ORGANOCHLORINE PESTICIDES IN KENYAN MOTHERS' MILK:

LEVELS AND SOURCES

LAETITIA WAKONYU KANJA, B Sc, M Sc., (University of Nairobi)

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



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DECLARATION

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

Alonga L. W. Kanja

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

Frangled Sho

Associate prof. Janneche Utne Skaare, M.Sc., Ph.D.

LOST LOGILLO OF ICCLOSING Achinik

Prof. C. K. Maitai, M. Pharm., Ph.D.

Par Lobken

Prof. P. Løkken, D.D.S., M.Sc., Ph.D.

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ABBREVIATIONS

DDT	1,1,1-trichloro-2-bis(p-chlorophenyl)ethane
DDE	1,1-dichloro-2-bis(p-chlorophenyl)ethylene
DDD	1,1-dichloro-2-bis(p-chlorophenyl)ethane
≪ −нсн	alpha-hexacyclohexane
β -нсн	beta-hexacyclohexane
δ -HCH(Lindane)	gamma-hexacyclohexane
НСВ	hexachlorobenzene
2,4,5-T	2,4,5-trichlorophenoxyacetic acid
PCB	polychlorinated biphenyls
HEOD	1,2,3,4,10,10 hexachloro-6,7 epoxy-1,4,4a,5,6,-
	7,8,8a-octahydro-exo 1,4,endo-5,8,dimethano-
	naphthalene
PIPD	3,3 exo-4,5,6,6 7-hexachloro-11,12-exo-epoxy-
	pentacyclo $(6.4.0^{2.10}.0^{3.7}0.^{5.9})$ dodecane
GLC	Gas liquid chromatography
ECD	Electron capture detector
TLC	Thin layer chromatography
R _F	Distance from starting point to the component
	divided by distance from starting point to the
	solvent front
MS	Mass spectrometer
HPLC	High pressure liquid chromatography
OC	Organochlorine compound
ADI	Acceptable daily intake
MRL	Maximum residue limit
ERL	Extraneous residue limit
^{LD} 50	Lethal dose, in milligrams per kilogram body
	weight needed to kill 50% of the test animals

AQA	Analytical quality assurance
FAO	United Nations Food and Agricultural
	Organization
WHO	World Health Organization
UNEP	United Nations Environment Programme

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"The unfolding of thy words gives light" Psalm 119:130

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To my husband, Kanja and children, Gatigi, Wambui and Waruguru.

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Human milk samples were collected from eight areas in Kenya with different agricultural activities. All the mothers had lived within the area for the last five years. They were healthy and breastfeeding their first or second child. In 264 mothers milk samples analysed, 13 organochlorine pesticide residues were detected in the following order of frequency: p,p'-DDT (100%), p,p'-DDE (100%), HCB (60%), aldrin (35%), lindane (30%), B-HCH (27%), dieldrin (20%), od-HCH (8%), transnonachlor (6%), heptachlor (4%), endrin (4%), heptachlorepoxide (0.4%), and oxychlordane (0.4%). No residues of PCBs were found. Great regional differences in the levels of these compounds were found, and the mean levels of sum DDT ranged from 1.69 mg/kg in milk fat of nomads from Loitokitok to 18.73 mg/kg milk fat in human milk from Rusinga Island. Regional differences were also found in the mean ratio of p,p'-DDT to its more persistent metabolite p,p'-DDE, with mean levels ranging from 0.7 in Karatina to 4.4 in Turkana. Similarly, the results demonstrated significant differences (P<0.05) in the mean levels of sum DDT and the ratio of p,p'-DDT to p,p'-DDE within the various sampling area depending on the agricultural activities. The results demonstrate 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 industrialized countries, and the estimated daily intake of a Kenyan infant exceeded the Acceptable Daily Intake set by the WHO/FAO. There was also a

positive correlation of sum DDT, and p,p'-DDT to p,p'-DDE ratio with the mother's age. The results were also examined in relation to differences in living conditions with regard to agricultural activities, dietary habits, and reported use of pesticides in the various sampling areas. Examination of commercial infant milk formulas on the Kenyan market revealed that only one contained organochlorine pesticides, and the levels were low, being 0.05 mg DDE/kg milk fat and 0.06 mg dieldrin/kg milk fat.

The time trend (1983-84) in DDT contamination in Turkana where DDT was extensively used in cotton growing, was also investigated. The changing from growing cotton to food crops within 9 months was followed by a reduction in the mean level of sum DDT from 7.79 to 4.49 mg/kg milk fat.

Human food samples were examined for organochlorine pesticides in order to determine the sources of these compounds in the human body. Of the 243 food samples examined, about 50% of them contained at least one organochlorine residue, but generally the levels were low. The DDT-group was most commonly found in fish, cow's milk, vegetables and cereals. Lindane had a high incidence of occurrence in all the food samples examined. Other organochlorines detected were aldrin, α' -HCH, β -HCH transnonachlor, oxychlordane, dieldrin. PCBs were not detected. The mean sum DDT found in fish was 128 ug/kg and p,p'-DDE was the major metabolite.

The dietary intake of the different organochlorines through food samples varied in the different areas. The occurrence of pesticide residues in food appeared to be closely associated with the use of similar pesticides in the area.

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Foods of animal origin contained higher levels of the DDT group as compared to other food groups in all areas. In general, a mothers daily intake of sum DDT was lower than the infants daily intake of sum DDT through breastfeeding, which demonstrates mobilization and excretion of stored pesticides in the mothers milk.

Samples of maternal blood, milk, subcutaneous fat, as well as umbilical cord blood were collected from mothers and their infants at Kenyatta National Hospital. The mothers were healthy but delivered by Caesarean operation. In the 41 samples analysed, the DDT-group occurred in all, dieldrin in 11, transnonachlor in 6, β -HCH in 5, endrin in 2 and lindane in 1. The mean levels (mg/kg fat) of sum DDT were 5.91 in subcutaneous fat, 4.86 in mothers milk, 2.75 in maternal serum and 1.87 in umbilical cord serum. β -HCH was only found in subcutaneous fat and milk with mean levels of 0.034 and 0.26 mg/kg fat, respectively. Dieldrin, endrin, and transnonachlor were detected in maternal serum but not in umbilical cord serum. Dieldrin detected in mothers milk and subcutaneous fat could not be quantified. There was a significant correlation between the levels of sum DDT in subcutaneous fat and milk fat (r=0.963), subcutaneous fat and maternal serum fat (r=0.843), and maternal serum fat and maternal milk fat (r=0.868), indicating the coherence of DDT in the body and demonstrates that human milk is a suitable indicator of monitoring organochlorine contaminants in the human body.

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CHAPTER ONE

INTRODUCTION

When God created man, he gave him the power to rule over the fish of the sea and the birds of the air, and over every living creature that moves on the ground (Genesis 1:26-28). Since creation then, man has interacted with his surroundings using what nature has provided. Initially, he lived as a hunter or nomad feeding off the land as he found it. When he tried to improve his way of life from hunting and food gathering to cultivation and harvesting, he created suitable conditions for the other consumers of his crops to proliferate. Some of these consumers later emerged as pests. His domestic animals and larger herbivores animals were potential pests of his crops but were restrained by use of fence and tether. However, there were other small animal pests, crop diseases and fungi which also needed his attention.

The discovery of chemical compounds which could be used effectively against many of the crop pests, must have looked like a final solution to all his problems. Pesticides, as these chemical compounds are known, are used against pests and diseases which compete with man for his agricultural land, his growing crops and his harvested produce. The early attempts to control pests chemically used naturally occurring toxic substances such as mercury, sulphur or plant extracts such as nicotine, pyrethrum or derris (rotenone insecticides). These were later followed by organic synthetic pesticidal compounds which dates from 1939 when the insecticidal properties of DDT (1,1,1-trichloro-2-bis (p-chlorophenyl)ethane were discovered
(Hill et al., 1978).

Mans endeavour to increase the food production to match that of human population and reduce crop losses resulting from pest attack by application of pesticides has resulted in undesirable consequences. After the second world war, there was extensive use of organochlorine compounds (OC), especially DDT, cyclodienes, aldrin, dieldrin and HCH (hexachlorocyclohexane). These compounds present a great threat to the environment. They are lipophilic and have the ability to persist in nature.

Due to their lipophility, they accumulate primarily in fatty tissues of fish, birds, and mammals (Brown, 1978). The prolonged use of these pesticides have also led to the establishment of resistant species, especially to cyclodienes. Also minor pests have become major pests owing to the elimination of their natural predators and new pests brought into being. In view of the potential health hazards many countries have banned the use of organochlorine compounds such as DDT and limited the use of others. It is important that such decisions and policy are based on scientific information rather than on emotional considerations. The ability to predict hazards, which may result from pesticide application in general, depends on proper judgement of the toxic properties of the compound like short and long-term toxicity, reproductive effects, mutagenicity and also the fate of chemicals in the environment, such as physico-chemical behaviour and biodegradability. The fate and the toxic potential of a pesticide may largely depend on the type of environment where the compound is applied. For the evaluation of the benefits relative to the

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potential adverse effects, it is imperative to know whether the use has resulted in the exposure of a given population to nonpermissible levels. If so, steps may be taken to reduce the levels. Many new pesticides, which are less likely to cause injurious environmental effects are continually being developed. However, a safer use of pesticides does not only depend on the development of new and more selective compounds. A considerable improvement may also be accomplished by better application and formulation techniques.

Despite the fact that organochlorine compounds have been used a lot in Kenya in the past and continue to be used, very few studies have been performed in this country to monitor the exposure and evaluate the potential adverse effects.

Human milk, due to its high fat content, is a suitable indicator for monitoring exposure and levels of persistent organochlorine compounds, and it is the main excretory route in lactating women. While the cow may excrete only 1.5% in the milk of its daily intake of DDT, women may excrete 125% of their daily intake (Knowles, 1974). Besides being a suitable material for assessing the exposure of lactating women and possibly the general population, special consideration should be given to the presence of organochlorines (particulary DDT) because of the possible deleterious effects on the developing infant (Farvar, 1974). Practically all organochlorine compounds are acutely neurotoxic and the accumulation in the central nervous system begins at a very early stage of its complex ontogeny. So far it has not been established whether this can impair the normal development and functioning of the nervous system. The main reason for this uncertainty is that with the

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presently available psychometric, biophysical and biochemical methods, the various functions of the central nervous system can be measured only very imprecisely. In contrast, several important functions of the liver can be measured with a sufficient degree of accuracy, and some of these are known to be affected by organochlorine compounds, e.g. they increase the activity of the microsomal enzymes that play an important role in the endogenous metabolism of, for example steroid hormones (Hayes, 1982).

Since the occurrence of DDT in human milk was first reported in 1951 (Laug <u>et al.</u>, 1951), numerous investigations on organochlorine pesticides in human milk have been carried out, but only few have been reported from developing countries where the use of organochlorine pesticides has been most extensive. From some places in developing countries, levels of DDT have been reported (Farvar, 1974), Hornabrook <u>et al.</u>, 1976, Winter <u>et al.</u>, 1976), which are two orders of magnitude higher than corresponding levels from industrialized countries of North America, East and West Europe. During the last 12-15 years the industrial chemicals PCRs (polychlorinated biphenyls) have been detected in most samples of human milk from the industrial countries (Jensen, 1983; 1987) while the developing countries so far have avoided the PCB contamination (Skaare <u>et</u> <u>al.</u>, 1988).

In an own pilot trial with five mother's milk samples collected randomly in a Nairobi hospital, the quantities of total DDT were found to range from 0.035 to 0.178 mg/kg milk. Compared with the WHO/FAO's Acceptable Daily Intake (ADI) of 0.005 mg DDT/kg body weight for humans and considering that a new born infant consumes about 0.6 liters milk per day and

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weighs no more than 3 kg, this corresponds to 7.1 times the ADI.

Other organochlorine compounds found in mother's milk include dieldrin, aldrin α - and β -HCH, lindane, HCB and PCBs. Because of the limited information on organochlorine compounds in human milk in developing countries, the urgent need for further studies has been stressed. In accordance with this, WHO/UNEP started a project to advance knowledge of the occurrence, distribution and significance of organochlorine compounds in humans with particular reference to the human milk from less developed parts of the world (WHO, 1979).

The objectives of the present research project were:

- To isolate, identify and quantify organochlorine pesticides in human milk.
- To generate reliable data on the contamination levels of organochlorine pesticides in human milk collected from selected Kenyan mothers living in different areas of Kenya.
- 3) To identify and evaluate the relative contribution of various sources for the occurrence of organochlorine pesticides in human milk, such as living conditions, agricultural activities and dietary habits. The sampling and analytical procedure were designed according to the recommendations for the WHO/UNEP project (World Health Organization/Food and Agriculture Organization. Report of Joint Expert Committee on Pesticide Residues. WHO Technical

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Report Series No.417, 1979). This should enable comparison and evaluation of the Kenyan results in relation to those from other parts of the world.

- 4) An investigation was also initiated to study the time trend of organochlorine pesticide contamination by collecting milk samples from the same mothers during a time period. Results of this kind should contribute to the knowledge of the biological half-lifes of persistent pesticides under tropical climatic conditions.
- 5) In addition, it was of importance to investigate if the levels of organochlorines in human milk really reflect the body burden of these pesticides, and to study the significance of organochlorine exposure of the infant <u>in utero</u>. Therefore, maternal adipose tissue, blood samples of mother and infant as well as milk sample were collected and analysed from 11 mother-infant pairs.

CHAPTER TWO

PESTICIDE USE AND PRACTICE IN KENYA

2.1 INTRODUCTION

Kenya encompasses an area of about 570.000 sq km with a population of about 23 million people. The estimated rate of growth is about 4% per annum.

The population of Kenya is dependent on agriculture to provide food and revenue. Most people are peasant farmers who grow their own subsistence crops with little plant protection being applied. Among the factors which limit food production are pests and diseases of crops and animals. This is influenced by natural and biological factors such as weather, soil conditions, parasites , predators, and by manipulation through cultural practices, such as planting time, cropping patterns and use of resistant varieties. Considering that pest damage may occur at any stage of crop growth and to any part of crop growth, a Kenyan farmer is faced with the difficult task of controlling these pests to avoid loss of quality and yield.

It is estimated that pests and diseases account for more than 30% yield loss in the field and 18% loss of stored products (Omamo, 1983).

Pesticides are used in an effort to increase the crop production, in food storage, and for control of vector-borne diseases with an objective to attain food self-sufficiency.

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Until recently, adequate attention has not been paid to their actual and potential side effects on human beings and other life forms. In fact the personal health of the farming community and consumers, and the risks of environmental pollution have been neglected to the benefit of food production per se. However, the fact that pesticides play a major role in increase of plant production cannot be denied. Table 2.1 indicates the average annual amounts of pesticide that have been used in Kenya from 1979 to 1983.

Table 2.1. Average annual amounts of pesticides used in Kenya (1979 - 1983)Pesticides Amount in Tons Insecticides 876 Fungicides 3610 . Herbicides 656 Acaricides 67 Nematocides 27 Fumigants 472 Rodenticides 12 Hormones 10 Insect attractants 1 Soil sterilants 18 Seed dressing 1 Biological insecticides 24

Source : UNEP Kenya state of Environment Report 2,1987.

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2.2 LEGISTLATION OF PESTICIDE USE IN KENYA

Modern agriculture depends heavily on use of agrochemicals. Most of these agrochemicals, e.g. pesticides, are not manufactured in Kenya and have to be imported. Legislation governing the use of pesticides in Kenya are applied through the Pest Control Product Act of 1982, which made provision for the Pesticide Control Products Board (PCPB) in 1983. This body regulates the importation, exportation, manufacture, distribution and use of products used for control of pests and/or of the organic function of plants and animals, and for connected purposes. Frior to the formation of PCPB, there was no registration of pesticides in Kenya. All pesticides to be registered are now studied and evaluated to ensure that it will not lead to unacceptable residue levels. The maximum residue levels (MRL) or tolerance limits for various crops are contained in Food, Drugs and Chemical Substance Act, Kenya Laws, Cap 254 (1980 revised).

Approximately 380 pesticidal compounds from major companies in Europe and United States have been considered for registration (Mutai, 1988, personal communication).

One of the major problems that face a developing country like Kenya with regard to the use of pesticides, is whether a chemical that has been banned from its country of origin should continue to be used. This is a difficult decision for those in authority especially where there is no toxicological data available to support the decision taken or where there is no appropriate substitute. One such chemical is DDT which has been banned in developed countries but is still in use in Kenya. Table 2.2 shows a list of pesticides that have been banned or restricted in other countries, but are still in use in Kenya.

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Table 2.2	PESTICIDES BANNED OR RESTRICTED IN OTHER COUNTRIES BUT STILL IN USE IN KENYA				
Name	Trade Name and use	Manufactured by	Effects	Regulation in Kenya	Countries where action taken
Azinphos Methyl	Gusathion Insecticide	Bayer	Tumorogen Toxic to beed Birds, fish and Wild life	Accepted	India
Endosulfan	Thiodan Insecticide	Hoechst	Suspect Carcinogen terratogen	Accepted	Phillipines
Methomyl	Lannate	Dupont	Embryo toxic Carcinogen	Accepted	Phillipines
Omethoate	Insecticide Folimat Insecticide	Bayer	condense seed	Accepted	Malaysia
Parathion	Parathion	Bayer	Damages Nervous System Embryotoxic	Accepted	Denmark, Finland India, Israel, Japan, Norway, N.Z., Phillipines, Sweden Turkey, U.K., USA
Parathion Methyl	Folidol Insecticide	Bayer	Teratogenic	Accepted	Hungary, Japan
Dinapracyl	Morocide Acaricide	Dupont	Carcinogenic	Accepted	Finland
Dimethoate	Rogor Insecticide & Acaricide	BASF	Mutagenic Affects Repro- ductive organs Fetotoxic	Accepted	U.S.A.
Oxythioguinox	Morestan Insecticide	Bayer		Accepted	India
Captan	Captan Fungicide	Chevron	Carcinogenic terratogen Mutagen	Accepted	Finland, Norway & Sweden
Methyl-Mercury	Arotane Fungicide	ICI	Persistent, Environmental impact Health Hazard	Accepted	European Community, Argentina, Denmark, Finland, Israel, N.Z. Sweden, Turkey
Thiophanate	Topsin+M	Nippon Soda (M)	Persistent, Environmental impact Health Hazard	Accepted	Finland

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Name	Trade Name and use	Manufactured by	Effects	Regulation in Kenya	Countries where action taken
Benomyl	Benlate Fungicide	Dupont	Carcinogenic	Accepted	Finland
Maneb	Dithane Fungicide	BASF Rohm & Hass		Accepted	USSR
Paraquat	Gramoxone	ICI	Poisoning hazards	Accepted	Denmark, Finland, Israel, N.Z., Phillipines Sweden, Turkey
2,4-D	2,4-D Amine	ICI	Affects liver Tumorogen Terratogen, Mutagen	Accepted	Colombia, Guatemala USA
Captafol	Captafol Fungicide	Chevron	Carcinogenic	Accepted	U.S.A. Tanzania, W.Germany, LV.K
DDT	DDT Insecticide	ICI Roger Foyd' herbe	Carcinogenic Accumulation in food chain	Accepted	Canada, Colombia, Cyprus, Denmark, Finland, Guatemala, Hungary, Israel, Japan, Mauritius, Norway, N.Z. Phill- ipines, Sweden, Thailand, Turkey, West Germany, U.S.A., USSR
Dieldrin	Dieldrin Insecticide	May & Baker	Oncogenicity Fetotoxic Terratogenic Toxic to Wildlife Harz -adous to humans	Accepted	Canada, Columbia, Cyprus, Denmark, Finland, Hungary, Israel, Japan Norway, Mouritus, N Pakistan, Phillipines Sweden, Turkey, W. Germany, USA, USSR
Methyl bromine	Methyl bromine Insecticide	Rentokil Robert Lemaire H.Henry & Co.	Highly toxic	Accepted	Phillipines
Phosphine generating HCN	Phosphine generating HCN	Rentokil H.Henry & Co	Highly toxic	Accepted	Phillipines
Dicrotophos	Dicrotophos	Health Hazard	Health Hazard	Accepted	India Malaysia
* Waiyaki et al	Disystcn . 1988	Bayer	Highly toxic	Accepted	India

cont: 9a

1.1

2.3 PESTICIDE TRADE IN KENYA

The most important types of pesticides imported for use in the country are insecticides, fungicides and herbicides. The import budget on pesticides has increased from K£ 13 million in 1981 to K£ 26 million in 1987 (Table 2.3), which reflects an increase in use of pesticides every year, as more farmers are becoming aware of the use of pesticides.

Most of the pesticides used in this country are imported from major companies in Europe and United States where they are already formulated. Only about 20% of all pesticide used are formulated locally.

The formulation and packing of agrochemicals in Kenya is undertaken by the major firms like May & Baker, Twiga Chemicals, Shell Chemical and Hoechst E.A. Ltd.

The Pesticides Chemicals Association of Kenya (PCAK), which was formed in 1959 to provide a forum for the agrochemical industry, maintains contact with the Government in order to put forward the views of its members and develop an acceptable strategy for the industry.

Table 2.3. Amount of pesticides imported directly into Kenya during the period 1983-1987^{*}.

Insecticides

Year	Quantity (Kg)	Value - Ksh
1983	2,074,877	152,107,512
1984	1,648,750	148,386,337
1985	1,615,931	156,154,090
1986	2,213,620	213,651,807
1987	1,588,994	189,245,834

Fungicides

Year	Quantity (Kg)	Value - Ksh
1983	2,255,657	120,577,547
1984	2,128,618	101,538,530
1985	1,982,813	115,616,402
1986	2,173,457	166,807,438
1987	3,174,738	211,133,270

Herbicides

Year	Quantity (Kg)	Value - Ksh
1983	1,306,825	77,338,915
1984	1,334,208	73,673,881
1985	943,657	87,759,418
1986	1,016,578	100,253,714
1987	1,068,590	123,553,602

Import Budget on pesticides from 1981 - 1987

1981	K£	13	million
1982	K£	20	million
1983	KE	17	million
1984	K£	16	million
1985	KE	18	million
1986	K£	24	million
1987	KE	26	million

*Source : Annual Trade Reports, 1981-1987. Customs and Excise Dept. Ministry of Finance.

2.4 PROPORTION OF TOTAL PESTICIDES USED IN VARIOUS MAJOR CROPS IN KENYA

Pesticides form an integral part of the current farming system and are used on a wide range of pest problems in Kenya, both on pests and disease causing agents.

About 33% of the farmers, predominantly in the large farm sector, use pesticides. In the small farms (mostly subsistence farming) there are minimal use of pesticides. Cash crops, such as coffee, takes about 50 per cent of the pesticides in use on the national scale, while horticultural crops receive about 20 per cent of the pesticides imported. Fungicides are the most important pesticide used in the production of coffee. In 1987 (Table 2.3) fungicides had the largest total percentage market share followed by insecticides and herbicides.

Other important crops that have taken a large share of pesticides are cotton, sugarcane, maize and tea.

Herbicides, which are used as a substitute to mechanical or hand weeding, has also been greatly used by coffee, maize, barley, wheat, sugarcane and tea farmers. The amount of pesticide used in coffee production seems to be proportional to the high coffee yields as it is the most

2.5 PESTICIDES USED IN LIVESTOCK IN KENYA

important crop in Kenyan economy.

The most important vectors of a variety of disease agents in domestic animals are ticks which cause the greatest loss of livestock. Different acaricides have been used to control ticks, but although they are all said to kill ticks, they differ in their rate of kill, residual periods, stripping rates, stability and safety. The use of acaricides in Kenya is well organized to avoid the influx into the market of all available acaricides which would otherwise result in development of resistance to these acaricides at the same time. Sodium arsenite was the only acaricide in use in Kenya, from 1912 to 1949, to control serious livestock diseases such as East Coast Fever (Keating, 1983). Benzene hexachloride (BHC, hexachlorocyclohexane, HCH) was introduced in 1949. The development of strains resistant to arsenic and HCH led to the increased use of toxaphene, a chlorinated camphene which was introduced in 1950, and by 1956 it was the major acaricide in use. This was due to its stability in dip washes and prolonged residue effect. Other organochlorine pesticides like DDT and dieldrin were introduced in 1956 and 1961, respectively, but due to the development of tick resistance, the organochlorine acaricides were banned in 1976. Organophosphorus compounds, such as Delnav (dioxathion) and coumaphos (Asuntol) were introduced in 1959. These organophosphorus compounds were often used in combination with arsenic, HCH, and toxaphene. Some acaricides which are still in use in Kenya, include carbaryl (Sevin), quintiofos (Bacdip), chlorfenvinphos (Supona), coumaphos and formamidines. Since some pests have also been reported to have become resistant to organophosphates, combinations with organochlorine pesticides have been introduced. Most of the farmers have their cattle washed in cattle dips since this is more economical and they are helped to maintain the correct strength of the chemical in the dip by testing the concentration of the particular acaricide used by Veterinary Research Officers.

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2.6 PESTICIDES USED IN PUBLIC HEALTH INCLUDING HOUSEHOLDS INSECTICIDES

Public health has benefited from effective control of vector borne diseases through effective control of pests, such as mosquitoes and water snails. The WHO programs to eradicate pests, such as mosquitoes, tsetse and other disease vectors of malaria, sleeping sickness, bilharzia etc. have made it possible for people to settle in such areas as Mwea/Tebere Settlement Scheme in the Central Province, the Kano Plains and the Lambwe Valley in Nyanza Province.

Also occasionally there have been attacks of army worms on vegetation which have been successfully controlled and eradicated by the use of pesticides.

In Kenya today, more than 30 different products are sold over the counter for the control of household pests. The most common household pests are flies, cockroaches, fleas, lice, ants, bedbugs, mosquitoes, rats and mice. The insecticides available include x-Pel, Niltox, Pips, Target, Killtox, Trig, and Strike (Trade names), among others. Some of these insecticides are in powder form, others as aerosols in bottles, while others are in solid form, e.g. mosquito coils. These household chemicals are used indiscriminately in the homes as most consumers do not know any other effect except that of

killing the nagging pest. It is not always easy to know the active component of the insecticide product, as not all the manufacturers indicate on the bottles or other containers what is the active chemical.

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2.7 DISTRIBUTION OF PESTICIDES IN KENYA

The agrochemical industries have mainly been responsible of distributing pesticides in Kenya. The main importers before independence included Mackenzie Kenya Ltd., BEA Corporation (affiliated to Overseas Trading Organisations), and the Kenya Farmers Association (which is presently known as Kenya Grain Growers Cooperative Union, KGGCU), (Mbatha, 1988). The chemicals were mainly used in plantations, estates and large farms owned by companies or individual farmers.

With the sub-division of these large farms after independence, the distribution of pesticides now involves more people and has become more complex. The representatives of the overseas manufacturers are involved in the importation of pesticides. They are also the main distributors and are responsible for supplying pesticides to the large scale and estate farmers and also provide continuous supply to the stockist shops in the country. There are also other distributors with a lower rank than the main distributors.

Most farmers in Kenya buy their pesticides from either stockists, distributors or through their Cooperative Societies and Unions. The latter is more favourable to the small scale farmer, especially if he is a member of the society, since there are credit facilities available to members on condition that their produce acts as security and is sufficient to cover the loan so acquired.

2.8 PESTICIDE HANDLING AND USE BY FARMERS IN KENYA

When buying pesticides, farmers are easily confused by the abundance of different chemicals which might be on the market

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at any one time. Differing advice and promotional literature are presented to him from various chemical manufacturers, each claiming its product to be the best. However, the farmers choice in many cases depends on the cost with some justification, that all the available products must work otherwise they wouldn't be on the market. In assessing the cost, he is not aware that different products contain different concentrations of active ingredient, and employ different usage rates, and it may be that the most expensive product is in fact the cheapest one to use. The other factors that influence the farmer's choice of product are what the other farmers are using, especially well informed and successful practising farmers, agricultural extension agents, size and nature of the packing materials, especially if the containers can be re-used, and availability of the product.

2.8.1 Delivery of pesticide chemicals to the farmer Most of the distributors do not deliver the chemicals to the farmers, especially if they are few. So the farmer has to make his own delivery arrangement. In many cases, public transport is used.

2.8.2 Storage

Most farmers do not have special storage facilities and thus it is not uncommon to find pesticides stored in a store where other household goods are kept or in a store where other farm produce are stored. Where there is no store, they are kept somewhere within the house.

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2.8.3 Applicators

Commonly used applicators in Kenya are tractor mounted sprayers and knapsacks. Aircraft mounted sprayers are used specially when controlling outbreak of army worms, tse tse flies or locusts. Cattle dipping is also widely practiced. Unorthodox applicators include flywisks and specially bound leaves/brooms (Nyaga, 1988). Since not every farmer can afford to buy an applicator, some farmers do borrow applicators from their neighbours.

2.8.4 Waste disposal

Although the containers should be burned or burried deep in the soil, most farmers find alternative use for them. Some of these containers have been used for foodstuffs, milk or drinking water.

2.9 DANGERS POSED BY USE OF PESTICIDES

When using pesticides the risks of adverse effects must be weighed against benefits which the pesticide produce. In some cases it may not be necessary to use the chemicals, especially where cultural or biological methods of control are effective. However, farmers are known to have been illusioned by some manufacturers that use of pesticides is a guarantee of good quality and high yield crop.

During spraying over wide areas, some drift and build up in the atmosphere, and may be hazardous to beneficial insects such as bees, wild animals which feed on the crop, and also to other smaller organisms. Other residues are washed by the rain into rivers, lakes, estuaries, and harbours and may adversely affect fish and other aquatic life. The problem of pesticide misuse in this country is aggravated by the fact that most of the farmers are illiterate and therefore instructions on a particular pesticide may not be followed. The language used on the labels do not always communicate to all the farmers. Swahili is mostly understood by many farmers but wherever possible vernacular language may be the best. Hence, interpretation of useful information on the labels may be difficult also for the farmer, especially where units of measure are not common. Furthermore, farmers may not be aware of changes of crop protection chemicals.

The actual figures of pesticide poisoning in Kenya may not be known, but death through ingestion of contaminated food and suicide by exposure to pesticides have been reported in our local newspapers. Here are some examples: Cases reported in Daily Nation, Friday, October 1986.

- A woman put a packet of powder pesticide on her food shelf. She mistakenly mixed up the content with maizemeal while preparing "Ugali" for her family. She and two of her children died.
- A family ate food previously stored in pesticide drums. They all fell seriously ill.
- Some people think that pesticides are medicine and try to use them to treat skin diseases.
- Unscrupulous fishermen use the chemicals to kill fish, which are then sold to unsuspecting consumers.
- A worker was overheard boasting to his friends that he knew all the pesticides kept in the store. All he did was identify them by their taste.
- In the field, plantation workers have been known to strip down to their shorts, leaving their arms, legs and chests bare while spraying pesticides.

Problems associated with pesticides use in Kenya.

- One of the major problems which has also been felt worldwide as a result of continued use of pesticides, is the emergence of super pests which are resistant to pesticides.
- Lack of personnel and funds to test pesticides locally and to ensure that private companies are adhering to the regulations as stated in the Pesticide Control Products Act.
- 3. Lack of proper knowledge about pesticides leading to indiscriminate use of them. Farmers may not be able to identify banned or restricted pesticides. Some of these chemicals find their way back to the shelves and are available to the farmers.
- Pesticides are used, which have been banned in other countries.
- 5. Lack of proper protective clothing during spraying.
- 6. Many a time, disease signs and symphtoms caused by pesticides are not diagonised, and if they are, the proper therapy or prophylaxis may not be instituted.

2.10 ENVIRONMENTAL PROTECTION

Bodies regulating the pesticide use in Kenya.

 Kenya Environmental Secretariat, which is a coordinating body with regard to all matters pertaining to the protection of our environment. This body provides the linkage between Kenya and various international organisations like UNEP, FAO and WHO, through which important information and policy guidelines are formulated. In this connection the agricultural industries are required to implement the FAO code of conduct on the distribution and use of pesticides.

- 2. Pesticide Chemicals Association of Kenya (PCAK). This body incorporates most of the manufacturers of agrochemicals in this country. The main objectives of the association are to ensure that members ascribe to the ethical objectives as they relate to safety, packaging, labelling and use of these products.
- 3. Pest Control Product Board of Kenya. This is a statutory organization of Kenya government established under the Pest Control Products Act, cap.346 of the Laws of Kenya. Its aim is to streamline and control the use of pesticides in the country at all stages viz. importation, transportation, labelling, formulation, storage, usage and disposal. It also investigates the role of pesticide and their impact on the environment and also collaborates with international organizations (e.g.WHO, FAO etc.).

Through mass media, people are becoming aware and concerned about the effects of pesticides both to the environment and public health. In Table 2.4 is listed the pesticides which have been banned or restricted for use in Kenya.

Chemical name	Trade name	Use	Action
Dibromochloropropane	Nemagon	soil fumigant	banned
(DBCP)			
Ethylene dibromide	EDB	soil fumigant	banned
(EDB)			
2,4,5-T	2,4,5-T	herbicide	banned
Chlordimeform	chlordimeform	acaricide/-	banned
		insecticide	
Chlordane	chlordane	insecticide	banned
Toxaphene	toxaphene	acaricide	banned
Endrin	endrin	insecticide	banned
Aldrin	aldrin	insecticide	restricted
Dieldrin	dieldrin	insecticide	restricted
DDT	DDT	insecticide	restricted
∦ -нсн	lindane	insecticide	restricted

Table 2.4 Pesticides banned or restricted in Kenya

The banning of these chemicals had been accentuated by their effects on the environment. The organochlorine compounds have been found to persist in nature and their residues have been detected in food and human samples. Others, such as DBCP, EDB and 2,4,5-T, have been banned because of carcinogenicity or due to high acute toxicity.

2.11 CONCLUSIONS

Pesticides have played a major role in the increase of crop production and in the control of vector borne diseases. At present the benefits versus risks may not be clearly understood. Since development is no longer only regarded as an increase in the national income of a country, all the factors that influence the quality of life and reduce absolute poverty should be incorporated. There is a great and urgent need to inform people at all levels about the benefits and hazards brought about by use of pesticides, which should be a concern for all those in the pesticide industry and the ministries involved. The language clearly understood by the local people should be used on the labels. Chemical screening, pesticide research and toxicological studies need to be more emphasized and practically demonstrated. All pesticides in use should be monitored and their toxic effects scrutinized. Presence of pesticide residues in horticultural crops indicate that preharvesting interval should be observed strictly for safe harvesting. With more rational use based on biological and economic studies, smaller quantities of pesticides could be used and the benefits could be correspondingly greater and the hazards correspondingly smaller.

CHAPTER THREE

ORGANOCHLORINE PESTICIDES I.ITERATURE REVIEW

3.1 INTRODUCTION

Pesticides are generally defined as chemical substances used to control pests. These include insecticides, herbicides, acaricides, fungicides, rodenticides, fumigants etc. The rising production of these substances is a measure of their value in agriculture in controlling disease-bearing pests and in other uses. Before the second world war pesticides were of two types: inorganic ones like arsenicals and fluorides, which were most commonly used, and those of plant origin, such as poisonous nicotine products. The development of organic synthetic pesticides began after the discovery of DDT as an insecticide by Müller in 1939. This stimulated the search for other synthetic chemicals with similar properties which led to the production of other organochlorine pesticides, viz aldrin, dieldrin, and other cyclodienes.

Organochlorine pesticides havebeen in use for more than three decades in agriculture and in health programs all over the world. Due to their persistence in nature, they have come into disfavour. Research done in many parts of the world has led to the knowledge about the presence, amount, and distribution of pesticides in the environment in general. An important feature of these lipophilic pollutants is their ability to become concentrated along food chains, reaching higher concentrations at higher trophic levels due to their great chemical stability, low aqueous solubility and high lipophilicity (Muthanna <u>et al.</u>, 1986). Thus, they may reach the human body through the daily diet (Adamovic <u>et al</u>., 1978; Noren, 1983; Kaphalia <u>et al</u>., 1984). Their tendency to accumulate in fatty tissues has caused significant residue burdens in adipose tissues (Wassermann <u>et al</u>., 1972; Saxena <u>et al</u>., 1981; Saigal <u>et al</u>., 1985), blood (Cariati <u>et al</u>., 1983; Kaphalia <u>et al</u>., 1983); and even human milk (Jensen, 1983; Slorach and Vaz, 1983). These chlorinated compounds have also been found to have access to the growing fetus through the placenta (Eckenhausen <u>et al</u>., 1981; Saxena <u>et al</u>., 1981; Lewerenz, 1982; Ando <u>et al</u>., 1985), and have been detected in cord blood (Eckenhausen <u>et</u> <u>al</u>., 1981; Cariati <u>et al</u>., 1983; Skaare <u>et al</u>., 1988). There are also reports on the presence of organochlorine pesticides in tissues of stillborn infants (Eckenhausen <u>et al</u>., 1981).

Because of these findings, use of many organochlorine compounds, especially DDT, has been banned in developed countries, viz Norway (Skaare, 1981), while other countries have restricted their use. However, organochlorine pesticides are still in use in many developing countries, including Kenya. The presence of organochlorine compounds in the adipose tissue of people from Kenya was first demonstrated by a study carried out by Wassermann <u>et al</u>. (1972). They reported that these chemicals were stored in the adipose tissue of people who had no occupational exposure to organochlorine insecticides, which indicate contamination through other sources. No further study has been done to find out if the organochlorine levels in Kenyan population has increased due to continued use of these chemicals. However, the influence on the levels of organochlorines in the human body by other factors, such as age

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(Wassermann et al., 1972; Hruba et al., 1984); sex (Wassermann et al., 1972; Ramachandran et al., 1984; Siddiqui et al., 1985), dietary habits (Norén, 1983; Kaphalia et al., 1985), and living conditions (Warnez et al., 1983; Siddiqui et al., 1985) has been demonstrated. The concern for the potential health hazard of organochlorine pesticide accumulation in the human body initiated intensive research activities on the toxicology of these compounds. Thus, these chemicals have been found to be potent hepatic microsomal enzyme inducers which may quantitatively alter the response to various drugs and toxic compounds as well as to naturally occurring substances in the body (Wassermann et al., 1974). This may lead to alteration of homeostasis of biochemical (endocrine, immunologic etc.) processes (Wassermann et al., 1974). Furthermore, the experimental evidence of the capacity of organochlorine pesticides to increase tumor incidence in laboratory animals has also been reported (Wassermann et al., 1974; Hayes, 1982). However, the only way the levels of these contaminants can be reduced in human bodies is by reducing the sources of environmental pollution. This is supported by the reports of reduction of levels of DDT in the environment and is also reflected by the low levels of DDT in mother's milk after this insecticide has been banned in industrialized countries (Skare, 1981, 1988; Slorach and Vaz, 1983). Since the effects of long term exposure of people to these contaminants is not certain, it is imperative that measures to reduce environmental contamination be undertaken.

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3.2 ORGANOCHLORINE PESTICIDES

3.2.1 DDT group

DDT has a molecular weight of 354.50. In pure form it is a white, tasteless, almost odourless crystaline solid melting at 108.5 ^O to 109 ^OC. Technical DDT which is commonly used as an insecticide is a waxy solid and has the following composition: p,p'-DDT, 77.1%; o,p'-DDT, 14.9%; p,p'-DDD, 0.3%; c,p'-DDD, 0.1%; p,p'-DDE, 4.0%; o,p'-DDE, 0.1%; and unidentified compounds 3.5%. The vapour pressure of DDT is 1.5×10^{-7} mm Hg at 20 °C. It is highly fat soluble but insoluble in water. DDT was first synthesized by Zeidler in 1874, but its insecticidal properties were discovered by Müller in 1939. After which it was extensively utilized due to its conspicuous benefits in terms of agricultural output and public health. This led the World Health Organization (WHO) to adopt strategies towards the eradication of malaria using DDT. Its success was due to its chemical stability, low cost and its low acute toxicity. The discovery in the 1960s of its accompanying environmental hazards caused by its high persistence, chronic toxicity and the ability to bioaccumulate resulted in restrictions or bans of the major uses in most industrialized countries by 1970 (WHO 1979^a), while its use continues in the developing countries. DDT is metabolised to DDE in many species including man. The detection of DDE in human fat was first demonstrated by Pearce, Mattson and Hayes in 1952 (Hayes, 1982). It is now known that some of our food contains DDE, but that man is also capable of forming the product from DDT (Hayes, 1982).

3.2.2 Cyclodiene group

Aldrin, dieldrin and endrin are persistent organochlorine insecticides with a higher acute toxicity than DDT. Dieldrin is an oxygenated metabolite of aldrin, and it is more persistent. The use of these compounds as insecticides have been banned in most industrialized countries, but they are still used in developing countries. These compounds have been detected in human milk (Jensen, 1983) and in human food samples (Ackerman, 1980; Mugambi, 1986).

Heptachlor, heptachlor epoxide and chlordane are closely related chlorinated insecticides. Heptachlor epoxide and oxychlordane are very persistent epoxy metabolites of heptachlor and chlordane, respectively. Heptachlor epoxide and transnonachlor both occur as impurities in technical grade chlordane. These pesticides have not been extensively used as compared to DDT, but their residues have been detected in human milk, adipose tissue, and food samples (Jensen, 1983).

3.2.3 HCH group

The commercial insecticide HCH is a mixture of the different isomers, viz: \ll -HCH, β -HCH, χ -HCH and δ -HCH, and other impurities. The χ -HCH isomer, also known as lindane, is the most acute toxic isomer, often used as a substitute for DDT. It is more volatile and significantly more soluble in water than DDT.

The β -HCH isomer, which is most stable and environmentally persistent, has the highest chronic toxicity(Jensen, 1983). Both β -HCH and χ -HCH have been detected in human samples, but

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 β -HCH has a higher ability to accumulate in fat tissues than has χ -HCH (Jensen, 1983). \sim -HCH and χ -HCH are rarely detected in human tissues and milk. The highest levels of β -HCH and lindane in mothers milk have been reported in Japan and India (Jensen, 1983).

3.3 INDUSTRIAL CHEMICALS (FCBs)

The polychlorinated biphenyls (PCBs) are mixtures in which chlorine substitutions can occur at ten sites in the biphenyl molecule. Up to 210 isomers are assumed to be possible to form in a mixture. They are widely used in industry as plasticizers, insulators, heat exchange fluids and lubricants. As with chlorinated hydrocarbons, the PCBs are chemically stable and lipophilic and are therefore persistent in the environment and also tend to accumulate in food chains. They were first detected in fish and wildlife by Jensen (1966). Since then, their residues have been reported in human fat (Brevik et al., 1978; Niessen et al., 1984; Ando et al., 1985), in foods (Yakushiji et al., 1977; Fytianos et al., 1985) and in samples of human milk (Jensen, 1983; Kimbrough, 1987). PCBs contamination are mainly found in industrial countries. Their occurrence in the environment in these countries presently seems to be at higher extent than chlorinated hydrocarbons.

3.4 TOXICOKINETICS OF ORGANOCHLORINE PESTICIDES

3.4.1 Absorption

The form in which the organochlorine pesticide occurs has a marked influence on its absorption, but all organochlorine

pesticides can be absorbed through the skin, the respiratory system and through oral routes. Absorption by means of the respiratory system depends on the concentration of the compound (in gaseous form) in the inhaled air, and rapid absorbtion through the alveolar epithelium to plasma may occur. Most insecticides are in form of mists or dusts including aerosols. The particle size determines the degree of retention of the inhaled compounds at different levels of the respiratory system. Large particles precipitate in the nose, in the trachea and in the bronchi, from where they are transported by ciliary epithelium in the direction of the throat and then are usually swallowed. Absorption may then take place from the gastrointestinal tract and not from the respiratory system. Dermal absorption is an important route of absorbing organochlorines into the body. Conditions, such as humidity of the skin, temperature, and contact between clothing and skin also influence absorption.

3.4.2 Distribution

After absorption the organochlorine compounds in the blood stream penetrate through the various lipid membranes by diffusion. Because of their high fat solubility they are distributed and concentrated in fat tissues throughout the body. Since the discovery of the environmental contamination with chlorinated compounds, DDT and its metabolite DDE have been detected in almost all the human samples studied.

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3.4.3 Storage depot/accumulation

The body tissues where these organochlorine pesticides are concentrated, are referred to as storage depots. The storage in subcutaneous fat may prevent high concentrations of these compounds in more vulnerable tissues. There is an equilibrium between the organochlorine substance in storage depot and the free substance in the plasma. When it is metabolized or excreted, more will be released from the storage site.

The tendency of the chlorinated insecticides to store in adipose tissue is determined by the rate of metabolism and subsequent excretion (Hayes, 1982). DDT and/or its metabolite DDE is stored in everyone studied unlike the cyclodienes. One of the highest reported levels of DDT in the fat of a healthy man (pesticide formulator) was 648 ppm (Laws et al., 1967). Storage levels of volunteers given daily dosages of 35 mg of DDT reached mean concentrations of 281 ppm after 21.5 months (Hayes Jr. et al., 1971). No volunteer complained of any symptoms or reported any adverse effects on health. Accumulation of these compounds which are resistant to metabolic degradation, such as DDT, aldrin and dieldrin, occurs as a result of chronic exposure to low level concentrations. Exposure to these chemicals can be direct e.g. occupational exposure, or can be indirect through consumption of foods containing residues of these chemicals. Accumulation along a food chain has been investigated and it has been noted that such accumulation can be fatal to humans. Accumulation of these compounds is a potential danger since they can be mobilized, especially during starvation. Thus, their concentrations can reach toxic levels in the plasma.

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3.4.4 Excretion

Since the organochlorine substances are highly lipophilic, they are not easily excreted. They are eliminated from the body by various routes viz through the kidneys, the liver, the biliary system, the lungs, and by milk excretion, partly after metabolism to more water soluble compounds. DDA has been found to be the major urinary metabolite of p,p'-DDT and o,p'-DDT in all mammals including man (Morgan <u>et al</u>., 1974; Spindler, 1983). Urine, collected from workers whose DDT intake was 35 mg/man/day, contained an average of 1.71 ppm DDA with a range from 0.12 to 7.56 ppm (Hayes, 1982).

Faecal excretion

Measurement of organochlorine loss through faecal excretion has not been achieved, perhaps because of formation of water-soluble conjugates that are difficult to isolate and measure (Morgan <u>et al.</u>, 1974), or they are not excreted to any important degree (Hayes, 1982). However, faecal excretion may include DDT remaining unabsorbed after ingestion as well as DDT and metabolites mobilized from storage.

In rats, faecal excretion of DDT exceeded the urinary excretion irrespective of the route of administration (Hayes, 1982).

Excretion with milk

Excretion of organochlorine substances into milk is important because of the transfer of the contaminants from the mother to the nursing child. Similarly these contaminants are passed from cows to humans.

Milk contains 3-5% fat, and is therefore a good solvent for the organochlorines which are lipophilic and hence can concentrate in milk. Thus, milk becomes a major route of excreting these compounds as well as PCBs from the body.

Women exposed to organochlorines excrete more DDT than those without any known exposure (Winter <u>et al</u>., 1976; Stacey <u>et al</u>., 1985). The concentrations of organochlorine insecticides and PCBs in the milk of women in various general populations have been reviewed by Jensen (1983) and Spindler (1983).

3.4.5 Biological half-lives

After the organochlorine compounds are absorbed into the body, they are retained to some extent. The time the compound takes to decrease to half its original concentration is known as the biological half-life for that compound. The organochlorines, which are lipophilic and undergo only very slow biochemical conversion have long half-lives. These compounds are stored in adipose tissues and are slowly released into the plasma and the level is thus sustained over a long time. The extent of exposure and concentration, and the half-life of the compound will determine whether a substance will accumulate in the body. 3.5 SOME TOXICOLOGICAL ASPECTS OF ORGANOCHLORINE PESTICIDES3.5.1 Acute toxicity

Acute poisoning by organochlorine pesticides is encountered when these chemicals are ingested directly in case of suicide or accidentally, or due to mishandling of the pesticides. All pesticides are poisonous but they vary enormously in their toxicity. The World Health Organization has classified pesticides according to their LD₅₀, which is the lethal dose in milligrams per kilogram of body weight needed to kill 50% of the test animals. DDT which has been widely used, is termed moderately toxic whereas others like endrin and dieldrin are extremely hazardous. There are increasing numbers of pesticide poisoning reported in our local newspapers, but unfortunately there appears to be no official figures available on the total number of deaths caused by pesticides. Waiyaki <u>et al</u>. (1988) report that there are at least two cases of pesticide poisoning daily at the Kenyatta National Hospital in Nairobi.

3.5.2 Chronic toxicity

Chronic toxicity occurs as a result of ingestion of small quantities of toxic chemicals over a long period of time leading to toxic concentrations and thus to symptoms of poisoning. Because of the chemical stability of the organochlorines, low level residues have been detected in the environment and in particular foodstuffs. This has led to bioaccumulation in various organisms including man. Of particular interest is exposure of foetuses to organochlorines, as well as infants and young children, because of the vulnerability and immaturity of their developing biological system which makes them to be at a higher risk than adults.

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3.5.3 Effects on reproduction/teratogenic effects

The effects of the various organochlorines on reproduction seems to vary with the different species of test animals investigated (Hayes, 1982). This variation makes it difficult to extrapolate animal results to humans. The foetus may be more susceptible to a pollutant than the mother. The effects of these chemicals on the foetus vary from lethality to malformations and retardation of growth. Thus, the effect on reproduction is manifested on fertility, gestation, and on the offspring. DDT had been connected with abortion in humans and dairy cattle. However, no significant relationship was established (Hayes, 1982). Saxena et al. (1983) also found higher levels of organochlorine insecticides in maternal blood, placenta, and umbilical-cord blood of stillborn cases than in liveborn cases. The results of animal experiments show aldrin/dieldrin and p,p'-DDT to be involved in stillbirth (Saxena et al., 1983). Organochlorine chemicals, such as DDT, have been found to adversely influence the development of the foetus in experimental animals leading to malformation of the foetus (Hayes, 1982). The foetus depends on the maternal organism for growth and maintenance. The placenta acts as a partial barrier to the transfer of organochlorines from the mother to the foetus (Lewerenz, 1982). The type of effects the chemical have on the foetus depend on concentration and duration of the organochlorine exposure. The effects may be modified by other factors, such as dietary deficiency, age, viral infection etc.

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3.5.4 Carcinogenic effects

DDT and other organochlorine insecticides have caused marked changes in the livers of various test animals, e.g. rodents and mice, and these changes progress to tumour formation (Hayes, 1982). Whether this also applies to humans, has not been undoubtedly confirmed.

3.5.5 Behavioral effects

When rats were fed DDT at levels too low to produce illness, ataxia in form of changes in gait was observed (Hayes, 1982). The animals were also less reactive to stress than normal ones.

The difference between DDT and its metabolites and other chlorinated organochlorine insecticides at toxic level, is that tremor is the first sign followed by convulsions while for aldrin, dieldrin, toxaphene, HCH etc., convulsion is the first sign.

The organochlorines have been demonstrated to stimulate the nervous system. The degree of stimulation is proportional to the concentration of the compound.

3.5.6 Enzyme induction

When insects are exposed to organochlorines, microsomal oxidase enzymes may be induced as an adaptive response. Thus, houseflies fed with sublethal levels of dieldrin or DDT develop

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increased levels of microsomal enzyme activity, so that they are capable of metabolizing not only the inducing insecticide, but those of chemically unrelated groups (Corbett, 1974). Induction of the microsomal enzymes is valuable to the insect in that it is protected against the insecticide which it encounters at sublethal concentrations. This response is different from the one which leads to resistant strains. The latter one is an inheritance of the ability to avoid the harmful effects of the insecticide, possibly by degrading it and passing this capacity on to their offspring.

3.6 ENVIRONMENTAL ASPECTS OF ORGANOCHLORINE PESTICIDES

The introduction of chemical weapons e.g., the organochlorines against insects, has affected the surrounding environment due to the non-selective nature of these chemicals. The pollution of air, water and soil and its effects on other living organisms including man that coinhabit the environment, is a cause for concern, as these chemicals are gradually accumulated in the ecosystem. The way in which a pesticide degrades after application is significant for its potent effects on the environment. The organochlorines are slowly metabolized and some of the metabolites (e.g.DDE, a metabolite of DDT), are extremely resistant to further degradation. Furthermore, some metabolites are more toxic than the parent compound (dieldrin, which is a metabolite of aldrin), is more toxic than aldrin (O'Brien, 1967). In addition, the partition coefficient of these organochlorine insecticides in fat soluble substances relative to aqueous media, is very high. Thus, they are concentrated through food chains as they tend to partition into lipoidal biological materials in increasing concentrations,

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reaching a potentially hazardous concentration at the top of the food chain. The impact the use of organochlorines have had on the environment in different parts of the world, has been documented by many researchers.

In Kenya residues of dieldrin were detected in the brain and liver of non-target wild animals in Lambwe valley after aerial spraying against tsetse flies (Allsopp, 1978). Lincer <u>et</u> <u>al.</u>, 1981) observed bioaccumulation of DDE in various lake food chains in lake Naivasha and Baringo in Kenya. Some people have complained of stomach problems after eating fish sold by vendors near Sagana bridge in Kenya. These fish are suspected to be contaminated with pesticide chemicals which are used in the surrounding coffee plantations which are drained eventually into the river (Waiyaki, personal communication). Although low levels of organochlorines has been reported in Kenyan environment (Koeman <u>et al.</u>, 1972; Wasserman <u>et al.</u>, 1972; Maitho, 1978; Lincer <u>et al.</u>, 1981), the use of these chemicals should be minimized not only because of their persistence in nature, but also because of insect resistance after prolonged use.

3.7 BENEFITS VERSUS RISKS OF ORGANOCHLORINE PESTICIDE USE When using pesticides, benefits should always be weighed against risk of injury to human health and to contamination of the environment.

Loss of food crops to insects or other pests with increase in population would obviously lead to starvation. The use of pesticides in Kenya indicates an advantage in that there has been increased food production and improved health of the people. In malaria prone areas, the use of DDT has saved many lives (Spindler, 1983). This also applies to animals, especially in the control of ticks. In some cases the use of pesticides has made areas habitable and recreational places more comfortable.

On the other hand, pesticides of various types have been used indiscriminately endangering the very lives they were intended to protect. Cases of poisoning of birds, fish and wildlife have been reported. Furthermore, elimination of desirable species may lead to ecological imbalance in nature which may be more difficult to handle, e.g. the continuous emergence of resistant species. There is also rather large use of pesticides by individual home owners in Kenya against nuisance flies, mosquitoes, cockroaches etc. This indoor spraying could even be found to be more hazardous than outdoor spraying if investigated and related to chronic disorders which are never diagnosed.

The rational use of pesticides can be achieved through the Integrated Pest Management, which utilizes various techniques to control pests and only uses pesticides when necessary. In this way the dangers to human health and to the environment are minimized.

3.8 THE RATIONALE FOR ASSESSING HUMAN EXPOSURE TO PERSISTENT ORGANOCHLORINE PESTICIDES THROUGH MONITORING THEIR LEVELS IN BREAST MILK

The organochlorine compounds are lipophilic in nature and once absorbed into the human body they tend to store in adipose tissues. The levels of these compounds in blood, hair and urine are relatively low and, in some cases, not detectable. However, their high levels in adipose tissue and breast milk (which also

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constitutes about 3.5% fat) provides a tool of assessing their concentrations in the human body which reflects the human exposure. Furthermore, breast milk is the major vehicle for excretion of these substances in lactating women. Adipose tissue can be obtained during surgery or at autopsy. Continuous monitoring of these substances in human milk would provide data which would detect trends in levels of exposure, especially in countries where these compounds are still in use. In developed countries, such a body of monitoring data is available and the newly generated data do reflect the effects of banning or restricting the use of these substances in their countries. In addition to providing a measure of the human exposure, the intake of the organochlorines by the breast-fed infant can be estimated.

3.9 THE RATIONALE FOR COLLECTING FOOD SAMPLES

All food crops grown are subject to attack by pests either in the field and/or in the store. The use of pesticides to protect foodstuffs from various pests have resulted in contamination of food with pesticide residues. Of particular interest are the organochlorine pesticides which are persistent in nature and have the ability to accumulate and concentrate in food chains. Small quantities of these residues ingested daily with food can build up to adverse high levels in the human body as they accumulate in adipose tissue. Food is thus considered to be the most important source of organochlorine compounds in the human body. Other sources like inhalation and absorption through the skin also contribute to the body burden. Foods, especially of animal origin, e.g. milk, milk products and meats, have been

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found to have the highest organochlorine pesticide concentrations. In addition to investigate the source of organochlorine pesticides in human body through ingested food, the data obtained would also demonstrate the environmental pollution by organochlorine compounds.

3.10 REVIEW OF ORGANOCHLORINE PESTICIDE RESIDUE STUDIES IN KENYA

Although the organochlorine pesticides have been used in Kenya for increased food production and vector control, the impact this has had on the environment has not been thoroughly investigated.

Kceman et al. (1972) studied the contamination of Rift Valley Lakes and found extremely low concentrations of DDT, DDE and dieldrin in the tissues of birds and fish collected in lake Nakuru. Lincer et al. (1981), found that African cormorants (Phalacrocorax africanus) collected in 1970, contained approximately eight to fifteen times as much DDE as the single whitenecked cormorant (Phalacrocorax carbo) from the same lake. The DDE level of a white pelican liver in 1970, was only half that found in same species in 1981, which indicates a possible increase of organochlorine contamination of the lake system. Other very low levels of DDE were detected in species in lakes Naivasha, Baringo and Elementaita. Greichus et al. (1978), also studied the contamination of lake Nakuru by organochlorines compound and found DDE, DDD, and dieldrin. The values were very low, although there was a slight increase from 1972 (Koeman et al., 1972). The effect of dieldrin, sprayed by aerial application for tsetse fly control, on game animals was also investi-

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gated by Allsop (1978) who reported that all tissues collected from the animals before and after spraying had low levels of HEOD (the active ingredient of dieldrin) and its photo-isomer (PIPD). Organochlorine residues have also been detected in various food commodities. Maitho (1978) investigated the pesticide residues in animal products and found low levels (below the tolerance level) of p,p'-DDT, p,p'-DDE, lindane, aldrin, and dieldrin in milk and fat of cattle. High levels of DDT and dieldrin in eggs from Embu District were detected in two consecutive studies by Kahunyo (1983) and Mugambi (1986). Residue analysis of food crops was initiated in the National Agricultural Laboratory at Kabete, but due to technical problems, the last analyses (not published) were performed in 1978 (Mutai; Ngatia, personal communications). Wasserman et al. (1972) investigated the storage of organochlorine insecticides in the adipose tissue of people from Kenya and found DDT to be the main contaminant. β -HCH, dieldrin and heptachlor epoxide were also detected. The interesting feature in this investigation was the positive age association with DDT-derived material stored in the adipose tissue of the age groups investigated. No further work has been done to investigate the extent of human exposure with the continued use of the organochlorine pesticides.

3.11 REVIEW OF PUBLISHED DATA ON ORGANOCHLORINE PESTICIDE IN HUMAN MILK

The benefit derived from breastfeeding both by the neonate and the mother cannot be overemphasized, but the finding that mother's milk can be contaminated by chemicals represent a problem. The first investigation of human milk contamination

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with organochlorine compounds was done by Laug et al. (1951), who found that milk from normal and healthy black American women contained considerable amount of the organochlorine insecticide DDT. Since then, many other investigations on human milk contamination have been reported from various countries (Jensen, 1983; Spindler, 1983; Slorach & Vaz, 1983). The idea behind these investigations is to elucidate the infant burden of these chemicals from nursing. Most contaminants found in human milk are fat soluble substances which are mainly detected in the fatty phase of the milk. DDT and its main metabolite DDE has been detected in almost every human milk sample analysed, but the PCBs have been mainly detected in human milk from industrialized countries. Other organochlorines that have been found are dieldrin, hexachlorobenzene (HCB), hexachlorocyclohexanes (HCH), heptachlor epoxide, aldrin and heptachlor (Jensen, 1983).

In many developed countries, organochlorines in human milk have markedly decreased during the last 20 years, due to restriction or ban of the use of these chemicals.

Table 3.1 shows the residues of sum DDT in human milk reported from various countries.

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| Table 3. | 1 MEAN/M | EDIAN | N LEVELS (| OF SUM DD | T (mg/kç | g fat |) IN HUMAN |
|----------|------------------|----------|------------|-----------|----------|-------------------|--|
| MILK FRO | M DIFFERE | NT CO | DUNTRIES | | | | Y |
| Country | No of
samples | %
fat | p,p'-DDT | p,p'-DDE | SumDDT | <u>DDT</u>
DDF | Ref. |
| Sweden | 41 | | 0.21 | 1.3 | 1.5 | 0.16 | Hofvander
<u>et al</u> .,
(1981) |
| Denmark | 57 | | 0.11 | 1.04 | | 0.11 | Anderson
<u>et al</u> .,
(1984) |
| Norway | 133 | 2.0 | | 1.27 | 1.44 | | Skaare,
(1981) |
| | 36 | 2.6 | | 0.82 | | | Skaare <u>et</u>
<u>al</u> .(1988) |
| U.K. | 102 | 2.7 | 0.11 | 1.6 | | 0.07 | Jensen,
(1983) |
| Germany | | | | | | | |
| | 374 | | | | 1.75/1. | 26 | Jensen,
(1983) |
| Japan | 1 | | 0.21 | 1.5 | 1.88 | 0.14 | Slorach
& Vaz,
(1983) |

U.S.A.							
(22 states)		<0.10	1.6	1.88		Slorach
							& Vaz
							(1983)
Belgium	20	2.64	0.35	1.59	1.94	0.22	Warnez
							et al.
							(1983)
Hungary			0.52	2.97	3.55	0.18	Jensen
							(1983)
Poland			1.20	8.7	9.98	0.14	
Turkey	163	3.2	0.65	4.61	5.84	0.14	Karakaya
							<u>et al</u> .
							(1987)
Townel	1.5						
Israel	15		0.66	1.25	3.94	0.53	Miller
							<u>et al</u> .
							(1979)
Tran		2 2	1 01	1 1 2	2 00	0 00	
11 dil		5.5	1.01	1.13	2.88	0.89	H.TONKA-
							bony <u>et</u>
							<u>al</u> .(19//)
China			1.8	4 4	6 71	0 41	Slorach
			.	2 . 7	0./1	0.41	
							(1983)
							(1)00)

India			1.2	4.8	6.55	0.25	Slorach
							& Vaz
							(1983)
Nigeria	44	2.79	2.37	1.33	3.83	0.56	Atuma
							et al.
							(1987)
Rwanda	75	3.13	1.68	2.36	4.16	0.71	Warnez
							<u>et al</u> .
							(1983)

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Tables 3.2-3.6 shows the residues of the other organochlorines from various countries (mean expressed as mg/kg fat).

FROM DIFFERENT	COUN'I	RIES					
Country (%	No of sampl posit	es	Percent fat	t Die	ldrin	Referen	ce
Sweden							
Uppsala 1978-9 (3 month group)	18		4.4	0.0	18	Hofvander (1981)	<u>et al</u> .
Uppsala 1978-9 (6 month group	23		4.0	0.0	16	"	
U.K. 1979-80	102		2.7	0.0	8/0.07	Jensen	(1983)
Germany 1979 (FRG)	374			0.0	6/0.04	"	"
Japan 1977 (Osaka)				0.0	52	"	11
Israel 1975	15			0.4	7	Miller (1979)	et al.
Iraq (Baghdad)	50	(66%)		0.030(wh	ole mi	lk) AL-Omar <u>al</u> .(198	<u>et</u> 5)
Iran	131	(36%))	0.33		H.Tonka et al.(bony 1976)

Table 3.2 MEAN LEVELS OF DIELDRIN (mg/kg fat) IN HUMAN MILK

Table 3.3 MI	EAN LEVELS (OF ALDRIN	(mg/kg fat) IN	HUMAN MILK FROM
DIFFERENT CO	UNTRIES			
Country	No of	Percent	Aldrin	Reference
	samples	fat		
	Positive)			
Italy 1975	30 (20%)	2.6	0.04	Jensen
(Milani)				(1983)
Spain 1979				
(Urban area)	24		0.004	Jensen (1983)
India				
Lucknow 1979	25		0.03	(Jensen 1983)
			(whole milk)	Siddiqui <u>et</u>
				<u>al</u> . (1981)

Iraq	
Baghdad	50 (68%)

0.017	Al-Omar et
(whole milk)	al. (1985)

Table 3.4	MENN LEVELS	5 OF HEPT7	CHLOR AND HEPTACHLO	R EPOXIDE
(mg/kg fat)	IN HUMAN N	MILK FROM	DIFFERENT COUNTRIES	
Country	No of l	Percent fat	Heptachlor/ heptachlor epoxide	Reference
(8	Positive)			
Spain	45		2.51	Jensen (1983)
Switzerland				
Basel 1971	50		0.07	Jensen (1983)
				0 0 1 1 2 1 2 2 3 7
Canada	147	2.7	0.13	
1967-8				
Guatemala				
rural area	46		0.004(whole milk)	deCampos et
1971				al. (1979)
				-
Mexico 1975	620		0.01	Jensen(1983)
U.S.A 1975	1436(61%)		0.09	
Japan 1979	33		0.0005 "	
Germany 1979	9 374		0.014/1.008	
Mexico 1976			0.01	
Israel			0.46	Miller <u>et</u>
				<u>al</u> .(1979)

Table 3.5 ME7 FROM DIFFERENT	N LEVELS	OF HCH-IS	OMERS (mg	/kg fat)]	IN HUMAN MILK
Country	No of samples	d-нсн	β-нсн	Х-исн	Reference
Sweden					
Uppsala 1978-9	18	0.006	0.065	0.003	Hofvander
(3 month group)				et al.
					(1981)
Uppsala 1978-9	23	0.006	0.084	0.003	
(6 month group)				
U.К.	102	-	0.22	0.03	Jensen
					(1983)
Germany 1979	374	0.068	0.45	0.05	
Israel	15	-	1	0.73	Miller et
					al.(1979)
Copenhagen (Deni	mark)	-	0.19	-	Jensen
					(1983)
					(2)00)
Czechoslovakia		0.15	0.50	0.35	
Norway		-	0.08	-	Skaare of
					al (1000)
					<u>ar</u> . (1988)

Japan	(Osaka)	40	-	4.83	1.00	Yakushiji
						et al.
						(1977)
Iran					0.24 H	.Tonkabony
						<u>et al</u> .
						(1977)
Turkey			0.01 ^a	1.02 ^a	<0.01 ^a	Karakaya
						et al.
						(1987)
Rwanda	(rural)		0.04		0.22	Warnez <u>et</u>
	(Urban)		-		0.07	<u>al</u> .(1983)
Nigeri	a(Benin)	35	0.04	0.47	0.05	Atuma <u>et</u>
						<u>al</u> .(1986)
Nigeri	a(Bendel)	44	0.015	0.610	0.087	Atuma <u>et</u>
						<u>al</u> .(1987)

a median

Table 3.6 MEAN	LEVELS OF H	EXACHLOROBENZENE	C (mg/kg fat) IN HUMAN
Country	No of	НСВ	Reference
	samples		
Nigeria(Bendel)	44	0.020	Atuma <u>et_al</u> .(1987)
Nigeria(Benin)	35	0.10	Atuma <u>et al</u> .(1986)
Mexico 1976	620	0.03(median)	Jensen (1983)
Hawaii 1979-80	50	0.04	Jensen (1983)
Japan 1979	38	2.5(median)	Jensen (1983)
Denmark 1982	57	0.13	Anderson <u>et al</u> . (1984)
Norway 1979	183	0.13	Skaare (1981)
U.K.	102	0.14	Jensen (1983)
West Germany ca	.2000	0.72	Anderson <u>et al</u> . (1984)
Sweden (Uppsala	1978-9)		
(3 month group)	18	0.098	Hofvander <u>et al</u> .
6 month group)	23	0 11	(1) (1)

3.12 LEVELS OF ORGANOCHLORINE PESTICIDES IN HUMAN MILK IN RELATION TO DIETARY HABITS OF THE MOTHERS

The residues of organochlorine pesticides in foodstuffs contribute to the contamination of the human body by these lipophilic substances. The food of animal origin may contain high levels due to biomagnification through the food chain. If good agricultural practice is not observed, high residues of organochlorine pesticides on harvested or stored food crops may through ingestion lead to high levels in fat depots of the human body.

In Sweden the major non-occupational source of the organochlorines studied is probably the diet, especially certain foodstuffs of animal origin, such as fish from inland waters and the Baltic sea (Hofvander <u>et al.</u>, 1981). Norén (1983) studied the levels of organochlorine pesticides and PCBs in milk from mothers with different dietary habits and found lowest mean levels of DDT, β -HCH, dieldrin, and PCBs in milkfat from lacto-vegetarians, and the highest levels in milk from mothers who regularly ate fatty fish. The vegetarian women who consume food low in the food chain, were also reported to have had lower levels of p,p'-DDT, dieldrin, β -HCH, p,p'-DDE, heptachlor epoxide and oxychlordane (Jensen, 1983).

Siddiqui <u>et al</u>., (1985), demonstrated that non-vegetarian mothers excreted relatively higher amounts of chlorinated pesticides through the placenta compared with vegetarian. Yakushiji <u>et al</u>., (1977) found no correlation between the concentration of organochlorines in mother's milk and the corresponding concentrations in the favorite foods of the mother.

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There are many other factors that affect the levels of organochlorine contaminants in the human body and the milk fat. Bradt <u>et al</u>. (1976) demonstrated that non-smokers had lower levels of DDT in their milk than mothers who smoke.

3.13 RELATIONSHIP BETWEEN ORGANOCHLORINE PESTICIDE RESIDUE LEVELS IN MATERNAL ADIPOSE TISSUE, BLOOD AND MILK, AND INFANT BLOOD

Many of the organochlorines, such as the DDT group of compounds, the cyclodienes and the PCBs, transfer from the mother to the fetus through the placenta and depending on their concentrations can have toxic effects on the fetus. However, because they are lipid soluble they tend to store in fat tissues and, as mentioned earlier this storage, removes the compound from critical reactive sites in the nervous system responsible for acute toxic effects (Murphy, 1980). Several investigators have reported the presence of organochlorine residues in placental tissue, cord blood, and maternal blood and discussed the relationship to corresponding residues found in body fat. Ando et al., (1985) found a significant correlation between HCB concentration in the placenta and HCB concentration in maternal blood and milk (r=0.668, r=0.736), and a significant linear correlation between HCB concentration in placenta and that in cord blood (r=0.549). Investigations by Saxena et al., (1983) also showed a significant correlation for total HCH, lindane, p,p'-DDE and p,p'-DDT between maternal blood and placenta, and also a significant correlation between maternal blood and umbilical cord blood for the insecticides, aldrin and p,p'-DDT. The presence of organochlorines in the

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placental tissue, and the finding of higher concentration of these compounds in maternal blood than in cord blood specimens (Saxena <u>et al.</u>, 1981), indicate partial transfer of such compounds to the foetus. Mothers age has been found to influence the accumulation of organochlorines in circulating blood and its subsequent transfer to the foetus (Saxena <u>et al.</u>, 1981). The evidence concerning the placental passage of FCBs is somewhat contradictory, and the relationship between PCB concentrations in maternal and cord blood has been found to differ for each PCB congener (Skaare et al., 1988).

3.14 REVIEW OF PUBLISHED DATA ON METHODS OF ANALYSING ORGANOCHLORINE PESTICIDES IN BIOLOGICAL MATERIALS

The determination of different pesticides in the soil, water, food and biological samples require analytical methods of high sensitivity and accuracy. The analysis for pesticide residues is often complicated by the fact that the spray history is often not known. Many pesticide residue analytical methods have been used in various laboratories. The choice of the method may be based on applicability to a wide range of pesticides, and commodities, speed, cost, availability of reagents and equipment etc.

Many of the fat extraction procedures from the sample matrix for the analysis of organochlorines involve the partition between two solvent system, e.g. acetonitrile, dimethyl sulphoxide, or dimethylformamide and hexane or light petroleum. The extract obtained from methods available in 1965 usually

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contained too much residual fat, which rapidly contaminated the chromatographic column and the detector (Norén et al., 1968). Norén et al., (1968) modified the dimethylformamide-hexane partition of the pesticides and the fat by adding water to the dimethylformamide to diminish the extraction of fat and obtained high recoveries of ()-HCH, lindane, heptachlor, heptachlor epoxide, dieldrin, and DDT group of compounds. However, the method was not suitable for aldrin. Multiresidue extraction procedures have been developed and tested in interlaboratory collaborative studies. These methods are contained in the Journal of Association of Official Analytical Chemists (JAOAC). These methods need continuous revision with the development and use of the pesticides. Before the identification of the compounds, column chromatographic separation of the coextractives is necessary to eliminate possible interferences, and prevent the contamination of the column and the detector. Thin layer chromatography (TLC) have been employed for general clean-up, for qualitative identification, and for semiquantitative estimation of pesticides.

There are also several chromogenic spray reagents for TLC, e.g. silver nitrate, which have been reported in the literature (Getz, 1980). The different classes of pesticides have been identified by their R_f values and colour. Gas liquid chromatography (GLC), which was introduced by James and Martin in 1952, is the most widely used technique for measuring pesticide residues (Mcnair <u>et al.</u>, 1969). The development in instrumentation techniques, such as glass capillary columns and selective detectors, have made it possible to assess the residue levels of the chlorinated pesticides in the environment and their effects on the ecosystem. Most of the organochlorine analysis utilise the electron capture detector (ECD), either ⁶³Ni or ³H, which respond selectively to compounds with high electronegativity. For confirmation of the identity of the pesticide residue in environmental samples, mass spectrometer (NS) when combined with GLC have been successfully utilized and found to be an ideal technique for residue analysis. However, its use in routine work is limited by cost, maintenance, and availability of the instrument. Chemical derivatization before glc analysis has been used as a confirmation technique. The use of high performance liquid chromatography (HPLC) for pesticide residue analysis has also been investigated.

Table 3.7 shows some of the published data on methods of analyzing organochlorine compounds in biological materials.

Table 3.7 METHODS OF ANALYZING ORGANOCHLORINE PESTICIDES IN BIOLOGICAL MATERIALS

specimen	solvent	clean-up	Identifi-	Ref.
	extraction		cation	

vegetable of	ils	8% water/dimethyl-	alumina	TLC/GLC	Norén <u>et</u>
margarine		formamide/hexane			<u>al</u> .(1968)
butter					
milk	1.	Potassium oxalate+			
		ethanol; ethyl ethe	r/		
		light petroleum			
	2.	Water/dimethyl-		**	
		formamide			
		-hexane			

fish	ethanol/water			
egg	ethyl ether/light			
	petroleum			
meat	water/dimethyl-			
	formamide		17	н
	-hexane			
milk,oils	acetonitrile/hexa	ne Florisil	GLC	Kaphalia
fat	H	н		<u>et al</u> .,
				(1985)
green vegetable	es "	 activate 	d "	
		charcoal		
animal tissue	acetone/petroleum	Florisil	GI	Allsopp
			ECD	(1978)
human blood	hexane(ultrasonic		GLC	MacCuiag
	treatment)		ECD	(1976)
				i
bird/fish	Petroleum ether/	partially	GLC	Koeman
tissue	soxhlet extraction	deactivate	d ECD	et al.
		alumina		(1972)
chickenfat	hexane/dimethyl	Florisil	GLC	Kahunyo
	formamide		ECD	et al.
				(1986)

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food samples	acetonitrile/	Florisil/		
milk,oils,fats	**	or H ₂ SO ₄	GLC/	Kaphalia
green vegetables "		activated	ECD	et al.
		charcoal		(1985)
placenta	formic acid/	H ₂ SO ₄	GLC/	Siddiqui
tissue and	hexane		ECD	et al.
human milk				(1985)
adipose	Petrol ether	Florisil	GLC/	Wasser-
tissue			ECD	man <u>et</u>
				<u>al</u> .(1972)
human fat	Hot hexane	gel perme-	GLC-MS	Van Dyk
		ation		<u>et al</u> .
				(1987)
adipose	Petroleum ether		HPLC/	Nielsen
tissue	<pre>soxhlet/hexane</pre>	S	ilica gel	et al.
				(1984)
human milk	20%CH ₂ Cl ₂ /hexane	Florisil	GLC/	Stacey
	hexane/aceton-		ECD,TLC	<u>et al</u> .
	itrile		Alumina	(1985)
			(AgNo ₃)	
human milk	acetonitrile/	Florisil	GLC/	Winter
	hexane		ECD	<u>et al</u> .
				(1976)

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human	milk	ethanol/diethyl	Florisil	GLC/	Adamovic
		ether/petrol		ECD	<u>et al</u> .
		ether			(1978)
human	milk	dimethyl	alumina	GLC/	Warnez
		formamide		ECD	<u>et al</u> .
					(1983)

CHAPTER FOUR

MATERIALS AND METHODS

4.1 EQUIPMENT

4.1.1 Gas chromatographic equipment

Gas chromatographs:

All samples were run on a Packard gas chromatograph, model DX 12362 series 428, equipped with a ⁶³Ni electron capture detector and a packed glass column, 2m x 4mm i.d., (Packard Becker R.V., Amsterdam, The Netherlands). Nitrogen, white spot, was used as carrier gas (East Africa Oxygen Co.,Ltd., Nairobi, Kenya). Gas-clean oxygen and moisture filters were used to clean the gas (Chrompack Co., Middelsburg, The Netherlands). The recorder used was Packard, Model 621 (Packard Becker B.V., Amsterdam, The Netherlands).

For confirmatory purposes and for interlaboratory analytical quality assurance testing purposes spiked cow milk samples as well as human milk samples were analyzed at The Norwegian Veterinary College, Department of Pharmacology and Toxicology, Oslo, Norway on 2 gas chromatographs: A Varian gas chromatograph, model 3700 (Varian instrument devision, Walnut Creek, California, USA), and a Carlo Erba gas chromatograph, model 2350 (Carlo Erba strumentazione, Milano, Italy), both equipped with ⁶³Ni electron capture detectors and packed glass columns, 1.5m x 2mm i.d. Nitrogen, 99.9% purity, was used as carrier gas (Norsk Hydro, Oslo, Norway), and the gas was cleaned using filters as in Kenya. Gas chromatographic column packing materials:

In Kenya the following packing materials were used: 1.5% OV -17 + 1.95% OV-210 on Supelcoport 100/120 mesh and 4% SE-30 + 6% OV-210 on Supelcoport 100/120 mesh (Supelco Inc., Bellefonte, Pennsylvania, USA).

In Norway the following packing materials were used: 1.5% SP-2250 + 1.95% SP-2401 on Supelcoport 100/120 mesh and 1.5% OV-1 + 1.5% OV-225 on Supelcoport 80/100 mesh (Supelco.Inc., Bellefonte, Pennsylvania, USA).

4.1.2 Other equipment

Equipment	Description	Supplier
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 ul, with 2"	S.G.E. PTY Ltd.
	long needle	Melbourne,Australia
SMI microlpetter	SMI digital adjust	Scientific Manufac-
	micro petter	turing Industries,
		Emeryville,
		California, USA.
Carlsberg pipettes	100ul, 1000ul J	ohn Poulten Ltd.
		Essex, England.
Pasteur pipettes		
Centrifuge	Gallenkamp	Gallenkamp & Co.Ltd.

London, England.

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disintegrator

Cell disruptor

Sartorius

sonicator

Heat Systems Ultrasonic Inc., Plainsview,New York, USA.

Sartorius-werke, Goettingen, West Germany.

Techne Cambridge Ltd., England.

Chrompack Co, Middelburg, The Netherlands.

Supelco, Inc. Bellefonte, Pennsylvania, USA

Moulinex, France Lab-line Instruments Melrose Park, California, USA.

Water bath

Balances

Tecam thermostated

Pestle and Mortars

Septa

chromsep septa(Red) No 10036

Glass wool

silane treated

Liquid dispenser sucorex dispenser Waring blender Whirl mixer

Glassware:

Sampling bottles 100ml, 40ml, 25ml, 7.4 ml Centrifuge tubes 15ml, 50 ml Extraction columns Custom-made Volumetric flasks 100ml, 10ul

4.2 CHEMICALS

Chemical	Brand name/Grade	Supplier
Hexane	Analytical Reagent	May & Baker
		Dagenham, England
Acetone	"	"
Methanol	Laboratory	Merck, Darmstadt,
		West Germany
Sulphuric Acid	Analytical	
Potassium Hydroxid	e "	
Diethyl ether		
Sodium chloride		
Magnesium sulphate	"	
Sodium sulphate	u	
Sand	Acid washed	Howse & Mc George
		Nairobi, Kenya
Snoop	liquid leak	Supelco Inc.,
	detector	Nupro company,
		Willoughby, Ohio,U

CPM standard

Willoughby, Ohio,USA Supelco Inc., Bellefonte,Pennsylvania, USA

4.3 ANALYTICAL METHODS

4.3.1 Cleaning of the glassware

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

- a) After removing the residues (if any) the glassware was rinsed with acetone.
- b) Soaked in hot water plus detergent.
- c) Rinsed in distilled water.
- d) Rinsed with redistilled acetone and dried at 150 °C.
- e) Rinsed just before using with the same solvent to be used in the analysis.

No plastics or fats were used in equipment for pesticide and PCB analysis.

4.3.2 Distillation of solvents

Hexane, acetone, and methanol were redistilled once or twice 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 the impurities. The initial 200ml of the distillate and about 400ml of the last portion of the solvent in the distillation flask were discarded. The initial portion serves to rinse the fractionating column and condenser while any impurities present in the solvent are more concentrated in the last portion.

4.3.3 Solvent purity check

Hexane

10ml of the redistilled hexane was concentrated twenty times to 0.5ml. 2-5 ul of this was injected into the gas chromatograph (GLC). If no peaks were observed then the hexane distil-

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late was suitable for pesticide analysis. If peaks of concern appeared then the redistillation was repeated.

Acetone and methanol

10ml of acetone or methanol were added to 5ml of redistilled hexane in a separatory funnel and shaken. To separate the two fractions 2ml of 2% sodium chloride were added and shaken. The hexane layer was taken out and concentrated to 0.5ml. This was checked in the GLC as above.

Cleaning of sulphuric acid

500ml sulphuric acid was added to 100ml redistilled hexane in a separatory funnel and shaken. The hexane layer was discarded and the cleaning repeated three times. 10ml of hexane was taken from the third rinse and concentrated to 1ml and tested by GLC.

Alkaline solution

Alkaline solution for base cleanup was prepared by dissolving 10g KOH/NaOH in methanol to 100ml volume.

This solution was also tested for possible contamination by washing a small volume with redistilled n-hexane and injecting the n-hexane wash into the GLC.

4.3.4 Preparation of standard solution

Stock solution

The CPM standard mixture obtained from "Supelco,S.A." is specifically made and tested for pesticide residue analysis.

The mixture contains thirteen organochlorine compounds in microgram concentrations supplied in a lml ampoule. This was emptied and rinsed with hexane into a 10ml volumetric flask, and the volume made up to 10ml. This was stored as the stock solution. lml of this solution was measured into another 10ml flask and volume made up to the mark using hexane. This gave a dilution of 1:100. All working standards were prepared from this dilution. The standards were kept in the refrigerator. The working standard was always taken out a few minutes before injected. Due to solvent evaporation, working standards were regularly prepared and checked against the initial chromatograms obtained after new preparation of the standards. Dates of preparation and dilution factors were noted on the bottle labels.

4.3.5 Preparation of gas chromatographic columns

The columns were cleaned according to the instructions given by Supina (1974) which involved treating the column with 5% dimethyldichlorosilane in toluene to seal any active site in the column, washing with acetone followed by methanol. The column was then oven dried at 150 ^OC for about 2 hours.

Packing columns

Prepared packing materials obtained from "Supelco S.A." were 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.

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The column was tapped or vibrated gently 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 40ml/min the oven was temperature programmed at 2[°]C/min to 240[°]C and held at this temperature over night. After cooling, the detector end was connected. The conditioning process gets rid of the volatile impurities, which would otherwise interfere with good separation giving rise to tailing of the peaks (column bleed).

4.3.6 Resolution and linearity of the gas liquid chromatograph The resolution of the GLC was checked by injecting 2-5 ul of 1:1000 dilution CPM standard and comparing the resulting elution pattern with those supplied by Supelco Inc. (Manufacturers of the CPM standard used). The linearity was checked by injecting different volumes of diluted CPM standard into the GLC and plotting a graph of concentration against peak heights. A straight line graph confirmed linearity.

4.3.7 Pesticide identification, quantitation and confirmation From the chromatograms obtained, the retention times of the peaks in the sample chromatogram were compared to the retention times of corresponding peaks in standard chromatograms. The peak heights were measured and related to the peak heights in the standard and the concentration of the compound of interest was calculated according to the formula:

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 $v/m \cdot h/h_s \cdot C_s \cdot 1000 = result in ng/g (ppb) on fresh weight basis.$

v = total extract volume (ml)
m = fresh weight of the sample (g)
h = peak height of the compound in sample (mm)
h_s= peak height of the compound in the standard (mm)
c_e= concentration of standard (ng/ul)

Confirmation of the identified pesticides in the sample was done by use of a different column of different polarity from the one that was used for the analysis. This was necessary to avoid identification of a contaminant peak as a pesticide peak.

4.4 ANALYTICAL QUALITY ASSURANCE (AQA)

In order to have valid and reliable data there was a need to check on the quality assurance of the method. There were two ways of looking into this:

 The intralaboratory and 2) the interlaboratory quality assurance.

4.4.1 Intralaboratory AQA testing on human milk

This was designed to provide frequent internal check on our laboratory performance and the analytical method used. This involved working through the method within the laboratory by regular repetitive analysis of fortified samples.

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Cows milk spiked with CPM mixture standard was used. Fortification levels chosen were within the range of the actual levels likely to be found in human milk. The cow's milk to be used was first checked and found to contain very low residues, if any. Analysis of fortified cow's milk were repeated until acceptable data on recovery, reproducibility, sensitivity and linearity of the method were obtained.

Preparation and handling of spiked samples

About 500 ml of cow's milk was prepared and homogenized using ultrasonic disintegrator. This was divided into two portions. One portion was spiked with 200 ul of 1:10 dilution CPM standard added to 220g cow's milk. The other portion was kept as control. Aliquots of 10g were measured into precleaned bottles with teflon lining and stored in a freezer. These were analysed at regular intervals during the monitoring phase and their chromatograms compared with the initial chromatograms. This was to check on the performance level as well as the reproduceability of the analytical procedure at any time.

The spiking levels were 0.005, 0.009 and 0.020 mg p,p'-DDE /kg milk.

4.4.2 Interlaboratory AQA testing WHO/UNEP samples

In order to evaluate the performance of our laboratory and to enable us to compare our results internationally, spiked milk samples were exchanged between our laboratory and a similar laboratory in Norway. We also received fortified milk samples from Sweden which were part of an ongoing WHO/UNEP pilot project on assessment of human exposure to pollutants through

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biological monitoring. These fortified milk samples were being used to test the performance of the participating laboratories. The participating laboratories were to be evaluated on the basis of their analytical results which were sent to the coordinating laboratory in Sweden (WHO/UNEP 1979).

Preparation of the samples

Samples from Norway had been prepared similarly to the intralaboratory AQA test samples made in Kenya (see p 69).

Samples from Sweden

These samples consisted of spiked or unspiked homogenized milk samples prepared from butter fat, skimmed cow's milk powder and water (Slorach and Vaz 1983). They were prepared in batches of 10-25 litres at a dairy pilot plant, heat sterilized and stored under refrigeration.

The fat content of the skimmed milk powder was 0.05% and the residue levels of organochlorine compounds found in the powder were below the limits of detection.

The organochlorine compounds (OC) of interest were dissolved in about 10 ml isooctane, and this solution was gently swirled for 1 hour with a weighed amount (about 500g) of butter fat held at 40 $^{\circ}$ C. Skimmed milk powder and water were mixed, and the mixture was allowed to stand overnight at 8 $^{\circ}$ C. The amount of fat necessary to prepare a batch of milk with a specified fat content was weighed out after warming the fat/OC mixture to about 50 $^{\circ}$ C. The mixture was then homogenized for 1 minute with an equal volume of skimmed milk heated at 50 $^{\circ}$ C. The resulting suspension was then poured into the remainder of the skimmed milk at 50 $^{\circ}$ C under continuous stirring. The spiked milk so prepared was then pasteurised at 75 $^{\circ}$ C for 15 seconds, homogenized (120-40 kp/cm²) and cooled at 8 $^{\circ}$ C. The first 1.5 1 of the homogenate were discarded and the remainder was collected in a container kept at 8 $^{\circ}$ C. The milk was stirred and transferred to 100 ml glass bottles. The bottles were sealed with butyl rubber caps (tested to ensure no contamination of the sample), held in place by aluminium closures.

The unspiked samples were prepared in an analogous manner using pure iso-octane in place of the solution of OCs in isooctane.

The unspiked and spiked cow's milk samples were sterilized in the following way. The temperature was raised from 20 $^{\circ}$ C to 120 $^{\circ}$ C for 15 minutes. It was then lowered to 80 $^{\circ}$ C during 3 minutes and held at this temperature for a further 3 minutes. Finally, the temperature was decreased to 20 $^{\circ}$ C during a period of 20 minutes.

The samples were stored at 4 ^oC prior to dispatch to the laboratories.

Handling of the samples

Once the samples were received in the laboratory, they were immediately stored at 4 ^OC until analysis. At the time of analysis, the milk sample in the bottle was homogenized using ultrasonic disintegrator for about 5 minutes. Then 10 ml aliquots were measured for extraction.

4.5 ANALYTICAL PROCEDURE FOR DETERMINATION OF ORGANOCHLORINE PESTICIDES IN MILK SAMPLES

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

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modified. 15 ml of acetone and 20 ml of hexane were added to 10 g of human milk. These were then homogenized using ultrasonic disintegrator for 5 minutes. The sample was then centrifuged at 2000 rpm for 5 minutes. The hexane layer was removed using a teat pipette immediately after centrifugation and transferred to a 15 ml centrifuge tube. The tube was then placed in a water bath at 40 °C and the evaporation process started under a steady current of nitrogen gas. 5 ml acetone and 10 ml hexane were added to the remainder of the centrifugate and the extraction process was repeated. The pipetted hexane layer was added to the centrifuge tube in the water bath and the evaporation process continued to a final volume of about 2 ml. This was then transferred to a graduated 10 ml centrifuge tube which has been weighed beforehand. The 15 ml centrifuge tube was rinsed 3 times with 2 ml hexane. The hexane was then evaporated to determine the fat content.

The fat was redissolved in hexane such that the final concentration was 0.05 g fat/ml hexane.

Two aliquots of the hexane extracts were cleaned by acid and base treatment, respectively.

4.5.1 Clean up

Acid cleanup

To 1 ml of the hexane extract was added 1.5 ml conc. sulphuric acid. After mixing using a swirl mixer, it was stoppered and allowed to stand for 1 hour at room temperature. The sample was centrifuged for 1 min. The hexane layer was removed and transferred to a sample bottle ready for GLC analysis.

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Base cleanup

1 ml of the hexane extract was evaporated and 1.5 ml 10% potassium hydroxide in methanol was added to the residue. After mixing, the sample was stoppered and placed on a water-bath at 40 ^OC and allowed to stand overnight. 3 ml of 2% sodium chloride solution and 1.0 ml hexane were added to the sample and mixed. After sentrifugation for 1 min. the hexane layer was removed into a sample bottle ready for GLC analysis.

4.5.2 Identification, quantitation and confirmation The identification and quantitation was carried out using a Varian model 3700, a Carlo Erba model 2350, and a Packard model DX 12362-Series 428 gas liquid chromatographs with the following instrument parameters and operating conditions: Detectors: ⁶³Ni electron capture Columns : glass length 1.5 m i.d. 2 mm (2 columns) length 2 m i.d. 4 mm

Column temperature:a)210 °C

b)temperature programme 190 °C (1 min)
to 230 °C, 5 °C/min.
c)205 °C

Carrier gas: Nitrogen

Nitrogen a)30 ml/min b)60 ml/min c)30 ml/min

Injection volume: 2-5 ul.

No isolation of PCBs from DDT compounds was made prior to GLC analysis because the levels of PCBs were such that they did not interfere with the analysis of other OC compounds. The organochlorine compounds were identified by analysing both acid and alkali-treated hexane fractions on one of the GLC columns and comparing the retention times of the OC components in the sample with those of the standards. The quantitation was achieved by measuring the corresponding peak heights of both the sample compounds and the standards. The confirmation of the identified peaks was done by use of a different column, which eluted the various OC components with slightly different retention times. In this study 4% SE-30 + 6% SP-2401 column was used for confirmation purposes while the other column 1.5% SF-2250 + 1.95% SP-2401 was used for analysis.

4.6 CRITERIA FOR EVALUATION OF RESULTS

In the UNEP/WHO project on the assessment of human Exposure to Pollutants Through Biological Monitoring (Report of a consultation on organochlorine compounds 1980), the following criteria were adopted for evaluating pesticide recoveries from spiked human milk.

 Δ = percentage deviation from spike concentration (calculated as 100 x reported value/spike concentration - 100)

- = + 10 excellent
- = + 20 good
- = + 30 acceptable
- $= \pm 40$ poor
- = + 50 unacceptable

In the present study the same criterion was adopted.

4.7 EVALUATION OF RESULTS

Evaluation by the coordinating laboratory of our laboratory performance was helpful in decision to proceed with the analysis of our samples.

4.8 TEST FOR PRESERVATION OF HUMAN MILK WITH FORMALIN

Freezing is one common technique used for preservation of samples but is not always possible under field conditions. When analysing for organochlorine compounds in samples, proper preservation is necessary to prevent possible metabolism of the compounds. The human milk samples collected from different areas in Kenya required immediate preservation before transportation to the laboratory. The main objective of this test was to find out if formalin used in the preservation of milk samples interferred with the analysis. The test was carried out according to the following procedure.

4.8.1 Preparation of the samples:

About 500 ml of cow's milk was prepared, homogenized and divided into two portions. The first portion of the milk was treated as given in the table below. The second portion of the milk was similarly treated, but was first spiked with a CPM standard containing thirteen organochlorine compounds and homogenized before dividing. 50ul formalin per 10g milk was used for preservation.

Table 4.1 Milk preservation treatment for organochlorine analysis.

	Contraction of the local division of the loc		- 1844		
Preservation	Time	Time of analysis			
Method	Fresh	After	After	After	
		2 days	8 days	2 weeks	
None	λ	-	в	-	
Formalin	С	-	D	-	
Frozen	-	Е	-	F	
Formalin-Frozen	-	G	_	Н	

Treatment A, B, C and D samples were from the same batch of homogenized milk. E, F, G and H were from another batch of homogenized milk, and after preparation they were kept in a freezer at -20 ^OC until analyses.

Treatment A, B, C and D samples were held at room temperature until analysis.

4.8.2 Extraction

The methods for extraction of the samples, cleanup and the GLC analyses was given in section 4.5.

4.9 ANALYTICAL PROCEDURE FOR DETERMINATION OF ORGANOCHLORINE FESTICIDES IN FOOD SAMPLES

Although this method had been established in the Kenyan laboratory previous to the present study (Mugambi, 1986), the efficiency of extraction was first tested by extracting food samples fortified with organochlorine pesticides. 4.9.1 Preparation of samples

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

Preparation of the spiked samples. 3g of the homogenized food sample was transferred to a mortar and spiked with 50ul of CFM standard dilution 1:10 and mixed. The following food samples were spiked and analysed: fish, maize flour and carrots.

4.9.2 Extraction and cleanup

3g of the homogenized sample was weighed into a mortar. 4.5g of anhydrous sodium sulphate and 4.5g of acid washed sand were added. The mixture was ground to a free flowing powder. 4g of the powder were weighed into a disposable extraction column (custom-made) 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 25 ml centrifuge tube. Small volumes of ether were added until 10-15 ml of ether was eluted. The eluent was centrifuged for 10 minutes at 75 rpm and then decanted into another clean and pre-weighed 15 ml centrifuge tube, which then was placed in a water bath at 40 ^oC. Following evaporation of the ether under a stream of nitrogen, the tube was weighed to determine the fat content.

The fat was redissolved in hexane to give a concentration of 0.05g fat/ml hexane and aliquots of the hexane extracts were treated with concentrated sulphuric acid and methanolic potassium hydroxide, respectively.

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An aliquot of 2-5 ul of the acid and base cleanup extracts were injected into the GLC. Carrier gas: N₂ 60 ml/min. Identification, quantitation and confirmation was done according to section 4.5.2.

4.10 FREQUENCY OF ANALYSING INTRALABORATORY AQA SAMPLESThere is need to check on the continuous level of performancewithin a laboratory because of the following changes:a) New solvents from different suppliers.

b) GLC sensitivity.

c) Calibration of the equipment and other inherent changes.

Due to these changes it was necessary to run intra-laboratory AQA samples regularly or whenever a new batch of samples was to be analyzed.

4.11 RESULTS AND DISCUSSION

4.11.1 Solvents and reagents used

In residue analysis, purity of solvents and reagents is of paramount importance because the electron capture detection mode senses any electron-capturing materials in the injection media, whether they are pesticides or contaminants. The contaminants may have retention characteristics as those of the pesticide on a particular column and therefore lead to erroneous interpretation of the chromatogram. The concentration of the solvent (during testing) to the same factor as used in the analytical procedure, helps to detect very low concentrations of the contaminants which also concentrate during analysis.
Milk fat is present as globules. The interior of the globule consists essentially of triglycerides whilst the outer membrane contains phospholipids, vitamin Λ and other components (Brevik, 1978). The addition of acetone to the milk during fat extraction and the homogenisation process using ultrasonic disintegrator, helps to break the membrane so that, hexane can penetrate through to the triglyceride phase.

4.11.2 Interlaboratory analytical quality assurance (AQA) The interlaboratory studies are important in that they help to check and approve of the analytical method and the performance of the laboratory as compared to other laboratories. The first attempt was to compare the results of milk samples analysed in our laboratory to those obtained in a laboratory in Norway, which was also using a similar analytical procedure. The results are shown in table 4.2, which demonstrates acceptable results from both laboratories. The small variation in the results was due to inhomogenity of the samples before distribution.

The results from WHO/UNEP samples are shown in table 4.3. The results obtained in our laboratory agree well with the expected values (results from Sweden). As described in the method of preparation, these samples were well homogenized before distribution to various laboratories. Our results therefore demonstrate acceptable analytical performance.

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4.11.3 Intralaboratory analytical quality assurance (AQA) In addition to the interlaboratory AQA tests, the intralaboratory AQA tests were performed in order to prevent or control the onset of any analytical problem and to detect them quickly when they arise. In this way the reliability of the results is sustained or improved throughout the study.

Milk samples

The results of average percentage recoveries of α -HCH, lindane, β -HCH, heptachlor, aldrin, heptachlor epoxide, p,p'-DDE, dieldrin, o,p'-DDD, endrin, o,p'-DDT, p,p'-DDD and p,p'-DDT from fortified cow's milk are presented in tables 4.4, 4.5, and 4.6. The fortification levels were within the range of levels which were likely to be found in the actual samples. The same evaluation criteria as that used in the WHO/UNEP samples was also applied to the intralaboratory AQA samples. The results show that all the organochlorine pesticides were well recovered, the best score seem to be from the lowest spiking level (Table 4.4). However, heptachlor and aldrin tended to be poorly recovered (Table 4.4 and 4.6, respectively). The reason for this could be that either they do not dissolve well in acetone or hexane or are partially metabolized during the cleanup procedure. Inhomogeneity of the fortified samples could also contribute to the low recoveries. In addition, through experience it was found that samples which were spiked with standards dissolved in hexane gave low recoveries. The recoveries were improved when using the more polar solvents acetone or methanol when preparing standards for spiking samples.

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Food samples

All the organochlorine pesticides were well recovered from fish samples (Tables 4.7 and 4.8). Aldrin had an average recovery of 68% at 0.020 ug/g spiking level which, according to WHO/UNEP evaluation criteria, was poor. Mugambi (1986) obtained high recoveries of aldrin from spiked eggs and feedstuffs by this method, however, he used isooctane instead of hexane.

Acceptable recoveries of lindane, β -HCH, heptachlor, p,p'-DDE, o,p'-DDD, o,p'-DDT, p,p'-DDD and p,p'DDT were obtained from spiked maize flour samples (Table 4.9) and tomato samples (Table 4.10), but the recoveries of α -, β -HCH, lindane and heptachlor, were poor from carrot samples (Table 4.11), but this could be due to low spiking levels.

4.11.4 Preservation of samples

The results of the effect of formalin preservation on the pesticide recoveries shown in tables 4.12a, 4.12b, 4.13a and 4.13b. Table 4.11a and 4.11b compares the results of samples treated according to treatment A,B,C,D. The formalin treatment tend to give slightly lower recoveries of the organochlorine pesticides added than the no-formalin treatment though this is not definitive. The reason for the variation could be due to inhomogenization of the samples during preparation.

Table 4.13b shows a comparison of results of samples treated according to treatment E,G,F and H, and includes a one week treatment too. It is interesting to note that in this case the formalin treatment together with keeping the samples frozen until analysed tend to give higher recoveries of most of the organochlorine pesticides investigated than freezing alone. However, one feature seems to be common to both treatments, there was a decrease of levels in all the compounds between 2 days and 1 week regardless of preservation method. Wiemeyer <u>et</u> <u>al</u>. (1984) found loss of DDE residues in blood samples which had been frozen compared to samples preserved with formalin and then kept frozen. Our results do not seem to favour formalin preservation alone.

4.11.5 Clean-up

During the extraction process, the fat is extracted together with other contaminants. It is therefore necessary to separate the compounds of interest from other coextractives. This is necessary in order to lower the detection limit and prevent contamination of column and detector. Acid and base clean up was used for this purpose.

Acid clean-up

Cleaned and tested for purity, concentrated sulphuric acid was used. Clean extracts were obtained, which were well demonstrated by gas chromatography of acid-cleaned samples.

During this process aldrin, heptachlor epoxide and endrin are metabolized, and thus are not recovered in this cleanup.

Base clean-up

Sodium or potassium hydroxide dissolved in 10% methanol and tested for purity was used. The base clean-up was found to be inadequate especially in samples with high fat content. Injection of base-cleaned extracts into the GLC led to gradual contamination of the column and detector. To minimize this problem, extracts were diluted to a concentration of 0.05g fat/ml. High recoveries of aldrin, heptachlor epoxide, dieldrin, and endrin were obtained as shown in the tables 4.4-4.13.

In base clean-up, DDT is converted to DDE.

4.11.6 FCBs

No isolation of PCBs from the DDT compounds was made prior to GLC analysis. The quantitation of PCBs and DDT compounds was achieved by comparing the acid and base clean-up chromatograms. The different PCB isomers and congeners all survive both acid and base clean-up. Following acid clean-up one PCB peak overlaps the p,p'-DDT, however, in base clean-up the p,p'-DDT is degraded to p,p'-DDE, and the peak appearing at the retention time of p,p'-DDT is a PCB isomer. In samples where both PCB and p,p'-DDT were present, the p,p'-DDT peak was obtained by subtracting the peak appearing after base clean-up (PCB peak) from the peak appearing after acid clean-up (i.e.p,p'-DDT peak + PCB peak).

4.11.7 Detection limits

The detection limits for organochlorines in milk using these methods were about 1 ppb for the DDT group and about 2 ppb for the other organochlorines.

4.12 CONCLUSION

The results demonstrate that the recoveries of the various pesticides through the analytical method was within the acceptable limits ranging between 70-115% for milk; 70-123% for fish samples; 58-116% for tomato samples and 83-104% for maize flour samples. Also the reproduceability of the method was good with standard deviation generally within ±20% for 30 parallel milk samples.

Choice of the methods

The methods used in this study were found to be suitable because they could be applied to a wide range of OC pesticides and samples. Mugambi (1986) evaluated the efficiency and cost of the present method used for food analysis and compared it to the previous method in the laboratory. He found the present method to be more efficient, cheaper and more samples could be analysed per unit time. An attempt to use a promising steam distillation method (multi-residue), which was studied by Njogu (1981), was initially curtailed by lack of water in the laboratory. However, both methods would be alternatively used successfully in the laboratory.

PCBs were not detected in Kenyan samples and therefore the problem of separating PCBs from other OCs did not arise.

			HCI	3	p,p'-	-DDE	PCBs	5
Sample	٩	Fat	whole	milk	whole	milk	whole	milk
no.			milk	fat	milk	fat	milk	fat
20/21	a	2.9	2.8	0.1	22.0	0.77	24.6	0.85
	b	3.0	1.8	0.06	17.0	0.57	11.0	0.36
29/30	a	1.1	1.4	0.13	6.3	0.6	8.2	0.79
	b	0.9	1.1	0.12	5.2	0.6	6.1	0.66

a. Results from Norway

b. Results from Kenya

Table 4.3 Interlaboratory analytical quality assurance (AQA). Levels (mg/kg) of organochlorine pesticides in spiked cow's milk samples provided by WHO/UNEP, Sweden.

Sample no.	% Fat	в-нсн	p,p'-DDE	p,p'-DDT	PCB
3A	2.7				
4A	2.8				
3B	2.6	0.60	1.57	0.63	2.0
	(2.5)	(0.50)	(1.5)	(0.50)	(2.0)
3C	2.5	0.64	1.76	0.15	2.2
	(2.5)	(0.60)	(2.0)	(0.66)	(2.5)
4B	2.7	0.87	2.7	0.84	1.9
	(2.7)	(0.8)	(3.0)	(0.81	(2.2)

Results are expressed as means of 4 parallel analysis. Swedish levels are listed in parentheses.

••••				
Pesticide	mg/kg added	<pre>% Recovery</pre>	±Δ	Evaluation ^a
⊲-HCH	0.001	79	-21	А
Lindane	0.001	75	-25	А
β-нсн	0.005	89	-11	G
Heptachlor	0.001	51	-49	Р
Aldrin	0.003	101	+ 1	E
Hept.epoxide	0.004	70	-30	Α
p,p'-DDE	0.005	77	-23	A
Dieldrin	0.006	112	+12	G
o,p'-DDD	0.010	78	-22	Α
Endrin	0.010	94	- 6	E
o,p'-DDT	0.011	76	-24	А

0.010

0.013

p,p'-DDD

p,p'-DDT

Table 4.4 Recoveries of organochlorine pesticides from spiked cow's milk.

Recovery results are expressed as means of 10 parallel analysis \pm percentage deviation ($\pm \Delta$) from spiked concentration. a : UNEP/WHO Criteria for evaluation of results (1980) E = Excellent (±10% of spiked amount) G = Good (±20%) A = Acceptable (±30%) P = Poor(±40%) $U = Unacceptable (\pm 50\%)$

86

78

-14

-22

G

Α

Table 4.5	Recoveries	of organochlor	rine pesti	icides from spike	d
cow's milk.			0		
Compound	mg/kg added	<pre>% Recovery</pre>	±Δ	Evaluation ^a	
d-нсн	0.002	91	- 9	E	
Lindane	0.002	95	- 5	E	
β-нсн	0.009	89	-11	G	
Heptachlor	0.002	89	-11	G	
Aldrin	0.005	110	+10	E	
Hept.epoxide	0.007	83	-17	G	
p,p'-DD	0.009	103	+ 3	E	
Dieldrin	0.011	115	+15	G	
o,p'-DDD	0.018	94	- 6	E	
Endrin	0.018	81	-19	G	
o,p'-DDT	0.020	81	-19	G	
p,p'-DDD	0.017	102	+ 2	F.	
p,p'-DDT	0.024	86	-14	G	
HCB	0.010	76	-24	А	
PCB	0.060	91	- 9	E	

Recovery results are expressed as means of 10 parallel analysis \pm percentage deviation ($\pm \Delta$) spiked concentration.

a : UNEP/WHO Criteria for evaluation of results (1980)

- E = Excellent (±10% of spiked amount)
- $G = Good \qquad (\pm 20\%)$
- $A = Acceptable (\pm 30\%)$
- $P = Poor (\pm 40\%)$
- $U = Unacceptable (\pm 50\%)$

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Compound	mg/kg	<pre>% Recovery</pre>	±Δ	Evaluation ^a
	added			
≪-HCH	0.005	88	-12	G
Lindane	0.005	85	-15	G
β-нсн	0.020	97	- 3	E
Heptachlor	0.005	71	-29	А
Aldrin	0.010	49	-51	U
Hept.epoxide	0.016	84	-16	G
p,p'-DDE	0.020	99	- 1	Е
Dieldrin	0.024	105	+ 5	Е
o,p'-DDD	0.040	96	- 4	Е
Endrin	0.040	97	- 3	Е
o,p'-DDT	0.045	82	-18	G
p,p'-DDD	0.038	111	+11	G
p,p'-DDT	0.052	84	-16	G

Table 4.6 Recoveries of organochloride pesticides from spiked cow's milk.

Recovery results are expressed as means of 10 parallel analysis \pm percentage deviation ($\pm \Delta$) from spiked concentration.

- a : UNEP/WHO Criteria for evaluation of results (1980)
- E = Excellent (±10% of spiked amount)
- $G = Good \qquad (\pm 20\%)$
- $A = Acceptable (\pm 30\%)$
- $P = Poor \qquad (\pm 40\%)$
- $U = Unacceptable (\pm 50\%)$

Table 4.7	Recoveries of	organochlorine	pesticides	from spiked
fish.				
Compound	mg/kg added	% Recovery	±∆ Ev	valuation ^a
≪-HCH	0.004	76	-24	Α
Lindane	0.004	81	-19	G

р-нсн	0.017	84	-16	G
Heptachlor	0.004	100	0	E
Aldrin	0.010	74	-26	А
Hept.epoxide	0.013	70	-30	Α
p,p-DDE	0.017	104	+ 4	Е
Dieldrin	0.020	76	-24	А
o,p'-DDD	0.033	100	0	E
Endrin	0.033	116	+16	G
o,p'-DDT	0.038	105	+ 5	E
p,p'-DDD	0.032	115	+15	G
p,p'-DDT	0.043	104	+ 4	Е

Recovery results are expressed as means of 6 parallel analysis \pm percentage deviation ($\pm \Delta$) from added concentration.

a : UNEF/WHO Criteria for evaluation of results (1980)

- E = Excellent (±10% of spiked amount)
- $G = Good (\pm 20\%)$
- $A = Acceptable (\pm 30\%)$
- $P = Poor \qquad (\pm 40\%)$
- $U = Unacceptable (\pm 50\%)$

0.008	101	1 +	* E	
0.008	99	- 1	E	
0.033	108	+ 8	E	
0.008	102	+ 2	E	
0.020	68	-32	Р	
0.027	71	-29	A	
0.033	108	+ 8	E	
0.040	81	-19	G	
0.067	110	+10	G	
0.067	100	0	Е	
0.075	113	+13	G	
0.063	123	+23	А	
0.087	112	+12	G	
	0.008 0.008 0.033 0.008 0.020 0.027 0.033 0.040 0.067 0.067 0.067 0.075 0.063 0.087	0.0081010.008990.0331080.0081020.020680.027710.0331080.040810.0671100.0671000.0751130.0631230.087112	0.008 101 $+ 1$ 0.008 99 $- 1$ 0.033 108 $+ 8$ 0.008 102 $+ 2$ 0.020 68 -32 0.027 71 -29 0.033 108 $+ 8$ 0.040 81 -19 0.067 110 $+10$ 0.067 100 0 0.075 113 $+13$ 0.063 123 $+23$ 0.087 112 $+12$	0.008 J01 + 1 - E 0.008 99 - 1 E 0.033 108 + 8 E 0.008 102 + 2 E 0.008 102 + 2 E 0.020 68 -32 P 0.027 71 -29 A 0.033 108 + 8 E 0.040 81 -19 G 0.067 110 +10 G 0.067 100 0 E 0.063 123 +23 A 0.087 112 +12 G

Recovery results are expressed as means of 6 parallel analysis
± percentage deviation (±A) from added concentration.
a: 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%)

Pesticide	mg/kg added	<pre>% Recovery</pre>	±Δ	Evaluation ^a
<i>о</i> -нсн	0.003	101	+ 1	Е
Lindane	0.003	102	+ 2	Е
β-нсн	0.011	96	- 4	Е
Heptachlor	0.003	83	-17	G
p,p'-DDE	0.011	96	- 4	Е
o,p'-DDD	0.021	<u>9</u> 3	- 7	Е
o,p'-DDT	0.024	98	- 2	Е
p,p'-DDD	0.020	98	- 2	Е
p,p'-DDT	0.28	104	+ 4	Е

Recovery results are expressed as means of 4 parallel analysis \pm percentage deviation ($\pm \Lambda$) from spiked concentration.

a : UNEP/WHO Criteria for evaluation of results (1980)

E = Excellent (±10% of spiked amount)

 $G = Good \qquad (\pm 20\%)$

maize flour.

- $A = Acceptable (\pm 30\%)$
- $P = Poor \qquad (\pm 40\%)$
- $U = Unacceptable (\pm 50\%)$

Table 4.9 Recoveries of organochlorine pesticides from spiked

condeces.				
Pesticide	my/kg	% Recovery	±Δ	Evaluation ^a
	added			
≪-нсн	0.01	58	-42	Р
Lindane	0.01	88	-12	G
β-нсн	0.03	116	+16	G
Heptachlor	0.01	87	-13	G
Aldrin	0.02	58	-42	Р
Hept.epoxide	0.03	87	-13	G
p,p'-DDE	0.01	88	-12	G
Dieldrin	0.04	8.0	-20	G
Endrin	0.07	77	-23	А
o,p'-DDD	0.07	72	-28	A
o,p'-DDT	0.08	70	-30	А
p,p'-DDD	0.06	80	-20	G
p,p'-DDT	0.09	79	-31	А

Table 4.10 Recoveries of organochlorine pesticides from spiked tomatoes.

Recovery results are expressed as means of 3 parallel analysis \pm percentage deviation ($\pm \Delta$) from added concentration. a : UNEP/WHO Criteria for evaluation of results (1980) E = Excellent (± 10 % of spiked amount) G = Good \cdot (± 20 %)

- A = Acceptable (±30%)
- $P = Poor \qquad (\pm 40\%)$
- U = Unacceptable (±50%)

Pesticide	mg/ky added	<pre>% Recovery</pre>	±Δ	Evaluation ^a
≪-IICH	0.04	39	-61	U
Lindane	0.04	42	-58	U
β-нсн	0.17	50	-50	U
Heptachlor	0.04	51	-49	P
Aldrin	0.10	73	-27	А
Hept.epoxide	0.13	92	- 8	Е
p,p'-DDE	0.17	89	-11	G
Dieldrin	0.20	89	-11	G
o,p'-DDD	0.33	76	-24	А
Endrin	0.33	62	-38	Р
o,p'-DDT	0.38	77	-23	А
p,p'-DDD	0.32	79	-21	А
p,p'-DDT	0.43	79	-21	А

Table 4.11 Recoveries of organochlorine pesticides from spiked carrots.

Recovery results are expressed as means of 4 parallel analysis \pm percentage deviation ($\pm \Delta$) from spiked concentration.

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Α

a : UNEP/WHO Criteria for evaluation of results (1980)

E = Excellent $(\pm 10\% \text{ of spiked amount})$

- G = Good(±20%)
- $\Lambda = Acceptable (\pm 30\%)$
- P = Poor (±40%)
- $U = Unacceptable (\pm 50\%)$

Table 4.12a Influence of means of preservation and storage on the levels (mg/kg) of organochlorine pesticides in mother's milk.

			in the sale.
Preservation	Ţ	ime of analysis	following collection
method		fresh	8 days
	p,p'-DDE		
None		93.02	91.42
Formalin		85.53	89.52
	p,p'-DDT		
None		51.87	48.48
Formalin		45.70	53.03

Results are expressed as means of two parallel samples.

Table 4.12b Influence of means of preservation and storage on the recoveries of organochlorine pesticides from spiked cow's milk.

	%Recovery				
Preservation	Time of analysis following collection				
method	fresh	8 days			
	<u>≪-нсн</u>				
None	98	102			
Formalin	96	94			
	Lindane				
None	106	99			
Formalin	102	99			
	<u>β-HCH</u>				
None	144	147			
Formalin	140	147			
	Heptachlor				
None	90	87			
Formalin	75	58			
	p,p'-DDE				
None	98	88			
Formalin	118	77			
	o,p'-DDD				
None	99	103			
Formalin	82	74			
	p,p'-DDD				
None	107	110			
formalin	94	98			
	p,p'-DDT				
None	70	96			
formalin	65	64			

Table 4.13a Influence of means of preservation and storage on the levels (mg/kg) of organochlorine pesticides in mother's milk.

Preservation	Time of analysis	following	collection
method	2 days	1 week	2 weeks
	Lindane		
Frozen	0.17	0.17	0.17
Formalin-frozen	0.25	0.26	0.22
	В-нсн		
Frozen	4.28	4.66	4.21
Formalin-frozen	5.35	5.20	4.21
	p,p'-DDE		
Frozen	50.59	51.65	54.14
Formalin-frozen	65.90	67.26	54.43
	p,p'-DDT		
Frozen	28.28	28.43	28.67
Formalin-frozen	37.84	39.60	28.70

Results are expressed as means of two parallel samples.

4.13b Influence of means of preservation and storage on the recoveries of organochlorine pesticides from spiked cow's milk.

Time of analys 2 days <u>~-HCH</u> 88	is followin 1 week	ng collectio 2 weeks	n
<u>2 days</u> <u>~-HCH</u> 88	<u>l week</u>	2 weeks	
<u>≪-нсн</u> 88			
88			
	83	81	
109	99	76	
Lindane			
88	78	88	
105	86	81	
β-HCH			
85	74	77	
84	69	68	
Heptachlor			
75	65	50	
87	77	39	
p,p'-DDE			
136	119	116	
191	135	99	
o,p'-DDD			1
96	95	84	
111	110	67	
p,p'-DDT		•	
104	94	76	
82	103	60	
p,p'-DDD			
105	104	94	
126	116 -	79	
	88 109 Lindane 88 105 96 136 97 136 97 136 191 126	88 83 109 99 Lindane 88 88 78 105 86 β -HCH 85 85 74 84 69 Heptachlor 75 75 65 87 77 $p,p'-DDE$ 136 191 135 $o,p'-DDD$ 95 111 110 $p,p'-DDT$ 104 82 103 $p,p'-DDD$ 105 105 104 126 116	88 83 81 109 99 76 Lindane 88 78 88 105 86 81 105 86 81 β – HCH 85 74 77 84 69 68 Heptachlor 75 50 $p, p' - DDE$ 39 9 136 119 116 191 135 99 $o, p' - DDD$ 96 95 84 111 110 67 $p, p' - DDT$ 104 94 76 105 104 94 126 116 79

Pesults are expressed as means of two parallel samples.

CHAPTER FIVE

ORGANOCHLORINE PESTICIDES IN MOTHERS MILK IN KENYA

5.1 INTRODUCTION

The increasing use of pesticides, mainly insecticides, fungicides and herbicides, in agriculture and public health creates conditions that expose the users to these chemicals. Some of these pesticides are known to persist for long periods in the environment exposing the entire population to some degree of chemical toxicity. The risk is greater to those involved in application with little or no knowledge of safe use of pesticides. Pesticide residues in human milk were first reported in 1951 by Laug et al., who found that the milk from normal and healthy black American women contained considerable amounts of the organochlorine insecticide DDT. Since then, DDT and related organochlorine pesticides have been found in human milk throughout the world (Jensen, 1983, Slorach and Vaz, 1983). Pesticide residues in milk have a special significance in that newborn and infants are involved since milk constitutes for them, for a certain time, almost the sole source of nourishment, hence the importance of assessing the levels of these residues in human milk cannot be overemphasized. Such studies have also been used to assess the levels of environmental pollution by organochlorines in different areas within or between countries.

The intention of this study was to generate reliable data on the contamination levels of organochlorine pesticides in human milk collected from selected Kenyan mothers living in different areas of Kenya.

5.2 MATERIALS

Eight Kenyan rural areas with varied climatic conditions were selected for the study. These were Karatina, Embu, Meru, Nanyuki,Loitokitok, Rusinga Island, Homa Bay and Turkana. The mothers represented various tribes having different conditions with regard to agricultural activities and dietary habits.

5.2.1 Description of sampling areas

Figure 1 shows the areas from where the milk samples were collected.

RUSINGA ISLAND

This island in Lake Victoria is about 45 km² and have about 12.000 inhabitants. There is a short stretch of dry road connecting it to the mainland at Mbita point. Fishing combined with small-scale farming is the major occupation of the inhabitants. Most farmers grow one crop per annum only, and the major food crop is millet. Some farmers keep local cows. Other food crops like maize, sorghum, groundnuts, beans, green grams, cassava, potatoes, sweet potatoes, cow peas, sugarcane and other vegetables are grown mostly for the farmer's own consumption. Sunflower and tobacco are grown on a small scale.

According to the residents of Rusinga Island, pesticides were not used in the agriculture; however, some mothers indicated use of commercial insecticidal sprays in their homes. At Mbita, pesticides were used including the organochlorines DDT, lindane and dieldrin. It was reported that DDT had been used intensively in the region up to 1981. DDT was also used in the tsetse-fly control from 1965 to 1967. Fish together with maize flour and kale were the major foods consumed daily. The milk samples were collected immediately following a crop failure due to prolonged drought. The nutritional and health status of the mothers were poor.

LOITOKITOK (KAJIADO DISTRICT)

Kajiado district covers an area of about 572,400 ha which is primarily a ranching area with little arable land. Agriculture is possible only on a limited area on the foot hills of mount Kilimanjaro and the economic activities are mainly concentrated on livestock. The population in the 1979 census was 149,000 people. The mothers milk samples were collected from nomads keeping cattle, sheep and goats. The staple foods of the mothers were milk, blood and meat. The nutritional and health condition of the mothers were poor.

NANYUKI (LAIKIPIA DJSTRICT)

Laikipia district extends from the North-eastern part of the Aberdares mountains to the Western part of mount Kenya, covering an area of 971,500 ha. According to the 1979 census nearly 135,000 people lived in the area, which consists mainly of a semiarid grassland formerly inhabited by a Masai sub-tribe. Other tribes have now moved in recently buying ranches cooperatively or just as squatters. The ranches have been subdivided into individual small holdings whose owners practice mixed farming. Food crops such as maize, potatoes, beans, onions, tomatoes and other vegetables are grown. Dairy cows are also kept. Most of the food grown is consumed within the district. Milk samples were collected from both farmers and nomads. The nutritional status of the mother engaged in farming was fair. Due to the recent drought, the diet of the nomadic people had constituted mainly of yellow maize imported from the USA, in addition to milk and meat. The nutritional and health status of the nomadic mothers were poor.

TURKANA DISTRICT

Turkana district is in the northern part of the Rift Valley Province. This is mainly a dry area with very little agricultural potential. Jt covers an area of 61768 km² with only 142,702 people according to the 1979 census giving a population density of 2 people per km². The milk samples were collected from exnomads now settled in an irrigation scheme in Katilu area. The scheme covers an area of approximately 2 km², with about 10,000 inhabitants living in 3 different villages within the area. Up to February 1984, cotton was the main crop. Thereafter cotton was replaced by food crops like maize, millet, sorghum, lemon, kale, tomatoes and aubergines, mostly for sale. From the information obtained, DDT was used extensively in the cotton growing and also when planting sorghum. Aldrin was used against termites. Due to drought in the region during the last 3-4 years, food, mostly maize, was imported from Kitale. Normally, staple foods would be milk, goat meat, maize flour and beans. The mothers were all both malnourished and undernourished.

HOMABAY (SOUTH NYANZA DISTRICT)

South Nyanza district covers an area of 5714 km². The population according to the 1979 census was 817.601 people. The inhabitants were engaged in fishing, keeping cattle, goats and poultry and small scale crop farming. Cotton was grown as a cash crop, and other crops included sorghum, maize, millet, ground nuts, kale, beans and sunflower. The organochlorine pesticides used in this region included aldrin, chlordane, DDT, dieldrin and lindane. DDT was extensively used in cotton growing, but has now been replaced with cypermethrin. The mothers milk samples were collected from the marginal cotton zone. The staple food of the mothers was fish, maizemeal and green vegetables. The condition of the mothers was fair.

KARATINA (NYERI DISTRICT)

Nyeri district is located on the western slopes of mount Kenya. The 1979 census showed that Nyeri is densely populated with 486,000 people on a total rural area of 201,000 ha (3284 km²) of which 158.900 ha (79%) is productive area. Mostly, people rely on agriculture for their income. The major cash crops grown are tea, coffee and pyrethrum, which provide the economic basis for the people and are also very important for the national economy. Other food crops grown include peas, cabbages, lettuce, potatoes, carrots, tomatoes, maize, beans, pyrethrum, bananas, sweet potatoes, onion, sunflower, arrow root and other vegetables and fruits. The staple food of the mothers is maize, beans and vegetables. The nutritional and health status of women was generally good. Dairy cows also provide milk which is another major source of income. All farmers use fertilizer and

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practice crop protection. All food produced is consumed within the district.

The mothers milk samples were collected from the following regions around Karatina (main town).

a) Tea - Dairy zone

b) Coffee - Tea zone

c) Main coffee zone

d) Forest zone (No cash crop area).

MERU DISTRICT

Meru district is on the south-eastern slopes of mount Kenya and it is one of the largest agricultural districts in Kenya covering an area of 9922 km². The population in 1979 was 830,279 people, of whom approximately 90% lived in the rural agricultural area. The agricultural area statistically available per household was 3.9 ha. The major cash crops within the area sampled were coffee, tea, cotton, tobacco, millet and livestock. The major and minor food crops are similar to those grown in Embu district. Most of the food produced is consumed in the district, but some crops like potatoes and cereals were sold to neighbouring districts. Farmers also keep dairy cows and poultry. Fertilizing and plant protection are widely practised. Generally, people used a lot of insecticides, the common ones being DDT, lindane and aldrin. Other organophosphorus compounds were used. The mothers milk and food samples were collected from the following regions. a) North Imenti Division - Coffe, tea, cotton. b) South Imenti Division - Coffee, tea, pyrethrum. c) Nithi Division - Coffee.

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The staple food of the mothers is maize, beans and vegetables. The nutritional status of the mothers was good.

EMBU DISTRICT

Embu district is one of the smallest districts of the Eastern Province and measures about 256,000 ha (2714 km²). According to the 1979 census, more than 260,000 people lived in the Embu district. One third of the district is productive area with an average individual holding of 2.2 ha. The major cash crops within the district are coffee, tea, sunflower, cotton, tobacco, while the major food crops are maize, beans, potatoes, bananas, sweet potatoes, cow peas, green grams, sorghum, millet and a wide varities of fruits and vegetables, among them mangoes, passion fruit, avocados, pawpaws, citrus and tomatoes. Minor food crops include cassava, arrow-roots, yams and pigeon peas. Dairy cattle and poultry were also kept by some farmers for milk and egg production. About 60-80% of food harvested is consumed within the district. The distribution of pesticide used varied in the different agroecological zones from where the milk samples were collected. Among the different pesticides used were DDT, lindane, dieldrin and the organophosphorus type of pesticides.

The milk and food samples were collected from the following regions.

- a) Runyenjes division
- b) Gachoka
- c) Siakago

The stable food of the mothers is similar to those in Meru and Nyeri (Maize, beans and vegetables). The health status of the mothers was good.

5.2.2 Criteria for selection of mothers for collection of milk samples.

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

- a) The mother should have been living in the rural area for the last five years or more.
- b) The 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 the latest pregnancy should have been normal.
- e) The mother should be breast-feeding one child only.
- f) The mothers milk sample to be collected between 1-4 months post partum.

All the above mentioned selection criteria could not be met during the sample collection in Turkana due to long lasting drought and hunger in the area. Health status and dietary habits were determined by personal interviews and questionnaires. Each mother was also asked to give information on the pesticide used. Additional information was obtained from the relevant authorities.

5.2.3 Collection and handling of human milk samples. Milk samples were similarly collected in all the areas. Basic information with regard to date and place of collection of sample, mothers age, parity, days post partum, occupation and staple food were also collected when each sample was taken.

The milk samples were obtained by manual expression and collected in prewashed containers with Teflon caps. When milk samples could not be frozen immediately, 50 ul formaldehyde solution (32-33% p.a.) was added to each 10 ml human milk sample as a preservative. Preliminary work (see 4.8) showed that this would not interfere with the analysis. Milk was frozen until analyzed. Care was taken to avoid contamination both during and after sample collection. The milk was rehomogenized with an ultrasonic homogenizer just before analysis.

5.3 ANALYTICAL PROCEDURE FOR DETERMINATION OF ORGANOCHLORINE COMPOUNDS IN HUMAN MILK.

This is given in section 4.5.

5.4. RESULTS

A total of 302 human milk samples were collected from eight areas in Kenya representing different geographical regions. Out of these 264 are included in the present study and the other 38 samples were spoiled either during transportation or during extraction. The milk was collected from those mothers who satisfied the conditions as set in the criteria for the selection of mothers as given in section 5.2.2. The mothers were assumed to be representative for the sampling location at the time of collection. Within each region the mothers were similar in their habits and general cultural traditions. However, it was not easy to sample the mother's milk in all the regions within the stated range post partum, and therefore this criterion had to be modified in certain areas. All other regulations were strictly adhered to, in order to allow direct comparisons of the analytical data obtained in the study.

The results are presented in Tables 5.3-5.8 and residue concentrations of the pesticides are presented both on a fat weight basis and wet weight basis. The former makes it possible to compare our results with corresponding results published from other countries and the latter facilitates the toxicological evaluation of the results. The main organochlorine contaminants found in all Kenyan human milk samples were p,p'-DDT and its more persistent metabolite p,p'-DDE. Great regional differences in levels of these compounds were found. The mean levels of sum DDT ranged from 1.69 mg/kg in milk fat of nomads from Loitokitok to 18.73 mg/kg milk fat in human milk from Rusinga Island. Similarly, the mean levels of p,p-DDT ranged from 0.47 mg/kg in milk fat of nomads from Loitokitok to 9.60 mg/kg in milk fat from Rusinga Island, and the same trend was observed for the p,p-DDE compound. Regional differences were also found in the mean ratio of p,p-DDT to its more persistent metabolite p,p'-DDE. The mean levels of p,p'-DDT-/p,p'-DDE ranged from 0.7 in Karatina to 4.4 in Turkana (Table 5.3). The statistical significance (p<0.05) of the differences in residue levels and ratio between the areas on the geometric means are also indicated in the table.

The meanpercentages of extractable milk fat ranged from 3.0% in Turkana to 5.1% in Karatina (Fig.5.1 and Table 5.14). Individual percentages ranged from 0.6 to 12.3 percent fat.

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The levels of p,p-DDT, p,p-DDE and sum DDT in human milk is presented in Table 5.3, with sum DDT ranging from 64 ug/kg in milk from Loitokitok to 615 ug/kg in human milk from Rusinga Island.

By subdividing the sampling areas according to the main agricultural activities, it is demonstrated that differences in the levels of sum DDT and the ratios of DDT/DDE exist within a main sampling area depending on the agricultural activities (Table 5.8). The statistical significance (p<0.05) in the differences in residue levels and ratio within a sampling area are indicated in Table 5.8. In Embu region the mean level of sum DDT ranges from 5.05 mg/kg in the cotton, tobacco, and coffee growing area to 21.38 mg/kg in the tea and pyrethrum growing area. In Karatina the range is from 1.04 mg/kg in an area where there is no cash crop grown to 6.87 mg/kg in the coffee growing area. The mean levels in Meru seems to be lower than those in Embu and Karatina, but ranges from 0.35 mg/kg in the coffee and pyrethrum growing area to 3.89 mg/kg in the cotton growing area. The results from the two areas in Nanyuki range from 3.50 mg/kg in the livestock area to 5.16 mg/kg where there is no cash crop but only subsistence crops grown.

Furthermore, residues of the other organochlorine pesticides and chlorinated biphenyls are given in Tables 5.4-5.7. Out of all the Kenyan human milk samples analyzed HCB was found in 60%, χ -HCH (lindane) in 30%, β -HCH in 27%, aldrin in 35%, endrin in 4%, dieldrin in 20%, α .-HCH in 8%, transnonachlor in 6%, heptachlor in 4%, endrin in 4%. Heptachlor epoxide in 0.4%, and oxychlordane in 0.4% of the samples analyzed. Some geographical differences were demonstrated in the residue levels in the human milk fat, the mean HCB levels ranging from 0.003 mg/kg in Meru to 0.011 mg/kg in Embu, the mean levels of 6-HCH ranging from 0.014 mg/kg in Meru to 0.164 mg/kg in Embu, the mean levels of lindane ranging from 0.010 mg/kg in Homa Bay to 0.139 mg/kg in Nanyuki, the mean aldrin levels ranging from 0.021 mg/kg in Meru to 0.151 mg/kg in Turkana, and the mean dieldrin levels ranging from 0.059 mg/kg in Karatina to 2.445 mg/kg in Loitokitok. \alpha -HCH, transnonachlor, heptachlor, and endrin had mean levels of 0.08, 0.05, 0.03 and 0.11 my/kg milk fat, respectively. Heptachlor epoxide was detected in only one milk sample from Turkana with a level of 0.007 mg/kg milk fat while oxychlordane was also detected in only one human milk sample from Meru with a concentration of 0.004 mg/kg milk fat. Pentachlor was on the other hand found in 97% of the milk samples analyzed from Turkana and had a mean level of 0.022 mg/kg fat. All samples analyzed from Loitokitok contained transnonachlor with a mean value of 0.069 mg/kg fat.

Residues of PCBs were not found in quantifiable amounts in any of the samples analyzed.

The other factors such as age, days post partum, parity and dietary habits, which seem to influence the levels of sum DDT in mothers milk, are indicated in Tables 5.9-5.14. A great individual variation in all the residue levels was demonstrated.

5.5 DISCUSSION

The present study is the first report on levels of organochlorine pesticides in human milk fat from an East African country based on analysis of individual samples.

5.5.1 Factors affecting the OC content of the mothers milk: The large individual variations in the present residue levels were not unexpected, since it is well known that many factors may influence the levels, e.g. fat content in the milk, maternal age, lactation period, parity of the mothers, exposure, age and weight of the mothers, dietary habits, tobacco chewing, and seasonal variations (Jensen, 1983).

Fat content of the milk: There are individual daily variations in milk production and fat content. The highest milk production usually occurs in early morning, and the lipid content reaches a maximum at mid morning, followed by a steady decline during the day (Muthanna et al., 1986). Furthermore, milk obtained at the end of a single breast feeding has a higher fat content than does milk obtained immediately before feeding. Fat content is also affected by maternal diet. The malnutrition of Turkana women could be partly responsible for the low mean fat percentage in their milk (Fig.5.1). The Karatina women were better nourished, which is also reflected in the higher percentage of fat in their milk. These variations in fat content have a particular importance in relation to maternal milk contamination with lipophilic environmental chemicals (Jensen, 1983). Both Polishuk <u>et al</u>. (1977) and Miller et al. (1979) reported that mothers weighing about 60 kg or less had higher levels of organochlorine residues in their milk fat than those who weighed more than 60 kg. The larger fat depots cause a dilution of the fat soluble contaminants.

<u>Maternal age</u>: Table 5.12 shows the organochlorine pesticide residues in Kenyan human milk by age group. Since the calculation is based on fat weight, the age association may not be very significant because of the variation of fat with time of sampling (Jensen, 1983). However, residues of the DDT group of compounds seems to show a positive age relationship, although the number of samples from age group 25-35 years are only 21 and those from age group 17-25 are 242. Rogan <u>et al</u>. (1986) and Hashemy-Tonkabony <u>et al</u>. (1977) demonstrated higher excretion of DDT and its metabolite by older mothers than younger ones. The comparison of other organochlorine compounds is more difficult since the numbers to be compared are much smaller. No α -HCH, dieldrin, pentachlor, and heptachlor were detected in mothers who were older, age group 26-35 years.

Older mothers are assumed to have had a longer contact with a more polluted environment including food than younger mothers, and hence increased accumulation of organochlorine residues with age can therefore be expected. This may be the case where there is stringent regulations with regard to use of chemicals in the environment and where there are food monitoring programs for these chemicals. Contradicting findings have been reported with regard to age and organochlorine accumulation in mothers milk. Jensen (1983) reports findings of some investigators who have found age association with accumulation of DDT in mothers milk and others who have found no correlation to age at all. Welseberg <u>et al</u>., (1980) found no correlation between the age of the mothers and the amount of organochlorine present in the milk.

From the statistical evaluation, there was no significant difference in mean values of sum DDT between the mothers milk in the different age groups. However, there was a positive correlation between mothers age and sum DDT (Fig.5.4).

Since the women who participated in the study are representatives of the areas and have lived there for more than five years, the rise in OC levels with age would be expected since body burdens of these chemicals are slowly accumulated with continued exposure.

Lactation period and number of children: As mentioned in the introduction, the organochlorine compounds are gradually accumulated in the fatty tissues, and during the lactation period they are mobilized and excreted through milk. Women have been found to contain higher levels of OCs during their first lactation period and in the earlier samples of a given lactation (Hofvander <u>et al</u>., 1981; Rogan <u>et al</u>., 1986). The OC levels then decline, both with time spent breastfeeding, and with the number of children nursed (Rogan <u>et al</u>., 1986). The change of organochlorine compounds in the milk fat with the length of lactation period was not investigated in this study. In the present study, 45% of the mothers sampled were breastfeeding their first child while almost an equal number, 46% were breastfeeding their second child. There was no significant difference (P>0.05) between the mean residue levels of sum DDT of mothers breastfeeding their first and their second child. However, in Nanyuki area there was a significant difference (P<0.05) between the mean sum DDT of mothers breastfeeding their first child and those breastfeeding their second child. The higher ratio of DDT to DDE of mothers breastfeeding their second child (Table 5.9), demonstrates the continued exposure of mothers to DDT. In countries where DDT is no longer used, the ratio of DDT to DDE was shown to decrease during the lactation period and with the number of children (Jensen, 1983).

The fat content of the milk from primaparae (Table 5.9) was higher than in milk from secundaparae for all areas, with the exception of Turkana. This is in agreement with Jensen <u>et</u> <u>al</u>. (1983) that the parity of the mother influences the milk fat concentrations with primaparae mothers having higher milk fat content than multiparae mothers. Kroger (1972) and Yakushiji <u>et al</u>. (1978b) have also reported that the levels of organochlorine residues in the mother's milk decrease with the number of previous deliveries of the mother. This will, however, be counterbalanced when the mothers are continuously exposed to organochlorine pesticides.

Other factors like dietary habits, chewing tobacco and other exposures, will be discussed in the following relevant sections.

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5.5.2 DDT group

Sum DDT: The great geographically based differences in the mean sum DDT levels in Kenyan human milk fat are believed to reflect differences with respect to the DDT environmental pollution load and a recent and/or present use of DDT. The lowest mean level of sum DDT, 1.69 mg/kg, was found in human milk fat of nomads living in the Ilbarma area in Loitokitok. This was similar and 50% higher than the corresponding mean levels in Norwegian human milk fat in 1979 and 1983, respectively (Skaare, 1981; Skaare et al., 1988). The Norwegian mean sum DDT residue levels are among the lowest reported (Jensen, 1983; Slorach and Vaz, 1983). On the other hand, the highest mean sum DDT residue level, 18.73 mg/kg, found in Kenyan human milk fat from Rusinga Island/Mbita, is among the highest mean levels found in any country after 1974, but is only about 20% of the sum DDT residue levels found in Guatemalan human milk in 1970-1971, which are the highest residues of DDT in human milk reported (Campos and Olzyna-Marzys, 1979). The main cause of the very high accumulation of DDT in Guatemalan human milk was indoor spraying of DDT for malaria eradication. The highest Kenyan mean sum DDT residue level found in human milk (Rusinga Island - 18.73 mg/kg), is about 70% of the level found in a malaria area in Guatemala in 1973-1974 (Winter et al., 1976) but exceeded levels in Beijing, China, 1981-1982 by 3 times (Slorach and Vaz, 1983); in India, 1981-1985 by 2 to 9 times (Slorach and Vaz, 1983; Ramakrishna et al., 1985); in rural areas of Rwanda, 1977-1979 by 3 times (Warnez et al., 1983), and in Bendel state of Nigeria by 4 times (Atuma and Okor, 1987). These countries have all reported continued

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use of DDT in vector control and in agriculture. The relatively high levels of sum DDT in Kenya together with the great geographical differences in levels are almost certainly due to the continuing use of DDT as an insecticide in agricultural and vector control. This is confirmed by the information obtained on the present use of organochlorine pesticides in the different sampling areas. DDT was reported presently used in all the sampling areas except in Nanyuki and Loitokitok.

As opposed to most industrialized countries who banned, or restricted severely, the use of DDT in the early 1970, many of the developing countries are still using considerable amounts of persistent pesticides.

DDT use was banned in Norway in 1970 and the earlier studies of Norwegian human milk (Bjerk, 1972; Brevik and Bjerk, 1978) revealed geographical differences in the levels of sum DDT, while the study done in 1979 (Skaare, 1981) showed that the levels of sum DDT were approximately the same in different parts of Norway.

<u>p,p'-DDT and p,p'-DDE</u>: p,p'-DDT was the main organochlorine contaminant in all the human milk samples analysed together with its more persistent metabolite p,p'-DDE. p,p'-DDE is the major metabolite of p,p'-DDT (Hayes, 1982). When the use of p,p'-DDT is banned or severely restricted, the level of this substance in foods of plant origin fall rapidly, but exposure to its metabolite p,p'-DDE still occurs through the consumption of foods of animal origin that continue to accumulate this substance from the environment long after the use of DDT has ceased. Furthermore, when food-producing animals are exposed to p,p'-DDT, they metabolize this substance and retain the metabolite. Contamination of human milk by DDT reflects relatively recent exposure of the mother to DDT indirectly from food or through direct exposure. Contamination with DDE reflects either earlier exposure of the mother to DDT which has been metabolized to DDE or exposure of the mother to DDE as such through consumption of foods of animal origin.

In most of the recent human milk studies made in industrialized countries, the levels of DDE in the milk have far exceeded the levels of DDT (Jensen, 1983; Slorach & Vaz, 1982). Direct DDT exposure stopped years ago in those countries, and the most important remaining exposures therefore are through contaminated foods of animal origin. In these countries, DDE is the major contributor to sum DDT. Mean levels of organochlorine pesticides in milk from industrialized countries have declined since the early seventies (Jensen, 1983; Skaare et al., 1986).

The mean levels of p,p'-DDT and p,p'-DDE reported in human milk fat in developing countries, including this study, are many times higher than those reported from industrialized countries who banned or restricted severely the DDT use in the early seventies. This is demonstrated in table 5.1.

Country	p,p'-DDT	p,p'-DDE	Sum DDT	Reference
Japan 1980/81	0.21	1.5	1.88	Slorach & Vaz
				(1983)
Poland 1979	1.20	8.7	9.98	Jensen (1983)
Norway 1979		1.27		Skaare (1981)
Norway 1983		0.82		Skaare <u>et al</u> .
				(1986)
Sweden 1981	0.10	0.81	1.00	Slorach & Vaz
				(1983)
USA (22 states)			
1979	<0.10	1.6	1.88	99
India 1982	1.2	4.8	6.55	
China 1982	1.8	4.4	6.71	н
Turkey	0.65	4.61	5.81	Karakaya <u>et al</u> .
				(1987)
Nigeria	2.37	1.33	3.83	Atuma et al.
				(1987)
Rwanda	2.01	3.69	5.83	Warnez et al.
				(1983)
Kenya	3.73	2.95	6.99	Fresent study

<u>Ratio of p,p'-DDT to p,p'-DDE</u>: The mean p,p'-DDT/p,p'-DDE ratio in Kenyan human milk fat from the different sampling areas are illustrated in Fig.5.3. These values are considerably higher than the p,p'-DDT/p,p'-DDE ratio found in Chinese, Indian and Mexican milk fat in 1981-1982, which were 0.4, 0.3 and 0.2, respectively (Slorach & Vaz, 1983). In comparison with the corresponding ratio in an industrialized country, it can be mentioned that in Norway, where the use of DDT was banned in 1970, the p,p'-DDT/p,p'-DDE ratios in human milk fat in 1970, 1976 and 1979 were 0.32, 0.27 and 0.15, respectively (Bjerk, 1972; Brevik and Bjerk, 1978; Skaare, 1981). Furthermore, only p,p'-DDE was detected in Norwegian human milk fat in 1983 (Skaare <u>et al</u>., 1988). However, in contrast the p,p'-DDT/p,p'-DDE ratio is higher for countries where p,p'-DDT is still being used (Table 5.1). The reason for this is as explained earlier that the ratio of p,p'-DDT/p,p'-DDE in human milk will normally increase following DDT exposure. An evaluation of this ratio is valuable in detecting the source and in asessing recent or previous exposure and direct or indirect exposure to organochlorines through food chains.

In the present study an attempt was made to identify important exposure routes by correlating the DDT/DDE ratio and sum DDT residue levels to the main agricultural activities in the sampling area, the staple foods, the pesticide use, and the living conditions in general.

Although there exists significant differences in the mean levels of sum DDT and the DDT/DDE ratio within a main sampling area depending on the agricultural activities (Table 5.8), no consistent pattern of differences in residue levels when considering the main agricultural activities could be found with respect to all sampling areas as discussed later.

5.5.3 Other organochlorines:

HCH-isomers: Technical HCH is used as an insecticide in Kenya. Relatively low levels of lindane, the X-isomer of HCH, together with the β -isomer of HCH were found in 30 and 27%, respectively, of the human milk samples. Technical HCH contains B-HCH as a minor component. The purity of the pesticide used presently and in the past may be partly responsible for the ratio of the HCH isomers found, since the different isomers have quite different biological properties. The (3-isomer is the environmentally most persistent HCH-isomer, and it is eliminated more slowly from the body than are lindane (Pfeilsticker, 1973). Also &-HCH has 10-30 times higher ability to accumulate in fat tissues than lindane (Heeschen, 1980). The ratio between different HCH isomer residues changes from the start of food chains until excretion in human milk fat, resulting in the more persistent β -HCH being the predominant isomer in human milk (Szokolay et al., 1977). The d- and γ -isomer may also isomerize into the ρ -isomer in living organisms (Jensen, 1983).

The mean levels of β -HCH and lindane in the milk fat were lower than the maximum residue limit set by WHO. The highest mean levels of β -HCH and lindane in the human milk were found in Embu district. This was one of the districts where pesticides, including organochlorines, were reportedly used in agriculture and in household spraying. Although not all the mothers excreted β -HCH or lindane through their breastmilk, technical HCH is one of organochlorine pesticide that has been easily available to the farmers from the agrochemical shops, and many of the mothers may have come into direct contact. However, in comparison with earlier reported HCH-isomers

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residue levels in human milk fat from other countries, the Kenyan levels were relatively low (Jensen, 1983; Slorach & Vaz, 1983). y-HCH has been used in Kenya against insects attacking maize on the cob since 1953 (Gough, 1977).

Aldrin, dieldrin, and endrin: Dieldrin is a metabolite of aldrin, and it is more persistent. While aldrin as a pesticide has been banned or restricted in most industrialized countries, the use is still permitted in most developing countries. The extent of the aldrin use in Kenya is probably much less than the use of DDT, since aldrin and dieldrin were found in only 35 and 20%, respectively, of the samples analyzed, while DDT was present in all samples. The presence of dieldrin residues in mother's milk reflects exposure to either aldrin or dieldrin, because aldrin is quickly converted to dieldrin in the human body (Ackerman, 1980, Hayes, 1982). It is interesting to note that despite of this, fact, more samples contained aldrin than dieldrin. This would be expected in areas where there is intense or medium horticulture, since aldrin is applied on certified seedlings obtained from the agrochemical shops. During planting, these seedlings are handled using bare hands and thus absorption through the skin could be one of the entry routes into the human body, in addition to inhalation. The mean level of dieldrin 0.37 mg/kg fat was about 2.5 times the ERL, (0.15 mg/kg fat (FAO/WHO, 1982), however, it is similar to the levels reported from other countries (Table 5.2).

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Table 5.2 Mean levels of dieldrin in human milk fat from other countries (Jensen, 1983).

Country	Dieldrin content	Reference
	in milk fat (mg/kg)	
Denmark (Aarhus)	0.41	Rodni (1968)
" (Randers)	0.37	"
Stockholm, 1974	0.38	Westoo % Noren (1978)
Israel, 1975	0.58	Polishuk <u>et al</u> .(1977)
Japan, 1970	0.38	Suzuki <u>et al</u> . (1973)
Australia (Brisban	ne	
1971-2)) 0.93	Miller & Fox (1973)

The use of organochlorine compounds have been restricted or banned in these countries, and the levels would be expected therefore to be significantly reduced to-day.

The result from Loitokitok include the highest dieldrin level in human milk (2.4 mg/kg fat) ever reported. The mother may have had direct contact with dieldrin since OCs are still in use in cattle dips in this area.

The mean level of aldrin, 0.05 mg/kg fat, was below the ERL, 0.15 mg/kg (FAO/WHO, 1982), and is comparable to the mean levels reported by Jensen (1983).

Endrin was detected in only eleven samples with a mean value of 0.183 mg/kg fat.

Heptachlor, Heptachlor epoxide, Transnonachlor: These compounds are also used as insecticides. Heptachlor epoxide is a metabolite of heptachlor and is more persistent. Heptachlor epoxide and transnonachlor occur as impurities in technical grade chlordane.

HCB and PCBs: The fungicide and industrial product and byproduct hexachlorobenzene (HCB), has since the early 1970s been recognized as an environmental contaminant comparable to DDT and PCBs in industrialized countries (Acker and Schultze, 1970; Taylor and Keenan, 1970; Stivje, 1971; Jensen, 1983). HCB contamination does not seem to be a serious pollution problem in Kenya. It was found in 60% of all human milk samples analysed, but the levels were very low. The mean levels in the various areas ranged from 0.003 to 0.011 mg HCB/kg milk fat, which are all below the ERL, 0.5 mg HCB/kg milk fat (FAO/WHO, 1982). The total mean (0.01 mg/kg fat) is about the same as reported from Sweden in 1981 (Slorach and Vaz, 1983), but only 1% of the level reported in German milk fat in 1983 (Slorach and Vaz, 1983). The industrial chemical polychlorinated biphenyl, PCBs, which are a major contaminant in industrialized countries (Jensen, 1983; Mes et al., 1984; Ando et al., 1985, were not detected in quantifiable amounts in Kenyan mother's milk.

5.5.4 DDT in mothers milk fat from different geographical regions.

In the present study an attempt was made to identify important exposure routes by correlating the DDT/DDE ratio and sum DDT

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residue levels to the main agricultural activities in the sampling area, staple foods, pesticide use and living conditions in general. Although there exist significant differences in the mean levels of sum DDT and the DDT/DDE ratio within a main sampling area depending on the agricultural activities (Table 5.8), no consistent pattern of differences in residue levels when considering the main agricultural activities could be found with respect to all sampling areas.

<u>Rusinga Island/Mbita region</u>: The highest mean sum DDT residue level was in human milk from this region (Fig.5.2). This was not unexpected, since this is a malaria- and trypanosoma-prone region, having a history of pesticide use in malaria and tsetse-fly irradication. It is also near to Lambwe valley where there has been aerial spraying with pesticides against tsetse flies. DDT was reportedly used previously in this area.

The relatively high DDT/DDE ratios reflect recent and present direct exposure to DDT. In addition, the diet which includes fish as an important food, may be a major source of sum DDT. In a Swedish investigation, Norén (1983) found the highest levels of PCB and sum DDT in milk from mothers who regulary consumed fatty fish from the Baltic.

Homa Bay: This is also a malaria prone area, with a history of DDT use in vector control and in cotton growing. The people living in this region are not agriculturalists, and depend mostly on fishing. The living conditions of the mothers in this region are similar to those of Rusinga Island/Mbita region. The

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lower mean value of sum DDT in the mothers milk fat from Homa Bay could partly be due to the smaller sample size. There was no significant difference between the mean ratio of p,p'-DDT to p,p'-DDE in Rusinga Island/Mbita area and Homa Bay region. In both areas p,p'-DDT was the major contributor of sum DDT.

Turkana: The relatively high mean sum DDT residue level combined with a high DDT/DDE ratio (Fig.5.3), found in the fat of Turkana human milk, reflect recent direct exposure to DDT. The fact that most of the mothers were emaciated, would also favour excretion of absorbed lipid-soluble pesticides in the milk fat. Furthermore, the mothers were exnomads, now engaged in farming within an irrigation scheme where DDT had been used extensively in cotton production until January 1984. Thereafter, DDT was only used in outplanting of sorghum. However, most mothers had access to the pesticide. In this area the reduction in DDT use, when changing from cotton to food crops, is reflected in a decrease of nearly 50% in the mean sum DDT residue level in the milk fat and a change in the DDT/DDE ratio from 22.6 to 1.8 during 15 months (Table 6.1). Thus, human milk proves to be a good indicator substance in monitoring the environment for contamination by DDT and DDE.

Several investigators have reported that tobacco smoking mothers have higher sum DDT levels in milk than do nonsmokers, probably due to the use of DDT in the tobacco-growing (Jensen, 1983). Tobacco smoking in Turkana has no tradition, while tobacco chewing is quite common, and 23 out of 30 mothers in this region were reported to do so. A significant lower mean

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sum DDT level was found in the milk from the tobacco-chewing mothers as compared to the non-tobacco users (Table 5.11). The major contributor to the sum DDT is p,p'-DDT and the ratio DDT/DDE is high, which reflect direct or recent exposure to DDT. The difference between smoking and chewing tobacco with respect to DDT levels in mothers milk is not easy to explain, since many factors may be involved. One factor may be the difference in metabolism capacity towards DDT in lungs versus liver and the fact that DDT entering the body through the lungs reach systemic circulation before passing the hepatic system, while DDT entering the body through the digestive system is transported through the liver before systemic circulation. Systemic circulation before liver passage make sequestration in fat depots possible.

<u>Embu</u>: The very high sum DDT contamination of human milk of mothers living in the tea- and pyrethrum-growing area of Embu district, probably mainly reflect earlier heavy exposure to DDT, since DDE constitutes the major contributor to sum DDT (Table 5.8). The vegetarian mothers in Embu had almost three times higher mean levels of sum DDT compared to non-vegetarians (Table 5.10). Siddiqui <u>et al</u>. (1981) found higher levels of DDT in cord blood from vegetarian mothers compared to non-vegetarian mothers, while Norén (1983) found lower levels of sum DDT in milk fat from lacto-vegetarian mothers compared to mothers eating mixed diet containing fatty fish.

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The second highest sum DDT contamination of mothers milk in Embu district came from the coffee producing area. The mean levels of p,p'-DDT and p,p'-DDE were almost the same, indicating both a direct and an indirect source of the DDT contamination of the mothers milk. Furthermore, a significant difference (P<0.05) between the mean milk levels of sum DDT in the cotton- and rice-growing area and the corresponding levels from the tea, pyrethrum- and coffee-growing area was found. The main contributor to sum DDT was p,p'-DDE. This, together with the low ratio of p,p'-DDT to p,p'-DDE, reflect an indirect exposure of mothers to DDT. This could mainly be through the foodstuffs. There was no significant difference (p>0.05) between the mean levels of sum DDT in mother's milk from the cotton, the tobacco and coffee, and the rice and cotton producing areas. The p,p'-DDE was also the major contributor to sum DDT, thus reflecting an indirect exposure of the mothers to DDT.

<u>Karatina</u>: There was relatively low level of DDT contamination in mothers milk from this area (Fig.5.2). This is one of the areas in Kenya where there is intense agriculture. The people living in this region are literate which may result in better agricultural practice.

The highest contamination of individual mother's milk in Karatina came from the coffee-producing area, which also had the highest mean level of sum DDT among the four areas studied (Table 5.8). No farmer indicated the use of DDT or any other organochlorines in their coffee fields.

The low levels of sum DDT and the ratio of DDT/DDE found in milk from the other areas reflect indirect exposure of the

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mothers to DDT. There was no significant difference (P>0.05) between the mean level of sum DDT in mothers milk from tea growing area and from areas where no cash crop were grown. The DDT exposure in this area is mainly from the foodstuffs.

<u>Meru</u>: The results from this area are comparable with corresponding results from Karatina (Fig.5.2). The farmers in this district have a well organised agricultural practice.

The highest mean sum DDT residue level in mothers milk in this district was found in the cotton growing area. However, there was no significant difference (P>0.05) between the mean values of sum DDT in this area and in tobacco-cotton growing area. The coffee-tea and coffee-pyrethrum growing areas also had low levels of sum DDT. The p,p'-DDE was the major contributor to sum DDT, and this together with low DDT/DDE ratios (except tea-pyrethrum area) reflect indirect exposure to DDT, probably through the foodstuffs.

<u>Nanyuki</u>: Although no use of pesticides was reported in this area, all milk samples from this area were contaminated with DDT. The high mean value of sum DDT residue levels in the human milk fat, and the fact that the mean value of p,p'-DDT was higher than the mean value of p,p'-DDE, indicate mother's recent or direct exposure to DDT. The milk was sampled from mothers who were settled and from mothers still living a nomadic kind of life (Table 5.8). The mothers who were settled grow mainly subsistence crops such as maize, beans, peas, potatoes etc. The nomads depended mainly on food of animal origin (meat, milk) and maize flour.

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Loitokitok: This is one of the regions where the mothers did not report any use of DDT. This agrees with the low mean level of sum DDT, 1.69 mg/kg, in the milk fat of mothers living in this area compared with other regions studied (Fig.5.2). The people in this area live a nomadic kind of life depending mainly on their cattle, sheep and goats for their foods. However, they do buy vegetables from the farmers settled in their neighbourhood. Their cattle are dipped once a week against ticks with Cooper tox, which contains no OC. This is the only area where all the mother's milk samples investigated contained HCB, but the levels were low.

Since the living conditions in general and the selection criteria with respect to parity and age of the mothers were not significantly different within the areas of Embu, Karatina and Meru, the present results demonstrates great variation in the practice of pesticide use within as well as between main sampling areas in Kenya.

5.6 TOXICOLOGICAL EVALUATION

The possible toxicological implications of the present levels of OC residues in Kenyan human milk are difficult to interpret. Until now, no recommendations specifically aimed at infants have been given by the international bodies. For adults, World Health Organization/Food and Agriculture Organization expert groups have estimated acceptable daily intake (ADI) for some chlorinated pesticides (FAO/WHO, 1978). Moreover, conclusions based on adult populations in well fed countries may be quite

reliable when evaluating acute and, even more important, subacute effects of such chemicals in developing countries, where there is usually a nutritional deficiency coupled with a multitude of other threats to health of the infant population. We can apply the ADI values established for adults to find the tolerance limits for infants. The ADI values for sum DDT is 5, lindane 2.5, aldrin 0.1, and dieldrin 0.1 ug/kg body weight. Assuming that the breastfed infant consumes about 130g milk/kg body weight per day and that the milk contains 3.5% w/w fat, the "tolerable" concentrations (mg/kg) would be 2.7 for lindane, 1.1 for DDT, 0.02 for aldrin and 0.02 for dieldrin (an average infant body weight of 5 kg was assumed in calculation). Thus, the present results indicate that the tolerable intake of sum DDT, aldrin, and dieldrin is exceeded severalfold by most children. The highest (mean) estimated daily intake (ug/kg body weight) of sum DDT exceeded the ADI by 17 times and the lowest average intake by 1.5 times (Table 5.15). The mean level of dieldrin, 0.37 mg/kg fat exceeded the ADI by about 18 times and the mean level of aldrin, 0.05 mg/kg fat exceeded the ADI by 2.5 times. No ADIs have been established for β -HCH and HCB (Slorach and Vaz, 1983). Since there is no firm evidence so far that even these high concentrations have deleterious effects on the health of the infant, breastfeeding should not be discouraged because of its well-recognized advantages. Rather the present results should be applied to introduce better practice in the use of organochlorine pesticides in developing countries.

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5.7 CONCLUSION

The data obtained in this study demonstrate human milk contamination with organochlorine pesticides, which also reflects the environmental contamination by these substances. All mothers milk samples contained DDT and metabolites, which reflects recent exposure of the mother to DDT from foodstuffs or other sources. There was a great variation of levels of sum DDT in all areas due to differences in age, dietary habits, fat content of the maternal milk, and agricultural usage of pesticides.

The infant dietary intake of DDT, aldrin, dieldrin through the mothers milk exceeded the ADI by several fold. The levels of organochlorines detected in the mothers milk in this study were higher than those reported from the industrialized countries. There is need to take steps towards reducing the environmental contamination by organochlorine pesticides by introducing better agricultural practice. Monitoring programs should be initiated and followed throughout the country.

Area Year of collection	No. of	p,p'-00T	p,p'-001				p,p'-00E				
lear of collection	sampies	No. positive	mg/kg mil mean (R	k fat (ppm)* ange)	ug/kg milk Mean (Rar	(ppb) uge)	No. positive	ng/kg Mean	<pre>silk fat (ppm)= (Range)</pre>	ug/kr Mea	g ∎i <u>lk (ppb)</u> n (Range)
Rusinga Island 1984/85	25	25	9.60 (1.	74 - 44.53)	320 (54 -	1459)	25	7.61	(1.38 - 21.43)	248	(27 - 702)
Embu 1985	48	48	3.63 (0.	60 - 28.08)	162 (20 -	1139)	48	5.23	(0.83 - 32.89)	243	(24 - 1963)
Homa Bay 1985	12	12	4.08 (0.	64 - 9.12)	126 (25 -	298)	12	3.48	(0.32 - 7.99)	100	(14 - 229)
Turkana 1983	30	30	7.38 (0.	29 - 26.23)	206 (2 - 2	(03)	30	2.17	(0.04 - 7.80)	57	(7 - 898)
Nanyuki 1984/85	42	42	2.47 (0.)	87 - 18,42)	377 (18 -	430)	42	1.52	(0.21 - 5.48)	51	(5 - 209)
Karatina 1983	50	50	1.59 (0.	12 - 24.17)	90 (6 - 1	847)	50	1.72	(0.37 - 15.60)	95	(6 - 1177)
Meru 1985	44	44	0.65 (0.	05 - 2.68)	33 (0.6 -	194)	44	1.41	(0.02 - 9.80)	69	(0.3 - 499)
Loitokitok 1984	13	13	0.47 (0.	16 - 0.83)	28 (8 - 6	8)	13	0.43	(0.11 - 1.64)	23	(4 - 51)
All areas	264	264	3.73 (0.0	05 - 44.53)	173 (0.6 -	1847)	264	2.95	(0.02 - 32.89)	117	(0.3 - 1963)
Area Your of collection	Sum DDT						p,p'-DDT/p,p)'-DOE			
lear of collection	No positive	ng/kg fat Mean (Ra	(ppm) ^b nge)	u <u>g/kg</u> milk (Mean (Range	ppb) Geo e) Nea	metric n⊂	Mean (Rar	nge)	Geometric Mean ^c		
Rusinga Island 1984/85	25	18.73 (3.	70 - 69.87)	615 (88 - 2	(289) 13.	49-	1.3 (0.4 -	2.6)	1.1=		
imbu 1985	48	9.76 (1.	71 - 51.54)	449 (50 - 3	6.	76 ¤	0.8 (0.2 -	2.0)	0.7		
ioma Bay 1985	12	7.94 (0.	99 - 16.63)	237 (41 - 5	6.	310	1.3 (0.4 -	2.3)	1.48.d		
furkana 1983	30	7.79 (0.	44 - 32.82)	271 (10 - 1	.123) 6.	76 ¤	4.4 (1.0 -	20.7)	3.2=		
anyuki 1984/85	42	4.32 (0.	60 - 23.02)	140 (23 - 5	37) 3.	55ª	2.0 (0.3 -	4.8)	1.6*		
aratina 1983	50	3.51 (0.	61 - 41.48)	195 (17 - 3	153) 1.	86•	0.7 (0.2 -	2.31	0.50.0		

2.20 (0.02 - 12.95) 108 (0.3 - 713) 1.05° 0.8 (0.2 - 2.2)

1.51 -- *

0.6

1.7 (0.2 - 3.8) 1.4^{-.d.}

1.6 (0.2 - 20.7) 1.2

Table 5.3 Regional differences in levels of p,p'-DDT, p,p'-DDE and sum DDT and the ratio of p,p'-DDT to p,p'-DDE in Kenvan human wilk.

- Below detection limit <0.015 mg/kg milk fat. - Extraneous Residue Limit : 1.25 mg/kg milk fat.

Meru 1985

All areas

Loitokitok 1984

44

264

13

- Geometric means in the same column without a common superscript letter are significantly different (p(0.05).

1.69 (0.44 - 2.60) 64 (16 - 137)

6.99 (0.02 - 69.87) 261 (0.3 - 3153) 4.73

Table 5.4 Regional differences in levels of aldrin and dieldrin in Kenyan human milk.

Area Year of collection	No. of	Aldrin	Aldrin				Dieldrin		
		No. positive	<u>ag/kg</u> a Hean (nilk fat (ppm) ^{m.c.d} Range)	ug/kg milk (ppb) Mean (Range)	No. Positive	mg/kg milk fat (ppm) ^{b.c.} Mean (Range)	a ug <u>/kg milk (ppb)</u> Mean (Range)	
Rusinga Island 1984/85	25	8	0.029	(0.012 - 0.060)	3.0 (0.6 - 2.4)	2	0.255 (0.215 - 0.295)	7 (4.3 - 9.7)	
Embu 1985	48	10	0.024	(0.012 - 0.042)	1.1 (0.5 - 1.9)	3	0.310 (0.057 - 0.576)	10 (2.6 - 25.9)	
Homa Bay 1985	12		<0.004				<0.050		
Turkana 1983	30	17	0.151	(0.067 - 0.388)	5.9 (1.3 - 15.6)	3	0.687 (0.520 - 0.815)	27.3 (26.1 - 29.6)	
Nanyuki 1984/85	42	6	0.033	(0.015 - 0.076)	1.5 (0.5 - 3.4)	8	0.657 (0.159 - 1.685)	17.6 (2.1 - 30.8)	
Karatina 1983	50	9	0.025	(0.008 - 0.064)	1.0 (0.3 - 2.0)	24	0.059 (0.008 - 0.156)	2.9 (0.3 - 7.3)	
Meru 1985	44	`32	0.021	(0.006 - 0.075)	0.9 (0.1 - 2.8)	13	0.166 (0.053 - 0.496)	8.4 (0.7 - 21.8)	
Loitokitok 1984	13	10	0.063	(0.047 - 0.101)	2.2 (0.7 - 4.3)	1	2.445 [°]	119.8 e	
All areas	264	92	0.05	(0.006 - 0.388)	2.2(0.1-15.6)	54	0.37 (0.008 - 1.685)	18.4(0.3-30.8	

Below detection limit : <0.004 mg/kg milk fat.</p>

c = Means of quantifiable levels.

Below detection limit: (0.050 mg/kg milk fat.

- " = Extraneous Residue Limit :\0.15 mg/kg milk fat."
- e =Not included in the mean.

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Table 5.5 Regional differences in levels of β +CH and lindane (γ +CH) in Kenyan human milk.

Area Year of collection	No. of	β-нсн			Lindane (74 HCH)			
	Samples	No. Positive	<u>mg/kg milk fat (ppm)</u> Mean (Range)	ug/kg miłk (ppb) Mean (Range)	No. Positive	mg/kg_milk_fat_(ppm)=-=-« Mean_(Range)	ug/kg_ailk_(ppb) Mean_(Range)	
Rusinga Island 1984/85	25	3	0.083 (0.024 - 0.177)	-2.7 (0.9 - 4.4)	7	0.053 (0.024 - 0.177)	2.1 (0.5 - 8.0)	
Empu 1985	48	18	0.164 - (0.077 - 0.270)	7.7 (2.3 - 13.8)	5	0.076 (0.033 - 0.014);	3.4 (2.4 - 5.2)	
Homa Bay 1985	12	5	0.034 (0.014 - 0.085)	1.7 (0.3 - 5.3)	6	0.010 (0.008 - 0.014)	0.3 (0.1 - 0.5)	
Turkana 1983	30	14	0.015 (0.002 - 0.093)	0.5 (0.1 - 2.8)	25	0.038 (0.005 - 0.213)	1.2 (0.1 - 9.7)	
Nanyuki 1984/85	42	4	0.136 (0.029 - 0.502)	4.9 (3.6 - 6.4)	6	0.139 (0.028 - 0.502)	0.3 (0.1 - 0.5)*	
Karatina 1983	50	10	0.016 (0.004 - 0.069)	1.1 (0.1 - 5.2)	16	0.030 (0.004 - 0.061)	1.3 (0.2 - 4.0)	
Meru 1985	44	6	0.014 (0.011 - 0.018)	0.9 (0.3 - 1.4)	15	0.018 (0.002 - 0.157)	0.7 (0.1 - 6.1)	
Lottokitok 1984	13	11	0.038 (0.017 - 0.065)	1.4 (0.3 - 3.1)	-		-	
All areas	264 -	71 ,	0.09 (0.002 - 0.502)	3.0 (0.1 - 13.8)	80	0.04 (0.002 - 0.502)	1.2 (0.1 - 9.7)	

Below detection limit : (0.002 mg/kg milk fat.

* = Means of quantifiable levels.

= Extraneous Residue Limit : 0.1 mg/kg milk fat.

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Table 50 Regional differences in levels of HCB and PCBs in Kenyan human milk.

Area	No. of samples	HC9	HCB				
Tear of collection		No. positive	ng/kg milk fat (ppm)***** Mean (Range)	ug/kg_wi7k_(ppb)_ Mean (Range)	No. positive ^b		
Rusinga Island 1984/85	25	23	0.010 (0.008 - 0.016)	0.3 (0.2 - 0.7)	0		
Embu 1985	48	14	0.011 (0.004 - 0.037)	0.5 (0.04 - 1.4)	0		
Homa Bay 1985	12	2	0.004 (0.004 - 0.005)	0.2 (0.19 - 0.2)	0		
Turkana 1983	30	27	0.005 (0.001 - 0.030)	0.5 (0.02 - 1.3)	0		
Nanyuki 1984/85	42	34	0.010 (0.002 - 0.049)	0.3 (0.04 - 1.2)	0		
Karatina 1983	50	42	0.004 (0.002 - 0.010)	0.2 (0.05 - 0.5)	0		
Meru 1985	. 44	4	0.003 (0.002 - 0.004)	0.2 (0.1 - 0.3)	0		
Loitokitok 1984	13	12	0.009 (0.004 - 0.013)	0.3 (0.1 - 0.7)	Û		
All areas	264	158	0.01 (0.001 - 0.049)	0.3 (0.02 - 1.4)	0		

= Relow detection limit : <0.002 mg/kg milk fat.</p>

= = Means of quantifiable levels.

b = Below detection limit : (0.020 mg/kg fat. d = Selow detection limit : 0.5 mg/kg fat.

^d = Extraneous Residue Limit : 0.5 mg/kg milk fat.

Area/year	N	α-HCH	Transnona-	Heptachlor	Heptachlor-	Pentachlor	Oxychlordane	Endrin
Rusinga					cpondic			
Island 1984/85	25	0.29 (0.22-0.36) 2/25						
Embu 1985	48	0.033 1/48						
Homa Bay 1985	12		0.014 (0.009-0.018) 3/12					
Turkana 1983	30	0.01 (0.005-0.012) 3/30			0.007	0.022 (0.002-0.085) 29/20		
Nanyuki 1984/85	42	0.059 (0.033-0.078) 3/42			1750	27/30		
Karatina 1983	50	0.016 (0.004-0.069 10/50		0.015 (0.007-0.028) 8/50				
Meru 1985	44	0.044	0.055 (0.019-0.091)	0.041 (0.025-0.028)			0.004	0.013
		1/44	2/44	2/44			1 / 4 4	* / * *
Loitokitok 1984	13		0.069				1/44	1/44
			(0.051-0.115) 12/13					0.2 (0.163-0.3 10/13

Table 5.7a Regional differences of other organochlorine pesticides in Kenyan human milk fat (mg/kg)

Results are expressed as means. Ranges are listed in parentheses. Ratios of the number of samples containing quantifiable amounts over the total number analyzed are also given.

Area/year	N	α-ΗCΗ	Transnona- chlor	Heptachlor	Heptachlor- epoxide	Pentachlor	Oxychlordane	Endrin
Rusinga Island								
1984/85	25	9.2 (8.7-9.7)						
		2/25						
Embu 1985	48	1.4 1/48						
Homa Bay 1985	12	-,	0.4					
			(0.1-0.7) 3/12					
Turkana 1983	30	0.4	-,		0.3	2.2		
		3/20			1/30	29/30		
Nanyuki 1984/85	42	18			1750	2730		
1411yun 1904,00		(1.1-3.1)						
Karatina 1983	50	0.5						
	50	(0.1-1.1) 10/50		0.6 (0.3-1.0)				
Mon 1085	4.4	0.4	2.0	8/50			0.2	1.2
WIELU 1900	44	0.4	(1722)	(1.0.4.2)			0.5	1.2
		1/44	(1.7-2.2)	(1.0-4.2)				
		1/44	2/ **	2/11			1/44	1/44
Loitokitok 1984	13		27				1/ 11	67
	10		(0.8-4.7)					(2.4-10.3

Table 5.7b Regional differences of other organochlorine pesticides in Kenyan human milk (ug/kg)

Results are expressed as means. Ranges are listed in parentheses. Ratios of the number of samples containing quantifiable amounts over the total number analyzed are also given.

Table 5.8 Intra-regional variations in the relationship between agricultural activities and levels of p.p'-DDT, p.p'-DDE and sum DDT in Kenyan human milk.

Area. year	Cash crops⁴	No. of	Fat. mean	p,p'-DDT	p.p'-DOE	Sum DDT	p,p'-DDT
		samples		ng/kg milk fat mean (Range)	mg/kg milk fat mean (Range)	ng/kg milk fat mean (Range)	p,p'-DDE
Embu	Tea. pvrethru∎	7	4.6	7.15 (3.17-14.22)	12.35 (4.70-32.89)	21.38 (8.70-51.54)	0.7•
1985	Coffee	16	4.3	5.11 (0.69-28.08)	5.03 (0.92-14.73)	11.10 (1.71-47.90)	1.00
	Rice. warginal cotton	9	4.7	1.73 (0.60-4.46)	4.29 (1.14-10.27)	6.79= (1.94-16.20)	0.4¢
	Cotton. tobacco. coffee	16	4.5	1.67 (0.81-2.66)	2.85 (0.83-6.13)	5.05= (2.43-9.51)	0.8 -
Karatina	Coffee	18	5.5	3.35 (0.29-24.17)	3.17(0.64-15.60)	6.87° (0.80-41.48)	0.8-
.983	Coffee, tea	6	5.0	1.63 (0.19-6.27)	1.54 (0.50-3.48)	3.34 (0.74-10.12)	0.8
	Теа	12	4.4	0.44 (0.17-0.80)	0.87 (0.51-1.66)	1.41 ^{b.c} (0.87-2.61)	0.5
	None	14	5.0	0.27 (0.12-0.60)	0.67 (0.37-1.28)	1.04 ^b (0.61-1.76)	0.5 6. c
leru	Cotton	12	4.1	0.87 (0.08-2.07)	2.72 (0.18-9.80)	3.89° (0.29-12.95)	0.4
985	Tobacco, cotton	12	5.0	0.96 (0.14-2.68)	1.95 (0.14-8.41)	3.12 (0.29-12.02)	0.8°
	Coffee. tea	10	6.5	0.51 (0.24-1.13)	0.42 (0.02-0.66)	0.92 ^b (0.02-1.69)	1.1 ^e
	Coffee, pyrethrum, tea	10	4.7	0.15 (0.05-0.62)	0.18 (0.08-0.53)	0.35= (0.13-1.21)	0.7•
lanyuki	None	21	3.6	3.05 (1.11-18.42)	1.83 (0.50-5.48)	5.16 (0.60-23.02)	2.5
.984/85	Grazing [®] (cattle, sheep,						
	goatsi	21	3.1	1.91 (0.87-11.79)	1.22 (0.21-5.37)	3.50° (1.20-19.05)	2.7

- a.b.c Mean values in the same column without a common superscript within a given region are significantly different (P(0.05) (Wilcoxon's two sample test)
- d other crops grown include various crops for own consumption and for sale at retail markets. Poultry, sheep and dairy cattle are kept by some farmers.
 Staple food are maize, beans, potatoes and green vegetables.
- e The mothers are nomads. Staple food were milk, meat and yellow maize (famine relief food).

Table 5.9 Sum DDT and ratio of p.p'-DDT to p.p'-DDE in milk from mothers breatfeeding their first or second child.

Area, year	No. of milk samples to child		Mean percent fat in milk to child		<pre>Sum DDT (mg/kg milk fat) in milk to child</pre>		**Child one Vs two	p.p'-DDT/p.p'-DDE in milk to child		**Child one Vs two
	One	Two	One	Тио	One	Тио		One	Two	
Rusinga Island 1984/85	7	15	3.7	3.3	23.1 (3.7-69.9)	17.2 (4.4-49.6)	P>0.05	1.6	1.1	P = 0.05
Embu 1985	27	18	4.9	3.8	9.2 (2.5-47.9)	8.6 (1.7-21.3)	P>0.05	0.8	0.8	p>0.05
Homa Bay 1985	6	2	3.2	2.5	9.2 (2.5-16.6)	4.7 (2.8-6.7)	P>0.05	0.9	1.5	P>0.05
Turkana 1983	9	18	2.8	3.2	11.8 (0.4-30.1)	9.5 (2.5-24.3)	P>0.05	2.8	13.2	P>0.05
Nanvuki 1984/85	22	17	3.6	2.9	4.7 (1.9-19.0)	4.1 (1.2-23.0)	P<0.05	1.4	2.6	P<0.05
Karatina 1983	28	22	5.6	4.6	4.9 (0.6-41.5)	1.7 (0.6-4.6)	P10.05	0.7	0.7	P>0.05
Meru 1985	20	22	5.7	4.8	3.0 (0.1-13.0)	1.5 (0.2-8.7)	P>0.05	1.4	3.3	P>0.05
Loitokitok 1984	0	6	-	4.0	-	1.7 (0.4-2.6)		-	1.7	
All areas	119	120	4.6	3.6	7.3 (0.1-69.9)	6.2 (0.1-49.6)	P>0.05	1.2	3.5	P>0.05

Mean (Range)

** P>0.05 (No significant difference)

Pesticide	Mg pesticide/kg milk fat.	Mean (Range)	Vegeterians
	Vegeterians (n=7)	Non-vegeterians (n=4)	Non-vegetarians
p,p'-DDT	7.15 (3.17-14.22)	3.02 (0.60-28.08)	P<0.05
p,p'-DDE	12.35 (4.70-32.89)	4.02 (0.83-14.73)	P<0.05
Sum DDT	21.38 (8.70-51.54)	7.81 (1.70-49.90)	P<0.05
<u>p.p'-DDT</u> p,p'-DDE	0.67 (0.40-0.90)	0.78 (0.20-2.00)	P<0.05
HCB	0.008 (0.007-0.010)	0.011 (0.001-0.037)	P>0.05
β-нсн	0.11 (0.10-0.11)	0.175 (0.077-0.273)	P = 0.05

Table5.10 Levels of pesticides in milk from vegeterian and non-vegeterian mothers in Embu.

P≥0.05 (No significant difference)

Pesticide	Mg. pesticide/kg milk fat. Mean (Range)							
	No. positive	Chewing tobacco	No. positive	Not chewing tobacco				
p,p'-DDT	23	6.23 (0.29-21.47)	7	11.29 (3.17-26.23)				
p,p'-DDE	23	2.12 (0.04-7.80)	7	2.29 (0.73-5.93)				
Sum DDT	23	8.73 (0.44-30.13)	7	13.84 (4.10-32.82)				
p,p'-DDT/p,p'-DDE	23	2.41 (1.0-20.7)	7	4.3 (2.2-13.40)				
o,p'-DDT	22	0.48 (0.07-1.57)	7	1.06 (0.48-2.29)				
o,p'-DDD	4	0.03 (0.01-0.07)	1	0.05				
p,p'-DDD	2	0.30 (0.21-0.40)	3	0.36 (0.18-0.55)				
НСВ	21	0.005 (0.001-0.030)	5	0.004 (0.002-0.005)				
α-нсн	1	0.012	2	0.009 (0.005-0.012)				
β-нсн	9	0.009 (0.001-0.029)	5	0.028 (0.008-0.093)				
Lindane	19	0.043 (0.005-0.196)	6	0.025 (0.005-0.055)				
Pentachlor	22	0.020 (0.002-0.081)	6	0.027 (0.003-0.027)				
Aldrin	2	0.018 (0.017-0.018)	2	0.015 (0.015)				
Dieldrin	1	3.77	0	-				
Heptachlor epoxide	0	-	1	0.007				

<u>Table 5.11</u>	Levels of pe	asticides in	n milk fr	om mothers	who chew	(n=23)	or not	chew	(n=7)
	tobacco in !	Turkana							

Table 5.12 (Organochlorine	pesticides	detected	in K	enyan	human	milk	by	age	group	Ь.
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	17-25 year old women (n=242)			26-35 year old women (n=21)				
	Percent	Mean conc.	(mg/kg milk)	Percent	Mean conc. (mg/kg milk)			
	samples	In all samples	In positive samples	positive samples	In all samples	In positive samples		
	100	2.01	2.01	100	7.04	7.04		
p,p	100	3.01	3.01	100	7.04	7.04		
p,p'-DDE	100	2.90	2.90	100	3.06	3.06		
o,p'-DDD	3	0.004	0.13	10	0.01	0.14		
o,p'-DDT	53	0.10	0.18	62	0.35	0.57		
p,p'-DDD	7	0.01	0.14	10	0.57	5.98		
Sum DDT	100	6.99	6.99	100	10.62	10.62		
p.p'-DDT p,p'-DDE	100	1.3	1.3	100	2.4	2.4		
α-нсн	4	0.004	0.098	0	0	0		
β-нсн	38	0.02	0.056	52	0.02	0.036		
Lindane	26	0.01	0.040	43	0.03	0.061		
Aldrin	21	0.01	0.027	14	0.01	0.063		
Dieldrin	17	0.07	0.42	0	0	0		
НСВ	58	0.005	0.008	81	0.004	0.005		
Endrin	4	0.01	0.15	10	0.03	0.28		
Transnonachlor	6	0.003	0.051	14	0.01	0.088		
Pentachlor	7	0.001	0.019	0	0	0		
Heptachlor	4	0.0008	0.020	0	0	0		

Area	Age (years)	No. of	Sum DDT mg/kg fat	p,p'-DDI	
		sampies	Mean (Range)	p,p'-DDE	
Meru	17 - 20	24	2.29 (0.13-12.02)	0.7	
	21 - 25	19	2.21 (0.14-12.95)	0.8	
Karatina	17 - 20	30	3.16 (0.64-25.72)	0.6	
	21 - 25	18	3.15 (0.53-29.52)	0.6	
Embu	17 - 20	18	7.42 (2.50-21.61)	0.7	
	21 - 25	25	10.64 (1.71-51.54)	0.8	
	26 - 30	5	13.85 (4.63-49.90)	1.0	
Nanyuki	17 - 20	29	4.50 (1.20-23.02)	2.3	
	21 - 25	10	4.0 (0.6-8.74)	1.3	
Turkana	17 - 20	4	9.93 (0.44-21.33)	5.4	
	21 - 25	13	8.18 (1.76-32.82)	4.2	
	26 - 30	6	11.30 (3.36-30.13)	3.6	
	31 - 35	7	11.56 (2.87-24.35)	2.5	
Rusinga Island	17 - 20	21	17.64 (3.70-69.87)	1.4	
	21 - 25	4	24.50 (5.41-49.58)	1.2	
Homa Bay	17 - 20	7	6.46 (3.75-12.57)	1.2	
	21 - 25	5	[.] 10.01 (0.99–16.63)	1.8	
Loitokitok	17 - 20	3	2.11 (1.19-2.67)	1.2	
	21 - 25	6	1.49 (0.89-1.80)	1.9	
	26 - 30	3	1.68 (0.44-2.60)	1.8	
All areas	17 - 20	132	6.21 (0.13-69.9)	1.3	
	21 - 25	90	6.31 (0.14-51.54)	1.4	
	26 - 30	14	10.15 (0.44-49.90)	2.3	
	31 - 35	7	11.56 (2.87-24.35)	2.5	

Table 5.13Residues of sum DDT mg/kg fat and the ratio of p,p'-DDT to p,p'-DDE
in Kenyan human milk from the different areas according to age.

Area year of collection	No. of	Sample collected	Percent fat	Sum DDT mg/kg (ppm) milk fat	p,p'-DDT		
	samples	Weeks post-partum Mean (Range)	Mean (Range)	mean (kange)	p,p'-DDE Mean (Range)		
Rusinga Island 1984/85	25	14 (2-30)	3.3 (1.6-4.5)	18.73 (3.70-69.87)	1.3 (0.4-2.6)		
Embu 1985	48	12 (1-94)	4.5 (1.0-9.3)	9.76 (1.71-51.54)	0.8 (0.2-2.0)		
Homa Bay 1985	12	9 (1-19)	3.5 (0.6-6.9)	7.94 (0.99-16.65)	1.3 (0.4-2.3)		
Turkana 1983/84	30	21 (3-33)	3.0 (0.9-8.8)	7.79 (0.44-32.82)	4.4 (1.0-20.7)		
Nanyuki 1984/85	42	43 (7-104)	3.3 (1.3-7.3)	4.32 (0.6-23.02)	2.0 (0.3-4.8)		
Karatina 1983	50	8 (2-18)	5.1 (2.4-8.7)	3.51 (0.61-41.48)	0.7 (0.2-2.3)		
Meru 1985	44	21 (2-88)	5.0 (1.2-12.3)	2.20 (0.02-12.95)	0.8 (0.2-2.2)		
Loitokitok 1984	13	12 (4-17)	4.6 (1.2-7.6)	1.69 (0.44-2.69)	1.7 (0.2-3.8)		

Table 5.14Levels of sum DDT and the ratio of p,p'-DDT to p,p'-DDE in Kenyan human milk from different areas with respect
to days post partum of collection.

Table 5.15 Estimated daily intake of sum DDT in breastfed children, assuming a consumption of 130 ml milk/kg body weight and 3.5% milk (w/w) fat, and the relationship between estimated daily intake and acceptable daily intake (ADI). A conditional ADI of 5 ug/kg body-weight has been established for the DDT complex by the Joint Meeting on Pesticide Residues (FAO/WHO, 1970).

Area / year	Sum DDT-estimated daily intake ug/kg body weight	Number of times the estimated daily intake exceeds the ADI				
	Mean (Range)	Mean (Range)				
Rusinga Island 1984/85	84 (17-314)	17 (3-63)				
Embu 1985	44 (8-232)	9 (2-46)				
Homa Bay 1985	36 (4-75)	7 (0.9-15)				
Turkana 1983	8 (0.2-35)	1.5 (0.04-7)				
Nanyuki 1984/85	19 (3-104)	4 (0.5-21)				
Karatina 1983	15 (3-187)	3 (0.5-37)				
Meru 1985	10 (0.1-58)	2 (0.02-12)				
Loitokitok 1984	8 (1.9-12)	1.5 (0.4-2)				
All areas	26 (0.1-314)	5 (0.02-63)				

Table 5.16 Data on the mothers from which the breast milk samples were collected.

Area / year	Mothers							
	Total	Age. years	Samples collected	Par	ity		Literacy	Staple foods
	NO.	Mean (Range)	Mean (Range)	1	2	3		
Rusinga Island/Mbita 1984/85	25	20 (16-25)	14 (2-30)	9	15	0	Medium	fish. kale. maize meal. cassava
-	÷.							and sorghum
Embu 1985	48	22 (18-30)	12 (1-94)	27	18		Medium	<pre>maize + beans + potatoes + vegetables. millet</pre>
Homa Bay 1985	12	20 (15-24)	9 (1-19)	6	2	4	Medium	fish, kale, maize meal, cassava. sorghum
Turkana 1983	30	25 (18-36)	18 (2-33)	9	18	0	Low	goat meat, cow milk. maize meal and kale, beans
Nanyuki 1984/85	42	19 (15-25)	43 (7-104)	22	17	3	Low	maize + beans + potatoes, goat meat, cow meat, kale, maize meal and cow milk.
Karatina 1983	50	20 (18-25)	8 (2-18)	28	22	0	High	maize + beans, potatoes. vegetables
Meru 1985	44	20 (17-25)	21 (2-88)	20	22	0	Medium	maize + beans + potatoes. sorghum, millet + vegetables
Loitokitok 1984	13	23 (18-28)	12 (4-17)	1	6	2	Low	Cowmeat. milk and vegetables , Maize mea



Fig.5.0 Map of Kenya indicating the various districts (numbered) where milk and food sampling was done:

1. Rusinga Island. 2. Kajiado. 3. Laikipia. 4. Turkana. 5. South Nyanza. 6. Nyeri. 7. Meru. 8. Embu.

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Fig. 5.1 Mean levels of the fat percentage in mother's milk from the different areas.



Fig. 5.2 Mean levels of the sum-DDT (mg/kg fat), in mother's milk from the different areas.


Fig. 5.3 Mean levels of the ratio p,p'-DDT to p,p'-DDE in mother's m the different areas.



Fig. 5.4 Relationship between the mean levels of sum-DDT in mother's milk fat and maternal age.

CHANGES IN ORGANOCHLORINE PESTICIDE RESIDUES IN MOTHERS BREAST MILK IN TURKANA FROM 1983 TO 1984

6.1 INTRODUCTION

As described in the chapter on methods and materials, the milk samples from Turkana were collected from exnomadic mothers who are now settled in an irrigation scheme. With the introduction of modern agricultural methods in the area (Katilu), the people have modified their way of life and instead of depending entirely on their diminishing livestock (due to drought and other diseases), they can now grow their own crops in their small shambas. Pesticides have been reported used on their shambas , especially DDT in cotton growing which was replaced by food crops before 1984. In August 1983, thirty mother's milk samples were collected from this area. The same criterion for selecting the mothers as outlined in 5.2.2. was followed, but days post partum and the age of the mothers were not strictly observed.

During the last 3-4 years, the people in this area depended on food brought from other areas due to continued drought. Since food has been reported to be a source of pesticide contamination in the milk (Norén, 1983), it was necessary to monitor the mother's milk during and after the drought. The change from cotton growing to food crops growing could also bring a change in the levels of pesticide residues in the mothers milk. Turkana was therefore found to be a suitable area to investigate the change in levels of pesticides in the mother's milk due to the changes also in the agricultural activities.

6.2 MATERIALS AND METHODS

30 mother's milk samples were collected in 1983 and a similar number in May 1984. Seven of the mothers who participated in August 1983 had their milk collected again in November 1984. The samples were preserved with formalin and then frozen until analysed. The analysis was carried out as given in section 5.3.

6.3 RESULTS AND DISCUSSION

The levels of p,p'-DDE, p,p'-DDT and sum DDT in the mother's milk fat from Turkana are given in Table 6.1. The mean level of sum DDT decreased from 10.47 mg/kg to 6.00 mg/kg milk fat within 15 months. At the same time, the ratio of p,p'-DDT to p,p'-DDE dropped from 22.6 to 1.8. By comparing the results of the 30 samples collected in August 1983 to an equal number of samples collected in May 1984, a similar trend as expressed above is noted. The mean level of sum DDT dropped from 7.79 mg/kg to 4.49 mg/kg milk fat within nine months and at the same time, the ratio of p,p-DDT to p,p'-DDE dropped from 4.4 to 3.2. DDT was used intensively in cotton production until January 1984. Thereafter DDT was only used in outplanting of sorghum. Thus, human milk proves to be a good indicator for monitoring environmental contamination by DDT and DDE. Table 6.11 Changes in levels of p,p'-DDT and p,p'-DDE, mg/kg. (ppm) milk fat, in Kenyan

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	human milk from 1	furkana.		
Area	Time of sampling	No. of samples	Percentage fat Mean (Range)	p,p'-DDT Mean (Range)
Turkana	August 1983	7	3.3 (1.6-4.3)	7.87 (2.37-21.47)
	November 1984	7	2.4 (1.3-4.0)	3.66 (0.98-9.10)
•	August 1983	30	3.0 (0.9-8.8)	7.38 (0.29-26.23)
	May 1984	30	3.6 (1.3-7.0)	3.18 (0.79-7.40)

<u>p.p'-DDT</u> p,p'-DDE Time of p,p'-DDE Sum DDT Area of sampling Mean (Range) Mean (Range) Mean (Range) 10.47 (3.22-30.13) 22.6 (2.3-140) Turkana August 1983 2.73 (0.04-7.80) November 1984 2.10 (0.48-5.04) 6.00 (1.51-14.70) 1.8 (1.3-2.1) August 1983 2.17 (0.04-7.80) 7.79(0.44-32.82) 4.4 (1.0-20.7) May 1984 . 1.18 (0.25-2.36) 4.49 (1.2-9.37) 3.2 (0.6-30.2)

ORGANOCHLORINE PESTICIDE RESIDUES IN FOODS FROM DIFFERENT REGIONS IN KENYA

7.1 INTRODUCTION

DDT and other organochlorine pesticides have been used in agriculture against pests such as caterpillars, army worms, maize stalk borer, aphids and others. Since the pest damage may occur at different stages of crop growth, the spraying of the crop with pesticides is done at all these stages. Residues of these pesticidal chemicals may remain in the crop until it is consumed and the effects on humans can be acute or chronic. In developed countries, there are programs to monitor the levels of pesticide residues in foods, and this has led to the setting up of tolerance levels for organochlorine compounds in food and beverages (FAO/WHO 1982).

In Kenya, there are about 121 pesticides which have been permitted for use in food crops and their tolerance levels are listed in the Food, Drugs and Chemical substances. Act. Cap. 254 (Revised 1980), Laws of Kenya. The increased use of pesticides in crop protection, increases the possibility of food contamination and the consequent exposure of humans to these products. The intake of organochlorine contaminated food, especially foods of animal origin, is significant because these compounds are lipophilic and will accumulate in the human body fat. There is a need for continuous analysis for pesticide residues in foods offered for sale in order to take steps to reduce the concentrations when the levels exceed the tolerances set. This can also help to determine whether the government restrictions on DDT reduce the residue levels.

The levels of organochlorine compounds reported in food samples by developed countries are generally low (FAO/WHO, 1982; Gartrell <u>et al.</u>, 1985;1986). In many of these countries, a decline in the levels of DDT complex in foods, especially of animal origin, has been observed (FAO/WHO,1982). Data in Norway show that the levels of DDT complex in different species of fish and in human milk have decreased after the use of DDT was banned (Skaare, 1981; Skaare <u>et al</u>., 1985). Levels of HCH isomers in foods of animal origin and in human milk in Japan have also declined since 1971, after the extensive use of HCH ceased (FAO/WHO, 1982).

Excessive mortality of poultry and contamination of poultry meat and eggs resulting from an incident of dieldrin contamination of the housing environment was reported by Bell <u>et al.</u>, (1983) who also found high levels of dieldrin in the blood of the workers.

However, there are not many studies done on food contamination in developing countries where the organochlorine pesticides are still in use.

In a recent study on organochlorine pesticides residues in 145 eggs and 105 chicken fat samples from 11 districts in Kenya, 12 different residues were detected (Kahunyo, 1983). The DDT-group showed both the highest incidence and the highest levels. This was followed up in a similar study where 367 eggs and 42 chicken feed samples were collected from Embu and Meru districts and analysed for organochlorine pesticide residues (Mugambi, 1986). All the eggs contained p,p'-DDT and/or its metabolite p,p'-DDE. Maitho (1978) found low residue levels of the DDT-group in cow's milk and body fat.

In the present study, 243 food samples were collected parallel to the collection of human milk samples from six different areas in Kenya and investigated for residues of organochlorine pesticides and PCBs. The aim of this study was to identify and evaluate the relative contribution of foods for the occurrence of organochlorine pesticides in human milk.

7.2 MATERIALS AND METHODS

7.2.1 Collection of food samples and sample handling Food samples which are representative of the staple food of the mothers were collected from each area where the mother's milk was also collected. Efforts were made to collect the food from the home where the mother was residing. Where this was not possible, it was bought from the nearest market. The food collected was wrapped in aluminium foil. Except for the dry cereals, all the other types of food, cooked or uncooked, were kept frozen until analysed.

7.2.2 Types of food collected

The types of foods collected from each area are given in Tables 7.1-7.6. It was not possible to collect food samples from Loitokitok. The food samples collected from Rusinga Island were considered representative for the kind of food consumed in the Homa Bay area.

7.3 ANALYTICAL PROCEDURE FOR DETERMINATION OF ORGANOCHLORINE PESTICIDES IN FOOD SAMPLES This was given in section 4.9.

7.4 RESULTS

Organochlorine pesticides found in food samples from different areas are listed in Tables 7.1 to 7.6. The residues whose identities were confirmed, but which were present at levels below the limit of quantitation, are reported as traces. The limit of quantitation varied with the chemical compound and the type of food. The ranges are given for the samples where the pesticide was detected. Table 7.7 indicates the percent of incidence of pesticide residue in food sample from each area. The mean levels of pesticide residues by food group from each area are given in Tables 7.8 to 7.12. In order to calculate the average daily intake of pesticide residues in food, the average weight of each food group taken by an adult from each area per day was estimated and is given in Table 7.13. Only the food group collected from each area was considered in calculating the average daily intake but where the weight of food group is considered to vary, it is indicated in the corresponding tables. 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 then adding the intakes determined for each food group. The calculated average daily intake in

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microgram per day (ug/day) of pesticide by food group from Rusinga Island, Turkana, Embu, Karatina and Meru are given in Tables 7.14 to 7.18, respectively.

Regional variations in food consumption are reflected in slight differences among the foods collected.

The concentration levels of pesticide residues found were generally very low. Of the 243 food samples examined for organochlorine pesticides, about 50% of them had at least one organochlorine residue present. About 10 different organochlorine pesticide residues were detected. PCBs were not detected in any food sample. In some food samples more than two organochlorine pesticides were detected. The DDT-group was most commonly found in fish samples from Rusinga Island, cow's milk from Embu and Meru, vegetables from Embu, and cereals from Meru, Turkana, and Embu. Lindane was found in all the samples from Turkana, in most of the fish samples from Rusinga Island, and at least one category of food from Embu and Meru, with a higher incidence of occurrence in vegetables (Table 7.7). Highest occurrence of aldrin was in cereals from Turkana. Dieldrin was found in fish from Rusinga Island. 🛛 - HCH was found in most of the food samples from Embu and Meru. Transnonachlor occurred in 15% and oxychlordane in 5% of food samples from Turkana (Table 7.7). β -HCH was detected in at least one sample from each area except Nanyuki, which had the lowest organochlorine residues in food. There were no residues in cash crops from Embu and Karatina (Tables 7.3 & 7.4).

7.5 DISCUSSION

7.5.1 The DDT-group

The DDT-group showed both the highest incidence and the highest levels in food analysed. Eggs collected from different districts in Kenya also showed the DDT group to be the most frequent contaminant (Kahunyo, 1983; Mugambi et al., 1989).

Rusinga Island

The highest mean level of sum DDT, 128 ug/kg, in food samples from this area was found from fish samples. The main contributor to sum DDT was p,p'-DDE, which was found in 91% of the samples. Since fish is part of aquatic food chains, it is capable of bioconcentrating a number of organochlorines. The fish consumed by the people from this area is obtained from Lake Victoria which receives water from streams, rivers and runoffs during the rainy season. Due to use of DDT and other organochlorines in agriculture and public health activities, these compounds are increasing amounts in the lake.

Fish metabolize these organic compounds similar to mammals (Sieber <u>et al.</u>, 1977). The finding of high levels of DDE in dried fish compared to fresh and smoked fish (Table 7.2), could be due to loss of p,p'-DDT through evaporation or conversion of p,p'-DDT to p,p'-DDE during the drying process. All mothers from this area consumed fish which is a staple food. The dietary daily intake of DDT from fish was found to be approximately 50 ug/person. The other common foods, cereals, pulses and tubers, also contained measurable amounts of sum DDT, while no DDT was detected in vegetables (Table 7.8). The dietary daily intake of DDT from cereals, pulses and tubers was found to be approximately 52, 6 and 2 ug/person, respectively. The calculated daily total dietary intake of DDT would be about 111 ug. This value is lower than that reported in other parts of the world (WHO 1982). Other investigators (Westoö <u>et al</u>., 1978; Hofvander <u>et</u> <u>al</u>., 1981; Norén, 1983) have attributed fish consumption to be a major source of DDT contamination in mothers milk. Only one sample of mixed staple food (cooked) was analysed and had a low level, 20 ug/kg of sum DDT.

The food intake in Homa Bay is similar to that in Rusinga Island since the people in both areas have similar food habits, and fish is obtained from the same source.

Turkana

The staple food of the Turkana people comprises meat, milk and cereals. DDT group was detected in all of the three food groups. The highest mean level of sum DDT, 54.6 ug/kg, was found in meat. Food of animal origin has been shown to contain higher levels of DDT because of bioaccumulation through food chain (Hayes, 1982).

Since DDT had been used in Turkana in cotton growing and also on food crops, the finding of its residues in food samples from this area are not unexpected. However, the locally grown

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maize and sorghum contained lower levels of sum DDT than imported maize (Table 7.1a). The calculated daily dietary intake of sum DDT from meat, milk and cereals was found to be approximately 22, 4 and 14 ug/day per person, respectively, giving a daily total dietary intake of about 40 ug of sum DDT. This value is also lower than some values of sum DDT intake in a total diet reported in other parts of the world (FAO/WHO, 1982). Because of the high levels of DDT found in mother's milk, food may not be the major source. However, the results do indicate that food also contributes significantly to the body burden of DDT. The major source in this area could have been direct exposure.

Embu

The highest mean level of sum DDT, 1235 ug/kg in vegetables and 185 ug/kg in cow's whole milk , were both found from this area. Mixed farming is the main agricultural activity of the people living in this area and crop protection by use of pesticides including DDT was reported. The relative high mean level of sum DDT, 54.5 ug/kg, in cereals from this area, reflects current use of DDT. Since it is a normal practice to feed animals with plant material, e.g maize stalks, peeling from potatoes etc., the cow's milk may become contaminated with pesticides if the feeds had been sprayed previously. Direct exposure may occur if the cows enter areas which have been sprayed with pesticides. The calculated daily dietary intake of sum DDT, ug/day per person,was approximately 0.3 from roots, 22 from cereals, 28 from milk and 247 from vegetables, which gives a daily total

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dietary intake of 297 ug. This value is about five times higher than the sum DDT in total diet reported from Guatemala (FAO/WHO 1982). As mentioned earlier, Kahunyo (1983) and Mugambi <u>et al</u>. (1989) found eggs from this area to have the highest levels of organochlorines. Furthermore, the finding of high levels of sum DDT in vegetables corresponds to the highest mean level of sum DDT in milk of mothers who are mainly vegetarians in this area (chapter 5). The mixed food, which is a mixture of the four main food groups viz. cereals, pulses, vegetables and roots, gives a lower total daily dietary intake of sum DDT, 48.6 ug/kg, the milk intake included. A part from the staple food which comprise the four major food groups considered, cow's milk was consumed by all the mothers. The daily consumption of foods which contains pesticide residues seems to contribute significantly towards the contamination of the mother's milk.

Karatina

The staple food of people in this area is very similar to that of Embu and Meru. This seems to be an area with the least contamination. As mentioned elsewhere (5.5.4) this is an area with good agricultural practice. It is likely that people in this area are aware of the effects of chemical contamination since farming is the main occupation, with crop protection being practiced. Furthermore, the people growing crops for their own consumption may not be applying chemicals. There were no DDT residues in pulses, fruits, roots, and tubers, but this could be due to the small sample size. Cow's milk, which is also part of total daily diet, was not sampled. The calculated daily dietary intake of sum DDT from cereals and vegetables were approximately 21 and 6 ug/day per person, respectively. The total daily dietary intake of Sum DDT was 27 ug. This value is similar to the 31 ug calculated from the mixed food. Mixed food comprised cereals, pulses, vegetables roots and tubers. These values are lower than the corresponding values obtained from Rusinga Island, Turkana and Embu and also reflects the lower levels of sum DDT found in mother's milk from this area (5.5.4).

Meru

Farming is the main agricultural activity of the people in this area. Plant protection by application of pesticides was reported in this area. The staple food of the people is similar to those in Embu and Karatina which comprises cereals, pulses, vegetables, roots and tubers. The mean levels of sum DDT in cereals was 12.8, vegetables 15.3, pulses 50.9, and milk 178 ug/kg. There were no DDT residues detected in roots and tubers. The calculated daily intake of sum DDT by food group was 3 from vegetables, 6 from cereals, 13 from pulses and 27 from milk ug/day, which gives a daily total dietary intake of 49 ug. The mixed food which comprised cereals, vegetables and pulses gave a total dietary intake of 34 ug (milk not included). The highest mean level of sum DDT from the cow's milk could be due to feeding the animals with contaminated plant materials which had been previously sprayed with pesticide chemicals.

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Nanyuki

The food samples collected from this area contained no DDT residues. No pesticides were reported used in this area. The food was collected only from those people who were agriculturalists. It was not possible to collect food samples from the nomads. However, one of their common food was cereals, mainly maize meal, which is an important daily food for almost all people in Kenya. It is a crop that requires protection from various pests both in the field and in the stores. DDT-group was found in most of the cereal samples analysed in all other areas, but in most samples the levels were below quantitation levels. However, in addition to the consumption of food containing low levels of pesticide residues there could be other sources of organochlorine in breast milk.

7.5.2 OTHER ORGANOCHLORINES

HCH isomers

 $\alpha_{\rm e}$, β -and γ -HCH isomers were detected in various food groups and at different levels. The highest mean level of α -HCH, 10.3 ug/kg, was found in milk samples from Embu with corresponding high levels, 11.1 ug/kg and 22.1 ug/kg, of β -HCH and β -HCH (lindane), respectively. The presence of these HCH-isomers in foodstuffs may be due to the use of commercial insecticide HCH, which contains a mixture of different isomers. The other food group found to contain low but measurable quantities of HCH, was meat from Turkana. The calculated daily total intake of β -HCH was 3.9 in Turkana, 3.1 in Meru, 2.2 in Karatina and 1.6 in Embu ug/day per person. Similarly the calculated total intake of lindane was 18.4 from Turkana, 15.8 from Embu, 14.2 from Meru and 7.3 ug/day from Karatina. The occurrence of these compounds in foods reflects, their use in agriculture. These levels are lower than those reported in India (Kaphalia <u>et al</u>., 1985), but higher than those reported in other countries (FAO/WHO, 1982). They reflect the corresponding levels found in mothers milk (Table 5.5). In Japan where HCH was used in rice fields, β -HCH was found to be the most important contaminant in milk and beef (Minagawa, 1979).

Aldrin and dieldrin

Aldrin and dieldrin were both detected mostly in cereals from Embu. This could be due to the intense use of both compounds in agriculture. In Rusinga Island dieldrin was detected only in fish, pulses and vegetables. Dieldrin might be an oxygenated metabolite of aldrin, but is likely to have come from aerial spraying against tsetse flies along the lake in this region. This may contaminate the vegetation around the lake and the lake waters, leading to dieldrin accumulation in fish. In Turkana, aldrin was found in meat, cereals and pulses, but no dieldrin. Aldrin was used as an insecticide in seed dressing. Maize seeds from Turkana contained high levels of this compound (Table 7.1). Apart from its use in seed dressing, it is also used against other insects in food crops. The consumption of aldrin contaminated plant materials by animals may lead to its accumulation in the animal fat. Dieldrin was also detected in cereals from Karatina and pulses from Meru, but the levels were low. The calculated daily total dietary intake of dieldrin in Embu was 4.4 ug and 24.8 ug in Rusinga Island, and the calculated daily total dietary intake of aldrin in Turkana was 3.7 ug. The presence of aldrin and dieldrin in mothers milk (Table 5.4) seems to be directly associated with their use in agriculture.

Heptachlor epoxide, oxychlordane and transnonachlor Heptachlor epoxide, which is a metabolite of heptachlor, was found in all food groups from Embu. The highest mean level was found in cow's milk samples, which again may be due to pesticide contamination of the feedstuffs since this was the area that reported intense use of a large number of organochlorine pesticides in agriculture. Only two cereal samples from Meru were found to contain low levels of heptachlor. The calculated daily total intake of heptachlor epoxide in food from Embu was 15 ug. However, no corresponding residues were detected in mother's milk. Oxychlordane and transnonachlor were only detected in meat samples from Turkana, but no residues of these compounds occurred in mother's milk.

7.6 CONCLUSIONS

1. The occurrence of pesticide residues in food samples were associated with the use of similar pesticides in the area.

2. Although pesticide contamination in foods was low the prolonged or consumption of large quantities of such foods, may

lead to accumulation in the human body as evidenced by excretion of organochlorine residues in mother's milk.

3. Food of animal origin usually contained higher levels of the DDT-group of compounds compared to other food groups.

4. Good agricultural practice and personal hygiene may reduce the daily total dietary intake of the pesticide residues.

5. The results of this study confirm that food is a major source of organochlorine in the human body.

6. It is important that studies of levels of organochlorine in food should be repeated at intervals in order to detect any increase in residue contamination levels, so that necessary steps may be taken.

Product	No. of samples	p,p'-DDT		p,p'-DDE		o,p'-DDT		p,p'-DDD)	Sum DDT
		No. positive	μg/kg Mean(Range)	No. positive	µg/kg Mean(Range)	No. positive	µg/kg Mean(Range)	No. positive	µg/kg Mean(Range)	µg/kg Mean(Range)
MEAT										
Cow	1	1	13.5	1	3.7.	1	6.2	0		23.8
Sheep	1	1	19.1	0	-	0		0		19.1
Goat	1	0		0	-	0		0		0
FAT										
Cow	1	1	49.8	1	33.9			1	31.0	121.0
MILK										
Cow	2	0		1	Trace	0		1	Trace	
Goat	2	2	3.0(T-3.0)	1	3.8	1	Trace	1	Trace	7.2(T-7.2)
Dried	1	0		0		0		0		
CEREALS *Maize										
(seeds)	1	1	6.4	0		0		0		6.4
*Maize	1	0		0		0		0		
*Maize-										
flour	3	3	75.8(8-82.7)	3	7.8(T-13)	3	14.7(T-16.2)	0		100.8(8-102)
Maize-										
flour	1	1	18.1	1	1.2	1	4.1	0		23.4
Sorghum	2	2	5.0(2.9-7)	1	7.2	0		0		13.0(2.9-14.9)
*Wheat	1	0		0		0		0		
PULSES										
*Beans Green	1	1	Trace	0		0		0		
grams	1	1	Trace	0		0		0		-

Table 7.1a Levels of compounds of the DDT-group in food samples collected from Turkana

*Imported from outside the district

Product	No. of	α - HCH		β - HCH		Lindane		Aldrin		Dielo	irin
	Sampioa	No. positive	µg/kg Mean(Range)	No. positive	μg/kg Mean(Range)	No. positive	µg/kg Mean(Range)	No. positive	µg/kg Mean(Range)	No. pos.	Mean(Range)
MEAT									6		
Cow	1	0		0		1	5.3	1	3.7	0	
Sheen	1	0		1	4.2	1 -	8.7	1	1.5	0	
Goat	1	0		0		1	7.1	0		0	
FAT											
Cow	1	1	5.6	1	15.1	1	2.5	0		1	1126.4
MILK											
Cow	2	0		0		2	1.1(0.7-1.5)	0		0	
Goat	2	0		0		2	0.3(0.2-0.4)	0		0	
Dried	1	0		0		1	0.1	0		σ	
CEREALS a*Maize											
(seeds)	1	0		0		1	1020.6	1	105.3	1	20.4
*Maize	1	0		0		0	41 9	1	2.7	0	
flour	3	0		0		3	(4.3-116.6)	3	5.9(5.6-6.5)0	
Maize-		-		0		4	2.2	4	3.0	0	
nour	1	0		0		2	1/0/2026	211	1.2	0	
*Wheat	2	0		0		1	3.1	1	4.2	0	
PULSES											
*Beans	1	0		0		1	28.7	1	4.0	0	
Green-		0		•							
grams	1	0		0		1	4.3	1	0.8	0	

<u>Table 7.15</u> Levels of α – HCH, β – HCH, lindane, aldrin and dieldrin μ g/kg (ppb) in food sample collected from Turkana

*Imported from outside the district. a not included in the calculation of the daily intake.

Product	No. of samples	HCB		Oxychlora	ine	Transnon	achlor	PCB	
		No. positive	μg/kg Mean(Range)	No. positive	μg/kg Mean(Range)	No. positive	µg/kg Mean(Range)	No. positive	µg/kg Mean(Range)
MEAT									
Cow	1	0		1	3.6	1	5.2	0	
Sheep	1	1	1.1	0		1	8.8	0	
Goat	1	0		0				0	
FAT									
Cow	1	1	3.1	0		1	8.9	0	
MILK									
Cow	2	2	1(0.5-1.5)	0		0		0	
Goat	2	2	0.2(0.1-0.2)	0		0		0	
Dried	1	0		0		0		0	
CEREALS *Maize									
(seeds)	1	0		0		0		0	
*Maize *Maize-	1	0		0		0		0	
flour Maize-	3	0		0		0		0	
flour	1	0		0		0		0	
Sorahum	2	0		0		0		0	
*Wheat	1	0		0		0		0	
PULSES									
*Beans Green-	1	0		0		0		0	
grams	1	0		0		0		0	

Table 7.1c Levels of some organochlorine compounds and PCBs in food samples collected from Turkana

*Imported from outside the district

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Product	No. of samples	p,p'-DDT		p,p'-DDE		o,p'-DDT		p,p'-DDD		Sum DDT
	1	No. positive	μg/kg Mean(Range)	No. positive	µg/kg Mean(Range)	No. positive	µg/kg Mean(Range)	No. positive	µg/kg Mean(Range)	µg/kg Mean(Range)
FISH										
Fresh	5	3	33.2(T-55.3)	5	56.4(14.9-122.4) 1	9.7	1	15.4	94(30.5-161)
Dried	5	0	,	4	130.6(14.4-330.	3) 0		1	15.6	149(33.3-367)
Smoked	1	1	11.1	1	92.0	0		1	26	142.1
Cooked	2	0		0		0		0		
COW MEAT	1	0		0		0		0		
COW MILK	1	0		0		0		0		
CEREALS						٠				
Maize	1	1	36.7	0		0		0		36.7
Sorghum	1	0		1	83.5	0		0		92.7
Millet	1	0		0		0		0		0
Rice	1	0		0		0		0		0
PULSES										
Beans	1	0		1	38.5	0		0		42.7
Greengrams	5 1	0		1	31.4	0		0		34.9
VEGETABLE	S									
Tomatoes	1	0		0		0		0		
Kale(fresh)	1	0		0		0		0		
Kale(cooked	3) 3	0		0		0		0		
ROOTS & TU	JBERS								51010-	
Cassava	1	0		1	Trace	1	Trace	1	Trace	
potatoes	1	1	36.7	0		0		0		36.7
	D (Cooked									
Maizemeal	+)								
kale	1	0		0		0		0		
Maizemeal	1	0		1	18.0	0		0		20.0

Table 7.2a Levels of DDT and metabolite residues in food samples collected from Rusinga Island

Table 7.2b

Levels of α – HCH, β – HCH, lindane, aldrin and dieldrin µg/kg (ppb) in food sample collected from Rusinga Island

Product	No. of samples	α-HCH		β-НСН		Lindane		Aldrin		Die	Idrin
	p	No. positive	µg/kg Mean(Range)	No. positive	µg/kg Mean(Range)	No. positive	μg/kg Mean(Range)	No. positive	µg/kg Mean(Range)	No. pos.	μg/kg Mean(Range)
FISH											
Fresh	5	3	2.5(2.1-3.3)	0		4	2.8(2.4-3.3)	0		2	44.5(15-73.9)
Dried	5	3	8.6(1-10)	0		2	4.5(2.5-6.4)	0		3	64.6(25.5-123.2
Smoked	1	1	1.3	0		1	2.5	0		1	17.4
Cooked	2										
COW MEAT	1										
COW MILK	1										
CEREALS											
Maize	1	0		1	203.4	0		0		0	
Sorahum	1	1	Trace	0		0	1	0		0	
Millet	1	0		0		0		0		0	
Rice	1	0		0		0		0		0	
PULSES											
Beans Green-	1	0		0		0	,	0		1	21.8
grams	1	0		0		0		0		0	
	s										
Tomatoes	1	0		0		0		0		0	
Kale(fresh)	1	0		0		1	3.8	0		1	9.1
Kale											
(Cooked)	3	0		0		1	33. 4	0		1	5.8
ROOTS & TUBERS											
Cassava Sweet	1	0		0		0		0		0	
potatoes	1	0		0		0		0		0	

Cont. Table 7.2b

Product	No. of sample	α - HCH		β-НСН		Lindane		Aldrin		Dielo	drin
		No. positive	µg/kg Mean(Range)	No. positive	µg/kg Mean(Range)	No. positive	μg/kg Mean(Range)	No. positive	μg/kg Mean(Range)	No. pos.	µg/kg Mean(Range)
MIXED FOO (Cooked) Maizemeal	•										
Kale	1	0		0		0		0		0	
Maizemeal	1	0		0		0		0		0	

Table 7.3a Levels of compounds of the DDT-group in food samples collected from Karatina

Product	No. of samples	p,p'-DDT		p,p'-DDE		o,p'-DDT		p,p´-DDD		Sum DDT
		No. positive	µg/kg Mean(Range)	No. positive	µg/kg Mean(Range)	No. positive	μg/kg Mean(Range)	No. positive	μg/kg Mean(Range)	μg/kg Mean(Range)
COFFEE(free	sh									
beans)	2	0		0		0		0		0
Tea(fresh										
leaves)	2	0		0		0		0		0
(processed)	1	0		0		0		0		0
CEREALS										
Maize	4	0		0		0		0		0
Maize flour	2	1	Trace	1	3.9	0		1	34	42.1
Sorghum	1	0		0		0		0		0
Sorghum flo	our+									
Maize flour	1	1	Trace	0		1	0	0		0
PULSES										
Beans	1	0		0		0		0		0
	s									
Kale	3	0		0		0		0		0
Tomatoes	2	0		1	26.5	0		0		29.4
Cabbage	1	0		0		0		0		0
leaves	2	0		0		0		0		0
ROOTS & TUBERS										
Potatoes	5	0		0		0		0		0
FRUITS Passion										
fruit	2	0		0		0		0		0
Banana	1	0		0		0		0		0

Cont. Table 7.3a

Product	No. of samples	p,p'-DDT		p,p'-DDE		o,p'-DDT		p,p'-DDD		Sum DDT
		No. positive	μg/kg Mean(Range)	No. positive	µg/kg Mean(Range)	No. positive	μg/kg Mean(Range)	No. positive	µg/kg Mean(Range)	µg/kg Mean(Range)
)									
Maize+										
beans Cooked mail	1 ze+	1	20.6	0		0		0		20.6
beans	1	1	15.3	1	15.4	0		0		32.4

Table 7.3b

Levels of α – HCH, β – HCH, lindane and dieidrin µg/kg (ppb) in food sample collected from Karatina

Product	No. of samples	α - HCH		β-нсн		Lindane		Diel	drin
2	Sampios	No. positive	μg/kg Mean(Range)	No. positive	μg/kg Mean(Range)	No. positive	µg/kg Mean(Range)	No. pos.	µg/kg Mean(Range)
COFFEE(fresh beans)	2	0		0		0		0	0
TEA(Fresh leaves)	2	0		0		0		0	0
(processed)	1	0		0		0		0	0
CEREALS									
Maize	4	1	1.4	0		1	3.2	1	4.1
Maize flour	2	0		0		0		0	0
Sorghum	1	0		0		0		0	0
Sorghum flour+									
Maize flour	1	0		1	4.4	1	5.3	0	0
PULSES									
Beans	1	0		0		0		0	0
VEGETABLES									
Kale	3	0		0		1	50.5	0	0
Tomatoes	2	0		0		1	2.3	0	0
Cabbage	1	0		0		0		0	0
Pumpkin leaves	2	0		0		0		0	0
BOOTS & TUBERS								1	
Potatoes	5	0		0		0		0	0
Maize+beans	1	1	1.9	0		1	Trace	1	4.4
Cooked maize+beans	1	0		0		0		1	4.4

Product	No. of	p,p'-DDT		p,p'-DDE		o,p'-DDT		p,p'-DDD		Sum DDT
	Jampies	No. positive	μg/kg Mean(Range)	No. positive	μg/kg Mean(Range)	No. positive	µg/kg Mean(Range)	No. positive	μg/kg Mean(Range)	μg/kg Mean(Range)
COWS MILK	8	2	186(140-231)	6	27(6-57)	2	22.4(22-22.9)	2	172(118-226)	185(56-570)
(beans)	2	0		0		0		0		
Tea(leaves)	2	0		0		0		0		
CEREALS Maize										
(green)	2	0		1	16.5	0		0		18.3
Maize(dry)	З	1	76.1	1	Trace	1	Trace	0		84.5
Maize		•		•		0		0		
(yellow)	E	0		0	20 1/12 7 25 41	0		0		22 3(15 2-28 2)
Sorgnum	2	0		3	20.1(13.7-23.4)	0		0		,
Millet fleur	0	0		0		0		0		
Willet flour	2	0		0	00.0	0		0		92.5
wheat nour	4	U		1	03.3	U I		v		02.0
PULSES	-									
Beans	5	0		1	Trace	0		0		
Cowpeas	8	0		3	Trace	0		0		
grams	4	0		0		0		0		
	•									
VEGETABLE	5		1100/75 1 0004	F	20 4/0 2 110 5)	5	217/25 0.016)	3	1314/71 5-346	8) 2458(114-5357)
Comatoes	0	5	1180(75.4-2694)	5	39.4(9.2-110.5)	5	317(25.9-910)	0	1014(71.0-040	0
Cabbage	2	0		0	Traca	0		0		0
Green years	4	0		•	Trace	0		v		•
tables	8	0		3	10.9(5.6-19.7)	0		0		12.1(6.2-21.9)
ROOTS &										
TUBERS English										
potatoes	3	0		1	5.6	0		0		6.2
Carrots	2	0		0		0		0		
Sweet										
potatoes	2	0		1	5.6	0		0		6.2

Table 7.4a Levels of compounds of the DDT-group in food samples collected from Embu

Cont. Table 7.4a

Product No. of samples		p,p'-DDT s		p,p'-DDE		o,p'-DDT		p,p'-DDD		Sum DDT
		No. positive	μg/kg Mean(Range)	No. positive	µg/kg Mean(Range)	No. positive	µg/kg Mean(Range)	No. positive	µg/kg Mean(Range)	µg/kg Mean(Range)
Arrow										
roots	2	0		2	Traces	0		0		
FRUITS										
Banana										
(green) Banana	1	0		0		0		0		
(roasted)	1	0		0		0		0		
Mangoes	1	0		0		0		0		
Lemon	2	0		0		0		0		
Passion										
fruit	4	0		0		0		0		
MIXED FOOI	OS									
Maize+bear	ns(not									
cooked)	2	0		0		0		0		
Maize+bear	ns									
(cooked/										
boiled)	7	0		2	11.5(9.9-13.1)	0		0		12.8(11-14.5)
Mashed ye	llow									
maize+cow	V-									
beans	1	0		0		0		0		
Mashed ma	nize+									
beans+										
potatoes	1	0		0		0		0		
Fried kale+										
carrots	1	0		1	21.3	0		1		23.6
Maize meal	1									
cooked	1	0		0		0		0		
Cooked gree	en									
vegetables	1	0		0		0		0		

<u>Table 7.40</u> Levels of α – HCH, β – HCH, lindane and heptachlor epoxide in food samples collected from	m Embu
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Product	No. of	α-HCH		Linda	เกอ	β- HK	ж	Dielo	Irin	Hept	achlor epoxide	Aldri	n
	samples	s										_	
	No.	μg/k pos.	g Mean(Range)	No. pos.	µg/kg Mean(Range)	No. pos.	µg/kg Mean(Range)	No. pos.	μg/kg Mean(Range)	No. pos.	µg/kg Mean(Range)	No. pos.	µg/kg Mean(Range)
COWS MILK	8	2	10.3(3.9-16.6)	1	22.1	1	11.1	0		2	38.5(19.4-57.6)	0	
COFFEE BEANS	2	0	,	0		0		0					
TEA (leaves)	2	0		0		0		0					
CEREALS													
Maize(green)	2	0		0		0		0		1	6.4	0	
Maize(dry)	3	0		1	6.8	0		0		0		0	
Maize(yellow)	1	0		0		0		0		0		0	T
Sorghum	4	1	5.6	1	9.0	0		0		0		1	Irace
Millet	3	1	5.6	1	Тгасе	0		1	7.7	0		0	40.0
Wheat flour	4	1	13.8	1	5.4	0		0		0		1	43.0
PULSES													
Beans	5	0		1	Trace	0		0		0		0	
Cowpeas	8	2	12,5	2	Traces	2	Traces	1	5.8	1	3.7	0	
Greengrams	4	0		0		1	Trace	0		1	6.4	0	
VEGETABLES													
Tomatoes	5	3	Traces	1	15.6	0		0		0		0	
Cabbage	1	0		0		0		0		0		0	
Kale Green	2	2	3.0(2.9-3)	1	9.05	0		0		0		0	
vegetables	8	4	10.8(2.2-32.4)	5	121(14.8-278)	0		0		1	19.4	0	
ROOTS & TUBERS													
English													
potatoes	3	0		0		0		0		1	14.6	0	
Carrots Sweet	2	0		0		0		0		0		0	
potatoes	2	0		. 0		0		0		0		1	Trace
Arrow roots	2	1	4.2	1	45.0	0		1	49.3	1	14.9	0	

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Product	No. of samples No.	α-HCH		Linda	Lindane		ж	Dieldrin		Heptachlor epoxide		Aldrin	
		μg/kg pos.	g Mean(Range)	No. pos.	µg/kg Mean(Range)	No. pos.	µg/kg Mean(Range)	No. pos.	µg/kg Mean(Range)	No. pos.	µg/kg Mean(Range)	No. pos.	μg/kg Mean(Range)
FRUITS													
Banana(green) Banana	1	0		0		0		0		0		0	
(roasted)	1	0		0		0	1	1	6.6	1	9.6	0	
Mangoes	1	1	6.4	0		0		0		0		0	
Lemon	2	0		0		0		0		0		0	
Passion fruit	4	4	Traces	2	Traces	0		0		0		0	
MIXED POODS Maize+beans (not cooked)	2	1	Тгасе	1	5.4	0		0		0			
Maize+beans (cooked/	-		11200										
boiled) Mashed yellow maize+	7	4	12.4(2.9-22)	2	9.0(5.4-12.5)	0		1	14.6	1	7.3	0	
cowpeas Mashed maize- beans+	1	0		0		0		0		0		0	
potatoes Fried kale+	1	0		0		0		0		0		0	
carrots Cooked maize	1	1	Trace	0		0		0		1	4.3	0	
meal Cooked green	1	1	11.2	0		0		0		0		1	Trace
vegetables	1	0		0		0		0		0		0	

Product	No. of samples	p,p'-DDT		p,p´-DDE		o,p´-DDT		p,p´-DDD		Sum DDT
		No. positive	μg/kg Mean(Range)	No. positive	μg/kg Mean(Range)	No. positive	μg/kg Mean(Range)	No. positive	μg/kg Mean(Range)	µg/kg Mean(Range)
MILK			2							
Cow's milk	6	5	28.4(19-38)	6	129(41.5-350)	4	7.6(T-10)	6	8.1(T-13.5)	178(65-442)
CEREALS										
Maize	5	2	9.1	0		0		0		9.1
Sorghum+										
millet	7	4	12.1(9.1-19.4)	3	7.8(4.8-12.2)	1	10.5	0		16,5(7,2-31)
PULSES										
Beans	4	0		0		0		0		0
Green-										
grams	1	1	50.9	0		0		0		50.9
Dolichos										
beans+								•		
pigeon peas	2	0		0		0		U		0

Table 7.5a Levels of compounds of the DDT-group in food samples collected from Meru

Cont. Table 7.5a

Product	No. of	p,p'-DDT		p,p'-DDE		o,p'-DDT		p,p'-DDD)	Sum DDT
-	sampies	No. positive	μg/kg Mean(Range)	No. positive	μg/kg Mean(Range)	No. positive	μg/kg Mean(Range)	No. positive	μg/kg Mean(Range)	μg/kg Mean(Range)
VEGETABLE	S									- 3
Green vege)-									
tabies	1	1	15.3	0		0		0		15.3
Cabbage	3	0		0		0		0		0
Tomatoes	2	0		0		0		0		0
ROOTS & TUBERS										
English										
potatoes	3	0		0		0		0		0
MIXED FOOD	xs									
Maize+bean	IS+									
green vege										
tables Maize+bean dolichos	4 15+	4	10.1(7.2-15.3)	0		1	Trace	0		10.1(7.2-15.3)
beans	1	1	9,3	0		1	Trace	0		9.3
Maize+doli-	-									-
chos beans	1	1	38.8	0		1	38,8	0		81.9
Maize+										
beans	13	7	10.7(5.4-23.2)	1	5.3	2	Traces	0		16.6(5.4-23.2)

Table 7.5b	Levels of other	organochlorine	compounds	in	food	samples	collected	from M	Meru
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Product	No. of	a-H	СН	β - Η	СН	Lind	ane	Aldrin		Dieldrin		Heptachlor		Heptachlor epoxide	
	ples	No. pos.	µg/kg Mean (Range)	No. pos.	µg/kg Mean (Range)	No. pos.	μg/kg Mean (Range)	No. pos	µg/kg Mean . (Range)	No. pos.	µg/kg Mean (Range)	No. pos	µg/kg Mean (Range)	No. pos	µg/kg Mean . (Range)
MILK Cows milk	6	0		0		2	Traces	0		0		0		0	
CEREALS Maize Sorghum+	5	2	6.2(T-6.2)	1	Trace	3	28.0(T-50.4)	0		0		1	Trace	0	
millet	7	7	3.3(1.3-8.4)	0		7	17.4(3-49.7)	2	Traces	0		1	1.8	0	
PULSES Beans Greengrams Dolichos beans	4	0		0 1	12.5	1	4.5 Trace	0		0 1	34.3	0		0	
pidgeon peas	2	0		0		1	1.3	0		0		0		0	
VEGETABLES Green															
vegetables	1	1	3.3	0		1	11.3	0		0		0		0	
Tomatoes	3	0		0		0		0		0		0		0	
ROOTS & TUBERS English potatoes	3	0		0		0		0		0		0	,	0	
MIXED FOODS Maize+beans+															
vegetables	4	3	2.7(2.4-3)	0		4	171(4.7-650)	0		0		0		1	3.8
Maize+beans+ dolichos beans Maize+dolichos	1	1	1.6	0		1	4.7	0		0		0		0	
beans Maize+beans	1 13	1 9	4.0 1.8(1.1-2.8)	1	2.5 Trace	1 11	6.5 7.5(3-13.5)	1 1	6.1 Trace	0		0 0		0 0	

Table 7.6a Levels of compounds of the DDT-group in food samples collected from Nanyuki

Product	No. of samples	p,p'-DDT		p,p'-DDE		o,p'-DDT		p,p'-DDO	Sum DDT	
		No. positive	µg/kg Mean(Range)	No. positive	μg/kg Mean(Range)	No. positive	μg/kg Mean(Range)	No. positive	μg/kg Mean(Range)	µg/kg Mean(Range)
Maize	3	0		0		0		0		
Beans Maize+bean	3	0		0		0		0		
(mixed)	1	0		0		0		0		
Potatoes	3	0		1	39.1	1	28.6	0		43.4

The mean was calculated only for those samples that were positive. The limit of quantitation varied with the compound and type of food.
Table 7.6b Levels of other organochlorine compounds in food samples from Nanyuki

Product	No. of samples	α-HCH		Lindane		Dieldrin		
		No. positive	μg/kg Mean(Range)	No. positive	µg/kg Mean(Range)	No. positive	μg/kg Mean(Range)	
Maize	3	0		0		0		
Beans	3	0		0		0		
Maize+beans(mixed)	1	0		0		0		
Potatoes	3	3	5.5(4.6-6)	0		1	2.8	

The mean was calculated only for those samples that were positive. The limit of quantitation varied with the compound and type of food.

Table 7.7.

The percent incident of samples with organochlorine residues from each area.

Sample collection area	N	p,p'- DDT	p,p'- DDE	o,p - DDT	p,p*• DDD	НСВ	α-ΗСΗ	β−НСН	Aldrin	Diel- drin	Hepta- chlor	Hept. epox- ide	Lin- dane	Oxy- chlor- dane	Transno- nachior
Turkana	20	70	45	30	15	30	5	10	60	10		•	95	5	15
Rusinga Island	30	20	50	7	13		27	3	0	30	-	•	30		-
Karatina	32	13	9	3	3	•	6	3	•	9	•	-	16	-	
Embu	98	8	34	8	5		32	4	4	5		11	22	-	-
Meru	53	49	19	19	11		45	8	8	2	4	2	62	-	-
Nanyuki	10		10	10			30			10				•	-

N: Number of samples

Table 7.8. Mean levels (µg/kg)of pesticide residues by food group from Rusinga Island

Pesticide	Fish	Meat	Milk	Cereals	Pulses
p,p'-DDT	25.8	0	0	36.7	0
p.p -DDE	89.6	0	0	83.5	35.0
Sum DDT	128.3	0	0	129.4	38.8
α-HCH	4.9	0	0	0	0
β-HCH	0	0	0	203.4	0
Lindane	3.2	0	0	0	0
Aldrin	0	Ō	0	0	0
Dieldrin	50.0	0	0	0	21.8
PCB	0	0	0	0	0

Vegetables	Roots & Tubers	Mixed foods (cooked)
0 0 0	36.7 0 36.7	0 18 20.0
0 0 18.6 0 7.5 0	0 0 0 0 0	0 0 0 0 0 0

	Pesticide	Meat	Milk	Cereals	Pulses	
1	p,p'-DDT	27.5	3.0	37.4	0	
	p,p'-DDE	18.8	3.8	6.0	0	
	Sum DDT	54.6	7.2	36.1	0	
	α-HCH	5.6	0	0	0	
	B - HCH	9.7	0	0	0	
	Lindane	5,9	0.6	23.1	16.5	
	Aldrin	2.6	0	4.7	2.4	
	Dieldrin	0	0	0	0	
	HCB	2.1	0.6	0	0	
	Oxychlordane	3.6	0	0	0	
	Transnonachlor	7.6	0	0	0	
	PCB	0	0	0	0	

Pesticide	Milk	Cereals	Pulses	Vegetables	Roots & Tubers	Fruits	Mixed foods
p,p'-DDT p,p'-DDE Sum DDT	186 27 185	76.1 40.0 54.5	0 0 0	1180 25.1 1235	0 5.6 6.2		0 16.4 18.2
α-HCH	10.3	8.3	12.5	6.9	4.2	6.4	11.8
β - HCH Lindane	11.1 22.1	0 7.1	0	0 48.5	0 45.0	0	0 7.2
Aldrin Dieldrin	0	43.0 7.7	0 5.8	0	0 49.3	0 6.6	0 14.6
epoxide	38.5	6.4	5.0	19.4	14.9	9.6	5.8

<u>Table 7.11.</u>	Mean levels	(µg/kg) of	pesticide	residues b	y food	group	from	Karatina
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Pesticide	Cereals	Pulses	Vegetables	Roots & Tubers	Fruits	Mixed foods
p,p'-DDT	0	0	0	0	0	18.0
Sum DDT	42.1	0	29.4	0	0	26.5
α-HCH	1.4	0	0	0	0	1.9
β - HCH Lindane Dieldrin	4.4 4.2 4.1	0 0 0	0 26.4 0	0 0 0	0 0 0	0 0 4.4
				1		

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<u>lable 7.12.</u> Mean le	evels (µg/	kg) of	pesticide	residues b	y food	group	from Mer	u
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Pesticide	Milk	Cereals	Pulses	Vegetables	Roots & Tubers	Mixed foods
p,p'-DDT	28.4	10.6	50.9	15.3	0	17.2
p,p'-DDE	129	7.8	0	0	0	5.3
Sum DDT	178	12.8	50.9	15.3	0	29.4
α-HCH	0	4.7	0	3.3	0	2.5
β - HCH	0	0	12.5	0	0	2.5
Lindane	0	22.7	2.9	11.3	0	47.4
Aldrin	0	0	0	0	0	6.1
Dieldrin	0	0	34.3	0	0	0
Heptachlor Heptachlor	0	1.8	0	0	0	0
epoxide	0	0	0	0	0	3.8

Table 7.13. Estimated average weight of each food group analysed in a Kenyan adult diet.

Food group	Average weight g/day
Fish	400
Meat	400
Milk	150
Cereals	400
Pulses	150
Vegetables	200
Roots and Tubers	50

Table 7.14. Calculated dally intake (µg/day) of pesticide by food group from Rusinga Island

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Fish	Meat	Milk	Cereals	Pulses	Vegetables	Roots & Tubers
51.3	0	0	51.8	5.8	0	1.8
2.0	0	0	0	0	0	0
0	0	0	81.4	0	0	0
1.3	0	0	0	0	3.7	0
0	0	0	0	0	0	0
20.0	0	0	0	3.3	1.5	0
	Fish 51.3 2.0 0 1.3 0 20.0	Fish Meat 51.3 2.0 0 1.3 0 0 20.0 0 0 0 0 0 0 0 0 0	Fish Meat Milk 51.3 0 0 2.0 0 0 0 0 0 1.3 0 0 0 0 0 20.0 0 0 0 0 0 0 0 0 0 0 0	Fish Meat Milk Cereals 51.3 0 0 51.8 2.0 0 0 0 0 0 0 0 1.3 0 0 0 0 0 0 0 20.0 0 0 0	Fish Meat Milk Cereals Pulses 51.3 0 0 51.8 5.8 2.0 0 0 0 0 0 0 0 0 0 1.3 0 0 0 0 0 0 0 0 0 20.0 0 0 0 0	Fish Meat Milk Cereals Pulses Vegetables 51.3 0 0 51.8 5.8 0 2.0 0 0 0 0 0 0 0 0 0 0 0 1.3 0 0 0 0 3.7 0 0 0 0 0 0 0 20.0 0 0 0 3.3 1.5

Table 7.15. Calculated daily intake (µg/day) of pesticide by food group from Turkana

Pesticide	Meat	Milk*	Cereals	Pulses	
Sum DDT α - HCH β - HCH Lindane Aldrin Dieldrin HCB Oxychlordane Transnonachlor	21.8 2.2 3.9 2.3 1.0 0 0.8 1.4 3.0	3.6 0 0.3 0 0.3 0 0.3 0 0	14.4 0 9.2 1.8 0 0 0	0 0 6.6 0.9 0 0 0	

*Milk: 500 g milk in the diet

Table 7.16. Calculated daily intake (µg/day) of pesticide by food group from Embu

Pesticide	Milk	Cereals ^a	Pulses ^b	Vegetables	Roots & ^C Tubers	Fruits ^d	Mixed foods ^e
Sum DDT	27.7	21.8	0	247	0.3	0	20.9
α-HCH	1.5	3.3	1.8	1.3	0.2	0.3	13.5
β - HCH	1.6	0	0	0	0	0	0
Lindane	3.3	2.8	0	9.7	0	0	8.2
Aldrin Dieldrin Heptachlor-	0	17.2 3.0	0 0.8	0 0	0 0.3	0 0.3	0 16.7
epoxide	5.7	2.5	0.7	3.8	1.9	0.4	6.6
							150 a/dau

Pesticide	Cereals ^a	Pulses ^b	Vegetables	Roots & ^c Tubers	Fruits	Mixed food ^d
Sum DDT α - HCH β - HCH Lindane Dieldrin	21.0 0.7 2.2 2.1 2.0	0 0 0 0 0	5.8 0 0 5.2 0	0 0 0 0 0		30.5 2.2 0 0 5.0
a:500 g/day;	b:25	0 g/day;	c:200 g/	day;	d:1150 g/da	y

<u>Table 7.18.</u> Calculated dally intake (μ g/day) of pesticide by food group from Meru

Pesticide	Milk	Cereals ^a	Pulses ^b	Vegetables	Roots & ^c Tubers	Mixed foods ^d
Sum DDT	26.7	6.4	12.7	3.0	0	33.8
α-HCH	0	2.3	0	0.6	0	2.8
β - HCH Lindane Aldrin Dieldrin Heptachlor Heptachlor	0 0 0 0 0	0 11.3 0 0 0.9	3.1 0.7 0 8.5 0	0 2.2 0 0 0		2.8 54.5 7.0 0 0
epoxide	0	0	0	0	0	4.3
a:500 g/day;	b::	250 g/day;	c:200 g	j/day;	d:1150 g/day	

CHAPTER EIGHT

ORGANOCHLORINE PESTICIDE RESIDUES IN INFANTS MILK FORMULAS

8.1 INTRODUCTION

Breastfeeding has important social and economic consequences, especially in low-income countries. It is a dependable means of providing infants with a nutritious and easily absorbed food, and it gives immunological protection against certain diseases. In addition, by prolonging post partum amenorrhea, it increases the interval between births.

Today there is a wide variation in the extent and duration of breastfeeding among the Kenyan women. Modernization is leading towards earlier weaning and discontinuation of breastfeeding particularly in the urban areas, where the mother has to work away from home. This, together with the fact that the maternity-leave for working mothers in Kenya is only two months, forces the working mother to give the baby breast-milk substitutes while she is away. Since it has now been established that Kenyan mothers milk may contain chemical contaminants, which could have adverse effects on the nursing infants, it was also found relevant to investigate if the commonly used breastmilk substitutes on the Kenyan market might contain similar contaminants.

No similar investigation has previously been reported in Kenya.

2

8.2 MATERIALS AND METHODS

Samples of three different brands of infant milk formulas were purchased from a supermarket in Nairobi, Kenya. The milk was prepared according to the instructions on the labels and parallel samples analyzed for organochlorine pesticides and PCBs as described in chapter 4.

8.3 RESULTS AND DISCUSSION

Table 8.1. Organochlorine pesticide residues found in infant milk formulas.

Pesticide	Mean levels in mg/kg milk fat					
	NAN	SMA	ISOMIL			
p,p'-DDE	0.04	0	0			
Total DDT	0.05	0	0			
dieldrin	0.06	0	0			
% fat	3.4	2.6	4.3			
PCBs	0	0	0			
No of samples	6	6	6			

Only Nan contained detectable organochlorine pesticide residues, namely DDE and dieldrin (Table 8.1). No organochlorine compound was detected in the other two brands, SMA and ISOMIL. No PCBs were detected in any of the three brands. Nan is the commonly used brand. The level of total DDT, 0.05 mg/kg milk fat, was much lower than the mean level in mothers milk, 6.99 mg/kg milk fat. The estimated intake of sum DDT was only 4% of the ADI for an infant, while the corresponding mean value for mother's milk was 5 times the ADI.

The level of dieldrin (0.06 mg/kg milk fat) detected in formula milk was also lower than the mean level (0.37 mg/kg milk fat) detected in mothers milk.

Although Kenyan mothers milk has been found to contain considerable amount of organochlorine compound residues, breastfeeding is still strongly recommended and should be encouraged. Mothers milk contain the right balance of nutrients required for an infant (Jensen, 1983). In addition to other benefits mentioned earlier, is the relationship that develops between mother and infant during nursing.

8.4 CONCLUSION

The infant formula milk investigated in this study was found to contain low levels of organochlorine pesticides. The present study has demonstrated that the infant milk formulas on the Kenyan market are acceptable for use when breast-feeding for one reason or another cannot possibly be continued. It is then, however, important to emphasize that the instructions on the labels must be strictly followed. - 203 -

A COMPARATIVE STUDY: ORGANOCHLORINE PESTICIDE RESIDUES IN MATERNAL ADIPOSE TISSUE, MATERNAL BLOOD, CORD BLOOD AND HUMAN MILK FROM MOTHER/INFANT PAIRS

9.1 INTRODUCTION

The concern about environmental contamination by persistent organochlorine insecticides used both in agriculture and vector control, has led many countries to investigate the magnitude of their own environmental pollution. Residues of these compounds have been found at every level of the food chains (Hayes, 1975). Human beings are placed at the top of most food chains and it is not surprising that high levels of these compounds have been found in human adipose tissue and milk fat (Jensen, 1983; Slorach and Vaz, 1983). Investigations on possible health and environmental hazards involved, have led many industrial countries to restrict or ban the use of these chemicals and to enforce the tolerance levels for the residues in food and feeds. Following the banning of these organochlorine compounds, for instance, DDT and PCBs in Norway, the levels in human milk have decreased (Skaare, 1981; Skaare et al., 1988), and this trend is expected to continue.

As more priority is now being given to this problem in developing countries more data on organochlorine residues in mothers milk, and adipose tissue are being reported (Warnez <u>et</u> <u>al</u>., 1983; Atuma, 1986; Mpofu, 1986). The correlation between the pesticide residue levels in mothers milk, adipose tissue and blood has also been studied in India (Saxena et al., 1981, 1983). In addition, pesticide residue levels have been determined in the infant blood collected at delivery to study the significance of pesticide exposure in utero versus during the lactation period (Siddigui <u>et al</u>., 1981; Skaare <u>et al</u>., 1988). In Kenya, storage of organochlorine insecticides in the adipose tissue of Kenyans was studied in 1972 (Wassermann <u>et al</u>., 1972).

The aims of the present study were to investigate if the pesticide levels in human milk in Kenya reflects the body burden of these chemicals by assessing the organochlorine contamination level in maternal adipose tissue, blood, and milk. Further to study the exposure of the infant in utero to organochlorine pesticides.

9.2 MATERIALS AND METHODS

9.2.1 Sampling and collection

A total of 11 women (18-30 years of age) participated in this study. The women were giving birth to their first or second child by caesarean operation in Kenyatta National Hospital in 1986. Specimens of subcutaneous fat (about 1-5g) were collected from the mothers during the caesarean operation. Immediately after the delivery, samples of maternal blood (5-10mls) and umbilical cord blood (5mls) were collected into heparinized bottles. The milk samples were obtained 6 days post partum. The milk samples were expressed manually directly into clean labelled glass bottles, and the samples were frozen immediately until analysis. All samples were taken from mothers with normal and healthy babies (no twins). A questionnaire including background data on mother, such as age, occupation, parity and dietary habits, as well as sex and weight of the child, was completed by each mother.

9.2.2 Sample analysis

Extraction, cleanup and analysis of milk and serum samples were made according to the method described in section 5.3. The subcutaneous fat tissue samples were prepared by a slight modification of the method described by Bjerk and Sundby (1970). The method comprised grinding the tissue with anhydrous magnesium sulphate and sea-sand, extraction with diethyl ether (10 ml), evaporation of the ether to determine the fat content, and redissolving in hexane (0.05g fat/ml hexane). Two aliquots of the different hexane extracts were treated with concentrated sulphuric acid and methanolic potassium hydroxide, respectively. Aliquots of the purified hexane extracts were made up to an appropriate volume and analysed by gas chromatography (GLC), using a Varian model 3700, and a Carlo Erba model 2350 with the following instrument parameters and operating conditions: Detectors: ⁶³Ni electron capture; columns: glass of 1.5m x 2mm i.d. packed with a mixture of 1.5% SP-2250 and 1.95% SP-2401 on Supelcoport 100/120 mesh (a), a mixture of 1.5% OV-1 and 1.5% OV-225 on Supelcoport 80/100 mesh (b); Column temperature: 205 ^oC (a); temperature progr. 190 ^oC (1 min) to 230 ^oC, 5 ^OC/min.(b); Carrier gas: Nitrogen 30ml/min and injection volume: 2-5ul. The organochlorine compounds were identified by analyses of both acid and alkali-treated hexane fractions on both GLC-columns after which the retention times of organochlorine chemical residues and standards were compared.

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The results expressed on fat and wet weight basis are presented in Tables 9.1-9.2. The means and the ranges together with the number of samples containing quantifiable amounts are listed. The detection limit varied with the type of compound. A compound which has been identified and confirmed but not quantified is indicated as below the detection limit. p,p'-DDT, p,p'-DDE were found in all the 41 samples and o,p'-DDT in 24 samples. No detectable residues of o,p'-DDT was found in umbilical cord blood.

Dieldrin was detected in 27%, transnonachlor in 15%, β -HCH in 12%, aldrin in 5% and lindane 2% of all the samples analysed. No PCBs were detected in any sample.

Of the HCH-isomers, the β -HCH was found in 4 samples of the subcutaneous fat with a mean level of 23.7 ug/kg wet weight, but was found in only one mother's milk sample with a level of 0.26 ug/kg milk fat. No quantifiable levels of lindane $(\lambda$ -HCH) were found in any of the samples. Dieldrin was not found in any quantifiable amounts neither in mothers milk nor in subcutaneous fat, but was found in two maternal serum samples with a mean level of 39 ug/kg wet weight. Dieldrin was not detected in umbilical cord serum samples, but was found in 2 maternal serum samples with a mean level of 16.47 mg/kg fat Endrin and transnonachlor were only found in two and six maternal serum samples with mean levels of 4.83 and 0.65 mg/kg fat respectively. There was also a wide variation between individual samples. There were significant differences between the mean level of sum DDT in subcutaneous fat and milk fat; subcutaneous fat and maternal serum; maternal serum and milk fat

(Table 9.2). A significant higher mean residue level of sum DDT were found in the maternal serum as compared to umbilical cord serum samples (Tables 9.1 and 9.2). On wet weight basis, the mean level of sum DDT in maternal serum was four times higher than the corresponding level in umbilical cord serum.

9.3.1 Correlation Analysis

The correlation graphs are shown in Figures 9.1-9.6.

There was a significant correlation between the levels of sum DDT in subcutaneous fat and milk fat of the mothers (Fig.9.1). (r = 0.963, y = 1.04x + 1730, n = 8 P<0.05). Similarly a significant positive correlation was found between the mean levels of sum DDT in maternal blood and maternal milk (Fig.9.2). (r = 0.868, y = 0.3x + 1437, n = 8, P<0.05), and also between the levels of mean sum DDT in adipose tissue and maternal serum, (Fig.9.3). (r = 0.843, y = 2.35x + 566, P<0.05). There was no correlation between the levels of sum DDT in adipose tissue and cord blood (Fig.9.4), maternal blood and cord blood (Fig.9.5), and maternal milk and cord blood (Fig.9.6). (P>0.05, n = 11).

9.4. DISCUSSION

9.4.1 DDT-group

The individual variations in the present residue levels were not unexpected since it is well known that many factors may influence the results, e.g. exposure, age and weight of the mothers, dietary habits, parity of the mothers, fat content of the milk which is also dependent on time of sampling during the meal, the day, and the lactation period, and seasonal variations, may influence the results (Jensen, 1983).

Subcutaneous fat had the highest levels of sum DDT, which is not unexpected due to the lipophilic nature of these compounds. During lactation these compounds are mobilized and secreted in the milk (Niessen et al., 1984). This fact is also supported by the significant difference found between the mean levels of sum DDT in subcutaneous fat, milk and blood. The significant correlation found between the sum DDT in subcutaneous fat and milk fat, which was 0.963, indicates a high degree of coherence of DDT in the body, which also corresponds with the results of other investigators (Acker, 1981; Rogan et al., 1986; Skaare et al., 1988). This demonstrates that either human milk or adipose tissue are suitable indicators for the human body burden of persistent lipophilic pollutants. The detection of DDT group of compounds in the cord blood, demonstrates the transfer of these compounds through the placenta. It is shown in this study that the placenta restricts the transfer of the compounds to a varying extent. This is illustrated by the low correlation coefficient between maternal and cord serum. Several investigators have reported the presence of organochlorine pesticides in perinatal samples demonstrating the placental permeability of these compounds and a partial barrier function of the placenta (Eckenhausen et al., 1981; Saxena et al., 1981). The presence of some organochlorine compounds in fetus and in stillborn and newborn babies, has also been recorded (Eckenhausen, 1981; Saxena, 1983).

The finding of no or negative correlation between the levels of sum DDT in adipose tissue and cord blood, as well as between the levels of sum DDT in mother's milk and cord blood, may be partly explained by the lower fat content of the blood as compared to adipose tissue and milk. Furthermore, the placenta acting as a partial barrier may also play a role.

The 4 times higher mean level of sum DDT in maternal serum compared to the cord serum, may also be due to the higher fat content in mothers serum, together with the placental barrier. The high levels of sum DDT in all samples investigated may be due to the continued exposure of DDT, which is demonstrated by the ratio of DDE to DDT, which are similar to those found in developing countries (Slorach & Vaz, 1983), but higher than in developed countries where DDT is no longer used (Skaare <u>et al</u>., 1988).

The mean level of sum DDT, found in this study, are slightly higher (1.3 times) than that found in adipose tissue of females 25-44 years of age in Kenya (Wassermann <u>et al</u>., 1972). The subcutaneous fat samples were collected from mothers between 18 and 30 years of age living in Nairobi. The slight increase of sum DDT and the mean ratio of p,p'-DDT to p,p'-DDE in subcutaneous fat, may indicate a gradual increase of DDT contamination in the environment. The high levels of sum DDT and the mean ratio p,p'-DDT to p,p'-DDE in human milk in Kenya, which also corresponds to other developing countries (Slorach and Vaz, 1983), also illustrates a recent exposure to these pollutants. Some of the mothers who took part in this study, came from the previous studied areas (chapter 5) where DDT was reported used but they are now living in Nairobi. Skaare <u>et al</u>. (1988) found significantly higher mean levels of the organochlo-

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rine pesticides DDT and HCH in the samples of immigrants to Norway.

9.4.2 Other organochlorines

Of the HCH-isomers only β -HCH was found in 4 subcutaneous samples and all the levels were below 0.1 mg/kg fat, but in mother's milk the level was higher. This is the most persistent of the HCH isomers and is reported to have 10-30 times higher the ability to accumulate in fat tissues than lindane (Heeschen, 1980). The finding of dieldrin, endrin and transnonachlor in maternal serum and not in cord serum may possibly be due to a selectivity of the placenta towards the transfer of organochlorines. However, other investigators have reported corresponding organochlorine residues in maternal and cord blood samples (Saxena <u>et al</u>., 1983; Skaare <u>et al</u>., 1988), but none has reported the presence of dieldrin.

9.5 CONCLUSION

The organochlorine pesticides are transferred from mother to fetus and newborn babies through placenta and milk. There is a significant correlation between the levels of sum DDT in subcutaneous fat and milk fat of the mothers. Human milk is a good indicator for monitoring the environment for organochlorine chemical contamination. Table 9.1Levels of organochlorine compounds and PCBs in samples from Kenyan mothers and their infants
delivered by caesarean operations (wet weight).

	Subcutaneous fat (n=11)		Maternal serum (n=11)		Umbilical cord serum (n=11)		Milk (n=8)	
	No. positive	μg/kg wet wt. Mean (Range)	No. positive	µg/kg wet wt. Mean (Range)	No. positive	µg/kg wet wt. Mean (Range)	No. positive	µg/kg wet wt. Mean (Range)
	11	1912 (522 7252)	11	47 (1 2-22)	11	0.98 (0.41-2.48)	g	50 (13-118)
	11	95 (25-301)	5	0.8 (0.5-1.2)	o'	0.50 (0.41-2.40)	8	5.6 (0.6-11)
no'-DDF	11	2283 (1059-4455)	11	6.6 (2.5-22.2)	11	1.85 (0.58-3.68)	8	80 (11-202)
Sum DDT	11	4277 (1606-11838)	11	12.4 (4.5-48.9)	11	3.01 (1.11-6.53)	8	154 (26-327)
p,p'-DDE	11	0.72 (0.39-1.65)	11	0.67 (0.38-1.04)	11	0.54 (0.28-0.90)	8	0.87 (0.48-1.32)
В-нсн	4	23.7 (4.9-52.3)	0		0		1	12
Lindane	0		0		0		1	<0.002
Dieldrin	4	<0.002	2	39 (9.5-68.4)	Ō		5	<0.002
Endrin	0		2	11.6 (3.4-19.8)	0		0	
Transnonachlor	0		6	3.2 (0.6-8.6)	0		0	
PCBs	0		0		0		0	

<0.002 = below detection limit (The compound was identified and confirmed but could not be quantified).

Table 9.2

Levels of organochlorine compounds and PCBs in samples from Kenyan mothers and their infants delivered by caesarean operations (fat weight).

	Subcutaneous fat (n=11)		Maternai serum (n±11) U		Umbilical	Umbilical cord serum (n=11)		Milk (n=8)	
	No. positive	mg/kg fat Mean (Range)	No. positive	mg/kg fat Mean (Range)	No. positive	mg/kg fat Mean (Range)	No. positive	mg/kg fat Mean (Range)	
	4.4	0.40 (0.75.0.99)	11	0.91 (0.21.2.62)	11	0.60 (0.15-1.77)	9	2 14 (0 54-7 18)	
	11	2.49 (0.75-9.88)	5	0.01 (0.01-2.00)	0	0.00 (0.15-1.77)	8	0.16 (0.08-0.35)	
	11	3 26 (1 47-6 07)	11	1 52 (0 63-2 52)	11	1 26 (0 28-2 63)	8	2 31 (1 06-6 04)	
Sum DDT	11	5.91 (2.27-16.3)*	ii	2.75 (1.10-5.56)*	11	1.87 (0.46-4.66)	8	4.86 (1.79-14.22)	
P.D. DDE	11	0.72 (0.39-1.65)	11	0.67 (0.38-1.04)	11	0.54 (0.28-0.90)	8	0.87 (0.48-1.32)	
B-BHC	4	0.034 (0.006-0.074)	0	0.01 (0.00 1.0 1)	0		1	0.26	
Lindane	ŏ	0.004 (0.000 0.07 1)	Ő		0		1	<0.002	
Dieldrin	4	<0.002	2	16.47 (1.86-31.07)	0		5	<0.002	
Endrin	0		2	4,83 (0,68-8.99)	0		0		
Transnonachlor	õ		6	0.65 (0.16-1.69)	0		0		
PCBs	Ō		0		0		0		

<0,002 = below detection limit (The compound was indentified and confirmed but could not be quantified). *Significant differences (p<0,05) between sum DDT in: Subcutaneous fat and milk fat,

subcutaneous fat and maternal serum, maternal serum and milk fat.



Fig. 9.1 Relationship between sum-DDT levels in adipose tissue and mother's milk.



Fig. 9.2 Relationship between sum-DDT levels in maternal blood and mother's milk.



Fig. 9.3 Relationship between sum-DDT levels in adipose tissue and maternal blood.













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CHAPTER TEN

GENERAL CONCLUSIONS

- Kenyan mother's milk was found to be contaminated with organochlorine pesticides.
- DDT and its more persistent metabolite DDE were the major contaminants.
- 3. Great regional differences were observed.
- 4. PCBs, which is a major pollutant problem of industrialized countries, were not found in Kenyan mother's milk.
- 5. The individual variations in the organochlorine residue levels in the milk were found to be influenced by age of the mother, dietary habits, fat content of the milk, parity, and pesticide exposure of the mother.
- 6. The infant dietary intake of DDT through the mother's milk exceeded the ADI by several fold. ADIs of dieldrin and aldrin were exceeded by some infants.
- A child fed on formula milk in Kenya will be exposed to very low levels of organochlorine pesticides.
- 8. The levels of sum DDT found in mother's milk in this study were higher than the corresponding levels reported from industrialized countries.

- Food was found to be one of the main sources of organochlorine pesticides in mother's milk.
- 10. There was an association between the organochlorine pesticides used in agriculture and the identified compounds in the mother's milk.
- 11.In general, lactating women had an average intake of about 105 ug of DDT per day. Their milk contained on average 6.99 mg/kg of DDT resulting in an infant dosage of about 26 ug/kg per day, which isapproximately 15 times higher than the mother's daily intake of DDT (assuming the mothers weight to be 65 kg). There is no firm evidence that infants have been harmed by these quantities.
- 12.Organochlorine pesticides are transferred from mother to foetus and new born babies through the placenta and milk.
- 13. Human milk is a suitable indicator for biological monitoring of environmental contamination by organochlorine pesticides.
- 14.The problem of pesticide contamination requires closer attention on the part of national authorities and more research is needed.
- 15. The indiscriminate use of pesticides and many accidents are due to ignorance; therefore education programs on the subject should be intensified.
- 16.Integrated Pest Control should be incoorporated in the agricultural practices.

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