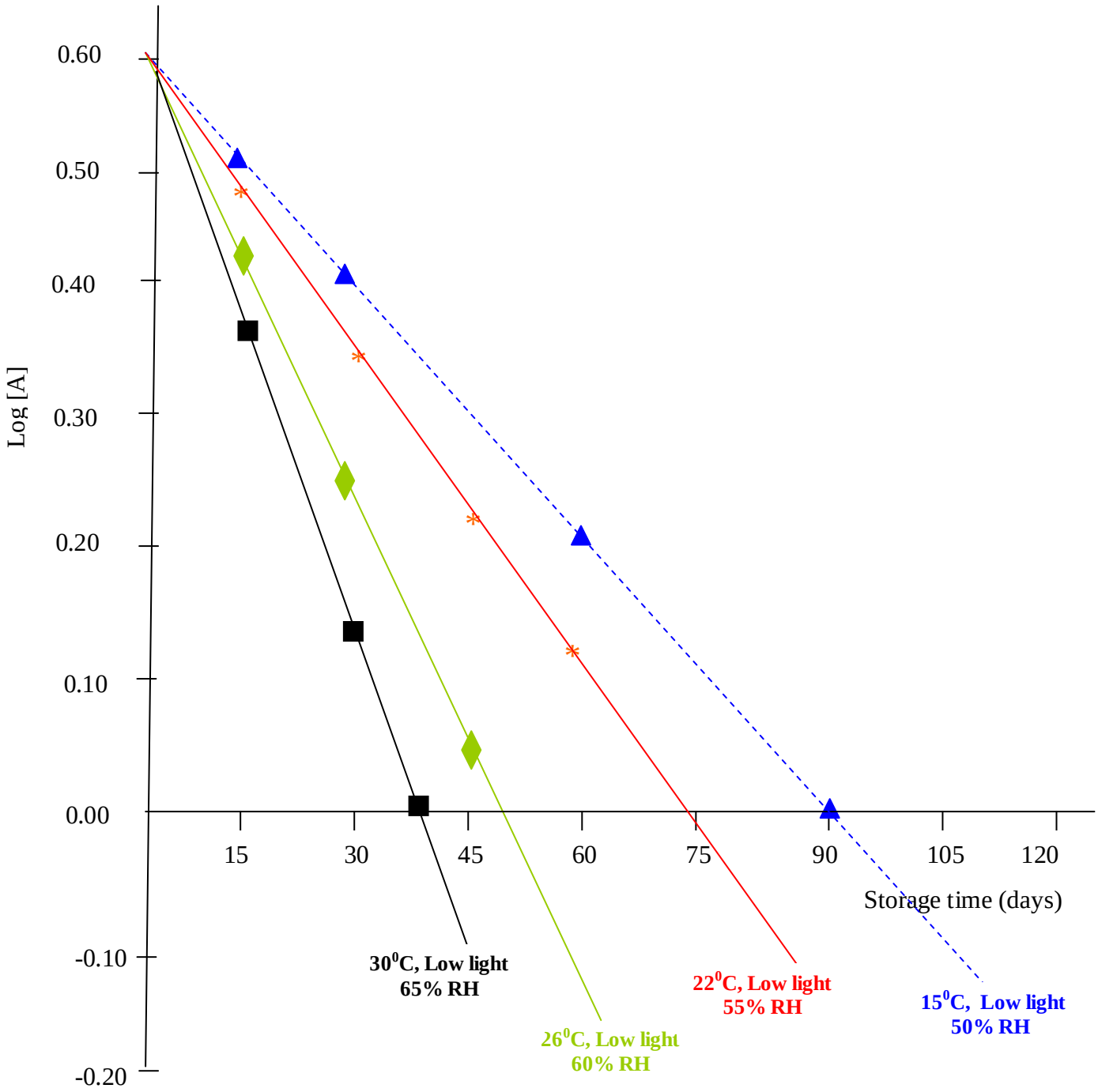


Fig 4.22: Log residue concentration against storage time (days) for different storage conditions of temperature and relative humidity at lowlight for malathion.

The two main processes involved in the malathion pesticide breakdown were hydrolysis and photodecomposition, but with hydrolysis taking the upper hand. (Bengston *et al.,* 1983). Hydrolysis was usually accelerated by the humidity (moisture) in the intergrain space. In Fig. 4.23 one water molecule (H_2O) provided a hydrogen atom to the ethan group (CH_3CH_2O) and the hydroxyl (OH) part of the water molecule combined with phosphorus atom of malathion molecule thus hydrolyzing into an inactive chemical with the release of ethanol (Bengston *et al.,* 1983). The faster the hydrolysis reaction, the less time the pesticide had in the environment for pesticidal activity, leaching or other movement.

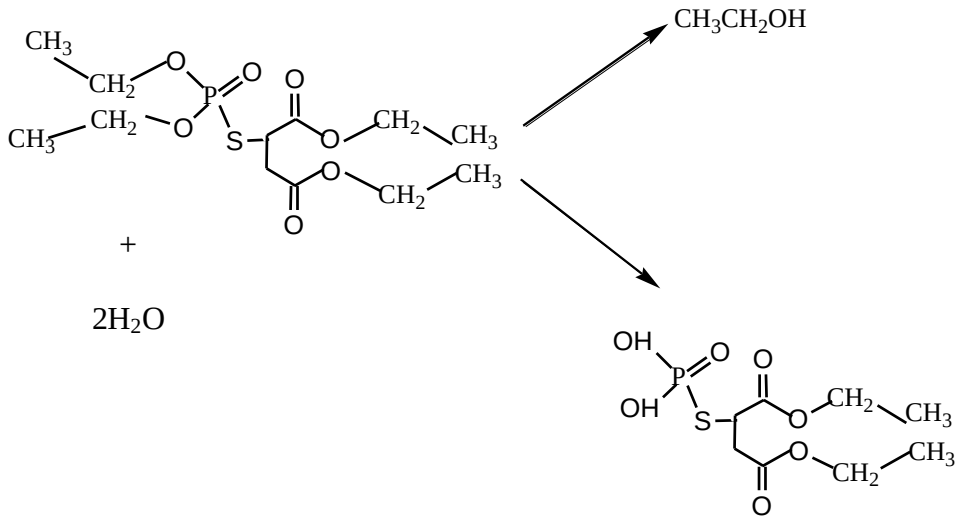


Fig. 4.23: Malathion and its hydrolysis products (Bengston *et al.,* 1983)

CHAPTER 5

5.0 CONCLUSIONS AND RECOMMENDATION

5.1 Conclusion

From the results of the field study, it can be concluded that the maize farmers of Chilchilla division, Kericho district of Rift valley Province in Kenya do not follow appropriate safety precautions with regard to pesticide formulation and application and therefore are exposed to dangerous levels of pesticides.Processing techniques of the grains before consumption always resulted in a reduction of pesticide residue levels. The most effective cleaning method in removing pesticides included a combination of hulling, water washing, milling and cooking. This method removed 71% malathion, 63% permethrin and 67% pirimiphomethyl. However in all cases the amount retained was still above the ADI levels and likely cause health risks. Conventional water wash removed the lowest amount of pesticides at 11.07% malathion, 10% permethrin and 10% pirimiphosmethyl. From the results obtained in this study it was deduced that toxic pesticide residues were present in maize grains mostly in trace amounts below the WHO maximum admissible levels (MRLs) of Malathion (8 $mg.kg^{-1}$), Permethrin (3 $mgkg^{-1}$) and Pirimiphosmethyl (5 $mg.kg^{-1}$). A study of the exposure dosages of the pesticides revealed that the consumer was exposed to residue levels above the ADIs of 0.02$mg.kg^{-1}.day^{-1}$ (Malathion) and 0.01$mg.kg^{-1}day^{-1}$ (Pirimiphosmethyl) confirming a health risk. Permethirn residues were below ADI level (0.05 $mg.kg^{-1}.day^{-1}$) thus suggesting no health risk from this pesticide residue.

The storage microclimate of temperature, highlight and humidity determined the rate at which a pesticide degraded into less toxic chemicals. Under high temperature and

high humidity, degradation occurred at a faster rate yielding a shorter half-life for the pesticide. Low temperature and low humidity led to a slow degradation rate and longer half-life. Low temperature, low light and low humidity led to the highest levels of pesticide residue, retention and persistence on grains.

5.2 Recommendations from the study

(i) Chilchila farmers should be given a health education program with regard to the safe use of pesticides. Health risk prevention should focus on reducing the volume of pesticides used on food crops (maize) and substitution the synthetic pesticides with safer natural compounds while establishing effective protective measures that would lower occupational exposure hazards.

(ii) Consumers of maize that has been treated with pesticides should ensure that they clean the grains with a lot of water that contains detergents then rinse them thoroughly with clean water before milling into flour or cooking the grains.

(iii) Farmers should store their maize harvest in well aerated, warm and properly lighted stores. High humidity should be avoided at all costs in the storage facility. Such storage conditions will facilitate pesticide degradation and lower residue levels on the maize grains.

5.3 General Recommendations

Farmers should be educated about alternative forms of pest control and incentives provided to encourage their use. Some of the recommended storage methods that would avoid pesticide use includes: irradiate the grains with sterilizing dosages

(16000 rad) of gamma rays from cobalt – 60 source or by electrons from an electron accelerator. This technique is too expensive. Cool conditions in store: Since many storage insects increase in number only slowly below 12°c, keeping commodities cool is a good method of control and does not entail the use of pesticides. This can be achieved by storing grains in mechanically ventilated store with unheated air. Refrigerated air can be used for grain of moisture content of up to 20%. Air tight underground pits (hermetically sealed stores) can be used to protect stored grains, without using pesticides. This is effective because in dry grain (<14 % moisture content) the insect depletes the oxygen to less than 2% and die. Fumigation with smoke: stored maize can be smoked periodically to discourage weevils by smoke, while the fire helps to keep the moisture content low such as. store harvested maize grain in the roof of the building used as kitchen . Mixing sand and woodash with threshed grains controls the breeding of insects. The sand scratches the cuticle of the insect body and the insect loses moisture through the scratches. If the grain is dry (13% moisture content) the insect will not be able to get enough moisture to replace the moisture lost through scratches and it will die. A thorough detergent washing of the grains is recommended as a means of reducing pesticide residues. A flour milling technique that removes the testa is recommended to reduce pesticide residues further.

5.4 Areas for further research.

There is need for quantazition of various pesticide residues in other food products such as vegetables, tomatoes, fruits, fish and animal meat and milk that compose the daily menu of most communities in Kenya while determining the storage conditions that enhance the reduction of these residues.

A clinicopathological study of effects of pesticide residues in various food products to help determine the extent of terminal health impacts on the consumer is necessary. Such a study should come up with a detailed and updated record of the pesticides in use, their MRL and ADI levels along with their environmental and health impacts as well as a control and warning system on misuse and dumping of pesticides banned in developed countries.

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APPENDIX A

QUESTIONNAIRE GUIDE FOR SMALL SCALE FARMERS

Your responses is highly appreciated. All information is this questionnaires will be confidential.

SECTION I

1. Farmers Name_____Age:_____

2. Location: Kokwet ( ) Chilchila ( ) Kunyak ( ) Kipteris ( )

3. Size of farm: 0 – 5 ha ( ) 5 – 10ha ( ) 15 – 20ha ( )

4. Main crops cultivated: Cash _____

   Food _____

5. How many people live in your home _____

6. Which months of the year do you harvest maize _____

7. Do you store this maize after the harvest yes_____No._____

8. How much maize do you store (in 100kg bags) for: Consumption_____

   Sale_____

9. Describe your storage facility:

   Traditional granary _____(ii) Hanging in house and smoking _____

   Sealed bins _____ (iv) Others: say how_____

10. Which is the most common maize storage pest(s)_____

11. What pesticides do you use to treat maize for storage (in order of preference)

    1_____ 2_____ 3_____

12. In which other way do you protect your stored maize from pest attack other than using the pesticides mentioned in (6) above. Give 3 different ways if any.

    1_____ 2_____ 3_____

13. Where do you obtain your pesticides from?

    Open air market ( ) Shops ( ) Chemist ( )

14. Have you ever changed from one pesticide to another? If yes why?

    Yes _____ No._____

    Reason:_____

15. Have you had any training in pesticide handling: Yes ( ) No ( )

16. Do you always read the label on pesticide before using Yes ( ) No ( )

17. When applying pesticides do you use any safety protective devices like

    groves when mixing ( ) Overall ( ) Goggles ( ) None ( )

18. How do you dispose the following materials.

    (I) Unused pesticide_____(ii)Empty pesticide containers_____

19. Do you observe the safety intervals stipulated on pesticide label before consuming the treated maize Yes ( ) No ( )

20. How is the maize prepared prior to milling and cooking

    Hulling ( ) Water washing ( )

    Detergent washing ( ) Removing outer cover testa ( )

21. (a) Is there any reported cases of illness after consuming treated maize

    Yes ( ) No ( )

    (b) What were symptoms of illness in 21 (a) above _____

22. Has any person been reported as poisoned by pesticides. Yes ( ) No. ( )

23. Do you feed livestock with the treated maize Yes ( ) No. ( )

24. In which other agricultural activity do you use pesticides.

	Activity	Pesticide Used
1.		
2.		
3.		
4.		

25. Have you ever noticed any environmental change after application of pesticides Yes ( ) No. ( )

If yes explain how: _____

26. In your opinion how should pesticide use be improved in this country:____

(Thank you for your cooperation)

APPENDIX B

STATISTICAL TREATMENT OF DATA

B – 1 Universal equation

The results of the data collected from a GLC chromatograms are calculated using the following Universal equation (B-1)

$$P_R = \left[\frac{P_S H_P D}{H_S W} \right] \text{----------} \quad \text{(B-1)}$$

P_R = Pesticide residue concentration in sample

P_s = Pg of pesticide standard

H_p = Peak height (or Area) of sample

D = Dilution factor

H_s = Peak height (or Area) of standard

W = Weight of original sample (in grams)

To determine the value of the dilution factor (D)

$$D = \frac{Initial\ extraction\ Volume(ml) X\ \ Final\ Volume\ (ml)\ before\ GC\ \ Injection}{Aliquot\ of\ \ initial\ extraction\ Volume\ (ml) X\ \ \mu l \quad Injected\ \ on\ GC}$$

B - 2. Mean

The mean levels of each pesticide residue for each location and each food product was carried out for pentaplicate determinations. This was done to ensure accuracy into the actual value of the levels. The mean was calculated using equation (B-2) below

$$\bar{X} = \frac{\sum_{i=1}^{i-n} Xi}{n} \text{ ---------- (B-2)}$$

$\overline{X}$ = is the mean

X_i = the nth term of the five determinations

n = is the total number of determinations (8)

B – 3 Standard deviation (s)

This was used to measure dispersion of obtained readings about the mean. The following equation was (C-3) used to calculate standard deviation.

$$S = \sqrt{\frac{\sum_{i=1}^{i=n} \left(Xi - \bar{X} \right)^2}{n-1}} \quad \text{---------- (B-3)}$$

B – 4 Correlation Coefficient (r)

Residues concentration from various storage conditions, locations or different food samples were correlated by the Pearson Correlation Coeff (r) to test whether the corresponding reading got for each, conditions, location and sample were comparable.

The following equation C-4 was used to correlate the above stated factors.

$$r = \frac{\sum_{i=1}^{i=n}\left(Xi - \bar{X}\right)\left(Yi - \bar{Y}\right)}{\sqrt{\sum_{i=1}^{i=n}\left(Xi - \bar{X}\right)^2\left(Yi - \bar{Y}\right)^2}} \quad \text{----------} \quad \text{(B-4)}$$

The degree of correlation of correlation between the two factors was treated as fair for 0.90<x<0.95 good for 0.95<r<0.99 and excellent for r>0.99

B – 5 **t – test**

This test is used to test whether an experimental factor will have a significant effect on a measurable variable or not

$$t_{cal} = \frac{\overline{X}_1 - \overline{X}_2}{\sqrt{\left(\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}\right)}} \quad \text{----------} \quad \text{(B-5)}$$

$\underline{\overline{X}_1}$ & $\overline{X}_2$ = mean of the two variables

S_1 & S_2 = standard deviation for factor 1 and factor n_1 & n_2

The t_{cal} values calculated were compared with tabulated t_{crit} values at 95% confidence level using a two tailed test and (n-2) degrees of freedom. If the t_{cal} $>t_{tab}$ the null hypothesis is rejected that is we conclude in such a case that a significant relationship or effect exists. When $t_{cal} < t_{tab}$ the null hypothesis is accepted, that is we conclude in such a case no significant relationship or effect exists. This is used in TUKEYS Multiple range test.

B - 6. **t-test on the r – value**

The statistical test used to test whether the correlation coefficient (r) obtained for two different factors compared is significant or not was the t-test given by the equation below.

$$t = |r|\sqrt{\frac{(n-2)}{1-r^2}} \quad \text{----------} \quad \text{(B-6)}$$

Where n is the number of data points.

The t – values calculated were compared with tabulated t_{crit} value at 95% confidence level using two tailed test and (n-2) degrees of freedom.

B – 7 Exposure dosage

$$\text{Exposure dosage} = \frac{[\text{Residue concentration in food of interest x food consumption rate}]}{\text{Body weight}}$$

B – 8 Hazard Index

$$\text{Hazard Index} = \frac{[\text{Exposure dosage}]}{\text{Reference dosage (ADI)}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (B-8)$$

B – 9 λ^2 test for charaterising the degradation data

$$\lambda^2 = \sum \frac{(P-O)^2}{(err/100 \times \bar{O})^2} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (B-9)$$

Where P = predicted value, O = observed value, $\bar{O}$ = mean of all observed values and err = measurement error percentage. If λ^2 is larger than tabulated value, then the model is not appropriate at the chosen level of significance. A fit that results in an error level of 15% is considered acceptable although this is not an absolute cut-off criterion and a visual assessment must always be made.

B – 10 % of residue removed by processing methods

$$\%\text{ of residue removed} = \frac{[\text{ Residue level before cleaning} - \text{Residue level after cleaning }]}{\text{Residue level before cleaning}} \times 100$$

APPENDIX C:

Compartment for controlling temperature and humidity regimes