SAFETY OF FEEDING CROP RESIDUES WITH PESTICIDES TO DAIRY CATTLE IN A MIXED FARMING IRRIGATION SCHEME IN CENTRAL KENYA.

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DECLARATION

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

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DEDICATION

TO MY DEAR PARENTS
MR AND MRS F.N. NJIRU

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

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ABSTRACT

The feeding of agricultural products previously sprayed with pesticides to dairy animals is a common practice in most intensive farming systems. This is due to diversification of agricultural practices in an attempt to increase food production for the growing human population which leaves limited land for grazing animals. Such animals are therefore supplemented with crop remains after harvest. The current study was undertaken in order to find out if pesticides used to spray crops fed to animals are eventually passed in milk which could pose a danger to consumers.

A total of 68 milk samples were collected from animals in Kibirigwi irrigation scheme since farmers often feed french bean remains previously sprayed with dimethoate and cypermethrin to their dairy animals. In addition, 34 samples of french bean remains were collected. The milk and french bean samples were analyzed for the above pesticides by use of a gas liquid chromatography and found to be negative. Another batch of 68 blood samples were collected from dairy cows and assayed for cholinesterase activity, before and after feeding on sprayed french bean haulms. There was no significant difference in cholinesterase activity pre and post feeding since the 95% confidence interval of the test statistic of the difference in activity included zero (-0.938, 1.291).

A total of 34 milk samples collected from the animals were analyzed for organochlorine pesticide residues. The samples were positive for eight compounds but their levels were low. These were ß-BHC, lindane, heptachlor, aldrin, heptachlor epoxide, P,P'DDE, dieldrin and O,P'DDD.

A small feeding experiment was conducted where twelve healthy animals were fed on french bean forage 1, 3 and 7 days after spraying with dimethoate. Milk from animals fed on foliage 1 and 3 days after spraying contained pesticide residues while that from animals fed on foliage 7 days after spraying did not. Blood samples were also withdrawn from the

experimental animals and assayed for cholinesterase activity before and after feeding. The difference in activity was found to be statistically significant since the 95% confidence interval of the test statistic of the difference excluded zero (-2.4289, -1.2377).

This study indicate that the level of contamination of french bean foliage is low after seven days postspraying. This important foliage is therefore safe to be fed to animals if withheld for at least seven days. The study also revealed low levels of contamination of cows milk with organochlorine pesticides.

CHAPTER ONE

1.INTRODUCTION AND OBJECTIVES

The population of Kenya will be approximately 32 million people by the year 2000 (Ministry of National Planning and Development, 1995). Land is a fixed resource, so that this growing human population will put increasing strain on the available land (Oguttu and Waelti, 1990). The effects of this have and will be most felt in the highlands, where most people farm small plots (< 1 ha) of land for their livelihood. These farmers will increasingly need to maximize the use of available resources through intensification (Oguttu and Waelti, 1990).

Keeping dairy cattle under an intensive zero- grazing system is one example of this trend to greater intensification. In addition to the usual grass fodder sources, animals kept under zero-grazing are supplemented with post-harvest crop residues. These include maize stover and residues of horticultural crops such as cabbages, tomatoes, and legumes. Usually, such crops have been sprayed with pesticides during various stages of growth and this provides a potential source of chemicals for food producing animals in zero- grazing systems (UNEP, 1991). Safety guidelines and safe use practices for the feeding of crop residues sprayed with the most widely used pesticides need to be systematically studied and documented, especially since chemical applications on crops are often improperly done (Mwathi and Kimani, 1989). This is the main motivation of this study.

This study was conducted in the Kibirigwi smallholder irrigation scheme, an area with small mixed crop and livestock (mainly dairy) farms. Kibirigwi is a typical highland area of Kenya lying between 1310-1486 m above sea level. It falls under agroecological zone 3, and although the rainfall is biannual, the amount and distribution is not usually sufficient for 2 cropping seasons. The Kenya Government, through the then

Ministry of Agriculture provided irrigation facilities in 1978 so that the farmers are able to grow crops on 0.5 ha plots throughout the year. These irrigated plots are usually planted with horticultural crops such as tomatoes, french beans, peas, peppers and cauliflower.

A preliminary study carried out in the scheme indicated that pests are a major problem on these plots and that farmers rely heavily on chemical pesticides to control these pests (Kimani and McDermott, 1994). These researchers also found that farmers misused pesticides, kept unlabelled compounds, used incorrect dosages, and improperly stored and disposed of pesticides and their containers. Such practices increase the chances of exposing man and animals to unsafe levels of chemicals.

On many of these farms, livestock, particularly dairy cattle, are fed on residues of pesticide sprayed crops. The most common crop residue fed is french bean haulms, which are sprayed with dimethoate during the main growing season and with cypermethrin closer to harvest. Farmers and agricultural extensionists are unsure as to what safety recommendations should be followed for feeding french bean haulms to lactating dairy cows. To determine if french bean haulms as currently fed in Kibirigwi are safe and what intervals between spraying and feeding should be observed, the present study was designed. The specific objectives of this study were:

1. To determine, under usual feeding practices, the levels of pesticide residues on french bean haulms fed to dairy cattle.
2. To determine if pesticides on french beans fed were absorbed by cows, as detected by decreased acetylcholinesterase levels in blood,
3. To determine if these pesticides could be detected in milk, and
4. To determine the days post-spraying that french bean haulms could be fed with no detectable residues either on the feed or in the cow.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 GENERAL USE OF PESTICIDES

2.1.1 Classification

"Pesticide "is a generic term which does not tell much about a particular chemical (Weinstein, 1984). In monitoring pesticides it is important to have a working knowledge of pesticide terms and the ways in which pesticides are classified (Weinstein, 1984). A pesticide can be defined as any substance intended to prevent, destroy, repel or mitigate pests (Derache, 1978; Hodgson *et al.*, 1988).

One way of classifying pesticides is by their main target pest or function (e.g.insecticides, herbicides, fungicides, rodenticides, molluscides, larvicides, ovicides, acaricides, defoliants, attractants, desiccants, disinfectants, repellents, and chemosterilants). Another mode of classification is by chemical makeup (Hubert, 1972). Almost all pesticides are combinations of 2 or more elements bound together by chemical bonds and can therefore be grouped by chemical type. The most important types are summarized in Table 1. Pesticides have also been classified by the WHO (1986) by hazard, which divides pesticides into three different categories based on their toxicity to laboratory animals (Table 2).

Table 1: Classification of pesticides by chemical makeup

Group	Constituents	Examples
Organochlorines	Carbon, Hydrogen, Chlorine	DDT, Lindane
Organophosphates	Phosphoric acid esters	Dimethoate, Parathion
Carbamates	Carbamic acid esters	Carbaryl, Aldicarb
Phenoxyherbicides	Phenoxyaliphatic acids	2,4-D, 2,4,5-T
Botanicals	Natural plant substances	Nicotine, Pyrethrin
Microbial	Microorganisms	*Bacillus thurigiensis*

TABLE 2: WHO classification of pesticides by hazard:

LD_{50} for the rat (mg/kg body weight)

CLASS	ORAL		DERMAL	
	Solids	Liquids	Solids	Liquids
Extremely hazardous	< 5	< 20	< 10	< 40
Highly hazardous	5-50	20-200	10-100	40-400
Moderately hazardous	50-500	200-2000	100-1000	400-4000

2.1.2 Production and use of pesticides

Early attempts to control pests chemically used natural toxic substances like mercury, sulphur or plant extracts such as nicotine and pyrethrins (Ramulu, 1979). Later organic synthetic compounds were developed, the first of which were organochlorines like Dichlorodiphenyltrichloroethane (DDT) whose pesticidal properties were accidentally discovered in the early 1940's (Hill and Wright, 1978). However, these were later discovered to cause environmental problems due to their persistence and toxicity to species other than man (Carson, 1962). This led to a search for more environmentally friendly substances. Less persistent organophosphate pesticides were developed. Subsequently other substances that mimic natural compounds, such as synthetic pyrethroids were development (Nakajima, 1982). These newer compounds, particularly the organophosphates and carbamates are highly toxic. This has led to numerous studies being undertaken to determine their dose-response relationships in various species (Derache, 1978).

Today, it is estimated that there are over 600 pesticides in use worldwide (Hayes, 1991). Since the 1950s, pesticide production in the developed countries has continued to rise. However, the demand in most of these countries began levelling- off in the 1980s, unlike developing countries, where pesticide usage increased five-fold over the late 1970s and early 1980s (Weir, 1985; Martin, 1988). Even worse, some pesticides which are banned, restricted or unregistered for use in developed countries are exported to and used in the developing world (Mbakaya *et al*, . 1994).

In 1981, a large-scale study found that 90% of pesticides used in the developing countries were applied to export crops like coffee, tea, sugar and vegetables or non-food crops like cotton and rubber (Spear, 1991). However, during the late 1980s chemical pesticides began to be applied to virtually all crops grown in Africa as well as a large

proportion of animals (Weir, 1985). Much of this use was indiscriminate based on a belief that the more pesticides were used the higher the production and quality of the product.

2.1.3 Benefits and problems of pesticides

Pesticides, when used properly, enhance health and agricultural production with minimal side effects (Spear, 1991). They contribute directly to health through the control of vector borne diseases like malaria, bilharzia, trypanosomiasis and leishmaniasis (WHO, 1990). It is estimated that over 10 million lives are saved per year in Africa through mosquito control for malaria alone (WHO, 1990). Pesticides also contribute directly to the economy via increased food and fibre production and through the protection of many materials during storage (WHO/FAO, 1990).

Notwithstanding their importance, pesticides pose significant environmental and occupational health hazards throughout the world (WHO/UNEP, 1987; Moses, 1983). Estimates by the WHO (1990) indicate that 3 million cases of severe poisoning occur annually of which 220,000 are fatal. In addition, 25 million agricultural workers in developing countries are estimated to suffer some symptoms associated with their use of or exposure to pesticides (WHO, 1990; Jeyaratnam, 1990).

The use of any biologically active compound poses potential problems of toxicity (Hayes, 1991). This can occur through direct contact with the compound during manufacture, formulation or use. If the compound is used in any stage of food production, residues of it or its derivatives may persist in food, thus, exposing the wider population, although usually at much lower levels than chemical and agricultural worker exposure (Hayes, 1991). However, the accidental contamination by pesticides of foods consumed by man has resulted in high morbidity and mortality rates in some cases (Ferrer and Cabral, 1991). Domestic and wild animals may also be affected depending on how and where the compound is used and its

persistence after use, but this is usually accidental (Cooper, 1991). Pests may also develop resistance to pesticides (McEwen and Stephenson, 1979).

The hazard caused by a specific pesticide is often proportional to the amount used (Hayes, 1991). Therefore, information on production and use constitutes an important factor determining both benefit and hazard. However, there is also wide variation in the toxicity of different compounds. Organophosphates are particularly toxic and are estimated to cause 40% of all pesticide related illnesses reported to United States poison control centers (Fuortes *et al.*, 1993). The total side effects associated with pesticide use were estimated at 839 million dollars in the U.S. alone in 1980. This was about 20% of the annual sales of pesticides by U.S. companies (Weir, 1985).

Pesticides have been associated with serious adverse effects in birds, man and animals. In Europe and America, some workers have reported that insecticide residues in birds caused harmful effects or even death. These include thin egg shells with low calcium content liable to breakage and reduced reproduction (Peakall, 1967, Edwards, 1970). Other suggested toxic effects in man and animals include carcinogenicity (Milham, 1971; Falck *et al.*, 1994), teratogenicity (Graham *et al.*, 1973), immunosuppression (Varshneya *et al.*, 1988), embrotoxicity, infertility and birth defects (Weintein, 1984). Spontaneous abortion and congenital malformations have also been reported in women exposed to organic pesticides (Kyrronnen, 1989, Taskinen *et al.*, 1989). Restrepo et al (1984) reported increased premature births and congenital malformations for pregnancies in floriculture workers who applied high levels of pesticides. Pesticides also pollute the environment (Hortung *et al.*, 1984; Iyaniwura, 1991). In Russia, researchers reported that pesticides were associated with the development of chronic pathology and temporary disability among female beet growers (Kundiev *et al.*, 1990). In SriLanka and Malaysia, symptoms of pesticide poisoning were reported in 7% of agricultural

workers (Jeyaratnam *et al.*, 1987). A study on pesticide poisoning in Costa Rica between 1980 and 1986, with special attention to agricultural and chemical workers estimated that over 3000 persons were hospitalized due to pesticide poisoning with 400 deaths (Wesseling *et al.*, 1993).

In East Africa, most cases remain unreported but a study by some workers on the status of pesticide usage in this region showed that pesticide poisoning may be contributing a great deal to most illnesses in our hospitals (Mbakaya *et al.*, 1994). These researchers carried out a descriptive epidemiological study using pretested questionnaires in 1989/90. They studied types of pesticides in current use with regard to procurement, distribution and utilization. Hospital records were examined for reported cases of pesticide poisoning as well as assessing the knowledge and awareness of health care providers on the recognition and potential of pesticide poisoning. They noted incidents of abuse, as in the use of organochlorine pesticides on food crops and reported pesticide poisoning cases in district hospitals where Kenya and Tanzania reported 455 and 736 cases respectively. Over 40% of health care professionals interviewed could not recognize pesticide poisoning cases. Therefore pesticide users, the general public and health care workers should be educated on pesticides.

2.2 SOURCES OF PESTICIDE RESIDUES IN ANIMALS

Chemical residues in animals may result from substances deliberately applied directly to the animal, feeding on contaminated feeds (either intentionally or unintentionally) or from contamination of the environment in which animals are raised (as in case of polychlorobiphenols (PCB) or wood preservatives) (Waltner and McEwen, 1994).

Contaminated feed either as pasture or harvested crop is considered an important source of intake for pesticide residues (Njau, 1988). For example, a study in France showed that some goats died suddenly after

grazing on a bait of toxaphene sprayed cassava leaves which had been put down to deter owners from allowing goats to trespass into farms of predominantly cassava and sweet potatoes which are an important source of green forage for livestock (Dufur, 1982). A dog that consumed blood from some of the carcasses also died. In another study organophosphate and organochlorine pesticides were associated with deaths of 125 out of 128 dairy cows in the coffee belt of Northern Tanzania (Njau, 1988). The majority of the poisoning cases occurred after the animals had consumed feed or water contaminated with pesticides. This was consistently confirmed by demonstrating the presence of pesticides in both the host and ingested material. In another incident, cases of poisoning of livestock after grazing on pasture treated with organochlorine pesticide, heptachlor, were reported in cattle body fat after grazing land uniformly treated with upto 1.1 kg/ha of commercial heptachlor (Gilbert and Lewis, 1982). A more serious exposure of horses and cattle to pasture treated with upto 6.5 kg/ha commercial heptachlor was reported by Dickson *et al* (1983). Concentrations of 180-550 mg/kg heptachlor were found in renal fat of animals that died from the poisoning.

Other sources include direct application on animals for control of external parasites where the pesticides are absorbed dermally and also through licking (Ushewokunze, 1991). The respiratory tract has not been considered an important route of exposure for domestic animals (Rosenberg and Quenon, 1988). However, illnesses following respiratory exposure to insecticides have been reported. In one example, permethrin aerosols applied for fly control were found to be harmful to lungs even though no residual amounts were detected in milk from the cows (Gandzyuk, 1989). Human error is commonly associated with pesticide poisoning of animals. Errors include improper dosage, use of improper compounds or formulations, use of treated seed as feed, improper spraying and improper storage and disposal of pesticide containers (UNEP,1991).

The disposition of pesticides or their metabolites in animals and

their products as a result of ingestion of treated feeds is not well documented. However, there are a number of case reports. In one example, a large outbreak of poisoning occurred in swine who were fed barley treated with ethylmercuric chloride for at least 23 days. All affected animals died (Howell,1969). The feeding of grains treated with mercury dicyandiamide to 1400 chicken killed 45 of them 45 days after feeding. The cause was determined by chemical analysis of eggs and meat which were withheld until the levels of residues were < 0.1 ppm (Howell, 1969). Poisoning was reported in a dairy herd, in which 9 of 18 cows died as a result of direct ingestion of endosulfan. For two affected cows which recovered from the poisoning, milk levels were > 1 ppb immediately after poisoning but declined to 1 ppb by 35 days (Braun and Lobb, 1976). In another incident, chlorinated hydrocarbon pesticide residues were detected in milk and milk products after their addition to the feed of cows (Smart *et al.*, 1972).

2.3 PESTICIDE EXPOSURE TO ANIMALS IN CENTRAL KENYA.

The major sources of pesticides that animals in central Kenya are exposed to are acaricides used to dip or spray livestock, the feeding of sprayed forage, grains and other feeds (Ministry of Livestock Development, 1994). The main acaricides are organophosphates although development of resistance by pests has led to introduction of combinations with organochlorine pesticides (Kanja, 1988). A study carried out at the University of Nairobi Veterinary Farm showed that significant amounts of organophosphates that animals are exposed to for tick control purposes are absorbed through the skin after dermal application on cattle (Ushewokunze, 1991). Other pesticides include herbicides whose exposure to animals is usually accidental, as a result of feeding near areas that have been recently sprayed (Mwathi and Kimani, 1989). A study by some researchers in an intensively farmed area of central Kenya showed that a wide variety of

pesticides are used on food crops ranging from organophosphates, carbamates, phenoxyaliphatic acids, pyrethroids and organochlorines in some cases (Kimani and McDermott, 1994). These chemicals may reach animals by accidental consumption or through feeding of sprayed foliage. Persistent chemicals like organochlorines and industrial wastes tend to accumulate in the food chains, eventually exposing man and animals who are at the top of most food chains (Kanja, 1988).

2.4 RESIDUES OF PESTICIDES IN ANIMALS AND ANIMAL PRODUCTS

2.4.1 Measurement methods

The analysis of any sample for pesticides involves taking a representative sample, extraction of the pesticide from the sample matrix using a suitable solvent, clean-up of the sample extract to remove co-extractants, and identification and quantification of the pesticide (Sherma, 1979). Proper sampling procedures should be followed to avoid biased and unrepresentative samples (Ambruse, 1978). In general, sampling is the major source of variability in pesticide residue analysis and may contribute a relative error, in terms of coefficient of variation, of 20% or more (Horwitz, 1978). A good sample should be large and unbiased (Martin *et al.*, 1987). Bias can be reduced through randomization and blinding (Martin *et al.*, 1987).

Extraction involves the removal of pesticide from a large bulk of sample matrix and the method and solvents used depend on the chemical and physical properties of the pesticide, type of substrate and final method of analysis (Keith and Stephenson, 1992). Non-bound residues are extracted directly by organic solvents while strongly bound residues are first released from substrate media by procedures often requiring derivatization for example silylation for phenolic metabolites of organophosphorus insecticides. A final step in extraction involves subjecting the sample

extracts to a cleaning procedure to remove co-extractives which interfere in the analytical determination and instrument performance. Various clean-up procedures have been used which include column chromatography, (Jensen, 1979), acid and base treatment (Bjerk and Sundby, 1970), gel permeation chromatography, silica gel and florisil clean-up (Bouwman et al., 1989) and thin layer chromatography.

The final stage in pesticide residue analysis is identification and quantification of the pesticide. Quantification methods are broadly classified into biological, chemical and physical methods (Keith and Stephenson, 1992). A review of different methods of pesticide residue analysis in food is described in Analytical Methods for Pesticides, Plant Growth Regulators and Food Additives (Zweig, 1963).

Biological methods measure the effect of pesticides in living organisms, for example, the depression of acetylcholinesterase by organophosphate and carbamate insecticides (Lawrays, 1983). Assays for acetylcholinesterase are based on hydrolysis of acetylcholine or a suitable synthetic ester (Wills, 1972). Four basic techniques have been used namely, potentiometric, colorimetric, titrimetric and manometric (Zweig, 1963, Derache, 1978). Bioassays are very sensitive, but not very specific, so they work well when used in conjunction with other analytical procedures to identify toxicants (Keith and Stephenson, 1992).

Most chemical methods are based on calorimetric reactions measured by spectrophotometry. The concentration of the compound is then related to absorbance of visible light, UV light, infrared light or fluorescence (Zweig, 1963). High detection limits and lack of sensitivity restricts the usefulness of these methods.

Physical methods include spectrophotometry, thin layer chromatography (tlc), gas liquid chromatography (glc) and high pressure liquid chromatography (hplc) (Zweig, 1963). Spectrophotometry detects light at specific wavelengths from infrared to ultraviolet. It has low sensitivity and specificity limiting its use. However it is still used to

scan wavelengths of compounds to find the most sensitive or specific wavelengths for the HPLC and GLC detection systems. TLC is used for separation, identification and quantification. It is good for metabolism studies using radiolabelled pesticides but is not very useful for routine analysis. GLC employs a chromatographic column to separate the analytes. The column terminates in a detection system for initial identification of analytes. It is simple, fast, sensitive, and capable of resolving complex mixtures. However it is limited in case of non-volatile samples and its high cost prohibits its use for routine analysis (Keith and Stephenson, 1992).

Immunoassay is a technique being adapted for pesticide residue analysis (Keith and Stephenson, 1992; Hall, 1996). It requires that an antibody be made to the pesticide. This antibody is then used in an assay such as ELISA or radioimmunoassay. Most pesticides have molecular weights < 500 so they are not strongly antigenic (Loitt, 1988). Thus, usually a protein-pesticide complex needs to be synthesized by covalently linking the pesticide to a protein such as serum albumin. This complex is then injected into rabbits or other animals and they form antibodies to the pesticide-protein complex, which can then be purified to isolate those that specifically bind to the pesticide molecule. ELISA assays can then be used as a preliminary screen to sort out samples containing no residues before applying other methods. Such assays are rapid and can be done in the field. They don't require any extensive cleanup or separation, hence, they are less complex, more rapid and less expensive. Immunoassays may suffer from lack of specificity, and even with monoclonal antibodies, cross-reactivity, although reduced, may still occur in chemicals with similar tertiary structures (Keith and Stepheneson, 1992).

2.4.2 Studies of residue occurrence and levels

There are two major types of epidemiological studies used in assessing residues. These are observational and interventional studies

(Martin *et al.*, 1987). Observational studies can be cross-sectional, cohort or case-control. In cross-sectional studies, a single sample is taken from the target population by means of a survey sampling technique and each unit is classified according to its current status for the factor and outcome at the time of sampling. Cohort studies involve sampling for units with and without the factor of interest and observing them for a period of time for development of the outcome as opposed to case-control studies where samples with and without the outcome of interest are selected and proportions that have been exposed to factor of interest estimated.

Interventional studies can be field trials, clinical trials or laboratory experiments. They involve manipulation and intervention in an attempt to explain a relationship between a factor and outcome of interest. Animal experiments are the most commonly used sources of information to characterize the hazards associated with chemicals (Crump *et al.*, 1989; Jelovsek *et al.*, 1989). Animal bioassay studies are advantageous since exposure can be specified and controlled and effects are not confounded by genetic or environmental factors (Waltner and McEwen, 1994).

2.4.2.1 Residues in Animal Products

Many compounds are stored in tissues or eggs and excreted in milk after administration to animals. However it is unusual for pesticide residues other than chlorinated hydrocarbons to be detectable in more than trace amounts in marketed food of animal origin (Spear, 1991). The major pesticide exposure for most people is through residues in food (Spear, 1991). Until recently, the literature on pesticide residues in food dealt principally with chlorinated hydrocarbons. The use of this class of compounds has been discontinued in most developed countries and this has gradually changed the residue picture (Spaulding, 1983). However,

chlorinated hydrocarbons are still used in many developing countries, especially on crops grown for domestic consumption (Albert, 1983).

Different kinds of foods require different pesticides in their production. There can be considerable variation in the retention and concentration of residues in foods when consumed. Therefore it is necessary to classify foods in connection with the residues of pesticides they contain.

2.4.2.1.1 Dairy products

These include milk, butter, cheese and processed milk. The pesticides most commonly found are chlorinated hydrocarbons. For example a survey in Canada in 1983 found detectable levels of α-BHC, DDE, dieldrin and heptachlor epoxide in 90% of milk sample but the levels were below tolerance levels (Frank *et al.*, 1985). No organophosphate residues were found. DDT and BHC residues have been found in dairy products at levels proportional to the intensity of pesticide use in malaria control (Kapoor *et al.*, 1980). In India, a survey of commercial samples of butter of nine separate brands showed that 90% of the samples contained DDT residues above the FAO/WHO guideline level (Dhaliwal and Kalra, 1978). In a similar study in Argentina, organochlorine pesticide residues were analyzed in 150 samples of butter collected from several parts of the country but most were within the FAO/WHO limits (Lenardon *et al.*, 1994). Organochlorine residues in dairy products generally show a downward trend with time but there appears to be a geographical variability mainly associated with the use of pesticides (Campanini *et al.*, 1980; Wandiga, 1995).

Most organophosphates, carbamates and sulphur containing compounds are not fat soluble and hence do not form fat soluble metabolites in dairy products (Spear,1991). Therefore very little if any of their residues are excreted in milk (Maitho, 1978). These chemicals may get into milk by drift from spraying or dusting for insect control in or near dairy barns

or processing plants. Studies carried out on the metabolism of organophosphates indicate that they are not deposited within body tissues to a large extent. For example Dauterman *et al* (1959) made a study of the bovine metabolism of organophosphates using dimethoate and found that most was excreted in urine and faeces within 24 hours and the chemical was not detected in milk. Another study using tagged phosphorus- malathion by O'Brien and others (1961) found that 90% of excreted malathion and derivatives was eliminated as malathion monoacid in urine and the milk contained no detectable malathion or malaoxon. In another study (Gyrisco *et al.*, 1961) which investigated the effects of feeding high levels of sevin to dairy cows, the concentration of sevin and its metabolites in milk was below the sensitivity of the analytical method even after 2 weeks of feeding.

Spraying tests have also been carried out using malathion. Claborn *et al* (1956) sprayed 4 cows twice per week with 0.5% and 1.0% suspensions and emulsions. One day following spraying only traces were found in the milk of all 4 cows. In seven days it was free of malathion. Residues found were higher in milk from cows sprayed with suspensions than from those sprayed with emulsion and also higher for the stronger concentration level.

Spraying tests using cypermethrin were carried out by Byazron *et al* (1989) in order to find out the accumulation and secretion of the pesticide in milk. Cows were sprayed three times at seven day intervals with a 0.01% emulsin and milked 7-144 hours later. No residues were found in any sample. This was in agreement to a study carried out to determine residues of an organophosphate, quintiofos, in cattle after dermal application to cattle (Ushewokunze *et al.*, 1991). Within 8-10 hours the levels of quintiofos were reduced to 0.01mg/l and the chemical was completely eliminated from milk between spraying intervals of 3 days.

2.4.2.1.2 Meat, Fish, Poultry and Eggs

The major residues found in these products belong to the chlorinated hydrocarbons and arsenicals. Since 1973, the U.S. Dept. of Agriculture has sampled meat products to estimate residue levels. In the mid 1970s, approximately 75% of sheep, swine, and cattle samples were positive for DDT and over 50% for Dieldrin. For chickens and turkeys these percentages were about 90% for both compounds. By 1981 most of the residues had declined (Spaulding, 1983). During the period 1986-1988, 602 samples of animal products were analyzed for organochlorine and organophosphate pesticides and industrial pollutants in Ontario (Frank *et al.*, 1990). Several organochlorines exceeding maximum residue limits were detected in abdominal fat from cattle, sheep, goats, pigs and rabbits but no residues of organophosphorus insecticides licensed for livestock were found in meat, fat or egg tissues. In another study, a number of residue violations were identified in Australian meat exported to the U.S.A. and the major pesticides involved were the organochlorines, dieldrin and heptachlor (Corrigan and Seneviratna, 1990).

Robertson *et al* (1990) studied sources of pesticide contamination from Australian livestock. Samples were analyzed for the presence of organochlorines by gas chromatography and as a result 673 herds were quarantined for having organochlorine residues above the maximum residue limits. Dieldrin was detected in 73% of herds while BHC, heptachlor and DDT occurred in 13%, 9% and 5% respectively. Analysis of 502 cases of organochlorine contamination showed that feed was the source in 26% cases, pasture in 24% cases, old cattle or sheep dips in 7% cases, yards treated for termites in 20% cases and careless use or storage of chemicals in 5% cases. Neumann (1988) also studied the occurrence and variations of organochlorine pesticide residues detected in Australian livestock at slaughter and found most were in excess of maximum residue limits. The most common sources of pesticides were crops and pastures, termite

treatment and feed/storage sites. Other studies on organochlorine pesticide residues in livestock products particularly meat and milk as well as in feedstuffs were done by Cho *et al* (1979) between 1976 and 1979. These workers measured pesticide residues in milk and tissues of goats fed different pesticide levels. They found that the secretion rate of pesticides in milk varied with dose.

Abdominal fat and other tissues from broilers fed with feed fortified with known levels of organochlorine pesticides and slaughtered at regular intervals showed presence of various residues (Devos *et al.*, 1972). Frying the meat reduced the levels of residues probably due to loss of fat. In another study, residues of DDT and Dieldrin were detected in eggs from chicken fed feed containing these pesticides (Delchev *et al.*, 1975). Laying hens were fed feed containing DDT and Dieldrin at 7ppm over a period of 4 months. During this time the contents of residues of DDT and Dieldrin in the yolk increased by 40.6ppm and 23.8ppm respectively. The contents of residues decreased gradually after the contaminated feed was withdrawn. In a similar study residues of pesticides were detected in meat of pigs and chicken fed with forage containing 25mg trichlorphon in the daily ration (Sergeeva *et al*,. 1975). Accumulation of residues in tissues stopped after the pesticide was withdrawn.

A study on residue levels in pigs and sheep after treatment with an organophosphate, phoxim, was conducted in Hungary. The treatment involved dipping and washing as well as pour-on formulations. Fourteen and twenty one days after treatment, a sample of animals were slaughtered from each group to assess their phoxim content in liver, muscle, kidneys and fat (Simon *et al.*, 1988). Fat tissues were found to contain high levels of phoxim even after 21 days. However, indications are that carbamates and organophosphates are metabolized more quickly, even those that contain chlorine in the molecule.

Ingestion of organophosphates by animals makes their products unfit for human consumption albeit for a period of time. For example, meat from

sheep fed with Formothion at 45mg/kg whose LD_{50} is 0.25mg/kg was unfit for human consumption upto and including 10 days after dosing, but was cleared by 20 days (Khaitov *et al.*, 1976).

2.4.2.2 Residues in animal feeds

Animal feeds constitute sprayed forage, grains, cereals and various types of meals. Occurrence of pesticide residues in animal feeds may be intentional as in the case of pesticide preserved grains or unintentional if pesticides are improperly stored near feeds (Waltner and McEwen, 1994). Residues on plants which are eventually consumed by animals are the result of direct application or absorption from the soil (Fikes, 1990). Chlorinated hydrocarbons are the main pesticides absorbed from the soil into the plant due to their persistence in the environment whereas the organophosphates, malathion and diazinon are the most commonly reported pesticide residues in grains and cereals (Gartrell *et al.*, 1986). Pesticides applied on fruits and vegetables to extend their lives and preserve quality during storage, transport and marketing may persist on the edible portions of such products and be passed to animals and individuals consuming them (Papadopoulou, 1991).

Pesticide contamination of animal feeds is important since the pesticides can eventually be passed to man through animal products such as milk, meat and eggs (Waltner and McEwen, 1994). Various studies have been done to assess animal feeds as a source of contamination of animal products. For example, organochlorine residues were found in 84 samples of raw milk, 24 samples of pasteurized milk and 15 samples of animal fodder (grass, hay and concentrates) from randomly selected farms in Valdivia province, Chile (Pinto *et al.*, 1990). Residues in animal fodder were much higher than in milk products and it is likely that the fodder was the source of contamination. Another study was carried out in Queensland on the relationship between organochlorine residues in animal feeds and

residues in tissues, milk and eggs (Noble, 1990). Higher than recommended levels of aldrin and dieldrin were found in feeds.

2.4.3 Residue studies in Kenya

Maitho (1978) investigated levels of organochlorine and organophosphate pesticide residues in cattle milk and meat and found that residue levels varied in different parts of Kenya. Kahunyo (1983) found that several organochlorine residues were present in chicken fat and eggs but their mean levels were lower than FAO Acceptable Daily Intakes (ADI). However more recent studies have found higher levels in animals and humans. For example Mugambi (1984) sampled eggs and found dieldrin residues above acceptable limits in about 30% of those analyzed. In the mid-1980's, Kanja (1988) investigated the levels of organochlorines in the breast milk of women in eight rural regions of Kenya and found high levels of DDT and its metabolite, DDE. A more recent study on milk from mothers in Nairobi shows that the situation is little better in urban settings (Kinyamu, 1992). A study by Wasserman *et al.*, (1972) found high levels of organochlorines in human adipose tissue from various Nairobi hospitals.

Other Kenyan studies indicate that pesticide residues are widespread. Kimani (1988) studied tomatoes from markets in Nairobi and found dithiocarbamate residues to be above set maximum limits. A study on flora and fauna of lakes Nakuru, Naivasha, Elmentaita and Baringo showed the presence of organochlorine pesticides in the studied samples (Lincer *et al.*, 1981). In another study, white pelicans and lesser flamingoes from Lake Nakuru were examined and found to contain high levels of organochlorine residues in their livers (Koeman *et al.*, 1972). An earlier study on fish eating birds from Lake Victoria had shown high levels of dieldrin (Koeman and Jennings, 1970). This corresponds to the widespread detection of organochlorine residues in Kenyan fish (Mugachia, 1990).

While organochlorines, because of their persistence are most

commonly detected, the current trend in pesticide usage shows a decrease in use of organochlorines and an increased use of organophosphates and synthetic pyrethroids. Thus it is expected that there will be a gradual decline in organochlorine residues. Since an organophosphate (dimethoate) and a synthetic pyrethroid (cypermethrin) are the compounds of interest in the current study, they will be reviewed.

2.5 ORGANOPHOSPHORUS PESTICIDES

Organophosphorus compounds are the largest and most diverse group of insecticides (McEwen *et al.*, 1979). They are esters of alcohols with phosphoric acid or anhydrides of phosphoric acid with another acid. Lassaigne first reported in 1820 that phosphoric acid could react with alcohols but it was not until 1930 that Schrader discovered the insecticidal applications of such a reaction (McEwen *et al.*, 1979). Organophosphates are easily degraded hydrolytically, enzymatically and biologically and require low application quantities to obtain the desired insecticidal activity, thus, reducing the danger of undesirable residues in harvested products (Buchel *et al.*, 1983).

2.5.1 Structure and activity relationships

The biochemical behavior of organophosphorus pesticides is largely determined by their ester character. The most important detoxification reaction in vivo is hydrolytic degradation. This forms water soluble degradation products which are excreted rapidly since they are incapable of penetrating lipophilic membranes. The insecticidal activity is therefore dependent on the quantity of insecticide taken up versus the amount hydrolysed in a given period of time. Therefore, a weak insecticide that penetrates rapidly is capable of reaching lethal concentration quickly at the site of action (e.g. dimethoate).

Organophosphorus insecticides are susceptible to hydrolysis and therefore do not persist for long in the environment. They are quickly hydrolysed on plant or soil surfaces, either by chemical reactions mediated by enzymes or after penetration into various parts of the plant. Duration of activity of most of these insecticides lasts from several days to a few weeks.

2.5.2 Mechanism of action of organophosphorus insecticides

Organophosphorus compounds owe their toxicity to their ability to inhibit cholinesterase (Garner, 1970; Fikes, 1990). This enzyme normally hydrolyses acetylcholine at the nerve endings after transmission of a nerve impulse. The signs of poisoning therefore are those which would result from the persistence of an excess of acetylcholine. In this reaction transesterification of the organophosphate occurs with the primary alcohol group of a serene molecule in the enzyme acetylcholinesterase. This is equivalent to phosphorylation of the active site on the enzyme. This is the primary mode of poisoning in insects and mammals (Morgan, 1989). The phosphorylated enzyme has a relatively long life compared to the acetylated enzyme. This causes accumulation of acetylcholine to lethal levels. The inhibition of cholinesterase by organophosphorus pesticides is irreversible (Murphy, 1986).

2.5.3 General metabolism

Alkylphosphates do not accumulate in the body and most phosphate insecticides are rapidly degraded and excreted in mammals. The major route of excretion for water soluble excretion products is via urine. Excretion via faeces is of less importance. Metabolizing enzymes in mammals are carboxyesterases, microsomal oxidases and carboxyamidases for amides, mainly in the liver (Sultatos *et al.*, 1984). With some organic

thiophosphates and dithiophosphates more highly toxic O-analogues are formed in the body of insects by oxidation of the pesticides by microsomes in the intestines, nerve cord and fat (Morgan, 1989).

Prior to the 1950, the chemical industry recognized that insecticides which were metabolized differently in insects and vertebrates (either substances which only insects can activate or active substances which only mammals can detoxify) would be of great value. This is already practical with some organophosphates. For example the mouse converts only a small proportion of malathion into toxic malaoxon, while the cockroach converts much more. Mammalian carboxyamidases are capable of removing the amide groups of organophosphates, but insects are not (e.g. dimethoate is deaminated in mammals but not in insects).

Reactions that inactivate insecticides or produce metabolites of equivalent or higher toxicity may be summarized into biochemical oxidations and degradation of phosphotriester bonds. The mechanisms of biochemical reactions involve desulfuration of P-S group, oxidation of thioether group, hydroxylation and dealkylation of N-alkyl substituents and oxidations of diphatic substituents. Mixed function microsomal oxidases play a role in such reactions and resistance to organophosphates has been associated with increased levels of microsomal oxidase activity (Morgan, 1989) .

2.5.4. Effects on cholinesterase and other enzymes

Esterases are enzymes that catalyses hydrolysis of carboxylic acid esters. Such enzymes are widespread in nature and exhibit a large number of different features which serve as basis for their classification. The pH optima for most mammalian esterases is 7.5-8.5.

Cholinesterases are a subgroup of esterases originally characterised as those that specifically catalyze the hydrolysis of acetylcholine. It later became apparent that substrate specificity was not absolute since

some hydrolyse noncholine esters. Cholinesterases can be divided into 2 groups: Group 1 ("true"or "specific" or "E-type" cholinesterases and Group 2 ("pseudo" or "nonspecific" or "S-type") cholinesterases (Fikes, 1990). Group 1 enzymes use acetylcholine, acetyl-ß-methylcholine and butyrylcholine as substrates but not benzoylcholine. They are present in high concentration in nervous tissues of vertebrates and invertebrates, and low levels in muscle tissue and erythrocytes of vertebrates and the electric organs of the eel. Group 2 cholinesterases have a higher affinity for butyrylcholine than for acetylcholine. They do not hydrolyse acetyl-ß-methyl-choline but they hydrolyse benzoyl- choline. They are located in the plasma of most animals. Cholinesterase inhibition is considered an adverse effect but its effect is uncertain since it can occur for long periods without harming the individual and at dosage levels well below those which cause acute or long-term harm.

Blood cholinesterase consists of plasma (pseudo) cholinesterase and erythrocyte (true) cholinesterase . Cholinesterases are also found in the nervous and neuro-muscular synapse levels, in nerve fibers, muscle spindles and tendons. Cholinesterases of central nervous system (CNS) are of 2 types. Extracellular cholinesterases, which are easily inhibited, and functional intracellular cholinesterases which are protected by cellular membranes and inaccessible to inhibitors. Mammals can become tolerant to abnormally high levels of acetylcholine in the brain caused by prolonged exposure to cholinesterase inhibition.

Other enzymes are also affected by organophosphates e.g. marked increase in serum glucuromidase activity was found after administration to rats of a single dose of parathion and paraoxon (Derache, 1978). Organophosphates also block a number of other hydrolytic enzymes like pseudocholine esterase, lipase, other esterases, trypsin and chymotrypsin. Malathion is a carboxyesterase inhibitor and when given repeatedly in low doses, it inhibits its own hydrolysis by inhibiting the malathion dehydrolysing esterase. Raised serum carboxyl esterases levels suppress

the inhibitory effects of organophosphates on cholinesterases.

2.5.5 Toxicity in Animals

Toxicity varies greatly between compounds. For example in rats the oral LD_{50} of chlorfenviphos is 10mg/kg while that of bromophos is more than 3,500 mg/kg. The LD_{50} values of a range of organophosphate insecticides for rats are given in Table 3 and a number of organophosphates are classified into toxicity classes in Table 4.

The classical signs of acute toxicity are salivation, watery and protruding eyes, tachycardia, hyperactivity, trembling, generalized muscular spasms, and convulsions followed by prostration. Often there are other signs like skin irritation, irritation of eyes, miosis, abdominal pain, diarrhoea and bronchial secretions.

Various short and long-term toxicity studies have been conducted in various species. According to FAO (1987) the most significant long-term effects in animals receiving organophosphates are growth impairment, signs of cholinesterase depression and micro- pathological lesions of tissues and organs.

Some organophosphates like triorthocresylphosphate exhibit neurotoxic effects following either single or multiple administrations to animals and man. Among the most important cresol substituted p-esters only those with ortho-cresol binding have neurotoxic effect. Lesions affect axons in the peripheral nerves, spinal cord and medulla oblongata. These lesions cause sensory disturbances and motor weakness (Marion, 1989; Morgan, 1989). Regeneration of peripheral axons occur but there can be permanent damage to the long spinal tracts. Clinical symptoms and signs of neural damage do not appear immediately following exposure; there is a delayed neurotoxicity (Marion, 1989; Fikes, 1990). This becomes manifest at least 8 days after exposure when blood cholinesterase levels have generally returned to normal.

2.5.6 Potentiation and protective action

Potentiation of organophosphates is of interest with respect to acute toxicity. Experimental studies have shown that some are capable of producing some degree of potentiation which is linked to non-linear transformation of the substance into a phosphorothioate compound. Some pesticides have a protective action by antagonizing the action of organophosphates (e.g. DDT, heptachlor and lindane have an antagonistic action on the acute effects of bromophos). Potentiation of the acute effects of bromophos occurs with bromophos-ethyl, diazinon, dichlorvos, dimethoate, malathion, mevinphos, parathion and carbaryl (WHO, 1990).

Concurrent administration of some drugs may increase animals susceptibility to organophosphorus insecticides. These include drugs with neuromuscular blocking effects or those that compete for target esterases for example phenothiazine, procaine, inhaled anaesthetics, depolarizing neuromuscular blocking agents like succinylcholine and decamethonium (Hatch, 1988). Antibiotics like aminoglycosides, polypeptides, lincomycin and clindamycin as well as central nervous system depressants have neuromuscular blocking effects (Hatch, 1988; Pittinger and Adamson, 1972).

2.5.7. Other effects

Organophosphates can cause meiotic damage as was demonstrated by administration of malathion and dimethoate at 0.2 micrograms per kg body weight /day for upto 10 days in mice. This led to decrease in division rate in the primary spermatocytes of mice (Hoda *et al.*, 1993). In another study in England it was demonstrated that dimethoate decreased the turnover of collagen in bone and skin of albino rats (Reddy *et al.*, 1991). Some organophosphates also affect on reproduction. Several experiments conducted on organophosphates have shown that some cross placental barrier and enter foetal tissues. Some also caused malformations of foetus and

reduced lifespan, loss of weight, reduced fertility, teratogenic effects while others showed no effects (Schardein, 1985).

Table 3. Acute oral LD_{50}* of some Organophosphates insecticides in the rat

Compound	Acute oral LD_{50} (mg/kg)
TEPP	1.12
Methamidophos	30.00
Dimefox	5.00
Phorate	2.00
Dimethoate	245.00
Ethion	96.00
Demeton-O-methyl	180.00
Demeton-S-methyl	40-60
Omethoate	50.00
Formothion	353.00
Parathion	6.40
Fenitrothion	500.00

* Is the statistical estimate of the dosage that would produce a specified effect in 50% of a large population of rats.

Source: Organophosphorus pesticides. Criteria (Dose effect relationships) by Derache R. 1978.

TABLE 4: Relative acute oral toxicities of organophosphorus pesticides

Highly toxic LD_{50} < 50mg	Moderately toxic 50mg < LD_{50} < 500mg	Slightly toxic LD_{50} > 500mg
Azinphos methyl	Diazinon	Bromophos
Chlorfenvinphos	Dimethoate	Malathion
Dichlorvos	Formothion	Trichlorfon
Parathion	Phosalone	Fenchlorfos
Phosphamidon	Thiometon	
Fenitrothion		
TEPP		

Source: Organophosphorus pesticides. Criteria (Dose effect relationships) by Derache R. 1978.

2.6 PYRETHRIN AND SYNTHETIC PYRETHROID INSECTICIDES

Pyrethrin is one of the oldest known, naturally occurring insecticide (Walker, 1994). Natural pyrethrins are extracted from Chrysanthemum spp (Fuchs and Schroder, 1983). The flowers of these plants have been dried and powdered since the beginning of the 19th century for control of household pests (Valentine, 1990). The first such products were imported over 100 years ago from Dalmatia and Iran (Fuchs and Schroder, 1983). Natural pyrethrins have low mammalian toxicity and good knockdown effect. Different naturally occurring pyrethrin products have been classified as pyrethrin I and II, cinerin I and II and Jasmolin I and II based on electrophysiologic responses, clinical signs and chemical structure (Valentine, 1990).

Synthetic pyrethroids are based on the naturally-occurring insecticidal components of pyrethrum extract and emerged in the 1970s as the fourth major chemical class of synthetic insecticides (James *et al.*, 1993). These compounds were developed due to high production costs of natural products. The first was allethrin, marketed in 1950. It is the allyl homologue of cinerin I. Since it had only a narrow spectrum of activity and the natural alcohol was difficult to obtain, it was replaced by other synthetic, more readily obtainable pyrethrin- alcohols. Newer synthetic pyrethroids also have improved light stability and have increased potency. They are increasingly replacing other groups of pesticides in the fields of agriculture, hygiene, household, wear- and building industry (Tippe, 1993). They include permethrin whose residual action lasts several weeks even under field conditions. It has an oral LD_{50} of 1500-2000 mg/kg in rat. It is used as a pesticide in cotton and in control of insects in public health. Decamethrin is one of the most active pyrethroids and was first synthesized by Elliott and others in 1974. It has an oral LD_{50} of 25-60 mg/kg in rat.

Other photostable pyrethrins are cypermethrin and fenvalerate. These

are widely used as pesticides on cotton and vegetables. They have oral LD_{50} of 500 mg/kg and 450 mg/kg in rat and i.v. LD_{50} of 50 mg/kg and 75 mg/kg in rats, respectively. These photostable pyrethroids were discovered through the work of Elliott and Janes in England (1973) and Ohno *et al* in Japan (1974). They are characterized by much lower application rates and lower mammalian toxicities compared to traditional insecticides. They are widely used in cotton for control of *Heliothis spp.*, fruit and vegetable growing, hygiene, and other possibilities are opening up in veterinary medicine and material preservation.

2.6.1 Mechanism of Action and Toxicity

Pyrethroids are purely contact poisons that penetrate rapidly into insects' nervous system. Voltage-dependent sodium channels in the nervous tissue are the major sites of pyrethroid action (Narahashi 1984; Vijverberg and Berken, 1982). They modulate the gating kinetics of the sodium channel, resulting in repetitive discharges or membrane depolarization (Vijverberg and Berken, 1982; Narahashi, 1987). They act in an all-or-none manner on individual sodium channels so that even a small fraction of sodium channels modified by a pyrethroid can result in neural dysfuntion (Narahashi, 1984). Another site of action of pyrethroids is at the gamma amino butyric acid (GABA) receptors which operates a chloride channel and is the major inhibitory receptor in vertebrate central nervous system (Crofton *et al.*, 1987; Lawrence and Casida, 1983).

Insects exhibit an initial phase of exceptional excitation followed by incoordinated movement, paralysis and finally death. The initial knockdown effect may not be lethal because natural pyrethroids are rapidly detoxified in the insect by enzymatic action and some recover. This enzymatic detoxification may be delayed by the addition of synergists. In practice organophosphates or carbamates are frequently added to pyrethroids for this purpose (Glickman and Casida, 1982). The

synergists competes with the insecticide and is oxidatively degraded by insecticidal enzyme instead. This prolongs the knockdown effect.

Toxicity towards mammals varies widely. For example, cismethrin has an acute oral LD_{50} of 100 mg/kg while bioresmethrin has an LD_{50} > 8000 mg/kg in rat (Fuchs and Schroder, 1983). The characteristic clinical signs of pyrethroid toxicosis are nerve and muscle disorders (Casida *et al.*, 1983; Cremer, 1983). These include profuse salivation, writhing convulsions and whole body tremors. The low toxicity of pyrethroids is due to the fact that most of the dose administered is not absorbed by mammals (James *et al.*, 1993).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Equipment

Equipment	Description	Supplier
Balances	Sartorius	Sartorius-werke
Waring Blender		Moulinex, France
Separatory funnels	1 l	
Filter funnels		
Whatman filter papers	33 cm	
Round bottomed flasks	500 ml	
Measuring cylinders	11, 500,200,50,25 ml	
Rotavapor	Buchii	
Glass columns	300 mm*22 mmid*25 mmod	Supelco
Glass vials with teflon stopcocks	10 ml,40 ml	Supelco
GLC	3400	Varian
Integrator	4400	Varian
Microliter syringe	5 ul	S.G.E PTY Ltd
SMI micropipetter	SMI digital adjust	SMI USA
Pasteur pipettes		
Centrifuge	Gallenhamp	Gallenhamp
Ultrasonic sonicator	Cell disruptor	Heat systemsInc.
Liquid dispenser	Sucorex dispenser	
Water bath	Thermostated	Memmert, W. Germany
Septa	Chromosep septa	Chromopack
Capillary column		
Glass wool	Silane treated	Supelco
Calsberg pipettes	100,1000,500,50 ul	John poulten

Vacuum pump	Air compressor 850	Corning England
Sampling bottles	250 ml	SchottGlaswerke, Germany
Vacutainers	10 ml	Becton Dicknson USA
Plastic bags		Kenya polythene Ltd.
Centrifuge tubes	15 ml, 50 ml	
Water distiller		
Volumetric flasks	10, 100, 1000 ml	
pH meter	Fisher 119	R.O.C.Taiwan
Whirl mixture		

3.1.2 Chemicals

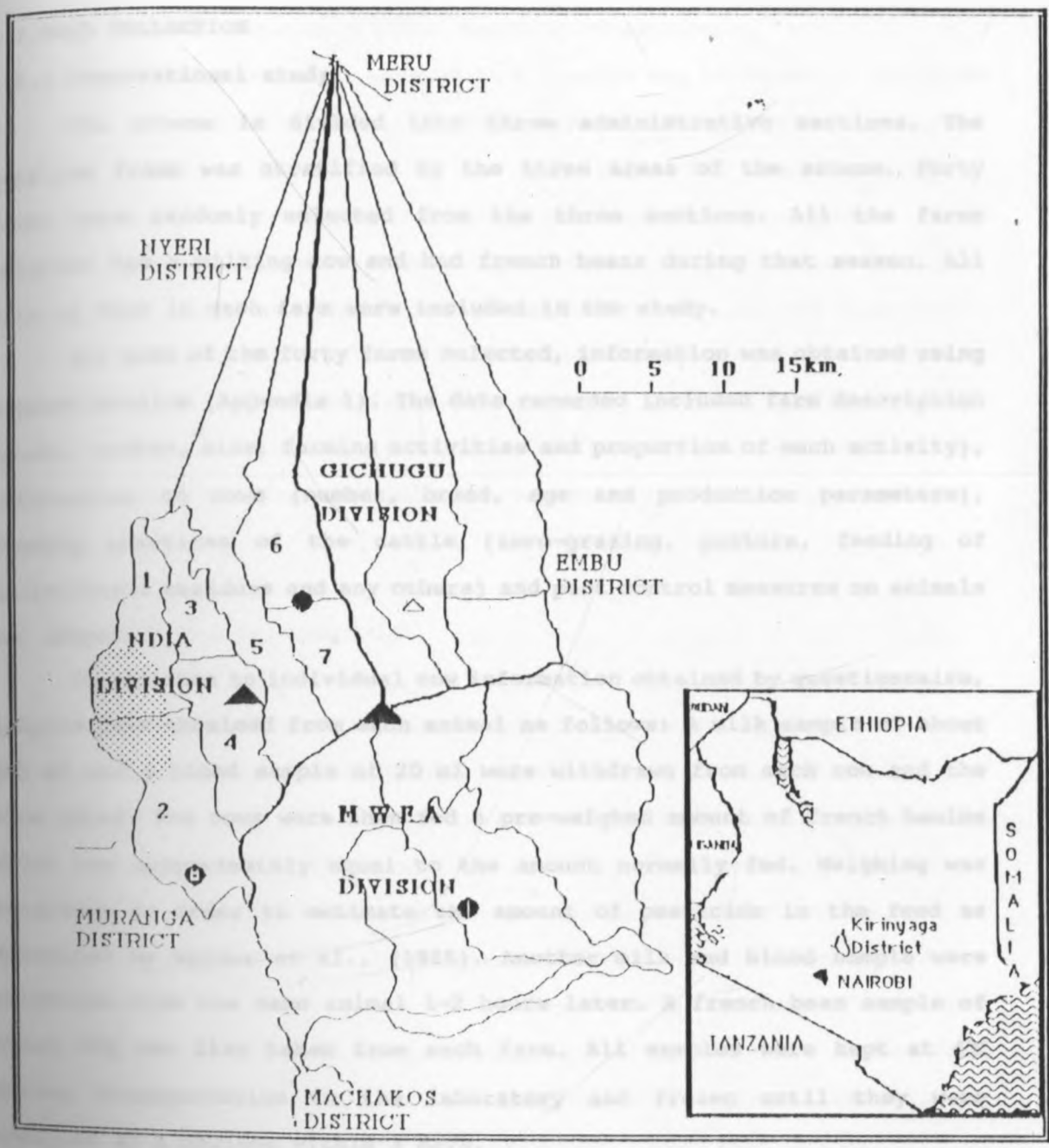
Chemical	Brand name/Grade	Supplier
Hexane	Analytical	BDH chemicals
Dichloromethane	Analytical	May & Baker
Acetonitrile	Analytical	May & Baker
Anhydrous sodium sulphate	Analytical	BDH chemicals
Sodium Chloride	Analytical	BDH chemicals
Florisil		Supelco
Acetone	Analytical	Howse & McGeorge
Ethanol	Analytical	Merck Darmstadt
Sulphuric acid	Analytical	BDH chemicals
Sand	Acid washed	Howse & McGeorge
Potassium hydroxide	Analytical	BDH chemicals
Snoop	Liquid leak detector	Supelco
CPM standard	1 ml ampoule	Supelco
Cypermethrin standard	1 ml ampoule	Supelco
Dimethoate standard	1 ml ampoule	Supelco
Omethoate standard	1 ml ampoule	Supelco
Nitrogen gas	99% pure and ordinary	

3.2 METHODS

3.2.1 Study area

The study area is located in the Kirinyaga District of Central Province of Kenya, on the slopes of mt. Kenya. The district is one of the most densely populated in Kenya with a population density of over 250 people per square kilometer (Ministry of Planning and National Development, 1995). It has a total rural area of 112,700 ha of which 95,500 ha (84.5%) is potential agricultural land (Jaetzold and Schmidt, 1983). It is situated on the North Eastern side of Nairobi (Figure 1).

The Kibirigwi Irrigation Scheme has an altitude of 1306-1486 m above sea level and covers an area of 1204 acres. Of these 208 acres (17.3%) is under coffee, 418 acres (34.7%) is under food crops, 84 acres (6.98%) is devoted to grazing and 362 acres (30.1%) is irrigated. The irrigated land is mainly under horticultural farming. The average farm size is 3 acres but this is decreasing due to subdivision. Majority of the farmers are smallholders practicing both livestock production, subsistence crop production and cash crop farming. The rainfall pattern is biannual with the long rains being experienced between March and June and the short rains between August and December. However, the rainfall is often inadequate so the area is irrigated for most part of the year.



Key:

Kibirigwi Irrigation Scheme

Figure 1: Map of Kirinyaga District showing the location of the study area

▲ -Border towns

● -Divisional headquarters

Numbers- Sublocation

3.3 DATA COLLECTION

3.3.1 Observational study

The scheme is divided into three administrative sections. The sampling frame was stratified by the three areas of the scheme. Forty farms were randomly selected from the three sections. All the farms selected had a milking cow and had french beans during that season. All milking cows in each farm were included in the study.

For each of the forty farms selected, information was obtained using a questionnaire (Appendix 1). The data recorded included farm description (owner, number, size, farming activities and proportion of each activity), information on cows (number, breed, age and production parameters), feeding practices of the cattle (zero-grazing, pasture, feeding of agricultural residues and any others) and pest control measures on animals and crops.

In addition to individual cow information obtained by questionnaire, samples were obtained from each animal as follows: A milk sample of about 200 ml and a blood sample of 20 ml were withdrawn from each cow and the time noted. The cows were then fed a pre-weighed amount of french haulms which was approximately equal to the amount normally fed. Weighing was necessary in order to estimate the amount of pesticide in the feed as described by Gelder *et al.*, (1985). Another milk and blood sample were withdrawn from the same animal 1-2 hours later. A french bean sample of about 10g was also taken from each farm. All samples were kept at 4°C during transportation to the laboratory and frozen until they were analyzed at a maximum within 3 days.

3.3.2 Feeding experiment

Twelve healthy animals were used in the experiment. The animals were divided into three groups of four animals each. One group was fed a preweighed amount of french beans haulms sprayed with dimethoate one day after spraying. The second and third groups were fed sprayed french beans

haulms three and seven days after spraying respectively.

The french beans were sprayed in a similar way to those in the farms used in the observational study. This was at the application rates of 40ml/20l to spray an eighth of an acre plot. The concentration of dimethoate used was 400g/l and spraying was done twice per week.
A milk sample of 200ml and a blood sample of 20ml were withdrawn from each animal. Each animal was fed 15kg of french bean haulms and the time noted. Another sample of 200ml milk and 20ml blood were taken from the same animal two hours after feeding. A bean sample of about 10g fed to each animal was also taken for analysis. Samples were transported to the laboratory and analyzed in the same way to those in the observational study.

3.4 DATA HANDLING AND ANALYSIS

Farming activities and individual cow data were entered and stored in separate data base files (Dbase IV) for handling. Each farm was treated as a separate record. After entering all information, the data files were examined for proper coding, missing results and errors in data entry. These were corrected before calculating descriptive statistics for various parameters to be analyzed. These parameters included average farm size, proportion of various farming activities, average number of cows per farmer, mean milk production, average weight of french beans haulms fed, average time between spraying and feeding, and average time between feeding and sampling. Summary statistics for the difference in cholinesterase activity before and after feeding sprayed french bean haulms were also calculated. Statistical analysis was done in the Statistix Analytical Software, version 4.0 1992). Graphing was done in the Freelance (Lotus Development Corp., 1988).

3.5 LABORATORY ANALYSES

3.5.1 Cleaning of glassware

Only glass apparatus was used in this study. Every piece of glassware was rinsed with hexane and then soaked in warm soapy water for 15 minutes, scrubbed thoroughly with a brush, rinsed in warm tap water, distilled water and acetone, and oven dried at 150° C. Just before use, the glassware was rinsed in distilled hexane.

3.5.2 Distillation of solvents

Hexane, acetone and dichloromethane were distilled twice in all glass fractionating column with a water cooled condenser. Glass beads were put in the fractionating flask to prevent super heating. The initial 200ml of the distillate was discarded since it served to rinse the system as well as the last 400ml portion which usually contains concentrated impurities. A small volume of solvent from each batch was injected into the GLC to check for purity and allowed to run for twenty minutes. No interfering peaks were noted hence the solvents were suitable for pesticide residue analysis.

3.5.3 Preparation of standards

All the pesticide standards were supplied in ampoules dissolved in 1ml of isooctane. The neck of each ampoule was broken and the contents emptied into a 10 ml volumetric flask. The ampoule was rinsed in hexane to capture any remaining standard. The stock standard mixture was then topped-up to 10 ml with additional hexane. This stock solution was stored at -20 ° C and working standards prepared from it by serial dilution up to 1:1000 at room temperature. Before GLC analysis, sample extracts and working standards were thawed to room temperature.

3.5.4 Detection of organophosphorus pesticides and cypermethrin in milk, bean and blood samples.

3.5.4.1 Milk samples

Pesticides were analyzed using a method described by Braun and Stanek (1982). 100 ml of milk were blended with 250 ml of 2:1 acetonitrile- water for 5 minutes at low speed. The sample was then divided into two portions. One portion was poured into a 1 liter separatory funnel together with 500 ml of distilled water, 25 ml of saturated sodium chloride and partitioned twice in 50 ml of dichloromethane. The mixture was shaken thoroughly and allowed to separate. The lower dichloromethane portion was passed through anhydrous sodium sulphate into a 500 ml round bottomed flask. The dichloromethane extract was evaporated to dryness in a rotary vacuum evaporator at 45°C and 4 revolutions per minute. The extract was redissolved in 5 ml of isooctane and divided into two portions. An aliquot of 0.5 ml portion was injected into a GLC (Varian 4400) fitted with a flame photometric detector (FPD) for organophosphorus pesticide detection. The other portion (4.5 ml) was cleaned in a florisil column for the analysis of cypermethrin using an electron capture detector (ECD) system. The operating conditions in the GLC for organophosphates and cypermethrin were as shown on Table 5.

Sample clean-up is necessary to reduce impurities which may introduce interfering peaks and reduce instrument performance. Florisil was used for this purpose since it is efficient and easy to use (Keith and Stephenson, 1992). The florisil was activated by heating at 350° C for 24 hours. 25 g of the activated florisil was introduced into a 300 mm x 22 mmID x 25 mmOD chromatographic column with frit and stopcock. The sides of the column were tapped gently to produce even packing. The column was topped with a 1 cm layer of anhydrous sodium sulphate and pre-washed with 50 ml hexane. The concentrated hexane extract was quantitatively

transferred into the column using three successive small rinses of hexane. As the last of the sample entered the top of the adsorbent, the inside of the column was rinsed with an additional 5 ml hexane. The sample was eluted sequentially at 5 ml/min with 200 ml of 1:4 dichloromethane-hexane and 200 ml of 0.35:50:50 acetonitrile-dichloromethane-hexane. The eluates were collected in 500 ml boiling flasks and the fractions evaporated to dryness with rotary vacuum evaporator at 45° C. It was redissolved in 5 ml isooctane for GLC analysis.

3.5.4.2. Bean samples

5 g of bean samples were weighed into a blender jar and processed in the same way as the milk samples.

3.5.4.3. Blood samples

Blood samples were assayed for cholinesterase activity, in order to measure organophosphate exposure. The cholinesterase activity was determined using a method originally developed for human blood by Michael (1949). Two stoppered test tubes were cleaned and dried. 2.5 ml buffer and 2.5 ml distilled water were added into each. The tubes were incubated at 25°C. 0.5 ml of acetylcholine bromide substrate was added into each tube and 0.05 ml of whole blood added into one tube. The pH in each tube was taken immediately and again after incubation for 1 hour at 25°C using a pH meter. The difference in pH between the incubated blood and the control x 100 gave the cholinesterase activity in pH units. The differences in cholinesterase activity before and after feeding were compared by means of paired t-test for statistical significance. Whole blood was used so as to measure both true and pseudocholinesterase.

Table 5: Operating conditions of GLC for dimethoate and cypermethrin detection

	Dimethoate	Cypermethrin
Injector temperature	230°C	250°C
Column temperature	150-250°C	150-250°C
Detector temperature	280°C	300°C
Injection volume	2μl	2μl
Column temperature rise	5°C/minute	5°C/minute
Column type	capillary J & W Scientific 15m long, 0.54mm id, 1.5 microns thick	capillary J & W Scientific 15m long, 0.25mmid,0.25 microns thick
Nitrogen flow rate	60ml/minute	60ml/minute

3.5.5. Detection of organochlorine pesticides in milk samples.

Milk samples were also tested for the presence of organochlorine pesticides. The samples were analyzed using a method described by Brevik (1978) with slight modification (Kanja *et al.*, 1986). The milk sample was removed from the deep freezer, thawed and homogenized. 10 g of the sample was weighed and 20 ml of hexane and 15 ml of acetone added. The mixture was put in a ultrasonic disintegrator and extracted for two minutes. It was then centrifuged for 5 minutes at 3000 rpm and the hexane layer transferred into a pre-weighed reagent tube with screw cap. This was placed on a sand bath at 50° C and evaporated to dryness under a gentle stream of nitrogen. The process was repeated with 10 ml hexane and 5 ml acetone and the fat content determined by reweighing the reagent tubes with the fat. The fat was redissolved in hexane to make a concentration of 0.05 g fat per ml of hexane. Subsamples of this extract were prepared for acid and base clean up.

In the acid clean-up, 1 ml of the extract was transferred to a 10 ml reagent tube with a glass stopper and treated with 2 ml sulphuric acid. The mixture was shaken and allowed to stand in the dark for atleast 1 hour and then centrifuged for 5 minutes at 3000 rpm. The top hexane layer was transferred into vials ready for GLC analysis.

For the base clean-up, 2 potassium hydroxide pellets were added to the tubes used for clean up and soaked in 0.1 ml of distilled water. 1 ml of 99.9% ethanol was added and the contents allowed to dissolve. 1 ml of the sample was added and the tube placed in a water bath at 50°C for 30 minutes. The mixture was then cooled and 5 ml of a solution of sodium chloride and orthophosphoric acid added and the contents were mixed. Cooling was done for a few minutes and the mixture centrifuged for 10 minutes at 3000 rpm. The hexane layer was removed into sample bottles and anhydrous sodium sulphate added to absorb moisture. The sample extract was transferred into vials ready for GLC analysis. The operating conditions of

the GLC for organochlorine pesticides are given on Table 6.

3.5.6 Pesticide recoveries

Standard curves were prepared using known amounts of organochlorine pesticides (Figure 2), dimethoate and cypermethrin (Figure 3).

The concentrations of the compounds in the cpm mixture are given on Table 7. The recoveries of all the thirteen compounds in the cpm standard ranged between 60% for heptachlor and 105% for dieldrin (Table 8).

The recoveries of dimethoate and cypermethrin in milk samples were 90% and 98% respectively. This was an average of ten parallel samples. The recoveries of dimethoate in french bean samples was 101% while that of cypermethrin was 89% . Table 9 shows the recoveries of dimethoate and cypermethrin in milk and bean samples plus the % deviations.

Table 6: Operating conditions of GLC for organochlorine pesticides detection

Detector	Electron Capture
Column temperature	210°C
Detector temperature	250°C
Nitrogen flow rate	50ml/min
Chart speed	10mm/min
Attenuation	128
Column contents	GP 1.5%, SP-2250/1.95%, SP- 2401 on 100/120 Supelcoport
Injection volume	1μl

3.5.7 Quantification of pesticides

The concentrations of the compound in the samples were obtained by employing the formula:

v/w. h1/h2. cs.1000 where

v = total extract volume (ml)

w= weight of the milk (g)

h1 = peak height of the compound in the sample (mm)

h2 = peak height of the compound in the standard (mm)

cs = concentration of the standard in (μg/μl)

The methods used in the study were found suitable for the analysis of pesticides since most recoveries were within the acceptable range. The limits of detection for all the compounds in this study was 0.001 mg/kg.

3.6 RESOLUTION AND LINEARITY OF GLC

Resolution of the GLC was checked by injecting 2 μl of a working standard and comparing the resulting elution patterns to those supplied by the manufacturer. The patterns were found to be similar for each compound.

Linearity was checked by injecting equal volumes of different concentrations of working standards for each compound and plotting standard curves of concentrations against the peak heights/area. These plots were found to be linear and all the analytical work was carried out within the linear range.

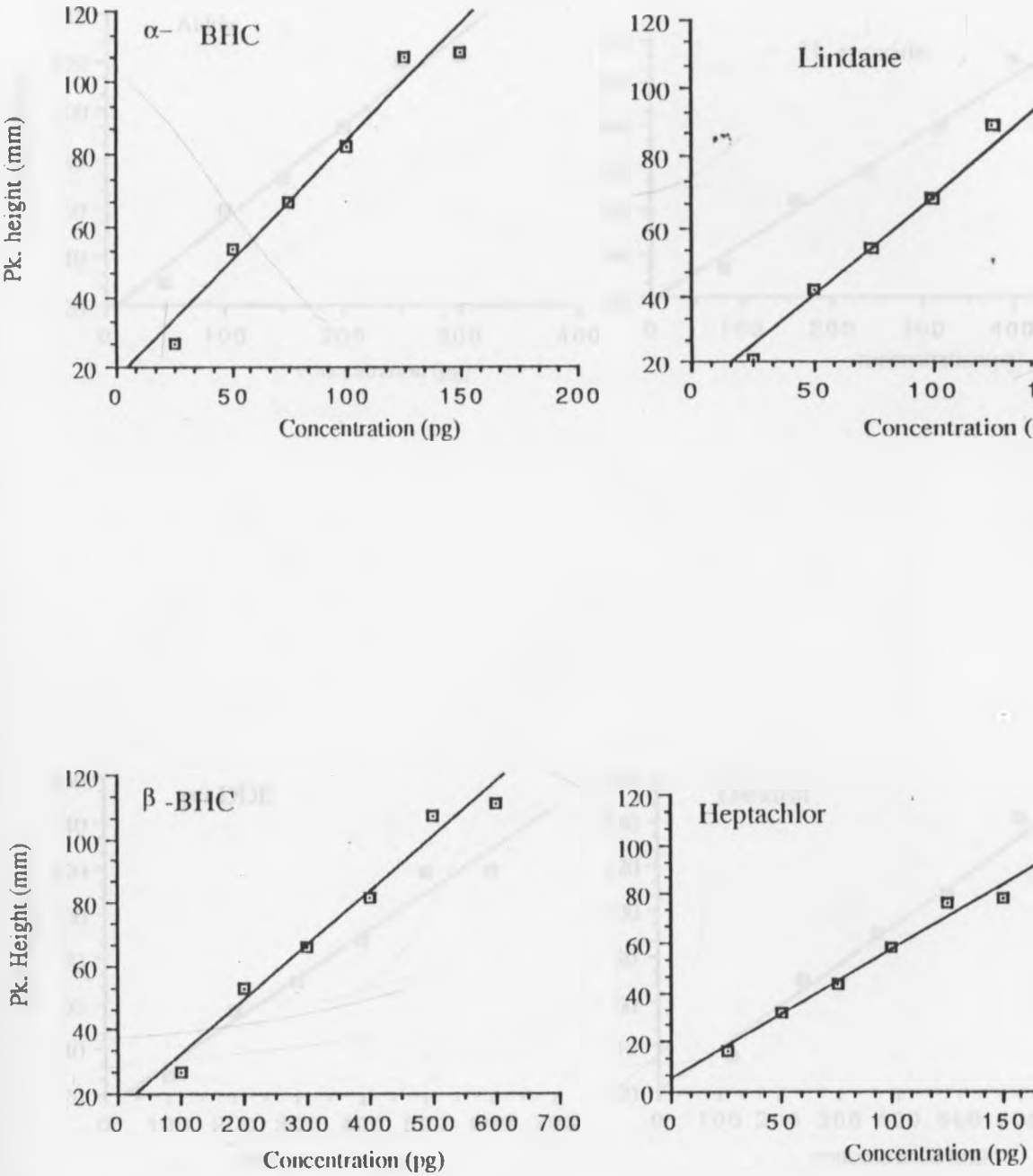
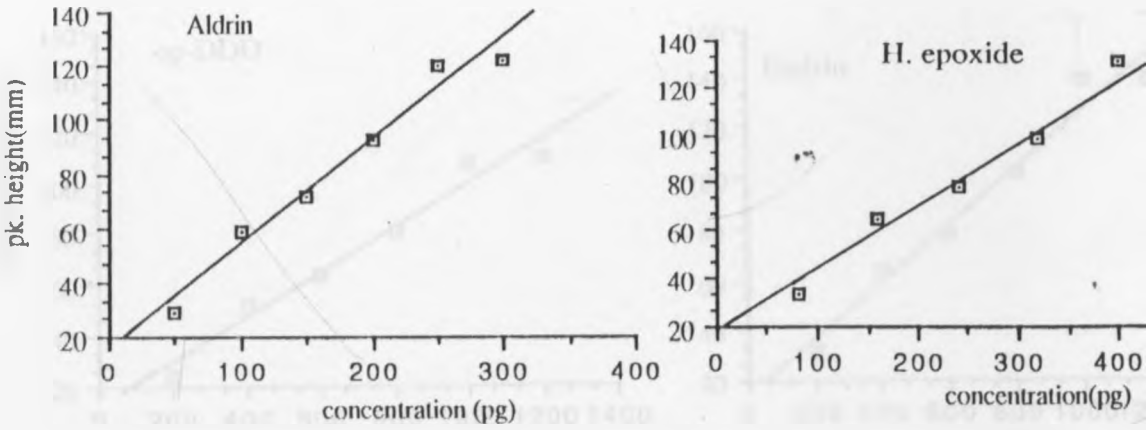


Figure 2: Standard curves for organochlorine pesticides



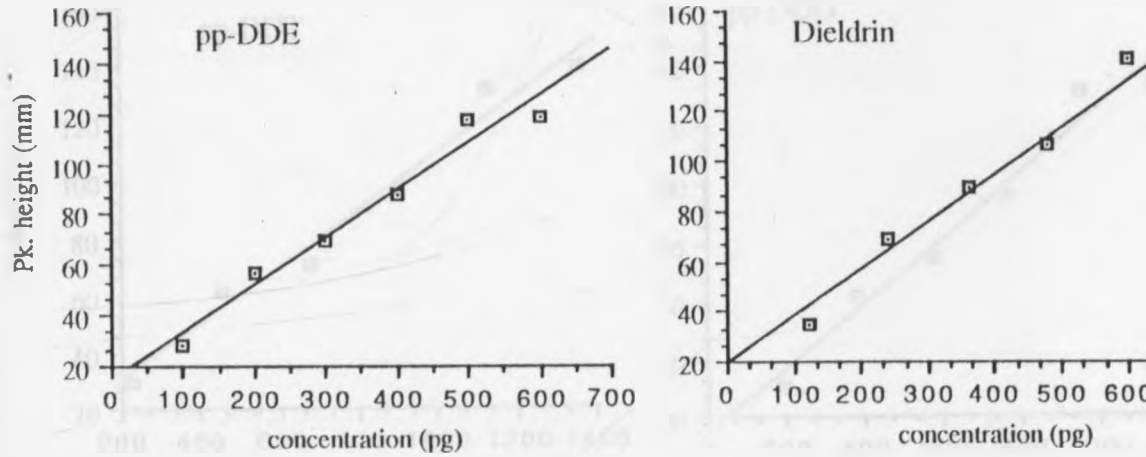
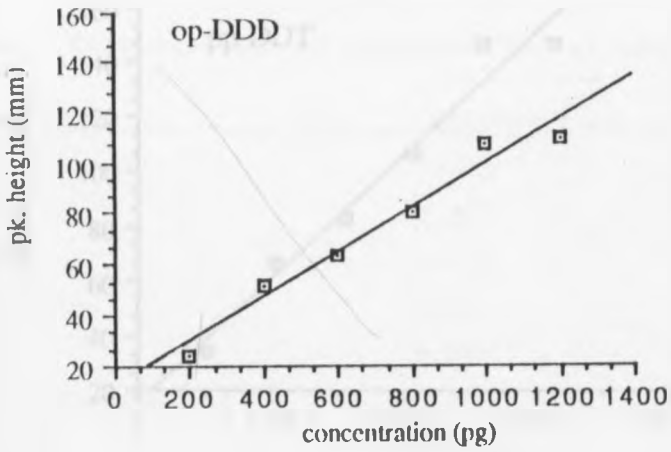
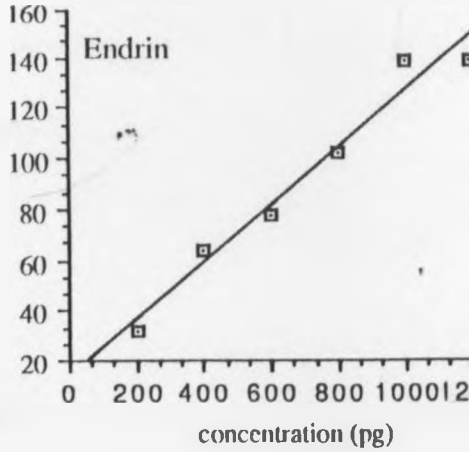
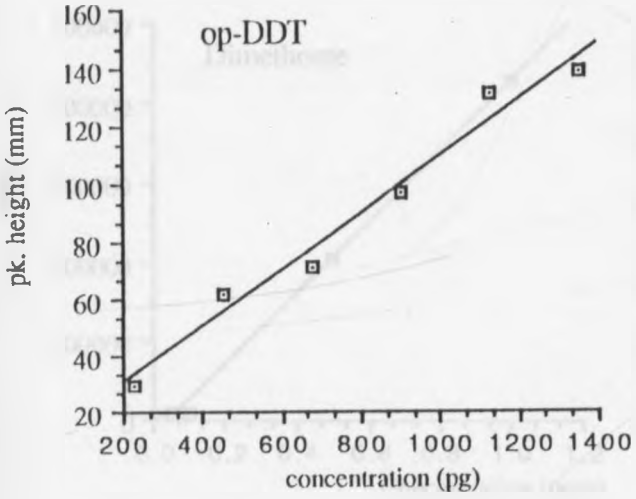


Figure 2 continued







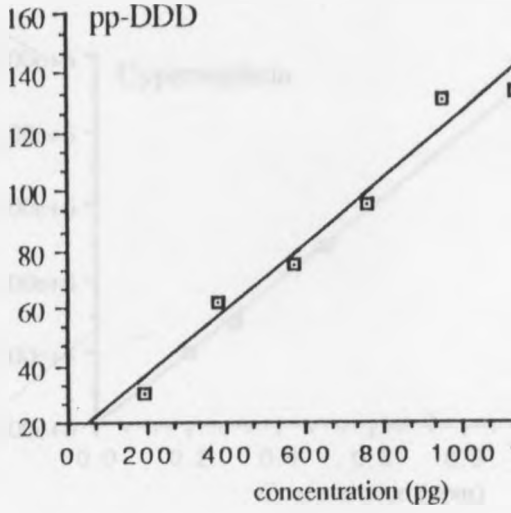


Figure 2 continued

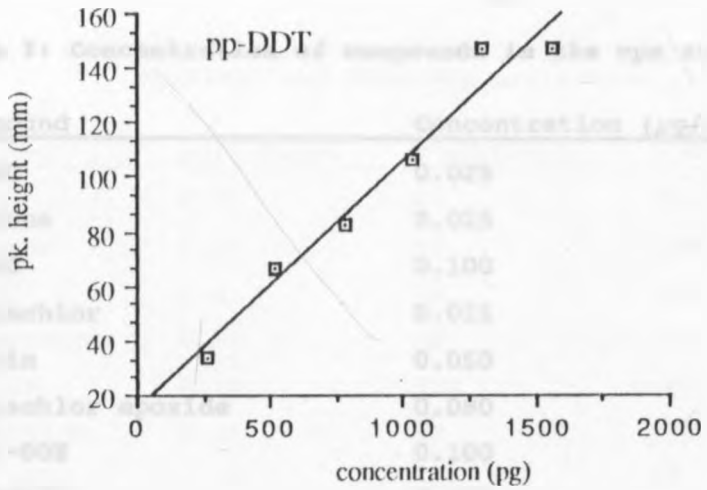


Figure 2 continued

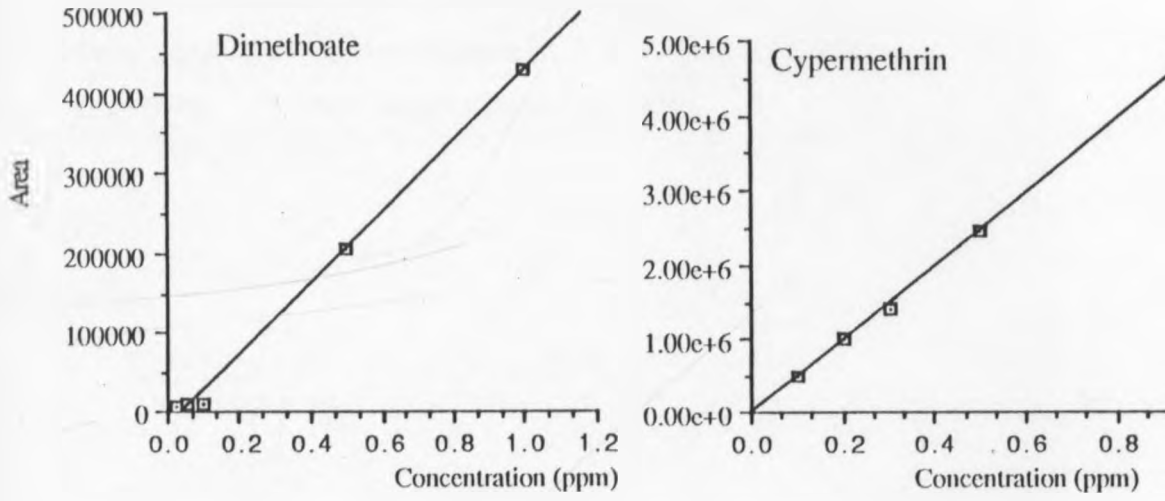


Figure 3: Standard curves for dimethoate and cypermethrin pesticides

Table 7: Concentration of compounds in the cpm standard.

Compound	Concentration (μg/μl)
α-BHC	0.025
Lindane	0.025
ß-BHC	0.100
Heptachlor	0.025
Aldrin	0.050
Heptachlor epoxide	0.080
p,p'-DDE	0.100
Dieldrin	0.120
o,p'-DDD	0.200
Endrin	0.200
o,p'-DDT	0.225
p,p'DDD	0.190
p,p'DDT	0.260

Table 8 (a): Recoveries of organochlorine compounds in spiked milk (high spiking) and UNEP/WHO evaluation (1980)

Compound	µg/g added	% recovery	% deviation	Evaluation
α-BHC	0.05	89	-9	E
Lindane	0.05	94	-10	E
ß-BHC	0.2	88	-18	G
Heptachlor	0.05	89	-18	G
Aldrin	0.10	104	-10	E
H.epoxide	0.16	83	-15	G
p,p'-DDE	0.20	102	+5	E
Dieldrin	0.24	105	+3	E
o,p'-DDD	0.40	90	-1	E
Endrin	0.40	80	-20	G
o,p'-DDT	0.45	80	-20	G
p,p'-DDD	0.38	100	-1	E
p,p'-DDT	0.52	85	+2	E

% deviation = 100 x (recovered pesticide/ spiked pesticide) -100

E = Excellent (+/- 10% spiked amount) G = Good (+/-20%)

P = Poor (+/- 40%) A = Acceptable (+/- 30%)

Table 8 (b): Recoveries of organochlorine pesticides in milk (low spiking) and UNEP/WHO evaluation (1980).

Compound	µg/g added	% recovery	% deviation	Evaluation
α-BHC	0.004	79	-20	A
Lindane	0.004	74	-15	G
ß-BHC	0.030	86	-21	A
Heptachlor	0.004	60	-23	A
Aldrin	0.011	101	-8	E
H.epoxide	0.015	73	-19	G
p,p'-DDE	0.010	76	-10	G
Dieldrin	0.033	102	-12	G
o,p'-DDD	0.030	88	-10	G
Endrin	0.030	84	-14	G
o,p'-DDT	0.035	76	-11	G
p,p'-DDD	0.028	86	-5	E
p,p'-DDT	0.042	76	-10	G

% deviation = 100 x (recovered pesticide/spiked pesticide)-100

E = Excellent (+/- 10% spiked amount)

G = Good (+/- 20%) P = Poor (+/- 40%)

A = Acceptable (+/- 30%) U= Unacceptable (+/- 50%)

Table 9: Recoveries of dimethoate and cypermethrin in milk and french bean samples and UNEP/WHO evaluation (1980).

a. Dimethoate

Sample	µg/g added	% recovery	% deviation	Evaluation
Milk	0.05	90	+3	E
	0.001	76	-5	E
Beans	0.05	101	+6	E
	0.001	76	-5	E
b. Cypermethrin				
Milk	0.02	98	-9	G
	0.005	77	-11	G
Beans	0.05	89	+5	E
	0.001	84	-11	G

% deviation = 100 x (recovered pesticide/spiked pesticide)-100

E = Excellent (+/- 10% spiked amount)

G = Good (+/- 20%)

A = Acceptable (+/- 30%)

P = Poor (+/- 40%)

U = Unacceptable (+/- 50%)

CHAPTER FOUR

4. RESULTS

4.1 FARMER RESPONSE

Of the 40 farms initially selected, six dropped out of the study. Two of them refused to have milk withdrawn from their animals on the second occasion, three sold their animals and one withdrew for no stated reason.

4.2 QUESTIONNAIRE RESULTS

From the questionnaire it was possible to gather information about the farm size and farming activities, proportion of farmland per activity, cow identification and production parameters and information on spraying and feeding of french bean haulms. A summary of the farming activities is given in Appendix 2. The average farm size was 3 acres and diverse farming activities were undertaken which included dairy farming, horticultural farming, cash crops and subsistence farming (Appendix 3). All farmers fed remains of agricultural products to their milking cows including those that had been previously sprayed (Appendix 4). Most farmers cited increased milk production as a result of feeding such remains, especially french bean haulms. Dimethoate and cypermethrin were the most commonly used pesticides to spray french beans. Dimethoate was used in the early stages while cypermethrin was used after the beans had flowered. Some farmers however used dimethoate throughout the growing period. The pesticides were sprayed twice per week at concentrations of 40ml/20l of water to spray one eighth acre plot. The concentrations of the pesticides used were 400 g/l and 50 g/l for dimethoate and cypermethrin respectively. The weight of french beans fed was on average 11 kg per day and ranged between 5 and 22 kg and the minimum time from spraying to feeding was 6 days (Table 10, Appendix 4). Most farms had only one milking cow plus one

or two heifer calves, goats and sheep in some cases and in some farms, a bull for draft and other purposes. The age of the cows sampled ranged from two to five years and the milk production was on average 7.4 kg per day (Table 11, Appendix 5). The most common breed of cows was Frisian (Appendix 5).

4.3 PESTICIDE RESIDUES IN MILK, BEAN AND BLOOD SAMPLES IN THE OBSERVATIONAL STUDY

4.3.1 Dimethoate/omethoate, and cypermethrin residues in milk and french bean haulms

All 34 bean samples and 68 milk samples tested for the above pesticides were negative after analysis with gas liquid chromatograph.

4.3.2 Blood samples

68 blood samples were assayed for cholinesterase activity. The difference in cholinesterase activity before and after feeding sprayed french bean haulms was not statistically significant ($p < 0.05$, Table 12). Most animals had cholinesterase activity between 66-80 pH units (Appendix 6, Figures 4 and 5).

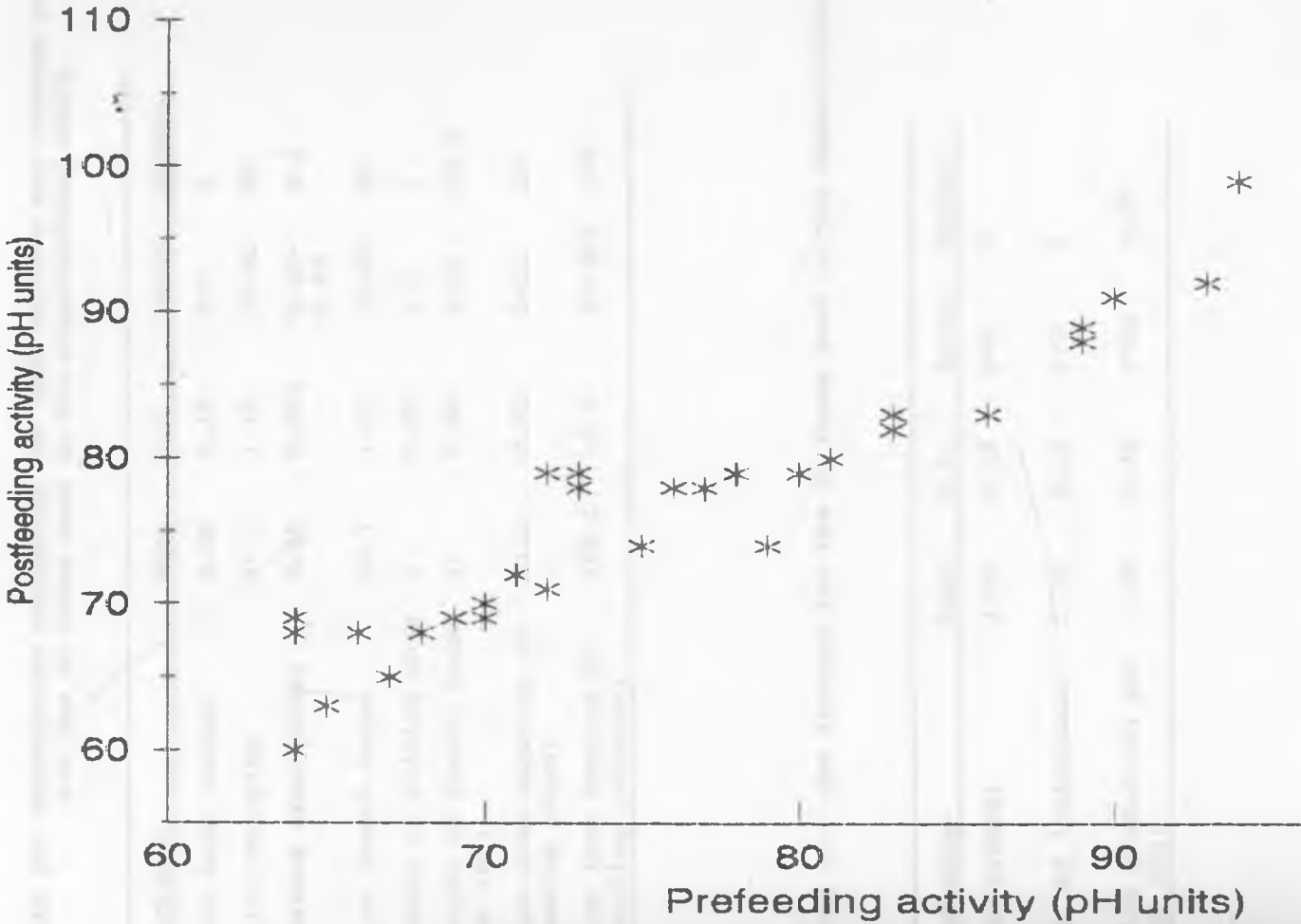


Figure 4. Scatter plot of cholinesterase activity before and after feeding sprayed fre bean haulms to cows in the observational st

Table 10: Descriptive statistics for farm factors and farming practices for the 34 farms used in the observational study.

Variable	Mean	S.E	Range	Median
Farm size (acres)	3.56	0.17	3-7	3
% for dairying	27.3	1.15	10-45	30
Pasture area(acres)	0.45	0.018	0.25-0.64	0.5
% on french beans	28.7	1.27	15-50	25
Number of milking cows	1	0.03	1-2	1
Weight of french beans fed (kg)	11	0.68	5-22	10.5
Time from spraying to feeding (days)	11.3	0.78	5-21	10
Time from feeding to sampling (minutes)	150.1	10.9	60-320	145

Table 11: Cow factors for the 34 farms used in the observational study

Variable	Mean	S.D.	Range	Median
Age (years)	2.59	0.13	2-5	2
No. of lactations	1.76	0.13	1-3	2
Milk production per day (kg)	7.38	0.33	4-12	7.50

Table 12: Descriptive statistics for the difference in cholinesterase activity before and after feeding sprayed french bean haulms in the 34 cows in the observational study.

Statistic	Prefeeding	Postfeeding	Difference
Mean	78.0	78.3	0.18
SD	11.4	10.7	0.55
SE Mean	1.95	1.83	0.094
Median	75.5	78	
Range	64-109	60-100	-9 to -7
t-test			0.32

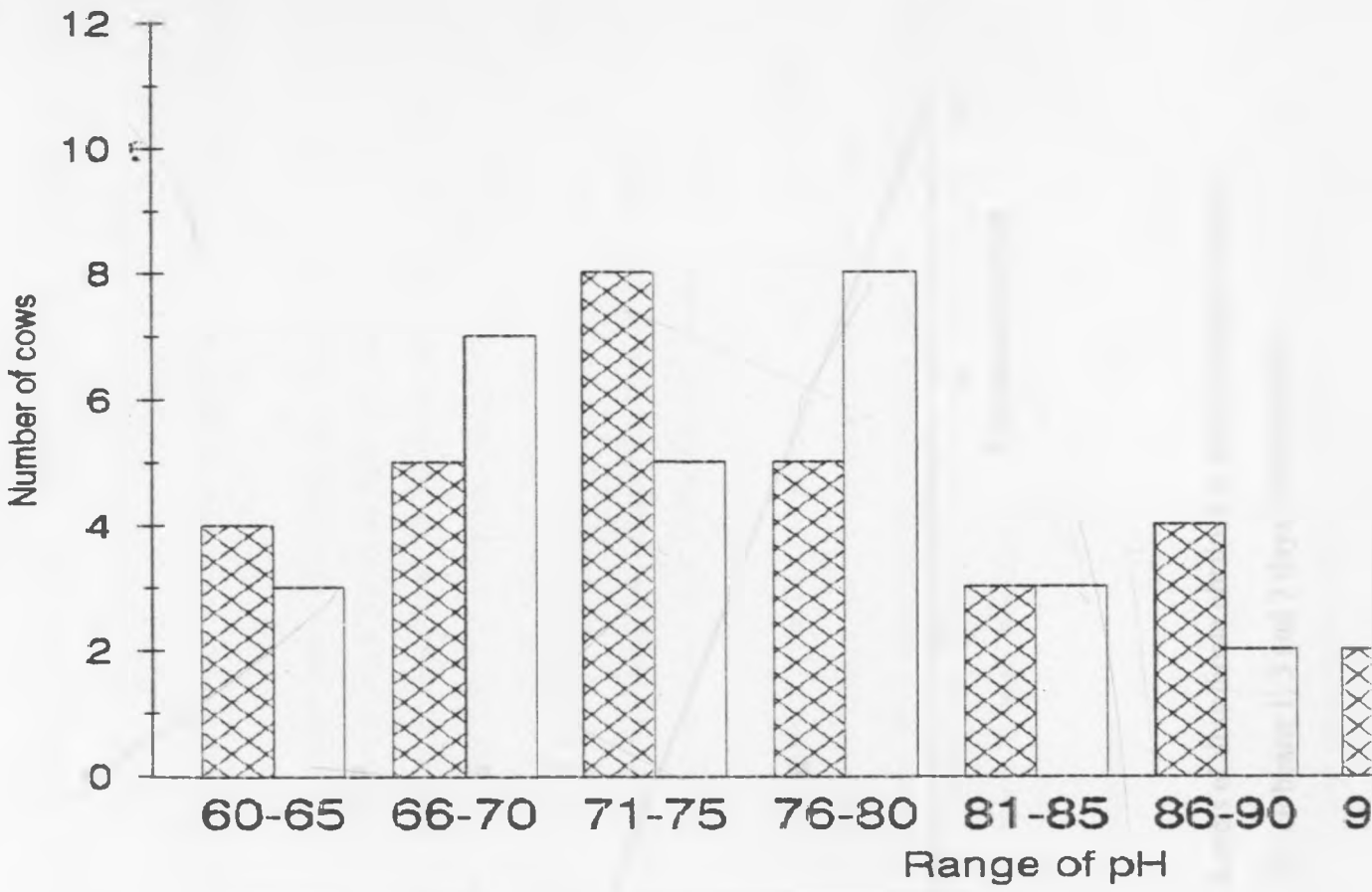


Figure 5. Frequency distribution of cholinesterase activity before and after feed sprayed bean haulms in the observational s

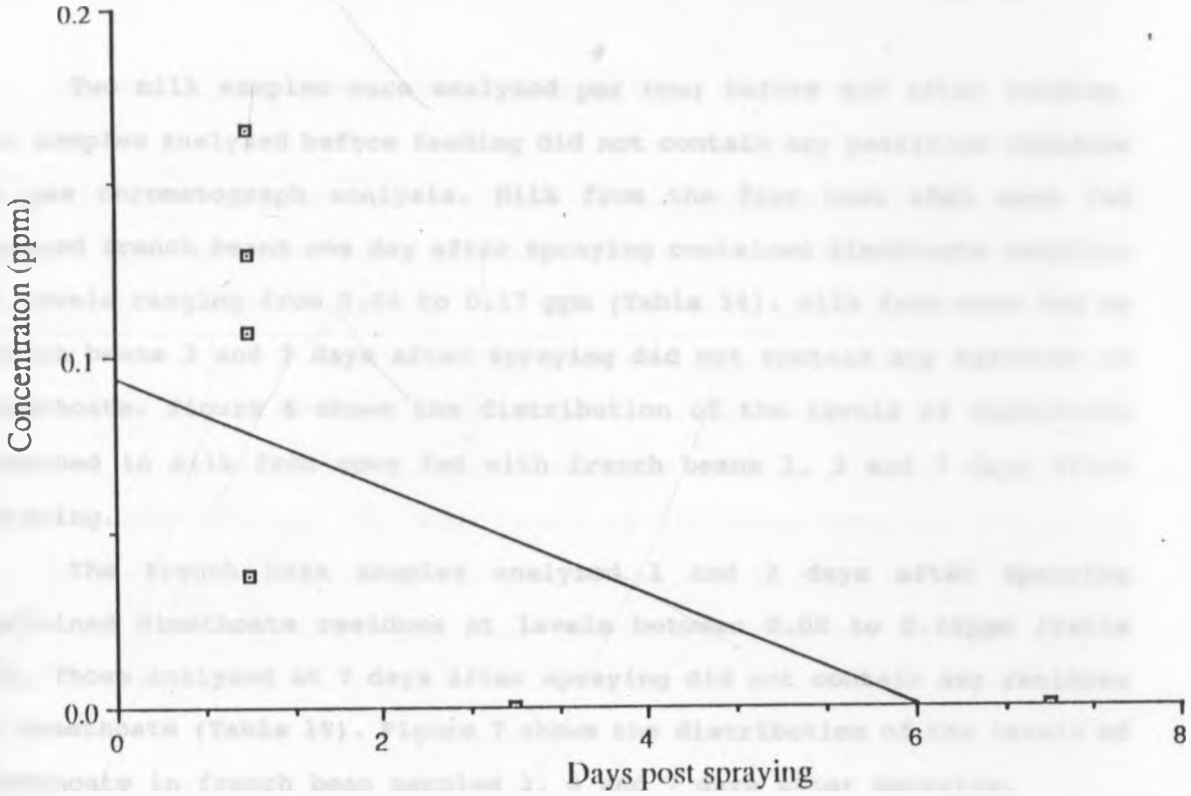


Figure 6: Levels of dimethoate obtained in milk from cows fed on french beans 1, 3 and 7 days after spraying

4.4 PESTICIDE RESIDUES IN MILK, BEAN AND BLOOD SAMPLES IN THE FEEDING EXPERIMENT

4.4.1 Dimethoate/omethoate residues in milk and french bean samples

Two milk samples were analyzed per cow; before and after feeding. All samples analyzed before feeding did not contain any pesticide residues on gas chromatograph analysis. Milk from the four cows that were fed sprayed french beans one day after spraying contained dimethoate residues at levels ranging from 0.04 to 0.17 ppm (Table 14). Milk from cows fed on french beans 3 and 7 days after spraying did not contain any residues of dimethoate. Figure 6 shows the distribution of the levels of dimethoate obtained in milk from cows fed with french beans 1, 3 and 7 days after spraying.

The french bean samples analyzed 1 and 3 days after spraying contained dimethoate residues at levels between 0.08 to 0.46ppm (Table 15). Those analyzed at 7 days after spraying did not contain any residues of dimethoate (Table 15). Figure 7 shows the distribution of the levels of dimethoate in french bean samples 1, 3 and 7 days after spraying.

4.4.2 Blood samples

24 blood samples were assayed for cholinesterase activity. 12 samples were withdrawn from the animals before feeding and 12 samples after feeding. The mean activity in pH units before feeding was 67.8 with a range of 60-76 while the mean activity after feeding was 65.9 with a range of 59-74 (Table 16, Figure 8). A Scatter plot of cholinesterase activity in the 12 animals used in the experiment is given in Figure 9. There was lower cholinesterase activity after (versus) before feeding sprayed foliage. This difference was statistically significant ($p < 0.05$) with the 95% confidence interval of the mean difference being -2.429 to -

1.238). Table 13 shows results of paired comparisons before and after feeding for the three categories of animals used in the feeding experiment.

4.5 ORGANOCHLORINE PESTICIDE RESIDUES IN MILK

34 milk samples were analyzed for organochlorine pesticides. The samples were positive for eight pesticides. These were ß-BHC, lindane, Heptachlor, Aldrin, Heptachlor epoxide, p,p'-DDE, Dieldrin, and o,p'-DDD. Table 17 shows the % of positive samples and mean levels for the detected compounds. The major pesticide found was the ß-isomer of HCH which occurred in 32.4% of the samples. The gamma isomer, lindane occurred in 23.5% of the samples. The mean levels of the other detected pesticides were lower than the maximum residue limits set by WHO. No DDT residues were found but p,p'-DDE, its major metabolite occurred in 8.8% of the samples (Table 17).

Table 13: Paired t-test results for difference in cholinesterase activity before and after feeding sprayed french bean haulms 1, 3 and 7 days post-spraying.

1 day post -spraying

Mean	S.E.	t-test	p value
-2.75	0.25	-11	0.002

Pooled 1 and 3 days post-spraying

Mean	S.E.	t-test	p value
-2.38	0.18	-13	0.00

7 days post-spraying

Mean	S.E.	t-test	p value
0.75	0.25	3	0.06

Table 14: Levels of dimethoate obtained in milk from cows fed on french beans 1, 3 and 7 days after spraying

Sample	Days postspraying	Concentration (ppm)
1	1	0.129
2	1	0.165
3	1	0.107
4	1	0.038
5	3	-
6	3	-
7	3	-
8	3	-
9	7	-
10	7	-
11	7	-
12	7	-

(-) Below limit of detection

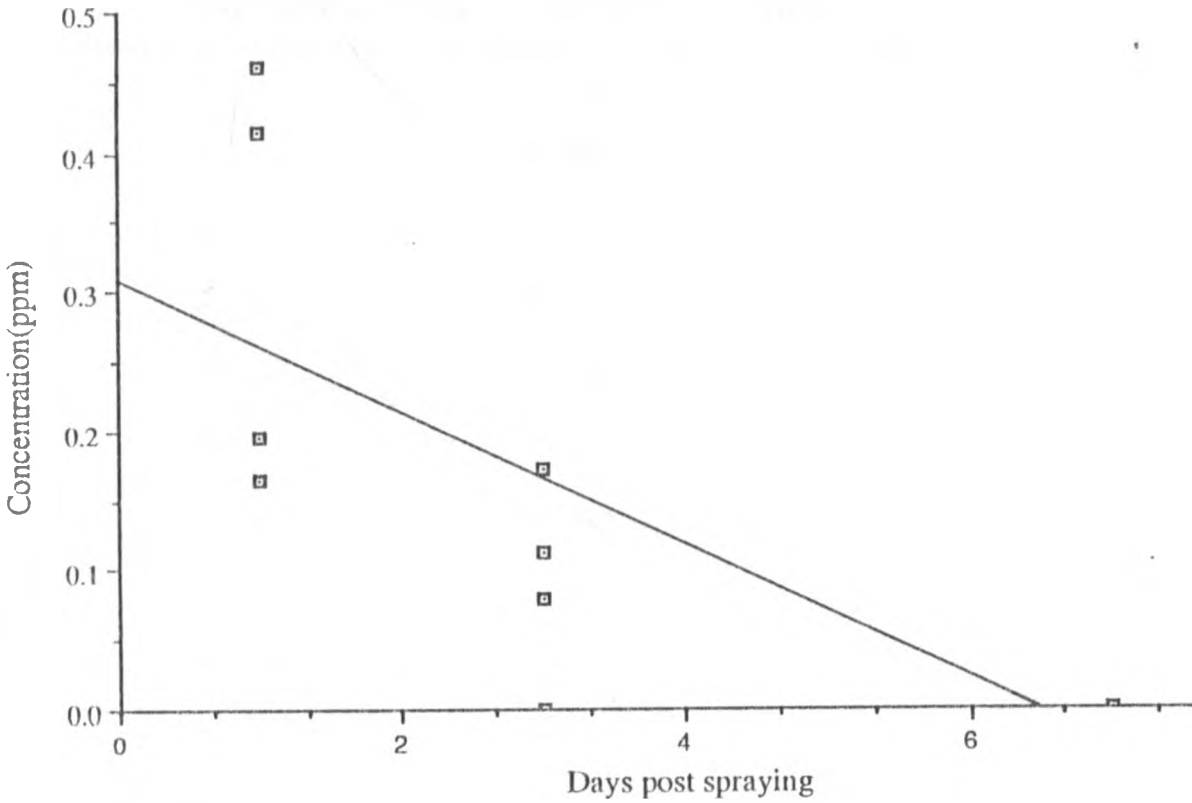


Figure 7: Levels of dimethoate obtained in french beans at 1, 3 and 7 days after spraying

Table 15: Levels of dimethoate obtained in french beans at 1, 3 and 7 days after spraying

Sample	Days postspraying	Concentration (ppm)
1	1	0.165
2	1	0.461
3	1	0.195
4	1	0.415
5	3	0.113
6	3	0.172
7	3	0.080
8	3	-
9	7	-
10	7	-
11	7	-
12	7	

(-) Below limit of detection

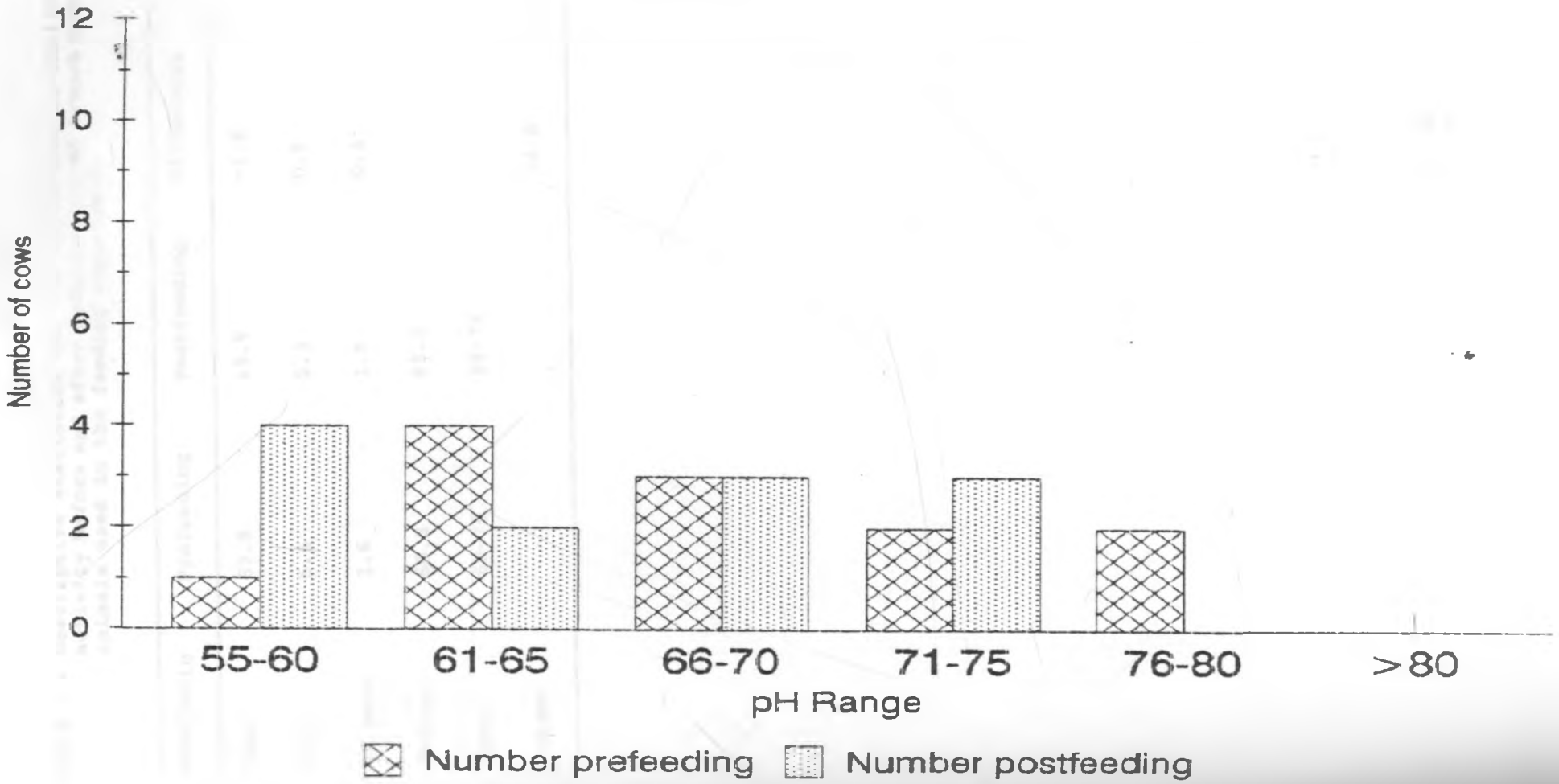


Figure 8. Frequency distribution of cholinesterase activity before and after feeding sprayed bean haulms in the experimental animals

Table 16: Descriptive statistics for the difference in cholinesterase activity before and after feeding sprayed french beans in the 12 animals used in the feeding experiment.

Statistic	Prefeeding	Postfeeding	Difference
Mean	67.8	65.9	-1.8
S.D.	5.6	5.3	0.9
SE mean	1.6	1.5	0.3
Median	66.5	65.0	
Range	60-76	59-74	
t-test			-6.8

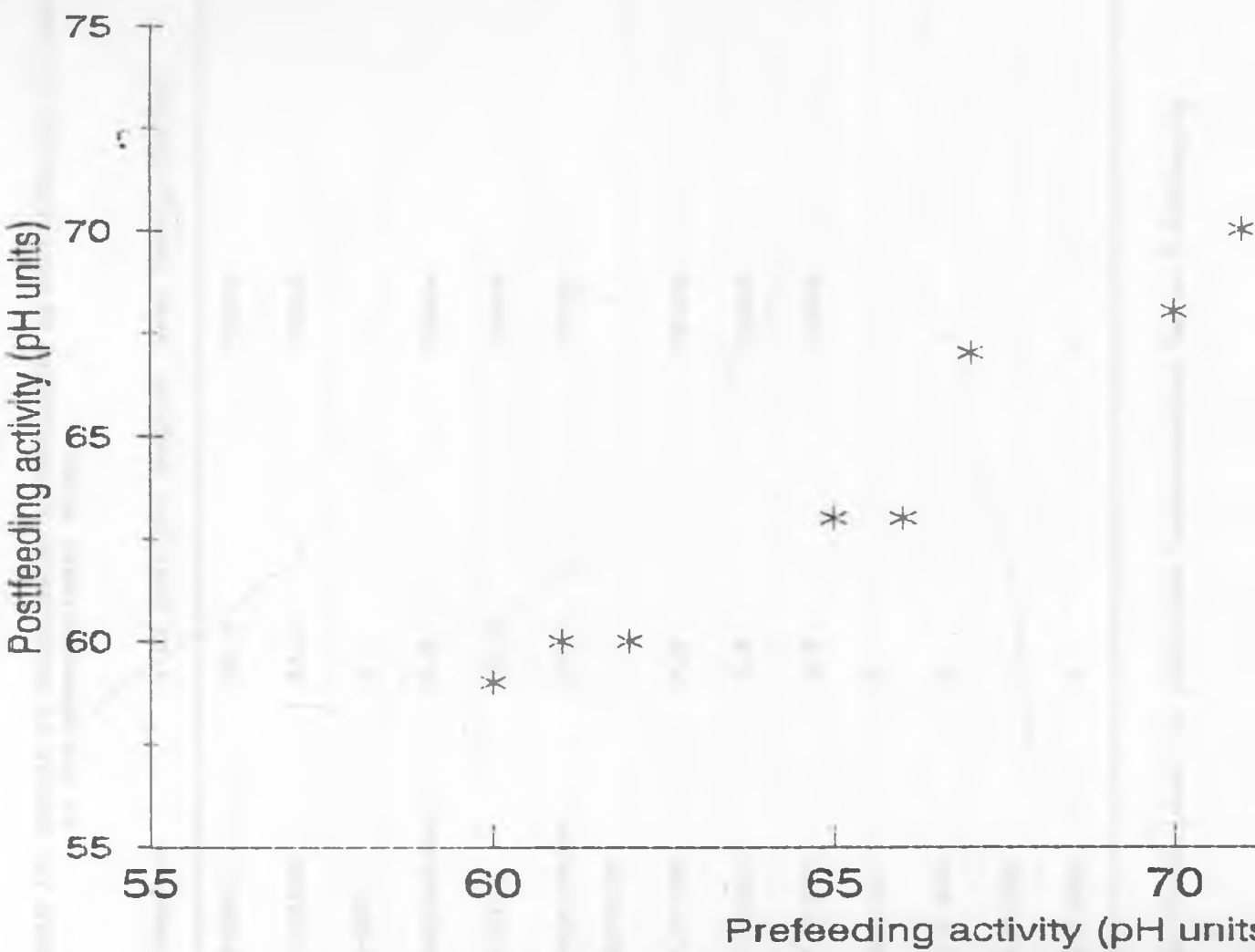


Figure 9. Scatter plot of cholinest
activity before and after feeding spray
bean haulms in the experimental a

Table 17: Levels of chlorinated hydrocarbons in milk from the 34 cows used in the observational study

Compound	% of positive samples	Mean levels (mg/kg)
ß-BHC	32.4	Trace
Lindane	23.5	Trace
α-BHC	0	-
Heptachlor	5.9	Trace
Aldrin	23.5	Trace
Heptachlor epoxide	14.7	Trace
P,P'DDE	8.8	Trace
Dieldrin	2.9	Trace
O,P'DDD	2.9	Trace
Endrin	0	-
O,P'DDT	0	-
P,P'DDD	0	-
P,P'DDT	0	-

NB: Trace level is pesticide concentration below 0.0001mg/kg

CHAPTER FIVE

5. DISCUSSION AND CONCLUSIONS

5.1 DISCUSSION

This study revealed high population pressure on limited land resources as evidenced by the relatively small farm sizes of < 1 ha supporting an average family of 5 members and 1 to 3 livestock. This has resulted in intensive zero-grazing systems where 1 or 2 dairy cows are kept and supplemented with various agricultural by-products. The trend to smaller farms is likely to continue due to subdivision.

In the study area, various horticultural by-products which have been previously sprayed with pesticides are important supplements fed to dairy cattle. Pesticide use within the Kibirigwi Irrigation Scheme was quite intensive with over 85% of farmers using pesticides on either crops or animals (Kimani and McDermott, 1994). Previous studies carried out within the scheme identified several unsafe handling practices for pesticides. These included use of leaking sprayers and unlabelled chemicals, application of pesticides without wearing protective clothing, improper storage of pesticides, use of higher than recommended dose, use of chemicals whose use has been discontinued or banned both in Kenya and in other parts of the world and improper disposal of pesticides and their containers (Kimani and McDermott, 1994). These findings are consistent with studies in other parts of the developing countries which have documented that pesticide handling and usage practices are poor hence exposing man and his animals to pesticide hazards (Mwathi and Kimani, 1989; Karembu, 1990; Mbakaya *et al.*, 1994).

Farmers and agricultural extensionists on the scheme wished to feed french bean haulms, the residue of an important cash crop in the area, to dairy cattle. However they were unsure of safe pre-harvest intervals. The

important objectives of this study, therefore, were to determine, under usual feeding practices, the levels of pesticide residues on french bean haulms fed to dairy cattle, to determine if such pesticides were eventually absorbed by the cows by assessing pesticide residues in milk and the depression of acetycholinesterase levels in blood, and to determine the days post-spraying that french bean haulms could be fed with no detectable residues either on the feed or in the cow.

The usual feeding practice is to feed the french bean haulms to cows a week after the end of pod picking. The french bean crop is usually sprayed until the farmers have exhausted picking the pods. Under these feeding practices, no residues of pesticides were detected in the french bean haulms or cows milk and neither was the cholinesterase activity of blood significantly depressed (Tables 12 and 14). This is consistent with studies carried out by Fetchner *et al.*, (1970) and Miles *et al.*, (1971) who found that organophosphorus and pyrethroid pesticides sprayed on foliage or the animals were hardly detected in milk. In order to establish some safety guidelines, a small feeding experiment was conducted to determine the number of days after spraying that french bean haulms would have detectable dimethoate and if this could be detected in milk. The dimethoate sprayed foliage posed no real danger to the animals since it was only 13% of the minimum oral toxic dose found in cattle (Gelder *et al.*, 1985).

Detectable residues of dimethoate were found in french beans 1 and 3 days post-spraying (Table 15; Figure 8) whereas detectable residues in milk occurred only on feeding sprayed french beans 1 day post-spraying (Table14; Figure 7). The levels in milk were far lower than the highest tolerable dose of 0.2 mg/kg daily in man (Worthing, 1979). Acetylcholinesterase levels were depressed by an average of 2.3%. No ill effects were noted in the animals as a result of feeding on the sprayed french bean haulms. This is in agreement with other studies which indicate that organophosphates on foliage are rapidly decomposed in the body with

very little residues being secreted in milk (Derache, 1978).

Milk samples were also screened for organochlorine pesticides. This was in order to give a general indication of the persistent nature of this group of pesticides and whether they appear to be used within the scheme. Detected levels were low (Table 17). The levels in milk showed a downward trend compared to other Kenyan studies indicating a general decline in their use (Maitho, 1978; Kanja, 1988). This is in agreement with studies carried out in other parts of the world (Frank *et al.*, 1985). No sample contained residues in excess of FAO/WHO Acceptable Daily Intake.

The detected pesticides included ß-BHC, lindane, Heptachlor epoxide, p,p'-DDE, dieldrin and o,p'-DDD. Table 17 shows the levels of each compound detected. Organochlorine pesticides like lindane and aldrin decompose in the soil and their presence in milk shows relatively recent use. Presence of DDT residues in animal products indicate recent contamination whereas presence of its metabolites like DDE reflects earlier exposure to DDT which has been metabolized. This indicates the persistent nature of organochlorine compounds. In this study, more samples contained aldrin than its more persistent metabolite, dieldrin. This interesting result was also found in the case of human milk from various parts of Kenya (Kanja, 1988).

Studies from other parts of the world have also revealed presence of banned chemicals in cow milk and milk products (Dhaliwal and Kalra, 1978; Kappor *et al.*, 1980; Frank *et al.*, 1985; Lawrie and Karen, 1988). Some Kenyan studies have also shown that organochlorine compounds are widespread in animal products as well as human milk and fat (Wasserman *et al.*, 1972; Maitho, 1978, Kanja, 1988; Kahunyo, 1983; Mugambi, 1984; Mugachia, 1990; Kinyamu, 1992).

Many organochlorine pesticides have been withdrawn or their agricultural uses severely restricted due to their persistence in the environment, damage to endangered species or potential to cause chronic health problems, reproductive system damage and cancer (Marion, 1989).

However this group of pesticides is often misused in the developing countries. For example in the study area, farmers were using carbosulfan and endosulfan on vegetables in disregard of regulations restricting their use to seeds and cotton respectively (Kimani and McDermott, 1994). Other banned chemicals are also used within the scheme and pre-harvest intervals for most pesticides are unknown and not observed since most farmers use unlabelled or mislabelled chemicals so they don't actually know what they are using (Kimani and McDermott, 1994).

Pesticide producing countries are partly to blame for this misuse because more often than not they do not inform importing countries of the full dangers of the chemicals (Sarojini, 1990). Several incidents of abuse by pesticide companies have been compiled by the Pesticide Action Network (PAN) since its inception in 1982. These include misleading advertisement, inadequate labelling and packaging, untrained sales people providing inappropriate and sometimes dangerous advice (Sarojini, 1990).

Moreover, many third world countries do not have the expertise or resources to ensure that dangerous chemicals are safely used (Marion, 1989). For example in the study area majority of the farmers applied pesticides without wearing any form of protective clothing and they rarely changed the clothes used during pesticide application which increases the chances of pesticide absorption through the skin (Kimani and McDermott, 1994). These practices result in over three million people being poisoned annually by pesticides (Sarojini, 1990).

A commendable effort to discourage the use of developing countries as dumping ground for chemicals was done by FAO (1987). A new enforcement on the Internal Code of Conduct on the Distribution and Use of pesticides adopted the principle of Prior Informed Consent (PIC) where a list of over 50 pesticides and chemicals that have been banned or severely restricted in five or more countries may not be sold abroad until the exporters notify importing countries of their health and environmental hazards and why they have been restricted or banned domestically. Importing countries

must then consent to the import of the chemicals.

The present feeding practices within Kibirigwi Irrigation Scheme of withholding french bean haulms from cattle for at least 7 days are adequate in preventing exposure of dimethoate to cows since no residues were detected on beans or in milk under usual feeding practices. However, immediate feeding of french bean haulms within 3-4 days post-spraying should be avoided, as detectable residues can be found upto 3 days post-spraying on the bean haulms.

5.2 MAJOR FINDINGS AND CONCLUSIONS

Within the limits of the data collected and information volunteered by the farmers, it can be concluded that:

1. French bean haulms previously sprayed can and are used as an important supplement to lactating dairy cattle under the prevailing zero-grazing systems within Kibirigwi Irrigation Scheme.

2. There are detectable residues upto 3-4 days post-spraying on french bean haulms. Milk from cows fed on sprayed french bean haulms did not contain any dimethoate residues after one day post-spraying.

3. Thus, the current waiting period of 7 days between spraying and feeding should be observed. This should allow an adequate safety margin for the feeding of previously sprayed french bean haulms to lactating dairy cattle.

4. Organochlorine residue levels in milk from cows within the scheme were generally low indicating a decline in their use. The presence of lindane residues in some of the milk samples is an indication that farmers within the scheme have used it recently despite the restriction on its use.

5. Some farmers used banned pesticides or pesticides that they did not know.

5.3 RECOMMENDATIONS

1. The fact that some farmers are currently using banned or severely restricted pesticides that they did not know suggests the need for action to prevent the importation and sale of these products in the country.

2. Farmers need to be better advised on proper labelling, storage, and use regulations in addition to current and planned efforts to improve pesticide application and the proper disposal of pesticides and their containers.

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APPENDICES

Appendix 1: Questionnaire

Circle or fill in the appropriate response where indicated
1. Date (day/month/year): ...../...../.....
2. Farmers name.....
3. Farm number.....
4. Section: 1.....
2.....
3.....
5. What farming activities do you undertake?
0) None
1) Cash crop farming Yes/No
If yes, which crops?.....
2) Dairy farming Yes/No
If yes, how many animals?.....
Their breeds.....
Their ages(Number of lactations).....
Milk yield per animal per day in kg.....
3) Horticultural farming Yes/No
If yes what crops are grown?.....
4) Others. Indicate.....
6. What area of land does your farm include?.....acres
7. What proportion is irrigated?.....acres
8. What is the proportion of each farming activity
Cash crops.....acres
Horticulture.....acres
Dairy farming.....acres
Subsistence.....acres
Others.....acres
9. Of your total farm area, what proportion is involved in dairying (housing, pasture and area of crops devoted to dairy forage)?.....%
10. Where do you take your animals during the day?
To pasture.....
Confine.....
Others. indicate....
11. How do your animals get access to forage?
grazing/pasture.....
Zero-grazing.....
Both..... Explain
12. If your animals graze on pasture, what size area do they have access to?.....acres
13. Do you feed remains of agricultural products to your animals? Yes/No
14. If you feed agricultural remains, which ones?.....
15. Do you feed remains which have been previously sprayed? Yes/No
16. If yes do you observe any interval between feeding and spraying? yes/No
17. if yes, how long?.....days
18. Which animals are given such agricultural remains?
lactating.....
Nonlactating.....
19. Indicate the approximate weight fed per day in kg.....kg
20. What benefits do you accrue to feeding agricultural remains?.....
21. Do you control ticks on your animals? Yes/No
22. If yes, what methods do you use?
Dipping.....
Spraying.....
Dusting.....
23. How often do you control the ticks?.....times/week

24. What chemicals do you use?.....
25. Are pests a major problem to your crops? Yes/No
26. If yes, how do you control them?
No control measures
Chemical control
Biological control
27. If chemical control measures are used, what chemicals are used?.....
28. How often are they applied?.....
29. Who applies the chemicals on the animals and plants? wife, son, daughter, employee, other- specify
30. How do you dispose of chemicals that remain and their containers?

Appendix 2: Farming practices in the 34 farms used in the observational study

Farm No.	Chemicals used on french beans	Frequency of spraying french beans	Chemicals used on animals	Frequency of dipping animals
396	dimethoate/ sherpa	2/ week	Triatix	1/ week
392	dimethoate/ sherpa	2/ week	steladone	1/ week
496	dimethoate/ sherpa	2/ week	supadip	1/ week
560	dimethoate/ sherpa	2/ week	-	-
756	dimethoate/ sherpa	2/ week	-	-
495	dimethoate/ sherpa	2/ week	Triatix	2/ week
465	dimethoate/ sherpa	2/ week	Triatix	2/ week
395	dimethoate/ sherpa	2/ week	-	-
1225	dimethoate	2/ week	-	-
1263	dimethoate/ sherpa	2/ week	supadip	1/week
369	dimethoate	2/ week	supadip	-
328	dimethoate/ sherpa	2/ week	-	-
470	dimethoate/ sherpa	2/ week	triatix	1/ week
370	dimethoate/ sherpa	2/ week	-	-
375	dimethoate/ sherpa	2/ week	-	-
378	dimethoate/ sherpa	2/ week	-	-
480	dimethoate/ sherpa	2/ week	supadip	1/ week
553	dimethoate/ sherpa	2/ week	-	-
665	dimethoate	2/ week	triatix	2/ week
571	dimethoate/ sherpa	2/week	supadip	1/ week
576	dimethoate	2/ week	-	-

377	dimethoate/ sherpa	2/ week	-	-
472	dimethoate/ sherpa	2/ week	supadip	1/ week
689	dimethoate	2/ week	supadip	1/ week
688	dimethoate/ sherpa	2/ week	-	-
542	dimethoate/ sherpa	2/ week	triatix	2/ week
1306	dimethoate/ sherpa	2/ week	-	-
356	dimethoate/ sherpa	2/ week	-	-
491	dimethoate/ sherpa	2/ week	-	-
501	dimethoate/ sherpa	2/ week	triatix	2/ week
555	dimethoate/ sherpa	2/ week	triatix	2/ week
383	dimethoate/ sherpa	2/ week	-	-
374	dimethoate	2/ week	-	-
1219	dimethoate/ sherpa	2/ week	triatix	2/ week

Appendix 3: Proportion of farming activities on the 34 farms used in the observational study

Farm	Farm size (acres)	Acreage on pasture	Percentage of land on french beans	Percentage of land for dairying
396	3.5	0.4	20	10
392	3	0.45	33	20
496	4	0.5	30	15
560	3	0.5	25	30
756	3	0.5	25	30
495	3	0.5	33	30
465	3.5	0.5	25	45
395	3.25	0.33	25	30
1225	3	0.33	25	30
1263	3	0.25	30	33
369	3	0.5	39	25
328	3	0.5	40	30
470	4	0.5	50	33
370	3.1	0.5	45	25
375	3	0.5	25	30
378	4.2	0.5	35	30
480	4	0.5	25	30
553	3	0.5	25	30
665	3	0.4	25	15
571	5	0.4	25	20
576	7	0.25	33	30
377	3	0.25	33	25
472	4	0.64	25	33
689	3	0.6	25	30
688	3	0.5	15	30
542	3	0.5	25	30
1306	3	0.52	25	30
356	3.5	0.52	33	30
491	3	0.5	33	30
501	3	0.5	33	30
555	4	0.41	33	15
383	3	0.25	25	20

374	4	0.25	25	25
1219	7	0.5	15	30

Appendix 4: Feeding practices for the 34 animals used in the observational study

Farm	Weight of french bean haulms fed	Time between spraying and feeding (days)	Time between feeding and sampling (minutes)
396	5.5	6	90
392	10	21	175
496	12	14	200
560	6	9	205
756	10	7	195
495	13	6	155
465	10	14	130
395	14	8	170
1225	7	21	105
1263	9	21	210
396	13	14	60
328	13	14	70
470	10	10	130
370	6	10	150
375	12	8	165
378	22	9	135
480	12	9	175
553	16	9	120
665	8	11	240
571	20	10	320
576	8	14	255
377	9	14	180
472	7	14	132
689	11	14	150
688	5	21	140
542	13	21	190
1306	6	28	140
356	9	28	62
491	16	14	60
501	10	14	75
555	12	13	70
383	11	13	90

374	15	10	100
1219	14	15	260

Appendix 5: Cow factors for the 34 farms used in the observational study

Farm of origin	No. of cows	Breed	Age (years)	No. of lactations	KG milk per day
396	1	frisian	3	2	7.5
392	1	frisian	5	3	6
496	1	frisian	2	1	5
560	1	frisian	2	1	7
756	1	frisian	2	1	4
495	1	frisian	3	2	4.5
465	1	frisian	3	2	6
395	1	frisian	2	1	7
1225	1	frisian	2	1	8
1263	1	guernsey	3	2	8
369	1	frisian	3	2	9.5
328	1	frisian	4	3	7.5
470	1	frisian	4	3	10
370	1	frisian x guernsey	4	2	6
375	1	frisian	3	2	6.5
378	2	jersey	2	1	7
480	1	frisian	2	1	8
553	1	frisian	2	1	7.5
665	1	frisian	2	1	8
571	1	frisian	2	1	9
576	1	frisian	2	1	9.5
377	1	frisian	2	1	7.5
472	1	frisian	3	2	6
689	1	frisian	3	3	5.5
688	1	frisian	2	2	6
542	1	frisian	3	3	6
1306	1	frisian	2	1	5
356	1	frisian	2	2	5
491	1	frisian	2	2	9.5
501	1	local x frisian	3	2	7
555	1	frisian	2	2	8
383	1	frisian	3	2	7.5

Appendix 6: Cholinesterase activity before and after feeding sprayed french bean haulms for the 34 animals used in the observational study

Cow No.	prefeeding activity	postfeeding activity	difference
1	64	69	5
2	68	68	0
3	69	69	0
4	73	78	5
5	72	79	7
6	97	96	-1
7	64	60	-4
8	64	68	4
9	90	91	1
10	70	70	0
11	81	80	-1
12	73	79	6
13	89	89	0
14	94	99	5
15	71	72	1
16	83	83	0
17	86	83	-3
18	89	88	-1
19	83	82	-1
20	71	72	1
21	66	68	2
22	77	78	1
23	98	97	-1
24	72	71	-1
25	79	74	-5
26	65	63	-2
27	80	79	-1
28	75	74	-1
29	109	100	-9
30	93	92	-1
31	78	79	1

32	67	65	-2
33	76	78	2
34	70	69	-1