# ORGANOCHLORINE PESTICIDES RESIDUES IN FISH AND SEDIMENT FROM LAKE NAIVASHA

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## DECLARATION

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

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## DEDICATION

**This report is dedicated to my wife Lydia, children and my parents**

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## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF APPENDICES

## ABBREVIATIONS

| | |
|---|---|
| FAO | Food and Agriculture Organization |
| WHO | World Health Organization |
| KMFRI | Kenya Marine and Fisheries Research Institute |
| GLC | Gas Liquid Chromatograph |
| DDT | 1,1,1 –trichloro-2, 2-di- (p-chlorophenyl) ethane |
| HCH | Hexachloro Cylohexane |
| ANOVA | Analysis of Variance |
| MRL | Maximum residue limit. |
| ADI | Acceptable daily intake |
| $LC_{50}$ | Lethal concentration 50%. |

**ABSTRACT**

The main objective of this study was to identify and determine the levels of organochlorine pesticides residues in fish and sediment from Lake Naivasha and to evaluate the toxicological implications of their presence. A total of 82 fish samples and 24 samples of sediment were analyzed using a Packard gas liquid chromatograph fitted with a $^{63}$Ni electron capture detector. The fish samples comprised of liver, eggs and muscle tissues. Out of 82 samples of fish analyzed 93.9% had detectable levels of at least one of the organochlorine compounds. Hexachlorocyclohexanes were the most frequently detected pesticides residues, α -HCH showing in 93.9% of the samples, β-HCH in 60.9% of the samples and lindane in 6% of the samples. The cyclodienes were also detected with 50% of fish samples having heptachlor which had a mean of 0.004mg/kg, dieldrin was detected in 26 samples with a mean of 0.023 mg/kg, heptachlor epoxide in 25 samples with a mean of 0.0052mg/kg, aldrin was detected in only one liver sample of a female black bass and endrin was also found in only one liver sample of a male Tilapia fish.

The 24 sediment samples analyzed, 62.5% showed presence of α -HCH with a mean of 0.003mg/kg, 37.5% samples had β-HCH with a mean of 0.00315mg/kg, 12 samples had lindane with a mean of 0.00175mg/kg; Heptachlor epoxide was detected in only one sample with a value of 0.0012mg/kg, dieldrin was found in one sample with a value of 0.0033mg/kg and aldrin in one sample with a mean of 0.0025mg/kg. No DDT or its analogue was detected in fish and sediment samples. This showed that there has not been any recent use of the pesticide in the surrounding areas. The presence of α -HCH, β-HCH and γ-HCH in both fish and sediment samples indicate continuous use of these chemicals in the numerous flower farms around Lake Naivasha.

**The organochlorine pesticide residues found in the present study are generally below the maximum residue limits (MRL) set by FAO/WHO bodies. This indicated that the organochlorine pesticide residues do not pose a health risk to the consumers of the fish from Lake Naivasha. However emphasis should be laid on a continuous monitoring programme to safe guard the consumer of fish and animal products from Lake Naivasha**

## CHAPTER ONE

### 1.0 INTRODUCTION

Since time immemorial pests have plagued man, bringing upon him diseases, loss of animals and crops. The discovery of chemical compounds that could be used to eliminate or control these pests was received with a lot of enthusiasm. In those early days pesticides commonly used were inorganic compounds such as arsenicals and fluorides or natural plant products such as rotenone and pyrethrum extract (Matsumura, 1975).

The organochlorine pesticides particularly DDT, came into the market during the Second World War to combat vector borne diseases such as malaria; bubonic plague and typhoid. The pesticides proved even more important in the protection of crops and crop products in order to feed the ever increasing human population, without the use of pesticides it is estimated that up to 50% of our agricultural food supply would be lost (Tschirley, 1979).

The initial use of organochlorine pesticides proved effective and popular because of long residual effect, low cost and low toxicity until 1960s when some studies showed their persistence in the ecosystem due to their long residual effect and resultant damage to the environment much more than it eased pest menace. Organochlorine compounds are highly lipophilic in nature and therefore tend to accumulate in the fatty tissues of fish and mammals in general, leading to a decline in fish populations due to poor reproduction success and early fly mortality. Other adverse effects of pesticides include, increase of resistant populations, minor pests becoming major pests due to the elimination of their natural predators as a result of intense pesticide application(Mc Ewen and Stephenson, 1979).

The ultimate effect of the pesticides is seen on the non-target organisms, especially those inhabiting the aquatic environment. The aquatic environment is the ultimate sink for pollutants and any compounds produced on an industrial scale are likely to reach this environment sooner or later (Murty, 1986). Pesticides reach the aquatic environment through rainfall run-off from agricultural fields, direct entry from spray operations, industrial effluents, spraying of cattle and dust storms.

Once in the aquatic environment, the pesticides are absorbed by aquatic organisms and concentrated in the trophic food 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 to the concentration in water fish samples (Chau and Afgan, 1982) .since organochlorine pesticides had been in use in Kenya for a long time, it is expected that the aquatic systems, are polluted to some extent. These pesticides are not easily biodegradable in nature and persist in the environment long after use and this becoming a health hazard to human consumers of a variety of products among them fish. The ultimate effect of organochlorine pesticides is seen on the non-target organisms, especially those inhabiting the aquatic environment.

Previous studies revealed the presence of organochlorine pesticides residues in fish and birds from some Rift valley lakes and Lake Victoria (Koeman *et al.,* 1972, Mitema and Gitau, 1990, Mugachia et al., 1992). Mugachia (1992) found α -HCH at a concentration of 0.014mg/kg from a composite sample of 10 fish from Lake Naivasha but did not check on the residues in sediment. There are about 250,000 people living around Lake Naivasha and inhabiting an area that has a non functional sewage system. Industrial effluents mainly from flash floods from the town and

industrial developments flow into the Lake and hence these activities increasingly pollute the lake.

Lake Naivasha has no outlet and therefore any incoming biocides or organochlorines are likely to build up in the lake more than in any other aquatic system in Kenya. The levels of organochlorine residues need to be evaluated in the fish biota and sediments because the level obtained indicates the extent of contamination to the lake with those chemicals. The sediments are of interest particularly because they may be reservoirs rather than ultimate sinks and may slowly release absorbed chemicals, thus maintaining the contamination of aquatic biota for prolonged periods of time as a variety of organisms including fish use the detritus as a source of food (Murty, 1986).

This study was initiated with the following objectives:

- To identify and quantify organochlorine residues in Tilapia fish, Black bass fish and Sediment from Lake Naivasha.
- To asses the toxicological implications associated with the observed levels of residues.

## CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1. General Definition of Pesticides

Pesticides are chemical substances used to control pests in an effort to increase crop production quality, and food storage, reduce energy input in form of labour and for control of vector borne diseases. In Kenya there are approximately 786 fully registered pest control pesticides, with commercial imports averaging around 7,300 tonnes annually as shown in table 1 and the government of Kenya spends about 700 million Kenya shillings in the importation of pesticides every year (Pest Control Products Board, 2008).

**Table 1: Importation of different groups of pesticides into Kenya (1986-1995), Quantity in tonnes.**

| Year | Insecticides + acaricides | Herbicides | Others | Fungicides | Total |
|---|---|---|---|---|---|
| 1995 | 1413.3 | 870.6 | 2323.0 | 501.9 | 5108.8 |
| 1994 | 1049.9 | 747.4 | 1671.8 | 563.3 | 4032.4 |
| 1993 | 839 | 882 | 1503 | 309 | 3533 |
| 1992 | 1670 | 1122 | 2634 | 1164 | 6590 |
| 1991 | 1072 | 844 | 1568 | 570 | 4054 |
| 1990 | 1572 | 1134 | 1330 | 857 | 4893 |
| 1989 | 1571 | 1148 | 4627 | 665 | 7711 |
| 1988 | 1089 | 2108 | 4259 | 801 | 8257 |
| 1987 | 1206 | 1311 | 715 | 697 | 10371 |
| 1986 | 1076 | 112 | 654 | 808 | 9597 |

**Source: Pest Control Products Board, 2008**

The exact date of the introduction of synthetic pesticides into Africa is not known. Synthetic pesticides were brought into the Africa continent by colonial powers at around the turn of the first or second decade of last century. From historical records, the earliest British government legislation, the public Health Act, which was intended to protect human beings and regulate the use of pesticides by farmers in Kenya, was enacted in 1921.Therefore; the toxic effects of pesticides were observed very early, soon after their application in the environment.

It must be concluded that the early observed adverse effects of pesticides on humans necessitated the regulation of their use and handling. Assuming that other colonial governments followed the same pattern of pesticide regulation as the British, then it may be concluded that synthetic pesticides have been used in the continent for about 80 years (Wandiga, 2001).

Although the importance of the use of pesticides cannot be over emphasized, man is also aware of the dangers associated with the pesticides and there is a growing public concern about effects pesticides has both on the environment and health of the people. The government of Kenya has enacted legislation for regulatory control of pesticides use as shown below (Table 2):

**Table 2: Legislation Relating to Pesticides in Kenya**

| YEAR | ACT | PURPOSE |
|---|---|---|
| 1921 | Public Health Act | Control the general use and handling of pesticides |
| 1937 | Cattle Cleansing Act | Prescriptions for tick control |
| 1937 | Plant Protection Act | Regulate plant protection practice |
| 1951 | Water fish samples Act, Cap 389 | Regulate water fish samples use and controls water fish samples additives. |
| 1952 | United Kingdom Act | Protection of employees against risks of poisoning by substances used in agriculture |
| 1954 | Poisonous Substances Ordinances | Protection of employees against risks of poisoning by substances used in agriculture |
| 1955 | Agriculture Act | Control pesticide use, distribution, and control |
| 1957 | Pharmacy and Poisons Act, UK | Control of the profession of pharmacy and the trade in drugs and poisons. |
| 1963 | Use of Poisonous Substances Act | Protect persons from the risk of poisoning by certain substances |
| 1965 | Food, Drug and Chemical Substance Act | Prevention of adulteration of food, drugs, and chemical substances |
| 1972 | Factories Act | Regulate factory working conditions with an aim at maximizing health protection of workers |
| 1972 | Fertilizers and Animals Foodstuffs Act, Cap 345 | Control fertilizers use and animal foodstuff contamination |
| 1983 | Pest Control Products Act | Regulation of importation, exportation, manufacture, and distribution of products used for pest control |
| 1999 | Environmental Management and Coordination Act | Manage and coordinate all statutes dealing with environment |

Pesticide compounds have been widely used for control of both crops and livestock pests and in the control of vector borne diseases for both humans and animals. Organochlorine Pesticides have high chemical stabilities, low aqueous solubilities and high lipophilicity that enables the chemicals to accumulate along food chains and reaching high concentrations at the top of the food chain. Organochlorine Pesticides are persistent and non-biogradable in the environment pesticides are therefore, potentially toxic to all forms of life and may present hazards to workers consumers, public at large and wildlife (Brooks, 1974).

Pesticide residue is any substance in human foods or animal feeds resulting from the use of a pesticide and includes any specified derivatives such as degradation and conversion products and impurities that are considered of toxicological significance (FAO/WHO, 1986)

Since the introduction of DDT in 1941 a number of halogenated organic compounds were synthesized and used as pesticides. These compounds generally referred to as organochlorines (OC) or chlorinated hydrocarbons include cyclodienes compounds, hexachlorocyclohexanes (HCH), toxaphene and others. These compounds have been of great concern.

The investigations of adverse effects of pesticide residues started in the early 1960s, resulting in strict restriction on the use of bioaccumulative organochlorine pesticides in some countries and others banning their use altogether (McEwen and Stephenson, 1979, EPA 1985). In Kenya, the Government created a Pest Control Products Board under the pest control products ACT of 1982 to control pesticides during manufacture, distribution and application. The Government of Kenya banned or restricted the use of environmentally persistent organochlorine pesticides from 1986(pest control products board records 2008). Lindane was restricted for use as a seed dressing in 1998. However the compound is still used as an insecticide in the control of insect pests of cotton. The other mixtures of HCH isomers were banned for all uses.

Endrin and heptachlor were banned for use as insecticides in 1986. Aldrin and dieldrin were also banned but restricted for use in termite control in the building industry in 2004. DDT was banned for use in agriculture and its use restricted to public health only in control of mosquitoes in 2004 (Pest Control Products Board, 2008).

### 2.2 Classification of organochlorine pesticides

The organochlorines can be divided into four groups namely, DDT and its analogues, hexachlorocyclohexanes (HCH), cyclodiene compounds and toxaphene group.

#### 2.2.1 DDT and Its Analogues

DDT was first prepared in the laboratory by Zeidler in 1874. The insecticidal properties of the compound were discovered in the Swiss laboratories of the Geigy Company in 1939 by Muller (Chau and Afghan, 1982). DDT has been widely used for control of vector borne diseases like malaria and in control of mites, lice and plant pests. Under most environmental conditions, DDT is persistent and resistant to complete biodegradation by either soil microorganisms or higher organisms. DDT is a white, tasteless and almost odorless Crystalline solid with melting point of 108.5$^{0}$C to 109 $^{o}$C. p, p′-DDT is the most important insecticide in this group. Commercial DDT also contains 0, p′-DDT metabolites. The 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.

The discovery in the 1960's that the application of DDT was accompanied by environmental hazards due to its high persistence, toxicity and bio-accumulation resulted in restriction or ban of its uses in many developed countries (Jensen 1983). Despite all these disadvantages DDT is still considered in many developing countries to have significant positive benefit cost ratios and

hence forms a major part in many programmes involved in preventing and controlling vector-borne diseases.

### 2.2.2 Cyclodienes

Cyclodienes are highly chlorinated cycle hydrocarbons with endomethylene bridged structures. Compounds in the group include chlordane, aldrin, dieldrin, heptachlor, heptachlor epoxide and endrin. Their discovery dates back to the 1940's and they have been widely used for insect control as the most effective insecticides known to date. The cyclodienes are very persistent in the environment and tend to accumulate in biological as well as non —biological systems. Aldrin and heptachlor are rapidly metabolized by organisms but their metabolites, dieldrin and heptachlor epoxide, respectively are more toxic and persistent than the parent material (Howard, 1991). The compounds in this group are classified as neurotoxins. In both humans and animals the primary acute toxic effects are on the central nervous system, including hyper excitability and tremors followed by convulsions and possibly death. Acute poisoning from them may be fatal. The compounds also affect the reproductive system, liver and kidney.

### 2.2.3 HexachloroCyclohexanes (HCH)

Hexachlorocyclohexanes are mixtures of stereoisomers, which differ in the relative position of the chlorine around the hexane ring. The commercial insecticide is a mixture of the different isomers, which include α -HCH, β-HCH, γ -HCH, ∂-HCH, and others.Lindane( γ -HCH) is the most toxic isomer and also the most commonly used insecticide whereas β -HCH is the most symmetrical and stable isomer and also the most environmentally persistent and chronically toxic HCH. β- HCH also has a higher ability to accumulate in the fat tissues than lindane.

### 2.2.4 Toxaphene Group

This group includes toxaphene which was discovered in 1947 and strobane which was introduced in 1955. Toxaphene was first used on cotton in combination with methylparathion an organophosphate. The chemicals in these groups are less persistent in the soil due to their volatility rather than their actual metabolism or photolysis. They are metabolised in mammals and birds and they are not stored in body fat. They are neurotoxic which results from the imbalances in sodium and potassium ions on the neurons.

## 2.3 Absorption and Accumulation of Organochlorine Pesticide in Fish

Fish absorb and accumulate xenobiotics from water and food particularly those with water solubility and high lipophilicity. 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, lipid content, size, stage of development, physiological activity and environmental factors such as temperature. Because of its great affinity to lipid material DDT is taken up quickly from food and water, metabolized slowly, and stored for an extended period.

Studies carried out with rainbow trout, bluegills, and gold fish showed that they took up DDT rapidly after a single exposure of a few hours. Generally the other organochlorine pesticides are less rapidly absorbed from water by fish than HCH. Dieldrin is 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).

## 2.4 Metabolism and Excretion of Organochlorine Residues in Fish

### 2.4.1 Metabolism.

Among all the pesticides, degradation of DDT and mirex by fish is very slow. Little excretion of DDT through urine or gill affiliate or conversion to metabolites was evident 48hrs after administering DDT to dogfish via the caudal vein, artery or stomach tube. (Murty, 1986). Metabolisms of organochlorine pesticides in fish mainly occur in the liver effected by the mixed function oxidate (MFO). Optimum temperature for fish MFO's is lower than that for mammals and is around 25°c. The system is confined to liver and is negligible in other tissues. Xenobiotic detoxification mechanisms comprise not only MFO's but certain other enzymatic processes as well, like the oxidative biotransformation, mediated by MFO's is one of the major metabolic, processes available for handling foreign compounds, which along with other processes like reductions, methylation, hydrolysis represent only the first in the detoxification process. The second step comprises the conjugation of the first step, with a molecule like glucoronic acid or amino acids provided by the body increasing polarity and likewise elimination. These biochemical pathways, although they often lead to detoxification, may sometimes result in the activation of a relatively nontoxic compounds or its lack in fish should be a welcome feature (Murty, 1986). The major metabolic product of DDT in fish is DDE and to some extent DDD. These two compounds are retained for long due to their low polarity (Addison *et al.*, 1978). Aldrin in fish is epoxidized to dieldrin, which is more toxic to the species (Janice *et al.*, 1976) Endosulfan is poorly metabolized by fish and is mainly converted to endosulfan sulphate. Endrin is metabolized by hydroxylation followed by conjugation in the liver (Murty 1986). Mirex $^{c}14$ administered in the diets of laboratory rats, Japanese quail and mosquito fish (Gambusia affanis) was not metabolized to any detectable extent. The maximum residue accumulation occurred in adipose tissue. The half-life of

the residues in the whole body of the fish was 130 days. The use of uniformly labeled mirex $^{14}$ in these studies permitted the detection of virtually any type of degradation product but hundreds of analyses over the 16 month period of this investigation showed no indication of mirex metabolism (Iwie et *al.*, 1974)

**2.4.2 Excretion of Organochlorine 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 path way also play 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 Killi fish 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 of Organochlorines in Fish**

Generally the potential impact of the pollutants is more on the aquatic organisms than on the terrestrial organisms because in the aquatic environment the body of the organism is bathed by the toxicant at high concentrations unlike the terrestrial environment where the pesticides and such other substances are transported to a greater distance and thus affecting organisms in low concentrations.

Organochlorine pesticides exhibit both acute and chronic toxicity to fish and are generally more toxic than organophosphorous compounds. However, some of the latter are as toxic as some of the highly toxic organochlorine compounds (Murty, 1986). As early as 1944 greater toxicity of DDT to fish than to mammals and the vulnerability of fish in laboratory tests, were reported (Murty, 1986).Chronic toxicity of organochlorine pesticides is the main concern of environmental 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**

Toxicity to fish varies in severity from harmful to highly toxic. 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 affanis is 0.32 mg/l as opposed to 1mg/l for methoxychlor (Murty 1986). The reported 96-hour $LC_{50}$ (The concentration that kills 50% of the test population in a given time) of lindane and technical hexachlorocyclohexane (HCH) to several species of estuarine fish ranges from 30 to l04ug/l (Murty 1986). Among the organochlorine pesticides endrin and other cyclodienes are highly toxic to fish. Dieldrin is highly toxic to fish and crustaceans, $LC_{50}$ ranging from 2.2 to 53 ug/l. The standard 96-hour toxicity tests with endrin yield $LC_{50}$ 1ug/l or less in the rainbow trout, Coho salmon, Chinook salmon, fat head minnoins, 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 2ppm. Grant (1978) 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 fresh water fish species ranges from 0.2 to 8.1 ug/l and 0.3 to 2.9 ug/l to saltwater fish (Ananda *et al*, 1981).

Heptachlor has a 96-hour LC5O range of 1 to 4ug/l for several species of estuarine fish (Schimmel *et al.*, 1976).

**2.5.2 Chronic toxicity and sublethal exposure**

It has become apparent that environmental risk assessments cannot be based solely on acute toxicity data. There is a range of subtle sub lethal or chronic effects on aquatic organisms which may be associated with low doses of pesticides ranging from behavioral aberrations to mutagenesis, teratogenesis, and carcinogenesis or associated with accumulation of residues to levels undesirable to the consumer (Murty 1986). Exposure of a predatory fish, *therapon jarbua* to 2 ug/l of DDT for l5days 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). 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). Chronic exposure of endrin to rainbow trout and gold fish is reported to cause hypersensitivity and growth inhibition (Grant, 1978).

A study of fish in the Laguna Madres area of Texas showed adult spotted sea trout are abundant, but a progressive decline in the numbers of juveniles was observed. DDT and its metabolites in the ovaries of spotted sea trout reached a peak of 8ppm prior to spawning in September 1968. There was no evidence of successful spawning later that year. One hundred miles away, the level of DDT and its metabolites were O.2ppm in sea trout ovaries and a normal number of young of the year was observed (Matsumura, 1975)

**2.6 Review of organochlorine pesticides residues in fish in 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 carried out in North America and Europe (Murty, 1986). Studies on residues in U.S. A. North America were carried out on museum specimens of six species of fish from Lake Michigan collected between 1929 and 1966 and stored in ethyl alcohol to determine the years in which the various contaminants started appearing in fish. The studies revealed that DDT and its metabolites appeared in fish samples for the first time 1949 i.e. within four years after the environmental application of DDT had commenced, while dieldrin appeared in 1955 (Murty, 1986).

Studies of organochlorine pesticide residue in fish from various aquatic systems, carried out from the early 1960s 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 showed far much lower concentrations than DDT. Studies centered on the great lakes in 1970, showed that fish from Lake Michigan had 2 to 7 times the residue load compared to fish from other great lakes. The great lakes occupy a unique position in the world because they represent the largest total mass of fresh water draining a large catchment area. The coho salmon in Lake Michigan played an important role in creating a general awareness of the environmental hazards of DDT group compounds. The coho in Lake Michigan was highly contaminated and unfit for human consumption (Murty 1986).

In Europe, major contributions to the environmental monitoring of pesticide residues in fish have come from countries near or around the Baltic Sea. Studies in the area revealed heavy use of DDT levels in the biota especially from southwestern coast cod caught in 1969 from Dasfjordan had total DDT content of 0.57 to 2.15 mg /kg (wet weight) whereas those caught from sonef jorden, near an intensive fruit growing area, had a residue level of 1.98 to 33mg/kg wet weight. (Stenersen, Kvalvag, 1972). Cod sample in 1971, had a 90 to 135 mg/kg (wet weight) in liver; on a fat weight basis the highest residue concentration was 576 mg/kg (Brevik *et al*, 1978).Reports of organochlorine pesticide residues levels have been published from other regions as well. In a survey conducted in 1974 in fish from two rivers in Iran, the sum DDT on fat weight basis, in barbus species was 60.6 mg/kg and 196mg/kg and Organochlorine residues in fish from lakes and reservoirs were generally less than 25mg/kg. (Murty 1986). DDT, main metabolite, and other organochlorines were analyzed in soils (n-6), fluvial sediments (n-10) that were collected in several areas of the Amazon region in Brazil. The samples were analyzed by capillary column gas chromatography coupled to electron capture detection. DDT residues were present in most of the collected sediments in concentrations of approximately 10 to 100 micro /kg (ppm, dry weight). Some urban topsoils were found to have more than 1mg/kg (ppm) in fish, as much as 0.5 mg/kg of total DDT (wet weight) was found in the edible parts. The presence of p,p'-DDT in most of the samples reflected the use of this insecticide against vectors of malaria between 1946 and 1993 which led to its ubiquitous presence in the environment of the Brazilian Amazon *(Torres et al., 2002).*

The levels and patterns for organochlorines including DDT group, HCH group and PCB group were investigated in sediments and Tilapias (Tilapia Moisambica) collected from Hong kong inland water fish samples systems. In the new territories of Hong Kong, sediment and tilapia samples

were also collected from two fishponds for comparison. The ranges of DDT group, HCH group, and PCB group in river sediments were 2.82-8.63 mg/g, 0.05-2.07 mg/g, and 43-46 mglg respectively. All these values were significantly higher ($p<0.05$) than the pond sediments. (Zhou et al., 1999). Hepatic levels of organochlorines were analyzed in deep-sea fish from the Nord Fjord in Norway. Levels of PCBs in the study exceed background levels in fish from Norwegian water fish samples by a factor of 1.5 to 50, and DDT group by one to two orders of magnitude. DDT group in fish from the NordFjord was attributed to DDT use in fruit orchards. The levels of DDT group showed that the decline usually found in biota Scandinavia since the 1970s was found in the deep-sea fish in Nord Fjord. There was no known local PCB source that could explain the elevated levels in the study. This indicated that Fjord efficiently accumulated atmospheric contaminants. Chlordanes and HCB were less important, and HCH group of compounds were not detected. (Bergu *et al.,* 1999). Levels and bioaccumulation of organochlorine pesticides (OCPS) and polybrominated diphenyl ethers (PBDES) was studied in fishes from the Pearl River estuary and Daya Bay, South China. Fifty fish samples were collected and were analyzed for DDTs,HCHs chlordanes and polybrominated biphenyl ethers (PBDES).Its was observed that except the high concentrations of DDT observed in fish ,the concentrations of HCHs, chlordanes and PBDES were low when compared to other regions(Lingli *et al.,* 2007).Many studies have reported on ensuing contamination of aquatic systems. The presence of organochlorine insecticides in the aquatic fauna is ubiquitous and can even be found in isolated regions. For example, Sakar *et al.,* (2003) reported the presence of $\gamma$ -HCH and DDT in two fresh water fish samples fish species, the putitor mahseer (*tor putitora*) and the snow trout (*schizorhorax richardsonii*) in the Himalaya Mountain.
In the republic of Benin a study of contamination of fish by organochlorine pesticide residues in the Oueme river catchment was done as it was felt that the aquatic ecosystems are subject to

poisoning risks due to the inappropriate use of pesticides, such as washing of empty bottles in rivers and using pesticides to catch fish .In some areas, cotton fields are located near river banks, increasing the probability of pesticides emission to the river. To assess contamination levels in Queme River catchment area, different fish species were collected from different geographical areas along the River.DDT, its metabolites and isomers were the most frequently identified pesticides in fish flesh, α -endosulfan, β-endosulfan,dieldrin,telodrin,lindane and octachlorostyrene were also detected . Concentrations of pesticides residues in fish ranged from 0 to 1364ng/g lipid. A preliminary risk assessment indicated that the daily intake of chlorinated pesticides by people consuming fish from the Oueme River still is rather low and does not present an immediate risk (Elisabeth *et al.,* 2006)

**2.7 Review of studies on organochlorine pesticide residues in Kenyan fish**

In the 1940s organochlorine pesticides were extensively used in control of mosquitoes and tsetse flies especially in the Lake Victoria Region. The country continued usage of these organochlorine pesticides until 1986 when the Pest Control Products Board restricted and banned the use of some of the pesticides.

Estimates indicate that Sub-Saharan African countries import about 50 billion Kenya shilling worth of pesticides each year. This accounts for about three percent of world pesticides imports. As a result of pressure to food production, pesticide use in the region between 1990 and 2000 increased by almost 200 percent compared to 20 percent in developed countries. Therefore this calls for frequent monitoring of the pesticides residues load in African countries. Investigations on the residue levels of the pesticides in fish in Kenya appear to be very little, while little has been done on sediments pesticide residues studies. Koeman *et al.,* (1972) carried out a preliminary survey of the possible contamination of Lake Nakuru in Kenya with some metals and

chlorinated hydrocarbons. They reported extremely low levels of dieldrin, p, p'- 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 to be 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) in studies of Kenya mothers milk, analyzed fish samples from Rusinga Island in Lake Victoria. The fish samples were analyzed 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 level ranged from 0.031 to 0.367 mg/kg. The smoked sampled showed the highest mean (0.149 mg/kg) of sum DDT. Mitema and Gitau, (1990) carried out an investigation on organochlorine residues in fish from Lake Victoria. They found the level of residues to be below the maximum residue limit. Mugachia *et al.,* (1992) carried out an extensive study of organochlorine pesticide residues in fish from Lake Naivasha, Tana River at Masinga dam and the estuary of Athi (Sabaki) River at Malindi. The residue levels compared well with those reported in other studies but lindane levels in some samples exceeded the maximum residue limits set by WHO and FAO.

## CHAPTER THREE

## 3.0 MATERIALS AND METHODS

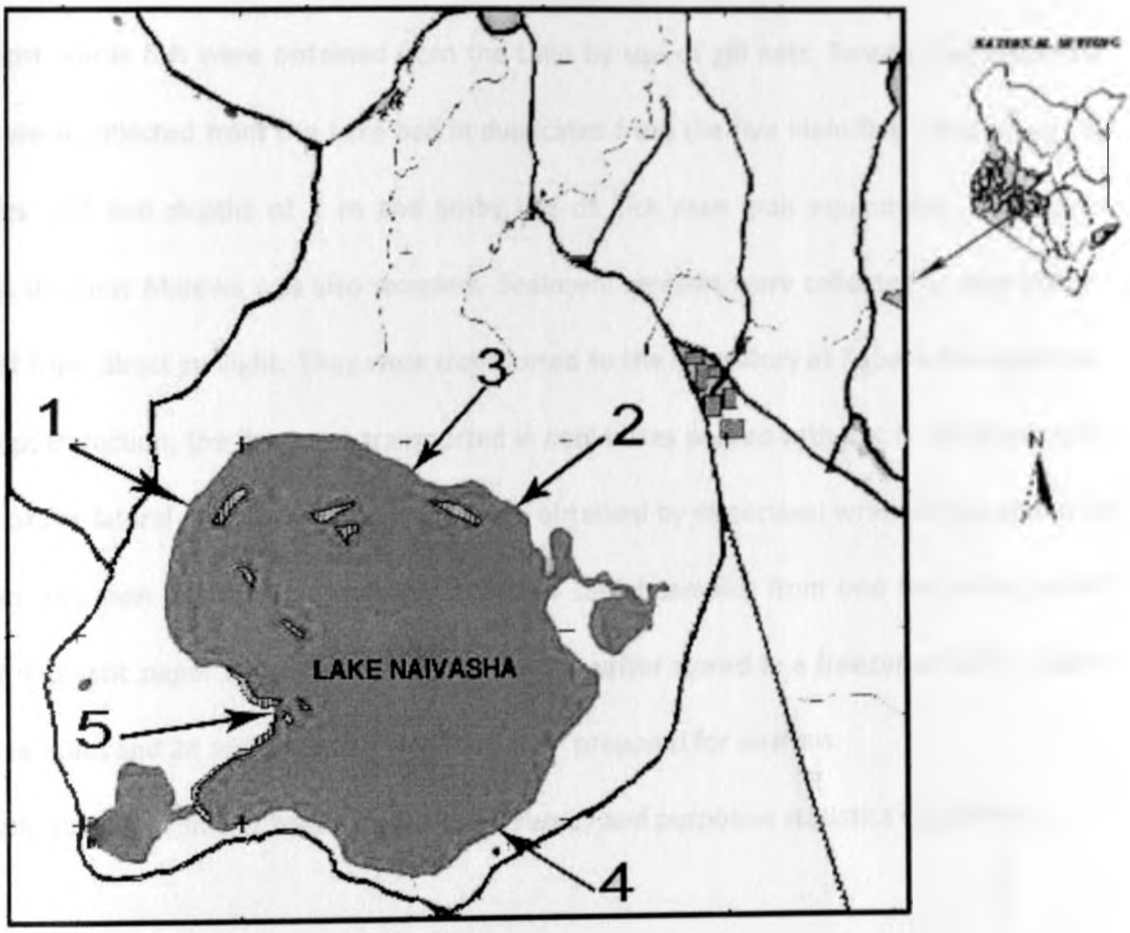
### 3.1 study site

The study was carried out at lake Naivasha, which is a fresh water lake, situated in the Southern region of the Great Rift Valley about 100km North of Nairobi, shown in Figure 1.

The lake covers an area of l50 kms and has a mean depth of 9 m; the pH of the water is around 7.3 and located at an altitude of 189m. It receives water from Malewa, Gilgil and Karati rivers, which enter the lake to the North. Of these rivers Malewa contributes about 90% of the discharge into the lake and most of the rest of discharge is contributed by Gilgil River. The lake has no outlet. The dominant vegetation types are belts of papyrus *(cyperus papyrush)* around the margins, stands of submerged macrophytes, of which the principal species is *Najas pectinata* and mats of floating plants comprising *sa/vinia molesta* and *Eichhornia crassies.*

Fig. 1 shows the locations of the various sampling sites in the study area

Figure 1: Map of Lake Naivasha showing Sampling Stations




LEGEND

1 - Flamingo Point
2 - Municipal Sewage Discharge Point
3 - Malewa River Mouth
4 - Sher Agencies
5 - Lake NAivasha Country Club

### 3.2. Sample collection

#### 3.2.1 Sampling procedure

Thirty eight whole fish were obtained from the Lake by use of gill nets. Twenty four sediment samples were collected from the Lake bed in duplicates from the five identified areas along the lakeshores and two depths of 1 m and 5mby use of Eick man grab equipment. Two points upstream the river Malewa was also sampled. Sediment samples were collected in clear bottles protected from direct sunlight. They were transported to the laboratory at Kabete the same day. To avoid putrefaction; the fish were transported in cool boxes packed with ice. In the laboratory, samples of the lateral muscle, liver and eggs were obtained by dissection; wrapped separately in aluminum 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 $-20^0$c . Eighty two fish samples and 24 sediment samples were then prepared for analysis.

The sample size decision was based on literature survey and purposive statistics calculation.

#### 3.2.2. Species of fish sampled

Black bass and Tilapia species of fish were targeted; they are the dominant species in Lake Naivasha. The two species of fish have different feeding habits. Black bass *(micropterus salmoides)*

is a carnivorous fish feeding on small fish and other small aquatic life forms. Small bass depend heavily on micronecta and large bass mostly take Cray fish.Tilapiines *(oreochromis leucostictus* and *Tilapia zilii)* are herbivorous in nature. Detritus predominates in the diet of *oreochromis leucostictus and Tilapia zilii* but the former also eats algae and the latter, micronecta and submerged macrophte.

## 3.3. METHODS

### 3.3.1 Cleaning of glassware

The glassware were 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 degrees centigrade overnight. Each piece was rinsed with redistilled hexane before use.

### 3.3.2 Purity check

To check the purity of hexane, l0 ml of the solvent was reduced to 1ml by evaporation in a water bath at 42 degrees Celsius 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. 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 diethyl ether) were checked as follows:

Ten milliliters of the solvent in a graduated centrifuge tube was evaporated to dryness in a water bath at 42 degrees Celsius 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 peaks were observed after a single distillation.

### 3.3.3 Washing of concentrated sulphuric acid with hexane

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 water bath 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.

### 3.3.4 Preparation of pesticide standards

A high quality organochlorine pesticide mixture (CPM) containing 13 compounds dissolved in 1ml isooctane in sealed ampoules was obtained from supelco, SA Gland, Switzerland. Working standards and stock solutions of standards were prepared by dilution of the 1ml concentrated pesticide mixture.

### 3.3.5 Gas liquid chromatograph (GLC)

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

### 3.3.6 Operating conditions

Carrier gas flow rate during the analysis was maintained a 30m/s /minute and attenuation of 128.

The GLC operating temperatures were:

Injection block 230°c

Oven (column) 210 °c

Detector 250 °c

### 3.3.7 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 in the region of 0.001mg/kg.

The process involved extraction, clean up, GLC injection and quantification.

### 3.4 Extraction of organochlorine residues

Three grams of thawed sample was weighed into a mortar containing 4.5 grams of anhydrous sodium sulphate and 4.5 grams of acid-washed sand. A pestle was used to crush and mix the three ingredients into a flowing powder. Four gram of the powder was weighed into a wad of hexane-washed cotton wool in a disposable extraction glass column. Enough diethyl ether was added to the column to wet sample. The set-up was then left to stand for 15 minutes following which small volumes of diethyl ether was added to the sample until 10 to 15 milliliters was eluted into a pre-weighed centrifuge tube.

The latter was placed in 42 degrees Celsius sand bath and evaporated to dryness under a stream of nitrogen. The centrifuge tube was cooled to room temperature and re-weighed. The difference between the initial weight of the tube and the final weight after evaporation of ether was taken as the weight of the fat extracted from the sample. The fat was redissolved in redistilled hexane to give a fat concentration of not more than 0.05 grams/milliliter.

### 3.4.1 Clean- up

Acid and base treatment was used to clean up the extracts. A modification of the method described by Bjerk and Sundby (1970) was used. Two 1-milliliter aliquots of the n-hexane fat

solution were put into different centrifuge tubes. The acid metabolizes aldrin, heptachlor epoxide, dieldrin and endrin. Thus these compounds were not recovered in the acid clean up but in the base clean up.

**3.4.2 Acid clean up**

One milliliter of hexane-washed concentrated sulphuric acid was added to one of the 1-milliliter aliquots of the n-hexane-fat solution and thoroughly mixed using a whirl mixer.

The mixture was later centrifuged for 2 minutes at 3,000 revolutions per minute (rpm). The clear hexane layer was transferred into an extract vial. The extract was then later injected into the GLC for analysis and used to identify the organochlorine residues other than aldrin, dieldrin, endrin and heptachlor epoxide, which are metabolized in acid clean-up.

**3.4.3. Base clean up**

Two potassium hydroxide pellets were added to 0.1 milliliters of distilled water in a graduated centrifuge tube and left for adequate time to soak.1 milliliters of 99.3% ethanol was then added to dissolve the ingredients. To this solution, 1 millitre of the h-hexane fat solution was added and thoroughly mixed.

The mixture was placed in a sand bath at 50°c for 30 minutes, and then cooled in a fridge to stabilize the hexane. Then 5 millitre of the solution of sodium chloride and orthophosphoric acid was added and mixed in whirl mixer. The mixture was cooled further in a freezer for a few minutes to obtain a clean separation. Then the mixture was centrifuged at 3000rpm for 10 minutes .The top hexane layer was transferred into a small tube and little anhydrous sodium sulphate added to dry off any water present. After setting, the top hexane layer was transferred into a small sample vial with a stopper and stored at -20°c awaiting analysis in the GLC.

### 3.4.4. Quantification of the pesticide residues

The extracts and the working standards were removed from the deep freezer and thawed at room temperature. Two ml of each extract was injected into the GLC using 10ml hypodermic syringes with a 7cm long needle. The syringe was rinsed several times with redistilled n-hexane before and after use. The detector response was observed for about 20 minutes. Identification was done by comparing the retention times of the sample peaks on the chromatograph to the ones of the standard chromatograph. Quantification was done by comparing the peak heights of the sample components with those of the corresponding components in standards of known concentrations. The amount of each compound in 1kg of sample was calculated by considering the final volume of the extract and correcting for the dilutions using the following formula:

$P=h/hs \times Cs \times v/m$

P=Concentration (ug/g) of pesticide residue in sample.

h= peak height of the residue in the chromatograph of the sample.

hs = Peak height of the pesticide in standard chromatogram

m= weight (g) of the sample used in the analysis.

v= Volume of n-hexane used to dissolve the ft extract.

Cs= Concentration of the standard in ug/ml.

### 3.4.5 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 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.

### 3.5 Toxicological Evaluation

Inorder to assess the toxicological significance of the results with regard to human health, the residue levels were evaluated against the maximum residue levels (MRL) and acceptable daily intake (ADI) in fish set by FAO/WHO.

### 3.6 Statistical analysis

Statistical analysis of data was mainly done by analysis of variance (ANOVA) and T test using SPSS programme.

Test of organochlorine pesticides residues differences between the fish species

**Table 3: Test of Homogeneity of Variances**

| | Levene Statistic | df1 | df2 | p-value |
|---|---|---|---|---|
| α -HCH | 16.990 | 1 | 30 | 0.000 |
| β -HCH | 13.507 | 1 | 21 | 0.001 |
| Heptachlor | [a,b] | 0 | | |
| Heptachlor epoxide | 2.980 | 1 | 9 | 0.118 |
| Dieldrin | 4.637 | 1 | 19 | 0.044 |

a. Groups with only one case are ignored in computing.

b. Test of homogeneity of variances cannot be performed for heptachlor because only one group has a computed variance

**Table 4: Anova for organochlorine Pesticide residues differences between the fish species**

| | | | | | | |
|---|---|---|---|---|---|---|
| α -HCH | Between Groups | .000 | 1 | .000 | 2.477 | 0.126 |
| | Within Groups | .006 | 30 | .000 | | |
| | Total | .006 | 31 | | | |
| β -HCH | Between Groups | .011 | 1 | .011 | 2.715 | 0.114 |
| | Within Groups | .088 | 21 | .004 | | |
| | Total | .099 | 22 | | | |
| Heptachlor | Between Groups | .000 | 1 | .000 | 1.093 | 0.405 |
| | Within Groups | .000 | 2 | .000 | | |
| | Total | .000 | 3 | | | |
| Heptachlor epoxide | Between Groups | .000 | 1 | .000 | .000 | 0.993 |
| | Within Groups | .000 | 9 | .000 | | |
| | Total | .000 | 10 | | | |
| Dieldrin | Between Groups | .001 | 1 | .001 | 3.157 | 0.092 |
| | Within Groups | .007 | 19 | .000 | | |
| | Total | .008 | 20 | | | |

There is no significant difference of organochlorine pesticides residues between the two species ($p>0.05$).

**Comparison of organochlorine pesticide residues in (liver. eggs, muscles) and sediment)**

**Table 5: Anova for the organochlorine pesticide residues in liver, eggs, muscles and sediment**

| | | | Sum of Squares | df | Mean Square | F | P value |
|---|---|---|---|---|---|---|---|
| α -HCH | Between Groups | (Combined) | .001 | 3 | .000 | 3.007 | .035 |
| | Within Groups | | .009 | 82 | .000 | | |
| | Total | | .010 | 85 | | | |
| β -HCH | Between Groups | (Combined) | .007 | 3 | .002 | 1.122 | .349 |
| | Within Groups | | .099 | 49 | .002 | | |
| | Total | | .106 | 52 | | | |
| γ -HCH | Between Groups | (Combined) | .000 | 1 | .000 | .004 | .949 |
| | Within Groups | | .000 | 8 | .000 | | |
| | Total | | .000 | 9 | | | |
| Heptachlor | Between Groups | (Combined) | .000 | 3 | .000 | 2.355 | .100 |
| | Within Groups | | .000 | 22 | .000 | | |
| | Total | | .001 | 25 | | | |
| Heptachlor epoxide | Between Groups | (Combined) | .002 | 3 | .001 | 1.714 | .195 |
| | Within Groups | | .008 | 21 | .000 | | |
| | Total | | .010 | 24 | | | |

a. Too few cases - statistics for dieldrin , cannot be computed.

The results show there were not significant differences (P>0.0 5) apart from α -HCH where there was a significant difference (p=0.035<0.05).

# CHAPTER FOUR

## 4.0 RESULTS

### 4.1 Levels of organochlorine pesticides residues in Fish samples

A total of 82 fish samples were analyzed for organochlorine pesticides residues.

The species of fish studied were tilapia and black bass.

93.9% samples had detectable levels of one or more of the 8 organochlorine pesticides residues.

The mean levels, ranges and standard deviation of the pesticide residues detected are given in tables 3 to 5 Table 3 covers pesticide residues levels in the two species of fish (black bass and tilapia), Table 4 has pesticide residues in the different sexes of the two fish species and table 5 pesticide residues levels in liver, muscle and eggs of the two fish species. The various residues detected are as explained below.

**HCH group**

This group had the most pesticides residues detected. Seventy seven samples showed α -HCH. 50 samples had β-HCH and lindane showed in 5 samples.

Their means were as follows:

The highest recorded organochlorine pesticide residue in this group was β – HCH with 0.22mg/kg from a tilapia fish liver sample (Appendix 7).

**Cyclodiene group**

Pesticides residues of this group detected were: Heptachlor in 41 samples or 50% of the total 82 samples of fish and a mean level of 0.004 mg/kg. Dieldrin was found in 26 samples (31.7%) a

mean level of 0.023 mg/kg and Heptachlor epoxide in 25 samples (30.49%) a mean level of 0.0052 mg/kg.

Aldrin was detected in only one liver samples of a female black bass fish with a weight of 0.00735 mg/kg. Endrin was also found in only one liver sample of a male tilapia fish.

The highest recorded pesticide residue in this group was dieldrin with 0.068 mg/kg from a black bass fish liver sample (appendix 2)

The organochlorine pesticide residues levels of individual samples are shown in appendix 2 to 7.

## 4.2 Levels of organochlorine pesticide residues in Sediment samples

Twenty four sediments samples were analyzed for organochlorine pesticides residues.

The results are given in table 6 (mean levels, standard deviation and ranges). The individual sample levels are given in appendix 8. The α -HCH, β-HCH, lindane, heptachlor epoxide, dieldrin and aldrin organochlorines pesticides were detected in the following order α -HCH 70.8%, β-HCH 37.5%, lindane 12.5%, heptachlor epoxide 4.1%, dieldrin 4.1%, aldrin 4.1%.

The mean levels were as follows: α -HCH 0.003 mg/kg, β-HCH 0.00315 mg/kg, lindane 0.00175 mg/kg. Heptachlor epoxide was detected in only one sample with a value of 0.0012 mg/kg. Dieldrin was found in only one sample with a value of 0.0033 mg/kg. Aldrin was also found in only one sample with a value of 0.0025 mg/kg.

### 4.3Toxicological results

The samples, showing presence of the organochlorine pesticide residues all were below the FAO/WHO maximum residue levels and acceptable daily intake (ADI) except one sample of black bass eggs having 0.022mg/kg α –HCH.

**Table 6: The MRL and ADI for the pesticide residues detected. Values (FAO/WHO codex Alimentarius commission 1986)**

| Compound | MRL ( mg/kg) | ADI (mg/kg) body weight |
|---|---|---|
| α -HCH | 0.2 | Not available |
| β -HCH | 0.2 | Not available |
| Lindane | 0.2 | 0.008mg/kg |
| Heptachlor | Not available | 0.0001mg/kg |
| Heptachlor epoxide | 0.01-0.5 | 0.0005mg/kg |
| Dieldrin | 0.2 | 0.0001mg/kg |
| Aldrin | 0.2 | 0.0001mg/kg |
| Endrin | Not available | 0.0002mg/kg |

**Table 7: Mean standard deviation (SD) and ranges of p... Naivasha.**

| Samples | | α - HCH | β -HCH | γ -HCH | Heptachlor | Heptachlor epoxide | Dieldrin | Endrin | Aldrin |
|---|---|---|---|---|---|---|---|---|---|
| Fish species | 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 |
| Tilapia | 36/37 | 0.0118 ±0.0143 (0.00126 - 0.0676) | 0.0069 ±0.0067 (0.00358 - 0.022) | 0.003 ±0.0016 (0.00122 -0.009) | 0.0027 ±0.0027 (0.0017 -0.014) | 0.0028 ±0.0048 (0.00179 -0.0219) | 0.004 ±0.0077 (0.00816 -0.0314) | 0.02* | |
| Black Bass | 42/45 | 0.0113 ±0.0064 (0.0015- 0.029) | 0.0053 ±0.0071 (0.0018- 0.0165) | 0.0001 ±0.0005 (0.0013- 0.00265) | 0.0015 ±0.0022 (0.00108- 0.0095) | 0.0012 ±0.0031 (0.0025- 0.015) | 0.0096 ±0.0196 (0.00334- 0.068) | | 0.00735* |

Residue levels are given on wet weight basis and Mean was calculated for positive samples only. *-Detected in only one sample.

Ranges are given in parentheses.

**Table 8: Mean, Standard Deviation (SD) and ranges of pesticide residue levels (mg/kg) in the different sexes of the two fish species (tilapia and black bass) two sexes sampled.**

| Sample | | α -HCH | β -HCH | γ -HCH | Heptachlor | Heptachlor epoxide | Dieldrin | Endrin | Aldrin |
|---|---|---|---|---|---|---|---|---|---|
| Sexes | 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 |
| Female | 29/30 | 0.0093 ±0.0068 (0.00225 - 0.025) | 0.0055 ±0.0069 (0.0013 - 0.01) | | 0.0012 ±0.00015 (0.0021 - 0.045) | 0.0008 ±0.0014 (0.00179 - 0.0219) | 0.0016 ±0.0052 (0.0023 - 0.034) | | 0.00735* |
| Male | 50/ 52 | 0.0127 ±0.0121 (0.00126 - 0.0676) | 0.0063 ±0.0069 (0.0013 - 0.026) | 0.0003 ±0.0014 (0.0013 - 0.014) | 0.0024 ±0.0029 (0.00108 - 0.014) | 0.0069 ±0.0067 (0.00358- - 0.022) | 0.0101 ±0.0184 (0.00334 - 0.068) | 0.02* | |



Residue levels are given on wet weight basis and Mean was calculated for positive samples only. * -Detected in only one sample.

Ranges are given in parentheses

**Table 9: Mean, standard deviation (SD) and ranges of pesticide residue levels (mg/kg) in the liver, muscle and eggs of the two species (tilapia and black bass).**

| Samples | | α -HCH | β -HCH | γ -HCH | Heptachlor | Heptachlor epoxide | Dieldrin | Endrin | Aldrin |
|---|---|---|---|---|---|---|---|---|---|
| Organs | 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 |
| Liver | 35/37 | 0.0116 ±0.0138 (0.00126 - 0.0676) | 0.0079 ±0.0088 (0.0018 - O.22) | 0.0005 ±0.0017 (0.00122 - 0.009) | 0.0028 ±0.0032 (0.00108 - 0.098) | 0.0026 ±0.0053 (0.0025 - 0.0219) | 0.0153 ±0.0205 (0.00334 - 0.068) | 0.02* | 0.00735* |
| Muscle | 34/36 | 0.0116 ±0.0073 (0.0025 - 0.031) | 0.0042 ±0.0042 (0.0013 - 0.013) | | 0.0014 ±0.0016 (0.0017 - 0.014) | 0.0015 ±0.0027 (0.00188 -0 0128) | 0.0005 ±0.0019 (0.0029 - 0.0035) | | |
| Eggs | 9/9 | 0.0111 ±0.0074 (0.00136 - 0.022) | 0.0055 ±0.0045 (0.0044 - 0.01) | | 0.0016 ±0.0017 (0.0016 - 0.0045) | 0.005 ±0.009 (0.00208 - 0.0022) | 0.003 ±0.008 (0.0016-0.018) | | |

Residue levels are given on wet weight basis and Mean was calculated for positive samples only. *-Detected in only one sample. Ranges are given in parentheses

**Table 10: Mean and standard deviation (SD) of pesticide residue levels (mg/kg) in Lake Naivasha sediment**
**Pesticide residue (mg/kg)**

| Sample | | α -HCH | β -HCH | γ - HCH | Heptachlor epoxide | Dieldrin | Aldrin |
|---|---|---|---|---|---|---|---|
| | Positive | Mean ±SD Range | Mean ±SD Range | Mean ±SD Range | Mean ±SD Range | Mean ±SD Range | Mean ±SD Range |
| Sediment | 1 7/24 | 0.00316 ±0.021 (0.0016 - 0.007) | 0.003 ±0.018 (0.0013 - 0.00375) | 0.00187 ±0.0062 (0.0013 - 0.0023) | 0.0012* | 0.0033* | 00025* |

Residue levels are given on wet weight basis and Mean was calculated for positive samples only. *-Detected in only one sample.

Ranges are given in parentheses

## CHAPTER FIVE

### 5.0 Discussion

The statistical analysis showed that there was no significant difference ($p>0.05$) between the two species of fish on the pesticide residues detected .Black bass fish is carnivorous feeding on small fish and other small aquatic life forms while tilapia fish is herbivorous in nature.

Therefore despite the difference in their diets there is no difference to the bioaccumulation of the pesticide residues in the fish tissues. The biomagnification is not noticed from the species probably because of heavy fishing in the lake where fish are caught while young before they bioaccumulate a lot of pesticides residues.

An inventory study of the pesticides in use around the lake implicated use of hexachlorocyclohexanes and cyclodiene group of pesticides (Werimo *et al.*, 2000). This agrees with findings of this study.

The pesticides uptake in fish is either through bioconcentration from water through gills or epithelial tissues and through bioaccumulation through water and through food leading to eventual biomagnification in different organisms, occupying successive trophic levels (Murty 1986). The result suggests the means of uptake to be bioconcentration considering that there was no difference despite the two species different diets.

The statistical analysis of the organochlorine pesticides residues detected in the two fish species(black bass and tilapia) organs and sediment show there were no significant differences ($P>0.0\ 5$) apart from α -HCH where there was a significant difference ($p=0.035<0.05$). The organochlorines pesticides in water are absorbed by particulate matter and sedimented to the bottom of such bodies on dams and lakes (Murty 1986).This could explain the difference as

regards α –HCH, however in this study the levels of pesticides residues found in sediments are lower than those in fish samples probably because Lake Naivasha is not static and there could be movement of these chemicals within the lake ecosystem.

### 5.1 Organochlorines contaminants

#### 5.1.1 Hexachlorocyclohexanes group

The commercial insecticide HCH is a mixture of different isomers mainly α, β and γ-HCH (Lindane). Other isomers in the group are delta and epsilon. Lindane (γ -HCH) has been used as an insecticide and is the most toxic. β -HCH is the most symmetric and stable isomer; it is also the most persistent in nature. β -HCH is eliminated five times more slowly from the body than other isomers and has a much higher ability to accumulate in the fat tissue than lindane (Howard 1991) The three isomers α, β and γ -HCH were detected in the study in that order of frequency and were the ones mostly encountered in the analysis of the pesticides residues, which show they are in use in the surrounding of the lake despite being banned in Kenya. However lindane use is restricted for use as seed dressing only. Lindane is highly volatile, and, when applied to field crops in particular a high proportion (up to 90%) of the pesticide enters the atmosphere and is later deposited by rain (pesticide trust 1995). Lindane is also rapidly degraded in the environment (Howard 1991) this explains the low levels of lindane in the analysis of the pesticide residues. However in an inventory of the pesticides done to establish the status of biological and chemical contaminants in use along the lake Naivasha catchment area the three isomers were indicated to be in use (Werimo *et al*, 2000).

Other studies (Mugachia *et al.*, 1992) and (Lincer *et al.*, 1981) detected little organochlorines pesticide residues in fish from lake Naivasha and the reason is probably because intensive farming had not started then, but this study shows a markedly increase in the levels of pesticide residues though still below the MRLs.

**5.1.2 Cyclodiene group**

This group includes aldrin, dieldrin, endrin, heptachlor and heptachlor epoxide. Aldrin and heptachlor are rapidly metabolized by organisms but their metabolites (dieldrin and heptachlor epoxide) respectively are as toxic and persistent as the parent material (Murty, 1986).

The pesticides in the group were banned in Kenya in 1986 with only aldrin and dieldrin restricted for use in termite control in the building industry. The cyclodienes detected in fish and sediment samples could be originating from discharges from domestic sewage since the Naivasha Municipal Council does not have a functional sewage system.

Again in the inventory of pesticides in use within Lake Naivasha indicated they were in use (Werimo *et al*, 2000) and this explains their presence and their identification in the samples. Heptachlor has a half-life of approximately six months in water and sediment whereas its derivative breaks down in about three to seven years (Howard 1991). Therefore the presence of heptachlor pesticide residue shows that the pesticide was in current use (appendixes2-8) at the time of sampling.

**5.4.3 DDT group**

The DDT groups of pesticides were not detected in the present study and were also not detected in inventory of the pesticides done to establish the status of biological and chemical

contaminants in use along the lake Naivasha catchment (Werimo *et al*, 2000). They are highly persistent in nature and their absence in the analysis shows that they have not been in use within the time of sampling.

**5.5 Maximum residue limits (MRL) and Acceptable Daily intake (ADI)**

The two terms are used in evaluation of the toxicological significance and pesticide residue data in food. The 'MRL' is the maximum concentration of a pesticide residue (expressed as mg/kg) recommended by the codex alimentarius commission to be legally permitted in food commodities and animals feeds. 'ADI' of a chemical is the daily intake that during an entire lifetime, appear to be without appreciable risk to the health of the consumer on the basis of all the known facts at the time of the evaluation of the chemical by joint FAO/WHO meeting on pesticide residues. It is given in mg/kg body weight (codex Alimentarius commission 1993).

**5.6 Toxicological significance of the results**

The pesticide residues levels detected in both fish and sediment samples were below maximum residue limits and acceptable daily intake and therefore toxicologically the residue levels found are low and therefore does not pose ecological environmental or health hazard at present. The presence of organochlorine pesticide residues however indicates the need for continuous monitoring of the Lake fish population to safeguard the health of the consumers. The data generated in this study will also enhance the status of fish industry in Kenya by demonstrating the quality of fish in Lake Naivasha as compared to fish from other water bodies.

### 5.7 Comparison of health results with others in different studies

The earliest study was done by Lincer *et al.*, (1981) on the lake Naivasha he 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.

Mugachia *et al.*, 1992 carried out an extensive study of organochlorine pesticide residues in fish from Lake Naivasha and Tana River. Forty fish samples were analyzed from Lake Naivasha and no individual sample had detectable levels of pesticide residues. A composite fillet sample of 10 fish had α -HCH at a concentration of 0.014mg/kg. The single value compares very well with the concentrations in the present study where the mean concentration of α -HCH was 0.118mg/kg.

Other organochlorines pesticides residues studies mostly in other countries have reported higher concentrations. Sum DDT values of 0.07 to 0.38mg/kg were reported in three species of fish from Lake Tanganyika (Deelstra *et al.*, 1976). In Jordan, in 1971, the sum ranged from 0.37 to 3.34mg/kg (Murty, 1986). Zorgani *et al.*, (1979) and Zorgan (1980) reported sum DDT of 0.0022 to 0.184mg/kg in different species of fish from other areas in Sudan. Sum DDT levels ranging from undetectable to 92mg/kg and 90 to 135mg/kg on wet basis have been reported in fish from the United States of America and Norway respectively. (Murty, 1986) .Generally organochlorine pesticide residue levels in the developed countries were higher because they were in use longer in those countries before they were banned.

## CHAPTER SIX

### 6.0 Conclusions and recommendations

### 6.1Conclusions

1. Lake Naivasha is contaminated with low levels of hexachlorocyclohexanes and cyclodiene organochlorines residues.
2. The organochlorines pesticides residues were found to be similarly distributed in the three fish organs, liver, muscle and the eggs.
3. There was no difference in the organochlorines distribution between the two species of fish (Black bass and Tilapia).
4. Fish from Lake Naivasha contributes to dietary intake of organochlorine pesticide by human beings since the residues were evidently found in their muscle, which are normally consumed.

### 6.2Recomendations

1. Continuous monitoring procedures need to be put into place for environmental safeguard.

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

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## APPENDICES

**APPENDIX I:**FORM USED DURING COLLECTION OF SAMPLES IN LAKE NAIVASHA

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DATE _____

**SAMPLE COLLECTION FORM**

TYPE OF SAMPLE _____

| Sample no. | Date | Site of collection | Species | Sex | Any other information |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |

**APPENDIX 2: Pesticide residue levels (mg/kg) in livers of black bass fish**

| SAMPLE N0 | α -HCH | β -HCH | γ -HCH | HEPTA-HLOR | HEPTA-CHLORE | DIELDRIN | ALDRIN | ENDR IN |
|---|---|---|---|---|---|---|---|---|
| BM5L | 0.011 | 0.0046 | | 0.00207 | 0.0091 | 0.031 | | |
| BF7L | 0.007 | | | | | 0.021 | | |
| BF8L | 0.0064 | 0.0065 | | | | 0.018 | | |
| BM9L | 0.011 | | | | | 0.00334 | | |
| BF1OL | 0.0015 | 0.0029 | | | | 0.004 | | |
| BM12L | 0.015 | 0.00585 | | 0.0039 | | 0.068 | | |
| BFIIL | 0.01 | | | | | 0.034 | | |
| BM15L | 0.014 | 0.0054 | | 0.00288 | | 0.066 | | |
| BM16L | 0.013 | 0.0165 | | 0.0078 | | 0.049 | | |
| BF14L | 0.0089 | 0.0058 | | | | | 0.00735 | |
| BM17L | 0.012 | | | | | 0.018 | | |
| BMI9L | 0.014 | | | | | | | |
| BF2OL | 0.0028 | | | | | | | |
| BM18L | 0.0097 | | | | | | | |
| BM2L | 0.0135 | 0.0206 | 0.0022 | 0.0095 | 0.015 | 0.07 | | |
| BMIL | 0.00289 | 0.00476 | 00.0013 | 0.00458 | 0.0086 | 0.0324 | | |
| BM3L | 0.00348 | 0.0139 | 0.00265 | 0.0033 | 0.00802 | 0.0112 | | |
| BM4L | 0.00488 | 0.0018 | | 0.00108 | | 0.036 | | |
| BM6L | 0.0095 | 0.0036 | | 0.001 08 | 0.0025 | | | |

**APPENDIX 3: Pesticide Residue Levels (mg/kg) in Eggs of Black Bass Fish**

| Sample no. | α -HCH | β -HCH | γ -HCH | Heptachlor | Heptachlor E. | Dieldrin | Aldrin | Endrin |
|---|---|---|---|---|---|---|---|---|
| BF8E | 0.0067 | 0.01 | - | - | | | | |
| BF7E | 0.0085 | | | 0.0016 | | | | |
| BEIIE | 0.018 | 0.0086 | | 0.0037 | | | | |
| BFI3E | 0.0027 | 0.0044 | | | | | | |
| BFI4E | 0.022 | | | | | | | |

**APPENDIX 4: Pesticide Residue Levels (mg/kg) in Muscle of Black Bass Fish**

| Sample | α -HCH | β -HCH | γ HCH | heptachlor | Heptachlor epoxide | Dieldrin | Aldrin | Endrin |
|---|---|---|---|---|---|---|---|---|
| BM6M | 0.021 | 0.013 | | 0.00297 | 0.0042 | | | |
| BF8M | 0.014 | 0.0087 | | 0.0027 | | | | |
| BF7M | 0.00225 | | | | | | | |
| BM9M | 0.00875 | | | | | | | |
| BFIOM | 0.015 | 0.0014 | | | | | | |
| BM12M | 0.0084 | 0.0049 | | | | | | |
| BF1IM | 0.025 | 0.0078 | | 0.003 | 0.0019 | | | |
| BFI3M | 0.011 | 0.0013 | | | | | | |
| BMI8M | 0.0197 | | | | | | | |
| BF14M | 0.019 | 0.00318 | | 0.0028 | 0.0035 | | | |
| BFI6M | 0.029 | | | | | 0.0029 | | |
| BMI5M | 0.0079 | | | | | | | |
| BM17M<br>BM19M | 0.012<br>0.016 | | | | | | | |
| BM18M | 0.0038 | | | | | | | |
| BM2M | 0.0071 | 0.00285 | | 0.004 | | | | |
| BM5M | 0.0095 | 0.0036 | | 0.00108 | 0.0025 | | | |

**APPENDIX 5: Pesticide residue levels (mg/kg) (mg/kg) in muscles of tilapia fish**

| Sample | α -HCH | β -HCH | γ-HCH | Heptachlor | Heptachlor epoxide | Dieldrin | Aldrin | Endrin |
|---|---|---|---|---|---|---|---|---|
| TM12M | 0.009 | - | | - | - | _ | _ | _ |
| TM13M | 0.014 | 0.0052 | | 0.0023 | _ | _ | _ | _ |
| TM14M | 0.00625 | - | | _ | _ | _ | _ | _ |
| TM15M | 0.019 | - | | _ | _ | _ | _ | _ |
| TM2M | 0.031 | 0.00625 | | _ | 0.0032 | _ | _ | _ |
| TM4M | 0.0065 | 0.00869 | | 0.00259 | 0.00546 | _ | _ | _ |
| TM5M | 0.00543 | 0.00846 | | 0.0017 | 0.0037 | _ | _ | _ |
| TM8M | 0.00695 | 0.012 | | 0.00517 | 0.0052 | 0.0035 | _ | _ |
| TF9M | 0.005 | 0.0092 | | 0.0038 | 0.0045 | _ | _ | _ |
| TM10M | 0.0046 | 0.0089 | | 0.00297 | 0.0128 | _ | _ | _ |
| TM11M | 0.00756 | 0.0045 | | - | _ | _ | _ | _ |
| TM1M | 0.00583 | 0.00387 | | - | _ | _ | _ | _ |
| TM6M | 0.00413 | 0.00538 | | 0.0024 | 0.0022 | _ | _ | _ |
| TF7M | 0.0074 | 0.0046 | | 0.0024 | 0.00446 | _ | _ | _ |

**APPENDIX 6: pesticide residue levels (mg/kg) in eggs of tilapia fish**

| Sample | α -HCH | β -HCH | γ-HCH | Heptachlor | Heptachlor epoxide | Dieldrin | Aldrin | Endrin |
|---|---|---|---|---|---|---|---|---|
| TFIE | 0.0136 | 0.006 | – | 0.0021 | 0.00208 | – | – | – |
| TF7E | 0.0028 | 0.01 | – | 0.0045 | 0.0022 | 0.0023 | – | – |
| TF9E | 0.0059 | 0.01 | – | 0.0022 | | – | – | – |

**APPENDIX 7: Pesticide residue levels (mg/kg) in livers of tilapia fish**

| Sample | α-HCH | β-HCH | γ -HCH | Heptachlor | Heptachlor epoxide | Dieldrin | Aldrin | Endrin |
|---|---|---|---|---|---|---|---|---|
| TM12L | 0.012 | 0.012 | _ | 0.00525 | _ | 0.019 | _ | _ |
| TM13L | 0.054 | 0.22 | _ | 0.0098 | _ | 0.0196 | _ | _ |
| TM14L | 0.014 | _ | _ | _ | _ | _ | _ | _ |
| TM17L | 0.0077 | _ | _ | _ | _ | _ | _ | _ |
| TM18L | 0.036 | 0.009 | _ | 0.0056 | 0.0035 | 0.0215 | _ | _ |
| TM3L | 0.0676 | 0.0152 | _ | 0.0061 | 0.0074 | | _ | _ |
| TM7L | 0.00289 | 0.0114 | _ | 0.00208 | 0.00259 | 0.0106 | _ | _ |
| TM5L | 0.00162 | 0.00565 | 0.00122 | 0.00288 | _ | _ | _ | _ |
| TM8L | - | 0.0233 | _ | 0.0085 | 0.0149 | 0.0089 | _ | _ |
| TM6L | 0.00287 | 0.00646 | _ | 0.0069 | 0.00179 | 0.0314 | _ | _ |
| TM4L | 0.00126 | 0.00358 | _ | 0.0024 | _ | 0.00816 | _ | _ |
| TM9L | 0.0025 | _ | _ | 0.0017 | _ | _ | _ | _ |
| TTMM11L | 0.0053 | 0.007 | _ | _ | _ | _ | _ | _ |
| TM10L | 0.013 | 0.26 | _ | 0.0084 | 0.0219 | _ | _ | _ |

**APPENDIX 8: Pesticide Residue Levels (mg/kg) in the Lake Sediment**

| Sample | α-HCH | β -HCH | γ -HCH | Heptachlor | Heptachlor epoxide | Dieldrin | Aldrin |
|---|---|---|---|---|---|---|---|
| SMP/M-2 | 0.0016 | _ | _ | _ | _ | _ | _ |
| SNP5M-1 | 0.0031 | _ | _ | _ | _ | _ | _ |
| SFFP-5M-1 | 0.0026 | _ | _ | _ | _ | _ | _ |
| SMP5M-1 | 0.00298 | 0.0013 | + | _ | _ | _ | _ |
| SNSPIM-1 | 0.0028 | _ | _ | _ | _ | _ | _ |
| SSPIM-2 | 0.00296 | _ | 0.0023 | _ | 0.0012 | _ | 0.0025 |
| SMP5M-2 | 0.0018 | _ | 0.0013 | _ | _ | _ | _ |
| SLCIM-1 | 0.0057 | _ | _ | _ | _ | 0.0033 | _ |
| SLCIM-1 | 0.0048 | 0.0032 | 0.0023 | _ | _ | _ | _ |
| SFFP5M-2 | 0.0054 | _ | 0.0019 | _ | _ | _ | _ |
| SSP5M-1 | 0.0031 | _ | 0.0021 | _ | _ | _ | _ |
| SFFP5M-2 | 0.0028 | _ | _ | _ | _ | - | _ |
| SSP5M-1 | 0.0028 | _ | _ | _ | _ | _ | _ |
| SMUP10-2 | 0.0034 | _ | 0.0013 | _ | _ | _ | _ |
| SMUP10-1 | _ | 0/00375 | _ | _ | _ | _ | |
| SMP10-1 | _ | 0.00375 | _ | _ | _ | _ | |
| SFFIM-2 | 0.0016 | - | _ | _ | _ | _ | |