STUDIES OF ORGANOCHLORINE PESTICIDE RESIDUES IN SOME FRESHWATER AND ESTUARINE FISH IN KENYA

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DECLARATION

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

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J. C. Mugachia

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

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DR. T. E. MAITHO, B. V. M., M. Sc., Ph. D.

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DR. L. W. KANJA, BSc., M. Sc., Ph. D.

to my wife mwikali
and sons
mugachia and malonza

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

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Abstract

Organochlorine pesticides persist in the environment and accumulate in aquatic organisms. Although the chemicals have been used in Kenya since the 1940's, information on the occurrence of their residues in fish is still scanty. The main objective of the present study was to identify and quantify organochlorine residues in freshwater and estuarine fish from selected areas in Kenya and evaluate the toxicological implications of the findings.

A total of 275 fish samples were collected from five locations in Kenya between October, 1988 and October, 1989. The samples were obtained from Tana River at Masinga Dam, Garsen and Tarasaa, the estuary of Athi (Sabaki) River at Malindi and Lake Naivasha in the Great Rift Valley. The fish were caught with gill nets, line and hook or fishing baskets. The liver, eggs and fillet (muscle) from each fish were analysed separately using a Packard gas liquid chromatograph, fitted with a ^{63}Ni electron capture detector. Nine organochlorine pesticide residues were detected in 22.5% of the samples in the following order of frequency: p,p' DDE (20.4%), p,p' DDT (12.7%), Lindane (4.7%), o,p' DDT (4%), p,p' DDD (3.6%), β-HCH (2.5%), α-HCH (1.8%), heptachlor (0.7%) and o,p' DDD (0.4%). Polychlorinated biphenyls (PCB's) and hexachlorobenzene (HCB) were not detected in the fish.

The residues with the highest levels were p,p'DDE and p,p'DDT with ranges of 0.027 to 1.241 and 0.011 to 2.674mg/kg on wet weight basis respectively. Sum DDT levels ranged from 0.03 to 3.148mg/kg. The range for residue levels of lindane and o,p' DDT were 0.004 to 0.295 and 0.031 to 0.133mg/kg respectively.

The results showed locational differences in the occurrence of the pesticide residues in the fish but no significant difference ($p > 0.1$) in the means of the residue levels. Out of the 67 samples from Malindi and 40 samples from Masinga Dam, 72.7% and 39.5% respectively had one or more of the 9 pesticide residues detected. Only one out of the 65 samples from Garsen had p,p' DDE at a low level of 0.033mg/kg. No residues were detected in the 65 samples from Tarasaa and 40 samples from Lake Naivasha.

There was a difference in the distribution of organochlorine pesticide residues in the fish body. Common carps obtained from Masinga Dam had lindane, p,p' DDE and p,p' DDT in fillet while fish from Malindi rarely had the pesticide residues in fillet. Lindane was only detected in fish from Masinga Dam and was more common than DDT and its metabolites in the fish. p,p' DDT was more frequent than p,p' DDE and other DDT metabolites in the fillet of common carps. This indicated a recent exposure of the fish to p,p' DDT or a slow degradation rate of the compound in this species. Common carps had significantly higher residue levels of lindane ($p = 0.008$) than catfish in Masinga Dam.

There was a positive correlation ($r = 0.74$) of sum DDT to weight in the seven species of fish with detectable levels of the residue. Sharks had significantly higher mean level of sum DDT ($p < 0.0001$) than catfish, common carps and breams. No difference was observed between males and females of various fish species and betwen different organs in the levels of the various pesticide residues detected in the fish. Nonetheless, the residues were most common in the liver and eggs (ovaries).

The residue levels found in the present study are generally below the maximum residue limits (MRL), for the respective

organochlorine pesticide residues, set by the National Food Administration (NFA) of Sweden. This indicates that the pesticide residues do not pose a health risk to the consumers of fish from the areas studied.

CHAPTER ONE

Introduction

In his pursuit for control and possibly eradication of pests, man has experimented and used a wide range of compounds both natural and synthetic in forestry, agriculture, veterinary and public health programmes. Prior to the advent of organochlorine compounds, pesticides were either inorganic compounds such as arsenicals and fluorides or natural plant products such as rotenone and pyrethrum extract (Matsumura, 1975).

Organochlorine pesticides were introduced in the market during the Second World War to boost food production and control vector- borne diseases. This led to extensive use and abuse of the pesticides, a practice that continued up to the early 60's, oblivious of the potential hazards of the pesticides to the environment. By then, organochlorine pesticide use had been extended to control of forest pests owing to the apparently low mammalian toxicity of the compounds.

Due to their persistence in nature and prolonged use organochlorine pesticides have led to environmental disturbances like establishment of resistant populations of arthropod pests and decline in populations of non-target organisms. Minor pests have in some cases become major pests due to elimination of their natural predators (McEwen and Stephenson, 1979).

Research done in many parts of the world has led to knowledge about the presence, amount and distribution of organochlorine pesticides in the environment in general. The aquatic environment is the ultimate sink for pesticides used in agriculture, forestry and both

human and animal disease vector control (Mason *et al* , 1986). Pesticides reach the aquatic environment through rainfall run-off from agricultural fields (Tangatz et al, 1976), atmospheric deposition, ground water contamination, discharge of effluents into waterways, cattle dips or cleaning of spraying equipment in rivers. The persistent nature of the pesticides and their long range in the environment greatly contributes to their concentration in water bodies.

Once in the aquatic system, the pesticides are absorbed by aquatic organisms and concentrated in the trophic chain, thus endangering the life of fish and other organisms. Fish are an integral link in the aquatic food chain and have been reported to accumulate organochlorines up to 800 times the concentration in water (Chau and Afghan, 1982). Holden in 1962 found that fish exposed to low organochlorine pesticide concentration in water over a long period of time accumulated the pesticide in their body up to 300 times the concentration in the gills (Muirhead-Thomson, 1971).

Mortality of fish has been reported following massive application of chlorinated hydrocarbon pesticides (Someren, 1946; McEwen and Stephenson, 1979; Murty, 1986). Chronic exposure of fish to sublethal concentrations of organochlorine pesticides causes decline in fish populations due to poor reproduction success and early fry mortality (McEwen and Stephenson, 1979).

In Norway, heavy use of DDT on fruit orchards resulted in high DDT levels in biota (Bjerk, 1973). Cod sampled in 1970 had 90 to 135mg/kg DDT in liver tissue on wet weight basis. The highest residue concentration recorded was 576mg/kg (Bjerk, 1973). Consequent to the banning of DDT in 1970, residue levels in cod showed a downward trend during the period 1972-82 (Skaare *et al*, 1985).

Lower levels of organochlorine pesticides in fish have been reported from Lake Tanganyika (Deelstra, 1976), Sudan (Zorgani, 1980), Canada and countries surrounding the Baltic (Murty, 1986). Murty (1986) has thoroughly reviewed reports of organochlorine pesticide residues in fish from various regions of the world. The residue data on fish reported reflects the intensity of pesticides use and the general awareness of environmental pollution in the reporting countries.

Continued use of organochlorine pesticides in Kenya implies that the aquatic systems continue being polluted by the compounds. Prior to this study, the accumulation of DDT and other chlorinated pesticides in Kenyan rivers was not yet known. Although some work has been done in Kenya concerning organochlorine pesticide residues in aquatic systems, this has mainly been focused on the Rift Valley lakes and Lake Victoria (Koeman *et al* , 1972; Frank *et al*, 1977; Lincer *et al*, 1981; Mitema and Gitau, 1990). There was therefore a need to evaluate the extent of pollution of the Kenyan aquatic environment by persistent organochlorine pesticides by determining the levels of the compounds in fish tissues. Tana River was selected for this study because of its present and future significance in fish production. The estuary of the Athi (Sabaki) River and Lake Naivasha were also included in the study to compare the findings with those of Tana River.

The main objectives of the study were:

- To establish a method for analysis of organochlorine pesticide residues in fish in the laboratory.
- To identify and quantify organochlorine residues in fish from Tana River so as to assess the extent of pollution of the river by residues of the compounds and evaluate the toxicological implications of the findings.

- To assess the natural distribution of the pesticide residues in fish liver, muscle and ovaries.
- To generate reliable data on the levels of contamination of organochlorine pesticide residues in Kenyan fish from Tana River.

CHAPTER TWO

Literature Review

2.1 Introduction

The use of organochlorine compounds in pest control dates back to 1939 when the insecticidal properties of DDT were discovered by Muller, in his search for a control for clothes' moths (Matsumura, 1975; McEwen and Stephenson, 1979). DDT had been synthesised in 1874 by Zeidler (Stetter, 1977) but its insecticidal properties had remained unknown. The broad-spectrum arthropodicidal activity of DDT led to its widespread use against insect vectors of important world diseases such as plague, yellow fever and most importantly malaria (McEwen and Stephenson, 1979). During the 1950's heavy use of DDT was extended from public health to forestry and agriculture leading to outstanding increases in food production. This massive use of DDT was favoured by the low cost of the chemical and its spectacular insect control. However, the possible effects of the compound on non-target populations was ignored due to its apparently low toxicity to avian and mammalian species.

DDT synthesis was soon followed in the 1940's by development of many other structurally related compounds in rapid succession mainly to provide a solution to soil pests. DDT had only been used for a few years when it was discovered that its marked stability and lipophilic nature led to environmental persistence and accumulation in tissues of non-target organisms. The chemical was also found to be excreted in eggs and milk thereby jeopardizing the consumers' health.

The discovery of these aspects of DDT led to investigation of

other organochlorine pesticides most of which were found to be bioaccumulative. Tests of acute toxicity of organochlorine pesticides in fish also proved that the compounds were highly lethal to the species (Muirhead-Thomson R. C., 1971). In some cases, high fish mortality was reported following aerial application of organochlorine pesticides for mosquito control (Van Someren, 1946; McEwen and Stephenson, 1979).

To date, the aftermath of these findings has been strict restriction on the use of bioaccumulative organochlorine pesticides in some cases while some countries have banned their use altogether (McEwen and Stephenson, 1979; EPA, 1985).

Kenya banned or restricted the use of environmentally persistent organochlorine pesticides in 1986 (Pest Control Products Board records, 1986; Kimani, 1990). The mixture of HCH isomers was banned for all uses. Lindane (γ – HCH) was restricted for use as a seed dressing only. However, the compound is still used as an insecticide in the control of insect pests of cotton. 17 metric tons of the pesticide were imported in 1989. Endrin and heptachlor were banned while aldrin and dieldrin were restricted for use in termite control in the building industry. 30 metric tons of aldrin and 10 of dieldrin were imported in 1989.

DDT was banned for use in agriculture and its use restricted to public health only in control of mosquitoes. Nonetheless, DDT may still be introduced into the aquatic environment through its impurities in dicofol (a close relative of DDT). Dicofol is accepted for agricultural use in Kenya and is recommended as a miticide in cotton farming. 20,000 litres of the compound were imported in 1989. The maximum recommended level of DDT impurities in dicofol is 0.1%. Endosulfan (Thiodan®) is accepted for use in Kenya

and is highly recommended for control of cotton pests.

2.2 Organochlorine pesticides

Organochlorine pesticides are divided into three groups depending on their chemical structure. The compounds have been put into different uses especially in agriculture and public health programmes.

2.2.1 DDT group:

p, p' DDT is the most important insecticide in this group. Commercial DDT also contains o,p' DDT. The DDT group also includes p,p' DDE and p,p' DDD, both of which are p,p' DDT metabolites. p,p' DDE, which is as persistent as p, p' DDT but has no insecticidal activity, is the major metabolite of p, p' DDT. In contrast, p,p' DDD has insecticidal properties and is commercially available as an insecticide. There are other compounds that are chemically and structurally related to DDT such as dicofol.

Common name*: DDT

Chemical name: 1,1,1- trichloro- 2,2- di- (p- chlorophenyl) ethane.

Uses*: Control of vectorborne diseases such as malaria, yellow fever and trypanosomiasis.

Control of such ectoparasites as mites and lice

Control of agricultural and forestry pests such as the spruce bud worm.

Common name: DDD (TDE)

Chemical name: 1,1,- dichloro- 2,2- di- (p- chlorophenyl) ethane

Uses: Same as for DDT

Control of different forms of adrenal hyperproduction of corticoids especially in dogs and man.

Common name: DDE

Chemical name: 1,1- dichloro- 2,2-di- (p- chlorophenyl) ethylene

Uses: None at present

2.2.2 Cyclodiene group

The main members of this group are aldrin, dieldrin, heptachlor, and isodrin. These are highly insecticidal cyclic hydrocarbons formed by the Diel's- Alder reaction (Chau and Afghan, 1982). Some of the chemicals can be epoxidized both *in vivo* and *in vitro* to give analogues that are also insecticidal. The epoxides of aldrin, isodrin and heptachlor are dieldrin, endrin and heptachlor epoxide respectively.

Common name: Aldrin

Chemical name: 1, 2, 3, 4, 10, 10 - hexachloro- 1, 4, 4a, 5, 8, 8a- hexahydro-1,4-endo, exo-5, 8 - dimethanonaphthalene.

Uses: Control of ectoparasites such as bedbags

Seed dressing

Mixed with fertilizer for the control of soil pests

Control of foliar, vegetable and fruit pests

Common name: Dieldrin

Chemical name: 1,2,3,4,10,10- hexachloro-6,7- epoxy- 1,4,4a,6,7,8,8a- octahydro- 1,4- endo, exo,5,8- dimethanonaphthalene

Uses: Control of ectoparasites such as lice,and ticks

Control of vector-borne diseases such as trypanosomiasis, yellow fever and malaria

Control of soil pests

Common name: Endrin

Chemical name: 1,2,3,4,10,10- hexachloro- 6,7- epoxy- 1,4,4a,5,6,7,8,8a-octahydro,1,4- endo, endo-5,8 dimethanonaphthalene

Uses: Control of crop pests

Common name: Heptachlor

Chemical name: 1,4,5,6,7,8,8- heptachlor- 3a,4,7,7a-tetrahydro-4,7-methanoindene

Uses: Control of soil and cotton pests

Control of grasshoppers

Common name: Heptachlor epoxide

Chemical name: 1,4,5,6,7,8,8-heptachloro- 2,3- epoxy- 3a,4,7,7a-tetrahydro- 4,7 - methanoindene

Uses: None at present

2.2.3 Hexachlorocyclohexane (HCH) group

HCH can exist as eight different isomers but the technical product has only five of them (2 optical alpha isomers, beta, gamma and delta isomers) with alpha, beta and gamma isomers being the most common. A specific steric configuration is necessary for activity since only the gamma isomer (lindane) is a powerful insecticide.

Common name: Lindane

Chemical name: gamma- 1,2,3,4,5,6- hexachlorocyclohexane

Uses: Control of ectoparasites such as fleas and mites

Control of soil and crop pests and wire worms

Control of grasshoppers

Common name: alpha- HCH (BHC)

Chemical name: alpha- 1,2,3,4,5,6- hexachlorocyclohexane

Common name: Beta- HCH (BHC)

Chemical name: beta- 1,2,,3,4,5,6- hexachlorohexane

* Information on the names and uses of organochlorine pesticides was obtained from Matsumura (1975); Soulsby (1978) and Chau and Afghan (1982)

2.3 Absorption and accumulation of organochlorine pesticides in fish

Fish absorb and accumulate xenobiotics particularly those with poor water solubility and high lipophilicity, from water and food. The uptake from water occurs because of the very intimate contact with the medium that carries the chemicals in solution or suspension and also because fish have to extract oxygen from the medium by passing enormous volumes of water over the gills (Murty, 1986).

The process of organochlorine pesticide absorption by fish either through food or from water, is influenced by many factors such as the chemistry of the molecule, physical condition of the medium and the fish itself: its lipid content, size, stage of

development and physiological activity (Murty, 1986). Absorption and accumulation factors of organochlorine pesticide residues in different fish species are proportional to the lipid content and age of the fish (Katsura *et al*, 1979). Using whole body autoradiography, Katsura *et al* (1979) showed that organochlorine pesticide residues are accumulated in fatty tissues including the liver, peritoneal fat, spinal cord and the brain.

Whether fish absorb organochlorine pesticides primarily through food or directly from the water through the gills is not yet clear. Some investigators have found that higher pesticide residue levels in fish tissues are achieved by direct uptake through the gills while others report that higher levels are obtained with intestinal absorption following ingestion of the pesticides in food. Holden (1962) reported that brown trout exposed to ^{14}C-labeled DDT rapidly removed the toxicant from water, absorption principally being through the gills, and most of the DDT was taken up and stored in body lipid. Fathead minows exposed to endrin in water absorbed and accumulated more amount of residues than those exposed through food with the highest concentration factor from water and food being 13,000 and 0.8 respectively (Jarvinen and Toyo, 1978). The uptake of toxicants through gills is substantial and rapid. Lockhart *et al* (1977) demonstrated that methoxychlor was rapidly absorbed by fish exposed to the chemical through water only.

Crawford and Guarino (1976) showed that DDT was absorbed from the intestine of killifish, *Fundulus heterochlictus*, into muscle and then redistributed to the liver, ovaries and testes and accumulated in body lipids.

Uptake of DDT

Due to its high lipid solubility, DDT is taken up quickly from food and water, metabolized slowly and stored for an extended period in fatty tissues. Studies with bluegills and goldfish showed that these fish species took up DDT rapidly after a single exposure of a few hours (Murty, 1986).Uptake of DDT by fish is enhanced by high temperatures (Murphy and Murphy, 1971).

The distribution pattern of absorbed DDT is independent of the dosage. The brain, liver and other fatty tissues have the highest levels while muscle shows the lowest (Crawford and Guarino, 1976; Katsura *et al*, 1979). In the tissues, DDT is stored as the parent compound and the metabolites DDD and DDE. However, an additional DDT metabolite, DDA, was reported by Crawford and Guarino (1976) in killifish exposed to DDT in water.

Uptake of other organochlorine pesticides

Generally, other organochlorine pesticides are less rapidly absorbed from water by fish than DDT. Dieldrin is absorbed faster than HCH. Dieldrin is slowly absorbed from the intestines and fish exposed to lower concentrations in water tend to absorb a higher percentage of the chemical than those exposed to higher concentrations (Murty, 1986).The uptake of endrin by channel catfish was demonstrated to be low and the compound was completely eliminated upon transfer to an endrin-free diet (Jackson, 1976). Schimmel *et al* (1976) found that heptachlor-exposed spot reached equilibrium levels within 72 hours, but eliminated the compound slowly in uncontaminated water.

periods in the body due to their low polarity (Addison and Willis, 1978). Intestinal micro-organisms in fish are, however, known to convert DDT to DDD rather than DDE (Wedemayer, 1968).

Aldrin in fish is epoxidized to dieldrin which is more toxic to the species (Janice and James, 1976). Fish MFO system is highly potent in this process of lethal synthesis. Endosulfan is poorly metabolized by fish and is mainly converted to endosulfan sulphate (Murty, 1986). Mirex and heptachlor are also poorly metabolized and more than 80% of the parent compound still remained in goldfish 10 days post exposure (Iwie *et al* , 1974; Feroz and Khan, 1979). Endrin is metabolized by hydroxylation followed by conjugation in the liver (Murty, 1986).

2.4.2 Excretion of residues by fish

The hepatobiliary path is the main route of excretion of organochlorine pesticide residues in fish. However, excretion through the gills and renal pathway also plays an important role as shown by Moore *et al* (1977). Compounds with higher polarity are eliminated faster than those with lower polarity. Katsura *et al* (1979) showed that lindane was excreted more rapidly than α and β-HCH. Lindane is eliminated faster than dieldrin which in turn, is eliminated more rapidly than DDT (Murty, 1986).

The reproductive route also serves as an excretion pathway for organochlorine pesticide residues in fish. Crawford and Guarino (1976) found that a large proportion of DDT absorbed by killifish was deposited in the ovaries and testes. This was then incorporated in the eggs in females and a significant amount shed during spawning. Males were found to shed more of their DDT burden in milt (semen) than did females in eggs.

2.5 Toxic effects in fish

Organochlorine pesticides exhibit both acute and chronic toxicity to fish and are generally more toxic than organophosphorus compounds. However, some of the latter are as toxic as some of the highly toxic organochlorine compounds (Murty, 1986). Toxicity of organochlorine pesticides to fish was noticed soon after the introduction of DDT when high fish mortality was observed following spraying programmes for control of agricultural pests or for malaria control (Someren, 1946; McEwen and Stephenson, 1979).

Chronic toxicity of organochlorine pesticides is the main concern of conservationists since the long-term effects of the compounds may either pass unnoticed or be recognized when it is already too late to save the situation.

2.5.1 Acute toxicity

Among the organochlorine pesticides, endrin and other cyclodienes are highly toxic to fish. Standard 96-hour toxicity tests with endrin yield LC_{50} of 1µg/l or less in bluegills, rainbow trout, coho salmon, chinook salmon, fathead minnows, brook trout and cut-throat trout (Grant, 1978). This is an extremely low LC_{50} and for this reason, the maximum amount of endrin recommended in water if aquatic organisms are to be protected is 2 parts per trillion (ppt). Grant also reported that younger fish were more susceptible to endrin toxicity than older ones and that higher temperature tended to enhance endrin toxicity.

Another cyclodiene, endosulfan, is also highly toxic to fish. Its toxicity to several species of freshwater fish species ranges from 0.2 to 8.1 µg/l and 0.3 to 2.9µg/l to saltwater fish (Ananda *et al*, 1981). Heptachlor has a 96-hour LC_{50} range of 1 to 4µg/l for several species

of estuarine fish (Schimmel *et al*, 1976).

DDT has a low acute toxicity to fish when compared to many other organochlorine pesticides. o,p' DDT is about one third as toxic to fish as p,p' DDT while methoxychlor is less toxic than DDT. The 96-hour LC_{50} for DDT to *Gambusia* is 0.32mg/l as opposed to 1mg/l for methoxychlor (Murty, 1986).The reported 96-hour LC_{50} of lindane and technical hexachlorocyclohexane (HCH) to several species of estuarine fish ranges from 30 to 104µg/l (Murty, 1986).

2.5.2 Chronic toxicity and sublethal exposure

Nowadays, mass mortality of fish due to pesticide exposure is rare and results only from accidents or direct spraying into water masses. Fish are nonetheless commonly subjected to long term stress from exposure to sublethal pesticide concentrations. With time, such exposure may prove to be even more hazardous than lethal challenge because the small prolonged effects on the fish may alter their behaviour, feeding habits or reproductive success, thereby resulting in a slow but sure death of the population.

Exposure of a predatory fish, *Therapon jarbua* to 2µg/l of DDT for 15 days resulted in darkening of the skin, formation of a brown spot on the forehead, swelling of the eyes and erosion of the fin margins (Murty, 1986). When chinook and coho salmon were fed DDT, they developed severe hyperplasia of the nose which progressed until one eye was lost (Buhler *et al*, 1969). DDT lowers the learning ability of fish. Brook trout fed DDT orally lost a reaction they had learnt; to avoid their preferred light or dark sides of the aquarium (Anderson and Peterson, 1969).

Aberrant swimming, gathering in unusual parts of the water column and unusual species association was observed in Canada

when pools containing trout and salmon were accidentally contaminated with endrin (Murty, 1986). Chronic exposure of endrin to rainbow trout and goldfish is reported to cause hypersensitivity and growth inhibition (Grant, 1978).

DDT and other organochlorine pesticides have been shown to cause a decrease in reproduction and an increase in fry mortality. Crawford and Guarino (1976) demonstrated that *Fundulus* eggs exposed to 0.1mg/l of DDT in water lagged two stages behind the controls in early embryo development. The DDT treated eggs 'caught' up with the controls in the later stages of development and hatching was normal. In another experiment, only 40% of the eggs of a winter flounder exposed to 1.21ng dieldrin and 4.6ng DDT were fertilized while those exposed to 1.74ng dieldrin failed to be fertilized (Murty, 1986). Larvae of the eggs that hatched showed vertebral deformities, their extent being dose dependent. In 1955, 346,000 fry hatched from eggs of Lake George trout failed to survive and the tragedy was traced to high concentration of DDT in the eggs. All the lots of eggs with a concentration higher than 2.95mg DDT per kg died (Murty, 1986).

Exposure of goldfish to endrin via food at 143 to 430µg/kg showed decreased gametogenesis and smaller size of testes in experimental than in control fish (Murty, 1986).

2.6 Effects of organochlorine pesticides in other aquatic organisms

Contamination of the aquatic environment with organochlorine pesticides also threatens the life of other aquatic organisms. Some of the organisms for instance algae, daphnids,

oysters and planktons are important fish foods and their survival and productivity indirectly influence the survival of those fish relying on them for food. It has been shown that most of the aquatic flora and fauna have a high ability of absorbing and concentrating organochlorine pesticides in their tissues (Murty, 1986).

Vance and Drummond (1969) demonstrated that algae concentrate endrin in water many fold and are less susceptible to its toxicity than other organisms above them in the food chain. Four species of algae exposed to endrin in water at concentrations ranging from 0.02 to 1ppm for 7 days accumulated the compound by factors ranging from 140 to 222 fold. High resistance to organochlorine pesticide toxicity by fish food organisms is detrimental to to the fish because they may consume lethal doses of the compounds from the fish food organisms. Buttler (1966) reported that fish fed on oysters which had earlier accumulated high levels of DDT from ambient water died within two days.

Oysters demonstrate a high ability to concentrate dieldrin and endrin. Mason and Rowe (1976) reported concentration factors of 1670 and 2780 for a 168-hour exposure of the eastern oyster to endrin at a concentration of 0.1µg/l and 50µg/l in water. A similar duration of exposure for dieldrin at a concentration of 0.5 and 9µg/l showed concentration factors of 2880 and 2070 respectively.

Blue crabs absorb and concentrate DDT in their body lipid. This has been shown to cause toxicity in these organisms during winter when they mobilize their fat depots for energy provision (Koenig *et al*, 1976).

In conclusion, the available data indicate that pollution of the hydrosphere with organochlorine pesticides could result in altered community structure due to the differences in the response of

various organisms to the pollutants. Heckman (1981) described the changes in the aquatic communities associated with the orchard ditches near Humburg that occurred as a result of 25 years of intense pesticide use. He reported that certain species ,especiallly predators, had completely disappeared, hence stimulating the growth of other species.

2.7 Review of organochlorine pesticide residue studies in fish from different regions of the world

Since the recognition of the hazardous effects of organochlorine pesticides to the environment , their persistence, accumulation in the food chain and detrimental effects on non-target organisms, most industrialized nations have thoroughly investigated and accumulated massive data on the residue status in their environment , especially the hydrosphere. Most of this work has been done in North America and Europe.

2.7.1 United States of America (U. S. A.)

In the U. S. A., studies done on museum fish specimens collected from Lake Michigan between 1929 and 1966 and preserved in ethanol, indicated that DDT and its metabolites appeared in fish samples for the first time in 1949 (Murty, 1986). This was only four years after commencement of environmental application of DDT. Dieldrin on the other hand appeared for the first time in 1955 (Neiddermyer and Hickey, 1976). Studies of organochlorine pesticide residues in fish from various aquatic systems, done from the early 1960's to 1985, showed sum DDT levels ranging from undetectable to 92.2mg/kg on wet weight basis (Murty, 1986). Other organochlorine

pesticides including HCH isomers, heptachlor, heptachlor epoxide, aldrin and dieldrin had far much lower concentrations than DDT.

In a three-year study conducted in Massachusetts from 1965 to 1967, sum DDT in individual fish varied from undetectable levels to 49.1mg/kg on dry weight basis (Murty, 1986). Total DDT of channel catfish collected from 18 sites in the watersheds of Nebraska in 1964 was 2.2 to 92.2mg/kg and that of dieldrin 0.1 to 6.7mg/kg (Stucky, 1970). Endrin was detected in largemouth bass from the Lost River system (California) at concentrations of 97 and 107µg/kg in 1967 and 1968 respectively (Godsil and Johnson, 1968). In 1967 and 1968, DDT and its metabolites were detected in 584 out of 590 composite samples of 62 fish species collected from all over the U. S. A. The DDT levels ranged from undetectable to 45µg/kg on wet weight basis. Dieldrin was detected up to 2mg/kg in 75% of the samples (Murty, 1986).

Lindane, heptachlor, heptachlor epoxide, dieldrin, DDT, DDE, DDD and toxaphene were recorded in 1968 to 1969 in Lake Poinsett in South Dakota. DDT and its metabolites were detected in all the 147 samples collected in 1969. In 1970, DDT was found in all the catfish samples obtained from 54 commercial fish farms sampled in Arkansas. Dieldrin, endrin and toxaphene were detected in 89, 76 and 95% of the samples (Crockett *et al.*, 1975). Various studies showed that between 1970 and 1974, the mean DDT levels in fish declined but high levels were still recorded in areas of intense use of the pesticide; dieldrin and endrin levels remained unchanged (Schimmitt *et al.*, 1981).

Studies of organochlorine pesticide residues reported in fish from the Great Lakes of America in 1970, showed that fish from Lake Michigan had two to seven times the residue load of fish from

the other Great Lakes. The coho salmon from Lake Michigan was found to be highly contaminated and unfit for human consumption (Murty, 1986).

Following the ban on use of environmentally persistent organochlorine pesticides in the U. S. A. in the early 1970's, the levels of these compounds in fish have continued to show a declining trend (Murty, 1986).

2.7.2 Canada

Reports from various organochlorine pesticide studies in Canadian fish indicate low levels of the compounds in fish. Fredeen *et al.* (1971) detected DDT, DDD and DDE at levels of 0.01, 0.015 and 0.01 to 0.06 mg/kg in nine species of fish from the Saskatchewan River where DDT had been used for 20 years (1948 to 1967) for control of the blackfly larvae. Zitko (1971) reported low levels of DDT, DDE and pp' DDD in marine and freshwater fish in New Brunswick and Nova Scotia. A downward trend in total DDT content was recorded in Lake Simcoe fish in central Ontario during the period 1970 to 1976 (Murty, 1986). Dieldrin showed declining trend while chlordane and heptachlor epoxide were recorded for the first time in 1975 and 1976.

2.7.3 Europe

Countries around the Baltic Sea have made substantial contribution to the environmental monitoring of the pesticide residues in fish in Europe. In Norway, cod sampled in 1969 from Dalsfjorden had total DDT content of 0.57 to 2.15mg/kg where as those caught from an intensive fruit growing area had a residue level of 1.98 to 33mg/kg on wet weight basis (Stenersen and

Kvalvag, 1972). Bjerk (1973) sampled cod in 1971 and detected DDT at concentrations of 90 to 135mg/kg on wet weight basis in the liver. The highest residue level on fat weight basis was 576mg/kg. In 1976, cod collected from 16 localities in Norway had total DDT of 0.1 to 1.9mg/kg with a single wayward value of 14.5mg/kg on wet weight basis (Brevik, 1978). Kveseth (1981) detected high DDT (14mg/kg) levels in 1975 to 1976 in southern Norway in a lake near a plant nursery school.

Following the ban of DDT use in Norway, the level of contamination of fish with this compound has shown a declining trend. Skare *et al* (1985) reported a declining trend of organochlorine pesticide residues in fish during the period 1972 to 1982. This indicated the effectiveness of the ban of DDT use in Norway in 1970. The highest mean level of sum DDT in 1982 was 1.237mg/kg on wet weight basis, with a range of 0.191 to 3.845mg/kg, about a third of the corresponding 1972 level. γ- and α-HCH were also detected at low levels.

Levels of organochlorine pesticide residues in fish in Sweden and Finland were found to be generally lower than those in fish from Norway in various studies done from the mid 60's to the 70's (Murty, 1986). A steadily declining trend in organochlorine residues in fish was observed following the ban of use of the compounds in agriculture.

A study conducted in Ireland in 1966 showed residues of lindane, aldrin and dieldrin that ranged from 0.01 to 0.6mg/kg and o,p' DDT ranged from 0.03 to 0.7mg/kg in salmon trout (Murty, 1986). In 1974 to 1976, low levels of sum DDT in fish were reported from Finland and German Democratic Republic (Murty, 1986).

2.7.4 Other regions

Reports of organochlorine pesticide residue levels lower than those in fish from North America and the Baltic have been published from Iran, Jordan, Sudan, Lake Nubia, Lake Tanganyika and South Africa (Murty, 1986). In a survey conducted in 1974 in fish from two rivers in Iran, the sum DDT on fat weight basis, in barbus species was 60.6mg/kg and 196mg/kg. Organochlorine residues in fish from lakes and reservoirs were generally less than 25mg/kg.

Sum DDT values of 0.7 to 0.38mg/kg were reported in three species of fish caught from Lake Tanganyika in 1971 (Deestra *et al.*, 1976). Dieldrin and DDT at levels of 0.25 and 0.75mg/kg respectively were recorded in fish from two man- made lakes in South Africa in 1977 (Greichus *et al.*, 1977). Zorgani *et al.* (1976) and Zorgani (1980) reported sum DDT of 0.0022 to 0.184mg/kg in Lake Nubia fish and 0.3 to 2.9mg/kg in different species of fish from elsewhere in the Sudan. In Jordan, in 1971, the sum DDT in three species of fish ranged from 0.37 to 3.34mg/kg (Murty, 1986).

Mexico, Australia and New Zealand have reported very low levels of organochlorine contamination in fish tissues (Rosales and Escalona, 1983; Murty, 1986).

2.8 Review of organochlorine pesticide residue studies in Kenyan fish

The use of organochlorine pesticides in Kenya dates back to 1946 when DDT was used in aerial spraying for control of mosquitoes in the Lake Victoria region. Despite the national and the international outcry over the environmental hazards linked to the use and abuse of persistent organochlorine pesticides, it was not

until 1986 that Kenya restricted the use of some organochlorine pesticides. Investigations on the residue levels of the pesticides in fish in Kenya also appears to have been ignored and data is still scanty.

Someren (1946) reported massive fish deaths in Lake Victoria following aerial spraying of DDT to control mosquitoes. During that time, there were no available facilities to quantify organochlorine pesticides in biological material.

Koeman *et al* (1972) reported extremely low levels of dieldrin, pp' DDE and DDT in fish from Lake Nakuru. The levels reported for the three compounds were all below 0.007mg/kg on wet weight basis. Greichus *et al* (1978) studied the contamination of Lake Nakuru by organochlorine pesticides and found their residue levels very low in *Tilapia grahami*. Lincer *et al* (1981) reported undetectable to very low levels of DDE in fish from Lake Naivasha in a study conducted to investigate organochlorine pesticide residue levels in Kenya's Rift Valley Lakes. A predatory fish from Lake Baringo showed the highest level (2.13mg/kg) of DDE in the study.

Kanja (1989), as part of her study to investigate levels and sources of organochlorine pesticide residues in Kenyan mothers' milk, analysed fish samples from Rusinga Island in Lake Victoria. The fish samples were analysed in four categories as fresh, dried, smoked or cooked. Pesticide residues were detected in all the four categories except cooked fish. The overall sum DDT levels ranged from 0.031 to 0.367mg/kg. Smoked fish showed the highest mean (0.149mg/kg) of sum DDT. The DDT compounds detected in the study were pp' DDT, p,p' DDE, o,p' DDT and p,p' DDD with p,p' DDE being most prevalent. Other organochlorine pesticide residues found in this study were α-HCH, lindane and dieldrin. The levels

were, however, low and ranged from 0.0013 to 0.123mg/kg for all the detected compounds.

An investigation on the pesticide residue levels in fillet and fat of nile perch from Lake Victoria, done in 1988, revealed low levels of the compounds in the tissues (Mitema and Gitau, 1990). The mean sum DDT was 0.45 and 0.099mg/kg in fillet and fat respectively. The highest sum DDT level was 4.51mg/kg, detected in a fat sample. Four compounds of the DDT group namely p,p' DDT, o,p' DDT, p,p' DDD and p,p' DDE were detected. α-, β- and γ- HCH, dieldrin and aldrin were also detected at low levels ranging from 0.003 to 0.22 and 0.005 to 0.26mg/kg in fat and fillet respectively.

The findings of the studies reviewed indicate that the Kenyan aquatic system has been exposed to organochlorine pesticides. There is therefore a need for more data due to the continued use of the chemicals in recent years.

2.9 Review of methods for analysis of organochlorine pesticide residues in fish

The analysis of organochlorine pesticide residues in samples is divided into three parts: extraction, clean-up and quantitation. At residue levels the analytical method is required to be of high sensitivity and accuracy to enable legistrative bodies to arrive at valid decisions with regard to residue limits in commodities within their jurisdiction.

Extraction entails removal of the pesticide residue from the sample matrix. Extraction systems that have been employed for organochlorine pesticide residues in fish include liquid-liquid partitioning, ethanol/water extraction (Noren *et al* , 1968), blending

with anhydrous sodium sulphate followed by extraction with acetonitrile (Hesselberg and Johnson, 1972), column extraction using diethylether (Bjerk and Sundby, 1970) and soxhlet extraction (Koeman *et al* , 1972).In most extraction procedures, the residue is removed with a host of co-extractives which may interfere with the final quantitation of the residue.

The clean-up procedure eliminates the interfering co-extractives. Some of the documented clean-up procedures for pesticide residues in fish are column chromatography using alumina, liquid-liquid partitioning and acid/base treatment.

Quantitation is the step at which the amount of pesticide residue in the sample is determined. Gas-liquid chromatography, introduced in 1952 by James and Martin, is presently the most widely used quatitation technique for organochlorine pesticide residues (Chau and Afghan, 1981). The electron capture detector used in gas liquid chromatography is selectively responsive to compounds with high electronegativity (Holden, 1981). Thin layer chromatography (TLC) is a qualitative and semi-quantitative technique for analysis of organochlorine pesticide residues in fish. However, its use has long been superseded by gas liquid chromatography owing to its low sensitivity especially at low (nanogramme) residue levels.

The choice of the method to be used for organochlorine pesticide residue analysis is commonly dependent on availability of reagents and equipment, applicability of the method to multiresidue analysis, precision of the method, speed and cost of analysis. Table 2.1 shows some of the published methods for analysis of organochlorine pesticide residues in fish samples.

2.10 Polychlorinated biphenyls (PCBs) and Hexachlorobenzene (HCB)

PCB's are industrial chemicals mainly used as fire retardants. They are interfering substances in the determination of organochlorine pesticide residues in fish especially marine and estuarine ones from industrialized nations (Murty, 1986). HCB is used as a fungicide in agriculture and the chemical is persistent in biological material (de Vos *et al*, 1972).

Table 2.1 Some published methods for analysis of organochlorine pesticide residues in fish.

Extraction	Clean-up	Quantitation	Reference
Ethanol/water	Alumina	TLC/GLC	Noren *et al* (1968)
Diethylether (column elution)	Acid/base treatment	GLC	Bjerk and Sundby (1970)
Blending with acetonitrile	-	GLC	Hesselberg and Johnson (1972)
Petroleum ether/ soxhlet extraction	Partially deactivated alumina	GLC	Koeman *et al* (1972)

CHAPTER THREE

Materials and Methods

3.1 Sampling

3.1.1 Area of study

The main area of study comprised upper Tana River (Masinga Dam) and the Tana Delta sampled at Garsen and Tarasaa. The lower part of the Tana Delta (Kipini) was not sampled due to floods following heavy downpour at the time of sampling. Lake Naivasha and the estuary of the Athi (Sabaki) River were studied for comparison of results with those obtained from the Tana River study.

Tana River originates from Mount Kenya and the Nyandarua (Aberdare) ridges and meanders through areas with different agricultural activities. It first flows through Nyeri, Muranga and Kirinyaga Districts, where organochlorine pesticides have been used in control of vegetable pests, before traversing Embu and Tana River Districts. The river has along its course several irrigation schemes. Cotton is mainly grown in the giant Bura Irrigation Scheme, Hola Irrigation Scheme and the Galole Pilot Project while horticultural farming is done in the recently established Masinga Irrigation Scheme. Rice is grown in the Mwea Rice Irrigation Scheme and water from the scheme drains into the Tana through the Thiba tributary. Majority of rivers from Embu and Meru also drain into Tana River.

Athi (Sabaki) River originates from the Ngong Hills and flows through Machakos, Kitui, Taita-Taveta and Kilifi Districts before

emptying into the Indian Ocean at Malindi. Rainfall run-off from Nairobi is emptied into the river through the Nairobi River. Some horticultural farming is done under irrigation at various points along the course of Athi River.

Lake Naivasha is a fresh water lake situated in the southern region of the Great Rift Valley. Malewa River which originates from the Nyandarua Ridges flows through Kipipiri, North Kinangop and Ol Kalau and empties into the lake.

Fig. 3.1 shows the locations of the various sampling sites of the study area.

3.1.2 Sampling procedure

Fish were randomly obtained from the study areas by gill nets, fishing baskets or line and hook. Fish from Naivasha and Masinga Dam were caught and transported to the laboratory at Kabete the same day. To avoid putrifaction, the fish were transported in coolboxes packed with ice or freezer packs (dry ice). In the laboratory, samples of the lateral muscle, liver and ovaries were obtained by dissection, wrapped separately in aluminium foil, then labeled appropriately and each set of samples from one fish were packed together in plastic paper bags. The samples were thereafter stored in a freezer at -20°C until the time of analysis.

Fish from Malindi and lower Tana River were first transported to the laboratories of the Fisheries Department at Malindi where fillet, liver and ovary samples were obtained and stored in a freezer at -20°C. The samples were later transported to Kabete and stored as described above.

3.1.3 Species of fish collected

Various species of fish with different feeding habits were caught from the five sampling sites.

Sharks (*Carcharinus spyraena*): These are highly migratory carnivorous marine fish which predate on the smaller estuarine fish such as catfish and Malindi herrings. Their average length was 55cm.

Catfish (*Clarius* species): This species was caught in Malindi, Masinga Dam, Garsen and Tarasaa. The fish is carnivorous and feeds on smaller fish and their fry, insects and their larvae.

Breams (*Motaxis grandocuris*): These are fairly large marine and estuarine fish weighing up to 5kg and feed on detritus. They are mainly localized at the estuary of Athi River.

Soles (*Solea solea*) and *monodactylus* species: These are flat marine and estuarine fish which feed on the bottom sediments of planktons and algae.

Malindi herring: This is a small marine and estuarine bottom feeding fish.

Tilapia: Three species of tilapia were caught from four of the five sites. *Tilapia zilli* and *leucostictus* were caught in Lake Naivasha while *Tilapia mossambicus* was caught in Masinga Dam, Garsen and Tarasaa.Tilapia is a herbivorous open water feeder.

Lungfish (*Protopterus amphibius*): This is a herbivorous fresh water fish and was only caught at Garsen.

Black bass (*Micropterus salmoides*): This is a carnivorous fish that feeds on frogs, clayfish and smaller fish such as tilapia. It was only caught in Lake Naivasha.

Common carps (*Cyprinus carpio*): This species was only caught at Masinga. It is a fresh water bottom feeder surviving on planktons and algae.

3.2 Materials

3.2.1 Glassware

Item	Description/Supplier
Beakers	150, 100, 50ml
Volumetric flasks	10ml
Conical flasks	500ml
Chromatographic columns	2m by 4mm ID and 1.8m by 4mm ID, all glass, Supelco S. A., Gland, Switzerland.
Centrifuge tubes	50ml, 15ml, Duran®
Voltex flasks	Duran®
Carlsberg's pipettes	50, 100, 500µl
Pasteur pipettes	140ml
Blow-out pipettes	1, 2, 5, 10ml
Screw cap vials	7.4ml, Supelco S. A., Gland, Switzerland

3.2.2 Equipment	Description/ Supplier
Gas liquid chromatograph (GLC)	Model 428, Packard
Detector	^{63}Nickel electron capture detector (ECD), Packard Instrument Co. Inc. Illinois, USA.
Recorder	Model 621, Packard, Kipp and Zonen Holland.
Balances	Sartorius
Centrifuge	Joh Achelis and John Bremen, West Germany.
Waterbath	Memmert®, West Germany
Whirl mixer	Lab-line Instruments Inc., Melrose, USA.
Pestles and mortars	Porcelain
Rubber teats and pipette fillers	Rubber

3.2.3 Chemicals

Chemical	Description/Supplier
Column packing materials	
Analytical	GP 1.5% SP-2250/1.95% SP-2401 on Supelcoport 100/120
Confirmatory	GP 4% SE-30/6% SP-2401 on Supelcoport 100/120, Supelco S. A., Gland, Switzerland.
Standards	Organochlorine pesticide mixture (CPM) containing 13 compounds, Supelco S. A., Gland, Switzerland.

Chemical	Description/Supplier
n-Hexane	Laboratory grade: May & Baker, Dageham, England or Analar®: BDH Chemicals Ltd., Poole, England.
Acetone	Analar® BDH Chemicals Ltd., Poole, England.
Anhydrous sodium sulphate	Analar® BDH Chemicals Ltd., Poole, England.
Sodium hydroxide pellets	Analar® BDH Chemicals Ltd., Poole, England.
Sodium chloride	Analar® BDH Chemicals Ltd., Poole, England.
Methanol	Analar® BDH Chemicals Ltd., Poole, England.
Concentrated sulphuric acid	East Anglia Chemicals, Hadleigh, England.
Diethylether	Laboratory grade: May & Baker, Dageham, England or Analar® BDH Chemicals Ltd., Poole,England.
Acid washed sea sand	May & Baker, Dageham, England
Distilled water	Was prepared in the laboratory
Nitrogen gas (purified 99% and ordinary nitrogen)	East African Oxygen, Nairobi, Kenya.

3.3 Cleaning of glassware:

The glassware was first rinsed with tap water and then scrubbed with a brush and warm water containing a liquid detergent. This was followed by a rinse in tap water, distilled water and finally acetone before drying in an oven at 150°C overnight. Each piece was rinsed with redistilled hexane before use.

3.4 Solvent preparation

3.4.1 Distillation:

Redistillation of solvents in pesticide residue analysis is an important step as it removes impurities that may interfere with identification and quantitation of pesticide residues.

Two and a half litre volumes of hexane, acetone and methanol were separately distilled once in an all-glass fractionating column fitted with a water- cooled condenser. Glass beads were used in the distillation flask to prevent superheating of the solvents. The solvents were heated slowly to prevent co-distillation of impurities. The first 200ml of the distillate and the last 400ml of the solvent in the distillation flask was discarded in accordance with recommendations by Maitho (1978).

3.4.2 Purity check

To check the purity of hexane, 10ml of the solvent was reduced to 1ml by evaporation in a waterbath at 42°C over a gentle stream of nitrogen. This was then injected into the GLC and the detector response was observed for 20 minutes. The detector response was observed for 20 minutes because this is the longest retention time

for the last compound (p,p' DDT) in the CPM standard under the GLC operating conditions used in the study. In some cases, minute peaks were observed close to the solvent front but they did not interfere with the analysis of α–HCH (the first peak in the CPM standard).

The purity of the other solvents (acetone, methanol and diethylether) was checked as follows. 10ml of the solvent in a graduated centrifuge tube was evaporated to dryness in a waterbath at 42°C over a stream of nitrogen. The tube was then rinsed with 2ml of hexane which was then injected into the GLC. The detector response was then observed for 20 minutes. No interfering peaks were observed after a single distillation.

3.4.3 Washing of concentrated sulphuric acid with hexane

Hexane and concentrated sulphuric acid at a ratio of 1:2 (volume by volume) was put in a separatory funnel, shaken thoroughly and then left to stand for 30 minutes. The acid layer was drained into an all-glass dispenser for storage while the hexane layer was discarded.

To check the purity of the sulphuric acid, 2 volumes of acid were washed with one volume of hexane. The hexane layer was drained into a graduated centrifuge tube, evaporated to 1ml in a waterbath at 42°C and an aliquot was injected into the GLC. Detector response was observed for 20 minutes. No interfering peaks were observed and hence the hexane-washed acid was found suitable for use in pesticide residue analysis.

3.5 Preparation of pesticide standards

A high quality organochlorine pesticide mixture (CPM 4-9151) containing 13 compounds dissolved in 1ml isooctane in sealed

ampoules was obtained from Supelco, SA Gland, Switzerland. The constituents of the CPM and their concentrations were:

Compound	Concentration (µg/µl)
α-HCH	0.025
Lindane (γ-HCH)	0.025
β-HCH	0.100
Heptachlor	0.025
Aldrin	0.050
Heptachlor epoxide	0.080
pp' DDE	0.100
Dieldrin	0.120
op' DDD (TDE)	0.200
Endrin	0.200
op'DDT	0.225
pp' DDD (TDE)	0.190
pp' DDT	0.260

Working standards and stock solutions of standards were prepared by dilution of the 1ml concentrated pesticide mixture. The CPM ampoule was broken at the neck and all its contents emptied into a 10ml volumetric flask using a 500µl Carlsberg's pipette. The ampoule was rinsed with redistilled hexane and all the rinsings transferred into the volumetric flask.

The contents of the flask were then topped up to the mark with redistilled hexane to obtain a solution of 1:10 dilution. Serial dilution of this solution was done to give 1:100, 1:1000, 1:2000 and 1:10,000 dilutions. The 1:10 and 1:100 dilutions were kept as the stock solutions while the 1:1000 and 1:2000 solution were used as

the working standards. The 1:10,000 dilution was used to test detector sensitivity at very low pesticide concentrations. All the dilutions were done at room temperature and the solutions were stored in a deep freezer awaiting analysis. Redistilled hexane was used as the the diluent since it was the final extraction solvent for the pesticides residues. Hexane, isooctane and benzene are suitable solvents for preparation and dilution of organochlorine pesticides standard solutions (Chau, 1982). The use of benzene is , however, discouraged due to its toxicity especially carcinogenicity.

3.6 Gas liquid chromatograph (GLC)

The instrument used was a Packard model 428 equiped with a ^{63}Ni electron capture detector (ECD) and a Packard recorder, model 621.

3.6.1 Operating conditions

Carrier gas flow rate during the analysis was maintained between 60 and 75ml/minute. The recorder was operated at 10mV with a chart speed of 10mm/minute and attenuation of 128. The GLC operating temperatures were:

Injection block	230°C
Oven (column)	210°C
Detector	250°C

3.6.2 Resolution and linearity

Resolution of the column and linearity of the detector were checked prior to the analysis of samples. To check resolution, aliquots of the working standard were injected into the GLC and

the resulting elution patterns were compared with those supplied by the CPM manufacturer.

Linearity was checked by injection of equal volumes of dilutions of the CPM standard solution into the GLC followed by plotting of calibration curves. The best curve of fit was determined by the method of least squares using a computer.

3.6.3 Detection limits

The modern ECD has a detection limit of 1pg for organochlorine pesticides (Chau and Afghan, 1982). In the present study the detection limit was about 0.001mg/kg.

3.7 Pesticide residues

3.7.1 Identification

The retention times of the components in the sample chromatogram were measured and compared with those of corresponding components in the standard chromatogram (Fig. 3.2)

3.7.2 Quantitation

This was achieved by comparing the peak heights of sample components with those of corresponding components in standards of known concentrations. The amount of each compound in 1g of wet sample was calculated by considering the final volume of pesticide extract and correcting for any dilutions done during analysis. Pesticide levels were expressed in mg/kg without correcting for the percent recovery of the pesticides. The

following formula was used for calculating the concentration of the pesticide residues in the sample:

$$P = \frac{PH(s)}{PH(std.)} \times W \times \frac{V(e)}{V(i)} \Big/ g$$

P = concentration (mg/kg) of pesticide residue in sample

PH(s) = peak height (mm) of the pesticide in the chromatogram of the sample.

PH(std) = peak height (mm) of the pesticide in the chromatogram of the standard solution

W = weight (µg) of the standard injected into the GLC

V(e) = final volume (µl) of sample extract

V(i) = volume (µl) of sample extract injected into the GLC

g = weight (g) of sample analysed.

3.7.3 Confirmation

Another column with a different packing material was used for the confirmation of residues. This is necessary because the retention time of a compound may coincide with that of a contaminant on the analytical column. Aliquots of the working standard and representative sample extracts were injected into the GLC and the retention times of the sample components on the confirmatory column were compared with those of the corresponding components of the standard.

3.8 Analytical quality assurance (AQA)

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

3.8.1 Preparation and handling of AQA samples

40g of fillet from the lateral muscle of a whole fish sample was weighed into a voltex flask. The sample was homogenized with an electric meat mincer. 3g of the homogenate were weighed into a mortar in 6 parallels. 100 or 600µl of CPM standard (1:100 dilution) were added to each 3g of sample to give a low or high spiking of the sample. Two blanks, one containing 3g of unspiked sample and the other sodium sulphate and sand only, were prepared.

Table 3.1 shows the pesticide concentrations attained in the spiked sample.

3.8.2 Analysis

This process involved extraction, clean-up, GLC injection and quantitation of the pesticides in the spiked samples. The method used in extraction and clean-up of pesticide residues in this study was adapted from a method developed by Bjerk and Sundby (1970) for analysis of organochlorine pesticide residues in aquatic organisms.

Table 3.1 Pesticide concentrations attained in the spiked sample

Pesticide	µg/g sample	
	Low spiking	High spiking
α-BHC	0.008	0.050
Lindane (γ-BHC)	0.008	0.050
β-BHC	0.033	0.200
Heptachlor	0.008	0.050
Aldrin	0.017	0.100
Heptachlor epoxide	0.027	0.160
pp' DDE	0.033	0.200
Dieldrin	0.040	0.240
op' DDD (TDE)	0.067	0.400
Endrin	0.067	0.400
op' DDT	0.075	0.450
pp' DDD (TDE)	0.063	0.380
pp' DDT	0.083	0.520

3.8.3 Extraction

4g of the contents of each mortar were weighed into glass minicolumns plugged with hexane-washed cotton wool and mounted on a stand. Diethylether was added to just wet the sample and the set- up was left on the bench for 15 minutes. Small volumes of diethylether were added to the sample and 10-15ml were slowly eluted into pre-weighed centrifuge tubes. The ether was evaporated to dryness in a waterbath at 42°C and the

tubes were re-weighed after cooling to room temperature.The difference between the initial weight of the tubes and their weight after evaporation of the ether eluate gave the weight of the fat extracted from the sample. This fat was contained in 1g of wet sample. The fat was redissolved in 4ml of redistilled hexane to give a maximum fat concentration of not more than 0.05g/ml.

3.8.4 Clean-up

The clean-up procedure involved acid and base treatment.Two 1ml aliquots of the hexane fat solution were withdrawn using 1ml blow-out pipettes and transferred into different centrifuge tubes. 1.5ml of the hexane-washed concentrated sulphuric acid was added to one of the aliquots and thoroughly mixed with a whirl mixer. The mixture was left on the bench for one hour, after which it was spun with a centrifuge for 2 minutes at 3000 revolutions per minute (r.p.m.). The clear hexane layer was transferred into extract vials with teflon caps using pasteur pipettes and was ready for injection into the GLC.

For the base clean-up, the other 1ml aliquot of hexane fat solution was evaporated to dryness in a waterbath at 42°C over a gentle stream of nitrogen. 1.5ml of 15% methanolic sodium hydroxide was added and thoroughly mixed with a whirl mixer. The mixture was left overnight in a waterbath at 42°C. The tubes were removed from the waterbath the following morning and 1ml of redistilled hexane and 3ml of 2% aqueous sodium chloride solution was added. The mixture was spun with a centrifuge at 3000 r.p.m. for 2 minutes and the hexane layer was removed as with the acid clean-up. The pesticide extract was always stored in a freezer at -20°C when not in use.

3.8.5 Injection of sample extracts into the GLC

The pesticide extracts were removed from the freezer and left to warm to room temperature. Between 1 and 5µl of the extract was injected into the GLC using 10µl graduated glass syringes. The detector response was observed for 20 minutes.

3.9 Results

3.9.1 Resolution

The elution pattern and resolution of the peaks for all the 13 components of the CPM standard were found to conform with the chromatogram supplied by the CPM manufacturer (Fig. 3.2a). The retention times were, however, different from those given by the CPM manufacturer. This was attributed to the use of GLC operating conditions different from those used by the CPM manufacturer.

3.9.2 Linearity

The detector showed a linear response over a wide range of pesticide concentrations (figs. 3.3 - 3.5). Pesticide residue analysis in the samples was carried out within this linear range of detector response.

3.9.3 Pesticide recoveries from spiked samples

The recoveries obtained in the low and high spiking levels for the 13 pesticides ranged from 79 to 102 %. This was found to be within the acceptable range for pesticide residue analysis according to the UNEP/WHO Criteria for evaluation of pesticide

recovery results (1980). Table 3.1 shows the pesticide recoveries from spiked samples and their evaluation.

Table 3.2 Percent pesticide recoveries from spiked samples

	Recovery (%)					
Pesticide	Low spiking	±Δ	Evaluation	High spiking	±Δ	Evaluation
α-HCH	81	-19	G	88	-12	G
Lindane	84	-16	G	97	-3	E
β–HCH	83	-17	G	96	-4	E
Heptachlor	88	-12	G	91	-9	E
Aldrin	79	-21	A	90	-10	E
Heptachlor epoxide	81	-19	G	91	-11	G
p,p' DDE	98	-2	E	102	+2	E
Dieldrin	87	-13	G	99	-1	E
o,p' DDD	88	-12	G	91	-9	E
Endrin	82	-18	G	95	-5	E
o,p' DDT	87	-13	G	89	-11	G
p.p' DDD	90	-10	E	96	-4	E
p,p' DDT	98	-2	E	101	+1	E

The recoveries are given as means of 6 parallels ± percent deviation (±Δ) from the added amount.

$$\Delta = 100 \times \frac{\text{recovered pesticide}(\mu g)}{\text{spiked pesticide}\ (\mu g)} - 100$$

E = Excellent (±10% of spiked amount)

G = Good	(±20%)
A= Acceptable	(±30%)
P= Poor	(±40%)
U= Unacceptable	(±50%)

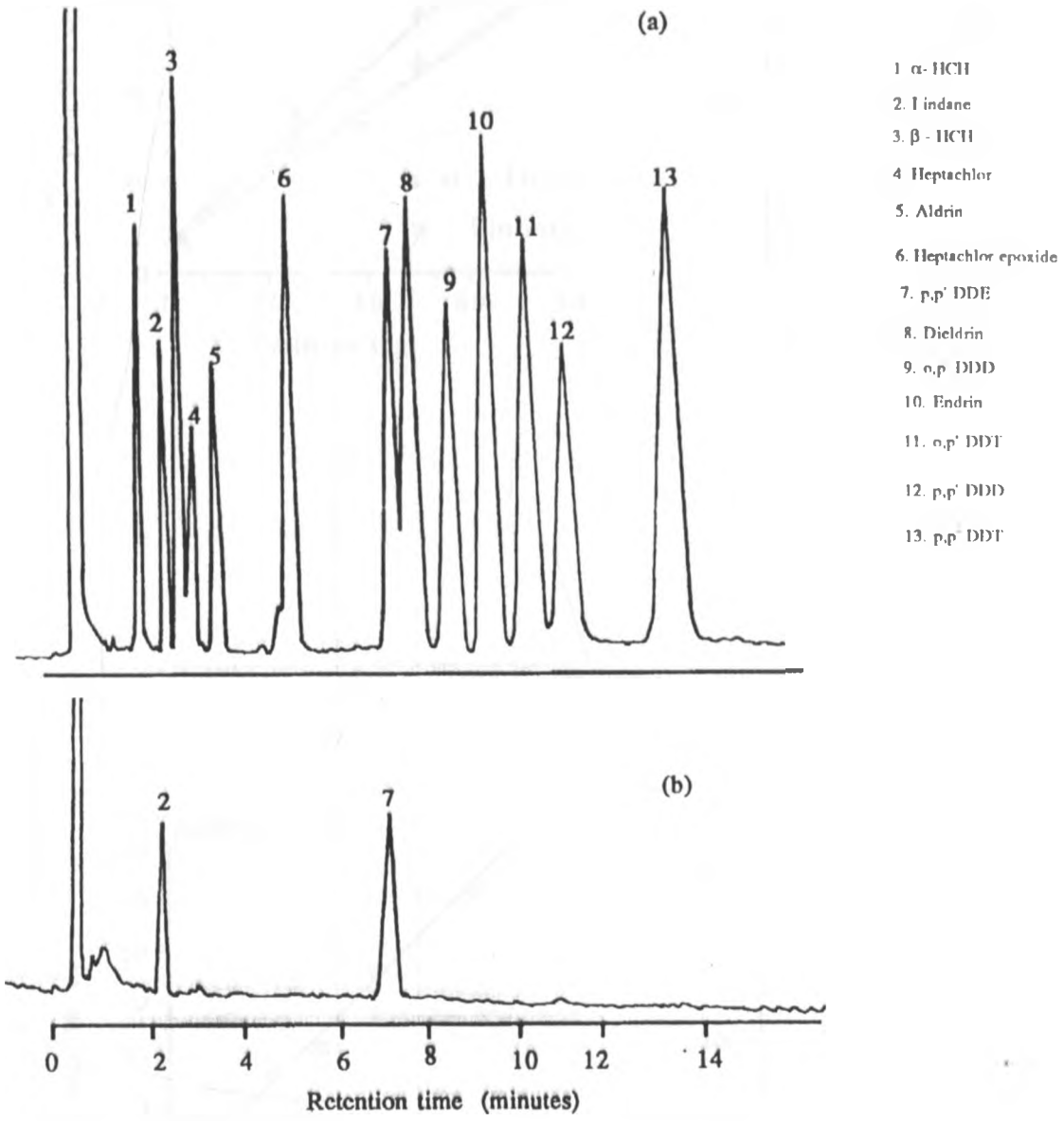

Fig. 3.2 Chromatograms of CPM standard (a) and a positive fillet sample (b) on the analytical column.Conditions: column, 2.0m x 4mm ID, all glass, packing GP 1.5% SP- 2250 / 1.95% SP- 2401 on 100 / 120 Supelcoport. Temperature: column 210°C, detector 250°C, injector 230°C. Carrier gas: N_2, flow rate 70ml / min.

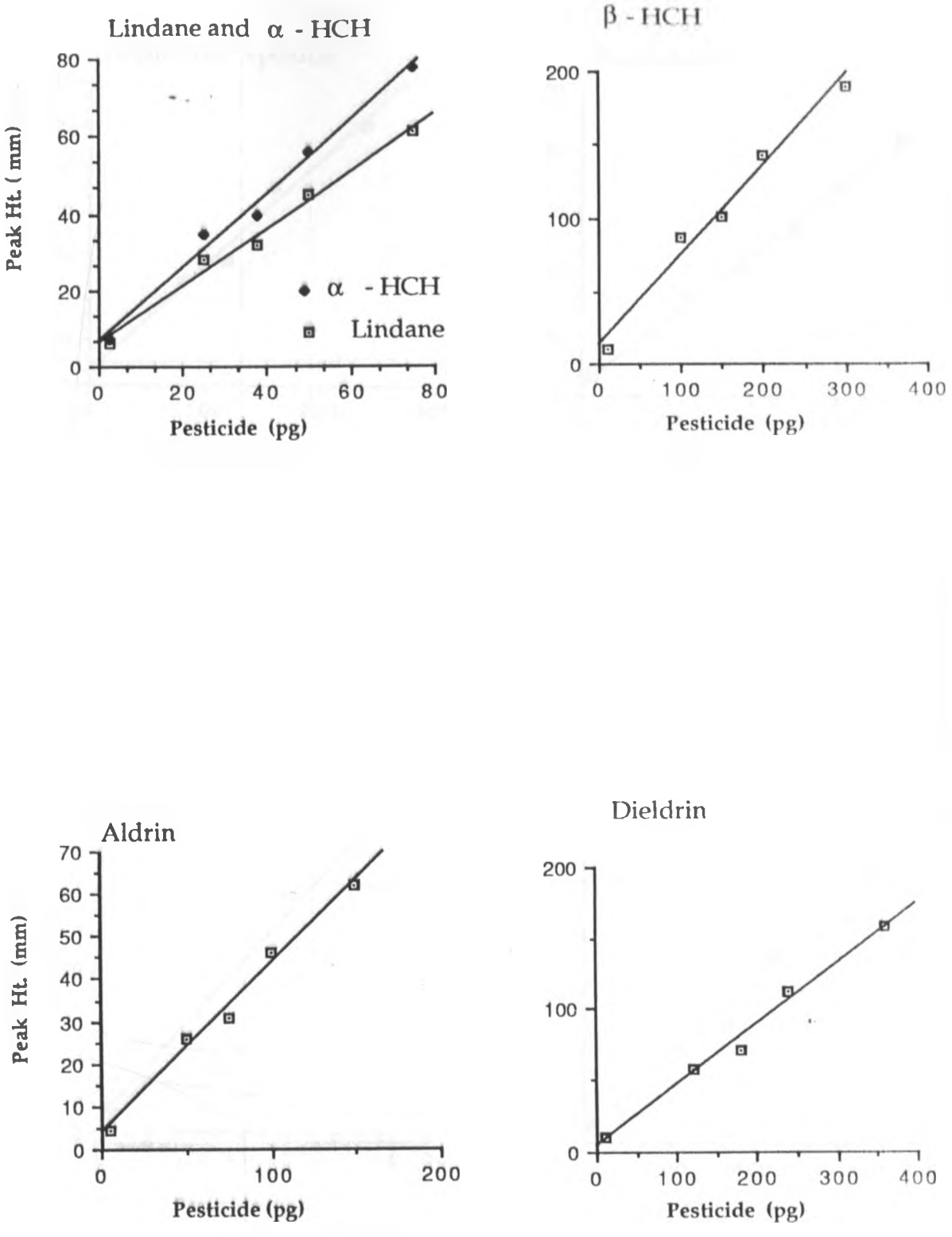

Fig. 3.3 Linearity of the ECD for α- HCH, lindane, β- HCH, aldrin and dieldrin

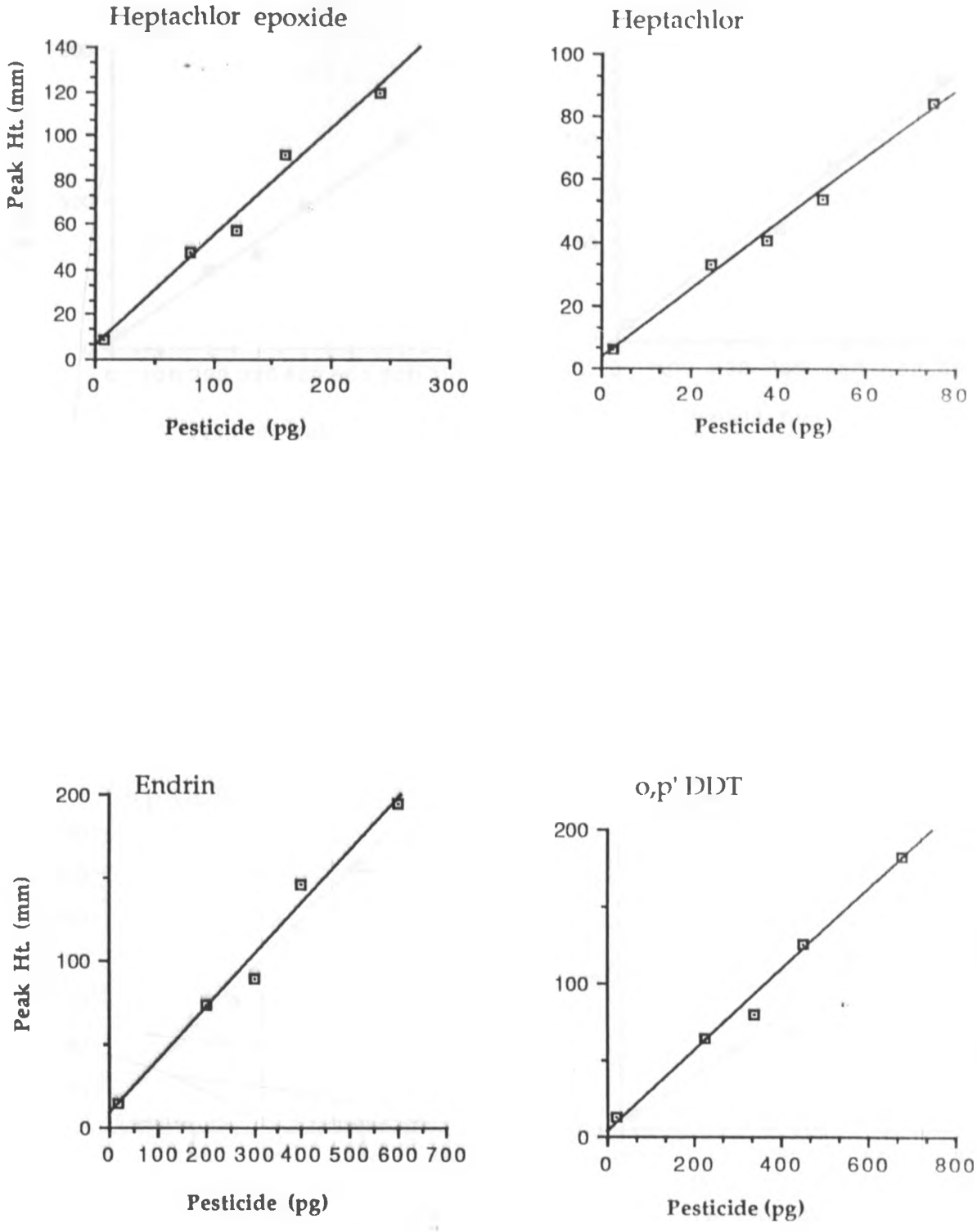

Fig. 3.4 Linearity of the ECD for heptachlor epoxide, heptachlor, endrin and o,p' DDT

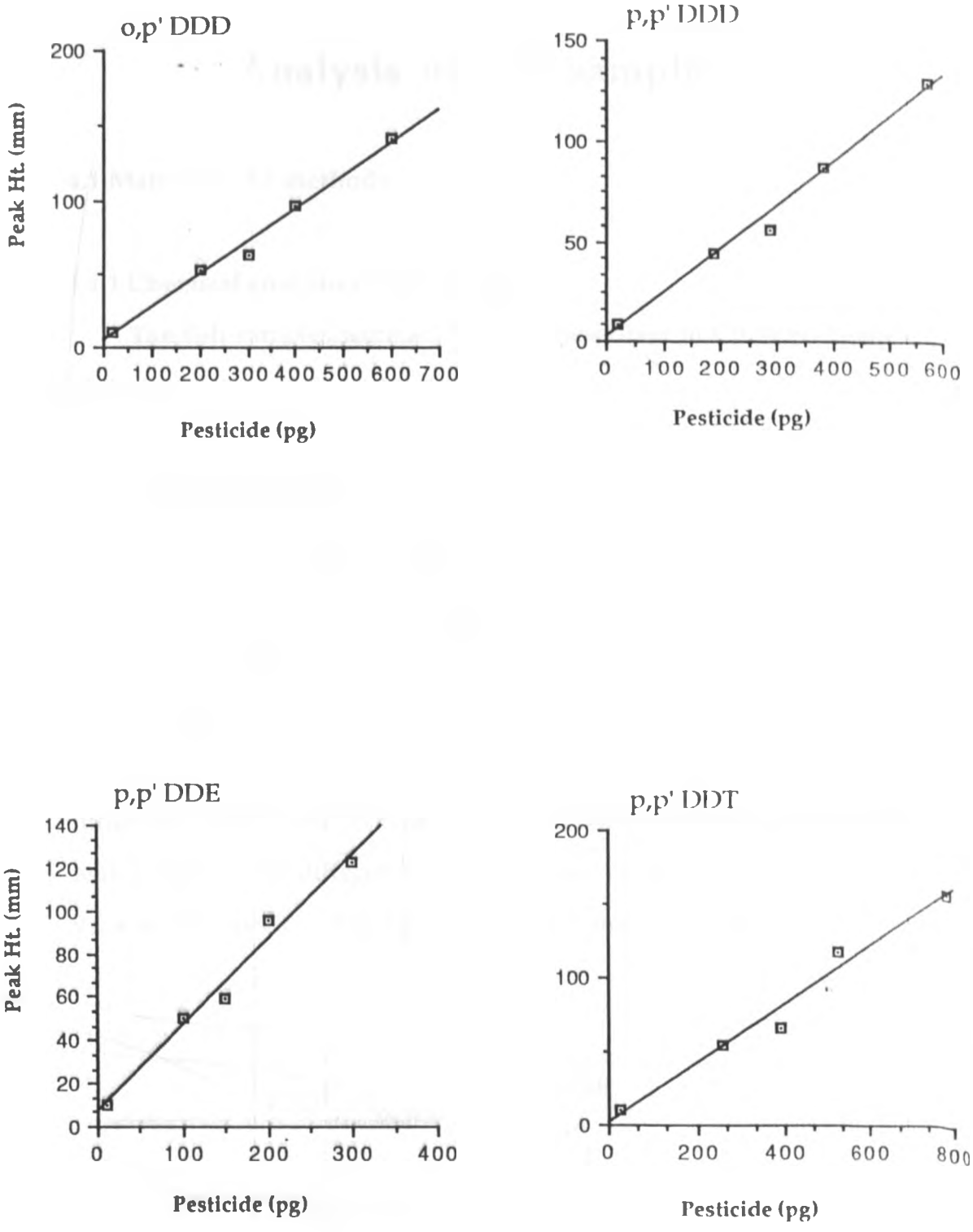

Fig. 3.5 Linearity of the ECD for o,p' DDD, p,p' DDD, p,p' DDE and p,p' DDT

CHAPTER FOUR

Analysis of fish samples

4.1 Materials and methods

4.1.1 Chemical analysis of fish samples

The fish samples were analysed as described in Chapter 3 page 41 to 44.

4.1.2 Statistical analysis

Statistical analysis of data was mainly done by analysis of variance (ANOVA) using an IBM computer with a PANACEA statistical programme. Linear regression and correlation relationships were used to determine the variation of pesticide residues with the weight of the fish. Turkey's highest significant difference (HSD) test (Wayne, 1983) was used to determine if there was a significant difference in the means of residue levels in the various fish species. The equation used in this case was:

$$HSD^* = q_{\alpha,\kappa,N-\kappa}\sqrt{\frac{MSE}{n^*_j}}$$

where: α = chosen level of significance

k = number of means in the experiment

N= total number of observations in the experiment

MSE = mean square error from the ANOVA table

q = obtained by entering a HSD statistic table

n^*_j = the smallest of the two sample sizes associated with the two sample means that are to be compared

4.1.3 Maximum residue limits (MRL) and acceptable daily intake (ADI)

The two terms are used in evaluation of the toxicological significance of pesticide residue data in food.

The MRL used in the present study is the maximum concentration of a pesticide residue that is recognized by the National Food Administration (NFA) of Sweden, as permissible in fish for human consumption (Andersson *et al*, 1984). It is expressed in mg/kg. The NFA is a collaborating centre for the Joint FAO/WHO Food and Animal Feed Contamination Monitoring Programme (Codex Alimentarious Commission, 1986).

ADI refers to the daily intake of a pesticide which during an entire lifetime appears to be without appreciable risk to the consumer's health on the basis of all the known facts at the time of evaluation of the chemical by the FAO/WHO meeting on pesticide residues. It is given in mg/kg body weight (Codex Alimentarius Commission, 1986).

4.2 Results

4.2.1 Summary of results

A total of 275 fish samples were analysed from the five sampling sites and 9 organochlorine pesticide residues were detected in 22.5% of the samples. p,p' DDT and its metabolite, p,p' DDE were the most frequently observed compounds occurring in 12.7% and 20.4% of the samples respectively. The other residues detected in order of decreasing frequency of occurrence were

lindane (4.4%), o,p' DDT (4%), p,p' DDD (3.6%), β- HCH (2.5%), α-HCH (1.8%), o,p' DDD (0.4%) and heptachlor (0.4%).

Statistical analysis of the data revealed some general findings with regard to all the fish species analysed for organochlorine pesticide residues. The levels of p,p' DDT and sum DDT were positively correlated (r= 0.74) to weight of the fish.

Mean levels of sum DDT in catfish, breams, common carps and sharks were significantly different ($p< 0.0001$) and their values in order of magnitude were, 0.144, 0.213, 0.234 and 0.702mg/kg respectively. There was no significant difference ($p> 0.05$) in the mean levels of the various DDT group residues between male and female fish and between different organs. Mean residue levels of DDT group compounds were not significantly different ($p> 0.05$) in fish from Masinga and Malindi.

Table 4.1 shows the mean and standard deviation of the pesticide residue levels found in four species of fish from the five sampling sites.

4.2.2 Pesticide residue levels in estuarine fish from Malindi

Sixty seven samples were analysed from this site and 71.6% had detectable levels of one or more of the 8 organochlorine pesticide residues detected. Tables 4.2 to 4.5 show the mean, standard deviation and range of residue levels of the DDT, HCH and cyclodiene groups of pesticides detected in the samples.

Table 4.1 *Mean and standard deviation (SD) of pesticide* residue levels in four species of fish obtained from the five areas of the study.

			Pesticide residue (mg/kg)									
	Samples		p,p' DDE	p,p' DDD	o,p' DDD	o,p' DDT	p,p' DDT	sum DDT	α-HCH	β-HCH	Lindane	Heptachlor
Fish species	total	positive	Mean±SD (Range)	Mean±SD (Range)	Mean±SD (Range)	Mean±SD (Range)	Mean±SD (Range)	Mean±SD (Range)	Mean±SD (Range)	Mean±SD (Range)	Mean±SD (Range)	Mean±SD (Range)
Shark	31	24	0.397±0.277 (0.053-1.257)	0.046±0.018 (0.026-0.078)	0.039*	0.071±0.041 (0.031-0.133)	0.281±0.574 (0.011-2.674)	0.702±0.646 (0.082-3.148)	0.003*	0.075±0.022 (0.042-0.095)	-	-
Catfish	90	19	0.124±0.071 (0.027-0.294)	0.039*	-	0.045*	0.065±0.037 (0.036-0.108)	0.145±0.091 (0.031-0.417)	0.104±0.026 (0.009-0.29)	0.025*	0.009±0.003 (0.004-0.013)	0.033** (0.024-0.042)
Breams	7	6	0.154+0.157 (0.028-0.422)	-	-	-	0.127** (0.108-0.146)	0.213±0.217 0.031-0.614)	-	0.158*	-	-
Common carps	19	9	0.03±0.02 (0.015-0.054)	-	-	-	0.233±0.34 (0.085-1.125)	0.234±0.358 (0.085-1.185)	-	-	0.152±0.086 (0.033-0.295)	-

Residue levels are given on wet weight basis
Mean was calculated for positive samples
*Detected in only one sample
**Detected in two samples
(-) Below the detectable limit

DDT group

Five residues of this group of pesticides were detected in the fish samples analysed.

p,p' DDE : The residue was detected in 73% of the samples and mainly occurred in the liver and egg samples. Only two fillet samples from a female shark and female soles showed detectable levels of p,p' DDE. Sharks had the highest mean level of p,p' DDE (0.397mg/kg) followed by breams (0.154mg/kg) and catfish (0.117mg/kg). The only liver sample from *Monodactylus* species contained 0.703mg/kg of p,p' DDE. The highest level of the residue recorded was 1.27mg/kg in a liver sample from a male shark. Sharks had significantly higher mean p,p' DDE level than catfish and breams ($p=0.05$). However, there was no significant difference in the mean levels of the residue in breams and catfish ($p=0.3$).

p,p' DDD : The residue occurred at low levels and mainly in the liver of sharks. The highest level recorded was 0.078mg/kg in the liver of a female shark (Appendix 3).

p,p' DDT : The compound was detected in 39% of the samples from Malindi and occurred mainly in the liver of sharks. No residues of the chemical were detected in egg samples. The highest level recorded was 2.674mg/kg in a liver sample from a female shark (Appendix 3).

o,p' DDT : The compound was detected at low levels in 16% of the samples and only occurred in liver samples.

o,p' DDD : This o,p' DDT metabolite was only detected in one liver sample of a male shark. It occurred at a concentration of 0.039mg/kg.

Sum DDT : Sharks had the highest mean level (0.702 mg/kg) of sum DDT. The mean total DDT levels in catfish and breams were 0.145 and 0.213mg/kg respectively. There was a significant difference (p=0.02) between the mean sum DDT levels in sharks, breams and catfish. The difference between the means of sum DDT in catfish and breams was, however, not significant (p= 0.07). The highest sum DDT level recorded was 3.148mg/kg in a liver sample from a female shark. The only liver sample from a fish of *Monodactylus* species had a sum DDT level of 1.077mg/kg.

HCH group :

Low levels of α- and β- HCH were detected in liver samples only. α- HCH occurred in only 3% and β- HCH in 10% of the samples (Table 4.5). The highest recorded levels of these residues were 0.29 and 0.158mg/kg for α- and β- HCH respectively.

Cyclodiene group :

Heptachlor was the only compound detected in this group of pesticides. The residue was detected in low levels (0.024 and 0.042mg/kg) in a liver and eggs sample from a catfish (Table 4.5). This represented only 1% of the samples analysed.

The residue levels for individual samples are shown on appendices 2- 6.

Table 4.2 Mean and standard deviation (SD) of residue levels of DDT compounds in sharks from Malindi

		Pesticide residue (mg/kg)					
		p,p' DDE	p,p' DDD	o,p' DDT	o,p' DDD	p,p' DDT	Sum DDT
species/sex/ organ	No. of samples	mean±SD range	mean±SD range	mean± SD range	mean±SD range	mean±SD range	mean± SD range
Male Sharks							
Liver	13	0.522±0.375 0.118-1.257 (11)	0.044±0.018 0.031-0.065 (3)	0.056±0.039 0.031-0.124 (5)	0.039 - (1)	0.154±0.114 0.011-0.331 (10)	0.522±0.375 0.118-1.257
Fillet	13	-	-	-	-	-	-
Female Sharks							
Liver	18	0.395±0.193 0.128-0.668 (12)	0.047±0.02 0.026-0.078 (5)	0.089±0.043 0.031-0.133 (4)	-	0.411±0.804 0.057-2.674 (10)	0.821±0.835 0.146-3.148
Fillet	18	0.074 (1)	-	-	-	-	0.082

Residue levels are given on wet weight basis
Mean was calculated for the positive samples
Figures in parentheses represent the number of positive samples for each residue
(-) Below the detectable limit

Table 4.3 Mean and standard deviation (SD) of residue levels of DDT compounds in catfish and breams from Malindi

		Pesticide residue (mg/kg)					
		pp' DDE	pp' DDD	op DDT	op' DDD	pp' DDT	Sum DDT
species/sex/ organ	No. of samples	mean±SD range	mean±SD range	mean± SD range	mean±SD range	mean±SD range	mean± SD range
Male Catfish							
Liver	2	0.119 (1)	-	-	-	-	0.132
Fillet	2	-	-	-	-	0.036 (1)	0.036
Female Catfish							
Liver	18	0.09±0.075 0.069-0.294 (15)	0.039 (1)	0.045 (1)	-	0.108±0 0.108 (2)	0.141±0.111 0.03-0.417
Fillet	18	-	-	-	-	-	-
Eggs	16	0.141±0.069 0.069-0.294 (16)	-	-	-	-	0.156±0.077 0.076-0.326
Male Breams							
Liver	5	0.179±0.162 0.028-0.422 (5)	-	-	-	0.127±0.027 0.108-0.146 (2)	0.25±0.221 0.031-0.614
Fillet	5	-	-	-	-	-	-
Female Breams							
Liver	2	0.028 (1)	-	-	-	-	0.031
Fillet	2	-	-	-	-	-	-
Eggs	2	-	-	-	-	-	-

Residue levels are given on wet weight basis Figures in parentheses represent the number of positive samples for each residue
Mean was calculated for the positive samples (-) Below the detectable limit

Table 4.4 Mean and standard deviation (SD) of residue levels of DDT compounds in Malindi herrings, soles and Monodactylus spp from Malindi

		Pesticide residue (mg/kg)					
		pp' DDE	pp' DDD	op DDT	op' DDD	pp' DDT	Sum DDT
species/sex/ organ	No. of samples	mean±SD range	mean±SD range	mean± SD range	mean±SD range	mean±SD range	mean± SD range
Male Malindi Herrings							
Fillet	6	-	-	-	-	-	-
Female Malindi Herrings							
Fillet	1	-	-	-	-	-	-
Female Soles							
Fillet	1	0.011 (1)	-	-	-	-	0.012
Monodactylus spp							
Liver	1	0.703 (1)	0.039 (1)	0.056 (1)		0.191 (1)	1.077

Residue levels are given on wet weight basis
Mean was calculated for the positive samples
Figures in parentheses represent the number of positive samples for each residue
(-) Below the detectable limit

Table 4.5 Mean and standard deviation (SD) of pesticide residue levels of α-HCH, β-HCH and Heptachlor in fish from Malindi.

		Pesticide residue (mg/kg)		
		α-HCH	β-HCH	Heptachlor
Species/sex/ organ	No. of samples	Mean ± SD Range	Mean ± SD Range	Mean ± SD Range
Male Sharks				
Liver	13	-	0.075±0.018 0.062-0.088 (2)	-
Fillet	13	-	-	-
Female Sharks				
Liver	18	0.003 (1)	0.075±0.029 0.042-0.095 (3)	-
Fillet	18	-	-	-
Female Catfish				
Liver	18	0.29 (1)	0.025 (1)	0.024 (1)
Eggs	16	-	-	0.042 (1)
Fillet	18	-	-	-
Male Breams				
Liver	5	-	0.158 (1)	-
Fillet	5	-	-	-

Residue levels are given on wet weight basis

Mean was calculated for positive samples

Figures in parentheses represent the number of positive samples for each residue

(-) Below the detectable limit

4.2.3 Pesticide residue levels in fresh water fish from Masinga Dam

A total of 38 fish samples were analysed from the dam and 36.8% of the samples had detectable levels of one or more of the four organochlorine pesticide residues found in fish from the area.

Table 4.6 shows the mean and standard deviation of residue levels of the pesticides detected.

DDT group :

Only p,p' DDT and p,p' DDE were detected in the fish samples from Masinga Dam.

p,p' DDT : The residue occurred in 26% of the samples analysed and was mainly detected in the fillet (muscle) of common carps. The mean residue levels for the three species of fish ranged from 0.052 to 0.223mg/kg. Liver samples from common carps were not obtained as the organ had putrified by the time the fish were eviscerated. The mean residue level of p,p' DDT in fillet of common carp was 0.223mg/kg and the highest level was 1.125mg/kg. A liver and fillet sample from the same catfish had 0.052mg/kg of p,p' DDT each.

p,p' DDE : Low p,p' DDE levels were detected in 18% of the samples and the residue occurred mainly in the liver of catfish and fillet of common carps. The mean levels of the residue ranged from 0.03 to 0.138mg/kg. The highest residue level recorded was 0.22mg/kg. Only one catfish fillet sample showed detectable levels (0.102mg/kg) of p,p' DDE. The residue also occurred in the only sample of eggs from tilapia.

Sum DDT : Common carps had mean sum DDT level of 0.234mg/kg while catfish had 0.138mg/kg. The difference between

the two mean levels was significant (p= 0.018). The highest sum DDT level (1.185mg/kg) was recorded in a fillet sample from a common carp.

HCH group :

Lindane (γ- HCH) : This was the most frequently occurring residue in fish samples from Masinga Dam and the mean levels ranged from 0.003 to 0.295mg/kg. It was detected in 34% of the samples analysed. The mean residue level in common carps (0.14mg/kg) was significantly higher than that in catfish (0.01mg/kg) (p= 0.008). The highest level of lindane (0.295mg/kg) was detected in fillet from a common carp.

α- HCH : Low levels of the residue, ranging from 0.009 to 0.021mg/kg, were detected in a liver and fillet sample from the same catfish and the only available egg sample from a tilapia.

Appendices 7 and 8 show the residue levels for individual samples from Masinga Dam.

4.2.4 Pesticide residue levels in freshwater fish from Garsen, Tarasaa and Lake Naivasha

Garsen : Out of the 65 samples analysed, only one liver sample from a lungfish showed detectable levels of pesticide residues. This contained 0.033mg/kg p,p' DDE equivalent to 0.037mg/kg sum DDT.

Tarasaa : A total of 65 samples were analysed but none showed detectable residue levels of organochlorine pesticides.

Lake Naivasha : Of the 40 fish samples analysed, no individual sample had detectable levels of pesticide residues. A composite fillet sample of 10 fish, however, had α- HCH at a concentration of 0.014mg/kg.

4.2.5 Polychlorinated biphenyls (PCB's) and hexachlorobenzene (HCB)

The method used could detect these chemicals but none was found in the samples analysed.

Table 4.6 Mean and standard deviation (SD) of pesticide residue levels in fish from Masinga Dam

		Pesticide residue (mg/kg)				
		pp' DDE	pp' DDT	Lindane	α-HCH	Sum DDT
Species/sex/ organ	No. of samples	Mean ± SD Range	Mean + SD Range	Mean + SD Range	Mean + SD Range	Mean + SD Range
Common Caps						
Fillet	19	0.03 ± 0.021 0.015-0.054 (3)	0.223 ± 0.34 0.085-1.125 (9)	0.14 ± 0.093 0.033-0.295 (8)	-	0.234 ± 0.358 0.085-1.185
Catfish						
Liver	8	0.138 ± 0.081 0.059-0.22 (3)	0.052 (1)	0.01 ± 0.001 0.009-0.011 (3)	0.009 (1)	0.163 ± 0.054 0.117-0.222
Fillet	11	0.102 (1)	0.052 (1)	0.009 ± 0.006 0.013-0.004 (2)	0.013 (1)	0.113
Tilapia						
Fillet	8	-	-	0.011 (1)	-	-
Eggs	1	0.068 (1)	-	0.009 (1)	0.021 (1)	0.075

Figures in parentheses represent the number of positive samples for each residue
Mean was calculated for the positive samples

Residue levels are given on wet weight basis
(-) Below the detectable limit

4.3 Discussion

The results obtained showed differences in the residue levels in fish from the five sampling sites and between the various species of fish. There were also variations in the frequency of occurrence of the different residues.

4.3.1 Locational differences

Fish from Masinga Dam and Malindi evidently had detectable levels of organochlorine residues while hardly any residue was detected in fish from Lake Naivasha and lower Tana River. The difference could be attributed to a number of factors. Silt deposition in Masinga Dam and the estuary of Athi River could lead to build up of pesticide levels in fish food (algae, planktons and detritus) resulting in increased intake of the chemicals by fish. At Garsen and Tarasaa, siltation occurs at a very low rate as the water is always in continuous flow. Organochlorine pesticides in water are adsorbed by particulate matter and sedimented to the bottom of such water bodies as dams and lakes (Murty, 1986).

Heavy fishing at Garsen and Tarasaa may also be a contributory factor as fish are removed from the water before they accumulate a lot of the residues in the tissues. Low levels of organochlorine pesticide residues were reported in fish from Lake Naivasha (Lincer et al, 1981). The low levels were attributed to low usage of the chemicals in the catchment areas of the lake.

Lindane was only detected in fish from Masinga Dam. The pesticide could possibly be in use in the recently established Masinga Irrigation Scheme which drains its water into the Dam. Attempts to find out, from the Tana Development Authority, the pesticides used in the scheme were unsuccessful.

4.3.2 Interspecies differences

Sum DDT and p,p' DDE residues exhibited bioamplification. Sharks, the highest species in the food chain, had the highest mean levels of the two residues (0.397 and 0.702 mg/kg respectively) and the widest range of residues as well (Table 4.1). The migratory behaviour of this species, however, makes it difficult to say whether all the residues could have been obtained from the same location.

Bottom feeding fish (breams, common carps and *monodactylus* had higher levels of sum DDT than the intermediate carnivore (catfish). The bottom feeders are likely to have higher residue deposits than the catfish as they are liable to consume high residue amounts in their diet of algae, planktons and detritus. Algae and planktons have been shown to accumulate organochlorine pesticides many fold (Holden, 1962) while detritus adsorbs the pesticides.

Frequent occurrence of lindane, p,p' DDE and p,p' DDT in the fillet of common carps and rarely in that of the other species was consistent with the higher percentage of fat observed in the fillet of this species. The mean fat percentage for the species was 2.6. Appendix 10 shows the percentage of fat in the fish samples positive for organochlorine pesticide residues. Common carp fillet mainly had p,p' DDT instead of p,p' DDE and other DDT metabolites. This indicated a recent exposure of the fish to p,p' DDT or a very slow DDT breakdown rate in the species. Some fish species are known to eliminate DDT at a slower rate than others. Skaare *et al* (1985) found that the cod and haddock eliminated sum DDT from their liver at a slower rate than did the sea scorpion and catfish species of fish.

4.3.3 Toxicological significance of the results

In order to assess the toxicological significance of the results with regard to human health, the residue levels were evaluated against the maximum residue levels (MRL) in fish, set by the National Food Administration (NFA) of Sweden and the FAO/WHO acceptable daily intake (ADI) for the compounds in carcass meat. Two fillet samples of common carp from Masinga Dam had residues of lindane above the NFA maximum residue level (Appendix 7).

Table 4.7 shows the MRL and ADI for the pesticide residues detected.

Table 4.7 MRL and ADI of sum DDT*, α- HCH, β- HCH, lindane (γ - HCH) and heptachlor

Compound	MRL (mg/kg)	ADI (mg/kg)
Sum (total) DDT	5	0.02
α- HCH	0.2	not available
β- HCH	0.2	not available
Lindane	0.2	0.01
Heptachlor	not available	0.0005

*Sum DDT was calculated as p,p' DDT + o,p' DDT + 1.11(p,p' DDD + p,p' DDE). 1.11 is a correcting factor for lower molecular weights of the DDT metabolites (Skaare *et al* , 1985).

The residues considered for ADI evaluation were those that occurred in the fillet since it is the edible part of the fish analysed. An adult man weighing 60kg and consuming 1kg of fish per day was chosen as the yardstick. The mean sum DDT level in common carps and catfish from Masinga was below the ADI of 0.02mg/kg body weight but the highest level (1.185mg/kg) in common carps was just equal to the ADI. It is nonetheless, unlikely that fish is consumed at this rate in the country and the chances of someone consuming sum DDT in excess of the ADI are thus low. Levels of lindane and heptachlor were below the ADI. The concentration of pesticide residues in cooked fish may also be lower than that in the raw fish. De Vos *et al* (1972) and Ritchey *et al* (1972) showed that cooking reduced the concentration of p,p' DDT, lindane and heptachlor epoxide in poultry tissue.

The residue levels found are low and therefore do not pose an ecological, environmental or health hazard at present. Since the country is in the process of phasing out the use of persistent organochlorine pesticides, the residue levels in the aquatic systems are expected to decline with time.

In comparison with the high organochlorine pesticide residue levels in the United States and Britain that resulted in decline in populations of fish and fish- eating birds (McEwen and Stephenson, 1979), the levels in the present study may not have detrimental effects on the organisms.

4.3.4 Comparison of the results with others in Kenya

Few workers have investigated organochlorine residues in Kenyan fish from other aquatic systems and generally reported very low levels of the compounds (Chapter 2, p23 to 25). DDT and its

metabolites especially p,p' DDE composed the largest proportion of organochlorine residues in the fish samples analysed. This is consistent with findings reported by other workers (Skaare *et al*, 1985; Murty, 1986). Aldrin and dieldrin which were reported by most of the other investigators were not detected in the present study. However, heptachlor was reported in Kenyan fish for the first time.

The residue levels are generally higher than those reported in the other studies (Chapter 2, p23 to 25) but compare fairly well with the findings of Mitema and Gitau (1990). In the present study, the mean sum DDT level in shark was 0.702mg/kg and the highest value was 3.148mg/kg. Mitema and Gitau (1990) reported mean sum DDT of 0.45mg/kg and the highest level recorded was 4.51mg/kg in fresh nile perch fat and fillet from Lake Victoria. The nile perch, like the shark, is carnivorous and is at the top of the food chain in Lake Victoria. Residue levels of the HCH group ranged from 0.003 to 0.295mg/kg while Mitema and Gitau reported a range of 0.001 to 0.11mg/kg in the nile perch.

From the results obtained in the present study, it is difficult to determine whether there has been an increase in the pesticide residues in the fish since the previous studies reported were concentrated on Lake Victoria and Rift Valley lakes.

4.3.5 Comparison of results with others reported from different parts of the world

The results compare well with those reported from other developing countries but the levels are lower than those reported from the industrialized countries such as Norway and the United States of America.

The range of sum DDT was 0.012 to 3.148mg/kg on wet weight basis in the present study. In Jordan, in 1971, the sum DDT in three species of fish ranged from 0.37 to 3.34mg/kg (Murty, 1986). El Zorgani *et al* (1979) and Zorgani (1980) reported sum DDT of 0.0022 to 0.184mg/kg in Lake Nubia fish and 0.3 to 2.9mg/kg in different species of fish from other areas in the Sudan. Sum DDT values of 0.07 to 0.38mg/kg were reported in three species of fish from Lake Tanganyika (Deelstra *et al* , 1976). While dieldrin, aldrin and endrin were reported in some of the studies, none was detected in the present investigation. This could be due to differences in use of the chemicals in different countries. Sum DDT levels ranging from undetectable to 92mg/kg and 90 to 135mg/kg on wet weight basis have been reported in fish from the United States of America and Norway respectively (Murty, 1986). The levels of lindane and heptachlor reported in the United States were also higher than those reported in the present study.

The difference in residue levels between industrialized and developing countries is attributed to intensive use of the pesticides in the industrialized countries. Intensive farming and mechanised pesticide application is practised in the industrialized countries, while it is rarely done in developing ones. For instance, in the United States, the period after the Second World War was marked by heavy use of organochlorines through aerial application using aircrafts (McEwen and Stephenson, 1979).

The results obtained from the present study show that, like in other countries, organochlorine pesticides used in agriculture and public health programmes in Kenya have resulted in contamination of the aquatic systems.

In view of the above findings, attempts should be made to use less persistent pesticides for public health programmes, agricultural and other purposes in order to minimize environmental contamination.

CHAPTER FIVE

Conclusions

The following conclusions were made from the present study.

1. The method used in the study was found suitable for analysis of organochlorine pesticide residues in fish since the recoveries (79-102%) obtained after spiking were within the acceptable range for pesticide residue analysis.

2. There was variation in the pesticide residue levels in the different species of fish. Sharks from Malindi had the highest mean levels of sum DDT and the widest range of pesticide residues.

3. Nine organochlorine pesticide residues were detected in 62 (22.5%) of the 275 fish samples analyzed.

4. The aquatic systems of the Tana and Athi Rivers are contaminated with organochlorine residues but the levels do not pose a health or environmental hazard at present.

5. DDT and its metabolites, especially p,p' DDE were the major organochlorine residues found in the fish studied and sum DDT levels were positively correlated to weight of the fish.

6. Organochlorine pesticide residues occurred more frequently in the liver and eggs of the species of fish studied. Fish from Malindi is not a major source of organochlorine pesticides in humans since the liver and eggs of the fish are not normally consumed.

7. Common carps from Masinga Dam may contribute to the dietary intake of organochlorine pesticides by humans since the residues occurred in the fillet of the species.

8. Fish is a suitable indicator for biological monitoring of pollution in aquatic systems contaminated with organochlorine pesticides .

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Appendix 1 Form used during collection of samples

University of Nairobi,
College of Agriculture and Veterinary Sciences,
Faculty of Veterinary Medicine,
Department of Public Health, Pharmacology and Toxicology,
P. O. Box 29053, Nairobi.

Date.....

SAMPLE COLLECTION FORM

TYPE OF SAMPLE.....

sample no.	date	site of collection	species	age	weight (g)	length (cm)	width (cm)	any other information

Appendix 2 Pesticide residue levels in livers of male sharks from Malindi

	Residue levels (mg/kg)								
sample no.	p,p' DDE	p,p' DDD	o,p' DDT	o,p' DDD	p,p' DDT	α-HCH	β-HCH	Heptachlor	Sum DDT
L85	0.314	-	-	-	-	-	-	-	0.349
L28	1.241	-	0.124	-	0.331	-	-	-	1.346
L40	0.041	-	0.043	0.039	0.178	-	-	-	0.736
L45	0.496	0.031	0.05	-	0.194	-	-	-	0.834
L80	0.101	-	-	-	0.053	-	0.062	-	0.165
L36	0.602	0.037	0.031	-	0.257	-	0.088	-	0.966
L50	0.8	0.065	-	-	0.297	-	-	-	1.257
L32	0.053	-	-	-	0.059	-	-	-	0.118
L41	0.098	-	-	-	0.045	-	-	-	0.154
L39	0.3	-	0.033	-	0.111	-	-	-	0.481
L33	0.291	-	-	-	0.011	-	-	-	0.334

Residue levels are given on wet weight basis
(-) Below the detectable limit

Appendix 3 Pesticide residue levels in livers of female sharks from Malindi

	Residue level (mg/kg)								
Sample No.	p,p' DDE	p,p' DDD	o,p' DDT	o,p' DDD	p,p' DDT	α-HCH	β-HCH	Heptachlor	sum DDT
L48	0.281	-	-	-	-	-	-	-	0.312
L54	0.422	0.078	-	-	0.311	-	-	-	1.342
L51	0.624	0.041	0.091	-	0.414	-	-	-	1.253
L86	0.668	0.026	-	-	0.158	-	-	-	0.928
L48	0.257	0.035	-	-	0.143	0.003	-	-	0.467
L30	0.195	-	0.031	-	0.097	-	0.095	-	0.348
L29	0.171	-	-	-	0.057	-	0.042	-	0.247
L83	0.590	-	-	-	-	-	-	-	0.655
L22	0.590	0.055	-	-	0.085	-	0.089	-	0.146
L27	0.128	-	-	-	0.074	-	-	-	0.216
L81	0.491	-	0.133	-	0.093	-	-	-	0.786
L26	0.327	-	0.1	-	2.674	-	-	-	3.148

Residue levels are given on wet weight basis
(-) Below the detectable limit

Appendix 4 Pesticide residue levels in livers of female catfish from Malindi

Sample no.	Residue level (mg/kg)								
	p,p' DDE	p,p' DDD	o,p' DDT	o,p' DDD	p,p' DDT	α-HCH	β-HCH	Heptachlor	sum DDT
L63	0.227	-	-	-	-	–	–	-	0.252
L70	0.184	-	-	-	-	–	–	-	0.204
L1	0.262	-	-	-	-	–	–	-	0.292
L6	0.078	-	-	-	-	–	–	-	0.087
L97	0.029	-	-	-	-	–	0.025	-	0.032
L87	0.194	0.039	0.045	-	0.108	–	–	-	0.417
L99	0.027	-	-	-	-	0.29	–	-	0.030
L12	0.079	-	-	-	-	–	–	0.024	0.08
L2	0.059	-	-	-	-	–	–	-	0.065
L95	0.056	-	-	-	-	–	–	-	0.062
L96	0.065	-	-	-	-	–	–	-	0.180
L64	0.06	-	-	-	-	–	–	-	0.067
L62	0.056	-	-	-	-	–	–	-	0.062
L65	0.151	-	-	-	-	–	–	-	0.168
L4	0.105	-	-	-	-	–	–	-	0.117

Residue levels are given on wet weight basis
(-) Below the detectable limit

Appendix 5 Pesticide residue levels in eggs of catfish from Malindi

	Residue level (mg/kg)								
Sample no.	p,p' DDE	p,p' DDD	o,p'DDT	o,p' DDD	p,p' DDT	α-HCH	β-HCH	Heptachlor	sum DDT
L1	0.294	-	-	-	-	-	-	-	0.326
L6	0.152	-	-	-	-	-	-	-	0.169
L92	0.152	-	-	-	-	-	-	-	0.164
L97	0.076	-	-	-	-	-	-	-	0.084
L99	0.133	-	-	-	-	-	-	0.042	0.149
L12	0.069	-	-	-	-	-	-	-	0.076
L95	0.083	-	-	-	-	-	-	-	0.092
L98	0.102	-	-	-	-	-	-	-	0.113
L96	0.205	-	-	-	-	-	-	-	0.228
L62	0.07	-	-	-	-	-	-	-	0.228
L65	0.14	-	-	-	-	-	-	-	0.140
L4	0.217	-	-	-	-	-	-	-	0.239

Residue levels are given on wet weight basis
(-) Below the detectable limit

Appendix 6 Pesticide residue levels in livers of male breams from Malindi

	Residue level (mg/kg)								
Sample no.	p,p'DDE	p,p'DDD	o,p'DDT	o,p' DDD	p,p' DDT	α-HCH	β-HCH	Heptachlor	sum DDT
L54	0.422	-	-	-	0.146	–	–	-	0.614
L92	0.028	-	-	-	-	–	0.158	-	0.031
L91	0.048	-	-	-	0.108	–	–	-	0.161
L56	0.249	-	-	-	-	–	–	-	0.276
L57	0.150	-	-	-	-	–	–	-	0.167

Residue levels are given on wet weight basis
(-) Below the detectable limit

Appendix 7 Pesticide residue levels in fillet of common carps from Masinga

Residue levels (mg/kg)

Sample no.	p,p' DDE	p,p' DDT	Lindane	α-HCH	Sum DDT
F15	0.054	1.125	-	-	1.185
F18	-	0.085	0.033	-	0.085
F19	-	0.085	0.233*	-	0.085
F20	-	0.102	0.295*	-	0.102
F21	-	0.205	0.075	-	0.205
F22	-	0.102	0.195	-	0.102
F23	0.015	0.096	0.10	-	0.113
F24	-	0.096	0.134	-	0.096
F25	0.022	0.112	0.151	-	0.136

Residue levels are given on wet weight basis
(-) Below the detectable limit
* Above the NFA maximum residue limit

Appendix 8 Pesticide residue levels in liver and fillet of catfish from Masinga

Sample no.		Residue levels (mg/kg) p,p' DDE	p,p' DDT	Lindane	α-HCH	Sum DDT
L1	27.7	0.059	0.052	0.011	0.009	0.117
L2	27.13	0.135	-	0.009	-	0.150
L3	27.2	0.220	-	0.009	-	0.222
*F3	27.1	-	-	0.013	-	-
*F1	27.6	0.102	-	0.004	0.013	0.113

Residue levels are given on wet weight basis
* Fillet sample
(-) Below the detectable limit

Appendix 9 Weight of fish, p,p' DDT and sum DDT levels in the samples

Sample No.	Weight (kg)	p,p' DDT (mg/kg)	Sum DDT mg/kg)
L48	2.45	-	0.312
L54	1.5	0.311	0.866
L51	2.0	0.414	1.253
L86	0.5	0.158	0.928
L49	2.75	0.143	0.467
L30	2.8	.097	0.348
L29	2.65	0.057	0.247
L83	1.4	-	0.655
L22	1.4	0.085	0.801
L27	2.85	0.74	0.216
L81	2.9	0.093	0.786
L26	2.55	2.674	3.148
F43	2.75	-	0.082
L85	1.95	-	0.349
L28	2.0	0.331	1.346
L40	1.8	0.178	0.736
L45	1.75	0.194	0.834
L80	1.9	0.053	0.165
L36	2.0	0.257	0.966
L50	1.75	0.297	1.257
L32	2.775	0.059	0.118
L41	1.7	0.045	0.154
L39	2.7	0.111	0.481
L33	2.5	0.011	0.334
L63	1.25	-	0.252
L70	0.3	-	0.204
L1	1.5	-	0.292
L6	1.5	-	0.087
L97	0.925	-	0.032
L87	1.19	0.108	0.417
L99	1.0	-	0.03
L12	1.0	-	0.08
L2	1.5	-	0.065
L95	0.65	-	0.062
L96	0.8	-	0.180
L64	0.7	-	0.067
L62	1.25	-	0.062
L67	0.925	-	0.168
1.0	1.0	-	0.117
L65	.925	-	0.132
F93	0.7	0.036	0.036
L1	1.5	-	0.326
L6	1.5	-	0.169
L92	2.25	-	0.164
L97	0.925	-	0.084
L99	1.0	-	0.149
L12	1.0	-	0.076
L95	0.65	-	0.092
L98	0.9	-	0.113
L96	0.8	-	0.228
L62	1.25	-	0.078
L65	0.925	-	0.155
L4	1.0	-	0.241
L54	1.5	0.146	0.614
L92	2.25	-	0.031
L91	2.45	0.108	0.161
L565	4.25	-	0.276
L57	2.8	-	0.167
L58	2.45	-	0,031
*F75	0.6	0.191	1.077
*F16	0.4	-	0.012
*F15	1.0	1.125	1.185
*F18	0.615	0.085	0.085
*F19	0.409	0.085	0.085
*F20	0.575	0.102	0.102
*F21	0.371	0.205	0.205
*F22	0.744	0.102	0.102
*F23	0.556	0.096	0.113
*F24	0.610	0.096	0.096
*F25	0.48	0.112	0.136
*L5	-	-	0.075
*L2	-	-	0.113
*L3	0.7	-	0.222
*F3	0.446	0.052	0.117
*F1	0.442	-	0.15

* Samples from Masinga Dam

Appendix 10 Fat percentage in fish samples positive for pesticide residues

		Fat %		
Fish species	Sampe no.	Fillet	Liver	Eggs
Shark	85	0.2	75	
	28	0.1	46.3	
	40	0.2	38	
	45	0.2	46	
	80	0.1	44	
	36	3.2	54	
	50	0.1	24	
	32	0.3	26	
	41	0.5	28.2	
	39	0.3	44.7	
	33	0.2	51.3	
	38	0.3	45	
	54	0.1	41.5	
	51	0.2	49.1	
	86	0.03	19.4	
	48	0.1	63.4	
	30	0.3	57	
	29	0.2	61	
	83	0.2	70	
	22	4.4	30.4	
	27	0.1	32	
	81	0.3	40.5	
	26	0.2	46.2	
Catfish	63	2.51	6.91	-
	70	0.06	4.25	-
	1	0.1	9.8	7.1
	6	0.01	8.4	45.2
	97	0.18	4.14	5.74
	87	0.22	31.31	-
	99	0.13	2.98	7.62
	12	0.11	4.37	4.24
	2	0.2	5.7	-
	95	0.22	5.71	7.21
	96	0.18	10.99	5.95
	64	0.02	11.26	-
	62	0.9	10.12	7.2
	65	2.1	11.89	7.14
	4	0.1	9.7	7.4
	92	0.44	5.37	4.9
	98	0.13	2.98	7.62
	1*	8.07	6.65	-
	2*	0.12	1.82	-
	3*	0.74	7.82	-
Breams	55	0.08	41.47	
	90	0.44	5.37	
	56	0.07	29.61	
	57	0.3	12.07	
	91	2.38	6.28	
Common carps				
	15*	0.5	-	-
	18*	2.3	-	-
	19*	3.0	-	-
	20*	4.5	-	-
	21*	2.0	-	
	22*	2.5	-	
	23*	2.7	-	
	24*	5.6	-	
	25*	1.1	-	

* Samples from Masinga Dam

(-) Tissue not available for analysis

No entry in the eggs column indicates the sample was obtained from a male or viviparous fish

The mean fat percentage in the fillet of shark, catfish, breams and common carps was 0.501, 0.827, 0.545 and 2.69mg/kg respectively.