ORGANOCHLORINE PESTICIDE RESIDUES IN BREAST MILK OF MOTHERS LIVING IN NAIROBI, KENYA.

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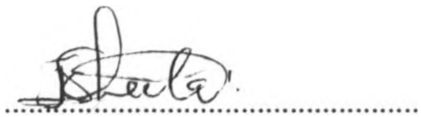
DECLARATION

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



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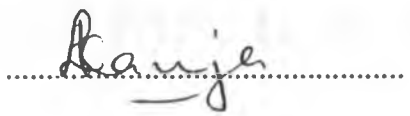

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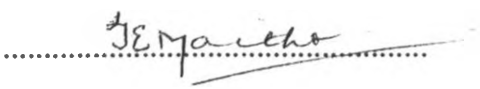

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To my parents
Mr and Mrs. B. N. Kinyamu

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Acknowledgements

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ABSTRACT

A total of 216 human milk samples were collected from mothers either attending post natal clinics or in the maternity wards of some hospitals in Nairobi. The samples were analysed for organochlorine pesticides by use of a gas liquid chromatograph equipped with a ^{63}Ni electron capture detector. Thirteen organochlorine pesticides were detected in the following order of frequency:- p,p'-DDE (99.5%), p,p'-DDT (78.2%), dieldrin (37.5%), β-HCH (16.2%), lindane (16.2%), o,p'-DDT (11.5%), α-HCH (11.1%), heptachlor (10.2%), p,p'-DDD (9.3%), aldrin (5.6%), heptachlor epoxide (4.2%), endrin (2.7%), and o,p'-DDD (0.44%).

The mean level (mg/kg milk fat) of sum DDT in all the human milk samples analysed was 0.47 and ranged from 0.004 to 6.32. Mean levels (mg/kg milk fat) of the other residues identified were as follows:- dieldrin 0.022, β–HCH 0.089, lindane 0.017, α-HCH 0.067, heptachlor 0.026, aldrin 0.03, heptachlor epoxide 0.017, and endrin 0.035..

There were significant differences ($p<0.05$) in the levels of sum-DDT and p,p'-DDE, in relation to the parity of the mother with higher levels being observed in mothers nursing their first child as compared to those nursing their second child. Mean level (mg/kg milk fat) of sum-DDT was 0.51 in primipara and 0.41 in secundipara while mean level (mg/kg milk fat) of p,p'-DDE was 0.32 in primipara and 0.28 in secundipara.The other factors found to affect the levels of organochlorine pesticides in this study were, maternal age and fat content of the milk. Diet, social class and race of the mother were not significant contributors ($p>0.05$) to the pesticide levels in the human milk. The mean ratio of p,p'-DDT to p,p'-DDE was low implying less exposure of the population to the parent compound p,p'-DDT.

The levels of the organochlorine pesticides in this study were lower than levels observed in an earlier study carried out in the rural areas of Kenya and also in some

cases lower than levels observed in earlier studies carried out in some industrialised countries.

The estimated daily intake of sum-DDT, dieldrin, aldrin and endrin by the infant was found to exceed the acceptable daily intake (ADI) set by the World Health Organisation.

Food samples collected from major markets in Nairobi were examined for organochlorine residues. Contamination of the food samples with organochlorine pesticides was low and only residues of dieldrin, p,p'-DDE, p,p'-DDT were detected. Food of animal origin was found to contain higher levels of organochlorines than food of plant origin. Although the contamination of food with organochlorine pesticides was low the presence of DDT, DDE and dieldrin in some food stuffs demonstrated that food could be one of the sources of these compounds in the human milk.

CHAPTER 1
INTRODUCTION

Pesticides are chemically active compounds intended mainly to kill pest populations by their toxic or other deleterious reactions. They are classified in accordance with their use e.g. insecticides, herbicides, fumigants, rodenticides and many others. Prior to world war two, the pesticides that were in use were mainly of two types, the inorganic ones like arsenicals and fluorides which were most commonly used and those of plant origin such as the poisonous nicotine products. The use of these was stopped because of the toxicity to both man and animals, and this therefore led to the development of less poisonous compounds.

During the second world war, the insecticidal properties of certain chlorinated hydrocarbons like DDT and lindane were discovered. These compounds then replaced the above as pesticides, they were widely used in control of agricultural pests and in public health programmes. The enthusiasm about the effectiveness of these compounds was however shaded by the alarming discovery of their persistence in the ecosystem due to their long residual effect.

Organochlorine compounds are highly lipophilic in nature and therefore tend to accumulate in the fatty tissues of the fish, birds and mammals. Prolonged use of these compounds has led to accumulation in the environment and undesirable effects in the ecosystem. An important feature of these lipophilic pollutants is their ability to concentrate along the food chain reaching higher concentration at higher trophic levels (Edwards, 1978) due to their great chemical stability, low aqueous solubility and high lipophilicity. Human beings are at the top of most food chains, thus these compounds reach the human body mainly through the daily diet and are deposited in human adipose tissues.

The hazards caused by these chemicals to living systems are under continuous investigation and evaluation, and many countries have accumulated sufficient information on environmental hazards due to these chemicals that has led to

banning or restricting of their use.

DDT was first reported in human milk by Laug et al, (1951), since then many other investigations have been carried out and DDT and other related organochlorine compounds have been found in human milk throughout the world (Jensen, 1983). Although these kind of studies have been carried out in industrialised countries, very few investigations have been carried out in developing countries where organochlorine pesticides have been used indiscriminately.

Human milk is the source of nutrition for the newborn babies and because of the possible deleterious effects on the developing infant, special consideration should be given to the presence of organochlorine chemicals in the mothers milk. Human milk has a high fat content and is therefore a main route of excretion of organochlorine pesticides. Apart from evaluating the levels of organochlorine pesticides in the individual mothers milk, the milk may be used to assess the levels of the environmental pollution by these compounds.

A recent study on the organochlorine pesticide residues in mother's milk from the rural areas in Kenya (Kanja, 1988), demonstrated large contamination of Kenyan human milk with organochlorine pesticides especially DDT and DDE. The levels of sum DDT obtained were higher than corresponding levels reported from the industrialized countries and the estimated daily intake of a Kenyan infant exceeded the acceptable daily intake set by the WHO/FAO.

Previous studies did not include the mothers living in urban centres of Kenya. The present study was therefore initiated in order to investigate the distribution of organochlorine pesticides in mothers living in an urban environment and evaluate the possible sources.

OBJECTIVES

1. To identify and quantify the levels of organochlorine pesticides residues in milk from mothers living in Nairobi, Kenya.
2. To compare the results of the present study to those obtained from the rural areas of Kenya in an earlier research which was undertaken in this department.
3. To compare the levels of organochlorine pesticides in different mother's based on their:-

   social class (rich versus poor),

   race of origin (Asian versus Africans), and

   their diet (vegetarians versus non-vegetarians).
4. Investigate the sources of the organochlorine pesticides residues in mothers milk by analysing samples of the common foods.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Organochlorine pesticides were among the first types of synthetic pesticides specifically developed for animal use, and in forms of DDT, hexachlorobenzene and dieldrin were used extensively for parasitic control (Brander *et al.*, 1960). DDT was a great success in the fighting of malaria-carrying mosquitoes during the second world war. Later it became one of the most important insecticides in agriculture. The use of these organochlorine compounds in public health and agricultural programmes has caused serious environmental problems due to their lipophilic nature. In spite of new restrictive measures of many governmental organs regulating use of organochlorine pesticides, their presence (especially DDT and its analogues) is still widely detected in the environment.

In many countries, the heightened awareness of the possible health and environmental hazards involved has led to the approval of laws and regulations for the production and use of organochlorine insecticides and to the enforcement of tolerance levels for the residues in foods. In Kenya use of DDT, aldrin, dieldrin, lindane and endrin has been restricted but previous studies in this country have shown presence of these organochlorine pesticides in both human and animal tissues (Wasserman *et al.*, 1972; Maitho, 1978; Kahunyo, 1983; Mugambi 1986; Kanja, 1988; Mitema *et al.*, 1990; Mugachia, 1990).

Organochlorine pesticides enter the environment from various sources, usually during manufacture or when in use, they are transported mainly by air and water (Matsumura, 1975). They have a high lipid solubility and low volatility (Brander *et al.*, 1960) and thus leave residues in crop and animal products (Hill, 1983). The residues are of great concern because they are potential hazards to the consumers and to the environment. Because of their persistence in nature the chemicals accumulate along food chains and endanger the species or individuals at the top of the contaminated ecosystem.

The accumulation of residues of non biodegradable pesticides in human and animal tissues has caused a great concern from the point of view of health. DDT and the other chlorinated hydrocarbon insecticides are suspected carcinogens (Crouch and Wilson, 1979) and have been implicated in a number of biological disorders (Hayes, 1985).

The first recognition that human breast milk may be contaminated by environmental chemicals came with the findings of Laug and coworkers (1951) showing that the milk from normal and healthy black American women contained considerable amounts of the organochlorine insecticide DDT. Since then, many investigations have been made in countries all over the world and DDT and it's metabolite DDE have been detected in most human milk investigations. Other organochlorine pesticides that have been detected in human milk include aldrin, dieldrin, endrin, hexachlorohexane and polychlorinated biphenyls (Jensen, 1983).

Human beings are at the top of most food chains (Gochfield, 1972), this explains why human adipose fat and milk fat have a higher level of persistent organochlorine pesticides as compared to milk fat from cows and other lower animals (Polishuk *et al* .,1977; Landoni *et al* .,1982).

Contamination of human milk by xenobiotics has a special significance since newborns and infants, are involved and milk constitutes for them for a certain time, almost the sole source of nourishment (Skaare, 1981). It is therefore important to monitor the amount of organochlorine pesticides in human milk not only to assess the levels in the milk and the significance but also as an indicator to environmental pollution.

2.2 Organochlorine pesticides

2.2.1 Chemical Properties

Organochlorines are aryl, carbocyclic or heterocyclic compounds of molecular weight ranging from 291 to 545. They may be divided into four groups viz:- DDT and its analogues, hexachlorocyclohexanes (HCHs) and related compounds, cyclodienes and related compounds and toxaphene and related compounds.

Representatives of each group may be identified by characteristic chemical structure (Hayes, 1982).

The chemical properties of chlorinated hydrocarbon pesticides are similar. They are poorly soluble in water but soluble in oils and organic solvents. They have slow vaporization and persistent in the environment because they resist chemical or microbial decomposition especially when protected by layers of soil (Booth, 1981). The half lives in years (temperate regions) of these insecticides that are worked into soil are aldrin 1-4, dieldrin 1-7 lindane 2, DDT 3-10, endrin 4-8, and heptachlor 7-12. These half lives may be reduced to weeks if the pesticides are fully exposed to the elements of the environment.

2.2.2 Toxicokinetics.

Absorption

Absorption of Chlorinated hydrocarbon pesticides occurs through the skin, oral and respiratory routes. The efficiency of the dermal exposure varies with different compounds, for example, DDT is poorly absorbed through skin whereas dieldrin is effectively absorbed. The compounds rarely reach unpermissible levels in the air in form of vapors. If they do, they are absorbed by the respiratory epithelium (Hayes, 1982).

Distribution

After entry into the bloodstream, organochlorine pesticides may be bound to serum proteins and are distributed to other tissues, with the highest concentrations being in the adipose tissue. This situation is permitted by the high degree of solubility of the compounds in fat (Hayes, 1982). Under constant pesticide intake, the concentration of the pesticide in the body reaches a plateau at which a balance is achieved in a state of equilibrium which is influenced by addition of pesticide into the blood stream and losses from the blood by distribution to body tissues, metabolism and excretion.

Metabolism and excretion

Metabolism mainly occurs in the liver with the parent compound and metabolites being excreted in urine, bile, milk and feces. When pesticide intake stops, the concentration in tissues decline at a rate dependent on the chemical nature, metabolism, excretion and the type of tissue. Fat is the main storage of organochlorines and since it is poorly supplied with blood it can retain these compounds for a long time. This explains the toxicity of these compounds and persistence in milk or tissues just after one exposure.

2.2.3 Toxicity.

Acute toxicity

The organochlorine pesticides mainly act on the central nervous system and elicit a variety of central nervous system symptoms among which are neuromuscular and behavioral. The compounds cause incoordination, ataxia, stiff gait, anxiety, confusion, aggressiveness, abnormal posturing and inability to concentrate. The group is not acutely toxic to man. Hayes *et al.* (1956), administered DDT to human volunteers at doses of 35 mg per day, which is 200 times the amount present in the average diet. Throughout the 18 months of administration, the human subjects exhibited no symptoms related to DDT intoxication.

Chronic toxicity

Clinical signs in chronic toxicity are similar to those of acute toxicoses, probably because of release of pesticide from the storage in the body. In human beings, chronic effects are not well established but in experimental animals many organ changes have been associated with presence of organochlorines.

The compounds are associated with enzyme induction, teratogenicity, mutagenicity, carcinogenicity and other behavioral effects in laboratory animals though this has not been observed in mammals.

2.2.4 DDT and it's analogues.

DDT is a white, tasteless, almost odorless crystalline solid, with melting point of 108.5° to 109° C. Technical DDT is a waxy solid and usually contains o,p'-DDT.

p,p'-DDT is the most important pesticide in the DDT group. p,p'-DDD and p,p'-DDE are both metabolites of DDT. p,p'-DDE has no insecticidal activity but it is more persistent in the environment than the parent compound (Jensen, 1983).

DDT has been used for control of vector borne diseases like malaria and in control of mites, lice and plant pests. It was the first environmental chemical to be detected in human milk (Laug *et al.*, 1951). DDE being the more persistent of the two is usually found to increase in milk following DDT exposure. An evaluation of this relation might therefore be valuable in source detection and in assessing recent exposure and direct or indirect exposure through food chains. The widespread contamination by DDT and it's potential health hazard to man have been of considerable public concern (Matsumura, 1984).

2.2.5 Cyclodienes.

Cyclodienes are highly chlorinated cyclic hydrocarbons with endomethylene bridged structures. Compounds in this group include chlordane, aldrin, dieldrin, heptachlor and endrin. From the mid 1940's to the mid 1960's, the organochlorine insecticides were used widely in agriculture, soil structure and, insect control, including malaria control programmes.

The cyclodienes are also very persistent in the environment and tend to accumulate in biological as well as nonbiological media. The compounds in this group are classified as neurotoxins. Acute poisoning from them may be fatal. The compounds affect the central nervous system, the reproductive system, liver and kidney.

Aldrin and dieldrin have been used widely for insect control. Aldrin is readily epoxidised to dieldrin. Degradation of dieldrin is slow and as a result, it has contaminated food, air and water in many areas of the world.

Heptachlor, heptachlor epoxide and chlordane are closely related chlorinated insecticides. Heptachlor epoxide is a persistent metabolite of heptachlor. Low levels of these pesticides have been detected in samples from countries like the U.S., Canada and Japan (Jensen, 1983).

2.2.6 Hexachlorocyclohexanes

Hexachlorocyclohexanes are mixtures of stereoisomers which differ in the relative position of the chlorine around the hexane ring. The commercial insecticide is a mixture of the different isomers which include, α-HCH, β-HCH, γ-HCH, δ-HCH and others. γ-HCH (lindane) is the most toxic isomer and most commonly used insecticide whereas, β-HCH is the most symmetrical and stable isomer and also the most environmentally persistent and chronically toxic HCH. β-HCH also has a higher ability to accumulate in the fat tissues than lindane. It is the isomer usually found in highest concentration in the human adipose tissues and milk. The α and γ isomers may isomerise into β-isomer in living organisms (Jensen, 1983).

2.3 Rationale for assessing human exposure to persistent organochlorine pesticides through monitoring their levels in breast milk.

Chlorinated pesticides due to their high lipophilic nature accumulate in fatty tissues. The levels in milk and adipose tissues are therefore high as compared to levels in other tissues like blood, hair, and urine. Human milk,.because of the high fat content (3.4%) can be used in assessment of the levels of the compounds in the general population. At the same time, the milk can be used in assessment of environmental pollution. Monitoring programs using human milk have been used widely in getting the trends of residue levels in many developed countries.

Large volumes of milk can be easily obtained from healthy lactating women, and by combining this with a questionnaire completed by the donor, it is possible to discover risk factors or chemical exposures of importance relating to levels found. This makes it possible to encircle pollution sources and prevent further exposure.

2.4 Rationale for collecting food samples.

Organochlorine pesticides have been used widely in the control of both animal and agricultural pests. These compounds are highly lipophilic and accumulate in the fatty tissues. Food is a major source of these pesticides to humans. Food of animal origin has higher levels of pesticides (Kanja, 1988).

Duggan and Weatherwax (1967), on the dietary intake of pesticide chemicals by man reported that meat, fish, poultry and dairy products were major sources of pesticides and account for more than half the intake of the residues. Foods of plant origin are also important sources especially when good agricultural practices are not followed.

A study of levels of organochlorine pesticides in food will serve as an indication of the amount of these pesticides ingested by man and also an indicator of environmental pollution.

2.5 Review of organochlorine pesticides residue studies in human milk in Kenya.

Kanja *et al* (1986), studied the levels of organochlorine pesticides in human milk in Kenya. Samples from the rural areas of Kenya at different altitudes and with different geographical conditions and different agricultural activities were analysed. The areas covered were Meru, Embu, Rusinga Island, Loitokitok, Nanyuki, Turkana, Homa Bay, and Karatina.

Kenyan mothers' milk was found to be contaminated with organochlorine pesticides. The major contaminants were DDT (100% positive samples) and its more persistent metabolite DDE (100%). Other organochlorine pesticides identified were:- HCB, γ-HCH, aldrin (3.5%), endrin (4%), dieldrin (20%), α-HCH (8%), transnonachlor (6%), heptachlor (4%), heptachlor epoxide(0.4%) and oxychlodane (0.4%). Residues of PCBs were not found in quantifiable amounts in any sample analysed.

Mean levels (mg/kg in milk fat) of sum DDT ranged from 1.69 in milk from nomads in Loitokitok to 18.73 in human milk collected in Rusinga Island. The mean range of ratio of p,p'-DDT to p,p'-DDE was 0.7 (Karatina) to 4.4 (Turkana). The levels of sum DDT obtained were higher than the corresponding levels from the industrialised countries.

The infant dietary intake of DDT through mothers milk exceeded the acceptable daily intake by several fold. ADIs of dieldrin and aldrin were exceeded by some

infants. In addition, levels of the sum DDT in mothers milk in this study were higher than the corresponding levels from the industrialised countries. Although the results were alarming, no further work has been done in this field.

2.6 Organochlorine pesticides in human milk in relation to the dietary habits of the mothers.

. Food is a major source of pesticides that accumulate in the human body. Other routes of exposure which are of less importance are inhalation and absorption through skin. Of importance is food of animal origin which probably due to it's high fat content, tends to have higher levels of organochlorine pesticides than that of plant origin (Kanja, 1988).

Foods of plant origin may have significant levels of pesticides since the chemicals are applied widely both in storage of grains and in control of agricultural pests. The contaminated food may be ingested by man especially if good agricultural and hygienic practices are not observed.

Noren (1983), studied levels of organochlorine pesticides in human milk in relation to the dietary habits of the mothers and found that the levels of DDT, DDE, and dieldrin were lowest in milk fat from lacto-vegetarians and highest in mothers who regularly ate fatty fish. In a study conducted by Walter (1986), women who consumed sportfish during pregnancy had higher levels of DDE than those who did not consume any fish at all.

Siddiqui (1986), demonstrated that non-vegetarian mothers excreted relatively higher amounts of organochlorine pesticides compared to vegetarian mothers. In Sweden it has been noted that the major non-occupational source of organochlorine compounds in human milk studied was probably the diet, especially certain foods of animal origin such as fish. Hergenrather *et al.* (1981), reported that vegetarian women who consume food low in the food chain had lower level of organochlorines.

In a study to find out what determines the levels of DDT in human milk, Bradt (1976), analysed breast milk samples and demonstrated that higher levels were

related to a diet high in calories. Other factors found to determine the levels were number of children nursed, smoking, and use of non-persistent pesticides.

2.7 Organochlorine pesticides in human milk in relation to the residential area of the mothers.

No uniformity is found in literature on the effect of area of residence of the mother on the levels of organchlorine compounds in human milk. Jensen (1983), suggests that, within a country, levels of organchlorine compounds are lower in mothers living in rural areas than those living in urban areas. On the other hand, levels in the agricultural rural areas may be very high in cases where these persistent pesticides are in common use. In Japan milk, from urban mothers had a greater contamination of organochlorines (DDT, HCH and dieldrin) than did rural mothers, probably due to a greater intake of animal protein and fat by the former group (Jensen, 1983).

Siddiqui (1981), demonstrated that the area of living of the mother during pregnancy influences pesticide excretion through milk and the placenta. In a study to demonstrate this phenomenon it was found that the total HCH and lindane were higher in blood of newborn babies born of mothers residing in urban areas compared to the neonates of rural mothers. The difference in total DDT by area of residence was not significant, however, slightly higher DDE level was found in urban subjects. In another study, it was found that levels of mean DDT were higher in rural subjects than in the urban subjects, the levels of HCH followed the reverse trend (Siddiqui et al., 1985). Kontek (1971), found no difference in pesticide residues between the city and rural samples

2.8 Review of organochlorine pesticide residue studies in Kenya.

A few studies have been carried out on the presence of organochlorine pesticide residues in different animal tissues in Kenya. As early as 1970, Koeman and coworkers did an orientational survey on the side effects and environmental distribution of insecticides used in Tse-Tse control in Africa and found higher levels of dieldrin and DDE as compared to other organochlorine pesticide in fish

collected along the shores of Lake Victoria. A similar study on levels of organochlorines in tissues of birds and fish collected in Lake Nakuru was carried out. DDT, DDE, and dieldrin were detected in trace amounts in some of the specimens (Koeman *et al.*, 1970).

Analysis of cow milk and fat samples for organochlorines and organophosphates revealed low levels of the chlorinated hydrocarbon pesticides, with DDT being the most frequent organochlorine residue in the samples (Maitho, 1978).

Kahunyo (1983), studied levels of organochlorine compounds in chicken fat and eggs and found a range of 12 organochlorines in the samples. The DDT group had the highest incidence followed by lindane, dieldrin and heptachlor. In a more recent study on the residues of organochlorines in domestic fowl eggs, 10 organochlorine pesticides were detected in various combinations, and as in the previous studies DDT was the most frequently occurring compound (Mugambi, 1988).

Lincer and coworkers (1981), carried out a study of organochlorine pesticide residues in Kenyan Rift Valley lakes and found low levels of DDE in the flora and fauna of these lakes. Chlorinated hydrocarbon pesticides were detected in 78% of the pectoral muscles of birds investigated by Frank in 1977. The most widespread contaminant was DDE and the related DDT compounds . The residues observed were substantially higher than those previously reported among the non-raptorial birds in the agricultural areas of East Africa. Studies of organochlorine pesticide residues in some fresh water and estuarine fish from different locations in Kenya revealed contamination of the liver, eggs and the fillet of fish by nine organochlorine pesticides (Mugachia, 1990).

Wasserman *et al.* (1972), analysed specimens of adipose tissue sampled from people without known exposure of organochlorines. He found low levels of DDT and it's metabolites, HCH, dieldrin and heptachlor epoxide.

In a study carried out on the levels of organochlorine pesticides in human milk, Kanja *et al.*, (1986) found that Kenyan human milk was contaminated with

organochlorine pesticides. DDT and it's more persistent metabolite DDE were the major contaminants while other organochlorines were found in much lower frequency.

A most recent study in this field was on organochlorine pesticide residues in fish from Lake Victoria. Nine organochlorine pesticide residues were detected, DDT and it's derivatives formed the largest proportion of the residues. This finding is consistent with the findings in other studies where high amount of DDT have been detected in other tissues (chicken eggs and fat, human milk) as reported in earlier studies (Kahunyo, 1983; Kanja *et al.*, 1986;.Mugambi, 1989). The levels of the various organochlorine pesticide residues in fish are still below the extraneous residue limit (Mitema *et al.*, 1990).

2.9 Analytical methods for organochlorine pesticides.

The basic steps in the analysis of organochlorine compounds in biological samples have been widely studied. However, there is not one standardised method. Individual workers prefer their own modifications of similar procedures.

Assessment of environmental pollutants in biological media usually involves analytical procedures capable of detecting very minute quantities of residues. Laboratories should maintain strict control of the environment in which the analysis are performed because of the very low concentration of the pollutants to be analysed. A thorough and reliable system of establishing and maintaining a high quality performance is essential. Procedures used to ensure quality control, accuracy and precision should be reported in the results. Such procedures include intralaboratory controls and interlaboratory checks (Berlin *et al.*, 1979; Slorach and Vaz, 1983).

In any methods used for pesticide analysis, there are five main steps (Sherma, 1979).

a) Sampling

b) Extraction

c) Clean-up

c) Identification and quantitation

d) Confirmation of the presence.

2.9.1 Sampling and storage.

In excising tissue and collecting body fluids for biological monitoring, necessary precautions must be taken to obtain adequate information relative to the purpose of the study and the substance to be analysed. Contamination of the sample during collection and preparation should be avoided (Sherma, 1979).

Methods of storage should ensure minimal degradation. Freeze drying, deep freezing and chemical preservation are some of the methods used for storage of samples (Berlin *et al.*, 1979).

2.9.2 Extraction

To ensure complete extraction of the lipophilic substances from biological materials, it is necessary to accomplish a dehydration and disruption of cells. This can be achieved either by homogenization with a dehydrating agent such as anhydrous sodium sulphate followed by continuous extraction in soxhlet apparatus, or by homogenization of the sample with a dehydrating solvent like acetone followed by batch extraction (Jensen, 1979).

Bouwman *et al.* (1990), explains a method of extraction by denaturation of proteins using mercaptoethanol and solubilising of fats by deoxycholic acid followed by liquid-liquid extraction. Partition between solvent system like acetonitrile, dimethyl sulphoxide or dimethyl formamide and hexane or light petroleum is also commonly used for extraction (Kanja, 1988).

2.9.3 Clean-up procedures

Clean up of samples removes co-extractives which interfere with residue determination and instrument performance. This can be done by column chromatography, before gas liquid chromatography (GLC) analyses (Jensen, 1979). Acid and base treatment (Bjerk and Sundby, 1970), gel permeation chromatography (Johnson, 1976), Silica gel (Bouwman, 1989) and thin layer chromatography have all been used in clean -up procedures.

2.9.4 Detection of residues

Final extract can be analysed with any GLC system having an electron capture detector (^{63}Ni, ^{3}H/Sc, or ^{3}H/Ti). The ^{63}Ni and ^{3}H/Sc detectors are generally recommended since they can be operated at higher temperature than ^{3}H/Ti and this causes less contamination of foils. Risk for decomposition of pesticides on the column is a common problem in residue analysis. Therefore, all glass columns are normally preferred. The decomposition problem is also avoided through deactivation of the active sites on the glass and column material with silylating agents (Jensen, 1979),

Other less sensitive methods include paper and thin layer chromatographic techniques (Mugambi, 1986).

2.9.5 Confirmation of residues.

To confirm the presence of residues, it is recommended that the sample extract is run in a different column, with a different column packing material (Jensen, 1979; Kanja, 1988; Mugachia, 1990). Thin layer chromatography (Maitho, 1978) and mass spectrometry have also been applied in confirmation of the residues.

CHAPTER THREE

MATERIALS AND METHODS.

3.1 Materials

3.1.1 Equipment

Equipment	Description	Supplier
Gas liquid chromatograph	Parkard model 428	Parkard Becker B.V, Amsterdam, The Netherlands.
Recorder	Parkard model 621	Parkard Becker B.V Amsterdam, The Netherlands.
Vacuum pump	Air compressor 850	Corning Ltd. Halstead Essex, England.
Water jet pump	Gallenkamp pxy 290	Gallenkamp &Co. Ltd Technico Hse, London, England.
Microsyringe	10 µl, with 2" long needle	S.G.E. PTY Ltd Melbourne, Australia.
SMI micropetter	SMI digital adjust micro petter	Scientific Manufacturing Industries, Emeryville, California, USA.
Calsberg pipettes	100 µl, 1000 µl 500 µl, 50 µl	John Poulten Ltd Essex, England.
Pastuer pipettes		
Centrifuge	Gallenkamp	Gallenkamp & Co. Ltd London, England.

Ultrasonic disintegrator	Cell disruptor sonicator	Heat Systems Ultrasonic Inc., Plainsview, New York, USA.
Balances	Sartorius	Satorius-Werke, Goettigen, West Germany
Water bath	Thermostated	Memmert, West Germany
Pestle and mortars	Porcelain	
Septa	Chromsep septa (red) No 10036	Chrompack Co. Middelburg, The Netherlands.
Glass wool	Silane treated	Supelco, Inc. Bellfonte, Pennsylvania, U.S.A
Liquid dispenser	Sucorex dispenser	
Waring blender		Moulinex, France.
Whirl mixer		Lab-line Instruments Melrose Park California, USA.

3.1.2 Glassware

Item	Description	Supplier
Sampling bottles	40 ml, 25 ml, 7.4 ml	Supelco Inc, Bellfonte, U.S.A
Centrifuge tubes	15 ml, 50 ml	
Extraction columns	Glass	
Volumetric flasks	1000 ml, 100 ml, 10 ml	

3.1.3 Chemicals

Chemical	Brand name/Grade	Supplier

Nitrogen gas	99% pure and ordinary	East Africa Oxygen Co. Ltd., Nairobi Kenya
Hexane	Analytical reagent	May & Baker Dagenham, England
Acetone	Analytical reagent	May & Baker Dagenham, England
Methanol	Laboratory	Merck, Darmstadt, West Germany
Sulphuric acid	Analytical	BDH chemicals LTD Poole, England.
Potassium hydroxide	Analytical	BDH chemicals LTD Poole, England.
Diethyl ether	Analytical	BDH chemicals LTD Poole, England.
Sodium chloride	Analytical	BDH chemicals LTD Poole, England.
Sodium sulphate	Analytical	BDH chemicals LTD Poole, England.
Sand	Acid washed	Howse & Mc George Nairobi, Kenya
Snoop	Liquid leak detector	Supelco Inc., Nupro Company, Willoughy, Ohio, USA.
CPM standard	1 ml ampoule	Supelco Inc, Bellfonte, Pennsylvania, USA.

Nitrogen gas	99% pure and ordinary	East Africa Oxygen Co. Ltd., Nairobi Kenya
Hexane	Analytical reagent	May & Baker Dagenham, England
Acetone	Analytical reagent	May & Baker Dagenham, England
Methanol	Laboratory	Merck, Darmstadt, West Germany
Sulphuric acid	Analytical	BDH chemicals LTD Poole, England.
Potassium hydroxide	Analytical	BDH chemicals LTD Poole, England.
Diethyl ether	Analytical	BDH chemicals LTD Poole, England.
Sodium chloride	Analytical	BDH chemicals LTD Poole, England.
Sodium sulphate	Analytical	BDH chemicals LTD Poole, England.
Sand	Acid washed	Howse & Mc George Nairobi, Kenya
Snoop	Liquid leak detector	Supelco Inc., Nupro Company, Willoughy, Ohio, USA.
CPM standard	1 ml ampoule	Supelco Inc, Bellfonte, Pennsylvania, USA.

Column Packing materials	a) 4% SE 30/6%SP-2401 on 100/120 Supelcoport. b) 1.5% SP-2250 /1.95% SP-2401 on 100/120 Supelcort.	Supelco Inc., Bellfonte, Pennsylvania, USA.

3.2 Analytical methods

3.2.1 Cleaning of glass ware

All the glassware used in this study were scrupulously cleaned to avoid the presence of the extraneous peaks resulting from contamination. The following procedure was used:-

After removing the residues if any, the glass ware was rinsed with hexane and soaked in hot water plus detergent. This was then scrubbed thoroughly and rinsed in tap water followed by another rinse in distilled water. The glass ware was then rinsed in distilled acetone and dried in the oven at 150°C. Just before use the glass ware was rinsed in the solvent to be used in the analysis (hexane).

3.2.2 Distillation of solvents.

Hexane and acetone were distilled once in an all glass fractionating column equipped with a water cooled condenser. Glass beads were used in the distillation flask to prevent the solvents from super heating. The rate of distillation was controlled to avoid co-distillation of impurities. The initial 200 ml of the distillate and about 400 ml of the last portion of the solvent in the flask were not used in the analysis. The initial portion serves to rinse the fractionating column while any impurities present in the solvent are more concentrated in the last portion.

3.2.3 Solvent purity check

Hexane

10 ml of the distilled hexane was evaporated in a water bath at 50°C and concentrated twenty times to a final volume of 0.5 ml. 1-3 µl of the aliquot was

injected into the gas chromatograph. The detector response was observed for fifteen minutes, a period equivalent to the retention times of compounds in the cpm standard.

No interfering peaks were observed and therefore the hexane distillate was suitable for pesticide analysis.

Acetone, ethanol and diethyl ether

10 ml of each reagent was put in a tube and evaporated to dryness in a water bath at 50°C under a gentle stream of nitrogen. The tube was then rinsed with 1 ml hexane. 2 µl of the hexane was injected into the GLC and the chromatogram observed for 15 minutes

Sulphuric acid

Concentrated sulphuric acid was cleaned using hexane. 500 ml sulphuric acid was added to 100 ml redistilled hexane in a separatory funnel thoroughly shaken and left to stand on the bench for 30 mins. The hexane layer was discarded and the cleaning repeated three times. 10 ml of the hexane was taken from the third rinse and concentrated to 1 ml by evaporation. 2 µl of this was tested by GLC.

Alkaline solution

Alkaline solution for the base clean-up was prepared by dissolving 2 pellets of potassium hydroxide in 1 ml redistilled ethanol. A solution of orthophosphoric acid and sodium chloride was prepared by dissolving 11.6 g of sodium chloride in 6.83 ml of orthophosphoric acid and made up to a volume of 1000 ml with distilled water. This solution was added to the base clean up to facilitate the separation of the hexane layer.

To check the purity of the solution of sodium chloride in orthophosphoric acid, 10 ml of the solution was shaken with 20 ml hexane and kept in a deep freezer for about 30 minutes. The hexane layer was removed into another tube and evaporated to a volume of 1 ml. 2 µl of this was injected into the GLC.

3.2.4 Pesticide standards

Chlorinated pesticide mixture (CPM) contains thirteen organochlorine

compounds in 1 ml of iso-octane. The CPM standard mixture was obtained from "Supelco, S.A." Bellfonte, U.S.A. The concentrations of the thirteen pesticides are as shown in the table 3.1.

The neck of the ampoule containing the cpm mixture was broken and the mixture emptied into a 10 ml volumetric flask. The ampoule was rinsed with hexane twice. Hexane was used to top up the mixture to the 10 ml mark. The prepared stock solution was stored in a deep freezer at -20 °C.

A working standard was prepared by serial dilution of the stock solution with hexane to a dilution of 1:1000. All preparation of the standard was done at room temperature and the solutions stored in deep a freezer until when required.

During analysis the working standard solution together with the sample extract to be injected on that day were removed from the deep freezer and left to thaw at room temperature before injection into the GLC. Due to solvent evaporation, working standards were regularly prepared from the stock solution.

3.2.5 Gas chromatographic columns

Preparation

The columns were cleaned by treating each with 5% dimethyldichlorosilane in toluene to seal any active site in the column. They were then washed with acetone followed by methanol. The column was oven dried at 150°C for about 2 hours.

Packing columns

Prepared packing material obtained from "Supelco S.A." Bellfonte, U.S.A.was used to fill the column. The outlet end of the column was plugged with a small wad of silanized glass wool and then connected to the vacuum source. A funnel was attached to the inlet end of the column and the packing was added very slowly. The column was tapped or vibrated gently with a mechanical vibrator while the packing was added.

Column conditioning

Each column used was conditioned while it was installed in the injection port only, to avoid the detector being contaminated during the conditioning process. At

a constant flow rate of about 40 ml/min, the oven temperature was programmed at 2°C/min to 240°C and held at this temperature overnight. After cooling, the detector end was connected. The conditioning process removes the volatile impurities, which would otherwise interfere with good separation giving rise to tailing of peaks.

3.2.6 Resolution and linearity of the ECD

Resolution of the GLC was checked by injecting 2 µl of 1:1000 cpm standard into the GLC under the operating conditions described below. These conditions were used in the analysis.The resulting elution pattern was compared to the elution pattern supplied by the manufacturers of the cpm (Supelco Inc).

Linearity of the GLC was checked by injecting equal volumes of different concentrations of cpm into the GLC and plotting the graph of concentration against the peak heights.

3.2.7 Detection limits

The modern ECD has a detection limit of 1 picogram for organochlorine pesticides. In this study the detection limit was about 0.001 mg/kg for all compounds.

3.2.8 Pesticide identification, quantitation and confirmation

Identification was carried out using a Packard model DY 12362 series 428 gas liquid chromatograph with the following instrument parameters and operating conditions.

Detectors: ^{63}Ni electron capture.

Packing materials

a) 4% SE-30/6%SP-2401 on 100/120 Supelcoport.

b) 1.5% SP-2250/1.95% SP-2401 on 100/120 Supelcoport.

Column temperature-210°C, Detector temperature-250°C, Injection port temperature-230°C, Carrier gas: flow rate-60 ml/min, Injection volume: 1-5 µl.

The retention times of the peaks of the sample chromatogram obtained were compared to the retention time of the corresponding peaks in the standard

chromatograms.

Peak heights in the sample were measured and related to the peak heights in the standard and the concentration of the compound calculated using the formula:-

V/W.H/Hs.Cs.1000=conc in μg/g on fresh weight basis.

where V=total extract vol (ml)

W=fresh weight of the sample (g)

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

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

Cs=concentration of the standard (mg/ml)

Confirmation of the identified components was done by use of the column packing material 4% SE-30/6% SP-2401 on 100/120 supelcoport. This eluted the various organochlorine components with slightly different retention times.

3.2.9 Analytical quality assurance (AQA)

This was done to check the efficiency of the analytical method and the reproducibility of the results. Milk and food samples were fortified with known quantities of the pesticides under investigation. Extraction, clean up and quantitation of the pesticides was done. The percent recoveries were calculated and evaluated according to the UNEP/WHO criteria for evaluation of results (1980).

Preparation and handling of the AQA samples

Milk samples

500 ml of cows milk was homogenized using an ultra sonic disintegrator. 50 g of the sample was weighed into beakers in three parallels. 1 ml or 100 ml of cpm standard (1:100 dilution) was added into the 50 g to give low or high spiking of the sample. A blank containing cows milk only was prepared. Aliquots of 10 g were measured and analysed for the pesticides.

Food samples

The food samples were first homogenized by a waring blender. 3 g of the homogenized sample was transferred into a mortar and fortified with 50 ml of 1: 10 dilution of cpm standard and mixed. Meat, onions, beans, and maize flour were

spiked. Table 3.2 shows the pesticide concentrations attained in the spiked samples.

Analysis

Extraction, clean up, GLC injection and quantitation of the pesticides in the spiked sample were carried out and the pesticide recoveries calculated according to the formula pr/pa x 100 % where pr is the pesticide recovered and pa is the pesticide added.

3.3 Analytical procedure for determination of organochlorine pesticides in milk samples

Extraction and clean up of the milk samples were carried out according to a method described by Brevik (1978), slightly modified.

The sample was extracted with a solvent mixture of hexane and acetone. The extract was cleaned up using acid and alkaline and final identification done by gas liquid chromatography.

3.3.1 Extraction

The milk sample was homogenized using a whirl mixer. 10 grams of the sample were measured for extraction and the extraction carried out by ultrasonic treatment. The first extraction involved addition of 20 ml hexane and 15 ml acetone with an extraction time of 2 minutes using an ultrasonic disintegrator. The mixture was centrifuged for 5 minutes at 3000 rpm and the hexane layer transferred to a preweighed reagent tube with a screw cap. The reagent tube was placed on a sand bath at 50°centigrade and the extract solvent evaporated to dryness under a gentle stream of nitrogen. The extraction process was repeated with 10 ml hexane and 5 ml acetone and the extract added to the reagent tube. The extract solvent was evaporated till dryness and the fat content determined by reweighing the reagent tubes with the fat. The tubes were then stored at -20°C until clean up was done.

3.3.2 Clean-up

The fat was redissolved in hexane to make a concentration of 0.05 gms fat per ml hexane. Sub-samples of this redissolved fat containing extract were prepared for

acid and alkaline clean-up

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. After shaking, the mixture was allowed to stand in darkness for at least 1 hour and then centrifuged at 3000 rpm for 5 mins. The top hexane layer was transferred into vials ready for GLC analysis.

Alkaline clean-up

To the tubes to be used for alkaline clean-up, 2 potassium hydroxide pellets were added and soaked in 0.1 ml of distilled water. 1 ml of 99.5% ethanol was added and the ingredients let to dissolve. 1 ml of the sample was added and the tube placed in 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. After mixing, the sample was cooled for a few minutes and centrifuged for 10 minutes at 3000 rpm. The hexane layer was removed into sample bottles and anhydrous sodium sulphate added to absorb the moisture. The sample was then transferred into vials ready for GLC analysis.

3.4 Analytical procedure for determination of organochlorine pesticide residues in foods.

3.4.1 Preparation of samples

The food samples were homogenized using a waring blender and put in prewashed glass containers.

3.4.2 Extraction

3 g of the homogenized sample was weighed into a mortar. 4.5 g of anhydrous sodium sulphate and 4.5 g of acid washed sand were added. The mixture was ground to a free-flowing powder. 4 g of the powder were weighed into an extraction column containing a small wad of hexane-washed cotton wool. The column was mounted on a stand and the sides tapped gently to obtain a uniform packing. Diethyl ether was added to cover the material, and the column left standing for 15 minutes. The ether was then eluted into a pre-weighed reagent

bottle. Small volumes of ether were added to the column until 10-15 ml was collected in the bottle. The ether extract was completely evaporated in a sand bath at 40°c under a gentle stream of nitrogen. The bottles with the extract were left to stand on the bench for about 20 mins to allow cooling after which they were reweighed. The difference between the weight of the empty bottle and the bottle after reweighing was taken as the weight of the fat. By proportions, the weight of the fat obtained in each tube was from 1 g of the sample. A blank was run through the entire method for every batch of sample analysed..

The fat was redissolved in hexane to give a concentration of 0.05 g fat /ml hexane. A standard volume of 4 ml was added to the extract. 1 ml of the extract was treated with concentrated sulphuric acid and ethanolic potassium hydroxide respectively for the acid and base clean up as described earlier.

3.4.3 Pesticide identification, quantitation and confirmation

Identification of the pesticides was done by use of the GLC. Retention times of components of the sample were measured and compared with those of corresponding components in the standard chromatogram .

Quantitation was done by comparing peak heights of sample components with those of corresponding components in the standard of known concentrations.

Confirmation was done by using a column with different packing material from that used for analysis.

3.5 Results and discussion

3.5.1 Solvents

The GLC has a very sensitive detector,.the electron capturing detector. Thus scrupulous cleaning of glassware, and the redistillation of solvents is necessary to reduce the amounts of the contaminants that may be in the extracts and which might have interfering peaks thus giving erroneous results.

3.5.2 Resolution of the GLC

The chromatogram obtained when 2 µl of 1:1000 cpm was injected into the GLC on the analytical column was similar to that presented by the manufacturers of the

cpm standard. All the thirteen compounds present in the mixture were adequately resolved, and their peaks easily identified. Only 11 out of the 13 peaks were resolved when 2 μl of 1:1000 cpm was injected into the GLC using the confirmatory column. Lindane and β-HCH eluted as one peak as did endrin and o,p'-DDT.

3.5.3 Linearity of the ECD

Linearity of the GLC is confirmed by a straight line graph when different concentrations of the cpm are injected. All the analytical work was done within the linear range of the detector. Fig 3.1 and 3.2 shows the linearity of the ECD.

3.5.4 Clean-up

This is done to remove the fat which would lead to fast contamination of the detector and lower the detection limits. In acid clean-up, aldrin, heptachlor epoxide and endrin are broken down and therefore not recovered. In the base clean-up, DDT is converted into DDE. There is a high recovery of aldrin, heptachlor epoxide, dieldrin and endrin. The pigment removal in the base clean-up was incomplete and negative peaks appeared in the samples that had so much of colour. These, however, did not interfere with measurements of the peak heights.

3.5.5 Pesticide recoveries of the methods

The percent recoveries for all the thirteen pesticides in the cpm standard ranged from 68% for recovery of heptachlor epoxide in spiked onions to 105% for recovery of p,p'-DDT in milk (Table 3.3-3.7). Apart from two compounds (heptachlor epoxide and aldrin) that had poor recoveries in onions and milk respectively all other compounds were adequately resolved in all samples analysed.

3.6 Conclusion

The methods used in this study were found to be suitable for analysis of organochlorines in both milk and food samples. Most of the recoveries were within the acceptable limits and the same procedure could be used for a wide range of compounds.

Table 3.1 Pesticide concentration in the cpm standard

Pesticide	Concentration (μg/μl)
α-HCH	0.025
Lindane	0.025
β-HCH	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 3.2 Concentration of pesticides in the spiked food and milk samples

	mg/kg added		
		Milk samples	
Pesticide	Food samples	High spiking	Low spiking
α-HCH	0.040	0.050	0.005
Lindane	0.040	0.050	0.005
β-HCH	0.170	0.200	0.020
Heptachlor	0.040	0.050	0.005
Heptachlor epoxide	0.130	0.160	0.016
Aldrin	0.080	0.100	0.010
p,p'-DDE	0.170	0.200	0.020
Dieldrin	0.200	0.240	0.044
o,p'-DDT	0.330	0.450	0.045
p,p'-DDD	0.320	0.380	0.038
p,p'-DDT	0.380	0.520	0.052
Endrin	0.330	0.400	0.040
o,p'-DDD	0.430	0.400	0.040

Table 3.3 % recoveries of organochlorine pesticides from spiked beans.

Compound	mg/kg added	% recovery	±Δ	Evaluation
α-HCH	0.04	86	-16	G
Lindane	0.04	80	-20	G
β-HCH	0.17	77	-23	A
Heptachlor	0.04	79	-21	A
Aldrin	0.08	82	-18	G
Heptachlor epoxide	0.13	79	-21	A
p,p'-DDE	0.17	90	-10	E
Dieldrin	0.2	84	-16	G
o,p'-DDD	0.43	92	-8	E
Endrin	0.33	90	-10	E
o,p'-DDT	0.33	92	-8	E
p,p'-DDD	0.32	95	-5	E
p,p'-DDT	0.38	103	+3	E

Recovery results are expressed as means of 3 parallels ±% deviation

Evaluation, UNEP/WHO criteria for evaluation of results (1980)

E=Excellent	±10% of spiked amount
G=Good	±20%
A=Acceptable	±30%
P=Poor	±40%
U=Unacceptable	±50%

Table 3.4 % recoveries of organochlorine pesticides from spiked maize flour.

Compound	mg/kg added	% recovery	±Δ	Evaluation
α-HCH	0.04	97	-3	E
Lindane	0.04	101	+1	E
β-HCH	0.17	90	-10	E
Heptachlor	0.04	82	-18	G
Aldrin	0.08	84	-16	G
Heptachlor epoxide	0.13	82	-18	G
p,p'-DDE	0.17	96	-4	E
Dieldrin	0.20	84	-16	G
o,p'-DDD	0.43	92	-8	G
Endrin	0.33	90	-10	G
o,p'-DDT	0.33	97	-3	E
p,p'-DDD	0.32	97	-3	E
p,p'-DDT	0.38	95	-5	E

Recovery results are expressed as means of 3 parallels ±% deviation

Evaluation , UNEP /WHO criteria for evaluation of results (1980)

E=Excellent +/-10% of spiked amount

G=Good +/-20%

A=Acceptable +/-30%

P=Poor +/-40%

U=Unacceptable +/-50%

Table 3.5 % recoveries of organochlorine pesticides from spiked lean meat.

Compound	mg/kg added	% recovery	± Δ	Evaluation
α-HCH	0.04	92	-8	E
Lindane	0.04	87	-13	G
β-HCH	0.17	90	-10	E
Heptachlor	0.04	82	-18	G
Aldrin	0.08	73	-27	A
Heptachlor epoxide	0.13	79	-21	A
p,p'-DDE	0.17	102	+2	E
Dieldrin	0.20	88	-22	A
o,p'-DDD	0.43	98	-2	E
Endrin	0.33	72	-28	A
o,p'-DDT	0.33	92	-8	E
p,p'-DDD	0.32	95	-5	E
p,p'-DDT	0.38	103	+3	E

Recovery results are expressed as means of 3 parallels ±% deviation

Evaluation, UNEP /WHO criteria for evaluation of results (1980)

E=Excellent	±10% of spiked amount
G=Good	±20%
A=Acceptable	±30%
P=Poor	±40%
U=Unacceptable	±50%

Table 3.6 % recoveries of organochlorine pesticides from spiked onions.

Compound	mg/kg added	% recovery	±Δ	Evaluation
α-HCH	0.04	90	-10	E
Lindane	0.04	92	-8	E
β-HCH	0.17	79	-21	A
Heptachlor	0.04	82	-18	G
Aldrin	0.08	79	-21	A
Heptachlor epoxide	0.13	68	-32	p
p,p'-DDE	0.17	98	-2	E
Dieldrin	0.2	89	-11	G
o,p'-DDD	0.43	90	-10	E
Endrin	0.33	78	-22	A
o,p'-DDT	0.33	87	-13	G
p,p'-DDD	0.32	95	-5	E
p,p'-DDT	0.38	97	-3	E

Recovery results are expressed as means of 3 parallels ±% deviation

Evaluation, UNEP /WHO criteria for evaluation of results (1980)

E=Excellent	±10% of spiked amount
G=Good	±20%
A=Acceptable	±30%
P=Poor	±40%
U=Unacceptable	±50%

Table 3.7 (a) Pesticide recoveries from spiked cow milk (high spiking)

Compound	mg/kg added	% recovery	± Δ	Evaluation
α-HCH	0.05	92	-8	E
Lindane	0.05	87	-13	G
β-HCH	0.20	80	-20	G
Heptachlor	0.05	82	-18	G
Aldrin	0.10	85	-15	G
Heptachlor epoxide	0.16	80	-20	G
p,p'-DDE	0.20	103	+3	E
Dieldrin	0.24	87	-13	G
o,p'-DDD	0.40	98	-2	E
Endrin	0.40	78	-28	A
o,p'-DDT	0.45	102	+2	E
p,p'-DDD	0.38	99	-1	E
p,p'-DDT	0.52	105	+5	E

Results are expressed as means of 5 parallels ±% deviation.

Evaluation, UNEP /WHO criteria for evaluation of results (1980)

E=Excellent	±10% of spiked amount
G=Good	±20%
A=Acceptable	±30%
P=Poor	±40%
U=Unacceptable	±50%

Table 3.7 (b) Pesticide recoveries from spiked cow milk (low spiking)

Compound	mg/kg added	% recovery	± Δ	Evaluation
α-HCH	0.005	79	-21	A
Lindane	0.005	82	-18	G
β-HCH	0.020	77	-23	A
Heptachlor	0.005	75	-25	A
Aldrin	0.010	69	-31	P
Heptachlor epoxide	0.016	75	-25	A
p,p'-DDE	0.020	91	-9	G
Dieldrin	0.044	83	-17	G
o,p'-DDD	0.040	89	-11	G
Endrin	0.040	72	-28	A
o,p'-DDT	0.045	92	-8	E
p,p'-DDD	0.038	97	-3	E
p,p'-DDT	0.052	96	-4	E

Results are expressed as means of 5 parallels ±% deviation.

Evaluation, UNEP /WHO criteria for evaluation of results (1980)

E=Excellent	±10% of spiked amount
G=Good	±20%
A=Acceptable	±30%
P=Poor	±40%
U=Unacceptable	±50%

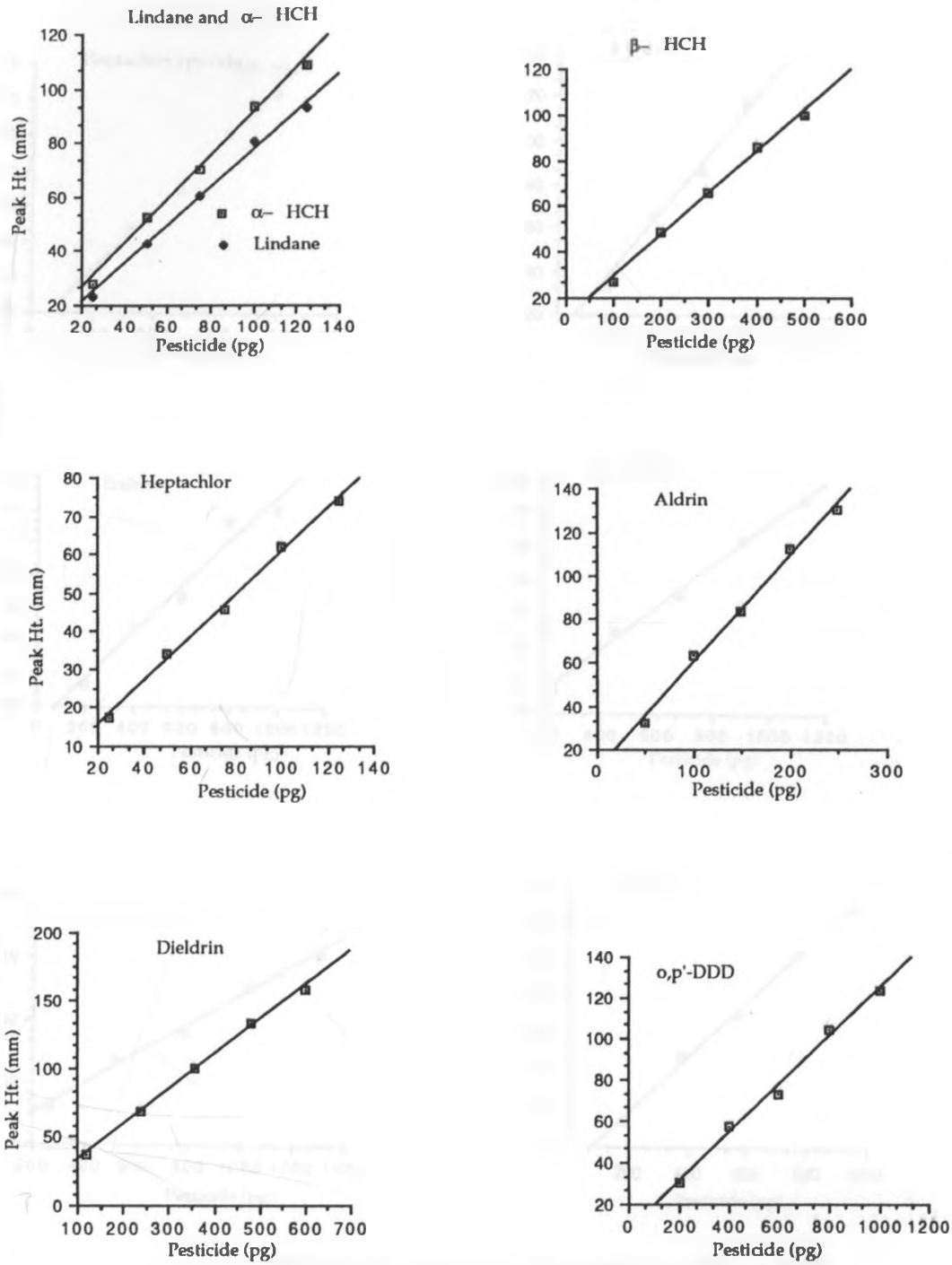

Fig 3.1 Linearity of the ECD for α-HCH, lindane, β-HCH, heptachlor, aldrin, dieldrin and o,p'-DDD

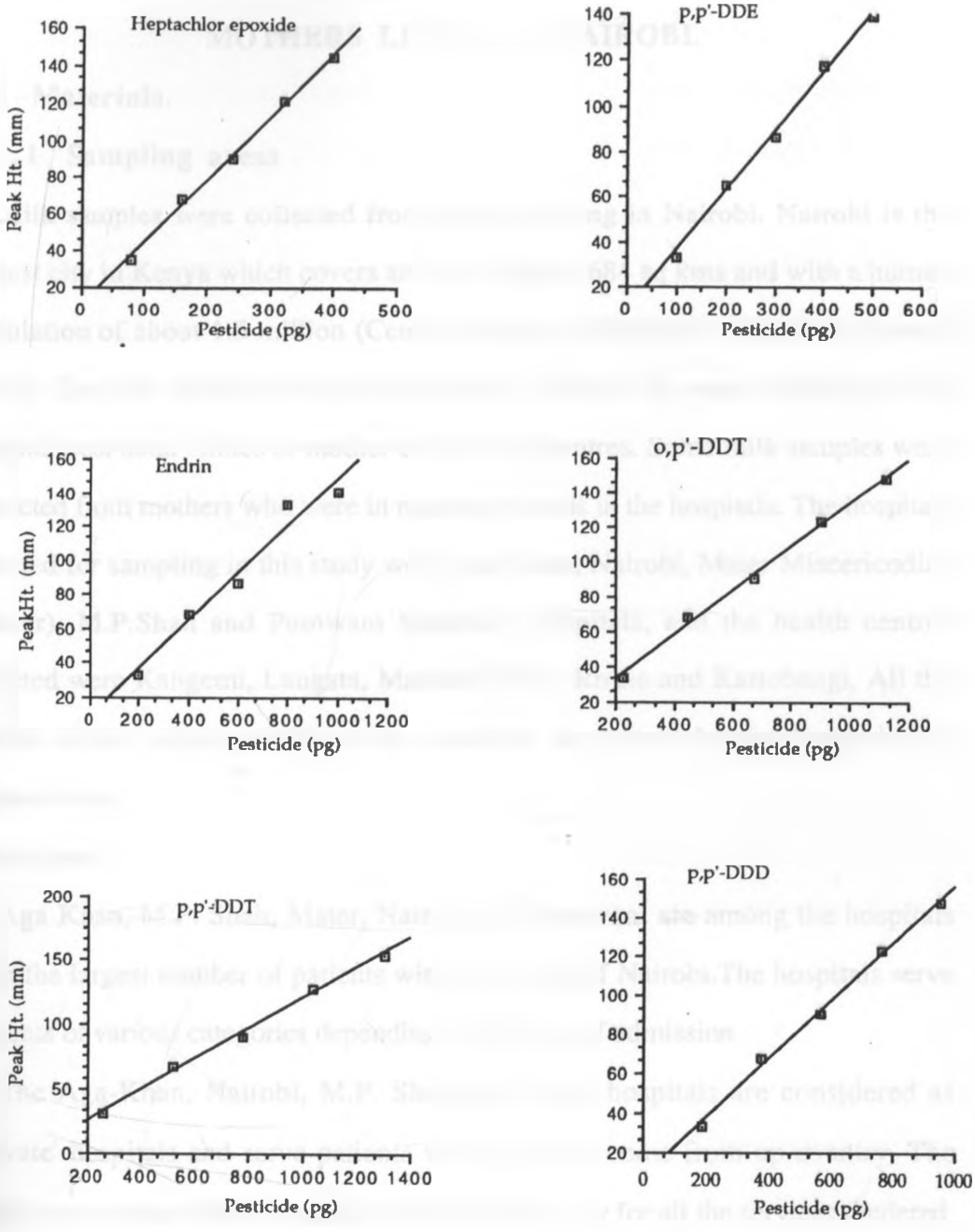

Fig 3.2 Linearity of the ECD for heptachlor epoxide, p,p'-DDE, endrin, o,p'-DDT, p,p'-DDT and p,p'-DDD.

CHAPTER FOUR

ORGANOCHLORINE PESTICIDE RESIDUES IN MILK OF MOTHERS LIVING IN NAIROBI.

4.1 Materials.

4.1.1 Sampling areas

Milk samples were collected from mothers living in Nairobi. Nairobi is the largest city in Kenya which covers an area of about 684 sq kms and with a human population of about 1.5 million (Central Bureau of Statistics, Economic Survey, 1991). The milk samples were collected from mothers who were attending either hospital post natal clinics or mother child health centres. Some milk samples were collected from mothers who were in maternity wards in the hospitals. The hospitals selected for sampling in this study were Aga-Khan, Nairobi, Mater Miscericodiae (Mater), M.P.Shah and Pumwani Maternity hospitals, and the health centres selected were Kangemi, Langata, Mathare North, Riruta and Kariobangi. All the health centers where samples were collected are owned by the Nairobi City Commission.

Hospitals

Aga Khan, M.P. Shah, Mater, Nairobi and Pumwani are among the hospitals with the largest number of patients within the City of Nairobi.The hospitals serve patients of various categories depending on the cost of admission.

The Aga-Khan, Nairobi, M.P. Shah and Mater hospitals are considered as 'private' hospitals and serve patients who may also come from up country. The patients who attend these hospitals are expected to pay for all the services rendered. Pumwani Maternity Hospital on the other hand is a City Commission hospital serving patients from all over Nairobi. Most of the patients who attend this hospital are those who cannot afford to pay private hospitals' charges. The charges are quite low and most of the times the wards are very congested.

Health centres

Most of the health centers in Nairobi are managed by the Nairobi City Commission. These are situated in different areas of Nairobi and mainly serve the mothers living in the surrounding estates. Milk samples were collected from mothers who were attending the postnatal clinic in these mother child health centres.

All the health centres where sampling was done are run by the City Commission and are less expensive than the privately owned ones. Most of the mothers attending these centres mothers are either housewives, self employed or those with low income jobs.

4.1.2 Groups of mothers sampled.

Mothers represented different groups in terms of their social class, income bracket, education and the race of origin. By selection of mothers from various hospitals and different health centres, the mothers living in Nairobi area were well represented.

Social class

The monthly income of the family together with the residential area, occupation of both the mother and the spouse, and hospital where they attended were used to evaluate the social class of the mother.

Race of origin

Samples were collected from Asian and African mothers. The samples from the Asian mothers were collected from Mater, M.P. Shah and Aga- Khan hospitals, while samples from African mothers were collected in all the sampling areas.

4.1.3 Criteria for selection of mothers for collection of milk samples

The following criteria was used in selecting the mothers from which samples were collected.

a) The mother should have been living in Nairobi for the last 2 years.

b) Mother should be between 18 and 30 years of age

c) The mother should be nursing her first or second child

d) Both mother and child should be apparently healthy and latest pregnancy should have been normal.

e) The mother should be breast feeding one child only

f) Mothers milk sample to be collected 1-4 months post partum.

4.1.4 Collection and handling of milk samples.

The samples were collected by manual expression directly into precleaned bottles with teflon caps. About 10 ml was collected from each mother. During collection of samples a questionnaire was completed by each mother, this gave information on the age of the mother, diet, residential area, parity, days post partum, occupation, income and the ethnic group.

The samples were then transported to the laboratory and stored in a freezer at -20 °C until analysis was done.

4.1.5 Analytical procedure for determination of organochlorine compounds in human milk.

This is given in section 3.3.

4.1.6 Statistical methods.

Data was analysed using a Harvard professional computer with 'Statistix' statistical programme. Analysis of variance (ANOVA), multiple regression and correlation relationships were used to determine the effect of diet, milk fat, race, parity and the social class of the mother on the levels of the pesticide residues in the mothers milk.

Since the data was not normally distributed, a logarithmic transformation was carried out on the dependent variables (p,p'-DDE, p,p'-DDT, sum DDT, %fat and the ratio of p,p'-DDT to p,p'-DDE) to make their distribution normal.

For regression, a step down (backward) method of regression was carried out, where y (the dependent variable) regresses on all x's (independent variables) with subsequent elimination of the x's with the highest p-values and the equation for best prediction of y variable obtained.

4.2 Results

Milk samples were collected from 10 different sampling locations in Nairobi These sampling areas were either hospitals or mother child health centres as described above. Within each sampling location, the mothers were of similar socio-economic status. In the health centres, mothers encountered were mainly young and most were house wives or lowly paid workers. This was the same in Pumwani maternity hospital. On the other hand, the mothers encountered in the other hospitals were mostly well to do working class women, Asians or housewives with high income husbands. Samples were collected from mothers who satisfied the conditions of the criteria as specified in section 4.1.3. In some cases all conditions stated were not always met especially when collecting samples from mothers of Asian origin who were few in number and rarely encountered in the sampling areas. It was established that most of the Asian mothers attended their family doctor private clinics. The criteria for days post partum and the age was slightly modified. Table 4.13 is a summary data of mothers from whom samples were collected.

A total of 216 samples were analysed for presence of organochlorine pesticides. 28 of the samples analysed were from Asian mothers and 188 were from the African mothers. The results are presented in tables 4.1-4.3 and appendices 2-5. The mean and ranges are presented both on fat weight and wet weight basis The results are not corrected for recoveries. The percent extractable fat in the milk is also demonstrated.

Out of all the milk samples analysed in this study, 13 organochlorine pesticide residues were detected (table 4.1-4.3), p,p'-DDT and p,p'-DDE (a metabolite of p,p'-DDT) were the most frequently encountered contaminants in all the human milk samples analysed. p,p'-DDE was detected in 215 samples (99.5%) and p,p'-DDT in 169 samples (78.3%). Other residues detected in order of decreasing frequency were:- dieldrin (37.5.%) β-HCH (16.2 %), lindane (16.2%), α-HCH (11.1%), Heptachlor (10.2%), aldrin (5.6%), heptachlor epoxide (4.2%), endrin (2.7%). Other metabolites of DDT like o,p'-DDT and p,p'-DDD were detected in

few of the samples analysed (appendix 5).

The mean level of sum DDT in all the samples analysed was 0.473 mg/kg milk fat and the range was from 0.004 to 6.321 mg/kg milk fat. The sampling area with the highest mean level of sum DDT (0.878 mg/kg milk fat) was Mathare clinic, while samples collected in M.P. Shah hospital had the lowest mean level (0.327 mg/kg milk fat) (table 4.1).

Mean level of p,p'-DDE in all samples analysed was 0.306 mg/kg milk fat with a range of 0.003-4.818 mg/kg milk fat in the individual samples. From the different sampling areas, the mean of p,p'-DDE ranged from 0.162 mg/kg milk fat in samples collected in M.P. Shah hospital to 0.575 mg/kg milk fat in samples collected in Mathare clinic (table 4.1).

The mean level of p,p'-DDT in all the samples analysed was 0.152 mg/kg milk fat and the range 0.002-2.58 mg/kg milk fat in the individual milk samples. Means of p,p'-DDT in the different sampling areas ranged from 0.042 mg/kg milk fat in samples collected in M.P. Shah hospital to 0.376 mg/kg milk fat in samples collected in Langata clinic (table 4.1)

The mean of the ratio of p,p'-DDT to p,p'-DDE in all the samples analysed was 0.58 and the range 0.013-11.99 in the individual milk samples. Means of this ratio in samples collected in different sampling areas ranged from 0.24 in samples collected in Nairobi hospital to 1.865 in samples collected in Langata clinic (table 4.1).

The percent extractable fat in all the milk samples analysed ranged from 0.59% - 8.4% in the individual milk samples with a mean of 3.749%. The highest mean percentage of milk fat (4.445 %) was recorded in samples collected in Mathare and Riruta clinics and the lowest (2.613 %) was recorded in samples collected in Nairobi hospital.

The mean levels and ranges (mg/kg milk fat) of compounds in the HCH group detected in the milk samples analysed were as follows:- α-HCH (0.017, traces-0.067) lindane (0.017, trace-0.134), β-HCH (0.089, trace-0.96) (table 4.2). The

same table gives the means of these compounds in samples collected in the different sampling areas.

Mean levels and ranges (mg/kg milk fat) of compounds in the cyclodiene group detected in the milk samples were as follows:- Heptachlor (0.026, 0.004-0.118), aldrin (0.033, trace - 0.102) heptachlor epoxide (0.022, trace-0.121), Dieldrin (0.022, trace-0.273) and endrin (0.035, trace -0.08) (table 4.3). The same table demonstrates the mean levels and ranges in samples collected in the different sampling areas.

The results are also presented in relation to factors such as race, parity, age and dietary habits of the mothers (tables 4.4-4.11). Some of these factors have been shown to significantly affect levels of organochlorine residues in human milk. In the present study lower levels of organochlorine pesticides were associated with, higher parity, older mothers and lower milk fat content. Dietary habits, race and social class of the mother had no significance influence on the levels of organochlorine pesticide residues in the human milk.

4.3.Discussion

4.3.1 DDT group

Sum-DDT

The mean of sum -DDT (0.473 mg/kg milk fat) observed in this study was lower than the lowest mean level (0.69) observed in an earlier study carried out on mothers living in the rural areas in Kenya (Kanja *et al.*, 1986) (Table 4.11). The criteria for selection of mothers used in this study was the same as that used in the previous study of the rural areas. Kanja, (1988) found out that mothers in the rural areas were exposed to these chemicals during their agricultural practices and also through consumption of contaminated foods. Warnez *et al* (1983) found a similar trend where levels of sum-DDT were higher in rural areas as compared to an urban area of Rwanda.

The differences in the mean levels of sum-DDT between the sampling areas were slight but not statistically significant. The highest mean level (0.878 mg/kg milk fat)

observed in Mathare was significantly higher than the others. This mean was however greatly influenced by two samples that had high levels of sum-DDT (6.3 and 5.5 mg/kg milk fat). The individuals from whom the samples were obtained did not seem to have any known exposure of the compound but may have been indirectly exposed. One of the individuals was a teacher while the other one was a house wife. Both frequently used insecticides for mosquito control as did many other mothers from whom samples were collected. On exclusion of these samples the mean level of sum-DDT in the samples collected in Mathare clinic would be 0.372 mg/kg milk fat which is close to the mean levels in other sampling areas.

The mean level in this study (0.473 mg/kg milk fat) was five times lower than that recorded in a similar study carried out in the urban areas of Rwanda (Warnez *et al.*, 1983). On comparison of the mean levels in this study with levels in other developing countries, it is noted that, this mean is 1/14 times the mean level observed in India (Slorach and Vaz, 1983), 1/12 times the mean level observed in Turkey (Karakaya *et al*, 1987), and 1/8 times the mean level observed in Nigeria. (Atuma *et al*, 1987).

The mean level of sum-DDT in this study was also lower than mean levels recorded in earlier studies carried out in some developed countries like Sweden-1.00 mg/kg milk fat, United States of America-1.88 mg/kg milk fat and Japan-1.88 mg/kg milk fat. (Slorach and Vaz, 1983). Although levels in this study are lower, it is not possible to make a direct comparison since there was no available literature on more recent studies carried out in the developed countries. The levels of DDT in mothers milk from these countries would be expected to be lower since use of DDT was banned.

The low levels of the concentration of DDT in mother's milk observed in this study as compared to the levels in the rural areas could be attributed to the restriction of DDT use and the collection of samples from an urban area where there is no wide application of DDT as an insecticide or in agricultural practices, however, the presence of DDT in this study reflects a continued use of this

chemical and thus its presence in the environment.

The higher mean levels in the rural areas possibly reflects a higher degree of exposure of rural mothers to these compounds as compared to the urban mothers. In addition the rural areas are intensive areas of agriculture where there has been wide application of DDT in pest control. In 1986, use of DDT was restricted for mosquito control in public health programmes and this may also have contributed to the presence of the low levels observed in this study.

On regression analysis levels of sum-DDT and p,p'-DDE in human milk in this study were found to be influenced by parity, percentage milk fat and maternal age. These factors are discussed further on.

p,p'-DDE

p,p'-DDE was the major contaminant of the human milk in this study. This compound is a major metabolite of p,p'-DDT (Hayes, 1982). With restriction or total ban of the use of DDT, levels of p,p'-DDT in foods of plant origin fall rapidly but exposure of humans to p,p'-DDE still occurs through consumption of foods of animal origin (Kanja, 1988). Contamination of human milk by DDT reflects relatively recent exposure of the mother to this compound, and contamination by p,p'-DDE reflects either earlier exposure of the mother to DDT which has then been metabolised to DDE or exposure of the mother to DDE through consumption of foods of animal origin. In this study, though p,p'-DDE and p,p'-DDT were present in most of the samples , the mean levels were low and comparable to the levels in developed countries for example Norway (1983), where the mean level observed was 0.82 mg/kg milk fat (Skaare,1986) and in Sweden (1981), where the mean level observed was 0.81 mg/kg milk fat (Slorach and Vaz, 1983). The mean level of p,p'-DDE (0.31 mg/kg milk fat) was lower than the lowest mean level (0.43 mg/kg milk fat) observed in an earlier study carried out in Kenya (table 4.8). A similar trend was observed in earlier studies carried out in Rwanda (Warnez *et al.*,1983), where mean levels of p,p'-DDE was found to be higher in the rural samples as compared to the urban samples. Levels of p,p'-DDT followed a similar

trend, with the mean level (0.15 mg/kg fat) in this study being lower in the urban area than in the rural areas (0.47 mg/kg fat) (Kanja, 1988).

Ratio of p,p'-DDT to p,p'-DDE

The ratio of p,p'-DDT to p.p'-DDE is higher in countries where DDT is still being used. With a ban of its use, this ratio decreases. In countries like Norway where use of DDT was banned in 1970 (Skaare, 1981), the ratio of p,p'-DDT to p,p'-DDE decreased over the years from 0.32 in 1970 to 0.15 in 1979 (Skaare, 1981) Evaluation of this ratio is valuable in detecting the source of the contamination and in assessing recent or previous exposure and direct or indirect exposure through the food chain.

In the present study the mean ratio was 0.584. This was much lower than the ratios obtained in other regions of Kenya in an earlier study carried out when DDT was still being widely used (Kanja *et al*, 1986) (Table 4.11). The lower ratio in the mothers of the urban area reflects less exposure of the urban mothers to the parent compound DDT as compared to the metabolite, DDE.

4.3.2 HCH group

The commercial insecticide HCH is a mixture of different isomers mainly α, β, and γ, HCH. γ-HCH (lindane) is the most important isomer that is used as an insecticide and is the most toxic. β-HCH is the most symmetric and stable isomer, it is also the most persistent in nature. β-HCH is eliminated five times more slowly from the body than the other isomers and has a much higher ability to accumulate in the fat tissues than lindane. Thus, β-HCH is usually found in highest concentrations in human adipose tissues and milk (Heeschen, 1980). The α and γ isomers may isomerise into the β-isomer in living organisms. In the present study, levels of the residues of the β-HCH isomer were higher than levels of the other two isomers.

The mean levels of β-HCH and lindane in the present study were lower than the corresponding mean levels observed in earlier studies on human milk in Kenya (Kanja *et al.*, 1986).

On comparison with the MRL set by WHO (0.5 for other HCH isomers and 0.2 for lindane in mg/kg milk fat), mean levels of these compounds were found to be lower than the MRL. Use of other HCH isomers as insecticides was banned in Kenya, but use of lindane is restricted for seed dressing. The low levels of these compounds are possibly because of the ban and restriction of their use as noted above.

Kanja, (1988), found low levels of α, β and γ HCH in few of the samples analysed. This was attributed to direct contact of the mothers with the technical insecticide, which was easily available from the Agro chemical shops (Kanja *et al*, 1988). Presently, although use of lindane is restricted, it has been noted that, products with lindane as the active component are available for pest control on vegetables and this may be a major contributor of the compound in the human body.

4.3.3 Cyclodiene group

This group includes aldrin, dieldrin, endrin, heptachlor and heptachlor epoxide. Dieldrin is an oxygenated metabolite of aldrin, and it is more persistent. Many of the industrialised countries have banned the use of these compounds as insecticides. In Kenya, dieldrin and aldrin are still being used in the building industry for termite control (Pesticide Products Control Board, 1991). Presence of dieldrin in milk samples reflects exposure of the mother to aldrin or dieldrin, since aldrin is quickly metabolised to dieldrin in the human body (Hayes, 1982).

In the present study, apart from compounds in the DDT group, dieldrin was the other major contaminant of the human milk samples analysed. The mean level of dieldrin (0.022 mg/kg milk fat) in the present study was comparable to the mean level of the same compound in samples from Embu but much lower than the common mean for the rural areas of Kenya (table 4.12). Aldrin was detected in very few samples in the present study.

Mean levels (mg/kg milk fat) of dieldrin (0.022) and aldrin (0.033) were lower than the ERL (0.15 mg/kg fat) set by the WHO/FAO (1982). Endrin was detected

in very few (2.7 %) of the samples analysed. Possibly this compound is not commonly used as an insecticide by many farmers. Heptachlor epoxide is a metabolite of heptachlor and it is more persistent. In the present study heptachlor epoxide was found with less frequency (4.2%) than the parent compound heptachlor (10.2%). The presence of heptachlor with a higher frequency than heptachlor epoxide, implies recent exposure of the mothers with the residues to the parent compound. The source of this compound is possibly contact with fruits like pineapples, since it is known that although there is a ban of its use, heptachlor, is still used in pest control in pineapple growing.

4.3.4 Variations of the organochlorine pesticides in the individual human milk samples.

The influence of factors such as fat content of the milk, race, diet, parity, maternal age, and social class were reflected in the present study by the demonstration of individual variation of the organochlorine pesticides in the mothers milk. Only residues of DDT were used in this investigation since they are the only ones that were detected in a large number of samples.

Fat content of the milk

Organochlorine levels are closely correlated to the fat content of the milk. This varies greatly during each feeding, being higher in hind milk than fore milk (Slorach and Vaz, 1983). Fat content is also affected by the maternal diet, women who were well fed have higher fat content than those with malnutrition (Kanja, 1988). These variations in the fat content have a particular importance in relation to the maternal milk contamination with pesticide residues. The mothers who have a high fat content seem to have higher concentration of DDT (Polishuk, et al., 1977; Miller et al, 1979; Kanja, 1988). In the present study, mothers with a higher fat content in the milk had higher levels of organochlorine pesticides as compared to those with low milk fat content.

Race

Samples were collected from 198 African and 28 Asian mothers.

All samples from mothers of Asian origin were positive for p,p'-DDE with a mean level of 0.38 mg/kg milk fat, p,p'-DDT was detected in 21 out of 28 samples. The mean level of p,p'-DDT in the milk was 0.07 mg/kg milk fat. Mean level of sum DDT in Asian mothers was 0.48 mg/kg milk fat and the mean ratio of p,p'-DDT to p,p'-DDE was 0.37. The mean percentage extractable fat in the milk was 3.97% (table 4.4).

In mothers of African origin similar observations were made. p,p'-DDE was the major contaminant with a mean residue level of 0.29 mg/kg milk fat. The mean levels (mg/kg milk fat) of p,p'-DDT, sum DDT, ratio of p,p'-DDT to p,p'-DDE and the % fat in the milk were 0.16, 0.47, 0.623 and 3.72%, respectively (table 4.4)

Analysis of variance revealed no significant differences in the means of sum DDT, p,p'-DDE, p,p'-DDT and the percentage fat content in the mothers in relation to the race of origin. This could possibly be because of the very few number of Asian mothers sampled as compared to the African mothers. However, there was a significant difference between the two races in the ratio of p,p'-DDT to p,p'-DDE ($p<0.05$) with the mean ratio being higher in African mothers than in Asian mothers. It was not possible to compare statistically the levels of other compounds detected in this study in terms of race because of the very few samples that were positive, however mean levels of the other compounds has been demonstrated (table 4.5).

In literature, only three earlier studies report racial effects on levels of organochlorine pesticides in human milk. Rogan (1986), found a substantial racial difference in DDE levels. Almost half of the blacks had over 6 ppm (fat basis), while only 5% of the whites reached this level. There are other studies where levels of DDT in the adipose tissue of black women was found to be higher than the levels in whites (Kutz *et al.*,1977). Woodard *et al.* (1976), attributes the differences in the

concentration of DDT in human milk from whites and blacks to socio-economic factors. These may include dietary habits, residential area, culture etc.

Dietary habits

Out of the 216 samples analysed 198 samples were from non vegetarian mothers who commonly eat meat, fish, and other foods of animal origin and only 18 samples were from vegetarian mothers. Most of the mothers on vegetarian diet were Asians (15 out of 18) and only very few were Africans (3 out of 18).

The mean level (mg/kg milk fat) of sum-DDT was 0.53 in vegetarian mothers and 0.47 in non-vegetarian mothers(table 4.6). From the mothers of Asian origin the mean level (mg/kg milk fat) of sum-DDT was 0.59 in the vegetarians and 0.35 in the non-vegetarian mothers. The ratio of p,p'-DDT to p,p'-DDE in the vegetarians was 0.32 and 0.44 in non vegetarians (table 4.7).

There was a significant difference in the mean level of the ratio of p,p'-DDT to p,p'-DDE ($p<0.005$) between the two groups. The ratio was higher in non vegetarian mothers as compared to vegetarian mothers. The ratio of DDT to DDE would be higher in vegetarian mothers when the main source of the compounds is primary eg from food of plant origin or when the mothers have higher levels of DDE (as would occur in cases of greater exposure to foods of animal origin or past exposure to DDT) as in this study. The higher ratio in vegetarian mothers as compared to non vegetarian mothers in the present study could be attributed to the higher levels of DDE in the milk samples analysed.

Diet has been reported to be a major source of exposure to organochlorine pesticides for the general population (Noren, 1986; Bradt, 1976). In Kenya Kanja (1988), found that food of animal origin contained higher levels of the DDT group of compounds as compared to food of plant origin and it was the major source of organochlorines in the human body.

Furthermore there is evidence that in some countries, the levels of the organochlorine pesticides in breast milk fat are related to the dietary habits of the mothers. Fish and other foods of animal origin are the main source of

organochlorine pesticides in countries where use of persistent organochlorine pesticides is severely restricted (Jensen, 1983). However, in many developing countries there is continued use of these compounds for pest control on edible plants and on grains in storage and therefore even foods of plant origin may be a source of exposure to the human population.

In the present study, the food of animal origin analysed was found to contain more organochlorines than found in the vegetables and other food crops. The levels in both food groups were in general very low (see chapter 5).

On studying the influence of the diet on the levels of organochlorines, there was no significant difference between the levels of the pesticides in the human milk from the vegetarian and non-vegetarian mothers. This could be explained by the fact that although all the individuals in this study fall into two major groups (vegetarians and nonvegetarians) a clear distinction between the two cannot be made since a large number of the mothers recorded as vegetarian also consume foods of animal origin like milk and eggs.

The statistical analysis was made only in the case of DDT, DDE, sum DDT and the ratio of DDT to DDE, since other organochlorines were found in very low frequencies and in some cases there were no values for some groups..

The influence of social class on individual levels of pesticides in human milk was also investigated in this study but found to have no significant effect. Social class largely determines the type of food that the family commonly eats, and since food of animal origin is known to contain higher amounts of organochlorine pesticides then it can be assumed that those who can commonly afford meats, eggs and other foods of animal origin (these are generally more expensive than foods of plant origin) would accumulate more pesticides than those who cannot afford such foods and hence eat mainly vegetables by virtue of being not able to afford any other kind of food. In this study, this may not be a true picture since the foods from Nairobi markets had very low levels of pesticides and there was very low correlation between income and the diet.

Parity

Studies on the individual mothers have shown that levels of p,p'-DDT and p,p'-DDE in breast milk fat are higher when the mother is nursing her first child than during subsequent nursing periods (Jensen, 1983). This observation only holds when there is no continued exposure to the compounds, with continous exposure, this observation may be reversed because of additional accumulation of the pesticides. Organochlorine pesticide levels have also been found to decline with the time spent breast feeding (Rogan *et al.*, 1986). This relationship was not investigated in this study.

Rogan (1986), found higher levels of organochlorine pesticide residues in mothers nursing their first child as compared to mothers with subsequent deliveries. Many other investigators have come up with similar observations (Noren, 1983; Yakushiji, 1978; Bradt *et al.*,1976; Bouwman 1990). Andersen (1982), found that contents of β-BHC and HCB in individual samples decreased with increasing number of parturations, though he found no relationship between levels of sum-DDT and dieldrin. In other studies, (Welsenberg, 1980) there doesn't appear to be any correlation between the number of children in the family and the amount of pesticides present in mothers milk. Mussalo-Rauhamaa *et al.* (1988), found an increase in levels of pesticides with increasing parturations.

In the present study, 134 were from mothers nursing their first child and 81 were from mothers nursing their second child. There was a significant difference ($p<0.05$) in the mean levels of sum DDT and p,p'-DDE in the human milk in relation to the parity of the mother. Mothers with one child had higher levels of pesticides than those with the second child. Although there was no significant difference in the levels of p,p-DDT ($p>0.05$) between the first and second child a similar trend was observed where mothers nursing their first child had higher levels than those nursing their second child. Differences in the ratio of p,p'-DDT to p,p'-DDE were not significant ($p>0.005$) (table 4.8 and 4.9).

Human milk is a major excretory route for organochlorine compounds. With

subsequent deliveries, the pesticide levels decrease due to the previous losses. This may not be so in all cases and especially with continued exposure to the pesticides. Kanja (1988), found no significant difference ($p>0.05$) between the mean levels of sum-DDT of mothers breast feeding their first or second child. The inverse relationship observed in the present study is consistent with the findings of other investigators as described above. A possible explanation of this is that since the mothers sampled were from an urban area where there is less or no use of pesticides, then there is continued loss of pesticides through breast milk during previous breast feeding periods. In addition the restriction of the use of DDT could bring similar effects.

Maternal age

The increase of the chemical concentration of organochlorine compounds in the human milk with age may be anticipated since these chemicals are lipophilic in nature and slowly accumulate in the fatty tissues over a lifetime. However, this effect may however be counter balanced by other factors like breast feeding patterns, slimming and changes in the use of these pesticides.

In the present study, there were weak negative correlations between age and levels of DDE, DDT and sum-DDT. However there was no significant difference in the levels of these compounds in the different age groups (table 4.10 and 4.11). This may be due to the decreased use of pesticides like DDT and the loss of the compounds through breast feeding. On regression analysis,maternal age had a significant influence on the levels of the organochlorines in human milk.

No unanimity from the the literature on the aspect of influence of maternal age could be found. Siddiqui (1981), Noren (1983), Rogan (1986), Mussalo-Rauhama *et al.* (1988), found positive relationships between sum DDT levels in breast milk and maternal age. Polishuk *et al.* (1977), reported a negative relationship and Welsenberg (1980), could find no correlation between age of mothers and the amount of organochlorines present in milk and weak correlations in some cases (Jensen 1983). It seems therefore that any of the above relationships may be found.

4.3.6 Toxicological evaluation.

No specific recommendations aimed at infants have been given by international bodies, but by applying the acceptable daily intake (ADI) established for adults the tolerance limits for infants can be calculated. The ADIs (μg/kg body weight) for adults set by the WHO/FAO experts for some of the chlorinated pesticides are as follows:- sum DDT-5, lindane-2.5, aldrin-0.1 and dieldrin-0.1.

Assuming the following parameters as standard for infants (WHO, 1983), the daily intake of these compounds via breast milk may be calculated.

Mean weight of the infant =5 kg

Mean daily intake of milk =130 g/kg body weight per day

Mean fat content of milk = 3.5% (WHO 1983)

The daily intake (μg/kg body weight) is calculated by multiplying the levels of organochlorine (mg/kg milk fat) in the mothers milk with a factor of 4.55 (130x3.5/100). Thus the daily intake by infants in the present study would range from 0.02 to 28 for sum-DDT. Infants whose mothers have the highest levels of lindane, aldrin and dieldrin would have a pesticide daily intake of 0.61, 0.4 and 1.2 respectively. These results indicate that some of the infants exceed the acceptable daily intake. Although this may be so, it is uncertain the effects these high levels may have on the infants since the acceptable daily intake is calculated for a life time and the infant gets exposed to pesticides from mothers only for a period.

Since there is no firm evidence so far that even these high concentrations have deleterious effects on the health of the infant, breast feeding should not be discouraged because of it's well recognised advantage.

4.4 Conclusion

The results of this study, show that breast milk from mothers living in Nairobi is contaminated with organochlorine pesticides. The major contaminant was p,p'-DDE followed by p,p'-DDT. The mean level of sum-DDT was lower than mean levels observed in earlier studies carried out in the rural areas of Kenya

The levels of pesticides in the human milk were influenced by the parity of the

mother. Levels were higher in mothers nursing their first child as compared to those nursing their second child. Other factors found affecting the levels of organochlorine pesticides in human milk were maternal age and fat content of the milk. Younger mothers and mothers with higher milk fat content had higher levels of organochlorine pesticides as compared to older mothers and those with lower milk fat content. Dietary habits, social class and the race of the mothers had no significant effect on the pesticide levels in the milk.

For some infants,the daily intake of sum-DDT, aldrin and dieldrin exceeded the acceptable daily intake set by WHO.

The presence of organochlorine pesticides in human milk shows continued exposure of these compounds from the environment through contact with contaminated material or through the diet. Steps should be taken to educate people on safe use of pesticides in order to reduce contamination. In addition, there should be a follow up on the compounds that are banned or restricted to ensure that they are not availed for other uses.

Table 4.1 Levels (mg/kg) of compounds of the DDT group, [illegible] and the percent fat in mothers milk samples collected in Nairobi.

	Pesticide residue							
	Sum-DDT		p,p'-DDE		p,p'DDT		DDT/DDE	% fat
Sampling area	Mean±S.E.M [a] Range positive	Mean±S.E.M [b] Range	Mean±S.E.M[a] Range positive	Mean±S.E.M [b] Range	Mean±S.E.M[a] Range positive	Mean±S.E.M [b] Range	Mean± S.E.M Range Number	Mean±S.E.M Range Number
Aga Khan Hospital n=64	0.411±0.079 0.013-3.471 64	0.142±0.046 0.006-2.811	0.302±0.068 0.012-2.99 64	0.106±0.04 0.003-2.422	0.081±0.013 0.006-0.542 55	0.027±0.006 0.002-0.249	0.416±0.054 0.013-2.29 55	3.705±0.239 0.59-8.4 64
Mater Hospital n=24	0.354±0.151 0.012-3.557 23	0.060±0.011 0.002-0.254	0.180±0.043 0.011-0.88 23	0.042±0.008 0.001-0.191	0.173±0.127 0.009-2.580 20	0.023±0.008 0.003-0.172	0.472±0.14 0.102-2.932 20	3.302±0.306 1.00-7.37 24
Nairobi Hospital n=15	0.581±0.294 0.018-4.605 15	0.041±0.008 0.004-0.116	0.463±0.223 0.016-3.5 15	0.029±0.005 0.003-0.07	0.143±0.097 0.023-0.72 7	0.019±0.01 0.003-0.078	0.241±0.081 0.09-0.718 7	2.613±0.314 0.7-4.48 15
Pumwani Hospital n=25	0.398±0.125 0.018-2.826 25	0.130±0.046 0.006-1.130	0.272±0.079 0.01-1.67 25	0.087±0.028 0.004-0.668	0.132±0.051 0.01-0.972 19	0.043±0.02 0.003-0.389	0.653±0.147 0.11-2.629 19	3.92±0.322 1.6-6.9 25
M.P. Shah Hospital n=14	0.327±0.108 0.034-1.743 14	0.061±0.012 0.006-0.137	0.162±0.035 0.014-0.50 14	0.081±0.033 0.011-0.5	0.042±0.11 0.002-0.09 8	0.017±0.006 0.001-0.048	0.325±0.094 0.017-0.888 8	4.104±0.532 1.4-8.18 14
Langata Clinic n=11	0.664±0.171 0.095-1.607 11	0.236±0.096 0.031-1.109	0.241±0.052 0.047-0.541 11	0.088±0.031 0.015-0.374	0.376±0.136 0.012-1.319 11	0.134±0.019 0.006-0.67	1.865±1.031 0.085-11.991 11	3.263±0.537 1.3-6.18 11
Kariobangi clinic n=13	0.488±0.227 0.033-3.061 13	0.111±0.035 0.006-0.490	0353±0.18 0.03-2.465 13	0.085±0.029 0.006-0.394	0.150±0.067 0.017-0.535 8	0.027±0.065 0.002-0.059	0.414±0.164 0.035-1.441 8	3.415±0.51 1.00-5.80 13
Kangemi clinic n=15	0.362±0.157 0.019-2.404 15	0.148±0.051 0.007-0.721	0.223±0.127 0.014-1.984 15	0.085±0.038 0.003-0.595	0.123±0.045 0.016-0.672 14	0.056±0.008 0.005-0.309	1.055±0.22 0.102-2.824 14	4.331±0.459 2.3-7.7 15
Mathare clinic n=22	0.878±0.342 0.015-6.321 22	0.209±0.089 0.008-2.086	0.575±0.25 0.014-4.818 22	0.160±0.074 0.005-1.59	0.307±0.153 0.014-2.554 17	0.084±0.033 0.003-0.394	0.555±0.092 0.09-1.367 17	4.352±0.388 1.2-8.03 22
Riruta clinic n=13	0.422±0.342 0.004-2.494 13	0.156±0.075 0.003-1.023	0.228±0.081 0.003-1.058 13	0.089±0.091 0.001-0.434	0.174±0.144 0.006-1.32 10	0.071±0.052 0.004-0.541	0.381±0.114 0.097-1.248 10	4.445±0.683 1.21-8.20 13
All areas n=216	0.473±0.059 0.004-6.321 215 (99.5%)	0.131±0.02 0.002-2.811	0.306±0.041 0.003-4.818 215 (99.5%)	0.091±0.015 0.001-2.422	0.152±0.026 0.002-2.58 169 (78.2%)	0.045±0.007 0.001-0.67	0.585±0.077 0.013-11.991 169	3.749±0.126 0.59-8.40 216

a- levels of pesticides on milk fat basis
b- levels of pesticides on fresh weight basis (milk)
Positive= number of samples with the residue
Means are calculated from the positive quantifiable levels only.
n=Number of samples from each area
S.E.M=standard error of the mean

Table 4.2 Levels (mg/kg) of compounds of the HCH group in mothers milk samples collected in Nairobi.

	Pesticide residue					
	α-HCH		Lindane		β-HCH	
Sampling area	Mean±S.E.M[a] Range positive	Mean±S.E.M[b] Range	Mean±S.E.M[a] Range positive	Mean±S.E.M[b] Range positive	Mean±S.E.M[a] Range positive	Mean±S.E.M[b] Range positive
Aga Khan Hospital n=64	0.008±0.003 T-0.018 6	0.004±0.001 T-0.007	0.012±0.003 T-0.02 9	0.005±0.001 T-0.011	0.172±0.073 T-0.96 16	0.042±0.020 T-0.282
Mater Hospital n=24	0.032 - 1	0.013 -	0.012±0.005 T-0.022 5	0.004±0.002 T-0.009 5	0.011±0.003 0.004-0.022 6	0.004±0.001 0.001-0.005
Nairobi Hospital n=15	0.067 - 1	0.001 -	-	-	0.027±0.008 0.01-0.049 4	0.005±0.001 0.003-0.009
Pumwani Hospital n=25	0.011±0.003 0.008-0.014 2	0.005±0.001 0.003-0.006	0.013 - 1	0.001 - 1	0.056±0.052 0.003-0.16 3	0.034±0.033 0.001-0.099
M.P. Shah Hospital n=14	0.01 - 1	0.003 -	-	-	0.028±0.021 0.006-0.07 3	0.01±0.006 0.003-0.022
Langata clinic n=11	0.002±0.001 0.002-0.003 2	0.001 0.001-0.001	0.007±0.002 0.003-0.011 3	0.002±0.0003 0.001-0.002	0.012 - 1	0.004 -
Kariobangi clinic n=13	0.006 - 1	0.003 -	-	-	-	-
Kangemi clinic n=15	0.007±0.002 0.004-0.012 5	0.002±0.001 0.001-0.004	0.024±0.018 T-0.134 8	0.011±0.009 T-0.062	-	-
Mathare clinic n=22	0.02 (0.005) 0.008-0.038 5	0.01±0.006 0.004-0.019	0.025±0.011 0.022-0.082 7	0.01±0.004 0.001-0.031	0.012 - 1	0.004 -
Riruta clinic n=13	-	-	0.004 - 2	0.002±0.001 0.001-0.003	0.009 - 1	0.001 -
All areas n=216	0.017±0.003 T-0.067 24 (11.1%)	0.005±0.001 T-0.019	0.017±0.005 T-0.134 35 (16.2%)	0.007±0.002 T-0.062	0.089±0.034 T-0.96 35 (16.2%)	0.024±0.010 T-0.282

a- levels of pesticides on milk fat basis
b- levels of pesticides on fresh weight basis (milk)
Means are calculated from the positive quantifiable levels only.
S.E.M=standard error of the mean

T- indicates positive peaks that were not quantifiable
- Below detection limits
n=number of samples from each area

Table 4.3 Levels (mg/kg) of compounds of the cyclodiene group in mothers milk samples collected in Nairobi.

	Pesticide residue									
	Heptachlor		Aldrin		Heptachlor.epoxide		Dieldrin		Endrin	
	Mean±S.E.M[a] Range positive	Mean±S.E.M[b] Range positive	Mean±S.E.M[a] Range positive	Mean±S.E.M[b] Range positive	Mean±S.E.M[a] Range positive	Mean±S.E.M[b] Range positive	Mean±S.E.M[a] Range positive	Mean±S.E.M[b] Range positive	Mean±S.E.M[a] Range positive	Mean±S.E.M[b] Range positive
Aga Khan n=64	0.028±0.013 0.006-0.116 8	0.011±0.005 0.002-0.045	0.035±0.014 0.01-0.074 4	0.01±0.002 0.004-0.016	0.006±0.0003 T-0.006 5	0.001 T-0.001	0.028±0.011 T-0.273 30	0.008±0.002 T-0.05	0.022 - 1	0.005 -
Mater n=24	0.028 1	0.011	0.034 T-0.034 2	0.02 T-0.02	0.007 - 1	0.002 -	0.008 T-0.008 4	0.006 T-0.006	0.08 T-0.008 2	0.026 T-0.026
Nairobi n=15	-	-	-	-			0.018±0.008 T-0.049 6	0.003±0.0005 T-0.004	-	-
Pumwani n=25	0.014 - 1	0.006 -	-	-	-	-	0.02±0.007 T-0.036 9	0.007±0.002 T-0.014	-	-
M.P. Shah n=14	0.006 1	0.002	0.068±0.034 0.034-0.102 2	0.016±0.006 0.01-0.022	0.121±0.06 T-0.121 2	0.004±0.001 T-0.004	0.012±0.002 T-0.018 8	0.004±0.0005 T-0.005	0.031 - 1	0.007 -
Langata n=11	-	-	-	-			0.029±0.018 0.006-0.082 4	0.004±0.002 0.001-0.009	-	-
Kariobangi n=13	0.01±0.01 0 2	0.004±0.001 0.003-0.004	-	-			0.047±0.032 0.01-0.144 4	0.03±0.017 0.001-0.075	-	-
Kangemi n=15	0.045±0.036 0.004-0.118 3	0.02±0.017 0.009-0.017	-	-	0.004 - 1	0.001 -	0.008±0.001 0.006-0.01 7	0.004±0.001 0.001-0.006	0.034 - 1	0.011
Mathare n=22	0.032±0.002 0.026-0.034 4	0.014±0.002 0.009-0.017	0.013±0.006 0.002-0.026 4	0.006±0.003 0.001-0.013			0.01±0.002 0.006-0.018 5	0.005±0.001 0.002-0.007	0.01 - 1	0.003
Riruta n=13	0.007±0.003 0.004-0.01 2	0.002±0.001 0.001-0.004	-	-			0.006±0.002 T-0.008 4	0.002 T-0.008	-	-
All areas n=216	0.026±0.007 0.004-0.118 22 (10.2%)	0.011±0.003 0.001-0.054	0.033±0.009 T-0.102 12 (5.6%)	0.01±0.002 T-0.022	0.022±0.017 T-0.121 9 (4.2%)	0.002±0.0004 T-0.004	0.022±0.005 T-0.273 81 (37.5%)	0.007±0.002 T-0.075	0.035±0.012 T-0.08 6 (2.7%)	0.01±0.004 T-0.026

a- levels of pesticides on milk fat basis
b- levels of pesticides on fresh weight basis (milk)
T- indicates positive peaks that were below detection limits.
- levels were not detectable
Means are calculated from the positive quantifiable levels only.
n=number of samples from each area
Positive=number of samples with residues.
S.E.M=standard error of the mean

Table 4.4 Levels of sum DDT, p,p'-DDE, p,p'-DDT, (mg/kg in milk fat) ratio of p,p'-DDT to p,p'-DDE and the % fat in relation to the race of the mothers.

	Pesticide residue				
	Sum-DDT	p,p'-DDE	p,p'-DDT	DDT/DDE	% fat
Race	Mean ±S.E.M positive	Mean ± S.E.M positive	Mean ± S.E.M positive	Mean ± S.E.M Number	Mean ± S.E.M Number
Asians n=28	0.479±0.109 28	0.380 ± 0.114 28	0.07 ± 0.03 21	0.374 ± 0.089 21	3.974 ± 0.416 28
Africans n=188	0.469 ± 0.063 187	0.294 ± 0.044 187	0.164 ± 0.03 148	0.615 ± 0.088 148	3.716± 0.131 188

Table 4.5 Levels (mg/kg milk fat) of other organochlorine pesticides in mothers milk fat in relation to the race of the mothers.

	Pesticide residue							
	α-HCH	Lindane	β-HCH	Heptachlor	H. epoxide	Aldrin	Dieldrin	Endrin
Race	Mean ±S.E.M positive	Mean ±S.E.M positive	Mean ±S.E.M positive	Mean ±S.E.M positive	Mean ±S.E.M positive	Mean ±S.E.M positive	Mean ±S.E.M positive	Mean ±S.E.M positive
Asians n=28	0.002 1	0.002 1	0.098±0.061 6	0.049±0.034 3	0.005 2	0.03 1	0.015±0.003 4	-
africans n=188	0.014±0.003 23	0.007±0.002	0.086±0.042 26	0.021±0.006 19	0.025±0.019 7	0.033±0.01 11	0.022±0.005 77	0.035±0.012 6

S.E.M = Standard error of the mean
n = Number of individuals
Positive = number of the individuals with the residues.

Table 4.6 Levels of p,p'-DDE, p,p'-DDT, sum DDT (mg/kg in milk fat) and the ratio of p,p'-DDT to p,p'-DDE in relation to the diet of the mothers.

	Non vegetarians n=198	Vegetarians n=18	Vegetarians vs non vegetarians
Compound	Mean ± S.E.M Positive	Mean ±S.E.M Positve	
p,p'-DDE	0.294 ± 0.042 197	0.438 ± 0.17 18	p>0.05
p,p'-DDT	0.160 ± 0.028 156	0.055 ± 0.013 17	p>0.05
Sum-DDT	0.467 ± 0.062 197	0.533 ± 0.188 18	p>0.05
DDT/DDE	0.609 ± 0.085 160	0.302 ± 0.098 13	p<0.05

p>0.05 No significant difference between the mean levels of the two groups.at 95% level
p<0.05 There is a significant difference between the mean levels of the two groups.at 95% level

Table 4.7 Levels of p,p'-DDE, p,p'-DDT, sum-DDT (mg/kg milk fat) and ratio the of p,p'-DDT to p,p'-DDE in Asian and African mothers in relation to the diet.

	Africans n=188		Asians n=28	
	Non vegetarians n=185	vegetarians n=3	Vegetarians n=15	Non vegetarians n=13
Compound	Mean ± S.E.M positive	Mean ± S.E.M positive	Mean ± S.E.M positive	Mean ± S.E.M positive
p,p'-DDE	0.297 ± 0.044 184	0.169 ± 0.092 3	0.489 ± 0.002 15	0.253 ± 0.077 13
p,p'-DDT	0.164 ± 0.030 147	0.054 ± 0.018 2	0.055 ± 0.015 11	0.085 ± 0.022 10
SUM-DDT	0.476 ± 0.006 189	0.223 ± 0.123 3	0.594 ± 0.222 15	0.347 ± 0.097 13
DDT/DDE	0.617 ± 0.09 147	0.089 ± 0.002 2	0.317 ± 0.116 11	0.436 ± 0.139 10

S.E.M = Standard error of the mean
n = Number of individuals
Positive = number of the individuals with the residues.

Table 4.8 Levels of p,p'-DDE, p,p'-DDT, sum-DDT (mg/kg milk fat) and ratio of p,p'-DDT to p,p'-DDE in relation to the parity of the mothers.

	Number of children		
	1 n=134	2 n=82	1 vs 2
Compound	Mean ± S.E.M positive	Mean ± S.E.M positive	
p,p'-DDE	0.324 ± 0.046 134	0.275 ± 0.077 81	p<0.005
p,p'-DDT	0.167 ± 0.038 107	0.128 ± 0.031 62	p>0.005
Sum-DDT	0.512 ± 0.072 134	0.407 ± 0.099 81	p<0.005
DDT/DDE	0.475 ± 0.051 107	0.775 ± 0.195 62	p<0.005

Table 4.9 Levels of p,p'-DDE, p,p'-DDT, sum-DDT (mg/kg milk fat) and ratio of p,p'-DDT to p,p'-DDE in Asian and African mothers in relation to the parity.

	Number of children				
	African mothers n=188		Asian mothers n=28		
Parity	1 n=118	2 n=70	1 n=17	2 n=11	1 vs 2
Compound	Mean ± S.E.M positive	Mean ± S.E.M positive	Mean ± S.E.M positive	Mean ± S.E.M positive	
p,p'-DDE	0.314 ± 0.05 117	0.263 ± 0.082 70	0.395 ± 0.125 17	0.356 ± 0.227 11	p<0.05
p,p'-DDT	0.184 ± 0.044 90	0.133 ± 0.033 58	0.077 ± 0.016 17	0.038 ± 0.011 4	p>0.05
Sum-DDT	0.51 ± 0.081 117	0.407 ± 0.109 70	0.525 ± 0.138 17	0.409 ± 0.251 11	p<0.05
DDT/DDE	3.707 ± 0.174 117	3.73 ± 0.198 71	0.411 ± 0.107 17	0.213 ± 0.053 4	p<0.05

p>0.05 No significant difference between the mean levels of the two groups at 95% level
p<0.05 There is a significant difference between the mean levels of the two groups.at 95% level
S.E.M = Standard error of the mean
n = Number of individuals
Positive = number of the individuals with the residues.

Table 4.10 Mean levels (mg/kg milk fat) and the standard error of the mean (S.E.M) of p,p'-DDE, p,p'-DDT, sum-DDT and ratio of p,p'-DDT to p,p'-DDE in relation to the age of the mothers.

	Age group			
	15-19 n=32	20-24 n=109	25-29 n=57	≥30 n=18
Compound	Mean ± S.E.M positive	Mean ± S.E.M positive	Mean ± S.E.M positive	Mean ± S.E.M positive
p,p'-DDE	0.287 ± 0.109 32	0.287 ± 0.045 108	0.342 ± 0.102 57	0.322 ± 0.116 23
p,p'-DDT	0.244 ± 0.117 22	0.17 ± 0.039 90	0.096 ± 0.022 46	0.053 ± 0.012 14
Sum-DDT	0.501 ±0.191 32	0.463 ± 0.072 108	0.465 ± 0.128 57	0.503 ± 0.178 18
DDT/DDE	0.815 ± 0.158 22	0.628 ± 0.139 90	0.474 ± 0.088 46	0.241 ± 0.035 11

Table 4.11 Mean levels (mg/kg in milk fat) and the standard error of the mean (S.E.M) of p,p'-DDE, p,p'-DDT, sum-DDT and ratio of p,p'-DDT to p,p'-DDE in Asian and African mothers in relation to the age of the mother.

	Asians n=28				Africans n=188			
Age group	15-19 n=1	20-24 n=9	25-29 n=10	≥30 n=8	15-19 n=31	20-24 n=100	25-29 n=47	≥30 n=10
Compound	Mean ±S.E.M positive	Mean ±S.E.M positive	Mean ± S.E.M positive	Mean ± S.E.M positive	Mean ± S.E.M positive	Mean ± S.E.M positive	Mean ± S.E.M positive	Mean ± S.E.M positive
p,p'-DDE	0.05 1	0.269 ± 0.039 9	0.358 ± 0.202 10	0.572 ± 0.316 8	0.295 ± 0.112 31	0.288 ± 0.05 99	0.339 ± 0.117 47	0.146 ± 0.028 10
p,p'-DDT	0.032 1	0.077 ± 0.022 9	0.059 ±0.021 7	0.081 ± 0.038 4	0.254 ± 0.122 21	0.18 ± 0.043 81	0.102 ± 0.026 39	0.045 ±0.007 7
Sum-DDT	0.087 1	0.375 ± 0.057 9	0.455 ± 0.221 10	0.676 ± 0.355 8	0.515 ± 0.196 31	0.471 ± 0.078 99	0.467 ±0.149 47	0.398 ±0.145 10
DDT/DDE	0.64 1	0.307 ± 0.081 9	0.543 ± 234 7	0.160 ± 0.027 4	0.823 ± 0.165 21	0.644 ± 0.154 81	0.451 ±0.053 39	0.288 ±0.045 7

n= number of individuals Positive= number of the individuals with the residues

Table 4.12 A comparison between the mean levels (mg/kg milk fat) of p,p'-DDT, p,p'-DDE, Sum DDT, p,p'-DDT/p,p'-DDE and dieldrin in samples from the rural areas (Kanja, 1988) and an urban area (Nairobi) in Kenya (Present study)

Area/ year of collection	p,p'-DDT	p,p'-DDE	Sum DDT	p,p'-DDT/ p,p'-DDE	Dieldrin
Rusinga island 1984/85	9.60	7.61	18.73	1.3	0.255
Embu 1985	3.60	5.23	9.76	0.8	0.01
Homa bay 1985	4.08	8.48	7.94	1.3	<0.05
Turkana 1983	7.38	2.17	7.79	4.4	0.687
Nanyuki 1984/85	2.47	1.52	4.32	2.0	0.657
Karatina 1983	1.59	1.72	3.51	0.7	0.059
Meru 1985	0.65	1.41	2.20	0.6	0.465
Loitoktok 1984	0.47	0.43	1.69	1.7	2.445
All areas	3.70	2.95	6.99	1.6	0.370
Nairobi 1990	0.15	0.31	0.47	0.6	0.022

Table 4.13 Summary data of the mothers from whom milk samples were collected

sampling area	Age	Income	Parity		Race		Diet	
	Range	Range	1	2	Asians	Africans	vegetarians	Non veg
Aga Khan n=64	18-35	5000-30000	40	24	20	44	14	50
Mater n=24	20-29	2000-13000	17	7	2	22	2	22
Nairobi n=15	21-32	3000-50000	9	6	4	11	1	15
Pumwani n=24	16-28	1000-8000	16	9	0	25	0	25
M.P. Shah n=14	19-32	500-20 000	9	5	1	13	1	13
Langata n=11	18-28	800-5500	5	6	0	11	0	11
Kariobangi n=13	15-28	500-10000	9	4	0	13	0	13
Kangemi n=15	18-24	600-6000	7	8	0	15	0	15
Mathare n=22	16-32	600-10000	13	9	0	22	0	22
Riruta n=13	18-26	1200-8000	9	4	0	13	0	13
All areas n=216	15-35	500-50000	134	82	28	188	18	198

n= Number of mothers from whom samples were collected in the sampling area.

CHAPTER FIVE

ORGANOCHLORINE PESTICIDES IN FOODS FROM NAIROBI MARKETS.

5.1 Sampling areas

Food samples were bought from market places and in different supermarkets in various localities of Nairobi City. The markets sampled in this study were the most centrally located ones where most of Nairobi residents buy their foodstuffs. These included Westlands market, City market, Kenyatta, Korogocho, Wakulima, Kariobangi, Ngara, Kawangware, Kangemi and Wangige.

Most of the foods found in these markets comes from all over the country. Wakulima market serves as a distribution center to the other smaller markets like Ngara and Westlands. Other markets like Wangige, Kangemi and Kawangware get their products from the nearby Limuru area and also from elsewhere in the country side.

5.2 Sampling procedure.

Food samples were collected in polythene bags from the markets and transported to the laboratory where they were wrapped in aluminium foil.These were then stored in a deep freezer at -20° C until analysis was done.

The cereals were also collected as above and on arrival in the laboratory these were ground using a waring blender, and stored in wide mouthed bottles with teflon caps until analysis was done.

5.3 Types of foods collected.

Different types of food were collected from each market. Vegetables, cereals pulses, roots and tubers and meats of different varieties were collected. Various samples were collected in an effort to cover most of the types of foods eaten by different ethnic groups living in Nairobi. Specifications of the types of foods collected are shown in tables 5.1 to 5.4.

5.4 Analytical procedure for determination of organochlorine pesticide residues in food.

This is as described in section 3.4

5.5 Results

The results from the analysed food samples are given in tables 5.1 to 5.4. The means of the residue levels are reported only for the positive samples. Residues with identifiable peaks, but with values below the detection limits are reported as trace. The mean levels of pesticide residues by food group are given in table 5.5

Only three out of the thirteen pesticides analysed for were present in quantifiable amounts in the samples analysed. Out of the 163 food samples analysed, 5.5% had residues of p,p'-DDT, 5.5% had residues of p,p'-DDE and 3.1% had residues of dieldrin. The DDT group showed the highest incidence and the highest levels in the samples. The mean level of the residues of p,p'-DDE ranged from traces in maize flour to the highest level of 0.46 mg/kg. Mean levels of p,p'-DDT ranged from traces in mutton to 0.26 mg/kg in ram meat. Dieldrin was detected in samples of meat, fish, rice and lettuce. Mean levels of this compound ranged from traces in the rice to 0.32 mg/kg in lean beef meat.

5.6 Discussion.

Levels of organochlorine pesticide residues detected in foods in the present study were generally low. Of the samples analysed, foods of animal origin like the meat of different animals and fish had the highest incidence of the residues. This is demonstrated in table 5.5. Kanja (1988), made the same observation on analysing food from different regions of Kenya. Other studies in Kenya have shown DDT as a major contaminant in the biological materials analysed (Maitho, 1978; Kahunyo, 1983; Mugambi, 1989; Mugachia, 1990).

Apart from the two samples of food of plant origin, all other samples that were positive for these residues were of animal origin. The two samples of plant origin that were positive for DDT were both of mixed spices (table 5.3).

Residues of some organochlorine pesticides in foods would be expected since

these compounds have in the past been widely used in agricultural practices for pest control and also in public health programs. Although use of these compounds has been restricted or banned, insecticide preparations with constituents from these compounds are still found in the market. It is not therefore surprising that residues of these compounds continue to appear in biological materials analysed. At the same time, these compounds persist in the environment for a long time, and only undergo slow degradation. They have a high ability to concentrate in the food chain and occur in higher concentration in animals higher in the food chain.

Use of DDT was banned in Kenya in 1986 (Mugachia, 1990) though some of the products used in pest control still incorporate it. Even after their ban residues of organochlorines are expected to remain in the animal tissues for several years because of their ability of bio-accumulation. Animals have the ability of accumulating these compounds in their fatty tissues, thus their occurrence in animal tissues more than in the plant tissues is expected.

The low levels may possibly be because of the restriction of the use of these compounds as explained above, in addition, the levels of the residues may reduce because of the much handling of the food substances since the collection from the farms to the time they are sold in the markets

The maximum residue limits (MRL) of DDT and dieldrin allowed in foods are presented in table 5.5. The same table also gives a comparison of the MRL with the residues detected in different foodstuffs in the present study. The levels observed in foods analysed in this study were generally lower than the MRL in most foods analysed and in some few cases the levels were higher than the MRL (table 5.5), as in the case of the mean level of sum-DDT in cereals and the level of dieldrin in meat. Sum-DDT levels were lower than levels observed in earlier similar studies carried out on food stuffs in India (Kaphalia *et al.*, 1985), where appreciable amount of sum-DDT was detected in eggs, wheatflour, meats, milk fats and oils to be 0.97, 0.32, 0.24, 0.22, and 0.15 mg/kg respectively. The levels in this study were higher than those observed for the same compounds in the United States

(Martell *et al.*, 1985).

In order to calculate the average daily intake of pesticide residues in food, the average weight of each food group taken by an adult per day was estimated and is given in table 5.6. The average daily intake of a particular pesticide was determined by multiplying the mean concentration of the pesticide in each food group by the weight of that food group consumed in a day and adding the intakes determined for each food group (Table 5.6). The calculated dietary intake (mg/day) of sum-DDT was 0.094 in the daily diet of non-vegetarians and 0.033 in vegetarians. That of dieldrin was 0.1 in non vegetarians and 0.015 in vegetarians. It appears from these studies that non vegetarians would have a higher intake of organochlorines in their daily diet as compared to vegetarians. The calculated daily intakes in this study were lower than those observed in India (Kaphalia et al, 1985) and in earlier studies in Kenya (Kanja, 1988)

5.7 Conclusions

1. Food samples collected in Nairobi markets had low level of contamination by p,p'-DDT, p,p'-DDE, and dieldrin. The levels of these compounds were lower than the MRL of the compounds in most food groups but exceeded the MRL in some others like cereals (DDT) and meat (dieldrin). The levels in this study were lower than those reported in earlier studies in Kenya.
2. Food of animal origin had a higher incidence of contamination than foods of plant origin and on calculation of the average daily intake of pesticide residues by human beings, it was observed that individuals who consume foods of animal origin would be exposed to higher levels of pesticides.
3. The presence of low levels of DDT and dieldrin indicates that food is a source of pesticide contamination in the human body.
4. It is necessary to monitor the levels of the pesticides in the foods sold in the markets and at the same time determine the source of the contaminated foods.

Table 5.1 Levels (mg/kg fresh weight) of organochlorine pesticides in meats and cereals sold in Nairobi markets.

		Pesticide residue					
Product	No. of samples	p,p'-DDT positive	mean Range	p,p'-DDE positive	mean Range	∑DDT mean Range	Dieldrin positive	mean Range
Cow fat	6	2	0.21 0.17-0.25	5	0.46 0.002-0.51	0.52 0.014-0.72	1	0.01
Beef	6	1	0.03	0	-	0.03	2	0.32 T-0.32
Mutton	3	1	T	1	0.12	0.133	0	-
Ram	4	2	0.26 T-0.26	0	-	0.26	0	-
Fish	8	3	0.074 0.025-0.11	1	T	0.074 T-0.111	1	0.21
Cereals								
Maize	4	0	-	1	T	-	0	-
Rice	6	0	-	0	-	0	1	T
Wheat flour	3	0	-	0	-	0	0	-
Maize flour	3	0	-	0	-	0	0	-

-below detection limits (0.001mg/kg) T- positive peaks present but not quantifiable

Means are only calculated from quantifiable levels

Table 5.2 Levels (mg/kg fresh weight) of organochlorine pesticides in pulses sold in Nairobi markets.

		Pesticide residue						
Product	No. of	p,p'-DDT		p,p'-DDE		∑DDT	Dieldrin	
	samples	positive	Mean	positive	Mean	Mean	positive	Mean
			Range		Range	Range		Range
Beans	7	0	-	0	-	0	0	-
Green grams	5	0	-	0	-	0	0	-
Cow peas	5	0	-	0	-	0	0	-
Pigeon peas	4	0	-	0	-	0	0	-

- below detection limits (0.001mg/kg)

T- positive peaks present but not quantifiable

Means are only calculated from quantifiable levels.

Table 5.3 Levels (mg/kg fresh weight) of organochlorine pesticides in vegetables and spices sold in Nairobi markets.

		Pesticide residue						
Product	No. of	p,p'-DDT		p,p'-DDE		∑DDT	Dieldrin	
	samples	positive	mean range	positive	mean range	mean range	positive	mean range
Kales	6	0	-	0	-	-	0	-
Cabbage	5	0	-	0	-	-	0	-
Tomatoes	6	0	-	0	-	-	0	-
Celery	5	0	-	0	-	-	0	-
Spinach	6	0	-	0	-	-	0	-
Lettuce	5	0	-	0	-	-	1	0.03
Eggplant	6	0	-	0	-	-	0	-
Cucumber	6	0	-	0	-	-	0	-
Cauliflower	5	0	-	0	-	-	0	-
Onions	3	0	-	0	-	-	0	-
Spices								-
Mixed spices	5	2	0.04* T-0.04	0	-	-	0.04* T-0.04	-
Chillis	2	0	-	0	-	0	0	-

- below detection limits (0.001mg/kg)

T- positive peaks present but not quantifiable

Means are calculated from quantifiable levels only.

*-only one sample had quantifiable amounts.

Table 5.4 Levels (mg/kg fresh weight) of organochlorine pesticides in roots and tubers and ready foods sold in Nairobi markets.

		Pesticide residue						
Product	No. of	p,p'-DDT		p,p'-DDE		∑DDT	Dieldrin	
	samples	positive	mean	positive	mean	mean	positive	mean
			range		range	range		range
English potatoes	5	0	-	0	-	0	0	-
Carrots	7	0	-	0	-	0	0	-
Turnips	4	0	-	0	-	0	0	-
Beetroots	5	0	-	0	-	0		-
Roti	3	0	-	0	-	0	0	-
Pitta bread	6	0	-	0	-	0	0	-

- below detection limits (0.001mg/kg)

T- positive peaks present but not quantifiable

Means are only calculated from quantifiable levels.

Table 5.5 Mean levels (mg/kg fresh weight) of pesticide residues by food group and the maximum residue limit of dieldrin and sum-DDT allowed for each food group.

pesticide	Meat	Fish	cereals	pulses	vegetables	roots and tubers	Ready foods	milk
p,p'DDT	0.145	0.074	0.006	0	0	0	0	0
p,p'DDE	0.12	T	T	0	0	0	0	0
Sum DDT	0.28 (7)	0.074	0.06	0	0 (7)	0 (1)	0	0 (1.25)
Dieldrin	0.32 (0.2	0.21	0.01 (0.02)	0	0.03 (0.1)	0 (0.1)	0	0

Figures in parenthesis indicate the MRL of the compounds in the respective food groups.

Table 5.6 Estimated average amount of food (gms/day) consumed daily by an adult.

Food group	Average weight	
	Non-vegetarians	vegetarians
Fish/meat/poultry	250	-
Milk	100	100
Cereals	400	550
Pulses	150	300
Vegetables	200	300
Roots and tubers	50	100

Table 5.7 Calculated daily intake (mg/day) of pesticides by food group and in the total diet.

	Calculated daily intake			
Food group	Non-vegetarian		non vegetarian	
	DDT	Dieldrin	DDT	Dieldrin
Fish/meat/poultry	0.07	0.08	-	-
Milk	-	-	-	-
Cereals	0.024	0.004	0.033	0.006
Pulses	-	-	-	-
Vegetables	0	0.006	-	0.009
Roots and tubers	-	-	-	-
Total	0.094	0.1	0.033	0.015

CHAPTER SIX

GENERAL CONCLUSIONS

The following conclusions were made from the present study:-

1. The methods used in this study were acceptable for analysis of organochlorine pesticides in human milk and food samples as observed in the results of recovery studies.
2. Breast milk of mothers living in Nairobi was contaminated by organochlorine pesticides. 13 organochlorine pesticides were detected in varying frequencies in human milk samples. p,p'-DDE was the major contaminant of the milk samples and also the main contributor to total DDT in the milk samples analysed.
3. Levels of pesticides in mothers milk in this study were lower than levels reported in earlier studies carried out in the rural areas of Kenya
4. Individual variations in the levels of the organochlorine pesticides in the breast milk were found to be influenced by parity, maternal age, and fat content of the milk. There was no relationship between levels of organochlorine pesticides in human milk and other factors studied such as diet, socio-economic status and the race of the mothers.
5. For some infants, the daily intake of pesticides through mother's milk of dieldrin, sum DDT and aldrin exceeded the ADI set by the WHO.
6. Food samples from the Nairobi markets that were analysed for pesticide contamination had very low levels. Foods of animal origin had a higher incidence and higher level of contamination than foods of plant origin.
7. It is necessary to monitor the levels of the pesticides in human milk and food continually in order to determine the trends of environmental pollution by pesticides.

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Appendix 1 Questionnaire completed during collection of human milk samples.

1. Sample number .....
2. Date when sample was collected (day\month\year) .....
3. Time of the day .....
4. Place where sample was collected (home, hospital, health-centre), etc.
5. Size of the sample collected (approximately) .....ml
6. Mother's name .....
7. How long has the mother lived in this area? .....Years.
8. Mother's age .....years, height .....weight .....kg.
9. Infant's sex .....and date of birth (day, month, year,) .....
10. Is it mother's first or second child? .....
11. Has the mother's residential place been treated with any pesticides at any time? e.g. against mosquito, flies, cockroaches, bedbugs, lice, etc. Yes/No, If yes give further information (by whom, how, when, how frequently, against what, and if possible name the product(s)
12. What are the main agricultural activities/crops in the area where the mother is living?.....
13. Does\has the mother used tobacco (smoked, chewed or as snuff)? Yes/No, If yes state how often.
14. What is the staple food of mother.....
15. Check the relevant boxes

    ☐ Mother eats virtually no food of animal origin including milk and eggs.

    ☐ Mother eats mainly vegetarian diet but also milk and eggs.

    ☐ Mother eats both animal and vegetable foods.

    ☐ Mother eats almost exclusively animal foods

16. Fish consumption

    ☐ Eats shellfish .....frequency

    ☐ Eats freshwater fish .....frequency

    ☐ Eats saltwater fish .....frequency

    ☐ No fish or shellfish

17. Does the mother eat any spiced foods? If so, give details .....
18. Name of the residential place of the mother .....
19. Any other comments .....

20. What is your current marital status?

☐ Married

☐ Stable union, but unmarried

☐ Separated or divorced

☐ Single

☐ Other (specify)

21. What level of formal education have you reached?
(tick one box only)

☐ Primary School

☐ Secondary School

☐ VocationaNtechnical

☐ University

22. Mother's contact address.....Tel:.....

23. What type is your occupation ? (give details) 24. What is your husband/cohabitor's or where applicable head of household's occupation? (give details) .....

25. What is your income..... Ksh.

Appendix 2 Levels (mg/kg milk) of sum-DDT, p,p'-DDE, p,p'-DDT, dieldrin, ratio of p,p'-DDT to p,p'-DDE and the percent fat in individual human milk samples.

Sample No	Sum DDT	p,p'-DDE	p,p'-DDT	DDT/DDE	Dieldrin	% Fat
0.3	0.01	0.00	0.01	1.80	t	4.20
0.4	0.07	0.05	0.01	0.24	t	2.00
0.5	0.01	0.01	2.00	124		3.65
0.6	0.01	0.00	0.00	0.56		2.60
0.7	0.03	0.01	0.02	1.58		3.00
0.8	0.04	0.02	0.01	0.65	t	1.90
0.9	0.06	0.03	0.03	0.97		4.20
0.10	0.03	0.02	0.01	0.29		4.20
0.11	0.03	0.02	0.01	0.38		3.20
0.12	0.11	0.09	0.01	0.15		6.82
1.1	0.00	0.00	0.00	0.00	t	1.40
1.2	0.11	0.07	0.04	0.52		4.20
1.3	0.17	0.10	0.06	0.66		4.00
1.4	0.01	0.01	0.00	0.33		3.10
1.5	0.02	0.01	0.00	0.38		1.70
1.6	0.03	0.01	0.02	2.63	t	1.82
1.7	0.03	0.02	0.01	0.30	0.001	1.80
1.8	0.04	0.03	0.00	0.00	t	3.28
1.9	0.26	0.15	0.10	0.67		6.20
1.10	0.06	0.04	0.01	0.19		3.80
1.11	0.08	0.06	0.01	0.13		3.20
1.12	0.03	0.01	0.01	0.90		6.90
2.1	0.12	0.05	0.05	0.89		6.60
2.3	1.13	0.67	0.39	0.58	0.014	4.00
2.4	0.06	0.05	0.01	0.21		3.80
2.5	0.08	0.06	0.01	0.17		4.00
2.6	0.22	0.09	0.12	1.36		1.90
2.7	0.04	0.03	0.01	0.25		2.92
2.8	0.10	0.08	0.01	0.17		5.20
2.9	0.03	0.02	0.00	0.17		2.60
2.10	0.50	0.04	0.44	12.0		3.30
2.11	0.01	0.01	0.00	0.42	t	1.00
2.12	0.06	0.04	0.02	0.41		2.40
3.1	0.03	0.02	0.01	0.93	0.003	3.30
3.2	0.11	0.06	0.05	0.88	0.003	1.70
3.3	0.13	0.04	0.08	2.07		1.30
3.4	0.04	0.03	0.01	0.34	0.001	1.90
3.5	1.11	0.37	0.67	1.79	0.009	1.30
3.6	0.07	0.05	0.02	0.30		6.18
3.7	0.09	0.08	0.01	0.09		5.30
3.8	0.23	0.16	0.05	0.31		4.61
3.9	0.17	0.12	0.04	0.34		7.70
3.10	0.01	0.01	0.00	0.00		1.90
3.11	0.09	0.05	0.03	0.68		1.90
3.12	0.03	0.03	0.00	0.00	0.075	5.20
4.2	0.02	0.01	0.00	0.00		6.40
4.4	0.11	0.10	0.00	0.00		5.36
4.5	0.03	0.03	0.00	0.00	t	1.43
4.6	0.39	0.29	0.06	0.21		4.20
4.7	0.02	0.01	0.01	1.40		3.10
4.8	0.06	0.04	0.02	0.46		5.10
4.9	0.02	0.01	0.00	0.23		1.60
4.10	0.14	0.11	0.02	0.19	0.005	6.00
4.12	0.06	0.04	0.01	0.14	0.005	2.90
5.1	0.21	0.14	0.05	0.35		3.30

Cont'd

5.2	0.15	0.12	0.01	0.11		4.20
5.3	0.10	0.09	0.00	0.00		4.20
5.4	0.06	0.05	0.00	0.00	0.003	2.60
5.5	0.02	0.01	0.00	0.10		2.63
5.6	0.14	0.12	0.00	0.00	t	4.67
5.7	0.01	0.01	0.00	0.00		1.40
5.8	0.08	0.04	0.03	0.59	0.005	2.90
5.9	0.15	0.11	0.03	0.25		5.40
5.10	0.03	0.03	0.00	0.00		4.00
5.11	0.06	0.05	0.00	0.00	0.011	3.10
6.1	0.11	0.04	0.06	1.44		1.10
6.2	0.49	0.39	0.05	0.13		1.60
6.3	0.07	0.05	0.02	0.39	0.004	3.70
6.4	0.07	0.06	0.00	0.00		3.30
6.5	0.07	0.05	0.01	0.25		5.20
6.6	0.03	0.02	0.01	0.33		3.90
6.7	0.09	0.07	0.02	0.27	0.026	5.90
6.8	0.01	1.20	0.00	0.00	0.002	4.00
6.9	0.48	0.11	0.25	2.29	0.006	4.60
6.10	0.00	0.00	0.00	0.00	t	1.21
6.11	0.05	0.04	0.00	0.10	0.035	2.50
6.12	0.06	0.04	0.01	0.26	0.006	5.60
7.1	0.26	0.20	0.04	0.21		5.10
7.2	0.05	0.03	0.01	0.26	0.003	4.20
7.3	0.14	0.09	0.04	0.46		5.70
7.4	0.04	0.02	0.01	0.77	t	3.60
7.5	0.11	0.07	0.03	0.37	t	8.20
7.6	0.14	0.09	0.03	0.38	0.003	4.00
7.7	0.09	0.06	0.02	0.41		1.60
7.8	0.07	0.03	0.03	1.03	0.002	2.70
7.9	0.16	0.12	0.03	0.27	0.05	3.30
7.10	0.16	0.05	0.10	1.91	0.005	6.00
7.11	0.08	0.07	0.00	0.00		4.10
7.12	0.27	0.15	0.11	0.75	0.006	7.20
8.1	0.02	0.02	0.00	0.00		5.80
8.2	0.07	0.03	0.02	0.50		6.50
8.3	0.08	0.06	0.01	0.15		7.57
8.4	0.07	0.05	0.01	0.22	0.010	5.10
8.5	0.06	0.02	0.04	2.20		3.80
8.6	0.18	0.14	0.02	0.17		7.20
8.7	0.01	0.01	0.01	0.69		2.30
8.8	0.11	0.09	0.02	0.18		2.70
8.9	1.02	0.43	0.54	1.25		4.10
8.10	0.06	0.05	0.01	0.13	0.013	5.40
8.11	0.01	0.00	0.00	0.75		6.00
8.12	0.01	0.00	0.00	0.00	0.002	2.10
9.1	0.10	0.07	0.02	0.22	t	3.95
9.2	0.22	0.19	0.00	0.04	0.001	1.00
9.3	0.05	0.04	0.01	0.23		6.30
9.4	0.06	0.04	0.01	0.23	0.004	2.40
9.5	0.03	0.02	0.01	0.53	0.004	5.30
9.6	0.04	0.03	0.01	0.42	0.024	3.30
9.7	0.01	0.00	0.01	1.71	0.001	2.40
9.8	0.01	0.01	0.00	0.00		3.06
9.9	0.03	0.03	0.01	0.23	0.002	1.40
9.10	0.43	0.11	0.31	2.82		4.20
9.11	0.72	0.60	0.06	0.10		3.00
9.12	0.08	0.06	0.02	0.10		1.87
10.1	0.13	0.09	0.03	0.35	t	4.80
10.2	0.20	0.50	0.39	0.78		8.03
10.3	0.08	0.04	0.04	0.88		4.90

Cont'd

10.4	0.83	0.40	0.38	0.95		1.50
10.5	0.10	0.05	0.05	1.12		2.60
10.6	0.06	0.04	0.01	0.30		4.29
10.7	0.24	0.17	0.06	0.34		4.00
10.8	0.02	0.02	0.00	0.00	0.007	7.47
10.9	0.07	0.03	0.04	1.37		4.90
10.10	0.15	0.11	0.03	0.23		4.20
10.11	0.10	0.07	0.02	0.22		2.20
10.12	0.13	0.10	0.01	0.11		5.00
10.13	0.12	0.06	0.05	0.76		3.90
10.14	0.10	0.08	0.01	0.09		2.70
10.15	0.08	0.05	0.02	0.47		4.60
10.16	0.03	0.03	0.00	0.18	0.006	3.70
10.17	0.05	0.03	0.01	0.33	0.007	3.80
10.18	0.05	0.03	0.02	0.58	0.005	5.60
10.19	0.01	0.01	0.00	0.00	0.009	6.00
10.20	0.02	0.01	0.01	0.46	0.002	2.70
11.1	0.59	0.39	0.15	0.38		8.15
11.2	0.11	0.09	0.01	0.10		4.44
11.3	0.05	0.02	0.02	1.09		1.80
11.4	0.00	0.00	0.00	0.00		5.80
11.6	0.04	0.03	0.00	0.15		2.80
11.7	0.02	0.01	0.01	0.69	0.002	3.61
11.8	0.02	0.02	0.00	0.21	t	1.00
11.9	0.01	0.01	0.00	0.00		3.90
11.10	0.06	0.03	0.02	0.83		3.98
11.11	0.07	0.02	0.05	2.93		1.19
12.1	2.81	2.42	0.12	0.05		8.10
12.2	0.06	0.04	0.02	0.48		3.50
12.3	0.01	0.01	0.00	0.00	0.010	5.00
12.4	0.04	0.03	0.01	0.32		3.30
12.5	0.25	0.19	0.04	0.22		3.42
12.6	0.03	0.03	0.00	0.00	0.003	5.40
12.7	0.17	0.11	0.06	0.51		5.10
12.8	0.14	0.10	0.02	0.22		6.30
12.9	0.02	0.01	0.00	0.15	0.001	1.00
12.10	0.06	0.06	0.00	0.00		2.80
12.11	0.04	0.04	0.01	0.16	0.001	2.40
12.12	0.07	0.05	0.02	0.30		1.25
12.13	0.12	0.03	0.08	0.23		2.02
13.1	0.06	0.03	0.01	0.36		5.67
13.2	0.01	0.00	0.00	0.58		1.90
13.3	0.01	0.01	0.00	0.00	0.002	2.01
13.4	0.05	0.05	0.00	0.00		8.40
13.5	0.06	0.03	0.02	0.51	t	6.56
13.6	0.08	0.06	0.17	0.29		4.30
13.7	0.01	0.01	0.00	0.14	t	4.00
13.8	0.04	0.04	0.00	0.00		6.60
13.9	0.09	0.06	0.02	0.31	0.006	0.59
13.10	0.13	0.09	0.04	0.44		4.21
13.11	0.20	0.15	0.04	0.24		7.82
13.12	0.01	0.11	0.00	0.00		4.90
14.1	0.01	0.01	0.00	0.00	0.005	5.00
14.3	0.03	0.02	0.01	1.35	t	1.30
14.4	2.09	1.59	0.31	0.19		3.30
14.5	0.01	0.00	0.01	0.33	0.002	1.80
14.6	0.14	0.08	0.04	0.46	t	2.85
14.7	0.02	0.02	0.00	0.00		3.62
14.8	0.26	0.14	0.07	0.54		5.31
14.9	0.03	0.02	0.00	0.00		4.90

Cont'd

14.10	0.03	0.02	0.01	0.32	0.001	1.70
14.11	0.06	0.04	0.02	0.64		7.20
14.12	0.32	0.26	0.03	0.13		1.20
15.1	0.01	0.50	0.00	0.00	0.004	3.27
15.2	0.09	0.08	0.00	0.02	0.005	8.18
15.3	0.04	0.02	0.01	0.59		3.50
15.4	0.01	0.01	0.00	0.00		1.90
15.5	0.02	0.02	0.00	0.10		4.66
15.6	0.03	0.06	0.00	1.38		1.87
15.7	0.10	0.08	0.02	0.26		4.02
15.8	0.07	0.04	0.02	0.41	0.001	1.80
15.9	0.02	0.02	0.00	0.00		1.50
15.10	0.04	0.03	0.01	0.35		1.70
16.1	0.08	0.07	0.01	0.14	0.001	2.60
16.2	0.07	0.05	0.01	0.15		1.90
16.6	0.09	0.07	0.01	0.21		1.16
16.7	0.17	0.13	0.03	0.20		6.91
16.8	0.06	0.05	0.01	0.14	0.006	7.37
16.9	0.07	0.04	0.03	0.73		3.90
16.10	0.02	0.01	0.00	0.00		1.65
16.11	0.06	0.04	0.02	0.72		4.48
16.12	0.05	0.04	0.01	0.16	0.004	2.70
17.3	0.04	0.04	0.00	0.00	0.004	2.94
17.5	0.01	0.01	0.00	0.00	t	0.70
18.2	0.09	0.07	0.01	0.18	t	3.35
18.3	0.06	0.05	0.01	0.15		2.70
18.5	0.02	0.02	0.00	0.14		3.36
18.9	0.06	0.05	0.00	0.09		1.25
20.1	1.02	0.90	0.01	0.01		4.20
20.2	0.13	0.08	0.04	0.57		6.03
20.3	0.04	0.03	0.00	0.00	0.002	4.00
20.4	0.16	0.14	0.01	0.08		2.80
20.6	0.14	0.12	0.01	0.07		6.14
20.7	0.04	0.02	0.02	0.80		1.00
20.8	0.03	0.02	0.01	0.31		4.83
20.9	0.02	0.02	0.00	0.00		2.85
20.10	0.02	0.02	0.00	0.00	0.003	3.70
20.11	0.02	0.02	0.00	0.00		3.75
21.1	0.00	0.00	0.00	0.00	0.002	3.70
21.2	0.01	0.01	0.00	0.00		2.48
21.3	0.05	0.03	0.01	0.40		3.10
21.4	0.03	0.03	0.00	0.00		1.28
21.5	0.09	0.07	0.01	0.14	t	4.70
21.6	0.13	0.10	0.03	0.30	0.003	4.90
21.7	0.16	0.11	0.02	0.20		3.20
i21.8	0.08	0.06	0.01	0.17		2.47
21.9	0.00	0.00	0.00	0.00	0.003	3.16
21.10	0.02	0.02	0.00	0.20		1.81
21.11	0.16	0.06	0.09	1.50		5.20
21.12	0.05	0.03	0.01	0.31		2.30

Appendix 3 Levels (mg/kg milk) of β-HCH (a) Lindane (b) and α-HCH (c) in the positive samples.

(a) β-HCH

Sample No.	Residue level
0.11	0.001
1.2	0.003
1.3	0.001
1.9	0.099
1.11	0.003
2.1	0.005
2.8	0.003
2.9	0.005
2.10	0.004
5.1	0.001
7.7	0.001
9.9	0.002
10.1	0.004
10.11	0.11
11.1	0.143
11.2	0.005
11.3	0.001
11.8	0.004
11.10	0.010
12.7	t
12.8	0.005
13.1	0.032
14.4	0.001
14.5	0.003
14.6	0.007
15.9	0.002
15.7	0.022
16.1	0.022
16.2	0.019
16.7	0.282
17.3	0.003
18.3	0.003
18.9	0.004
20.3	0.009
20.4	0.006
20.6	0.017
20.10	0.004
21.3	t
21.7	0.002

(b) Lindane

Sample No.	Residue level
0.7	0.001
2.10	0.002
2.11	t
2.12	t
3.1	0.001
3.3	0.002
3.9	0.002
4.7	0.004
6.3	0.001
7.5	0.003
7.6	0.002
7.7	0.001
7.8	t
8.5	0.004
8.7	0.001
8.10	0.001
9.10	0.062
10.3	0.011
10.5	0.009
10.7	0.008
10.9	0.015
10.10	0.002
10.14	0.001
10.17	0.031
11.4	0.002
12.1	0.011
12.10	0.002
14.1	0.001
14.3	0.002
14.8	0.004
14.11	0.004
15.3	0.007
16.9	0.009
20.4	0.002
20.7	0.002
21.3	t
21.7	0.002

(c) α-HCH

Sample No.	Residue level
0.9	0.006
3.2	0.001
3.7	0.001
4.7	0.004
5.4	0.003
5.11	0.003
6.9	0.003
7.6	0.004
7.11	0.003
8.5	0.002
8.7	0.001
9.11	0.001
10.3	0.019
10.9	0.004
10.15	0.009
10.17	0.008
11.1	0.002
12.1	0.007
14.8	0.003
14.11	0.009
15.3	0.006
15.9	t
16.9	0.013
17.3	0.001

t- positively identified peaks but below detectable limits.

Appendix 3 Levels (mg/kg milk) of β-HCH (a) Lindane (b) and α-HCH (c) in the positive samples.

(a) β-HCH

Sample No.	Residue level
0.11	0.001
1.2	0.003
1.3	0.001
1.9	0.099
1.11	0.003
2.1	0.005
2.8	0.003
2.9	0.005
2.10	0.004
5.1	0.001
7.7	0.001
9.9	0.002
10.1	0.004
10.11	0.11
11.1	0.143
11.2	0.005
11.3	0.001
11.8	0.004
11.10	0.010
12.7	t
12.8	0.005
13.1	0.032
14.4	0.001
14.5	0.003
14.6	0.007
15.9	0.002
15.7	0.022
16.1	0.022
16.2	0.019
16.7	0.282
17.3	0.003
18.3	0.003
18.9	0.004
20.3	0.009
20.4	0.006
20.6	0.017
20.10	0.004
21.3	t
21.7	0.002

(b) Lindane

Sample No.	Residue level
0.7	0.001
2.10	0.002
2.11	t
2.12	t
3.1	0.001
3.3	0.002
3.9	0.002
4.7	0.004
6.3	0.001
7.5	0.003
7.6	0.002
7.7	0.001
7.8	t
8.5	0.004
8.7	0.001
8.10	0.001
9.10	0.062
10.3	0.011
10.5	0.009
10.7	0.008
10.9	0.015
10.10	0.002
10.14	0.001
10.17	0.031
11.4	0.002
12.1	0.011
12.10	0.002
14.1	0.001
14.3	0.002
14.8	0.004
14.11	0.004
15.3	0.007
16.9	0.009
20.4	0.002
20.7	0.002
21.3	t
21.7	0.002

(c) α-HCH

Sample No.	Residue level
0.9	0.006
3.2	0.001
3.7	0.001
4.7	0.004
5.4	0.003
5.11	0.003
6.9	0.003
7.6	0.004
7.11	0.003
8.5	0.002
8.7	0.001
9.11	0.001
10.3	0.019
10.9	0.004
10.15	0.009
10.17	0.008
11.1	0.002
12.1	0.007
14.8	0.003
14.11	0.009
15.3	0.006
15.9	t
16.9	0.013
17.3	0.001

t- positively identified peaks but below detectable limits.

Appendix 4 Residue levels (mg/kg milk) of heptachlor (a), Aldrin (b), and Heptachlor epoxide (c) in the positive samples

(a) Heptachlor

Sample No.	Residue level
0.9	0.006
5.4	0.002
6.4	0.003
6.9	0.004
7.6	0.006
7.11	0.004
8.7	0.001
8.8	0.001
8.9	0.004
9.6	0.002
9.10	0.054
10.3	0.017
10.5	0.009
10.7	0.008
10.9	0.013
10.15	0.016
12.1	0.016
16.9	0.011
20.1	0.045
20.2	0.011
20.4	0.002
20.7	0.003

(b) Aldrin

Sample No	Residue level
2.11	t
5.6	0.022
5.8	0.010
10.5	0.005
10.7	0.004
10.9	0.013
10.14	0.001
10.15	0.003
11.4	0.020
14.3	0.010
15.3	0.009
21.11	0.016

(c) Hept. epoxide

Sample No	Residue level
2.7	0.002
4.7	0.001
5.8	0.004
5.9	0.001
12.12	0.001
13.3	t
14.5	0.001
15.7	t
15.8	0.001
16.1	0.001
18.2	t

t- positively identified peaks but below detectable limits.

Appendix 5 Residue levels (mg/kg milk) of Endrin (a) o,p'-DDT (b) and p,p'-DDD (c) in the positive samples

(a) Endrin

Sample No	Residue level
0.11	0.026
1.2	t
4.7	0.011
5.6	0.007
9.4	0.005
10.14	0.003
18.2	t

(b) o,p'-DDT

sample No	Residue level
1.2	0.001
1.3	0.022
1.9	0.020
1.10	0.002
2.1	0.012
2.3	0.103
2.6	0.005
2.10	0.052
3.2	0.005
3.3	0.020
3.5	0.062
4.10	0.011
5.1	0.009
6.1	0.003
6.2	0.006
6.9	0.2036
7.5	0.013
7.7	0.003
9.4	0.001
10.2	0.186
10.4	0.011
11.1	0.035
12.7	0.008
12.12	0.23
14.4	0.015
14.6	0.001

(c) p,p-DDD

Sample No	Residue level
1.3	0.001
2.1	0.007
2.6	0.003
2.10	0.021
3.2	0.001
3.3	0.002
3.5	0.022
3.11	0.002
5.1	0.003
6.1	0.003
6.9	0.096
7.5	0.005
7.7	0.001
9.4	0.001
10.2	0.006
10.4	0.002
11.3	0.001
14.4	0.011
14.6	0.002
14.8	0.029
21.7	0.009

t- positively identified peaks but below detectable limits.