DETERMINATION OF ORGANOCHLORINE PESTICIDES IN CATTLE LIVER OBTAINED FROM ZANGO, ZARIA-NIGERIA.

BY

Oluwagbenga ANIFOWOSE, B.Sc. Chemistry (B.S.U.)
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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTERS DEGREE IN CHEMISTRY.

DEPARTMENT OF CHEMISTRY,
FACULTY OF SCIENCE
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NIGERIA

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DECLARATION

I declare that the work in this dissertation entitled "Determination of Organochlorine Pesticides in cattle liver obtained from Zango, Zaria-Nigeria" was carried out by me in the Department of Chemistry. The information derived from the literature has been duly acknowledged in the text and in the list of references provided. No part of this thesis was previously presented for another degree in this or any other institution.

Oluwagbenga ANIFOWOSE	----------	----------
Name of Student	**Signature**	**Date**

CERTIFICATION

This dissertation entitled "DETERMINATION OF ORGANOCHLORINE PESTICIDES IN CATTLE LIVER OBTAINED FROM ZANGO, ZARIA-NIGERIA" by OLUWAGBENGA ANIFOWOSE meets the regulations governing the award of Masters degree in Analytical Chemistry of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

Prof. C.E. Gimba		
Chairman, Supervisory Committee	Signature	Date
Prof. G.I. Ndukwe		
Member, Supervisory Committee	Signature	Date
Prof. V.O. Ajibola		
Head of Department	Signature	Date
Prof. A.Z. Hassan		
Dean, School of Postgraduate Studies	Signature	Date

DEDICATION

This work is dedicated to God Almighty, the author and the finisher of my faith.

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ABSTRACT

The presence of Organochlorine pesticide residues in nine (9) Liver samples collected from slaughter house in Zango, Zaria-Nigeria was determined. All samples were analyzed for the residual contents of α-Benzenehexachloride, β-Benzenehexachloride, Lindane(γ-Benzenehexachloride), endrin, Aldrin, chlorothalonil, δ-Benzenehexachloride, heptachlor, heptachlor epoxide, endosulfan, dieldrin, endosulfan II, p,p'-Dichlorodiphenyldichloroethane, endosulfan sulfate, p.p'-Dichlorodiphenyltrichloroethane, lambda cyhalothrin and permethrin by Gas Chromatography with Electron Capture Detector (GC-ECD). Sample extraction was carried out by warm soxhlet extraction followed by column clean-up with Florisil. The range of concentration was α-BHC 0.02 – 0.21 mg/kg, β-BHC 0.09 – 2.57 mg/kg, Lindane 0.06 – 0.24 mg/kg, chlorothalonil 0.05 – 0.32 mg/kg, δ-BHC 0.07 – 0.90 mg/kg, heptachlor 0.13 – 1.16 mg/kg, Aldrin 0.27 – 0.61 mg/kg, heptachlor epoxide 0.13 – 1.16 mg/kg, endosulfan 0.02 –3.79 mg/kg, dieldrin 0.12 – 1.72 mg/kg, endrin 0.04 – 7.88 mg/kg, endosulfan II 0.12 – 1.56 mg/kg, p,p'-DDD 0.14 – 0.73 mg/kg, endosulfan sulfate 0.12 – 1.56 mg/kg, p.p'-DDT 0.12 – 1.20 mg/kg, lambda cyhalothrin 0.07- 0.37 mg/kg and permethrin 0.02 – 0.28 mg/kg. All the samples analyzed were found to contain these OCPs with higher concentrations observed in cattle above 24 months. Higher concentration of DDT than DDD suggests a more recent usage of the pesticides and calls for strict and effective measures to stop the sale and usage of the pesticides. This study has provided the preliminary information on the concentration of OCPs in liver of cattle for the first time in Zaria. The result will help in a scientific assessment of the implications of these pesticide residues with regards to human risk in Zaria.

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ABBREVIATIONS

OCP- Organochlorine Pesticides

FAO- Food and Agricultural Organization

WHO- World Health Organization

NAFDAC- National Agency for Food and Drug Administration and Control

DDT- Dichlorodiphenyltrichloroethane

DDD-dichlorodiphenyldichloroethane

HCB- Hexachlorobenzene

BHC-Benzenehexachloride

HCH- Hexachlorohexane

COP- Conference of Parties

UNEP-United Nations Environment Programme

POPs- Persistent Organic Pollutants

PCBs- Polychlorinated biphenyls

S.D.- Standard Deviation

CHAPTER ONE

1.0 INTRODUCTION

Organochlorine pesticides (OCPs) are synthetic organochlorines which are lipophilic and hydrophobic. Their lipophilicity, hydrophobicity, stability to oxidation, low vapour pressure and low chemical and biological degradation rates have led to their accumulation in biological tissues and the subsequent magnification of concentrations in organisms, progressing through to the food chain (Helberg *et al*., 2005). They can be recycled through food chains and produce a significant magnification of the original concentration at the end of the chain (Doong *et al*., 2002). They are resistant to natural breakdown processes and are extremely stable and persistent, highly toxic and bioaccumulate in the fatty tissues of animals and humans (Forget *et al*., 2001). Bioaccumulation is the ability of a pollutant to accumulate in living tissues at levels higher than those in the surrounding environment. When chemical pollutant enters into the body of an organism and is not excreted but rather collected in the organism's tissues, bioaccumulation sets in (Zweig *et al*., 1999).

Persistent organic pollutants (POPs) are ubiquitous contaminants and have been detected far from their sources of origin because of long-range transport stemming from atmospheric exchange, water currents, animal migration and other pathways (Zhang *et al*., 2007). Efforts at minimizing and eventually phasing out POPs globally gave rise to the Stockholm Convention in 2001. OCPs namely aldrin, dieldrin, endrin, chlordane, dichlorodiphenyltrichloroethane (DDT), heptachlor, mirex, toxaphene, hexachlorobenzene (HCB) and industrial chemicals and byproducts, including polychlorinated biphenyls (PCBs), dioxins and furans, constitute the twelve chemical substances called the "dirty dozen" and defined under the Stockholm Convention. However, at its fourth meeting held in 2009, the Conference of the Parties (COP) adopted the amendments to annexes A (elimination), B (restriction) and C (unintentional

production) of the Stockholm Convention to list nine additional chemicals as persistent organic pollutants, resulting in the "dirty twenty one". The nine additional chemicals include chlordecone, alpha hexachlorocyclohexane, beta hexachlorocyclohexane, lindane, pentachlorobenzene, octabromodiphenyl ether, pentabromodiphenyl ether, perfluorooctane sulfonic acid and perfluorooctane sulfonyl fluoride. Residues and metabolites of many POPs are very stable, with long half-lives in the environment (UNEP, 2002).

The manufacture and use of chlorinated pesticides have been banned or restricted in developed countries. Although these bans and restrictions were enacted during the 1970s and 1980s, some developing countries are still using OCPs for agricultural and public health purposes because of their low cost and versatility in controlling various pests (Xue *et al*., 2006). Again, they are being used in most developing countries, including Nigeria, due to a lack of appropriate regulatory control and management of the production, trade and use of these chemicals (Darko and Acquaah, 2007). There is also a paucity of data on the use of pesticides in the country, a reflection of the lack of a mechanism and planning programme in place for chemicals management as well as a low level of understanding of the environmental and public health hazards of pesticide use (Osibanjo *et al*., 2002).

Pesticides are chemicals used to kill or control pests. They are classified according to their chemical class or intended use (function). According to functions, we have acaricides, algicides, antifeedants, avicides, bactericides, repellants (fumigants), fungicides, chemosterilants, herbicides, insecticides, molluscides, nematicides, plant activators, growth regulators, Rodenticides and virucides (Nick, 2012). Chemical classification may vary from organochlorinates to organophosphates, polybiphenyls, carbamates, benzophenones, bipyridilium, carbazates, dicarboximides, fluorine rich pesticides etc. OCPs are basically insecticides composed primarily of carbon, hydrogen and chlorine and are classified thus;

Hexachlorobenzenes (which include alpha, beta, delta and gamma(Lindane) HCBs), Cyclodienes (which include Aldrin, Eldrin, Endrin, cis-Chlordane, trans-Chlordane, Heptachlor etc.), Endosulfan (alpha- and beta-endosulfan), Diphenyl-Aliphatics (o,p'-DDT, p,p'-DDT, o,p'-DDD, p,p'-DDD, o,p'-DDE, p,p'-DDE) etc.

They are found in the environment as a result of human activities. Insecticides act by poisoning the nervous system of target harmful insects. The use of OCPs takes many forms, ranging from pellet application in field crops to sprays for seed coating and grain storage. OCP residues enter vegetative environments through effluent release, discharges of domestic sewage and industrial wastewater, atmospheric deposition, runoff from agricultural fields, leaching, equipment washing, and disposal of empty containers and direct dumping of wastes into the vegetation systems (Yang *et al*., 2005). The distribution of various chlorinated contaminants in the land and vegetative environment depends on the physicochemical properties of the ecosystem as well as the partition coefficients of individual chlorinated hydrocarbons (Sarkar *et al*., 1997). OCPs could distribute among the components of the ecosystem, such as water and sediment, and accumulate in the biota. As a result of their persistence, OCPs in water can be transferred into the food chain and accumulate in aquatic organisms like plankton. Different pesticides pose varying degrees and types of risk to water quality. It is reported that approximately three million people are poisoned and two hundred thousand die each year around the world from pesticide poisoning, the majority of them from the developing countries (FAO, 2000). It is also believed that the incidence of pesticide poisoning in developing countries may be greater than reported due to under-reporting, lack of data and misdiagnosis (Cocco *et al*., 1997). Some of the symptoms of pesticides poisoning include irritation, dizziness, tremor, tonic and chronic convulsion (Winter, 1992). OCPs have been linked to human breast and liver cancers, testicular tumours and lower sperm counts in humans (Davis and Bradlow, 1995; Cocco *et al*., 1997).

Studies have also suggested that OCPs may affect the normal function of the endocrine system of humans and wildlife (Xue *et al*., 2006). OCPs are among the most commonly detected pesticides around the world. Although most of them were banned in the 1970s and 1980s, they can still be found in the environment in several matrices such as water, soil, marine sediments and thus animal tissues (Sarkar *et al*., 2008; Guan *et al*., 2009; Essumang *et al*., 2009**)**.

Pesticides can be bioconcentrated through biogeochemical processes and can be scavenged from the water through sorption onto suspended material before they get deposited to the bottom substrate (Xue *et al.*, 2006). The sediment component of aquatic ecosystem deposits pesticides. Sediment is one of the principal reservoirs of environmental pesticides, representing a source from which residues can be released to the atmosphere, groundwater and living organisms (Xue *et al*., 2006). Persistence of these organic pollutants in sediment is possible due to their low solubilities and tendency to associate with suspended particulate matter. As a result of their low water solubility, OCPs have a strong affinity for particulate matter. They are hydrophobic compounds that tend to adsorb to suspended particulate matter and benthic sediments in aquatic ecosystems (Ademoroti, 1996). Sediments serve as ultimate sinks for them. Indirect exposure to contaminated sediments takes place when fishes feed on benthic invertebrates that are ingesting particulate matter. Direct exposure through the sediment takes place by release of contaminated particulate matter into the water column by both natural and anthropogenic processes.

1.1 Background of the Study

The contamination of the environment, food and animal feed by chlorinated organic pesticides has become a topical issue of considerable concern in many parts of the world, and has led many researchers to investigate their occurrence, distribution and concentrations in several ecosystems (Sankar *et al*., 2006; Yang *et al*., 2007; Poolpak *et al*., 2008). The toxicity of pesticides varies greatly with their intrinsic properties, the species being studied and factors in

the environment. Important factors that influence the impact of a pesticide on the aquatic environment are its persistence, the partitioning of the pesticide between the particulate and aqueous phases, toxicity to aquatic organisms and their tendency to bioaccumulate. All pesticides are toxic to some forms of life. Many modern pesticides are developed to be as selective against target organisms as possible, but it is rarely possible to achieve perfect control of one organism without the wider environment being exposed and susceptible non-target species being affected. Anthropogenic activities provide the primary point source of chlorinated hydrocarbon input into the aquatic environment (Malik *et al*., 2008).

OCPs enter the environment by deliberate application or by accident. These substances are sometimes applied directly to animals, vegetation or water bodies to control pests. OCPs have been extensively used for agriculture and vector control purposes in Nigeria. The pesticides applied on land eventually find their way to the aquatic environment, thus contaminating it. Being lipophilic, OCPs can be concentrated to harmful levels in the environment through bioaccumulation and bio magnification (Toft *et al*., 2003). Consequently, aquatic organisms that are commercially exploited for human food may pose a risk to man. It has also been recognized that the persistence and bioaccumulative tendency of these substances, their metabolites and residues in the environment make them not to remain where they are applied but to be partitioned between the major environmental compartments in accordance with their physicochemical properties. Such environmental distributions may lead to the exposure of living organisms, including man, that are far removed from intended targets.

OCPs are among the first set of pesticides to be used in Nigeria (Amakwe, 1984). They are still in use in Nigeria. Despite the difficulties associated with the analysis of organochlorine compounds, especially at the low levels normally found in tissue samples, there is evidence that they are major long-term contaminants of the environment (Yang *et al*., 2005; Malik *et al*.,

2008). Concern about exposure to organochlorines among humans has arisen mainly because of the proven carcinogenicity of these chemicals in experiments with animals and their ubiquity, bioconcentration and persistence in human tissues (Skaare *et al*., 2000). Many POPs, which pollute the environment, become incorporated into food webs. Humans, being the final links in the food chain, are the most affected. Consequently, the general public has become increasingly concerned about the potential risk to human health from the consumption of such polluted biota. The ill effects of pesticides may arise from short- or long-term and low- or high-level exposure through dermal absorption, inhalation and oral ingestion.

The toxicity of pesticides could be acute and chronic. There is growing evidence of cancer, neurological damage, endocrine disruption and birth defects arising from exposure (IARC, 2001). In view of the negative health effects of OCPs on humans and the nonexistence of a government policy on the regulation and monitoring of OCP levels in fishes in Nigeria, it is very important to evaluate the levels of chlorinated pollutants in commercially available cattle liver. Persistent organic pollutant levels are usually monitored in inorganic ecosystem compartments such as water, air and sediment or in biota. Monitoring in inorganic compartments has the advantage of producing an immediate, geographically localized measure of contamination, while biological monitoring provides information on the extent of biotransformation and bioaccumulation processes that the contaminants have undergone during their passage through biological systems. Biological monitoring, therefore, provides a more realistic view of the contaminant distribution in the environment site (Rissato *et al*., 2006).

1.2 Statement of the Problem

The occurrence of organochlorine pesticides in biological species even at trace levels is not desirable as they have toxic effects. Cattle meat and liver as good source of protein are at the top of the food chain and do bioaccumulate these OCP residues, leading to many diseases like

Cancer, Endocrine disruption, Immune system dysfunction, Parkinson's disease, Cytotoxic defects, Child development disorder and Diabetes among others in man.

1.3 Justification for the Study

Pesticides are known to be toxic to man (Ademoroti, 1996). The use of pesticides introduces some risks to the environment, the degree of risk depending upon the pesticide persistence, mobility, non-target toxicity and volume of use. The toxicity level of a pesticide also depends on the deadliness of the chemical, the length of exposure, the health status of the recipient and the route of entry into the body. OCPs contribute to many acute and chronic health effects, including cancer, neurological damage, birth defects, tremors, headache, dermal irritation, respiratory problems and dizziness (Garry, 1996; Mathur *et al.*, 2002; Toppari, 2002). Animal studies by Garry (1996) have also shown the potential for reproductive and developmental effects and disruption of normal hormone function. Long-term exposure to sub-lethal levels of OCPs and their metabolites through various pathways in the aquatic environment may cause far reaching ecological damage and health problems to man. Residues of these toxic chemicals found in water, sediments and aquatic biota pose a risk to aquatic organisms, predators and humans.

Cattle meat and liver are suitable indicators for environmental pollution monitoring because they concentrate pollutants in their tissues directly from their diet, thus enabling the assessment and transfer of pollutants through the trophic web (Fisk *et al*., 2001; Lanfranchi *et al*., 2006). The low activity of the mono-oxygenase enzymes in cattle liver and tissue limits their ability to metabolize organochlorines (Dearthand Hites, 1991). Hence, animal tissues generally reflect the levels of organochlorine pollution in their diet (Muir *et al*., 1990). This offers the opportunity to study the influence of environmental and biological factors on the bioaccumulation of pollutants (Porte and Albaiges, 1993; Sarkar *et al*., 2008). Besides analyzing

OCPs in the muscle tissues of cattle, it is vital to investigate their distribution in organs which could provide more information about the pathways along which bioaccumulation occurs, and thus reflect the environmental conditions. This is because they do possess pronounced ability to concentrate persistent organic pollutants from their diet (Yang *et al*., 2006; Zhou *et al*., 2008).

It has been reported that the consumption of contaminated liver, meat and fishes is one of the important pathways of human exposure to OCPs (Mwevura *et al*., 2002; Zhou *et al*., 2007; Muralidharan *et al*., 2008). Indeed, studies have related the presence of organochlorine residues in breast milk to the consumption of contaminated fishes and cattle parts (Fitzgerald *et al*., 2001). Data on the presence and distribution of OCPs in cattle liver are, therefore, important from the ecological and human health perspectives. The transport, dispersion and the ultimate effects of pesticides in marine systems depend upon their persistence, bioaccumulation and biodegradation. OCPs could be associated with organic components of soils, biological tissues and dissolved organic carbon in living systems (Xue *et al*., 2006; Vagi *et al*., 2007; Imo *et al*., 2007; Zhou *et al*., 2007). The indiscriminate use of pesticides in Nigeria has resulted in the occurrence of the residues in biota and other abiotic compartments (Osibanjo and Bamgbose, 1990; Ize-Iyamu *et al*., 2007; Adeyemi *et al*., 2008). It is necessary to ascertain the distribution, behaviour and fate of these compounds in various animal compartments. The determination of POPs existing in biota could indicate the extent of contamination and the accumulation characteristics.

There is need for continuous monitoring to identify the occurrence and the levels of OCPs in fishes, water and sediment of aquatic ecosystems in Nigeria as literature data on the concentrations of OCP residues in the Nigerian environment are inadequate. Most institutions and researchers in Nigeria lack the analytical facilities/equipment for the detection and quantitative analysis of OCPs in environmental samples. Lack of adequately trained experts in trace organic analysis, as well as funds for the purchase of chemicals, are some of the main

limiting factors for conducting research and monitoring OCP residues in biota and other media in the environment. Data gaps have thus been identified. There are gaps in evaluating the accumulation of OCPs in the organs of cattle and the daily intake of pesticide residues which the present study aims to bridge. This research serves to provide data on the prevailing levels of these persistent pollutants in cattle liver obtained in Zaria metropolis.

1.4 Aim of the Study

This research was aimed at investigating the occurrence, concentration and distribution of organochlorine pesticides in cattle liver obtained in Zaria, Kaduna state with a view to assessing the current state of contamination and exposure to these toxicants.

1.5 Objectives of the Study

The research was designed to achieve the following objectives:

i. Determination of the physicochemical properties of liver of cattle.
ii. Determination of organochlorine pesticide residues in the liver of cattle.
iii. Comparison of bioaccumulation levels of the organochlorine pesticide residues in the male cattle using age and the physicochemical parameters determined as a factor of categorization. Bioaccumulation of the OCPs are expected to increase as the duration of exposure increases hence, higher concentration of OCPs are expected as the age of the cattle increases.
iv. Evaluation of the intake of OCP residues through cattle liver to human beings per kilogram consumed.
v. To compare results obtained to Maximum Residual Limits as set by FAO (Food and Agriculture Organization) and WHO (World Health Organization)

1.6 Scope of Study

The scope of this work included

i. The determination of proximate parameters such as moisture content, ash content and Lipid content of the liver samples.

ii. The qualitative and quantitative determination of Endosulfan, DDT and metabolites, Heptachlor and isomers, Lindane, Hexachlorohexane and isomers, Chlorothalonil, Aldrin, Dieldrin, Cyhalothrin, Permethrin and Endrin. using GC-ECD.

iii. The analysis of results obtained using the Tukey range test (ANOVA), correlation of results obtained with some proximate parameters using Pearson's correlation and comparison with MRLs as established by FAO/WHO.

1.7 Limitations of the Study

i. The research work was limited to Zaria, Nigeria. All samples were taken from Zaria.

ii. Only the liver organs of the cattle were analyzed.

iii. A Single specie of cattle was used for the analysis (the N'dama). There are other cattle types that have not been analyzed in the present study.

iv. Cattle liver has been reported as a bioindicator.

v. Only male cattle liver was used in the research. This is because, OCP level in female animals have been known to fluctuate due to lactation.

vi. Not all the OCPs were determined. The OCPs of interest were those commonly used by farmers in Zaria and these are Endosulfan, DDT and metabolites, Heptachlor and isomers, Lindane, Hexachlorohexane and isomers, Chlorothalonil, Aldrin, Dieldrin, Cyhalothrin, Permethrin and Endrin.

CHAPTER TWO

2.0 LITERATURE REVIEW

Various investigations have been conducted in various countries to understand the status of organochlorine pesticides contamination in foodstuffs and its exposure to human. In previous decades, organochlorine pesticide had been used intensively for agricultural and industries purpose both in developed and developing countries, and caused significant contamination of food products.

2.1 Review of Previous Studies on OCPs in Fruits and Vegetable

In Nigeria, Adeyeye and Osibanjo (1999) determined residue levels of organochlorine pesticides in raw fruits, vegetables and tubers from markets in Nigeria. In the fruits, total HCH, aldrin and total DDT were detected in 77, 38 and 30% of all samples, respectively. In the vegetables, total HCH, HCB, total DDT and aldrin were detected from 95, 53, 50 and 30%, respectively, of all samples. Aldrin + dieldrin, total HCH, and total DDT were detected from 98, 79 and 49%, respectively, of all tuber samples. Other pesticides were below their detection limits. The average levels were generally low and none were above the FAOs maximum residue limits.

In China, a study by Odhiambo *et al*. (2007) determined residual levels of organochlorine pesticides (OCPs) in 34 samples of 19 varieties of vegetables collected from selected sites around Deyang city and Yanting County, Southwest China. The determinations were done using a gas chromatograph with electron capture detector (GC-ECD). The results indicated that all the vegetable samples had some levels of one or more OCPs in them. Residues of DDTs were found in 94.12% while HCHs were in 91.18% of all the samples analyzed indicating high incidence of these xenobiotics in the vegetables from the areas investigated. Among the HCH isomers, γ-

HCH was the most prevalent but β-HCH was the most abundant indicating both old and fresh inputs of HCHs. DDT metabolites p,p-DDE and p,p‘-DDD were more prevalent than the parent material, p,p-DDT suggesting minimal fresh inputs of DDT. The OCPs residue levels in the vegetables were generally low (≤ 1.3 ng/g wet weight) except in one sample of green pepper (*Capsicum annum L*) in which the concentrations (ng/g wet weight) of o,p‘-DDT (82.59), p,p‘-DDE (61.41) and total DDT (148.44), all exceeded the Chinese Extraneous Maximum Residue Limit of 50 ng/g for DDTs in vegetables according to the guidelines of the Chinese quality standard for food (GB 2763-2005).

In Albania, a preliminary study by Marku and Nuro (2005) determined the concentrations of chlorinated pesticides in the sediments and some fish species of Shkodra Lake. Seven sediment stations were chosen to represent different conditions of the lake. Biota samples were taken from the fat tissue of nine fish species. The concentrations of the chlorinated pesticides (except DDTs and Lindane) were found to be generally low. The total concentration of chlorinated pesticides in sediments was lower than the average values reported for the sediments in the Adriatic Sea.

A study conducted by Barkat (2005) in Pakistan determined residue levels of insecticides in fresh fruit and vegetable samples. Six hundred and eight samples of vegetables and fruits were analyzed using high performance thin layer chromatography (HPTLC) methods. The most commonly detected residues were those of methamidophos (9.8% of 608 samples), cypermethrin (8.5%), endosulfan (4.9%), chlorpyrifos (4.4%), trichlorfon (3.3%), methidathion and methomyl (2.8%), dimethoate (2.6%) and λ-cyhalothrin(1.8%) depending on the type of insecticide being used by the grower. Of all analyzed fruit and vegetable samples (608), 250 samples (41%) contained detectable residues. Of these, 13.8% had residues that exceeded Codex maximum

residue limits (MRLs). For individual crops, contaminated samples ranged from 10 to 100% of the number of samples analyzed.

In Mauritius, Lee and Seeneevassena (1997) monitored the amount of some insecticide residues in seasonal vegetables and fruits at the market level throughout the year. Locally produced vegetables and fruits were purchased from main urban and rural markets and analyzed by gas liquid chromatography for pesticide residues. A total of 126 samples of vegetables and 2 samples of fruits were extracted and analyzed for insecticide residues. 115 samples of the vegetable and fruit extracts were analyzed for the presence of the pyrethroid insecticides cyfluthrin, cypermethrin and deltamethrin. Fifty-two samples of the vegetable and fruit extracts were analyzed for the presence of organophosphorus insecticide dichlorvos, diazinon, fenitrothion, methamidophos, profenofos, phosphamidon, malathion and parathion. The data showed that most of the vegetable and fruit samples analyzed did not contain residues of the monitored insecticides above the accepted maximum residue limit (MRL) as adopted by the FAO/WHO Codex Alimentarius Commission (CAC), although some insecticide residues have been detected in certain samples only. The following insecticides have been detected in some of the samples of vegetables and fruits analyzed, but they have been mostly detected below the MRL: cypermethrin, deltamethrin, methamidophos, profenofos and malathion. The results obtained showed that 61.5% of the vegetable and fruit samples analyzed contained no detectable level of the monitored insecticides, 36.2% of the samples gave results with levels of insecticide residuesbelow the MRL, while 2.3% of the samples showed results above the MRL. Only three samples contained levels of insecticide residues above the MRL.

2.2 Review of Previous Studies on OCPs in Water, Sediment and Fishes

Organochlorine pesticides have been detected and determined in different compartments (water, sediments and biota) in the country using a gas chromatograph coupled with an electron capture detector (Nwakwoala and Osibanjo, 1990; Ize-Iyamu *et al*., 2007). Nigerian fishes have been reported to contain all the commonly encountered OCPs (Osibanjo and Bamgbose, 1990; Osibanjo *et al*., 2002; Unyimadu and Udochu, 2002; Adeyemi *et al*., 2008). From these studies, coastal waters are as contaminated as freshwaters. Most freshwater sediments in Africa are contaminated with OCPs (Calamari and Naeve, 1994). Based on the reported levels of dieldrin, lindane, endosulfan and total DDT, the water and sediments of the North African lakes in Egypt are more polluted, followed by those of West African rivers particularly in Nigeria. The East and Southern African waters are the least polluted by these organic pollutants. However, the trend for fish contamination is reversed. Fishes from East Africa are the most contaminated, followed by fishes from North Africa, while fishes from West Africa are the least contaminated (Calamari and Naeve, 1994). This disparity may be linked to a number of factors, including the relative fat contents in fishes, fish size and feeding habits, as well as biogeochemical transformations in different aquatic ecosystems.

Amakwe (1984) detected 10 organochlorine pesticides and HCB in 40 freshwater fish samples collected from various locations in Oyo and Ogun States. The relative occurrences of some of the residues identified were: lindane 100%, endosulfan 97%, DDT and metabolites 75%. The concentration ranges in ng/g fresh weight were: lindane 7.0-106.0; p,p'-DDE 2.0-30.0; p,p'-DDD 2.0-60.0; p,p'-DDT 3.0-18.0; total DDT 3.3-161.0; heptachlor 1.0-300.0; endosulfan 3.0-904.0; HCB 9.0-130.0 and α-HCH 0.2-5.0. Fish samples from freshwater were found to contain higher concentrations of these residues than sediments and water. Agunloye (1984) and Tongo (1985) studied the occurrences and levels of OCPs in water from 17 rivers, 2 lakes and one dam

in Southern Nigeria. The overall range of values (ng/L) of the major OCPs found were: lindane ND-167.0, aldrin ND-190.0, endosulfan ND-750.0, HCB ND-9.2 and heptachlor ND-96.0. DDT and metabolites were not detected. In the report, the OCP levels (ng/L) in River Ogun, which traverses three states and discharges into Lagos Lagoon, were: lindane 1.4-41.9, aldrin 5.1-49.0, endosulfan ND-260.0 and heptachlor ND-0.8. Okonna (1985) confirmed the presence of pesticide residues in Lagos Lagoon water with concentrations in ng/L as: lindane 85.3, aldrin 19.3, DDE 12.0, HCB 1.9, endrin 12.5 and dieldrin 28.0. Fayomi (1987) also detected and quantified 9 OCPs in South Eastern Nigeria. The relative occurrences of some of these compounds were aldrin, lindane, α-HCH (100%), endosulfan (44.4%), δ-HCH (16.7%), ρ,ρ'-DDD (33.3%), ρ,ρ'-DDE (61.1%) and heptachlor (72.2%). The concentration ranges in ng/g fresh weight were: α-HCH 0.2-7.4, lindane 0.6-13.0, heptachlor ND-1.0, aldrin ND-14.9, endosulfan ND-89.6, ρ,ρ'-DDE ND-4.2 and DDD ND-8.0.

Osibanjo and Bamgbose (1990) have reported the contamination of Nigerian marine fishes and shellfishes by OCPs, based on their analyses of 94 samples of 25 marine fish species between 1983 and 1985 and of 14 samples of 7 shellfish species from Lagos Lagoon. The concentration ranges in ng/g fresh weight were found to be as follows: HCB 0.04-9.48, lindane ND-5.30, endosulfan ND-4.95, DDT 0.15-18.6 and aldrin ND-54.60. Fishes contained higher concentrations of aldrin, heptachlor, HCB and lindane than shellfishes while the reverse was observed for DDT and PCB. The levels of residues obtained were found to be lower than those reported in the literature for industrialized countries. There was a positive and significant correlation coefficient between fish weight, fish length, fish fat and residue levels. Predator fish species were found to concentrate more residues in muscle tissue than plankton feeders. The DDT/PCB ratios were less than 1, indicating a predominance of industrial activities over agricultural activities as the source of contamination of the marine environment. The fish

Galeoides decadactylus was proposed as a potential bio-indicator organism for OCP pollution monitoring in the study area.

Sunday (1990) also analyzed 20 sediment samples from streams and rivers in lbadan, Oyo State. The concentration ranges in ng/g dry weight were: dieldrin ND-6.0, α-HCH ND-1.6, γ-HCH ND-2.0, aldrin ND-0.04, DDE ND-50.0 and PCB ND-14.0. Heptachlor, endosulfan and endrin were not detected. Ojo (1991) investigated the occurrence and levels of OCPs in 23 bottom sediment samples from Lekki Lagoon in Lagos State. Eleven organochlorine pesticides and HCB were detected. The ranges of concentration, reported in ng/g dry weight were: lindane 0.11-4.9, aldrin ND-347.0, ρ,ρ'-DDE 11.0-555.0, o,p'-DDD ND-348.0, endosulfan 7.0-1,155.0, heptachlor ND-1845.0, β-HCH ND-260.0, α-HCH ND-116.0, HCB ND-3.3, endrin ND-129.0, dieldrin 190.0-8,460.0. Compared with other parts of the world, the sediments were considered to be fairly contaminated with organochlorine pesticides. Nwankwoala and Osibanjo (1992) reported the detection of 10 OCP residues, including PCBs, in surface waters in Ibadan. The concentration ranges in ng/L of some of the residues quantified were α- and β-HCH 1.0-302.0, lindane 7.0-297.0, aldrin ND-40.0, dieldrin 17.8-657.0, endrin ND-19.0, heptachlor 4.0-202.0, endosulfan ND-430.0, HCB ND-92.0 and total DDT ND-1,266.0. The study showed that organochlorine pesticide residues were widely distributed in the surface waters, even at sites remote from point sources. The problem of ground water contamination by OCPs has also been identified in Nigeria (Osibanjo and Ayejuyo, 1994; Ayejuyo *et al*., 2008).

Fisheries research in Lagos Lagoon has revealed four species of decapod shellfishes in three families (Oribhabor and Ezenwa, 2005). The lagoon is reported to contain about one hundred and fifteen (115) species of fin fishes in seventy-nine (79) genera, forty-nine (49) families, seventeen (17) orders, two (2) classes and one (1) super-class (Gnathostomata). Recently, levels of organochlorine pesticide residues in water and fishes from some rivers in Edo

State have been determined (Ize-Iyamu *et al*., 2007). In all the water samples analysed from Ovia, Ogba and Ikoro Rivers, lindane, aldrin, ρ,ρ'-DDE, o,p'-DDD**,** p,p'-DDD, o,p'-DDT, and p,p'-DDT were present, except in Ikoro River where the water samples exhibited non-detectable levels for p,p'-DDE and p,p'-DDT. The level of p,p'-DDT, 0.74 ppb, was the highest in Ogba River, followed by lindane, 0.71 ppb, and aldrin, 0.60 ppb. Lindane, 0.79 and 0.59 ppb was found to be the highest in Ovia and Ikoro Rivers respectively followed by aldrin, 0.77 and 0.49 ppb. The other organochlorine pesticides were present at various levels: 0.31-0.49 ppb in Ogba River; ND-0.31 ppb in Ikoro River; and 0.30-0.56 ppb in Ovia River. The OCP residues detected in water were also detected in fishes at higher concentrations. The concentrations of these OCP residues in the feeders increased in the following order: bottom > middle > top. These findings agree with the earlier report by Tongo (1985). Lindane levels of 0.06 ng/g, 0.05 ng/g and 0.04 ng/g were detected in fishes from Ovia, Ogba and Ikoro Rivers while aldrin levels were 0.06 ng/g and 0.03 ng/g in the bottom and top feeders from Ovia River. These levels are high compared with the allowable limits of the Federal Environmental Protection Agency (FEPA), now the Federal Ministry of Environment.

Studies have also been carried out in other parts of Africa and the world. Water, sediment, red swamp crayfish (*Procambarus clarkii*) and blackbass (*Micropterus salmoides*) from Lake Naivasha, Kenya were analysed for selected organochlorine pesticide residues (Gitahi, 1999). The mean p,p'-DDT, p,p'-DDE and o,p'-DDT residue levels (ppb) recorded in blackbass and crayfish were 28.3, 34.2, 16.1 and 4.6, 3.2, 1.4 respectively. The mean lindane, dieldrin, endosulfan and aldrin concentrations in blackbass were 100.5, 34.6, 21.6 and 16.7 ppb while lower concentrations of 2.0, 2.0, 2.0 and 1.9 ppb were detected in crayfish. The higher fat content of 3.7 g in blackbass compared to 0.6 g in crayfish accounted for the significantly higher residue concentration in blackbass. There is also increasing interest in South America on organic

pollutants from the coastal marine environment. Menone *et al.* (2001) studied the occurrence and distribution of persistent organochlorine compounds in the Mar Chiquita, Argentina coastal lagoon watershed. The levels were measured in sediments and associated crabs. Pesticide concentrations were higher than PCBs as a result of agricultural activities in the area. Heptachlor epoxide, DDT and its metabolites, and % HCH were the predominant OCPs in sediments as well as in crabs. OCPs in sediment, water and biota from the coastal marine environment of Mumbai, India have been analyzed to assess their distribution in various environmental compartments (Pandit *et al*., 2002). HCH isomers, DDT and its metabolites were the predominantly identified compounds in all the samples. High ratios of DDT to DDE were found in seawater samples, indicating the presence of a significant source of DDT. The levels of organochlorines in fishes obtained from the investigation were found to be lower than the levels of organochlorines in fishes in temperate regions. Coat *et al*. (2006) measured the levels of chlordecone in various freshwater and marine species. The results showed a heavy contamination of many carnivorous and detritivorous species of fishes and prawns.

The levels of OCPs in edible biota from the coastal area of Dares Salaam city, Tanzania, have been reported (Mwevura, 2002). Samples were collected from the Msimbazi and Kizinga Rivers and from the coastal marine environment receiving waters from these rivers. The samples were analyzed for various OCPs using GC-ECD and the results were confirmed with GC-MS. Dieldrin, p,p'-DDT**,** p,p'-DDE**,** p,p'-DDD, o,p'-DDT and α-HCH were the only organochlorines detected at concentrations above the method detection limits. The samples showed significant differences in levels of residues, depending on location, mode of feeding and age/size of analyzed biota. The biota from mangrove areas showed the highest level of residues, followed by those from fresh water. The lowest concentrations were detected in biota from marine coastal water. In Uganda, Nile tilapia and Nile perch samples from Lake Victoria have been analysed for

lindane, aldrin, endosulfan, dieldrin, DDT and DDE (Kasozi *et al*., 2006). No significant difference in the residue levels between fish types was observed for lindane, endosulfan, p,p'-DDT, p,p'-DDE and dieldrin. The aldrin levels in Nile perch (*Lates niloticus*) were significantly higher than the levels in Nile tilapia (*Oreochromis niloticus*). No difference was observed in the distribution of residues in the different organs of Nile tilapia, although a difference for p,p'-DDE was observed in the Nile perch. No significant difference was also observed in the average fat content of the tissues of Nile perch and Nile tilapia. However, the distribution of fat was significantly different in the different organs of the fishes, with the abdominal portion having the highest amount of fat. There was no correlation observed between fat contents and organochlorine concentrations.

2.3 Review of Previous Studies on OCPs in Dairy and Meat Products

A study conducted by Nida *et al.* (2009) showed the presence of Orgaochlorine pesticide residues in milk, butter, cheese, labaneh and yoghurt owing to their use in sanitary and agricultural purposes. A total of 233 dairy product samples obtained from different areas in Jordan during 2001–2007 were analyzed for their residual contents of aldrin, DDT and metabolites (DDTs), dieldrin, endosulfan isomers, endrin, hexachlorocyclohexane isomers (HCHs), heptachlor and HCB. Levels of these compounds were determined by gas chromatography with electron capture detector (GC-ECD). The results indicated that 9% (21/233), 8.5% (20/233), 6% (14/233) and 2.1% (5/233) of the examined samples were contaminated with b-HCH, p'p'-DDE, α-HCH and γ-HCH, respectively. Heptachlor and endosulfan were only present in less than 2% of the analyzed samples. None of the samples revealed the presence of aldrin, o,p'-DDD, p,p'-DDD, o,p'-DDE, o,p'-DDT, p,p'-DDT, dieldrin,

b-endosulfan, endrin and HCB at their detection limits of which 48 (20.6%) samples were found to be contaminated with different OCP residues.

Luzardo *et al.* (2012) evaluated the level of OCPs and PCBs in 26 commercially available brands of milk (16 conventional and 10 organic brands) present in the market of Canary Islands. The Island is known for its high milk-intake in Spain and Europe. The aim of the research was to estimate the relevance of milk as a source of these chemicals for the Canary population. Their findings showed that hexachlorobenzene and transchlordane were present in almost all the samples with independence of the type of milk. For both types of milks, the concentration of OCPs was very low, showing organic milk's lower levels than conventional ones. As a consequence, the estimated daily intake for OCPs was lower than the tolerable daily intake (TDI) established by International Agencies. In conventional milk brands, results obtained were as follows; alpha-HCH; ND-11.21 ng/g fat, beta-HCH; ND-7.37 ng/g fat, delta-HCH; ND-4.35 ng/g fat, gamma-HCH (Lindane); ND-9.28 ng/g fat, Aldrin; ND- 1.26 ng/g fat, Dieldrin; ND-12.43 ng/g fat, Endrin; ND-10.08 ng/g fat, cis-chlordane; ND- 3.98 ng/g fat, trans-chlordane; 0.16-8.71 ng/g fat, Heptaclor; ND-7.77 ng/g fat, alpha-endosulfan; ND- 4.33 ng/g fat, beta-endosulfan; ND-3.53 ng/g fat, pp'-DDE; ND-30.22 ng/g fat, o,p'-DDE, o,p'-DDT, p,p'-DDT, o,p'-DDE, p,p'-DDE were ND. In organic milk brands, results obtained were as follows; alpha-HCH; ND-0.74 ng/g fat, beta-HCH; ND-1.86 ng/g fat, delta-HCH; ND-0.51 ng/g fat, gamma-HCH (Lindane); ND-1.86 ng/g fat, Aldrin; ND- 0.32 ng/g fat, Dieldrin; ND-7.60 ng/g fat, Endrin; ND, cis-chlordane; ND- 1.53 ng/g fat, trans-chlordane; ND- 4.51 ng/g fat, Heptaclor; ND-1.23 ng/g fat, alpha-endosulfan; ND- 1.00 ng/g fat, beta-endosulfan; ND, pp'-DDE; ND-20.49 ng/g fat, o,p'-DDE, o,p'-DDT, p,p'-DDT, o,p'-DDE, p,p'-DDE were ND.

Letta *et al.* (2013) determined residue levels of organochlorine pesticides (OCP) in a total of 90 cattle samples comprising meat, liver and kidney collected from carcasses slaughtered in

six towns in West Shoa Zone, Ethiopia, (Ambo, Guder, Ginchi, Gedo, Holeta and Tikur Inchini), were determined. The pesticides were extracted by solid phase extraction (SPE) and quantification was carried out using gas chromatography-mass spectrometry (GC-MS). A good linearity (r(2) > 0.998) was found in the range 0.001-7.00 mg/kg for the samples studied. Most of the pesticides had recoveries in the range 81-99% and values of relative standard deviation (RSD) <7.2% for repeatability and reproducibility, showing good accuracy and precision of the method. The concentration level of the studied organochlorines followed the order: p, p' dichloro-diphenyl-trichloroethane (DDT) > endosulfan>o,p'-DDT >lindane>dieldrin> endrin> aldrin>chlorothanolin while the order of contamination in the analyzed organs was liver > kidney > meat. Heat treatment of the meat, kidney and liver samples (boiling for 90 min.) produced an overall reduction of 62.2%, 44.5%, 37.7%, 29%, 31%, 34.3% and 30.8% in lindane, o, p'-DDT, endosulfan, p, p'-DDT, chlorothanolin, aldrin, dieldrin, and endrin, respectively. Although the residual contents of the organochlorines detected in all the contaminated samples analyzed from the six cities were below the respective maximal permissible levels set by international organizations, samples from Holeta town were more contaminated and may necessitate effective monitoring as bioaccumulation of these residues may pose health problems in human beings

2.4 Review of Previous Studies on OCPs in Soil

OCP analysis on 19 soil samples and one sediment core near the banks and in estuary of the Minjiang River showed that the soils and cores in the area were highly polluted by DDT. The concentrations of OCPs in the samples were determined by GC-ECD with the internal standard method. The results showed that the concentrations of OCPs, HCHs, DDTs in soils near the banks of Minjiang were in the ranges of 4.31-877.80 ng/g (with a mean 74.13 ng/g, 1.32-24.01 ng/g 7.50 ng /g, 1.67-876.49 ng/g and 66.64 ng/g, respectively. Through the study of the vertical distribution characteristics of OCPs in the area, the pollution history of OCPs in the Minjiang

estuary was discussed. The concentrations of HCHs and DDTs in the core ranged from 0.34-11.72 ng/g and 1.12-31.04 ng /g respectively. The upper section of the sediment core showed o,p'-DDT as its main pollutant, which indicated that new DDTs may be still used recently in the Minjiang area. The vertical changes of OCPs reflected basically their production and application history in banks of Minjiang River, Fujian Province (Zhang *et al*., 2011)

A study carried out in Mexico to monitor organochlorine pesticide concentrations and to compare its levels among soil, rye straw, and rye grains (of rye plant) using GLC-ECD indicated the following contamination levels distributed among soil, straw, and grains: HCB (0.7 - 1.2 - 0.7 microg/kg), alpha-HCH (0.6 - 3.4 - 1.2 microg/kg), gamma-HCH (1.8 - 27.3 - 4.4 microg/kg), Sigma-HCH (2.5 - 30.7 - 5.6 microg/kg), p,p'-DDE (1.0 - 7.8 - 5.5 microg/kg), o,p'-DDT (16.1 - 20.4 - 17.0 microg/kg), p,p'-DDT (38.0 - 41.7 - 49.6 microg/kg), and Sigma-DDT (54.2 - 63.2 - 72.1 microg/kg). The study verified the presence of organochlorine pesticides in the Mexican agricultural environment and their migration from soil to the growing rye plants. The contamination of growing plants occurs by adhesion of volatile substances from the air to the plant surface and by the migration of contaminants through xylem in inner ascendant transport. The determined organochlorine pesticide levels in rye plants are low, at residual levels they are below Codex Alimentarius Commission Maximum Residue Limits (Waliszewski *et al*., 2004).

Ruey-An *et al*. (2001) showed that the best extraction method for OCP analysis was soxhlet extraction. An analytical procedure was developed using headspace solid-phase microextraction (HS-SPME) for the determination of organochlorine pesticides (OCPs) and their metabolites in sandy soil samples. The developed procedures involving fiber selection, temperature effect, absorption time, soil matrix and the addition of solvents of different polarity were optimized. Also, the results were compared to those achieved using Soxhlet extraction

standard method. The 100-μm polydimethylsiloxane (PDMS) and 65-μm PDMS–divinylbenzene showed good extraction efficiency for 18 organochlorine pesticides. An increase in the extraction efficiency of organochlorine pesticides and the metabolites was observed when the temperature increased, and an optimum temperature of 70°C for extracting OCPs was obtained. The application of other hydrophilic solvents had different effects on the extraction of organochlorine pesticides and the metabolites. Higher responses of OCPs were obtained when 5 ml of water was added to the soil. Good linearity of OCPs between 0.2 and 4 ng/g soil was observed. The relative standard deviation was found to be lower than 25%. Also the limits of detection were between 0.06 and 0.65 ng/g, which were lower than those obtained using Soxhlet extraction.

A field study conducted in the Taihu Lake region, China in 2004 revealed the organochlorine pesticide concentrations in soils after the ban of these substances in the year 1983. Thirteen organochlorine pesticides (OCPs) were analyzed in soils from paddy field, tree land and fallow land. Total organochlorine pesticide residues were higher in agricultural soils than in uncultivated fallow land soils. Among the pesticides, DDD, DDE and DDT had the highest concentration for all the soil samples, ranging from 3.10 ng/g to 166.55 ng/g with a mean value of 57.04 ng/g and followed by HCH, ranging from 0.73 ng/g to 60.97 ng/g with a mean value of 24.06 ng/g. Dieldrin, endrin, HCB and α-endosulfan were also found in soils with less than 15 ng/g. Ratios of *p,p*'-(DDD+DDE)/DDT in soils under three land usages were: paddy field > tree land > fallow land, indicating that land usage influenced the degradation of DDT in soils.DDT in soils. Ratios of *p,p*'-(DDD+DDE)/DDT >1, showing aged residues of DDTs in soils of the Taihu Lake region. The results were discussed with data from a former study that showed very low actual concentrations of HCH and DDT in soils in the Taihu Lake region, but according to the chemical half-lives and their concentrations in soils in 1980s, the concentration of DDT in soils seemed to be underestimated. In any case, the resent data showed that the ban on

the use of HCH and DDT resulted in a tremendous reduction of these pesticide residues in soils, but there are still high amounts of pesticide residues in soils, which need more remediation processes (Wang *et al*., 2006).

2.5 Chemistry of Organochlorine Pesticides

Organochlorine pesticides belong to three chemical classes and these include the dichlorodiphenylethanes, the chlorinated cyclodienes, and the chlorinated benzenes and cyclohexane-related structures. They are persistent and are biotransformed at a slow rate due, in part, to the complex aromatic ring structures and the extent of chlorination. These ring substitutes are exceedingly difficult to remove by enzymatic processes available in body tissues. The molecular formula of DDT is C_{14}–H_9–Cl_{l5} and the formula shows the extent of chlorination. This pesticide has very low solubility in water (12 μg/kg) and a high solubility in fat (10 g/kg). This is why the pesticide is lipophilic and easily absorbed in fat. Once absorbed in the body tissues of a living organism, DDT is concentrated in the adipose tissue/fat depots (Leighty *et al.*, 1980). DDT is a potent, non-systemic contact pesticide and its storage in the fat tissue is important because it decreases the amount of the pesticide at the site of toxic action (Sharma, 2001).

2.5.1 Toxicokinetics of organochlorine pesticides

The lipoprotein, the chlorine bonds and the stable covalent bonds of the OCPs are the precursors that lead to their persistence and toxicity. The toxicity is evidenced in the different stages of the time course of OCPs disposition (Toxicokinetics) that include their absorption, distribution, metabolism and excretion in the whole living animal (Medinsky and Klaassen, 1996).

2.5.2 Absorption

The absorption of OCPs involves the direct transportation to the liver via the hepatic-portal vascular system of the body. OCPs are lipid-soluble; they are readily absorbed by the skin and mucous membranes of the gastrointestinal tract (Riviere, 1994; Roberts *et al*., 1997). Other lipophilic compounds such as PCBs can also get into the systemic circulation by passive transportation of lipids via the micelles and subsequent biological processes related to lipid metabolism (Klaassen and Rozman, 1996). The human skin comes into contact with many toxic agents; fortunately the skin is not very permeable and therefore is a relatively good barrier. When the skin absorbs these chemicals in sufficient quantities they can elicit systemic effects. For example, DDT is much less toxic to mammals than to insects when it is applied to the skin. DDT passes more readily through the chitinous exoskeleton of insects than through the skin of mammals (Albert, 1965; Hayes, 1965).

2.5.3 Distribution

After administration, DDT is transported into the blood stream by absorption. For lipid soluble toxicants, the major determinant of the rate of movement across capillaries is the partition coefficient. DDT accumulates in certain parts of the body as a result of protein binding, active transport and its high solubility in fat. The alpha and beta lipoproteins are very important in the transport and distribution of lipophilic xenobiotics such as DDT with the interactions being non-covalent (Wilkinson 1983; Oliyai and Stella, 1993). DDT binds to proteins that have hydrophobic regions such as lipoproteins and albumin. Chlordane, DDT and PCB, are highly lipophilic and become localized in adipose tissue, where they seem to exert little effect until mobilized by starvation of the animal. Distribution of the residues in tissues may be a protective mechanism if the tissue is not the target site for toxicity, and it also reduces toxic effects in other organs. Although sequestration into adipose tissue reduces exposure of other tissues,

mobilization of the fat in the adipose tissue may cause a sudden release of the organochlorine into the bloodstream with a dramatic rise in concentration and toxic consequences (Timbrell, 1991a). Sequestration into tissues occurs due to the action of an endogenous substance such as the protein glutathionetransferase 1,2,3 (GST 1, GST 2 GST 3). This protein binds and transports both endogenous and exogenous compounds into the liver. These toxicants accumulate in fat by dissolution in neutral fats, which constitute about 50 % and20 % of the body weight of obese individuals and lean athletic individuals respectively. Storage lowers the concentration of the toxicants in the target organ (Ecobichon, 1996; Rivero *et al.*, 1997). A study of the perch showed that the body burden of OCP residues was lowered by storage in adipose tissue (Olson *et al.*, 1999).

2.5.4 Metabolism

The metabolism of DDT and other organochlorine compounds converts these lipophilic pesticides into more polar substances and hence into more excretable metabolites (Redetzke and Applegate, 1993). Sometimes transformed substances (metabolites) may be more toxic than the parent compound and this is the case with DDT, where the metabolite p,p'-DDE is more persistent and toxic than the parent DDT (Stevens *et al.*, 1993; Timbrell, 1991b). Xenobiotic metabolism is divided into two main phases, which are phases I and phase II (Nebert, 1994). These sometimes, but not always, occur sequentially as in an experiment by Pratap *et al*. (2000) in which he observed that the reaction of an ethanolic extraction of the leaves of *Adhatoda vesica* (Acanthaceae) sequentially influenced phases I and II enzyme systems in Albino Swiss mice. However, xenobiotic metabolism is now well recognized as three phases where the elimination from the cell is regarded as the phase III (Bernard *et al*., 2003).

Phase I reactions commonly involves hydrolysis, reduction or oxidation (Otto *et al*., 1999). The reactions expose the original compound so that the functional group (-OH, - NH_2, -

SH, or -COOH) can be added and then conjugated in phase II reactions (Williams, 1983; Nichols *et al*., 1994). Phase II stage, involves conjugation reactions; these include glucoronidation, sulphonation, acetylation, methylation, conjugation with glutathione, or conjugation with amino acids. Enzymes catalyse metabolism of xenobiotics, and most of these enzymes are very specific in metabolizing particular compounds. Studies have been carried out to show catalytic activity of P-450 enzymes as expressed in bacterial, yeast or mammalian cells (Gonzalez and Korzekwa, 1995). These enzymes are localized in the sub-cellular environments such as the smooth endoplasmic reticulum and in the cytosol and a few are found in such organelles as the mitochondria. Most oxidation reactions are catalyzed by one enzyme system, which is the cytochrome P-450 mono-oxygenase or the mixed function oxidases (Farrell, 1987; Krishna and Klotz, 1994). Cytochrome P-450 system ranks first in terms of catalytic versatility in the numbers of xenobiotics it detoxifies or activates. (Guengerich, 1994; Werck-Reichhart and Feyereisen, 2000). Studies have been done in-vitro using human cells on the use of antipeptide antibodies directed against defined regions of human cytochrome P-450 enzymes to show the function of the enzyme on compounds (Raunio *et al*., 1995; Boobis *et al.,* 1996).

All cytochrome P-450 enzymes are haeme-containing proteins. The haeme iron in the cytochrome is usually in the ferric (Fe^{3+}) state. When reduced to the ferrous (Fe^{2+}) state, cytochrome P-450 can bind ligands such as oxygen (O_2) and carbonmonoxide (CO). The complex between ferrous cytochrome P-450 and CO absorbs light maximally at 450 nm, from which cytochrome P-450 derives its name (Guengerich, 1987). The preferred method for the analysis of the enzyme cytochrome P-450 is the microsomal fractioning method, followed by the colour complexing of the enzyme, which is calorimetrically determined on a scanning spectrophotometer. Quantitation of the enzyme is based on determination against microsomal proteins (Wilson, 1995). Human liver microsomes contain 15 or more different P-450 enzymes

(isoenzymes) such as CYP1A1, 1A2, 2A6, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, 3A4, 3A5, 3A7,4A9, and 4A11 (Guengerich, 1994; Yoshitomi *et al*., 2001).

The P-450 enzymatic system is known to exist in a multiplicity of forms (isoenzymes) with overlapping substrate specificities. Recent studies have shown that the definition of families and subfamilies has shifted toward lower cut-offs for inclusion. A more realistic view today is that a cut-off window is better than an absolute value like the original 40% identity or higher for family inclusion (Estabrook, 1999). More problematic is the merging or blurring of subfamilies that used to be distinct, and the CYP4 family is a prime example. It is now so full of subfamilies that they are hard to keep from overlapping (Daly *et al*., 1996). Cytochrome P-450 research is a field that has benefited by having a systematic standardized nomenclature (Nelson *et al*., 1996).

The CYP induction response occurs mainly in the liver, depending on the nature of the xenobiotic, but also in lungs, kidney and placenta. The induction is characterized by increased expression of the CYP genes and associated catalytic activities for one or more isoforms (Okey, 1990). The exposure to organochlorine pesticides leads to the induction of CYP 2B1, CYP 2B2 isoforms and also the CYP 3A4 subfamily (Lubet *et al.*, 1990; Kocarek *et al.*, 1991). These soluble cytochrome P-450 enzyme systems catalyze the pesticide DDT by dehalogenation. The overall reaction is a dehydrogenation and the formation of the metabolite pp'-DDE (Nims, *et al*., 1998). Studies have shown that DDT and its major metabolites pp-DDE and pp-DDD, as well as many other organochlorine pesticides, are phenobarbital (PB)-type cytochrome P-450 inducers in the rat (Li *et al*., 1995a; Nims and Lubet, 1995).

Thus, like administration of PB, exposure to such organochlorine pesticides results in the induction in rat liver or cultured rat hepatocytes of CYP2B selective catalytic activities. In addition, DDT and other organochlorine pesticides elicit in the rat many of the other manifestations of the hepatic pleiotropic response caused in rat by PB including hepatomegaly

and induction of the CYP3A subfamily, NADPH cytochrome P-450 oxidoreductase, and certain phase II activities and genes such as microsomal epoxide hydrolase (Campbell *et al*., 1983; Yashioka *et al*., 1984; Kocarek *et al*., 1991; Lubet *et al*., 1992).

2.5.5 Excretion

Toxicants are eliminated from the body through several routes. The kidney is one of the most important organs for the excretion of polar/water soluble xenobiotics as more chemicals are eliminated from the body by this route than by any other. Removal of a toxic compound may help to reduce the extent of damage to the organs and body tissue. The rate of elimination of foreign compounds is reflected by the parameters such as plasma half-life ($t_{1/2}$), elimination rate constant (Ke1) and total body clearance of the area under the curve (AUC). The half-life of some diphenyl aliphatics such as DDT and the cyclodienes such as aldrin may range from days to weeks or months. Residual elimination of organochlorine pesticides may be described by a two compartment model, where the first phase is relatively rapid, and the second phase is prolonged (Osweiler, 1996). Many xenobiotics have to be biotransformed to water-soluble products before they can be excreted in urine. However, most body secretions appear to have the excreted chemicals thus some toxicants have been found in sweat, saliva, tears and milk (Drummond *et al*., 1988 and Veberk *et al*., 1990). The biliary route of elimination is probably the most important route of elimination in as far as the chlorinated ethane derivatives such as DDE are concerned. The major excretory route is from bile to the digestive tract where some enterohepatic recycling may take place. The liver removes the toxic agents from the blood after absorption from the gastrointestinal tract. Some of the metabolites that are formed are excreted directly into the bile (Rozmanand Klaassen, 1996). The metabolites are slowly released from lipid storage depots until equilibrium with blood is achieved. DDT is metabolised to 1,1-dichloro-2-bis-(4-chlorophenyl) ethane (p,p'-DDD) and p,p,-DDE; then p,p'-DDD is further

metabolised to bis-(4-chlorophenyl) acetic acid (p,p'-DDA), which is water-soluble and is excreted in the urine (Fraklin *et al*., 1986 and He, 1999).

However, there is no efficient elimination mechanism for non-volatile highly lipophilic chemicals such as polyhalogenated biphenyls and chlorinated hydrocarbons insecticides such as DDT, more so if the pesticides are not easily biotransformed (Okonkwo *et al*., 1999). These chemicals are excreted very slowly and tend to accumulate in the body upon chronic exposure (Gladen and Rogan, 1995 and Czaja *et al*., 1997). However, there are a few rather slow processes for elimination and excretion of these lipophilic pesticides, such as excretion by the mammary gland after the pesticides have been dissolved in milk lipids (Bouwman *et al.*, 1992 and Barkatina *et al*., 1998).

The secretion of organochlorine pesticides such as DDT and HCH through the breast milk is a form of excretion or a form of elimination processes (Hernandez *et al*., 1993 and Hooper and Petreas, 1997). Research has demonstrated that for suckling infants, milk is their major source of the body burden of OCPs (Lederman, 1993 and Franchi and Farcardis, 1993). The health importance of secretion of DDT and other organochlorine pesticides in human breast milk depends entirely on the dosage received by suckling babies (Skaare *et al*., 1988 and Kanja *et al.*, 1992). Studies have demonstrated that milk as a route of excretion can be used as a biomarker for measuring and monitoring environmental exposure levels of DDT and HCH (Nair and Pillai 1992; Johansen *et al*., 1994).

2.5.6 Toxicity of organochlorine pesticides

The major toxic action of organochlorine pesticides is on the central nervous system (CNS), and the impact of the pesticide is its action on the membranes in the nervous system especially axonal membranes. Studies have shown that the nervous system both central and

peripheral and the cells that make up these systems are particularly susceptible to damage by these compounds (Handerson *et al*., 1989; Silbergeld, 1993; Mutti, 1999 and Aitio, 2002).

Endocrine disruption has been shown in animals exposed to OCPs. Studies in mice have suggested that the oocytes in the reproduction system of the female mice can be damaged or destroyed by the metabolic action of the organochlorine pesticides (Carson *et al*., 1989; Savitz and Harlow, 1991). Other studies haves shown that organochlorine pesticides can cause an estrogenic response in rats, leading to the thickening of the endometrium and an increase in the uterus weight. This is due to competitive inhibition of OCPs to binding of estradiol to receptor sites in the uterus cells (Alisan and Morgan, 1987; Carson *et al*., 1989; Safe, 2000).

2.6 Mechanism of Toxicity of Organochlorine Pesticides

Organochlorine Pesticides alter the porous channels through which sodium ions pass by interfering with the active transport of sodium out of the nerve axon during repolarization. Studies have demonstrated that at the level of the neuronal membrane, OCPs affect the permeability to K^+, reducing K^+ across the membrane. It was also observed that, OCPs inhibits neuronal adenosine triphosphatase (ATPase), particularly the Na^+, K^+, -ATPase and Ca^{2+} -ATPase that play a vital role in neuronal repolarization. It was observed that there is the inhibition of (ATPase) enzyme by exposure to OCPs when a preparation of this enzyme from a nerve ending fraction of the rat or rabbit brain was exposed to OCPs concentration of 10^{-5} mol in vitro (Matsumura, 1985; Guillette *et al.,* 1999). Finally, it was also observed that the pesticide inhibits the ability of calmodulin, a calcium mediator in nerves, to transport calcium ions essential for neuro transmitters (Ecobichon, 1996). Heptachlor and lindane may inhibit the binding of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) (Elderfrawi *et al.,* 1985; Fonseca *et al.,* 1993).

Acute and sub-acute toxicity of OCPs in humans and animals is rare, from an environmental situation. Chronic toxicity of OCPs is the main, since it is from an environmental exposure set-up.

2.7 Brief Description of Some Organochlorines and their Metabolites

2.7.1 Aldrin

Aldrin is an insecticide primarily used against soil and crop pests, such as corn rootworm, wireworm, rice water weevil and grasshoppers. Aldrin is most commonly used on corn, potato and cotton crops. It is used also used to protect wooden structures from termites. Aldrin readily converts to dieldrin in plants and animals and therefore, residues of this chemical are usually found in small amounts. Aldrin has low toxicity to plants, but has adverse effects on aquatic invertebrates, particularly insects. Acute exposure to aldrin has caused death in waterfowl, shorebirds, fish and humans. The International Programme on Chemical Safety (IPCS) estimates the fatal dose for humans to be five grams. The most common human exposure pathway is via food, particularly dairy products and meat. Aldrin has been banned or severely restricted in many countries (RFI, 2001). The molecular structure of aldrin is shown in figure 2.1

Fig 2.1: (1R,2R,3R,6S,7S,8S)-1,8,9,10,11,11-Hexachlorotetracyclo[6.2.1.1^{3,6}.0^{2,7}]dodeca-4,9-diene (Aldrin)

2.7.2 Chlordane

Chlordane is a broad-spectrum agricultural insecticide used on crops, such as vegetables, grains, maize, potatoes, sugarcane, fruits, nuts, cotton and jute. It is also used to combat termites. Chlordane can kill aquatic invertebrates, fish and birds. Bioconcentration, rather than bioaccumulation, seems to be the process of greatest concern. Chlordane is suspected of damaging the human immune system and is listed as a possible human carcinogen. The most common human exposure pathway is through the air, particularly from indoor environments. Chlordane has been banned or severely restricted in dozens of countries (RFI, 2001). Figure 2.2 shows the molecular structure of chlordane.

Fig 2.2: 1,3,4,7,8,9,10,10-Octachlorotricyclo[5.2.1.$0^{2,6}$]dec-8-ene

2.7.3 DDT

DDT was broadly used during World War II to control insects (namely mosquitoes) that spread diseases such as malaria, dengue fever and typhoid. After the War, DDT was also used on agricultural crops such as cotton. DDT is still used in many countries to control insect vectors that carry disease. Concerns about the serious environmental effects of DDT, particularly on birds, resulted in many countries banning or severely restricting its use in the 1970s. DDT's breakdown products include DDD/TDE and DDE, each of which possesses its own toxic characteristics. DDT is highly toxic to fish, causing severe behavioural changes and death. Acute toxicity of DDT in birds has been observed to affect reproductive success (for example, it causes

eggshell thinning in birds of prey). The long-term chronic health effects of DDT on humans include immune system depression and estrogen-like alterations during reproductive development. DDT and its metabolite DDE are listed as possible human carcinogens, particularly as regards hormonal cancers, such as breast cancer. Humans are mainly exposed to DDT through their food; in fact DDT has been detected in food and breast milk all over the world. To date, 34 countries have banned DDT and 34 have severely restricted its use (RFI, 2001). The molecular structure of DDT and its metabolites (DDE and DDD) is shown in figure 2.3, 2.4 and 2.5 respectively.

(DDT)

Fig 2.3: 1,1'-(2,2,2-Trichloro-1,1-ethanediyl)bis(4-chlorobenzene)

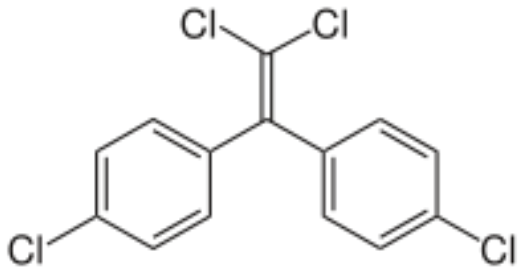


Fig 2.4: p,p'-DDE
(Dichlorodiphenyldichloroethylene)

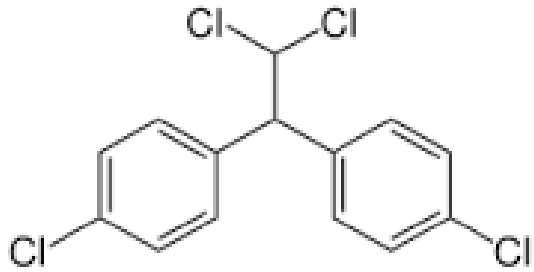


Fig 2.5: p,p'-DDD
(Dichlorodiphenyldichloroethane)

2.7.4 Dieldrin

Dieldrin is an insecticide used against termites, textile and agricultural pests, and insect vectors of disease. The main crops treated with dieldrin include corn, cotton and potato. The use of dieldrin as a vector control has been banned in some countries because of concerns related to the environment and human health. Dieldrin has low toxicity to plants but high toxicity to insects, fish and aquatic animals (frog embryos, for example, have been noted to develop spinal deformities when exposed to dieldrin). Toxic effects on birds and mammals vary. Dieldrin is suspected of negatively affecting the immune response in humans. Humans are exposed to dieldrin through their food (mainly dairy products and animal meats). It is important to note that because aldrin easily converts to dieldrin in plants and animals, levels of dieldrin reflect total concentrations of both chemicals (RFI, 2001). Fig 2.6 is a chiral molecular structure of Dieldrin.

Fig 2.6: (1R,2S,3S,6R,7R,8S,9S11R)-3,4,5,6,13,13,13-Hexachloro-10-oxapentacyclo [6,3,1,13,6.02,7.09,11]tridec-4-ene
(Dieldrin)

2.7.5 Endrin

Endrin is an insecticide used against pests of cotton, rice and corn. It has also been used as a rodenticide to combat mice and voles. Many animals can metabolize Endrin. Therefore, it does not accumulate as severely as some of the other persistent organic pollutants. However, endrin is highly toxic to fish and other aquatic organisms. Endrin is suspected of suppressing the human immune system. As with many of the other POPs, humans are exposed to endrin through their diet, although intake levels are usually noted to be very low (RFI, 2001). Figure 2.7 is a chiral molecular structure of Endin.

Fig 2.7:

2.7.6 Heptachlor

Heptachlor is an insecticide used to combat soil and crop pests (particularly of cotton), termites, grasshoppers, fire ants, and mosquitoes (to control malaria). Heptachlor is metabolized to heptachlor epoxide (Figure 2.8 and 2.9), which has a similar toxicity level to heptachlor. Heptachlor is toxic to wildlife even at low concentrations. In birds, exposure to the chemical has induced behavioural changes, reduced reproductive success, and mortality. Heptachlor is listed as a possible human carcinogen. The main exposure pathway for humans is through their diet, namely meat. Several countries have banned or restricted the use of Heptachlor (RFI, 2001).

Fig 2.8: 1,5,7,8,9,10,10-Heptachlorotricyclo[5.2.1.$0^{2,6}$]deca-3,8-diene

(Heptaclor)

Fig 2.9: 1,6,8,9,10,11,11-Heptachloro-4-oxatetracyclo[6.2.1.$0^{2,7}$.$0^{3,5}$]undec-9-ene

(Heptachlor expoxide)

2.7.7 Mirex

Mirex is an insecticide used against fire ants, western harvester ants and yellow jacket wasps in the United States, leaf cutter ants in South America, harvester termites in South Africa, and mealy bugs in Hawaii. Mirex is also used as a fire retardant in plastics, rubber, paint, paper and electrical goods. Mirex is one of the most stable and persistent POPs. The chemical is toxic to plants (affecting their development and growth), aquatic organisms (crustaceans are the most sensitive), fish (affecting their behaviour) and birds. Mirex is listed as a possible human carcinogen. Humans are exposed to mirex through their diets, particularly meat, fish and wild

game, although intake levels are generally below residue tolerances (RFI, 2001). Fig 2.10 shows the molecular structure of Mirex.

Fig 2.10: 1,1a,2,2,3,3a,4,5,5,5a,5b,6-dodecachlorooctahydro-III-1,3,4- (methanetriyl) cyclobuta [cd] pentalene

(Mirex)

2.7.8 Toxaphene

Toxaphene is an insecticide used to protect cotton, cereal grains, fruits, nuts and vegetables. Toxaphene is also used to combat the ticks and mites that are parasitic to livestock. Toxaphene is a mixture of up to 670 chemicals. It is non-toxic to plants, highly toxic to fish and has been noted to cause reproductive disorders in birds. Toxaphene has been listed as a possible human carcinogen. Humans are exposed to the chemical through their diets, but levels detected in food are usually very low (RFI, 2001). Molecular structure of Toxaphene is shown in Fig 2.11

Fig 2.11: Toxaphene

2.7.9 Hexachlorobenzene (HCB) or Hexachlorohexane or Benzenehexachloride

They are broad-spectrum insecticides used on fruits, vegetables, and forest crops**.** Hexachlorobenzene (BHC) was first manufactured in 1945 for use as a seed treatment, especially for control of bunt wheat (Ritter *et al.,* 1995). By the mid 1980s most nations had ceased its manufacture. Hexachlorobenzene is also generated as an unintentional by-product of the manufacture of pesticides, industrial chemicals (carbon tetrachloride, perchlorethylene, trichloroethylene and pentachlorbenzene, etc.); industrial processes (chlor-alkali plants that utilize graphite in their electrolytic cells, aluminium manufacture, pyrotechnic production, etc.); and as a result of incomplete combustion (burning of municipal waste). BHC has been detected in emissions of a variety of industrial processes, most likely reflecting use of products contaminated with BHC. BHC often appears as a contaminant in chlorinated pesticides (including pentachlorophenol, pentachloronitrobenzene dicloram, dacthal, chlorothalonil, picloram, simazine, and atrazine). Other documented uses include as a raw material for synthetic rubber, a PVC plasticizer, a rubber-peptizing agent in the production of nitroso- and styrene-rubbers, and a chemical intermediate in dyes, and wood preservation.

In humans, acute exposure to BHC has resulted in photosensitive skin lesions, unusual hair growth, colic, severe weakness, kidney and liver damage, central nervous effects (including seizures), circulatory collapse and respiratory depression, debilitation, urinary, arthritic, neurological and metabolic disorders, and death. BHC has been linked to spontaneous abortions in humans and is listed as a possible human carcinogen. Foods of all types, but particularly dairy products and animal meat, from all over the world have been found to contain BHC. Even at low levels, BHC has been observed to produce a variety of adverse affects in aquatic animals, fish, birds and small mammals, such as fatigue and skin irritation, reproductive disorders, kidney and liver damage, cancer and death. BHC has been found in Arctic air, snow, seawater, vegetation

and biota. BHC can be re-released from incineration of waste contaminated with it, while virtually 100 percent of BHC contaminant in pesticides is released to the air, posing a health risk to applicators (RFI, 2001)

They are available as two commercial formulations. Technical-grade BHC is a mixture of isomers containing mostly 64% α-BHC, 10% β-BHC, 13% γ-BHC, 9% δ- BHC, and 1% ε-BHC. The other commercial formulation contains more than 99% γ- BHC (lindane) (FAO/WHO, 2013). Micheal Faraday discovered BHCs in 1825 and Bender determined their insecticidal properties in 1935. Slade showed that γ-BHC was the only active insecticide of technical-grade BHC in 1942. Technical-grade BHC has been commercially produced since 1947. China and India, the two main users of technical-grade BHC globally, stopped its agricultural use in 1983 and 1990, respectively. The half-life of β-BHC in blood is 7 years, whereas γ-BHC has a half-life of only 20 hours (Cox *et al.*, 2013). Molecular structures of BHCs are shown in the figures below.

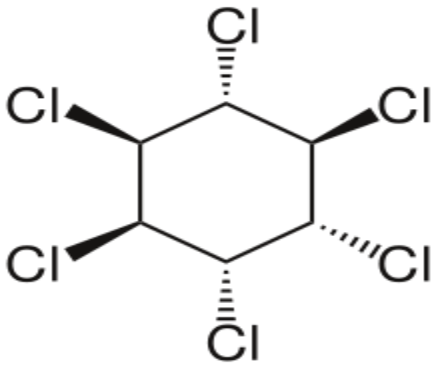


Fig 2.12: α-Benzenehexachloride

(α-BHC)

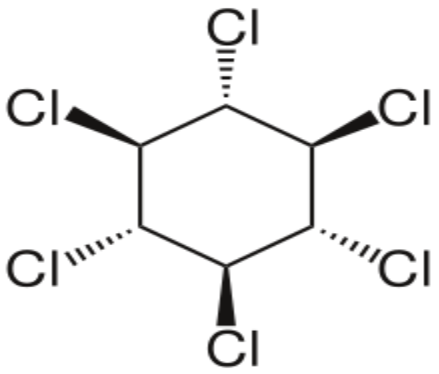


Fig 2.13: Beta- BHC (β -BHC)

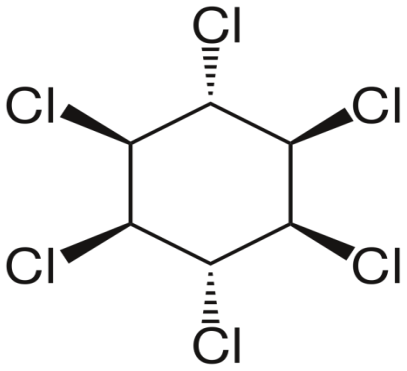


Fig 2.14: Lindane = gamma- BHC (γ -BHC)

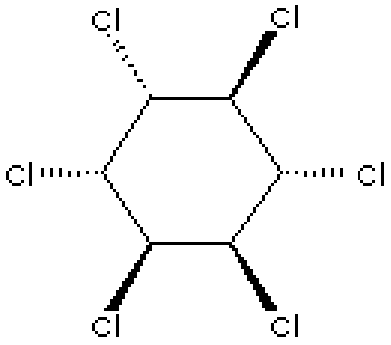


Fig 2.15: Delta – BHC (δ-BHC)

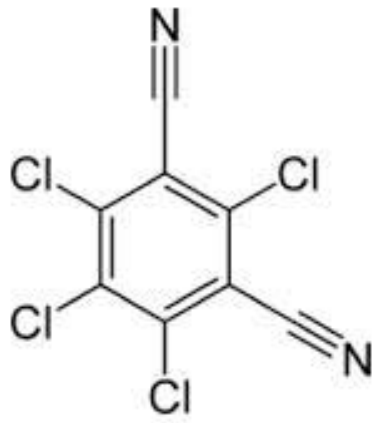


Fig 2.16: Chlorothalonil

2.8 Effects of Organochlorine Pesticides on Human Health

Over 300 foreign chemicals including several known carcinogens have been identified in the adipose tissue and other organs including brain cells and nervous system, the brain and the endocrine (hormonal) glands are the target site for the fat-soluble toxins to accumulate. Continued exposure to these chemicals for a long period may result in symptoms of mild cognitive dysfunction (including problems in identifying words, colours or numbers and unable to speak fluently) and hormonal imbalances leading to infertility, breast pain, menstrual disturbances, adrenal gland exhaustions and early menopause. Eventually these toxins are stored in the fatty body tissues and in cells of the brain. These stored toxins may be slowly released and recirculated in the blood, contributing to many chronic illnesses. Whenever the body is under stress, the stored fat is released along with the toxins and circulates freely throughout the body. The resulting exposure can target various organs and body systems, contributing to many chronic illnesses. The nature of health effects depend on the type of pesticide, dose, timing, duration of exposure and the susceptibility of the exposed individual (Xavier *et al*., 2004).

2.8.1 Cancer

Case–control studies have linked pesticide exposure to childhood cancer (Zahm *et al*., 1997). A number of studies have demonstrated that maternal employment in agriculture has a link with leukemia. The most convincing evidence that herbicides are human carcinogens comes from epidemiological studies (Hoar and Blair, 1986). It is reported that the population living around the active agricultural regions are highly prone to cancer. Thyroid and bone cancers are prevalent in agricultural regions where fungicides are extensively used (Schreinemachers *et al.*, 1999). Recent studies have shown that the incidence of hormone related organ cancers or hormonal cancers have increased among farmers. Exposure to endocrine disrupting pesticides, particularly to DDT and phenoxy herbicides, is the suspected cause in some of these hormonal cancers (Burananatrevedh and Roy, 2001). The association between different types of pesticides and prostate cancer shows moderate risk among farmers exposed to organochlorine insecticides and acaricides specifically DDT and Dicofol (Settimi *et al*., 2003). Over the last 10 years, breast cancer in women has increased worldwide by 33%. Various studies have linked our environment and the substances we are exposed to as prime suspects. There is growing evidence that the breast cancer epidemic is related to exposure to a wide range of environmental contaminants including DDT, other carcinogenic pesticides and oestrogenic stimulants. Organochlorine pesticides such as DDT and its metabolites DDD and DDE, dieldrin, heptachlor, HCH and its isomers were detected in the blood of breast cancer patients, irrespective of age, diet and geographic locations when compared to normal women (Mathur *et al*., 2002).

A study was conducted in New York by Wolff et al. (1993) to determine whether exposure to PCBs and to DDE [1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene], the major metabolite of DDT, is associated with breast cancer risk in women. The study analyzed sera from the stored blood specimens of 14,290 participants enrolled between 1985 and 1991 in the New

York University Women's Health Study, a prospective cohort study of hormones, diet, and cancer. Cohort members who developed breast cancer were included as case patients in a nested case control study.DDE and PCBs were measured by gas chromatography in the sera of 58 women with a diagnosis of breast cancer 1–6 months after they entered the cohort and in 171 matched control subjects from the same study population who did not develop cancer. The results showed that mean levels of DDE and PCBs were higher for breast cancer case patients than for control subjects, but paired differences were statistically significant only for DDE (P = 0.031). After adjustment for first-degree family history of breast cancer, lifetime lactation, and age at first full-term pregnancy, conditional logistic regression analysis showed a fourfold increase in relative risk of breast cancer for an elevation of serum DDE concentrations from 2.0 ng/mL (10th percentile) to 19.1 ng/mL (90th percentile). For PCBs, the relative risk for a change in serum levels from 3.9 ng/mL (10th percentile) to 10.6 ng/mL (90th percentile) was less than twofold, a non-significant association that was further reduced after adjustment for DDE. Therefore, breast cancer was strongly associated with DDE in serum but not with PCBs.

2.8.2 Effect on reproductive system

There is a growing concern that pesticides, having estrogenic property may cause a variety of reproductive disorders in wild life and human populations. Recent in vitro data suggest that the interaction between some weak estrogenic organochlorines, dieldrin, endosulfan, toxaphene and chlordane cause a synergistic increase in their estrogenic potency, an effect due to joint action on estrogenic receptors (Wade *et al*., 1997).

Exposure to pesticides may play a role in adverse pregnancy outcomes (Fowler, 2001). It has been shown that there is detectable levels of 2, 4-D and MCPA ([4 chloro-2 methylphenoxy]

acetic acid in the semen of farmers who recently used the pesticides. As these pesticides can be excreted in the semen, they could be toxic to sperm cells and be transported to the woman and developing embryo/foetus (Tye *et al*., 1994). Farm workers attending the plant protection operations and persons working in the pesticide manufacturing units are more prone to pesticide toxicity. In brief, exposure to pesticides with endocrine disrupting potential raises a particular concern for male fertility because of the possible occurrence of effects at low concentrations and additive interactions with other environmental risk factors. Epidemiological studies have confirmed an increased risk of delayed conception associated with exposure to pesticides. Moreover, an increased risk of spontaneous abortion has been noted among wives of exposed workers (Petrelli and Mantovani, 2002).

Birth abnormalities were reported in the offspring of registered users of pesticide as well as the general population living around agricultural areas (Garry, 1996). Studies show a stronger association between fetal deaths due to congenital abnormalities and residential proximity to applications of pyrethroid and observed elevated risk when the exposure occurred during the third – eighth week of pregnancy (Bell *et al*., 2001). In a study, umbilical cord blood was analyzed in a new-born, whose parents had been exposed to pesticides. The results indicated the presence of detectable DDE, the main metabolite of DDT. There was a positive correlation between maternal DDE and the consumption of fish (Sarcinelli *et al*., 2003). A cohort study of serum shortly after delivery indicated that DDE and other organochlorine pesticides may pose a risk to preterm birth in countries that continue to use such insecticides for malaria control (Torres- Arreola *et al*., 2003).

2.8.3 Endocrine disruption

Endocrine disruptors are exogenous chemical substances that cause adverse effects in the endocrine system. These chemicals mimic the action of hormones and can damage or disrupt the

normal functioning of an organism. In humans, endocrine glands which include adrenal, thyroid, pancreas, ovary and testis produce hormones which are distributed to receptors through the blood stream. Many pesticides acts as endocrine disruptors and affect sperm quality and reproductive development. There is now considerable evidence that male reproductive function is declining in human and wild life populations (Petrelli and Mantovani, 2002), that the mechanism of action may be disturbed testicular apoptosis and altered hepatic biotransformation of steroids. Sexual differentiation is regulated by reproductive hormones. Diethylstilbestrol is the best known endocrine disrupter and has caused abnormalities of sexual differentiation in both exposed female and male human foetuses (Toppari, 2002). Organochlorine contaminants in the human diets relate to the potential ability of many of these chemicals at low doses to act as endocrine disruptors (Smith and Gangolli, 2002). Such chemicals are capable of disrupting the normal functioning of endocrine system and may pose a growing threat to human and wild life health. These compounds can modulate both the endocrine and immune systems resulting in alteration of homeostasis, reproduction, development and behavior. The chemicals in the environment cause endocrine disruption and result in pathological effects on the male and female reproductive system, thyroid function and the central nervous system (Amaral-Mendes, 2002).

2.8.4 Immune system dysfunction

Experimental and epidemiological studies show that many pesticides in widespread use around the world are immunosuppressive. Organochlorine pesticides that are known to alter the immune system are DDT, chlordane, aldrin, lindane, hexachlorobenzene, mirex, and arochlor (a PCB) Frequent exposure of multiple toxins causes the detoxification system to be overloaded and inefficient, leading to the accumulation of toxins, dead cells and microorganism build up in the blood. To combat these foreign bodies, the immune system will produce excessive inflammatory chemicals. Under a hyper excited state, the immune system will produce auto

antibodies. This may lead to symptoms of immune dysfunction such as allergies, inflammatory states, swollen glands, recurrent infections, chronic fatigue syndrome and auto immune diseases (Freidman, 1967; Glick, 1974; Street and Sharma, 1975; Loose *et al*., 1977; Desi *et al*., 1978; Giurgea *et al.*, 1978).

2.8.5 Parkinson's disease

Parkinson's disease (PD) is the most common neurodegenerative disorder. It is now proposed that environmental factors in conjunction with genetic susceptibility may form the underlying molecular basis for idiopathic PD (Uversky *et al*., 2002). Epidemiological and experimental data suggest the potential involvement of specific agents as neurotoxicants such as pesticides (organochlorine and organophosphorus) in the pathogenesis of nigrostriatal degeneration, supporting a relationship between the environment and Parkinson's disease (Di Monte *et al*., 2002). Recent studies show clearly that genetic factors play a minor role in determining whether an individual develops this disease, rekindling an interest in the etiological significance of environmental factors (Lockwood, 2000). *In vitro* studies have provided proof that several pesticides including rotenone stimulate the formation of alpha-synuclein fibrils (one of the principal constituents of Lewy bodies). Moreover, a meta analysis of all case control studies so far showed a positive, statistically significant association between pesticide exposure and Parkinson's disease (Vanacore *et al*., 2002).

2.8.6 Cytotoxic defects

The potential genetic hazard of pesticides to human beings is of great concern. Results from the biological monitoring or cytogenetic methods for the detection of health risks to pesticides have showed DNA damage in peripheral lymphocytes among workers employed in municipality. The observed DNA damage was found to be significantly lower in workers taking some of the necessary safety precautions during their work (Undeger and Basaran, 2002).

Malaoxon is the first and main metabolite that is more toxic than the parental compound, Malathion. Malaoxon can damage DNA in human lymphocyte, by various mechanisms including oxidative damage. Hydrogen peroxide and reactive oxygen species may be involved in the formation of DNA lesions induced by Malaoxon. Malaoxon can also methylate DNA bases (Janusz and Dorota, 2001).

Increased chromatid breaks and chromosomal aberrations in human lymphocytes were observed in workers occupationally exposed to pesticides. Bolognesi et al. (2002) observed micronuclei frequency in peripheral blood lymphocytes among the farm workers, which was more evident among workers who avoided protective measures.

2.8.7 Childhood developmental disorders

A study in India (Kuruganti, 2005) assessed the effect of pesticide exposure on the abilities of children to perform developmental tasks. In all, 1,648 children were individually tested using a rapid assessment tool that measured children‘s memory, stamina, analytical, motor, and tactile perception abilities through various games and activities. The study controlled for major confounding factors such as socio-economic background, maternal education, schooling, media exposure, and the like. In more than 80 percent of the tasks tested, children heavily exposed to pesticides performed significantly worse than the less-exposed children.

Sagiv *et al.* (2010) found higher risk for attention deficit hyperactivity disorder (ADHD) at higher levels of PCBs and p,p‘-DDE in a longitudinal cohort study including 788 mother–infant pairs. The PCB and p,p’-DDE levels in cord serum were moderately associated with attention deficit hyperactivity disorder (ADHD) in children aged 7–11 years born in 1993–1998 in a PCB-contaminated area (ORs = 1.76). These results support the view that low-level prenatal organochlorine exposure is associated with attention deficit hyperactivity disorder (ADHD)-like behaviors in childhood.

A study in Mexico (Guillette *et al*., 1998) compared Yaqui children who were exposed to large amounts of pesticides with children who lived in the foothills, where pesticide use is avoided, found that functionally, the exposed children demonstrated decreases in stamina, gross and fine eye-hand coordination, 30-minute memory and the ability to draw a human figure.

2.8.8 Diabetes

The prevalence of diabetes has been increasing globally over the past few decades (King *et al*., 1998). Recent epidemiologic studies have shown that background exposure to persistent organic pollutants, especially organochlorine pesticides, is strongly associated with type 2 diabetes. Lee *et al*. (2006) demonstrated a very strong relationship between the levels of persistent organic pollutants in serum, particularly oxychlordane and transnonachlor, and the risk of type 2 diabetes in the general American population by extensive analysis of the National Health and Nutrition Examination Survey 1999–2002 data (Lee *et al*., 2006). This association was higher in obese people than among the non-obese. The associations between the serum concentrations of organochlorine pesticides and the prevalence of diabetes were examined in the Mexican-American population (Cox *et al*., 2007) and Korean population (Son *et al*., 2010). Exposure of *p, p*‘-DDE was related to the incidence of diabetes in a cohort of Great Lakes sport fish consumers from 1994 to 2005 (Turyk *et al*., 2009). Lee *et al.* (2010) also investigated whether several persistent organic pollutants predict the risk of type 2 diabetes within the Coronary Artery Risk Development in Young Adults (CARDIA) cohort (Lee *et al*., 2010). Some persistent organic pollutants, such as trans-nonachlor and highly chlorinated PCBs, were associated with the incidence of type 2 diabetes over an 18-year period, especially in obese people. Persistent organic pollutants showed strong associations at relatively low exposures, resulting in inverted U-shaped dose–response curves instead of the traditional dose – response relationship with diabetes.

2.9 Extraction Methods and Clean-up of OCPs

Animal feed as well as animal fat are considered a very complex matrices with large number of components especially lipids. Consequently, the development of sensitive methods for its analysis with elimination of interferent compounds and enough efficiency in term of analyte recovery represents an interesting task. (Weichbrodt *et al*., 2000; Suchan *et al*., 2001) The most intricate step in these procedures is represented by the sample extraction and clean-up that should be efficient enough to allow a reliable screening of contaminated samples. The selection of suitable solvent (s) and extraction method is critical for obtaining satisfactory recovery of OCPs from the food matrix. Of course, if co-extracted materials are minimized in the extract, the clean-up procedure would become simpler. Owing to the lipophilicity of OCPs, organic solvent (s) normally can extract OCPs form food efficiently but lipids are also co-extracted. Solid-liquid extraction method was applicable for extracting OCPs from various types of food samples including vegetables, liver, meats and its products, fish, eggs and animal fats. In addition, several standardized methods, including AOAC 970.52, EN 1528 and EN 12393, have employed such solid-liquid or liquid-liquid extraction techniques for the determination of OCPs in both fatty and non-fatty foods.(Vetter *et al*., 1998; Barriader-Perreira *et al*., 2008; Wilkowska and Biziuk, 2010) In some occasions, sonication or Polytron was also applied to improve the extraction efficiency and recoveries.

2.9.1 Clean-up methods

Matrix constituents can be co-extracted and later co-eluted with analyzed components and can consequently interfere with analyte identification and quantification. Moreover, co-extracted compounds, especially lipids, tend to adsorb in GC system such as injection port and column, resulting in poor chromatographic performance. A thorough clean-up minimizes such

matrix issues, improves sensitivity, permits more consistent and repeatable results, and extend the capillary column lifetime. Several approaches have been attempted to eliminated co-extracted interferences from extracts, including freezing centrifugation or filtration, liquid-liquid partitioning, gel permeation chromatography(GPC), solid phase extraction (SPE) and solid-phase micro-extraction (SPME). The simplest approach to remove the fatty co-extracted is by freezing centrifugation. (Yague *et al*., 2001; Karasova *et al*., 2003) The logic behind is that fatty substances (mainly lipids) have lower melting point than the solvent so that frozen lipids can be removed by centrifugation or filtering while OCPs remain dissolved in the solvent. Different freezing temperatures ranged from -24^{o}C to -70^{o}C have been used. However, the solubility of lipids in solvent not only depends on the temperature but also the solubility product. Therefore this technique can remove significant amount of lipids for some food matrix but not for every matrix. Certain amount of lipids would remain in the solvent after the freezing centrifugation step and hence, further cleanup is required.

Using materials with large surfaces area for absorption of lipids have been employed since early 1970s. These materials include, Florisil, Lipid Removal Agent (LRA) media from Supelco, micro Cel E and Calflo E from Johns-Manville. Micro Cel E and Calflo E and LRA are synthetic Calcium silicate while Florisil is magnesium silicate with high specific surface area. (Schenck *et al*., 1995; Yague *et al*., 2002] They can be applied to remove lipids either in sample preparation, solid phase extraction step or during sample clean-up step, with minimal effect on non-lipid chemicals. When food sample is mixed with these lipids absorbing materials, edible fat could be removed. Therefore, it is common to conduct a clean-up step by solid phase extraction (SPE) nowadays. Both, conventional glass column packed with sorbent(s) and ready-to-use cartridges have been utilized and the common used phases are silica, Florisil, alumina and C18-bounded silica. Doong and Lee compared the cleaning efficiency of ready-to-use cartridge filled

with three different adsorbents for shellfish extract. (Long *et al*., 1991; Lotte and Barker, 1993; Schenck *et al*., 1996) Their results demonstrated that out of 14 OCPs tested, two were retained in the C18-cartridge. As for alumina and Florisil SPE, though all 14 pesticides tested could be recovered, Florisil provide better results in term of recoveries, repeatability and removal of interfering substances. Similarly, Long *et al*., also showed that Florisil had better cleaning efficiency of fatty acids in fish extract when compared with C18. Besides, recoveries of some OCPs were poor with hexane as eluent and these more polar OCPs could be eluted out from the column with acetone.

Bazulic et al. (1998) reported also that the quality of Florisil was important in avoiding possible interference and misinterpretation of results. Even though GC-MS was employed as the detection system, poor quality Florisil could introduce false positive results for lindane and dieldrin (Long *et al*., 1991; Valsamaki *et al*., 2006) To sum up, the combination of sorbent(s) and eluting solvent(s) have to be chosen very carefully. Otherwise, some OCPs or their metabolites/derivatives would be lost during the clean-up step (Rogers, 1972; Porter and Burke, 1973; Bong, 1975). These OCPs could either break down or adhere to the sorbent material, leading to low or even no recovery. Finding of the optima clean-up conditions is an art itself. As the targeted OCPs might cover a wide range of polarities, it is quite difficult to find the best combination of SPE column material and eluting solvent, which permits recovering the polar OCPs (but leaving the polar interferents behind on the column), as well as recovering the non-polar OCPs (without eluting any residual oil present in the extract from the column).

2.10 Detection Techniques of OCPs

A number of different selective detectors can be coupled with GC for analyzing OCPs, including electron capture detector (ECD), halogen specific detector (XSD), electrolytic conductivity detector (ELCD) and atomic emission detector (AED). GC-ECD is the most

commonly used detection method with low detection limits. It is particularly useful for detecting halogen containing molecules. However, other organic molecules, such as aromatic compounds, would also give positive signal. Users have to confirm the presence of OCPs by another confirmative technique. Even though the above-mentioned selective detectors can be used for quantification, it is unlike to fulfill the European Commission's stringent requirements as set for pesticides analysis. Confirmation with GC-hyphenated with mass spectrometric (MS) detector is normally required. Single quadruple MS detector running in electron ionization (EI) mode with target analytes monitored by selective ion monitoring (SIM) becomes a routine monitoring tool for OCPs nowadays. Since some OCPs are electronegative in nature, GC-MS detector under negative chemical ionization mode with methaneas reagent gas could provide better sensitivity. [Goodspeed and Chestnut, 1991; Bazulic *et al*., 1998; Beyer *et al*., 1998; Rosenblum *et al*., 2001) To further increase confidence in confirmative analysis, GC coupled with tandem MS is one of suitable techniques. Besides providing a more definitive detection tool, tandem MS also decrease matrix interferences, improves selectivity and achieves higher signal-to-noise ratio and subsequently improves the detection limit. Both tandem-in-time (ion-trap) and tandem-in-space (triple quadruples) detectors have been applied for OCPs residues analysis in different matrices. The determination of pesticides residues in the environment and in food is necessary for ensuring that human exposure to contaminants, especially by dietary intake, does not exceed acceptable level for health. Consequently, robust analytical methods have to be validated for carrying out both research and monitoring programmes, and thus for defining limitations and supporting enforcement of regulations. In this field, reproducible analytical methods are required to allow the effective separation, selective identification and accurate quantification of pesticides analyses at low levels in food-stuff including food of animal origin.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

Blender

Analytical balance (range 0 - 120g, precision ± 0.001g) (Denver Instrument xp-200)

Analytical balance (range 0 - 500g, precision ± 0.1g) (Denver Instrument xp-600)

Balance (range 0 – 1,200g, precision ± 0.1g) (Denver Instrument xp-2000)

Soxhlet apparatus and Soxhlet thimble

Round bottom flasks: volumes 10ml, 50ml, 100ml, 250ml

Pasteur pipette: length 150mm and 250mm

Glass bottles (sample vials with Teflon seal)

Rotary evaporator (Buchi, Switzerland)

Desiccators

Florisil (Merck)

Oven (Gallenkamp, England)

Pipette

Muffle furnace (Carbolite, England)

Quartz fiber crucible

Glass funnels

3.2 Reagents

All reagents used in the extraction and determination of the OCPs were purchased from Sigma - Aldrich.

Petroleum ether (60-80^{O}C)

Petroleum ether (40 – 60 ^{o}C).

Petroleum ether /acetone (80:20% v/v)

Acetonitrile

Potassium Oxalate solution

Anhydrous Sodium Sulphate

Methanol

Diethyl ether

n- Hexane

Dichloromethane

Pesticide standard of Analytical grade 10ng/µL (from Dr. Ehrenstorfer, Germany)

3.3 Study Area

Zaria is located at approximately 11^{o}3’ north of the equator and on longitude 7^{o} 42’ East of the Greenwich meridian and at about 660m above sea level. It is the second largest city in Kaduna state after Kaduna town north ward along Kaduna-Kano highway.

The climate of Zaria is a tropical savanna climate, with distinct wet and dry seasons (Aw climate Koppens classification). Zaria experiences six (6) months of rainy season and six (6) months of dry season. The rainy season is from May to late October, while the dry season is from early November to April, this is as a result of the interplay of the two dominant air masses within the region i.e. the tropical continental air messes (cT) and Tropical maritime air masses (mT) (Iguisi and Abubakar, 1998).

The region generally falls within the Guinea Savannah vegetation. The climax climatic vegetation of the area ought to be northern Guinea Savannah, but because nearly all vegetation within the stream system has been degraded due to man's activities such as intense cultivation, fuel wool felling, the real climax vegetation is almost absent. What is seen presently are few scattered trees interspaced with tall tree grasses about 1-15m and 2-5m respectively.

The drainage system focuses on River Galma and Kubanni River. River Galma is a major tributary of River Kaduna and Kubanni River, on which Ahmadu Bello University Dam is situated.

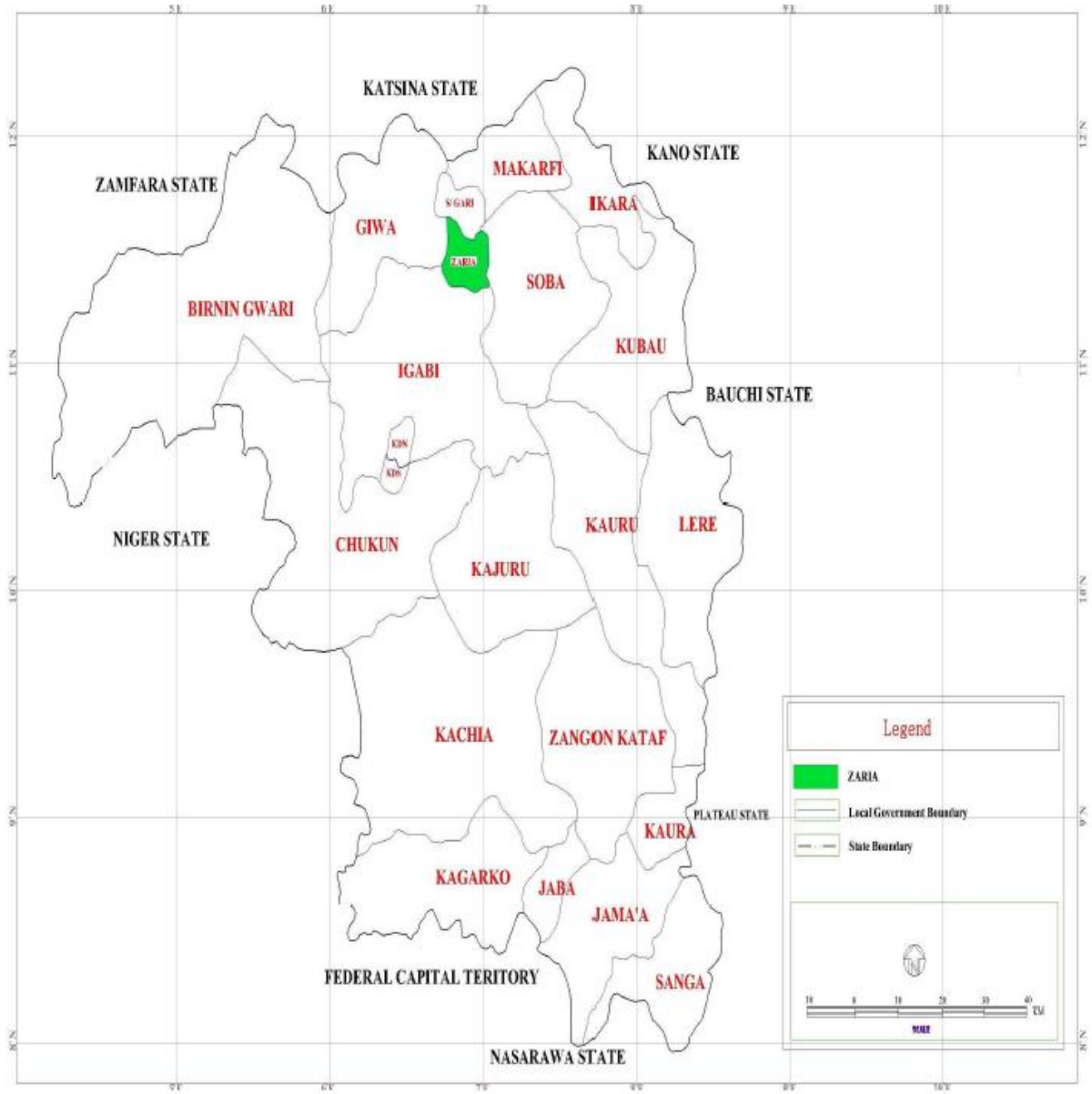


Fig 3.1: Map of Kaduna showing zaria.

3.4 Sampling

Fresh samples of liver were collected from the central slaughter house in Zango, Zaria. Only the Liver of Male cattle (age groups: <11 months, 12-20 months and ≥24 months) sourced from Zaria were collected (this is because, the OCP concentrations in female cattle vary during lactation and gestation). It is important to vary the age as bioaccumulation studies have shown that OCP concentration varies with the duration of exposure of the animal (Forget *et al.*, 2001). Four (4) potions of each Liver sample were collected and nine (9) liver samples were collected making a total of thirty-six (36 samples). The samples were washed with distilled water and temporarily stored in a clean plastic container. The containers were then transported immediately to the laboratory for the preparation of the Liver samples (this was to prevent decomposition). Proximate analyses of the representative Liver samples were also carried out. Only liver samples from the N'dama specie were used for the analysis.

3.5 Sample Preparation

Each sample was individually blended. Three portions were used for proximate analysis (moisture content, ash content and lipid content) while the fourth portion was used for OCP determination.

3.5.1 Pre-treatment of sodium sulphate

Three batches of 1000 mg of sodium sulphate were dehydrated for 8 hours at 600^0C, each in a porcelain dish (diameter 180mm). They were allowed to cool and transferred to the original storage bottles.

3.6 Sample Extraction

From the homogenized sample, an exact weight of 50g was mixed with 25g anhydrous sodium sulfate in a mortar. This mixture was then transferred to a clean thimble and extracted with petroleum ether (60-80^{O}C) in a soxhlet apparatus for about ten hours. The extracts was transferred to a weighed round bottom flask and the solvent was evaporated using a rotary evaporator at 35ºC and 350 mbar almost to dryness (~3cm^{3}) , then to dryness using a steam bath. The flask was left overnight in a desiccator and finally weighed to the nearest 0.1 mg (AOAC, 2001).

3.7 Column Clean-up

A column was developed using Florisil. This Florisil was activated overnight at 550ºC, then cooled in a desiccator and kept in a closed container. The needed amount was heated once again at 130ºC for 5 hours, cooled in the desiccator and mixed with doubly distilled water to give 3%(w/w) water in Florisil. This mixture was shaken for 30 minutes.

Exactly 40g Florisil (3% w/w water content) was used to develop a chromatographic column (Length= 50 cm, internal diameter = 20mm with Teflon stopcock) which contains 100 ml petroleum ether (40 – 60 ºC). As the Florisil settles down, the remaining petroleum ether was drained to about 1 cm above the Florisil level.

The residue extracted was dissolved in 10 ml petroleum ether and transferred with a Pasteur pipette to the Florisil column which was eluted with 300 ml petroleum ether /acetone (80:20% v/v) at a maximum flow rate of 5ml/min. The eluate was then evaporated using a rotary evaporator at 35ºC and 350 mbar almost to dryness, then to dryness using a steam bath. The residue was then dissolved in 2 ml n-hexane.

3.7.1 Preparation of stock solution

0.5ml of the standard solution was transferred into a 10ml flat bottom flask which was previously rinsed and dried with n- hexane. The solution was made up to 10ml with n- hexane resulting in a concentration of 5ng/µL.

From the resulting stock solution, five quantification standards were prepared for multiple calibration using serial dilutions preparing 1:10, 1:100, 1:1000 and 1:10000 potions resulting in a concentration range between 5 – 500 pg/µL.

3.8. Proximate Composition

3.8.1 Determination of moisture content (AOAC, 2011)

A clean crucible was dried to a constant weight in an oven at 105^0C, cooled in a desiccator and weighed (W_1). Two grams of the sample was weighed into the crucible (W_2) and dried in the oven at 105^0C for 8 hours. The crucible and its content were cooled in a desiccator and reweighed. The procedure was continued until a constant weight was obtained (W_3). The moisture content was calculated by the formula:

$$\% \text{ Moisture} = (W_2 - W_3) / (W_2 - W_1) \times 100$$

3.8.2 Determination of ash content (AOAC, 2011)

Exactly 10g of the finely ground sample was weighed into a previously weighed clean crucible (W_1) which had been ignited and cooled in a desiccator. The sample was then pre-dried over a steam bath until a constant weight was attained (W_2). The crucible was then transferred to a muffle furnace which was maintained at 550^0C for 8 hours, the crucible and its residual ash

was allowed to cool in a desiccator. The procedure was continued until a constant weight was obtained (W_3).

The ash content was calculated thus:

$$\% \text{ Ash} = (W_3 - W_1) / (W_2 - W_1) \times 100$$

3.8.3 Determination of lipid content (AOAC, 2008)

Exactly 200cm^3 of petroleum ether was transferred into a clean dry 250ml round bottom flask fitted with soxhlet extraction unit. Some boiling chips were also added. Fat free extraction thimble (W_1) containing 10 grams of sample (W_2) was then fixed into the soxhlet extraction unit with forceps and the cold water circulation was kept on. The heating mantle was switched on and heating rate was adjusted to between 40-60^{0}C until the solvent reflux at a steady rate. The extraction process was carried out for 8 hours after which the heating mantle was switched off. The thimble was then removed and dried to a constant weight in an oven at 70^{0}C and reweighed (W_3).

The extractable Lipid of the sample was calculated using:

$$\% \text{ Lipid} = \text{Weight of Lipid extracted} / \text{Weight of direct sample} \times 100$$

$$= (W_2 - W_3) / (W_2 - W_1) \times 100$$

3.9. Determination of Pesticide Residue

The determination was carried out using Gas Chromatography coupled with Electron Capture Detector (GC-ECD) at National Agency for Food and Drug Administration and Control (NAFDAC), Oshodi, Lagos. The Injection syringe used was 5µL while separation capillary was

25m long and 0.25mm internal diameter packed with 5-%-Phenyl-95-%-methylpolysiloxane. Injection and separation conditions are; Helium (He) carrier gas at a flow velocity was 35 cm/s, gas flow at split outlet of 50 ± 10 mL He/min at injector temperature of 280^0C. GC/ECD interface temperature was 260^0C. Hot needle injection of 1µL samples with a 2 minutes waiting period before opening of the split valve. Split ratio was 1:10. Temperature programme was adjusted as follows; 60^0C with a two minutes waiting period. It was then increased to 180^0C at a rate of 30^0C/min and finally to 280^0C at 3^0C/min.

3.10 Statistical Analysis

Results obtained were subjected to the following statistical analysis:

i. Analysis of variance (ANOVA) using the Tukey range test (one-way) at $P<0.05$ to compare the OCP concentrations in the liver samples obtained from the three groups.

ii. Two-tailed Pearson's correlation of the OCP concentrations with some proximate parameters to identify the factors that promotes the bioaccumulation of the OCPs.

CHAPTER FOUR

4.0 RESULTS

4.1. Proximate Parameters of Liver Samples

4.1.1 Percentage lipid content

Tables 4.1 shows the results for the average percentage lipid content of the liver samples collected in the age groups. All results were expressed in mean ± Standard Deviation (mean ± S.D.).

Table 4.1: Percentage lipid content of liver Samples

Samples	Below 11 months	Between 12 – 20 months	Above24 months
Sample 1	3.89 ± 0.005	5.06 ± 0.006	6.19 ± 0.071
Sample 2	4.06 ± 0.057	5.12 ± 0.021	5.87 ± 0.012
Sample 3	3.74 ± 0.025	4.71 ± 0.015	5.78 ± 0.038

Values are means ± S.D, n=3

4.1.2 Percentage ash content

Tables 4.2 shows the results for the average percentage ash content of the liver samples collected in the age groups. All results were expressed in mean ± S.D.

Table 4.2: Percentage ash content of liver Samples

Samples	Below 11 months	Between 12 – 20 months	Above24 months
Sample 1	2.62 ± 0.028	2.86 ± 0.006	3.19 ± 0.071
Sample 2	2.62 ± 0.057	2.72 ± 0.021	3.17 ± 0.012
Sample 3	2.05 ± 0.025	2.71 ± 0.015	3.12 ± 0.038

Values are means ± S.D, n=3

4.1.3 Percentage moisture content

Tables 4.3 shows the results for the average percentage moisture content of the liver samples collected in the age groups. All results were expressed in mean ± S.D.

Table 4.3: Percentage moisture content of liver Samples

Samples	Below 11 months	Between 12 – 20 months	Above24 months
Sample 1	67.29 ± 0.068	69.27 ± 0.009	69.03 ± 0.214
Sample 2	70.10 ± 0.105	70.90 ± 0.125	68.38 ± 0.530
Sample 3	70.12 ± 0.025	69.40 ± 0.290	70.08 ± 0.053

Values are means ± S.D, n=3

Table 4.4 is the Detection Limits of the seventeen (17) OCPs of interest as obtained by the GC-ECD. The results obtained were expressed in 6 decimal places and in mg/kg of samples used.

Table 4.4: Result of the concentrations of OCPs in liver samples using GC-ECD

PESTCIDES (OCPs) ($\times 10^{-1}$mg/kg)	Below 11 months			12 – 20 months			above 24 months		
	sample 1	sample 7	sample 8	sample 2	sample 3	sample 9	sample 4	sample 5	sample 6
Alpha- BHC	0.475343	**ND**	0.001017	1.02223	2.28785	0.171252	4.88288	1.32841	0.203455
Beta- BHC	0.269376	1.55151	0.974023	1.09281	2.31858	2.6743	4.87707	16.4729	5.58072
Lindane	0.441445	1.04573	0.269461	1.0543	2.30791	0.458793	4.87183	1.84741	0.468776
Chlorothalonil	0.428643	0.352138	0.59759	1.02052	2.25981	1.0824	4.89911	3.40571	1.28073
Delta-BHC	0.424605	1.46152	0.353206	1.01412	2.30329	1.36022	4.8834	5.44977	1.58396
Heptachlor	0.438931	1.86876	1.05022	1.07046	2.35123	5.06897	4.85152	19.7629	9.01063
Aldrin	0.440603	3.68755	3.90465	1.05048	2.3431	9.0736	4.8586	13.4541	0.112339
Heptachlor epoxid	0.438456	3.40189	0.128724	1.07634	2.35092	8.48702	4.85052	21.0545	8.99254
Endosulfan	0.440216	0.201208	0.118725	1.06998	2.34938	0.424615	4.85223	108.6605	0.251028
Dieldrin	0.437042	3.36134	5.23035	1.07066	2.34558	0.198991	4.85393	46.6452	0.216229
Endrin	0.434524	0.5203	0.162453	1.01697	2.31596	0.791628	4.87677	129.0016	102.489
Endosulfan II	0.433562	4.47041	2.07583	1.0622	2.33126	0.100711	4.8617	30.0761	**ND**
p, p' - DDD	0.400152	2.45223	1.47404	1.16703	2.32549	4.71813	4.84638	11.7731	5.35009
Endosulfan sulfate	0.459803	0.169513	9.94474	0.86727	2.28844	0.338044	4.91482	36.1977	0.161105
p, p' - DDT	0.417697	0.120226	8.807	1.06672	2.37782	0.239011	4.84376	23.0525	8.24662
Lambda- cyhalothrin	0.373642	0.801462	1.03609	0.86727	2.3499	2.55151	4.89922	4.98807	1.31668
permethrin	0.555056	**ND**	**ND**	1.44711	2.3736	0.520416	4.75563	**ND**	0.891922

ND: Not Detected

Table 4.5: Mean concentrations of the OCPs in Liver samples

The average concentrations of organochlorine pesticides in the Liver samples are shown in Table 4.5. A comparison of the concentrations in the three categories (below 11 months, between 12 – 20 months and 24 months) is expressed as mean ± S.D is shown in Table 4.5.

PESTICIDES (OCPs)	Below 11 months	12 – 20 months	24 months Above
Alpha - BHC	0.02 ± 0.027	0.11 ± 0.106	0.21 ± 0.244
Beta - BHC	0.09 ± 0.064 [a]	0.20 ± 0.082 [a]	2.57 ± 1.669[b]
Lindane	0.06 ± 0.041	0.13 ± 0.094	0.24 ± 0.225
Chlorothalonil	0.05 ± 0.012 [a]	0.13 ± 0.070[a]	0.32 ± 0.018[b]
Delta - BHC	0.07 ± 0.062 [a]	0.16 ± 0.067 [a]	0.90 ± 0.209 [b]
Heptachlor	0.10 ± 0.062 [a]	0.28 ± 0.204 [a]	1.12 ± 0.569 [b]
Aldrin	0.27 ± 0.194	0.42 ± 0.431	0.61 ± 0.676
Heptachlor epoxide	0.13 ± 0.180	0.40 ± 0.396	1.16 ± 0.842
Endosulfan	0.02 ± 0. 016[a]	0.13 ± 0.097 [a]	3.79 ± 0..613[b]
Dieldrin	0.20 ± 0.197 [a]	0.12 ± 0.107 [a]	1.72 ± 0.566 [b]
Endrin	0.04 ± 0.019 [a]	0.14 ± 0.082 [a]	7.88 ± 6.537 [b]
Endosulfan II	0.22 ± 0.167 [a]	0.12 ± 0.112 [a]	1.56 ± 1.014[b]
pp'- DDD	0.14 ± 0.102 [a]	0.27 ± 0.081 [a]	0.73 ± 0.390 [b]
Endosulfan sulfate	0.35 ± 0.460	0.12 ± 0.101	1.38 ± 1.960
pp-DDT	0.31 ± 0.493 [a]	0.12 ± 0.108 [a]	1.20 ± 0.126 [b]
Lambda-cyhalothrin	0.07 ± 0.033 [a]	0.19 ± 0.092[ab]	0.37 ± 0.209 [b]
Permethrin	0.02 ± 0.026	0.14 ± 0.930	0.18 ± 0.252

Values are means ± S.D, n=3, values with different superscripts across the row are statistically significantly different (p□ 0.05)

Fig 4.1 shows the graphical comparison between the distribution of the 17 OCPs in the liver samples and their respective MRLs as set by FAO/WHO. It gives a 3- dimensional presentation for the OCP concentration in all three categories of the Liver samples collected from Zaria.

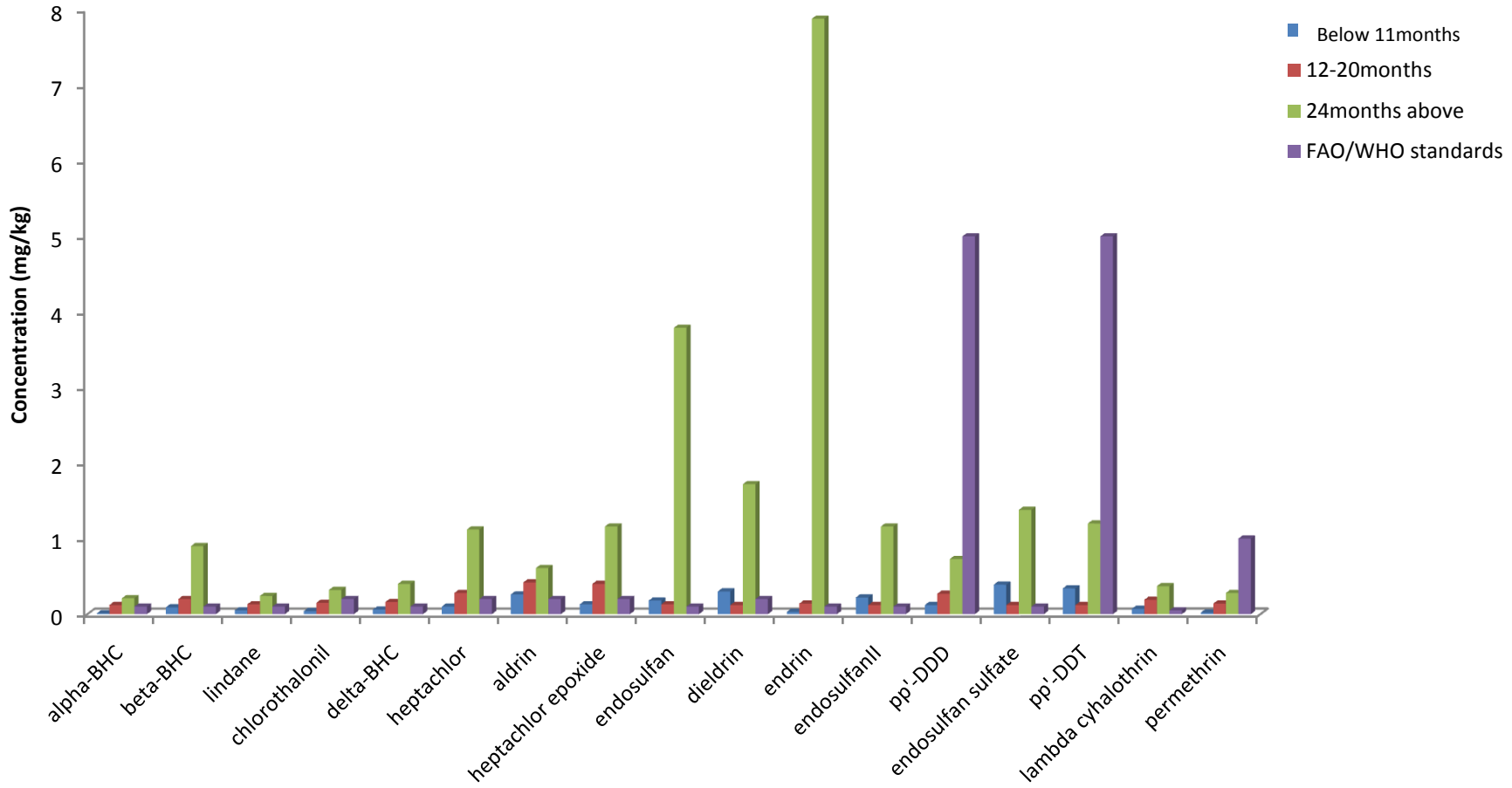


Fig 4.1: A comparison of the concentration of OCPs from samples to FAO/WHO standards

Table 4.6: Correlation matrix of different parameters associated with OCP contamination.

	Alpha- BHC	Beta- BHC	Lindane	Chloro-thalonil	Delta-BHC	Hepta-chlor	Aldrin	Heptachlor epoxide	Endosulfan	Dieldrin	Endrin	Endosulfan II	p, p' - DDD	Endosulfan sulfate	p, p' - DDT	Lambda-cyhalothrin	permethrin	lipid	moisture	ash
Alpha- BHC	1																			
Beta- BHC	0.288	1																		
Lindane	0.904**	0.31	1																	
Chlorothalonil	0.879**	0.614	0.917**	1																
Delta-BHC	0.698*	0.82**	0.791**	0.930**	1															
Heptachlor	0.235	0.985**	0.211	0.54	0.755*	1														
Aldrin	-0.006	0.754*	0.211	0.452	0.647*	0.736*	1													
Heptachlor epoxide	0.164	0.955**	0.17	0.492	0.727*	0.983**	0.800**	1												
Endosulfan	0.049	0.932**	0.163	0.447	0.690*	0.887**	0.782**	0.863**	1											
Dieldrin	0.072	0.925**	0.194	0.476	0.700*	0.871**	0.787**	0.836**	0.993**	1										
Endrin	0.17	0.872**	0.011	0.338	0.542	0.916**	0.431	0.862**	0.745*	0.723*	1									
Endosulfan II	0.101	0.923**	0.259	0.501	0.745*	0.861**	0.789**	0.835**	0.985**	0.987**	0.698*	1								
p, p' – DDD	0.314	0.968**	0.331	0.632	0.843**	0.979**	0.803**	0.979**	0.843**	0.833**	0.836**	0.838**	1							
Endosulfan sulphate	0.033	0.877**	0.161	0.442	0.962**	0.817**	0.774**	0.770**	0.962**	0.985**	0.673*	0.958**	0.777**	1						
p, p' – DDT	0.13	0.899**	0.122	0.445	0.891**	0.872**	0.637*	0.796**	0.891**	0.917**	0.835**	0.873**	0.813**	0.938**	1					
Lambda-cyhalothrin	0.667*	0.764*	0.763*	0.932**	0.637*	0.709*	0.737*	0.703*	0.637*	0.658*	0.442	0.674*	0.804**	0.626	0.586	1				
Permethrin	0.924**	-0.035	0.886**	0.748*	-0.222	-0.102	-0.151	-0.146	-0.222	-0.196	-0.207	-0.161	0.007	-0.222	-0.197	0.505	1			
Lipid	0.810**	0.655*	0.692*	0.839**	0.697*	0.654*	0.315	0.613*	0.697*	0.391	0.585*	0.407	0.707**	0.309	0.622*	0.766**	0.764**	1		
Moisture	-0.38	-0.277	-0.08	-0.175	-0.356	-0.259	-0.228	-0.257	-0.356	-0.336	-0.177	-0.322	-0.23	-0.282	-0.173	-0.193	-0.243	-0.132	1	
Ash	0.685*	.635*	0.571	0.696*	0.426	0.641*	0.254	0.63	0.426	0.377	0.601	0.418	0.657*	0.253	0.33	0.462	0.432	0.512	0.456	1

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

CHAPTER FIVE

5.0 DISCUSSION

5.1 Discussion

Table 4.1 shows the average percentage lipid content of the liver samples for the three age groups (below 11months, between 12 -20months and above 24months) used in the research. Samples from cattle above 24months had the highest percentage lipid content. The steady increase in lipid content observed within the groups was important in explaining the observed increase in OCP concentrations observed in Table 4.5.

Table 4.2 and Table 4.3 shows the average percentage ash and moisture content of the liver samples for the three age groups (below 11months, between 12 -20months and above 24months) used in the research. Although no correlation was observed between the OCP concentrations and the percentage moisture and ash content (Table 4.6), it was important to see if a relationship existed between them.

Appendix II shows a significant difference for the percentage lipid content across the groups at 95% confidence level. The increase in the percentage lipid content is expected with increase in age of the cattle and this is important in explaining the observed changes in the OCP concentrations.

Table 4.4 showed the GC-ECD results for concentration of OCPs in the liver samples for all three groups. The results are expressed in mg/kg of the sample used in the analysis. From the table, fourteen (14) OCPs were found in all nine samples while α-BHC and Endosulfan II were found in eight samples. Permethrin was found in six samples. The table also shows the Maximum Residual Limits (MRLs) as approved by FAO/WHO.

Table 4.5 shows the concentration expressed in mg/kg of seventeen OCPs isolated, identified and quantified from ten liver samples using GC-ECD. From the results, endrin has the highest concentration of 7.88 mg/kg while α-BHC had the lowest concentration of 0.21 mg/kg. β-BHC, δ-BHC, γ-BHC, chlorothalonil, heptachlor, heptachlor epoxide, aldrin, cis-endosulfan, endosulfan sulphate, endrin, dieldrin, DDT and DDD and lambda cyhalothrin were found in all sample while α-BHC and trans-endosulfan where found in nine (9) samples. Permethrin was isolated from seven (7) of the ten samples.

There was asignificant difference (p□0.05) between cattle of 12-20m0nths and above 24months as seen in the table 4.5. The result also shows the concentrations of these OCPs to be greater than the MRLs as set by FAO/WHO. This may be due to the high usage of these pesticides in Zaria or their continuous usage despite their ban. The high concentration may also be due to their bio-accumulation (ability to be present in living tissues at concentrations higher than obtainable in the surrounding environment).

The high concentration of endrin and endosulfan can best be explained by the wide usage of these pesticides by farmers in Zaria before its ban and the loose but continuous usage in present day to eliminate lice, ticks and crop pest. In Zaria, Rice, Corn, Potato and Cotton crops are cultivated in commercial qualities and endrin and endosulfan are the primary pesticides used against the soil and crop pest associated with these crops. Aldrin is also majorly used in Zaria for these purposes as well but, Aldrin readily converts to Dieldrin in plants and animals and therefore, residues of Aldrin are found in small amounts (John, 2007; Fred, 2013). This accounts for the low concentration of Aldrin detected in the liver samples.

In Nigeria, Organochlrorine pesticides is been regulated by National Agency for Food and Drug Administration. NAFDAC upholds the MRLs for the various Organochlorines as set

by food and Agricultural Organisation (FAO) and World Health Organization (WHO). The MRLs for meat as set by FAO/WHO are thus; 5 mg/kg DDT and its metabolites, 0.2 mg/kg Aldrin and Dieldrin, 0.2 mg/kg Chlorothalonil, 0.05 mg/kg Cyhalothrin, 0.1 mg/kg Endosulfan, 0.1 mg/kg Endrin, 0.2 mg/kg Heptachlor, 0.1 mg/kg Lindane and 1.0 mg/kg Permethrin. Hence, meat with more than these concentrations are deemed contaminated and unfit for human consumption.

As shown in Table 4.4, seventeen (17) OCPs were determined in nine (9) liver samples obtained from male cattle reared and slaughtered within Zaria, Nigeria. The OCPs of interest were chosen from interaction with farmers, timber sellers, market vendors of these pesticides and interaction with Veterinarians.

In this study, all samples analyzed showed the presence of OCPs. Regardless of the age of the sample, there was significant isolation from the samples. Samples from cattle above 24 months were found to contain higher concentration of OCPs than samples from younger cattle. The higher concentrations could be explained as a result of the bio- accumulation resulting from their low chemical and biological degradation rates, stability to oxidation and high lipophilicity coupled to the prolong exposure of the cattle to these OCPs (Forget *et al.,* 2005).

5.2 Incidence of Contamination with The Different Organochlorines

Table 4.5 summarises the average concentrations of the OCPs in cattle liver. Generally, the incidence of contamination of the examined samples by the OCPs followed the order; endrin > endosulfan > dieldrin > endosulfan sulfate > p,p'-DDT > heptachlor epoxide > heptachlor > endosulfanII > β-BHC > p,p'-DDD > aldrin > δ-BHC > lambda cyhalothrin > Chlorothalonil > permethrin > lindane (γ-BHC) > α-BHC. The high incidence of endrin, endosulfan and dieldrin results from their high usage in past decades by farmers and timber markets in Zaria. High

incidence of dieldrin and low incidence of Aldrin results from the conversion of aldrin to dieldrin hence, levels of dieldrin reflects the total concentration of dieldrin and aldrin (John, 2007; Fred, 2013).

Although, the residual contents of some Organochlorines detected in samples analyzed were significantly low, the concentrations of a majority appear to be higher than the Maximum Residual Limits as set by FAO/WHO codex Alimentarius commission. The result obtained however agrees with those described by Letta *et al.* (2013) which used liver, kidney and meat matrices collected from slaughter houses in West Shoa Zone, Ethiopia. Letta *et al*. obtained concentration range of 0.001 – 7 mg/kg.

5.2.1 Benzenehexachlorides (BHCs) or Hexachlorohexanes (HCHs)

All five members of the BHCs (α-BHC, β-BHC, δ-BHC, lindane or γ-BHC and chlorothalonil were found to be present in all samples collected regardless of the age group of the sample with highest mean values of 0.21 mg/kg, 2.57 mg/kg, 0.9 mg/kg, 0.24mg/kg and 0.32mg/kg respectively. The level of α-BHC and γ-BHC were not significantly different in all age groups as expressed in Table 4.5. However, level of β-BHC, δ-BHC and chlorothalonil were significantly higher in samples from cattle above 24 months indicating that with higher % lipid contents, the bio-accumulation of the Benzenhexachlorides is evident. Thus, we could infer the high exposure of the cattle to these OCPs (β-BHC, δ-BHC and chlorothalonil) and thus their subsequent bio-accumulation.

All BHC isomers were present at levels lower than their respective MRLS in samples below 11 months and 12 - 20 months however, significant bioaccumulation was observed in older samples (above 24 months) with OCP levels above the MRLs. This higher % lipid contents

as reported in Table 4.1 and corresponding high OCP levels is more evidence that shows the lipophilicity of the OCPs and the subsequent bioaccumulation.

The present result revealed that the frequency of different isomers of BHC residues in the liver samples on fat basis was in the order of β-BHC > δ-BHC > chlorothalonil > γ-BHC > α-BHC. With regards to mean concentrations for samples above 24 months, β-isomer was highest (2.57mg/kg) while α-isomer was lowest (0.21mg/kg). The ratios and levels of BHC isomers are often used as evidence of the past or current technical application of the BHCs (Jafari *et al.,* 2008). However, the low abundance of the isomers suggests rather older than current usage of the OCPS

5.2.2 Chlorinated Cyclodienes

Residues of the isomers of cyclodiene group of OCPs (Cis- and trans- endosulfan, aldrin, dieldrin, endrin, haptachlor and heptachlor epoxide) were detected in all samples analysed. Residues of aldrin, dieldrin, endrin, heptachlor, heptachlor epoxide and Cis-endosulfan were detected in all ten (10) samples while, trans-endosulfan was detected in nine (9) samples. Mean concentration for samples above 24 months of age was found to be higher than the MRLs as set by FAO/WHO indicating that for samples with high % lipid contents (above 24 months) there is bioaccumulation of the OCPs.

5.2.3 Diphenyl-aliphatics (DDT and its Metabolites)

Dichlorodiphenyltrichloroethane and one of its metabolites Dichlorodiplenyl-dichloroethane (DDD) were determined. Both DDT and DDD were isolated and detected in all samples at levels below the MRLs as set by FAO/WHO in 2013 and at lowest and highest means concentrations of 0.17 - 0.73 mg/kg for p,p'-DDD and 0.12 - 1.20 mg/kg for p,p'-DDT.

The detection of DDT in higher concentration than DDD suggests the continuous usage of DDT in recent times. DDT was used in the control of insects like mosquitoes and in the control of cotton pest. Its toxicity to fish modified its usage by fishermen in fishing. DDT and its metabolites has been listed as possible human carcinogens particular in regards to hormonal cancer such as breast cancer and human are exposed to DDT mainly through their food (Janet, 2015).

5.3 Proximate Parameters and OCPs

Table 4.6 shows the correlation of the OCPs with some physical parameters such as lipid, moisture and ash content. Alpha-BHC, Chlorothalonil, p,p'-DDD, Lambda cyhalothrin and Permethrin were strongly positively correlated with lipid content at 99% confidence level while β-BHC, lindane, δ-BHC, heptachlor, heptachlor epoxide, endosulfan, endrin and p,p'-DDT were also positively correlated with lipid content at 95% confidence level. No good correlation was observed with ash content while OCP concentrations were negatively correlated with moisture content. This indicates that high lipid content is a factor that promotes bioaccumulation of the OCPs. This result agrees with the literature as reported by Helberg *et al.,* (2005) and Toft *et al.,* (2003).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

The result obtained showed the presence of the OCPs in the Liver samples and their respective quantities. Also, proximate analysis carried out revealed the physicochemical parameters of the Liver samples.

The concentration of the OCPs were α-BHC 0.01 – 0.21 mg/kg, β-BHC 0.09 – 0.90 mg/kg, Lindane 0.05 – 0.24 mg/kg, chlorothalonil 0.04 – 0.32 mg/kg, δ-BHC 0.06 – 0.40 mg/kg, heptachlor 0.10 – 1.12 mg/kg, Aldrin 0.26 – 0.61 mg/kg, heptachlor epoxide 0.13 – 1.16 mg/kg, endosulfan 0.13 – 3.79 mg/kg, dieldrin 0.12 – 1.72 mg/kg, endrin 0.03 – 7.88 mg/kg, endosulfan II 0.12 – 1.116 mg/kg, p,p'-DDD 0.12 – 0.73 mg/kg, endosulfan sulfate 0.12 – 1.38 mg/kg, p.p'-DDT 0.12 – 1.20 mg/kg, lambda cyhalothrin 0.07- 0.37 mg/kg and permethrin 0.02 – 0.28 mg/kg. This result shows the high concentrations of these OCPs to be greater than their respective MRLs (5 mg/kg DDT and its metabolites, 0.2 mg/kg Aldrin and Dieldrin, 0.2 mg/kg Chlorothalonil, 0.05 mg/kg Cyhalothrin, 0.1 mg/kg Endosulfan, 0.1 mg/kg Endrin, 0.2 mg/kg Heptachlor, 0.1 mg/kg Lindane and 1.0 mg/kg Permethrin) as set by FAO/WHO in 2013.

The result also showed an increase in the concentration of the OCPs as the age of the cattle increases. This suggests that the concentration of the OCPs increases with prolonged exposure of the cattle the OCPs. Also, a direct correlation between the concentrations of the individual OCPs and the proximate parameters showed a linear relationship between the % lipid content and the concentrations of the OCPs. There is an observed increase in concentration as & lipid of the Liver samples increases.

6.2 Conclusion

Agrochemicals are generally recognized as a significant factor in enhancing the ability to meet Nigeria's need for sufficient, safe and affordable food. However, increased usage has led to environmental deterioration and the contamination of crops and lifestock. The review of agrochemical monitoring and exposure clearly indicate that not much have been done in the area of OCP studies in Nigeria. There is lack or absence of corrective and preventive measures, presence of persistent, bioaccumulative and toxic agrochemicals in soil, water, lifestock and human feed and there is strong evidence that accidents of pesticide exposure in Nigeria may occur due to lack of awareness on the health hazards resulting from the use of Organochlorine pesticides.

The ban on the usage of OCPs in Nigeria is over two decades and the sanction resulted in tremendous decrease of the OCP usage. Nevertheless, high residues of OCPs like DDT and Aldrin suggests a more recent than older usage of these pesticides. It is therefore important to intensify the enforcement of their ban and to employ remediative measures that will help reduce thir levels in the soil.

6.3 Recommendations

1. The findings of this research (High levels of OCPs in cattle liver which is greater than the acceptable limits) shows there is need for awareness creation on OCPs and its health effects especially in the Northern Region of Nigeria. This is to ensure a total halt in the importation, sales and usage of these pesticides. To achieve this, the Ministry of Agriculture in collaboration with Ministry of Health, National Food and Drug Administration Control, Standards Organization of Nigeria and Ministry of Education should educate both farmers and market vendors of these pesticides.

2. In order to protect consumers against the hazards of OCPs, FAO/WHO has fixed maximum tolerable limit of the various toxic OCPs in meat and lifestock products. However, the biggest challenge in regulating what is sold and purchased in Nigeria is the lack of enforcement of legislation partly attributed to the presence of non-formal food market system operating in the country. Under this structure, raw agricultural produce from farm and abattoir are sold directly to consumers without being screened for OCPs neither are they subject to inspection to verify the levels of the OCPs in them.

3. It is strongly recommended that extensive awareness creation for safe use of agrochemicals be introduced, epidemiological studies and impact of agro-chemical usage in the country be instituted. This will provide information on biological indices of OCPs and also provide a foundation for effective monitoring of exposure of farmers and lifestock to these OCPs.

4. Surveillance teams should be constituted and trained to use inexpensive rapid test kits to verify the safety of lifestock products available in the market for effective control on the usage of these pesticides.

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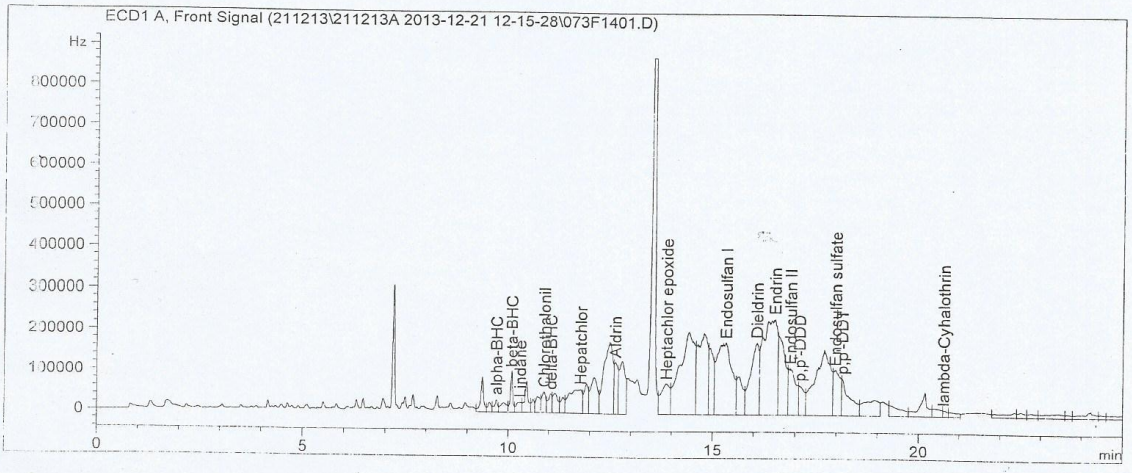
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External Standard Report

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Use Multiplier & Dilution Factor with ISTDs

Signal 1: ECD1 A, Front Signal

RetTime [min]	Type	Area [Hz*s]	Amt/Area	Amount [ng/ul]	Grp	Name
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10.078	VV S	4.37066e5	3.76898e-6	1.64729		beta-BHC
10.293	VV S	2.30867e5	8.00204e-7	1.84741e-1		Lindane
10.877	VV S	3.58285e5	9.50558e-7	3.40571e-1		Chlorothalonil
11.039	VV S	3.26460e5	1.66935e-6	5.44977e-1		delta-BHC
11.770	VV S	1.35433e6	1.45924e-6	1.97629		Hepatchlor
12.617	VV S	8.14511e5	1.65181e-6	1.34541		Aldrin
13.869	VV S	1.15853e6	1.81735e-6	2.10545		Heptachlor epoxide
15.321	VV S	4.61844e6	2.35275e-6	10.86605		Endosulfan I
16.070	VV S	2.61904e6	1.78101e-6	4.66452		Dieldrin

rument 1 12/31/2013 8:11:42 AM Analyst Page 1 of 2

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16.882	VV S	1.48796e6	2.02129e-6	3.00761		Endosulfan II
17.104	VV S	6.57008e5	1.79193e-6	1.17731		p,p'-DDD
17.968	VV S	1.31822e6	2.74596e-6	3.61977		Endosulfan sulfate
18.137	VV S	1.24771e6	1.84758e-6	2.30525		p,p'-DDT
20.612	VV S	1.11712e5	4.46514e-6	4.98807e-1		lambda-Cyhalothrin
21.337		-	-	-		Permethrin

Totals : 47.31707

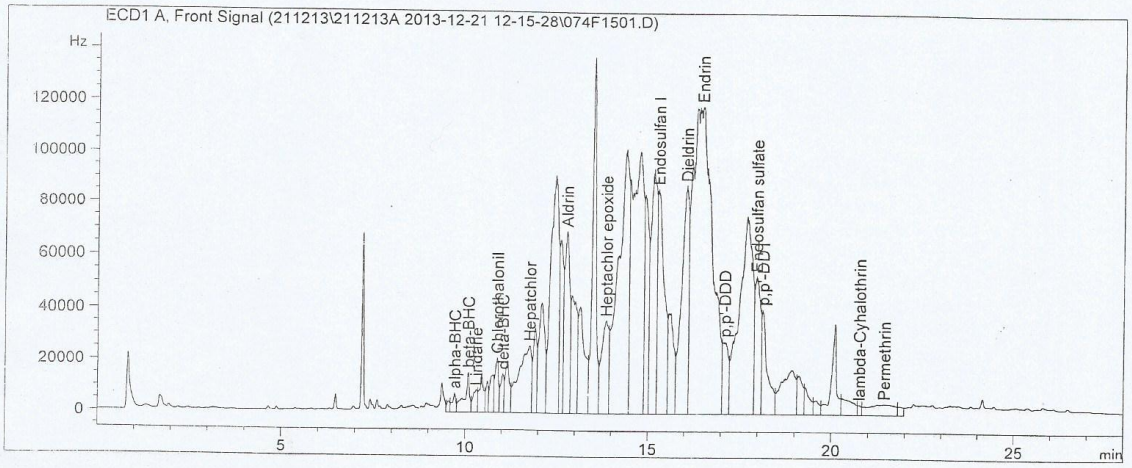
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Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

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External Standard Report

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Use Multiplier & Dilution Factor with ISTDs

Signal 1: ECD1 A, Front Signal

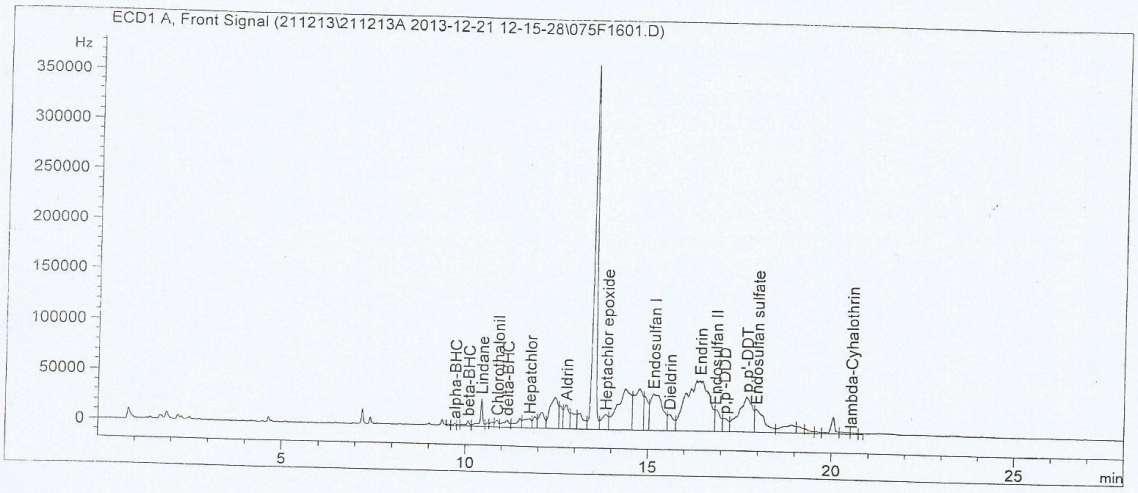
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10.294	VV S	7.35277e4	6.37550e-7	4.68776e-2		Lindane
10.868	VV S	1.41611e5	9.04401e-7	1.28073e-1		Chlorothalonil
11.023	VV S	1.00094e5	1.58248e-6	1.58396e-1		delta-BHC
11.756	VV S	6.25313e5	1.44098e-6	9.01063e-1		Hepatchlor
12.764	VV S	6.81995e5	1.64721e-6	1.12339		Aldrin
13.849	VV S	5.01563e5	1.79290e-6	8.99254e-1		Heptachlor epoxide
15.272	VV S	1.07386e6	2.33762e-6	2.51028		Endosulfan I
16.039	VV S	1.22030e6	1.77194e-6	2.16229		Dieldrin

rument 1 12/31/2013 8:23:25 AM Analyst

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Analysis Method	: C:\CHEM32\1\METHODS\OCPMETHODQUANT.M		
Last changed	: 12/23/2013 9:21:27 AM by Analyst		
Additional Info	: Peak(s) manually integrated		



External Standard Report

Sorted By	:	Signal
Calib. Data Modified	:	Monday, December 23, 2013 9:19:50 AM
Multiplier:	:	1.0000
Dilution:	:	1.0000
Sample Amount:	:	1.00000 [ng/ul] (not used in calc.)

Use Multiplier & Dilution Factor with ISTDs

Signal 1: ECD1 A, Front Signal

RetTime [min]	Type	Area [Hz*s]	Amt/Area	Amount [ng/ul]	Grp	Name
9.703	VV S	1.51292e4	0.00000	0.00000		alpha-BHC
10.074	VV S	4.25501e4	3.64630e-6	1.55151e-1		beta-BHC
10.444	VV S	1.39374e5	7.50306e-7	1.04573e-1		Lindane
10.865	VV S	4.69266e4	7.50401e-7	3.52138e-2		Chlorothalonil
11.142	VV S	9.29240e4	1.57281e-6	1.46152e-1		delta-BHC
11.757	VV S	1.41087e5	1.32454e-6	1.86876e-1		Hepatchlor
12.762	VV S	2.31596e5	1.59223e-6	3.68755e-1		Aldrin
13.846	VV S	1.97064e5	1.72628e-6	3.40189e-1		Heptachlor epoxide
15.142	VV S	8.62523e5	2.33278e-6	2.01208		Endosulfan I
15.590	VV S	1.99481e5	1.68504e-6	3.36134e-1		Dieldrin
16.418	VV S	2.29794e6	2.26420e-6	5.20300		Endrin

Data File C:\CHEM32\1\DATA\211213\211213A 2013-12-21 12-15-28\075F1601.D
Sample Name: Ext 3

Acq. Operator : Analyst Seq. Line : 16
Acq. Instrument : Instrument 1 Location : Vial 75
Injection Date : 12/21/2013 8:18:07 PM Inj : 1
Inj Volume : 1 µl
Acq. Method : C:\CHEM32\1\DATA\211213\211213A 2013-12-21 12-15-28\ORGANOCHLORINE METHOD.M
Last changed : 12/21/2013 9:42:27 AM by Analyst
Analysis Method : C:\CHEM32\1\METHODS\OCPMETHODQUANT.M
Last changed : 12/23/2013 9:21:27 AM by Analyst
Additional Info : Peak(s) manually integrated

RetTime [min]	Type	Area [Hz*s]	Amt/Area	Amount [ng/ul]	Grp	Name
16.840	VV S	2.29065e5	1.95159e-6	4.47041e-1		Endosulfan II
17.080	VV S	1.47200e5	1.66591e-6	2.45223e-1		p,p'-DDD
17.681	VV S	9.20409e5	1.84171e-6	1.69513		p,p'-DDT
17.953	VV S	4.38612e5	2.74107e-6	1.20226		Endosulfan sulfate
20.521	VV S	1.77616e4	4.51234e-6	8.01462e-2		lambda-Cyhalothrin
21.337		-	-	-		Permethrin

Totals : 12.55791

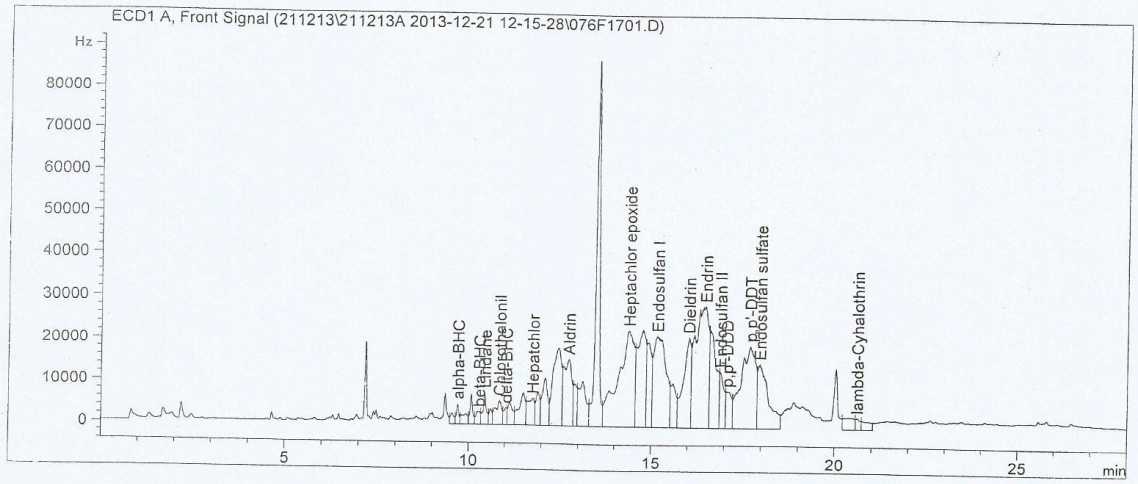
4 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Elution order of calibrated compounds may have changed
Warning : Negative results set to zero (cal. curve intercept), (alpha-BHC)

*** End of Report ***

ata File C:\CHEM32\1\DATA\211213\211213A 2013-12-21 12-15-28\076F1701.D
ample Name: Ext 4

Acq. Operator : Analyst
Acq. Instrument : Instrument 1
Injection Date : 12/21/2013 8:50:13 PM
Seq. Line : 17
Location : Vial 76
Inj : 1
Inj Volume : 1 µl
Acq. Method : C:\CHEM32\1\DATA\211213\211213A 2013-12-21 12-15-28\ORGANOCHLORINE METHOD.M
Last changed : 12/21/2013 9:42:27 AM by Analyst
Analysis Method : C:\CHEM32\1\METHODS\OCPMETHODQUANT.M
Last changed : 12/23/2013 9:21:27 AM by Analyst
Additional Info : Peak(s) manually integrated



External Standard Report

Sorted By : Signal
Calib. Data Modified : Monday, December 23, 2013 9:19:50 AM
Multiplier: : 1.0000
Dilution: : 1.0000
Sample Amount: : 1.00000 [ng/ul] (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs

Signal 1: ECD1 A, Front Signal

RetTime [min]	Type	Area [Hz*s]	Amt/Area	Amount [ng/ul]	Grp	Name
9.704	VV S	2.18388e4	4.65723e-8	1.01708e-3		alpha-BHC
10.283	VV S	2.72818e4	3.57023e-6	9.74023e-2		beta-BHC
10.443	VV S	5.07805e4	5.30639e-7	2.69461e-2		Lindane
10.839	VV S	7.19543e4	8.30514e-7	5.97590e-2		Chlorothalonil
11.024	VV S	2.80257e4	1.26029e-6	3.53206e-2		delta-BHC
11.758	VV S	8.55898e4	1.22704e-6	1.05022e-1		Hepatchlor
12.735	VV S	2.44554e5	1.59664e-6	3.90465e-1		Aldrin
14.383	VV S	7.12881e5	1.80568e-6	1.28724		Heptachlor epoxide
15.163	VV S	5.12623e5	2.31602e-6	1.18725		Endosulfan I
16.029	VV S	3.03958e5	1.72075e-6	5.23035e-1		Dieldrin
16.478	VV S	7.21854e5	2.25050e-6	1.62453		Endrin

rument 1 12/31/2013 8:25:22 AM Analyst

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Data File C:\CHEM32\1\DATA\211213\211213A 2013-12-21 12-15-28\076F1701.D
Sample Name: Ext 4

Acq. Operator : Analyst Seq. Line : 17
Acq. Instrument : Instrument 1 Location : Vial 76
Injection Date : 12/21/2013 8:50:13 PM Inj : 1
Inj Volume : 1 µl
Acq. Method : C:\CHEM32\1\DATA\211213\211213A 2013-12-21 12-15-28\ORGANOCHLORINE METHOD.M
Last changed : 12/21/2013 9:42:27 AM by Analyst
Analysis Method : C:\CHEM32\1\METHODS\OCPMETHODQUANT.M
Last changed : 12/23/2013 9:21:27 AM by Analyst
Additional Info : Peak(s) manually integrated

RetTime [min]	Type	Area [Hz*s]	Amt/Area	Amount [ng/ul]	Grp	Name
16.885	VV S	1.11336e5	1.86447e-6	2.07583e-1		Endosulfan II
17.070	VV S	9.36982e4	1.57318e-6	1.47404e-1		p,p'-DDD
17.700	VV S	5.44541e5	1.82626e-6	9.94474e-1		p,p'-DDT
17.963	VV S	3.21612e5	2.73840e-6	8.80700e-1		Endosulfan sulfate
20.608	VV S	2.30268e4	4.49950e-6	1.03609e-1		lambda-Cyhalothrin
21.337		-	-	-		Permethrin

Totals : 7.67175

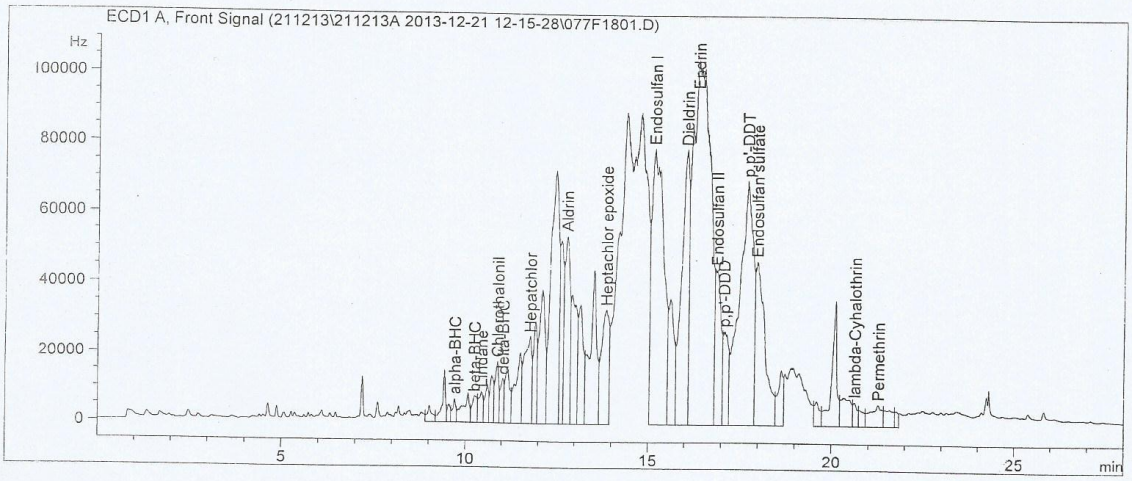
3 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Elution order of calibrated compounds may have changed

*** End of Report ***

ta File C:\CHEM32\1\DATA\211213\211213A 2013-12-21 12-15-28\077F1801.D
mple Name: Ext 6

Acq. Operator : Analyst
Seq. Line : 18
Acq. Instrument : Instrument 1
Location : Vial 77
Injection Date : 12/21/2013 9:22:14 PM
Inj : 1
Inj Volume : 1 µl
Acq. Method : C:\CHEM32\1\DATA\211213\211213A 2013-12-21 12-15-28\ORGANOCHLORINE METHOD.M
Last changed : 12/21/2013 9:42:27 AM by Analyst
Analysis Method : C:\CHEM32\1\METHODS\OCPMETHODQUANT.M
Last changed : 12/23/2013 9:21:27 AM by Analyst
Additional Info : Peak(s) manually integrated



External Standard Report

Sorted By : Signal
Calib. Data Modified : Monday, December 23, 2013 9:19:50 AM
Multiplier: : 1.0000
Dilution: : 1.0000
Sample Amount: : 1.00000 [ng/ul] (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs

Signal 1: ECD1 A, Front Signal

RetTime [min]	Type	Area [Hz*s]	Amt/Area	Amount [ng/ul]	Grp	Name
9.705	VV S	4.15496e4	4.12164e-7	1.71252e-2		alpha-BHC
10.246	VV S	7.22363e4	3.70215e-6	2.67430e-1		beta-BHC
10.441	VV S	7.23884e4	6.33793e-7	4.58793e-2		Lindane
10.874	VV S	1.21388e5	8.91685e-7	1.08240e-1		Chlorothalonil
11.022	VV S	8.69924e4	1.56361e-6	1.36022e-1		delta-BHC
11.763	VV S	3.58064e5	1.41566e-6	5.06897e-1		Hepatchlor
12.767	VV S	5.53060e5	1.64062e-6	9.07360e-1		Aldrin
13.848	VV S	4.74030e5	1.79040e-6	8.48702e-1		Heptachlor epoxide
15.161	VV S	1.81023e6	2.34564e-6	4.24615		Endosulfan I
16.049	VV S	1.12393e6	1.77048e-6	1.98991		Dieldrin
16.343	VV S	3.49297e6	2.26635e-6	7.91628		Endrin

rument 1 12/31/2013 8:25:54 AM Analyst

Page 1 of 2

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Data File C:\CHEM32\1\DATA\211213\211213A 2013-12-21 12-15-28\077F1801.D
Sample Name: Ext 6

Acq. Operator : Analyst
Seq. Line : 18
Acq. Instrument : Instrument 1
Location : Vial 77
Injection Date : 12/21/2013 9:22:14 PM
Inj : 1
Inj Volume : 1 µl
Acq. Method : C:\CHEM32\1\DATA\211213\211213A 2013-12-21 12-15-28\ORGANOCHLORINE METHOD.M
Last changed : 12/21/2013 9:42:27 AM by Analyst
Analysis Method : C:\CHEM32\1\METHODS\OCPMETHODQUANT.M
Last changed : 12/23/2013 9:21:27 AM by Analyst
Additional Info : Peak(s) manually integrated

RetTime [min]	Type	Area [Hz*s]	Amt/Area	Amount [ng/ul]	Grp	Name
16.864	VV S	5.04421e5	1.99656e-6	1.00711		Endosulfan II
17.063	VV S	2.71134e5	1.74015e-6	4.71813e-1		p,p'-DDD
17.698	VV S	1.82451e6	1.85280e-6	3.38044		p,p'-DDT
17.956	VV S	8.70807e5	2.74471e-6	2.39011		Endosulfan sulfate
20.620	VV S	5.70336e4	4.47369e-6	2.55151e-1		lambda-Cyhalothrin
21.268	VV S	1.37565e5	3.78305e-5	5.20416		Permethrin
Totals :				29.69878		

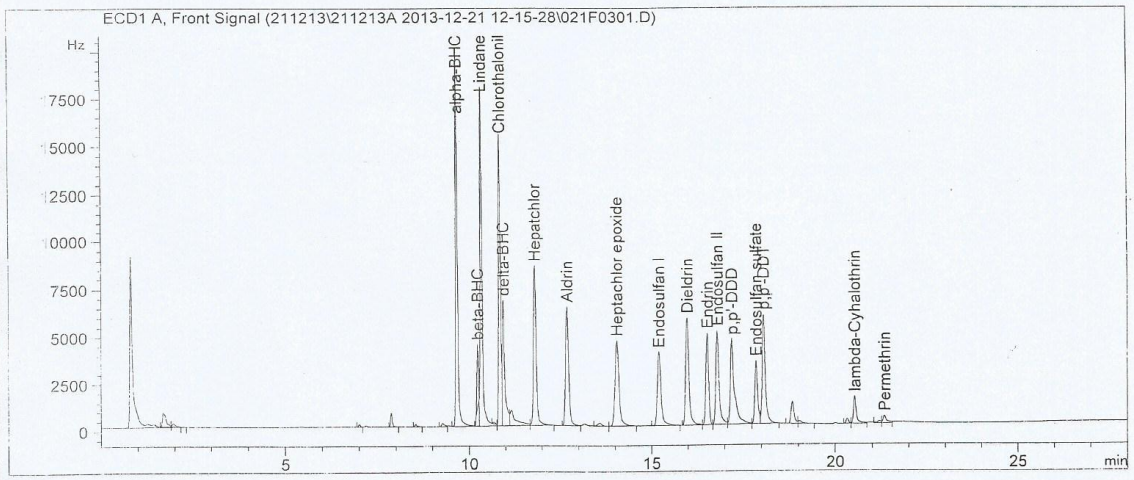
2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Elution order of calibrated compounds may have changed

*** End of Report ***

Data File C:\CHEM32\1\DATA\211213\211213A 2013-12-21 12-15-28\021F0301.D
Sample Name: OCPmix 0.05ppm

Acq. Operator	: Analyst	Seq. Line	: 3
Acq. Instrument	: Instrument 1	Location	: Vial 21
Injection Date	: 12/21/2013 1:21:13 PM	Inj	: 1
		Inj Volume	: 1 µl
Acq. Method	: C:\CHEM32\1\DATA\211213\211213A 2013-12-21 12-15-28\ORGANOCHLORINE METHOD.M		
Last changed	: 12/21/2013 9:42:27 AM by Analyst		
Analysis Method	: C:\CHEM32\1\METHODS\OCPMETHODQUANT.M		
Last changed	: 12/23/2013 9:21:27 AM by Analyst		



External Standard Report

Sorted By	:	Signal
Calib. Data Modified	:	Monday, December 23, 2013 9:19:50 AM
Multiplier:	:	1.0000
Dilution:	:	1.0000
Sample Amount:	:	1.00000 [ng/ul] (not used in calc.)

Use Multiplier & Dilution Factor with ISTDs

Signal 1: ECD1 A, Front Signal

RetTime [min]	Type	Area [Hz*s]	Amt/Area	Amount [ng/ul]	Grp	Name
9.672	VV S	7.87596e4	6.03537e-7	4.75343e-2		alpha-BHC
10.239	BV T	8651.23047	3.11373e-6	2.69376e-2		beta-BHC
10.343	VV S	7.04085e4	6.26976e-7	4.41445e-2		Lindane
10.846	VV S	5.47275e4	7.83232e-7	4.28643e-2		Chlorothalonil
10.940	VV S	3.22065e4	1.31838e-6	4.24605e-2		delta-BHC
11.811	VB S	4.41437e4	9.94323e-7	4.38931e-2		Hepatchlor
12.694	BV	3.78039e4	1.16550e-6	4.40603e-2		Aldrin
14.060	VB	3.56583e4	1.22960e-6	4.38456e-2		Heptachlor epoxide
15.213	BB	2.76593e4	1.59157e-6	4.40216e-2		Endosulfan I
15.984	BV	3.60138e4	1.21354e-6	4.37042e-2		Dieldrin
16.535	VV	2.54901e4	1.70468e-6	4.34524e-2		Endrin

JF

Data File C:\CHEM32\1\DATA\211213\211213A 2013-12-21 12-15-28\021F0301.D
Sample Name: OCPmix 0.05ppm

Acq. Operator : Analyst Seq. Line : 3
Acq. Instrument : Instrument 1 Location : Vial 21
Injection Date : 12/21/2013 1:21:13 PM Inj : 1
Inj Volume : 1 µl
Acq. Method : C:\CHEM32\1\DATA\211213\211213A 2013-12-21 12-15-28\ORGANOCHLORINE METHOD.M
Last changed : 12/21/2013 9:42:27 AM by Analyst
Analysis Method : C:\CHEM32\1\METHODS\OCPMETHODQUANT.M
Last changed : 12/23/2013 9:21:27 AM by Analyst

RetTime [min]	Type	Area [Hz*s]	Amt/Area	Amount [ng/ul]	Grp	Name
16.803	VV	3.05944e4	1.41713e-6	4.33562e-2		Endosulfan II
17.204	VV	3.49616e4	1.14455e-6	4.00152e-2		p,p'-DDD
17.864	VV	1.79009e4	2.56860e-6	4.59803e-2		Endosulfan sulfate
18.069	VB	3.34584e4	1.24841e-6	4.17697e-2		p,p'-DDT
20.534	VV	8161.04883	4.57836e-6	3.73642e-2		lambda-Cyhalothrin
21.337	VV	3098.63647	1.79129e-5	5.55056e-2		Permethrin
Totals :				7.30910e-1		

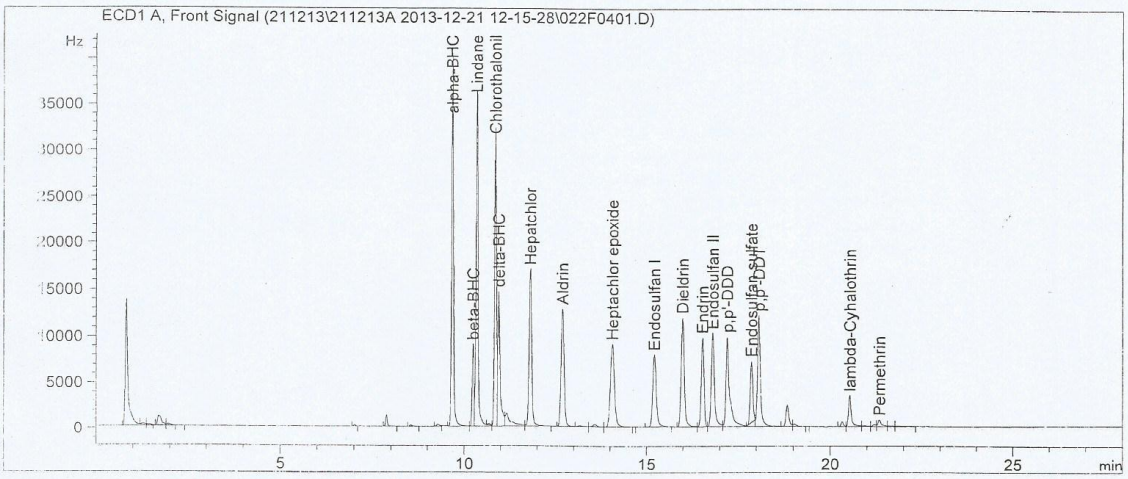
1 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)

*** End of Report ***

a File C:\CHEM32\1\DATA\211213\211213A 2013-12-21 12-15-28\022F0401.D
ple Name: OCPmix 0.1ppm

Acq. Operator : Analyst Seq. Line : 4
Acq. Instrument : Instrument 1 Location : Vial 22
Injection Date : 12/21/2013 1:53:14 PM Inj : 1
Inj Volume : 1 µl
Acq. Method : C:\CHEM32\1\DATA\211213\211213A 2013-12-21 12-15-28\ORGANOCHLORINE METHOD.M
Last changed : 12/21/2013 9:42:27 AM by Analyst
Analysis Method : C:\CHEM32\1\METHODS\OCPMETHODQUANT.M
Last changed : 12/23/2013 9:21:27 AM by Analyst



External Standard Report

Sorted By : Signal
Calib. Data Modified : Monday, December 23, 2013 9:19:50 AM
Multiplier: : 1.0000
Dilution: : 1.0000
Sample Amount: : 1.00000 [ng/ul] (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs

Signal 1: ECD1 A, Front Signal

RetTime [min]	Type	Area [Hz*s]	Amt/Area	Amount [ng/ul]	Grp	Name
9.672	VV S	1.45679e5	7.01698e-7	1.02223e-1		alpha-BHC
10.239	VV S	3.04223e4	3.59212e-6	1.09281e-1		beta-BHC
10.342	VV S	1.40352e5	7.51184e-7	1.05430e-1		Lindane
10.846	VV S	1.15079e5	8.86804e-7	1.02052e-1		Chlorothalonil
10.940	VV S	6.67259e4	1.51982e-6	1.01412e-1		delta-BHC
11.812	VB S	8.69621e4	1.23095e-6	1.07046e-1		Hepatchlor
12.693	BB S	7.42044e4	1.41566e-6	1.05048e-1		Aldrin
14.061	PB T	7.04011e4	1.52887e-6	1.07634e-1		Heptachlor epoxide
15.214	VB	5.43743e4	1.96780e-6	1.06998e-1		Endosulfan I
15.985	BV S	7.14330e4	1.49884e-6	1.07066e-1		Dieldrin
16.531	VV S	5.11430e4	1.98848e-6	1.01697e-1		Endrin

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Data File C:\CHEM32\1\DATA\211213\211213A 2013-12-21 12-15-28\022F0401.D
Sample Name: OCPmix 0.1ppm

Acq. Operator : Analyst
Seq. Line : 4
Acq. Instrument : Instrument 1
Location : Vial 22
Injection Date : 12/21/2013 1:53:14 PM
Inj : 1
Inj Volume : 1 µl
Acq. Method : C:\CHEM32\1\DATA\211213\211213A 2013-12-21 12-15-28\ORGANOCHLORINE METHOD.M
Last changed : 12/21/2013 9:42:27 AM by Analyst
Analysis Method : C:\CHEM32\1\METHODS\OCPMETHODQUANT.M
Last changed : 12/23/2013 9:21:27 AM by Analyst

RetTime [min]	Type	Area [Hz*s]	Amt/Area	Amount [ng/ul]	Grp	Name
16.805	VV S	6.15013e4	1.72712e-6	1.06220e-1		Endosulfan II
17.203	VV S	7.69060e4	1.51747e-6	1.16703e-1		p,p'-DDD
17.862	BV T	3.27940e4	2.65025e-6	8.69123e-2		Endosulfan sulfate
18.068	VB S	6.82757e4	1.56238e-6	1.06672e-1		p,p'-DDT
20.536	VV	1.92383e4	4.50803e-6	8.67268e-2		lambda-Cyhalothrin
21.336	VV	5428.39697	2.66581e-5	1.44711e-1		Permethrin

Totals : 1.80383

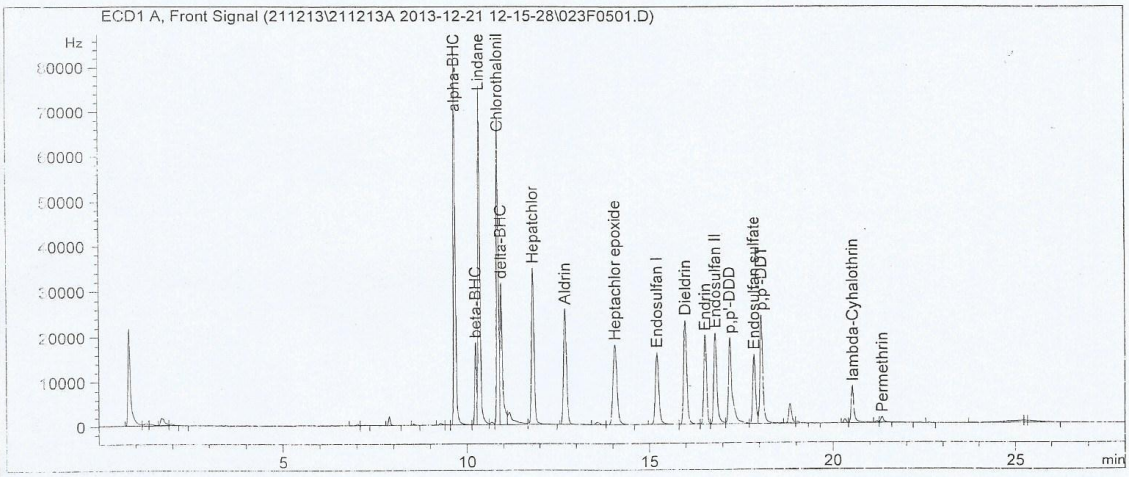
1 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)

*** End of Report ***

File C:\CHEM32\1\DATA\211213\211213A 2013-12-21 12-15-28\023F0501.D
ple Name: OCPmix 0.2ppm

Acq. Operator : Analyst Seq. Line : 5
Acq. Instrument : Instrument 1 Location : Vial 23
Injection Date : 12/21/2013 2:25:21 PM Inj : 1
Inj Volume : 1 µl
Acq. Method : C:\CHEM32\1\DATA\211213\211213A 2013-12-21 12-15-28\ORGANOCHLORINE METHOD.M
Last changed : 12/21/2013 9:42:27 AM by Analyst
Analysis Method : C:\CHEM32\1\METHODS\OCPMETHODQUANT.M
Last changed : 12/23/2013 9:21:27 AM by Analyst



External Standard Report

Sorted By : Signal
Calib. Data Modified : Monday, December 23, 2013 9:19:50 AM
Multiplier: : 1.0000
Dilution: : 1.0000
Sample Amount: : 1.00000 [ng/ul] (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs

Signal 1: ECD1 A, Front Signal

RetTime [min]	Type	Area [Hz*s]	Amt/Area	Amount [ng/ul]	Grp	Name
9.673	VV S	3.00547e5	7.61228e-7	2.28785e-1		alpha-BHC
10.238	VV S	6.28313e4	3.69017e-6	2.31858e-1		beta-BHC
10.343	VV S	2.83422e5	8.14299e-7	2.30791e-1		Lindane
10.847	VV S	2.41443e5	9.35960e-7	2.25981e-1		Chlorothalonil
10.939	VV S	1.42214e5	1.61959e-6	2.30329e-1		delta-BHC
11.811	VB S	1.73799e5	1.35284e-6	2.35123e-1		Hepatchlor
12.693	BV S	1.51354e5	1.54810e-6	2.34310e-1		Aldrin
14.059	VB S	1.39822e5	1.68136e-6	2.35092e-1		Heptachlor epoxide
15.215	VV S	1.08647e5	2.16239e-6	2.34938e-1		Endosulfan I
15.984	VV S	1.42700e5	1.64371e-6	2.34558e-1		Dieldrin
16.530	VV S	1.08355e5	2.13737e-6	2.31596e-1		Endrin

Data File C:\CHEM32\1\DATA\211213\211213A 2013-12-21 12-15-28\023F0501.D
Sample Name: OCPmix 0.2ppm

Acq. Operator : Analyst
Acq. Instrument : Instrument 1
Injection Date : 12/21/2013 2:25:21 PM
Seq. Line : 5
Location : Vial 23
Inj : 1
Inj Volume : 1 µl
Acq. Method : C:\CHEM32\1\DATA\211213\211213A 2013-12-21 12-15-28\ORGANOCHLORINE METHOD.M
Last changed : 12/21/2013 9:42:27 AM by Analyst
Analysis Method : C:\CHEM32\1\METHODS\OCPMETHODQUANT.M
Last changed : 12/23/2013 9:21:27 AM by Analyst

RetTime [min]	Type	Area [Hz*s]	Amt/Area	Amount [ng/ul]	Grp	Name
16.805	VV S	1.23894e5	1.88165e-6	2.33126e-1		Endosulfan II
17.201	VV S	1.40268e5	1.65789e-6	2.32549e-1		p,p'-DDD
17.864	VV S	8.44355e4	2.71028e-6	2.28844e-1		Endosulfan sulfate
18.068	VB S	1.38610e5	1.71547e-6	2.37782e-1		p,p'-DDT
20.536	VB S	5.25094e4	4.47520e-6	2.34990e-1		lambda-Cyhalothrin
21.337	VV X	7848.09375	3.02443e-5	2.37360e-1		Permethrin
Totals :				3.95801		

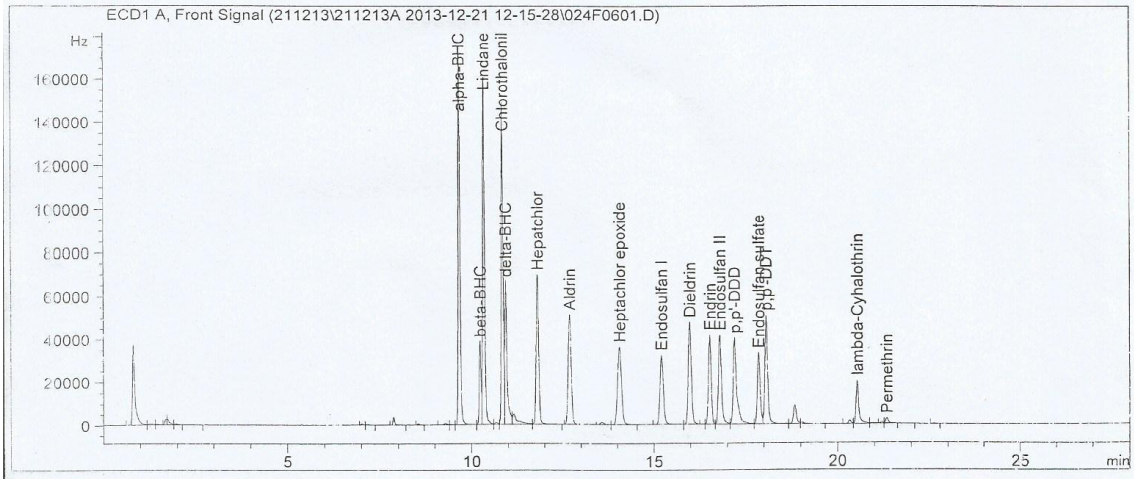
1 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)

*** End of Report ***

File C:\CHEM32\1\DATA\211213\211213A 2013-12-21 12-15-28\024F0601.D
le Name: OCPmix 0.5ppm

Acq. Operator : Analyst Seq. Line : 6
Acq. Instrument : Instrument 1 Location : Vial 24
Injection Date : 12/21/2013 2:57:22 PM Inj : 1
Inj Volume : 1 µl
Acq. Method : C:\CHEM32\1\DATA\211213\211213A 2013-12-21 12-15-28\ORGANOCHLORINE METHOD.M
Last changed : 12/21/2013 9:42:27 AM by Analyst
Analysis Method : C:\CHEM32\1\METHODS\OCPMETHODQUANT.M
Last changed : 12/23/2013 9:21:27 AM by Analyst



External Standard Report

Sorted By : Signal
Calib. Data Modified : Monday, December 23, 2013 9:19:50 AM
Multiplier: : 1.0000
Dilution: : 1.0000
Sample Amount: : 1.00000 [ng/ul] (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs

Signal 1: ECD1 A, Front Signal

RetTime [min]	Type	Area [Hz*s]	Amt/Area	Amount [ng/ul]	Grp	Name
9.672	VV S	6.18088e5	7.89997e-7	4.88288e-1		alpha-BHC
10.237	VV S	1.30477e5	3.73789e-6	4.87707e-1		beta-BHC
10.342	VV S	5.76036e5	8.45752e-7	4.87183e-1		Lindane
10.845	VV S	5.10560e5	9.59556e-7	4.89911e-1		Chlorothalonil
10.939	VV S	2.93295e5	1.66501e-6	4.88340e-1		delta-BHC
11.811	VB S	3.43321e5	1.41311e-6	4.85152e-1		Hepatchlor
12.694	BV S	3.01490e5	1.61153e-6	4.85860e-1		Aldrin
14.060	VB S	2.75965e5	1.75766e-6	4.85052e-1		Heptachlor epoxide
15.213	VV S	2.14820e5	2.25874e-6	4.85223e-1		Endosulfan I
15.984	VV S	2.82917e5	1.71568e-6	4.85393e-1		Dieldrin
16.532	VV S	2.21143e5	2.20526e-6	4.87677e-1		Endrin

APPENDIX II

Comparison of the average percentage Lipid content

A comparison of the average percentage lipid content of the three groups. Values with similar superscripts means no significant difference at 95 % confidence levels.

Samples/ groups	Below 11months	12-20 months	24 months Above
Sample 1	3.74 ± 0.029	4.71 ± 0.029	5.77 ± 0.029
Sample 2	3.89 ± 0.027	5.08 ± 0.027[a]	5.87 ± 0.027[a]
Sample 3	4.05 ± 0.012	5.12 ± 0.012	6.19 ± 0.012

The mean difference is significant at P□ 0.05 level