ANALYSIS OF PESTICIDE RESIDUES IN MAIZE AND BEANS TYPES IN LAGOS MARKETS

BY

OGAH, CELINA ONOTSE

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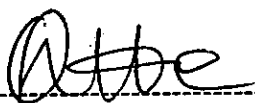
THESIS

ORIGINAL RESEARCH WORK SUBMITTED TO THE POSTGRADUATE
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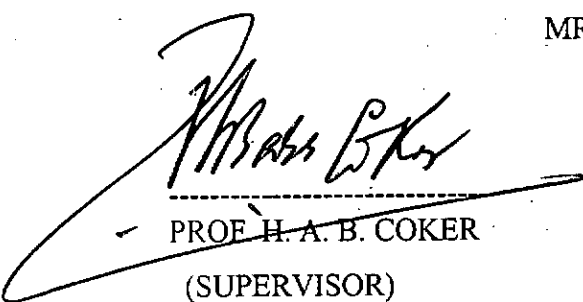REQUIREMENT FOR THE AWARD OF DOCTOR OF PHILOSOPHY DEGREE

BY

OGAH, CELINA ONOTSE

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY, FACULTY OF
PHARMACY, UNIVERSITY OF LAGOS

MRS CELINA O. OGAH
(AUTHOR)

PROF. H. A. B. COKER
(SUPERVISOR)

DR (MRS.) A. A. ADEPOJU-BELLO
(CO-SUPERVISOR)

MAY, 2008

SCHOOL OF POSTGRADUATE STUDIES
UNIVERSITY OF LAGOS

CERTIFICATION

This is to certify that the Thesis:

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By
OGAH, CELINA ONOTSE
in the Department of Pharmaceutical Chemistry

Mrs C. O. Ogah	_(signature)_	_15/05/08_
AUTHOR'S NAME	**SIGNATURE**	**DATE**
Prof. H. A. B. Coker	_(signature)_	_15.05.08_
1ST SUPERVISOR'S NAME	**SIGNATURE**	**DATE**
DR (MRS) ADEPOJU-BELLO, A A	_(signature)_	_15/5/08_
2ND SUPERVISOR'S NAME	**SIGNATURE**	**DATE**
Dr (Mrs) O. T. Asetun	_(signature)_	_15/5/08_
1ST INTERNAL EXAMINER	**SIGNATURE**	**DATE**
Dr. (Mrs) G. O. Ajayi	_(signature)_	_15/5/08_
2ND INTERNAL EXAMINER	**SIGNATURE**	**DATE**
Dr C. J. Eboka	_(signature)_	_15-05-08_
EXTERNAL EXAMINER	**SIGNATURE**	**DATE**
Dr (Mrs) O. O. Adeyemi	_(signature)_	_15/5/2008_
SPGS REPRESENTATIVE	**SIGNATURE**	**DATE**

DEDICATION

TO THE MEMORY OF MY LATE FATHER, JOHN BRAIMAH GBAGHIDI

ACKNOWLEDGEMENT

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GLOSSARY OF ABBREVIATIONS

Ach	=	Acetylcholine
AChE	=	Acetylcholinesterase
ADI	=	Acceptable daily intake
ATP	=	Adenosine triphosphate
CF	=	Calibration Factor
2, 4-D	=	2,4-dichlorophenoxyacetic acid
DCPA	=	3, 4-dichloropropionilide
DDD	=	Dichlorodiphenyldichloroethane
DDE	=	Dichlorodiphenyldichloroethylene
DDT	=	Dichlorodiphenyltrichloroethane
DFDT	=	Diflorodiphenyltrichloroethane
DNOC	=	Dinitro-o-cresol
ECD	=	Electron capture detector
EDI	=	Estimated daily intake
EHC	=	Environmental health criteria
EI	=	Electron Impact
ETDI	=	Estimated total diet intake
FAO	=	Food and Agriculture Organisation
FDA	=	Food and Drug Administration
FID	=	Flame Ionization Detector
FPD	=	Flame Photometric Detector
GAP	=	Good Agricultural Practice

GC	=	Gas Chromatography
GPC	=	Gel Permeation Chromatography
HCH	=	Hexachlorocyclohexane
HPLC	=	High Performance Liquid Chromatography
HPTLC	=	High Performance Thin Layer Chromatography
IDI	=	Insect Development Inhibitor
IGR	=	Insect Growth Regulator
IPM	=	Integrated Pest Management
LD_{50}	=	Lethal Dose to 50% of test population
LOD	=	Limit of Determination
MCPA	=	2-methyl-4-chlorophenoxyacetic acid
MPI	=	Maximum Permissible Intake
MRL	=	Maximum Residue Limit
MRM	=	Multiresidue Methods
MS	=	Mass Spectrometer
NAFDAC	=	National Agency for Food and Drugs Administration and Control
NPD	=	Nitrogen Phosphorus Detector
PAN	=	Pesticide Action Network
PCP	=	Pentachlorophenol
RBM	=	Rollback Malaria
SEC	=	Size Exclusion Chromatography
SFE	=	Supercritical Fluid Extraction
SIM	=	Selected Ion Monitoring

SPE	=	Solid Phase Extraction
SRM	=	Single Residue Method
2,4,5-T	=	2,4,5-trichlorophenoxyacetic acid
TEPP	=	Tetraethylpyrophosphate
TID	=	Thermoionic Detector
TLC	=	Thin Layer Chromatography
UNEP	=	United Nations Environment Programme
USEPA	=	United States Environmental Protection Agency
WHO	=	World Health Organization

ABSTRACT

Pesticides used in agriculture for the control of various pests often leave residues in foodstuffs and these have been shown to pose health hazards. Analysis of pesticide residues in food is one way to determine the level of human exposure to these chemicals and hence their potential human health hazards.

Maize (*Zea mays L.*) and beans (*Phaseolus vulgaris L.*) samples purchased from different markets in Lagos State were analyzed for residues of organochlorine, organophosphate and carbamate pesticides. Analysis was done using gas chromatograph with mass spectrometric detector (GC-MS) after careful extraction and cleanup.

Most of the maize samples (96%) were found to contain residues of one or more pesticides with concentrations ranging from 2.2-3951.0 µg/kg. The white types of maize contained higher concentrations of residues than the yellow types. Three classes of pesticides were detected in maize. All the samples of beans analyzed contained at least one pesticide residue (100% incidence). White beans were found to contain higher concentrations of residues than the brown types. The concentrations of organochlorine, organophosphate and carbamate pesticides residues in beans ranged from 2.3-1480.5µg/kg.

The most commonly found residue among both maize and beans samples was the organophosphate, pirimiphos-methyl. Its percent occurrence was 43 in maize and 54 in beans. There was a decline in the mean levels of organochlorine pesticides in both maize (6.9-41.3µg/kg) and beans (4.8-39.7µg/kg) compared to the results of a previous similar study (10.0-93.0µg/kg for maize and 25.0-303.0µg/kg for beans).

Maximum residue limits (MRLs) of the various pesticides were exceeded in up to 10% of samples of both maize and beans. The incidence of pesticide residues in maize and beans was found to be higher in some markets than others but mean concentrations were not different from market to market. The pesticide residue contents were decreased by boiling. Percent reduction ranged from 9 to 100. The extent of reduction was higher in the organophosphates (24-100%) and carbamates (20-100%) than in the organochlorines (9-32%).

The estimated total diet intakes (ETDIs) for most of the pesticides were well below their maximum permissible intakes (MPIs). On the other hand, the ETDIs for aldrin, dichlorvos and dieldrin exceeded their MPIs by 100%, 363% and 17% respectively.

Conclusively, most of the maize and beans in Lagos markets contain pesticide residues at different levels and maximum residue limits were exceeded in about 10% of samples. There is therefore a need for more stringent monitoring of the use of pesticides in agriculture and food storage in Nigeria.

CHAPTER ONE

INTRODUCTION

1.1 INTRODUCTION

Pesticides are chemical agents capable of destroying or controlling the growth and reproduction of pests. They have gained widespread use since their introduction in the early 1940s (Lever, 1990; Guo-Fang et al., 2006). They are used in agriculture to boost food supply by protecting crops against destructive pests both in the field and during storage. The mode of use varies from dressing of seeds, treatment of soil and spraying of fields to post-harvest treatment of farm produce. Fruits and vegetables, for instance, are often washed with fungicides or waxed with pesticide-containing emulsions to preserve their quality during transportation and handling, that is, to reduce perishability. Pesticides are also applied to grains during storage to prevent pest infestation.

The activities of agricultural pests, if unchecked, may lead to food spoilage, decreased food value, reduced germination, poor crop yield and post-harvest food losses (Collins, 2006). It has been estimated in the United States of America that about 40% of crop yield is lost to pests and that 20% of pest damage occurs after harvest (Reece, 1985). Also, world crop yield is reduced by 20-30% annually as a result of pest activities (Guo-Fang et al, 2006). These figures may be higher under tropical and subtropical conditions, hence the growing use of agricultural pesticides, otherwise known as agrochemicals.

Apart from their role in agriculture, pesticides are also used in public health services to eradicate certain veterinary and human diseases through domestic and industrial application. In the developed countries of the world, it is estimated that at least 1kg of pesticide is used per person per year (Taylor, 1990). This figure is likely to be higher in developing countries with a huge population of domestic pests as well as agricultural

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pests. In addition, low level of awareness of the negative effects of pesticides on human health and environment due to low literacy rates among pesticide users in developing countries may be a contributing factor to excessive use. Most (75%) of the world's usage of pesticides however, is in agriculture for increased food production as shown in Figure 2.11.

1.2   PESTICIDES, ENVIRONMENT AND HUMAN HEALTH

Global concern about environmental pollution is growing because of the huge number of chemical toxicants being discharged daily into the environment. There is the fear that human health is potentially endangered by these agents. This pollution has grown with urbanization and industrialization. As a result of their widespread use, pesticides have become a major contributor to present environmental contamination (Ezugwu and Okonkwo, 1999; Osibanjo, 2001; Koprucu et al., 2006).

Useful as they may be in food production and eradication of disease vectors, most pesticides are poisons. Once used, they enter into the environment and accumulate in the food chain (Turgut, 2007; Borrell and Aguilar, 2006). There is, consequently, a chronic exposure of the general population to low doses of these chemicals through air, water and food. This may lead to chronic toxicity due to accumulation in the human body over a long period of time and this, in turn, poses numerous health hazards. The public health risks of pesticides depend not only on the toxicity of the chemicals but also on the number of people exposed to them. Hence there is a need to ensure that residues in food and environment are at minimum levels to protect the general population. Possible health problems associated with pesticides include cancers, congenital malformations,

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neurotoxic disorders, infertility, blood dyscrasias and many others. These hazards may occur even with chemicals not associated with high acute toxicity (PAN, 2001).

Apart from chronic toxicity, there is the risk of acute and sub-acute toxicity to people who are directly involved in the manufacture, formulation, mixing and application of pesticides through dermal contact, inhalation and accidental ingestion. In addition to the quantities discharged into the environment, some pesticides, notably the organochlorine compounds have the attributes of being persistent in the environment by virtue of their high chemical stability and lipid solubility. Hence, such compounds may remain in soil and in the tissues of various organisms long after their use (WHO/UNEP, 1990b; Borrell and Aguilar, 2006).

The numerous environmental and health problems associated with pesticide use have generated concern about the quantity and quality of pesticides in use. To minimize the risks posed to humans while deriving optimum benefits, the use of pesticides is regulated by governments all over the world. Safety levels have been established for most pesticides by the FAO/WHO technical committee on pesticide residues (Handa, 1999; WHO, 2001). Effective regulatory control would help to minimize human exposure to these chemicals and reduce potential health hazards posed by pesticide use. In Nigeria, the National Agency for Food and Drug Administration and Control (NAFDAC) is the body with the mandate to regulate and control the importation, distribution, sales and use of chemicals, including pesticides (Ugbeye, 2004). Effectiveness of control measures cannot be assessed without analysis of pesticide residues in food and environmental media, hence the need for pesticide residue data base.

1.3   BACKGROUND OF STUDY

Agriculture plays a very important role in the provision of food for human consumption. In addition, agriculture provides raw materials for clothing and shelter which are also basic needs of man, next only to food. In the developing countries, a substantial segment of the rural and urban population depends on agriculture for subsistence. In order to meet the growing food demand of an expanding world population, there is a need to increase agricultural food production. Increased yield has been achieved over the years through technological inputs such as improved irrigation techniques, cheaper and better quality fertilizers, development of high-yielding seeds, new farm machinery and better farm management techniques (Naylor, 2003; Bomford and Langley, 2003). Crops produced using these techniques need to be protected against the destructive effects of pests both in the fields and during storage. Crop protection chemicals are therefore used as aid to adequate food production.

Although, there are other methods of pest control as discussed in section 2.10, chemicals remain less time-consuming and easier to use than their alternatives (Taylor, 1990; Naylor, 2003). Alternative methods require long-term research and planning. This makes their use difficult where there is low level of literacy among farmers and users. Hence, pesticides have continued to play a major role in agricultural food supply as well as in disease vector control.

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1.4   STATEMENT OF PROBLEM

In spite of World regulatory control of pesticide use, some countries, especially in the developing world, lack adequate facilities for monitoring importation, production and application of pesticides. Therefore, toxic and persistent pesticides which have been banned in developed countries remain on sale and are probably still in use in such countries that do not monitor pesticide use (Mansour, 2004). While legitimate use of authorized pesticides may not produce toxic residues in food and environment, misuse/abuse and the use of banned or substandard products may cause serious environmental contamination, leading to high human exposure with associated danger to health. In a survey of pesticide samples from countries that have no quality control facilities, one-third of the samples tested were found to be substandard (WHO, 2001).

Also, pesticide misuse leading to high residues in food may be widespread in countries that fail to monitor pesticide use. An example of acute toxicity arising from probable misuse in Nigeria is the "killer beans" incident of 1996 which resulted in the death of tens of people in Lagos and environs after consuming beans/bean products. Although the exact chemical involved in the incident was never identified, it was highly suspected that toxic concentrations of pesticides, not recommended for foodstuffs were used on the beans. Monitoring of pesticide residues in food is therefore an important approach to minimizing the potential hazards of pesticides to human health (WHO/UNEP, 1990b).

Factors that may lead to excessive use of pesticides among farmers include the following:

i. Fear of harvest failure.

ii. Eagerness to protect crops.

iii. Use of substandard products.

iv. Wrong timing of applications.

v. Lack of awareness of the negative effects of pesticides on health and environment.

vi. Ignorance about possible development of pest resistance to pesticides.

vii. Pesticide misuse (similar to drug misuse) in which farmers resort to pesticides even

when their use may not be absolutely indicated.

Under the above circumstances, there is a likelihood that hazardous concentrations of these chemicals are present in the environment, especially in agricultural products which have been treated with pesticides. Food is therefore a readily available agent of human exposure to pesticides. Sources of pesticide residues in food include absorption from soil, application of pesticides to crops in fields, drifts from other locations of pesticide use and post-harvest addition of pesticides to foodstuffs to avoid pest infestation during storage and transportation.

One category of foodstuffs likely to contain high levels of pesticide residues is cereal grains. Cereal grains are a major source of food for the world population as they are easily grown in many parts of the world and are accepted across age, social status, tribes, cultures and race. They provide feeds for livestock, which in turn provide meat, dairy products and eggs. Beans are a major source of protein especially in the developing world where other sources of protein are often too expensive for widespread consumption. Beans and cereal grains are major foodstuffs to which direct addition of

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chemicals is often made to avoid heavy pest infestation during storage. This increases

the risk of high levels of residues in these food items. The question therefore is:

How much pesticide residues are there in the food we consume?

1.5 SIGNIFICANCE OF THE STUDY

Analysis of pesticide residues in food is one of the ways of ensuring food safety. It is

very important for several reasons including the following:

1) To protect the health and well-being of consumers by preventing incidence of unsafe

   levels of pesticides in food.

2) To promote development of trade in food and food products between countries as

   foods exported from one country to another have to meet the residue limits of the

   receiving country.

3) To check compliance with national and international standards.

4) To enforce national and FAO/WHO regulations on pesticide use.

5) To get early warnings of future problems and prevent them.

1.6 OBJECTIVES OF STUDY

1.6.1 General Objective

This study was carried out to determine the incidence and quantity of pesticide residues in maize and beans types in Lagos markets.

1.6.2 Specific Objectives

Specific objectives of this study are the following:

1. To identify and quantify pesticide residues in maize and beans samples from various markets in Lagos State.

2. To compare values obtained with maximum residue limits (MRLs) established by FAO/WHO and thereby assess the level of compliance of pesticide users with national and international regulations on pesticide use.

3. To estimate daily intakes of pesticides by residents of Lagos State through consumption of maize and beans and compare values obtained with acceptable daily intakes (ADIs) established by FAO/WHO and thereby highlight possible health implications.

4. To investigate the possible effect of heat on pesticide residue contents of beans.

5. To contribute to data base on pesticide residues in Nigerian foods.

CHAPTER TWO

LITERATURE REVIEW

2.1 DEFINITIONS

2.1.1 Pest: - A pest is any living thing that successfully competes with humans for food, space or other essential needs or is injurious to human health (Purdom & Anderson, 1983). Some pests also transmit diseases from one living thing to another. These are known as vectors.

Pests may be animal or plant species; they may be domestic or agricultural pests. They are usually unwanted forms of life as their activities are generally directly or indirectly troublesome to man. Common pests include insects, fungi, bacteria, rodents, birds, nematodes, algae, weeds, etc.

2.1.2 Pesticide: - A pesticide may be defined as a chemical agent capable of destroying unwanted forms of life called pests (Parry, 1976). It may also be defined as any agent of chemical or biological origin that kills pests or otherwise prevents them from engaging in behaviors deemed destructive (Ware and Whitacre, 2004). Pesticides may be natural or man-made and are applied to target pests in different types of formulations and delivery systems.

The term pesticide has also been extended to include agents capable of controlling the growth and reproduction of pests (Sowunmi and Agboola, 1982). These include:

i. Growth Regulators – chemicals that stimulate or retard growth of plants.

ii. Defoliants – those that cause leaves of plants to die and fall off.

iii. Desiccants – those that speed up the drying of plants.

iv. Repellents – those used for keeping pests away.

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v. Attractants – chemicals for attracting pests.

vi. Chemosterilants –those for sterilizing pests.

Pesticides effect their actions through various mechanisms. A pesticide should ideally be selective in its toxicity. This means that it should be toxic only to the pests against which it is targeted. It should be non-toxic to crops, man, non-target animals and other forms of life, some of which may be beneficial to man. Also, a pesticide should be effective, safe, easy to apply and generally economical for use. It is, however, difficult to achieve this ideal state for any one pesticide and so most pesticides have advantages and disadvantages by which they are considered for use. Examples of pesticide groups and their target pests are shown in Table 2.1.

2.1.3 **Pesticide Residue**: - This is any substance occurring in food or agricultural commodity as a result of the use of pesticides on the commodity. Residues include degradation products, metabolites and reaction products of toxicological importance as well as parent compounds (Handa et al., 1999).

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Table 2.1 – Some Pesticide Groups and their Target Pests.

Pesticide	Target Pest
Insecticides	Insects
Fungicides	Fungi
Herbicides	Weeds
Rodenticides	Rodents
Bactericides	Bacteria
Avaricides	Birds
Nematocides	Nematodes
Molluscicides	Molluscs

2.2   CLASSIFICATION OF PESTICIDES

Pesticides may be classified in several ways (Sowunmi and Agboola, 1982; Evans, 1996; Valles and Koehler, 2003). The common modes of classification include the following:

a. Chemical classification

b. Classification based on target organism

c. Classification based on route of entry of the compound in the target pest

d. Hazard classification

2.2.1   Chemical Classification

In this form of classification, compounds with certain chemical groups in common are grouped together as one irrespective of their target pests. The main chemical classes of pesticides are the following:

1.   Organochlorines

These are compounds that usually contain carbon, hydrogen and chlorine. They are also known as chlorinated hydrocarbons or chlorinated organics. They were introduced in the 1930s and have a wide range of insecticidal activity (Lever, 1990). They are therefore used mainly as insecticides though some have fungicidal and herbicidal properties. Organochlorines are generally insoluble in water but soluble in organic solvents. Due to their chemical stability and lipophilic nature, they are very persistent in the environment and fatty tissues as they are not easily degraded or metabolized. As a result, they are concentrated as they pass from prey to predator in the food chain, such that animals such as fish, reptiles and birds can accumulate lethal amounts over time (Borell and Aguilar, 2006). These compounds are therefore hazardous to the environment and human health. Hence,

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they have been largely replaced by newer and less persistent compounds like organophosphates, carbamates and pyrethroids. Organochlorines are, however, still in use in most developing countries because they are cheaper and broader in spectrum of activity than their substitutes (WHO, 2001).

Classification of Organochlorine Pesticides

Organochlorine pesticides may further be classified into four chemical groups as follows:

(a) Chlorobenzene Derivatives (Diphenyl Aliphatics) – The first and best known member of this group is dichlorodiphenyltrichloroethane (DDT). DDT has been used widely in agriculture for the control of various species of insects that destroy crops and in public health against disease vectors like mosquitoes, flies, lice and fleas.

DDT has a negative temperature coefficient, that is, the lower the ambient temperature, the more toxic it is to insects. In man, DDT is slowly metabolized by dehydrochlorination to dichlorodiphenyldichloroethylene (DDE) or to dichlorodiphenyldichloroethane (DDD) by dehalogenation. The major metabolic pathway of DDT in man is shown in Figure 2.1. Its use has, however been banned in most countries because of its environmental hazards and as less persistent pesticides have been developed (Ware and Whitacre, 2004). Other members of the group are methoxychlor and diflorodiphenyltrichloroethane (DFDT).
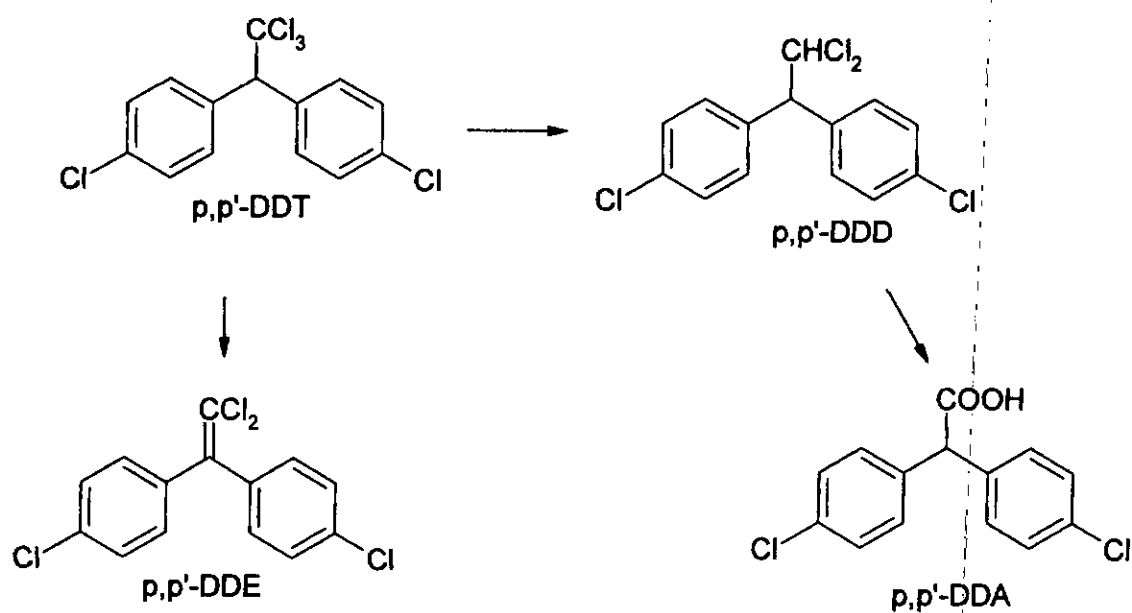
Figure 2.1: Major Metabolic Pathway of p,p'-DDT in Man

(b)   Benzene Derivatives – The first member of this group is hexachlorocyclohexane (HCH). HCH has five isomers (alpha, beta, gamma, delta and epsilon) depending on the configuration of the chlorine atoms in the six positions of the benzene ring. It has been found that only the gamma isomer has insecticidal properties while the other four isomers are inactive components, and that the active gamma isomer is only about 12% of a normal mixture of HCH (De Brun, 1976a). Hence, a commercial product containing 99% gamma isomer was developed and is commonly called Lindane. It is an effective insecticide for both domestic and agricultural use, but like most organochlorine pesticides, its use has been either banned or highly restricted in many countries due to potential hazard to man and useful animals.

Another member of this group is pentachlorophenol (PCP). This compound is used mainly for protection of timber against termites and wood-boring insects. Apart from being active against insects, PCP also has fungicidal and herbicidal properties.

(c)   Cyclodienes – Compounds in this group are more toxic than DDT and better absorbed through the skin. They therefore pose a higher risk of occupational exposure. The first member of this group is chlordane which was introduced in 1945. Other members include aldrin, dieldrin, heptachlor, endrin and endosulfan. They are effective against insects especially termites and soil-borne insects and are usually applied to soil for the control of these pests. They are equally toxic to mammals, birds and fish, and are highly persistent in the environment. Wood structures treated with Cyclodienes have been known to remain protected for more than 55 years (Ware and Whitacre, 2004). Therefore, their use is either banned or highly restricted in most countries.

(d) Chlorinated Camphenes (Polychloroterpenes) – This group is comprised of Toxaphene and Strobane. They are less persistent and less toxic than other organochlorine compounds. Toxaphene is the more widely used of the two compounds, usually on cotton. It is usually formulated with other insecticides as its toxicity to insects is low. Fish is susceptible to toxaphene poisoning. Structures of commonly used organochlorines are shown in Figure 2.2.

Mode of Action of Organochlorines

The exact mode of action of organochlorine pesticides has not been clearly established but there is indication that they disrupt the delicate balance of sodium and potassium within the axon of the neuron, thereby preventing normal transmission of nerve impulses (Ware and Whitacre, 2004). This causes hyper-excitability of nerves and muscles resulting to muscle twitching which may lead to convulsions and death. Organochlorines are therefore neurotoxic with DDT and its analogues affecting mainly the peripheral nervous system while γ-HCH and aldrin groups have pronounced effects on the central nervous system.
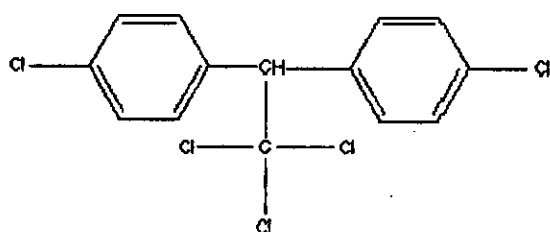
Dichlorodiphenyl-
trichloroethane
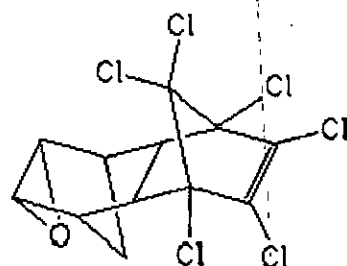
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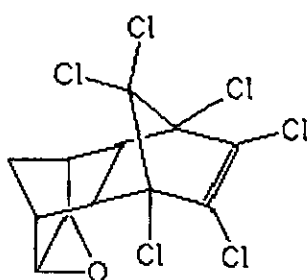
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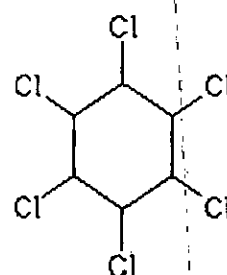
Hexachlorocyclohexane
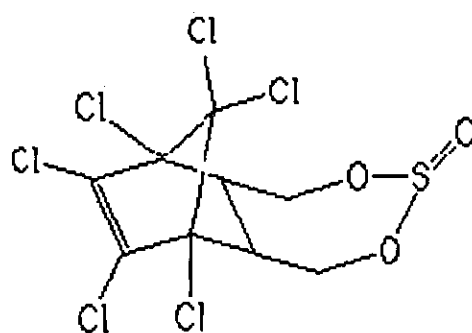
Endosulfan

Figure 2.2: Structures of Some Organochlorine Pesticides.

2. Organophosphates

These are chemically related compounds possessing the organic phosphate radical in their structure as shown in Figure 2.3. They are derivatives of phosphoric acid. They are also often referred to as organic phosphates, phosphoric acid esters or organophosphorus compounds.
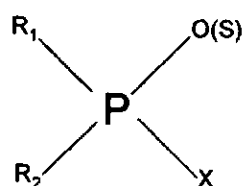
Figure 2.3: Basic Structure of Organophosphates.

R_1, R_2 = alkyl, alkoxy, phenoxy, amino or substituted amino groups.
X = any displaceable group e.g. halogens, chlorophenol, nitrophenol, phosphoric anhydride, etc.

Organophosphates have become a very important class of insecticides as they combine their feature of high potency with non-persistence and higher selectivity (De Brun, 1976$_a$). They have therefore superseded the persistent organochlorines especially in the control of storage insects in food crops.

Organophosphates are, however, highly toxic to vertebrates including man, most of them exhibiting lethal dosage (LD$_{50}$) values of as low as 0.1 – 10mg/kg body weight in mammals compared to 7 – 40mg/kg for most organochlorine compounds (Purdom and Anderson, 1983). This has resulted in organophosphorus compounds recording a higher incidence of poisoning or acute toxicity than other commonly used pesticides as shown in Table 2.2. Their degrees of toxicity vary widely among compounds and between animal

species mainly due to variation in the rate of biotransformation. They also vary in physico-chemical properties and stability.

Classification of Organophosphate Pesticides

Organophosphorus compounds may be sub-grouped as follows:

(a) Aliphatic Derivatives – These are compounds with linear carbon chains attached to the phosphate radical. They are quite unstable and hydrolyze quickly in water. This reduces their level of contribution to environmental contamination when used. Examples include tetraethylpyrophosphate (TEPP), Malathion, Trichlorfon, Monochrotophos, Dichlorvos, Mevinphos and Dimethoate.

(b) Phenyl Derivatives – These compounds contain a phenyl ring with one of the hydrogen atoms displaced by attachment to the organic phosphate moiety. They are generally more stable than the aliphatic derivatives, leaving longer lasting residues when used. Examples are Parathion, Fenitrothion and Crofomate.

(c) Heterocyclic Derivatives – Members of this group have an heterocyclic ring as part of the displaceable group (X) attached to the phosphate radical. Examples are Diazinon, Azinphos-methyl and Chlorpyrifos. They are more stable than members of groups (a) and (b) above.

Table 2.2 – Poisoning in the Ribeira Valley, Brazil in 1985 (PAN, 2001).

Group of Active Ingredients	Number of Poisonings	Fatal Poisonings	% of Poisonings per Ingredient
Organophosphates	45	8	37.5
Carbamates	36	—	30.0
Paraquat (a herbicide)	12	5	10.0
Chlorinated Hydrocarbons	8	2	6.7
Others	19	1	15.8

Mode of Action of Organophosphates

Organophosphorus pesticides exert their toxic action by inhibiting the enzyme acetylcholinesterase (AChE). This enzyme is responsible for the hydrolysis of acetylcholine (ACh), a neurotransmitter that conducts nerve impulses across neuromuscular junctions in the nervous system of vertebrates (as well as insects). Hydrolysis of Ach to choline and acetic acid ensures removal of the transmitter after electrical conduction, leading to cessation of muscle contraction. With AChE inhibitors like organophosphorus pesticides, the enzyme is rendered unavailable for the removal of ACh. This causes accumulation of ACh leading to generalized cholinergic action and resulting in rapid, uncontrolled twitching of voluntary muscles which eventually leads to paralysis, respiratory failure and death (De Brun, 1976b; Podolska and Napierska, 2006).

Enzyme inhibition by organophosphates is by covalent combination of the enzyme with the inhibitor. This is thought to be through a link between the organic phosphate radical of the compound and the active site of the enzyme molecule. Organophosphate compounds thus compete with ACh for available enzyme protein. This process known as phosphorylation results in the formation of a stable enzyme-inhibitor complex which is unable to remove ACh from electrical conduction sites. The anti-cholinesterase action of organophosphorus compounds is exerted not only in insects but also in higher animals, hence, their high acute toxicity in man and other vertebrates. Human exposure to organophosphorus compounds can therefore be monitored by determining erythrocyte and plasma cholinesterase activity (Guilhermino et al., 2004).

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Organophosphates inhibit both central and peripheral AChE. In insects, however, organophosphate poisoning affects mainly the central nervous system since the insect neuromuscular junction is not cholinergic. Although, the enzyme-inhibitor complex formed by phosphorylation may be spontaneously hydrolyzed to regain normal enzyme activity, the rate of hydrolysis is so extremely slow that AChE inhibition can be said to be irreversible. The mechanism of phosphorylation of an organophosphorus compound is shown in Figure 2.4.
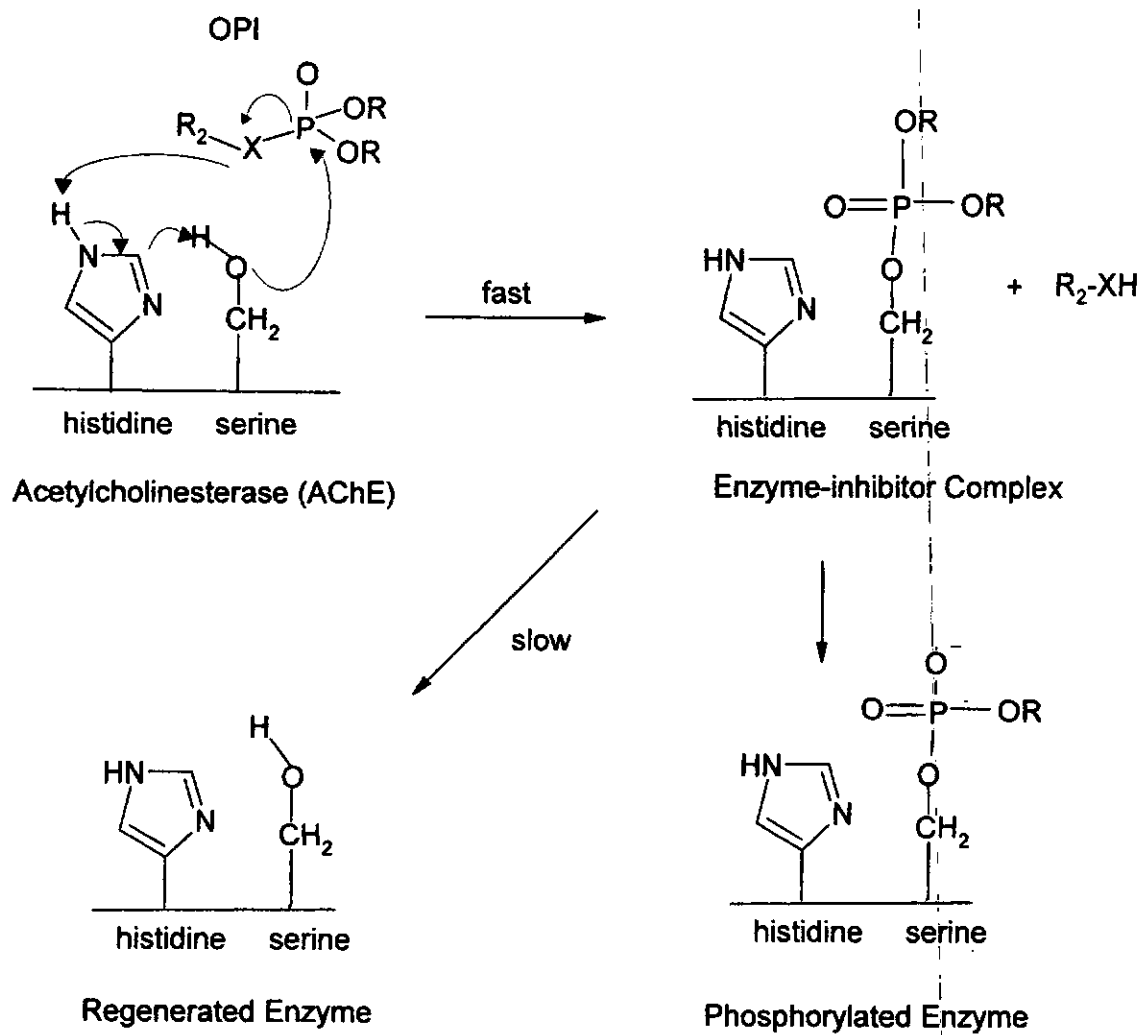
Figure 2.4 – Phosphorylation of the Active Site of AChE by an Organophosphorus Compound.

3. Carbamates

These are N-substituted esters of carbamic acid having the general formula shown in Figure 2.5. The carbamoyl group [OC(O)NC] is essential for pesticidal activity.

A   B

Figure 2.5: Structure of Carbamic acid (A) and General Structure of the Carbamates (B).

[R = methyl, aromatic or benzimidazole group;   X = aromatic or aliphatic moiety.]

Carbamates vary widely with regard to chemical structures of individual compounds as shown in Figure 2.6. Their toxicity depends to some extent on structural properties. They are crystalline solids with variable water solubility but highly soluble in polar organic solvents. They possess the advantage of combining low mammalian toxicity with full insecticidal effectiveness. They have therefore gained extensive use in agriculture as insecticides, fungicides, herbicides, nematocides and sprout inhibitors. They are also used as biocides in industrial applications and household products.

Carbamates undergo metabolism in plants, insects and mammals. The metabolites are generally less toxic than the parent compounds. They do not accumulate in the mammalian body but are rapidly excreted via the urine.

Classification of Pesticidal Carbamates

The various carbamates may be further classified into three sub-groups according to the nature of the N-substituents as follows:

(a) Carbamate Insecticides – These are carbamate compounds in which R is a methyl group (N-methyl carbamates). They are used mainly as insecticides and nematocides and are relatively less toxic to mammals than the other carbamate pesticides. The degree of cholinesterase inhibition of individual compound however, depends on the nature of the leaving group X (Fig. 2.5). Examples of carbamates in this group are carbaryl, carbofuran, propoxur and aldicarb.

(b) Carbamate Herbicides – In this group, the R is an aromatic moiety. The compounds are used mainly as herbicides and sprout inhibitors. Examples are Pyrolan and Dimetilan.

(c) Carbamate Fungicides – These are compounds with benzimidazole moiety in position R. They are used as fungicides. Examples include Benomyl, Carbendazim and the Thiophanates.

Mode of Action of Carbamates

The pesticidal action of carbamates, like the organophosphates, is due to the inhibition of cholinesterase enzyme with subsequent cholinergic effects. In the carbamate inhibition, the electrophillic carbamoyl group binds to the esteratic site of the enzyme in a process known as carbamylation. Unlike in phosphorylation, the process of carbamylation is competitive and reversible and the rate of regeneration of the active enzyme from the carbamoyl-enzyme complex is relatively rapid compared to regeneration of a

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phosphorylated enzyme (De Brun, 1976b). Hence, carbamates are less toxic than organophosphorus compounds. Also, carbamates are more slowly absorbed through the skin than organophosphates. As with organophosphates, exposure to carbamates can be monitored by measuring plasma cholinesterase activity.

Susceptibility of different insect and animal species to a given carbamate varies due to differences in the rate of in-vivo detoxication and routes of biotransformation. For example, carbamates accumulate in fish due to slow metabolism. They are therefore highly toxic to fish. Structures of Some organophosphate and carbamate pesticides are shown in Figure 2.6.

4. Pyrethroids

These are synthetic analogues of natural pyrethrins. They are more photostable than the natural product and are effective against most insect pests at low concentrations. They are used against flying as well as storage insects such as mosquitoes, cockroaches, aphids, ticks, lice, fleas, etc. They are less toxic to mammals due to more rapid metabolic deactivation in the latter. Some pyrethroids are volatile and non-persistent (e.g. tefluthrin) while others are non-volatile and moderately persistent (e.g. cypermethrin).
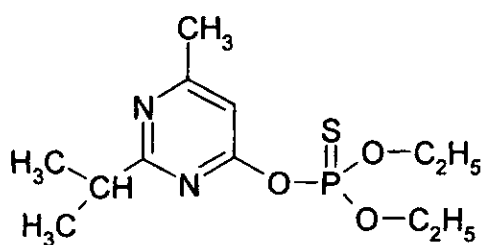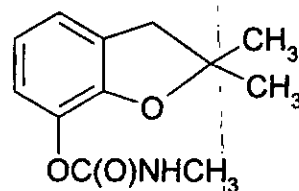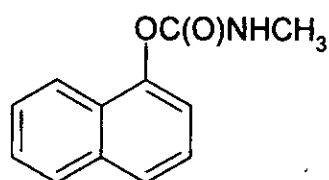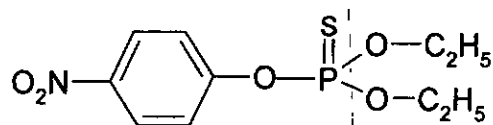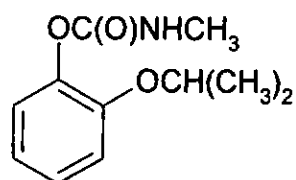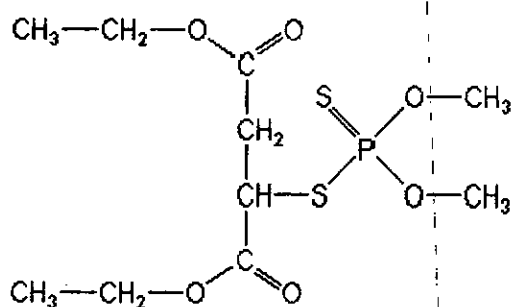
Figure 2.6: Structures of Some Organophosphate and Carbamate Pesticides.

The pyrethroids are said to be axonic poisons because they act by keeping sodium channels in neuronal membranes open, thereby causing a continuous slow depolarization which eventually blocks nerve conduction and causes paralysis (Ware and Whitacre, 2004). They affect both the peripheral and central nervous system of the insect. Pyrethroids are of two types depending on the effect of temperature on insecticidal activity. Type I pyrethroids have a negative temperature coefficient, showing increased activity with decrease in ambient temperature. Type II pyrethroids, on the other hand, have a positive temperature coefficient (that is, increased kill with increase in ambient temperature).

Synthetic pyrethroids in common use include permethrin, cypermethrin, cyfluthrin, deltamethrin and lambda cyhalothrin. Structures of pesticides from various classes are shown in Figures 2.7 and 2.8.

5. Organosulphur Compounds

These are compounds containing two phenyl rings with sulphur as their central atom. Sulphur itself is a good acaricide especially in hot weather but the organosulphurs are by far superior in activity. They are used mainly as acaricides and fungicides since their toxicity to insects is low. Their mechanisms of pesticidal activity are as varied as the chemical structures of member compounds. Groups of compounds included in this class are:

     (a)   Dithiocarbamates and thiocarbamates

     (b)   Trichloromethylsulphenyl compounds

     (c)   Tetrachlorodiphenylsulphides
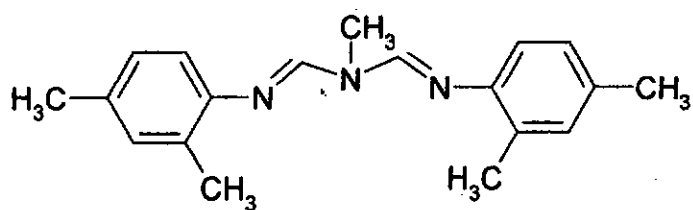
Amitraz
(A formamidine)
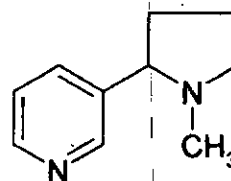
Nicotine
(A natural product)
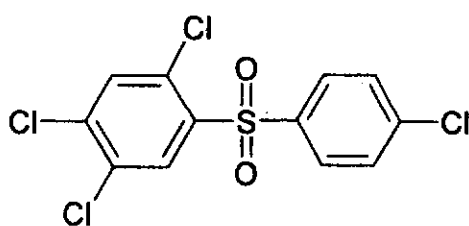
Tetradifon
(An organosulfur)

Figure 2.7: Structures of Some Pesticides

6. Formamidines

This is a small group of compounds used mainly as insecticides against organophosphate and carbamate resistant pests. Their mode of action has been proposed to be the inhibition of the enzyme monoamine oxidase (Sowunmi and Agboola,1982). This inhibition results in the accumulation of biogenic amines such as norepinephrine and serotonin. Examples of insecticides in this group are chlordimeform and amitraz.

7. Dinitrophenols

These compounds have been used as herbicides, insecticides and fungicides. They were very effective pesticides but their use has been limited due to high toxicity. They act by uncoupling oxidative phosphorylation, that is, preventing the formation of adenosine triphosphate (ATP) and hence preventing the utilization of nutritional energy. Examples of compounds in this group are Dinitrocresol (DNOC) which is now used as a total herbicide and Dinocap, an acaricide and fungicide found particularly effective against powdery mildew.

8. Thiocyanates

These are insecticides that are highly toxic to flying insects but relatively safe for man and animals. Their mode of action is not well established but they have been said to interfere with cellular respiration and metabolism. Two members of this group are Lethane 60 and Thanite.

9. Organotins

These are compounds containing tin (Sn) as their central atom. They are used as acaricides and fungicides. They act by inhibiting oxidative phosphorylation, just like the dinitrophenols. An example is cyhexatin.

10. Phenylpyrazoles (Fiproles)

The only member of this group is Fipronil which is an insecticide used for the control of many soil and foliar insects. It is a systemic pesticide with contact and stomach activity and is effective against insects resistant or tolerant to organophosphates, Carbamates and pyrethroids.

11. Pyrroles

The first and only member of this group is Chlorfenapyr which is a contact and stomach insecticide. It is used mainly on cotton.

12. Pyrazoles

The pyrazoles are non-systemic contact and stomach miticides. They give fast knockdown and long residual activity. Examples are Tebufenpyrad and Fenpyroximate.

13. Pyridazinones

The only member of this group is Pyridaben which is a selective contact insecticide and miticides. Like the pyrazoles, it has rapid knockdown and long residual control.

14. Quinazolines

Fenazaquin is the only member of this group. It is a contact and stomach miticide with ovicidal activity. It is used on cotton and citrus fruits.

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15. Natural Derivatives

These are compounds derived from plants and have been in use for longer than any other group of pesticides. Three main classes are known as follows:

(a) Nicotinoids – These are derivatives of the alkaloid nicotine from tobacco. Nicotine is effective mainly for the control of sucking insects such as aphids and soft-bodied insects like caterpillars. It mimics acetylcholine in the central nervous system of insects resulting in twitching, convulsions and death. Nicotine is used in its sulphate form. Synthetic derivatives include Imidacloprid, Acetamiprid and Thiamethoxam.

(b) Pyrethrum – This a broad-spectrum insecticide extracted from the flowers of a chrysanthemum plant. It is a mixture of four compounds: pyrethrins I and II and cinerins I and II. The pyrethrins are the oldest and safest group of insecticides and are effective against flying as well as storage insects. In addition, they have a fast knockdown, that is, the ability to rapidly disable the insect. They are however not suited for agricultural use because they are photolabile. They are less toxic to mammals due to more rapid metabolic deactivation in these animals. For instance, the pyrethrins are easily degraded by stomach acid.

Pyrethrum, like the pyrethroids, is an axonic poison. It affects electrical impulse transmission in the insect by keeping sodium channels in the axon open, thereby causing a continuous slow depolarization which eventually blocks nerve conduction and causes paralysis (Ware and Whitacre, 2004).

Pyrethrins are about the only natural insecticides still in use, usually in combination with synergists (such as piperonyl butoxide) or synthetic insecticides. Natural pyrethrum has been largely superseded by the synthetic derivatives which possess improved pesticidal properties and more photostability.

(c) Rotenoids – The active ingredient in this group of compounds is rotenone which is derived from the roots of plants of the genus Derris. It is effective against all leaf-eating caterpillars. It is a respiratory enzyme inhibitor and results in failure of the respiratory functions of the organism. It is a stomach and contact insecticide and also a piscicide, being toxic to all fish at low doses.

16.   Inorganics

This is a group comprising pesticides that do not contain carbon. Many of them were in use before the advent of other groups of pesticides and are still used in integrated pest management programmes. They are used mainly against insects, mites and fungi. Examples include sulfur, copper (mainly the acetate and sulphate), boric acid, sodium fluoride and compounds of mercury, boron, arsenic and selenium.
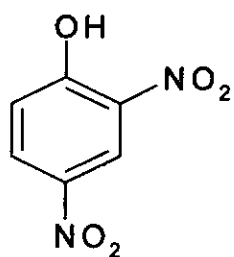
2,4-Dinitrophenol
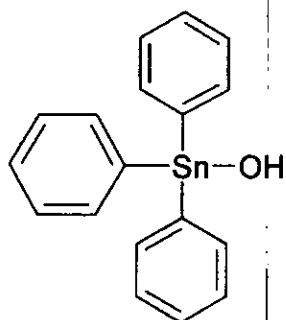
Cyhexatin
(An Organotin)
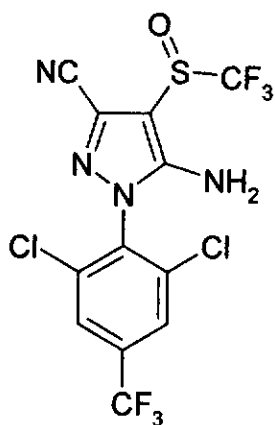
Fipronil
(A phenylpyrazole)
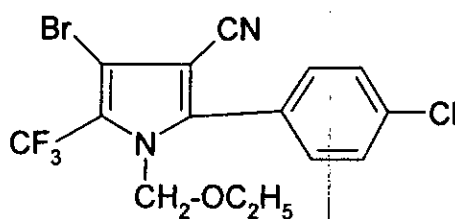
Chlorfenapyr
(A pyrrole)
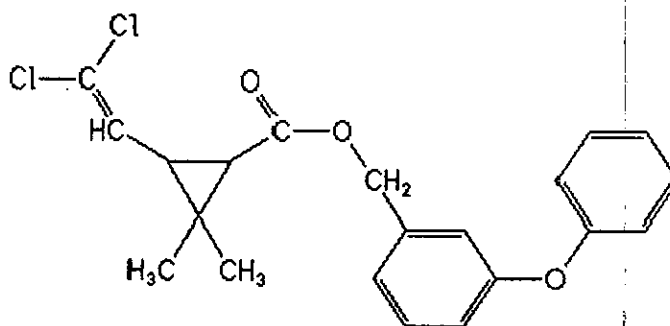
Permethrin (a pyrethroid)

Figure 2.8: Structures of More Pesticides

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2.2.2 Classification Based on Target Pests

1. Insecticides

These are compounds used because of their capability to kill or control insect pests. They are used widely in public health and in agriculture against insects such as mosquitoes, flies, lice, fleas, termites, cockroaches, ants, weevils, caterpillars, beetles, moths, budworms, and others. Members of this group are drawn from different chemical classes as discussed in section 2.2.1 above. Examples of insecticides are Chlorpyrifos, lindane, carbaryl, permethrin, etc.

Some compounds act as insect growth regulators (IGRs) and insect development inhibitors (IDIs). These act mainly on the immature forms, preventing them from developing into adult forms. The IGRs mimic the juvenile growth hormone which is what keeps the insects from developing into more mature forms. Therefore the immature forms fail to molt and death occurs. The IDIs inhibit the synthesis of a substance called chitin. Chitin is necessary for the formation of the hard outside skin (cuticle) of the insect, hence the adult is not formed.

These groups of chemicals are used widely against fleas in pets like cats and dogs. Since they are designed to mimic insect hormones or alter a unique insect process (the making of chitin, which mammals do not make), they are very safe for man and other animals. Examples are fenoxycarb (IGR) and diflubenzuron (IDI).

Common agricultural pests are shown in Figure 2.9.

Black Cutworm
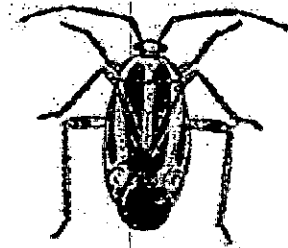
Fourlined Plant Bug
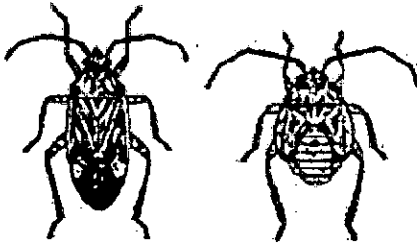
Tarnished Plant Bug
Adult and Nymph

Millipede

Common Slug
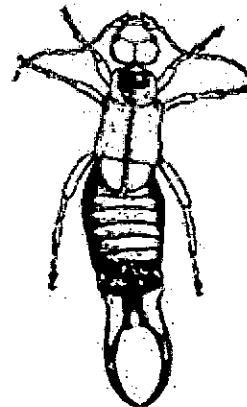
Male Earwig

Japanese Beetle
Adult
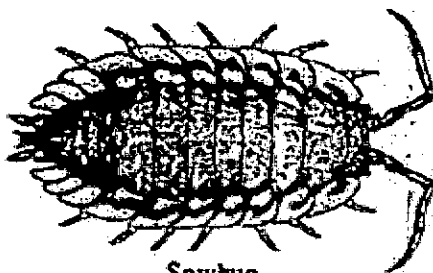
Sowbug
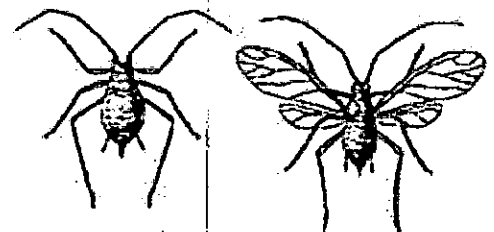
Nonwinged and
Winged Aphids

Figure 2.9: Some Common Agricultural Pests

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2. Herbicides

These are compounds used in the control of weeds. Weeds are unwanted plants which compete with crops for space, nutrients, light and moisture, thereby reducing the quantity and quality of crop yield in agriculture. Some weeds are parasitic, deriving all their requirements from the host plant. Weeds may create unwanted shade for crop plants thereby preventing them from getting needed sunlight while others bind themselves round crops and literally strangle them.

Herbicides are the most commonly used agricultural pesticides in the developed world (as shown in Table 2.5) but in developing countries, insecticides are the most often used (WHO/UNEP, 1990b; Mansour, 2004). This is probably due to the fact that mannual control of weeds (such as hoe weeding and hand pulling) is still widely practiced in developing countries. Herbicides are generally less toxic to man than most insecticides. Also, emergence of herbicide-resistant weeds is not common, unlike resistance to insecticides.

Herbicides may be selective or total in their action. In herbicide selectivity, the target pest (to be selectively killed) is closely related to the crop (to be protected), whereas with insecticides, selectivity is easier to achieve as insect pests are biologically very different from crops.

Herbicides comprise a variety of structural types and act on plant metabolism in different ways. They may affect plant growth by inhibiting cell division, cell elongation and enlargement, tissue and organ differentiation, seed germination and seedling growth (Igbedioh, 1991). Herbicides may be classified in two ways as follows:

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(a) Classification Based on Chemical Groups

The chemical groups of herbicides are:

i. Phenoxyalkanoic acid Derivatives – These are powerful herbicides having structural resemblance to plant growth hormones. They are relatively harmless but often contain the contaminant, dioxin (inadvertently formed during the manufacturing process) which is highly poisonous to animals and humans. Examples are 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2-methyl-4-chlorophenoxyacetic acid (MCPA). 2,4,5-T being the most heavily contaminated with dioxin is no longer used in most industrialized countries (Purdom and Anderson,1983).

ii. Bipyridylium Compounds – These are quaternary ammonium compounds which exhibit their herbicidal action by interfering with photosynthetic processes in the plant. Commonly used members of this group are paraquat and diaquat. They possess high dermal toxicity which is often higher than their oral toxicity. Paraquat is selectively accumulated in lung tissue and is therefore highly toxic to that organ.

iii. Triazines – These herbicides are widely used by application to soil, unlike (a) and (b) above which are applied to foliage. Examples are atrazine, propazine, prometone and ametryne.

iv. Phenylureas – These are effective but slow-acting weed killing agents. They are also applied through the soil. Examples of this group of herbicides are fenuron, monuron and diuron.

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v. Acylanilides – These are aniline-based selective herbicides. The main member of this group is 3, 4-dichloropropionanilide (DCPA).

(b) Classification Based on Type of Action on Plants

Herbicides may also be classified according to their type of action on plants as follows:

i. Contact Herbicides – These are fast acting compounds which kill plant parts through direct contact with foliage. Examples are atrazine and pentachlorophenol.

ii. Systemic Herbicides – These herbicides can be absorbed either through foliage or roots of plants and translocated through the entire plant system. They have long-term effect and only slightly toxic to animals. Examples of compounds in this group are 2,4-D and 2,4,5-T.

iii. Soil Sterilants – These are compounds that remain in the soil and exert herbicidal action for up to 24 months (Purdon and Anderson, 1983).

Some herbicides can fall into more than one category, e.g. a systemic herbicide can remain in soil as a soil sterilant. Structures of some herbicides are shown in Figure 2.10.

41
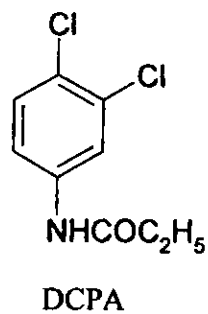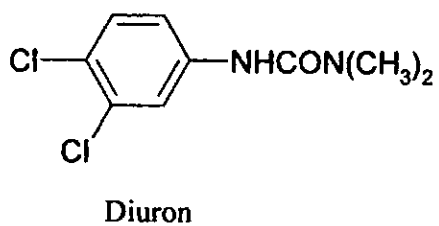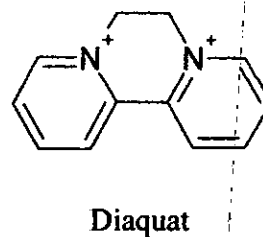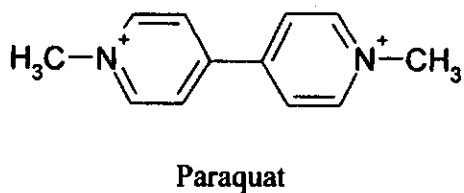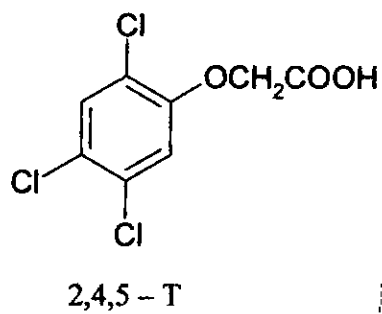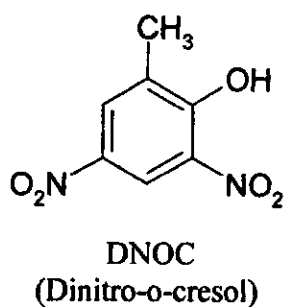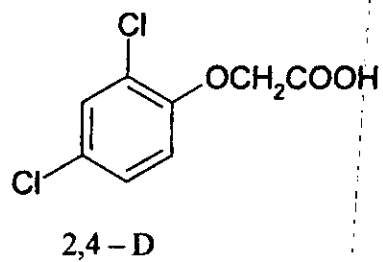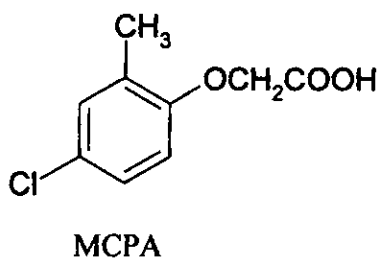
Figure 2.10: Structures of Some Herbicides

3. Fungicides

These are pesticidal agents used in the control of fungal diseases such as powdery mildew on tree plants and potato blight. They are drawn from various chemical classes and are generally less toxic to humans than insecticides. Fungicides may be used in the field as sprays on plants or in storage as seed dressings. Commonly used members of this group are the dithiocarbamates and the fungicidal carbamates.

4. Rodenticides

These are chemicals designed for the control of mice and other rodents. This class of pesticides comprises a variety of chemicals used in form of baits, tracking powders or fumigants (Crouse, 2001). Baits are used both in farms and in residential areas to attract rodents to feed while tracking powders are usually placed along rodent runways to be picked up by the fur as the animal passes by. Fumigants on the other hand are formulated to kill rodents in their burrows.

Types of rodenticides include the anticoagulants warfarin and diphacionone, cholecalciferol (vitamin D_3), bromethalin, zinc phosphide, strychnine and methyl bromide. Since rodenticides are designed to kill mammals, they are also very toxic to humans.

5. Others

Other classes of pesticides include bactericides, nematocides and avaricides for the control of bacteria, nematodes and birds respectively. These are however not as widely used in agriculture as classes 1 – 4 above. Some pesticides and their trade names are shown in Table 2.4.

2.2.3 Classification Based on Route of Entry into the Pest

1. Contact Pesticide

This is one that needs to penetrate the skin or cuticle of the pest before it can act. This type is usually effective against pests which move about a lot or which are likely to be on the plant at the time of application.

2. Stomach Pesticide

This type is only active after it has been consumed by the pest. It is therefore a kind of stomach poison and is effective against actively feeding pests.

3. Respiratory Pesticide

This is taken in by inhalation and acts by causing physiological disturbances in the respiratory system of the pest. Fumigants are a common example in this class.

4. Systemic Pesticide

This type of pesticide is generally able to penetrate the tissues of the pest and be translocated to parts remote from the site of application. A systemic pesticide can exert its pesticidal effect in parts other than that to which it was applied and is therefore effective against sap feeders and biting pests.

2.2.4   Classification Based on Degree of Hazard

This is a WHO classification in which LD_{50} is used as a measure of the degree of health risks posed by a pesticide compound. The compounds are then grouped into four classes as shown in Table 2.3.

Table 2.3 - Classification of Pesticides According to Degree of Hazard (WHO/UNEP, 1990$_a$).

Hazard Class	Description	LD_{50} (rat) (mg/kg body weight)			
		Oral		Dermal	
		Solid	Liquid	Solid	Liquid
la	Extremely Hazardous	5 or less	20 or less	10 or less	40 or less
lb	Highly Hazardous	5-50	20-200	10-100	40-400
ll	Moderately Hazardous	50-500	200-2000	100-1000	400-4000
lll	Slightly Hazardous	Over 500	Over 2000	Over 1000	Over 4000

Table 2.4 - Some Common Pesticides and their Trade Names

Common Name	Chemical Name	Trade Name	Chemical Class
Aldicarb	2-Methyl-2-(methylthio) propionaldehyde O-methyl Carbamoyloxime	Temik	Carbamate
Carbaryl	1-Naphthyl methylcarbamate	Sevin	Carbamate
Carbofuran	2,3-Dihydro-2,2-dimethyl benzofuran-7-yl methylcarbamate	Furadan	Carbamate
Chlorpyrifos	O,O-Dimethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate	Dursban, Lorsban	Organophosphate
Cypermethrin	α-Cyano-3-phenoxybenzyl-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropanecarboxylate	Barricade, Cymbush, Cynoff	Pyrethriod
Dichlorvos	2,2-Dichlorovinyldimethyl phosphate	Vapona	Organophosphate
Fenitrothion	O,O-Dimethyl O-4-nitro-m-tolyl Phosphorothioate	Sumithion	Organophosphate
Lambda-cyhalothrin		Demand, Karate, Warrior	Pyrethriod
Lindane (γ-HCH)	1,2,3,4,5,6-Hexachlorocyclohexane	Gammalin 20	Organochlorine
Monocrotophos	3-Dimethoxyphosphinyloxy-N-methylisocrotonamide	Azodrin	Organophosphate
Permethrin	(3-phenoxyphenyl)methyl-3-(2,2-dichloroethenyl)-2,2-dimethyl cyclopropanecarboxylate	Ambush, Dragnet, Prelude	Pyrethriod
Pirimiphos-methyl	O-2-Diethylamino-6-methyl pyrimidin-4-yl O,O-dimethyl Phosphorothioate	Actellic dust	Organophosphate
Propoxur	2-isopropoxyphenyl methyl Carbamate	Baygon	Carbamate
Tetradifon		Tedion	Organosulfur
Sodium borate	Disodium octaborate tetrahydrate	Tim-Bor, Bora-Care	Inorganic

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2.3 PESTICIDE FORMULATIONS

Pesticides are generally not used in their technical grade or pure form as they are either not usable or pose greater hazards in this form. This is because they are usually active in very small quantities and need to be bulked up with inactive excipients. They are therefore processed or formulated into products that can be applied directly or diluted just before application. Formulation therefore is the preparation of the pure active ingredient into a suitable form for use (PAN, 2001). This serves to improve the properties of the compound for better storage, handling, application, tissue permeation, effectiveness and safety. Choice of formulation for use is based on procurement and application costs, convenience, type of application equipment available, local conditions and grower preferences. The different types of formulations available for pesticides include the following:

2.3.1 Dry Formulations

These include:

1. **Dusts**: - This formulation is made up of fine dry particles usually less than 30μm in diameter. Dusts may be dilute or undilute. Undilute dusts consist only of the active agent in dry powdered form to be applied as such. An example of undiluted dust formulation is sulphur dust used in the control of mite.

Dilute dusts are those containing the active ingredient with an inert diluent. They usually contain 0.5 to 10 percent of the active ingredient although more concentrated dusts have been formulated for further dilution to the required strength. They are prepared by either coating diluent particles with the pesticide or mixing and grinding the pesticide and a diluent in a suitable mill. This forms a concentrate which is then mixed with the same

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diluent to the strength required for field use. Suitable materials for use as diluents include attapulgite, kaolin, diatomite and talc.

Dusts are usually applied using special dusting machines but they can be sold in small containers with perforated tops to serve as applicators. Dusts have to be kept dry to avoid deterioration of the active ingredient and to allow for free flow and uniform application. Their main advantage is that they are generally manufactured in field strength specifications and are ready for direct and immediate use. They are also of advantage in areas where water is scarce as they do not require water for application. Their use, however, is limited by several disadvantages. They are not suitable for use under such weather conditions as high wind, heavy rain or excessively dry conditions since such conditions would lead to the problems of particle drift, inhalation hazard and poor deposits. Dusts deteriorate rapidly under adverse conditions and are usually required in large quantities due to their low content of active ingredient, which increases transportation costs. Dusts are therefore not used much under field conditions but for specialized treatments such as treatment of seeds prior to sowing or admixture with grains during storage. An example of dilute dust commercially available in Nigeria is Actellic dust containing 2% Pirimiphos-methyl.

2.   Granules: - These are large discrete dry particles used especially for highly toxic pesticides which should not be applied in spray form. The pesticide is dissolved in a suitable solvent and impregnated on to a carrier material similar to the diluent used in dust formulation. They are manufactured to specifications of size and concentration of active ingredient and are meant to disintegrate on contact with moisture in the soil or plant. Granule application eliminates the problem of splash and skin contamination with

concentrated liquids. Precise placement of granules allows spot treatment of individual plants and reduces the amount of active ingredient required thereby reducing hazard to non target organisms. Granules also allow for controlled release of the active ingredient for longer periods of effectiveness.

Granules may be applied by hand wearing rubber gloves but are better applied using equipment with an accurate metering device. Like dusts, they usually do not require dilution or mixing on the farm.

3. **Dry Baits**: - These are pesticides mixed with edible products or inert materials in the form of dry pellets attractive to pests. This formulation method has been used against pests like locusts, hoppers, leaf-cutting ants and rodents. The main limitation with the use of dry baits is that they can be eaten by animals other than the target pest and in wet weather, they disintegrate and contaminate the environment. Also, mammalian pests may develop bait-shyness especially if bait is dispersed and dead animals left in the infested area.

4. **Dry Fumigants**: - In this type of formulation, the pesticide is compressed together with suitable additives into tablets or pellets designed to release the fumigant gas on exposure to moisture. Dry fumigants are often used to protect grains against pest infestation during storage. In this case, the tablets are evenly distributed throughout a mass of grain and allowed an exposure period of three days or more. Examples of dry fumigants are aluminum phosphide, methyl bromide, naphthalene crystals and ethylene dichloride.

2.3.2   Formulations for Application as Sprays

These are preparations meant for spraying on the target area in the form of solutions (for water-soluble pesticides) or suspensions (for water-insoluble ones). They include the following:

1.   Dispersible Powders: -   These consist of finely divided pesticide particles intended for dissolution in water before application. In the case of water-insoluble pesticides, surface active agents are included in the formulation to ensure the formation of a stable homogenous suspension of the powder in water. The powder concentrate usually contains up to 50% or more by weight of the active ingredient. Dispersible powders are also referred to as wettable, sprayable or soluble powders. To avoid puffing up of powder into the spray operator's face, the powder concentrate may be compacted into dispersible grains.

2.   Emulsifiable Concentrates (EC): -   In this type of formulation, a pesticide is dissolved in a suitable solvent and an emulsifier added for easy and uniform dispersion in water to form a stable and homogenous emulsion before application. These are oil-in-water emulsions. The usual concentration of the active ingredient is about 25%.

Invert emulsions which are water-in-oil have been formulated but they are not widely used as they are very viscous and require specially designed application equipment. They are however used for the application of some pesticides.

3.   Liquid Concentrates (LC): -   These are preparations in which the pure pesticide is dissolved in a light mineral oil ready for direct use as aerosol dispensed through fogging machines. They cannot be diluted with water as they do not contain emulsifiers or other additives.

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The oily liquid concentrates may also be encapsulated into gelatin microcapsules of about 10μm or less in diameter. This permits controlled release of the pesticide and reduces exposure of beneficial organisms.

4. Ultra-Low-Volume (ULV) Formulations: - These are formulations designed using suitable solvents to produce very small droplets when sprayed through a nozzle. The main aim is usually to achieve wide and effective coverage of target area.

5. Others: - Other types of spray formulations are fogs, smokes and pressure packs.

2.4 USE, MISUSE AND ABUSE OF PESTICIDES

2.4.1 Use

Pesticides are used world-wide in the control of various pests to improve human welfare. Well-known areas of use include the following:

A. **Agriculture** – Pesticides are used in agriculture to increase the quantity and quality of food produce. This they do by protecting crops against destructive pests and diseases, both in the field and during storage, transportation and handling (Reece, 1985). In the field, pesticides are sprayed on crops or applied to soil to protect crops against the various pests before harvest.

After harvest, pesticides are applied to food commodities such as grains, cereals, nuts, tubers, fruits and vegetables to prevent pest infestation during storage and transportation to the end-user. Pest infestation may cause spoilage, reduce germination and decrease food value (Collins, 2006). Fruits and vegetables are often washed with fungicides to

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preserve their quality during transportation and handling, that is, to reduce perishability (Sowunmi and Agboola,1982).

Pesticides are used in fish farming as antifoulants and in the control of sea lice (fish ecto-parasites) (Davies, 1995).

Pesticides used in agriculture include herbicides, insecticides, fungicides, nematocides, rodenticides, etc. Herbicides are used in the control of weeds which compete with crops. They therefore help to improve crop yield and reduce human effort. Globally, herbicides are reported to be the most widely used group of pesticides, followed by insecticides and fungicides as shown in Table 2.5. In Nigeria and many other developing countries, however, insecticides are more widely used than herbicides and other classes of pesticides (Mansour, 2004). This is due to high insect population and the fact that a lot of the weeding is still being done mechanically in this region.

Table 2.5 – World Agricultural Use of Pesticides (Reece, 1985).

Class of Pesticide	% Use
Herbicides	46
Insecticides	31
Fungicides	18
Others	5

B. **Public Health** – Pesticides are used in homes and public health programmes for

the control of disease vectors such as mosquitoes, tsetse flies, black flies, houseflies,

cockroaches, etc. This is to eradicate diseases spread by these pests. Such diseases

include malaria, trypanosomiasis, schistosomiasis, filariasis, onchocerciasis, plague

cholera and others. For instance, the Rollback Malaria (RBM) Programme of the WHO

advocates the use of insecticide-treated nets to prevent mosquito bites in the ongoing

fight against malaria.

Pesticides are also used in veterinary care to rid pets and other animals of ectoparasites

such as fleas, lice, mites and ticks. They are applied as sprays, shampoos, washes or dips.

C. **Forestry/Horticulture** – Pesticides are used in forestry and horticulture for the

control of weeds and for the protection of timber, forest and garden trees, ornamental

plants, lawns, flowers, fruit and vegetable trees.

D. **Industry** – Weeds and other pests in industrial premises and drains can be

controlled by the use of pesticides.

The main use of pesticides, however, is in agriculture because of perpetual need to

increase food supply. Figure 2.11 shows a pie chart of world pesticide usage.

The type of pesticide to be used in any pest control programme and the mode of

application depend on the target pest, size and location of the target area, pesticide

formulation and type of application equipment available.
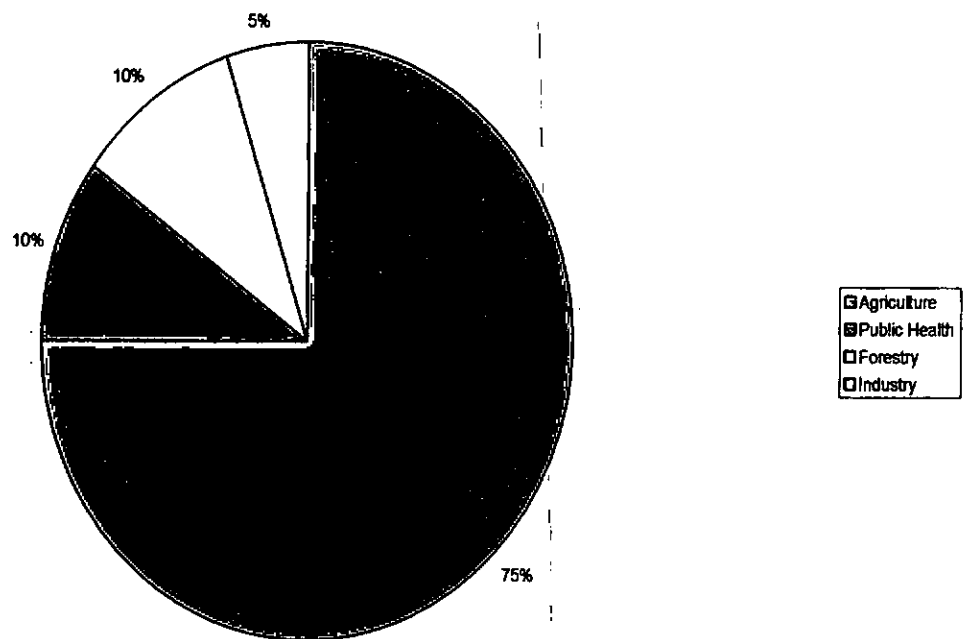
Figure 2.11: Pie Chart of Estimated World Pesticide Usage (Taylor, 1990).

2.4.2 Misuse and Abuse

These occur wherever pesticides are in use but more commonly in developing countries including Nigeria (Ezugwu and Okonkwo, 1999). Misuse and abuse of pesticides may lead to adverse consequences on health and environment. Reasons for misuse and abuse of these chemicals include illiteracy, ignorance, lack of awareness, fear of harvest failure, eagerness to protect crops, economic advantage among others. Examples of pesticide misuse and abuse include:

i. Unnecessary use of pesticides in situations where such use is not absolutely indicated.

ii. Use of high doses of pesticides and/or increased frequency of application in attempts to increase effectiveness.

iii. Use of highly toxic chemicals in preference to safer alternatives in the belief that the more toxic the chemical, the higher the level of pest control.

iv. Indiscriminate use of highly selective compounds against pest species which are not sensitive to them.

v. Use of non-specific agents leading to destruction of target and non-target pests.

vi. Continued use of persistent compounds (because of their low price) in preference to newer, more environment friendly alternatives.

vii. General failure to adhere to Good Agriculture Practice (GAP). This includes failure to observe waiting periods between pesticide application and harvest, and re-entry times after application.

viii. Abnormal use of toxic chemicals such as Gammalin 20 (gamma HCH) for fish catches and for preservation of kolanuts.

ix. Use of DDT on dry fish to protect it against vermin infestation.

2.5 HUMAN EXPOSURE TO PESTICIDES

Pesticides are used in several areas of human endeavor and so people are exposed to these chemicals in several ways. The severity of damage to human health varies with the type, source and route of exposure. Three main types of exposure are:

(1) Acute – This is the form of exposure in which a person is exposed once to a large dose with immediate manifestation of toxic effects (usually within 24 hours). Death may occur in the absence of prompt and effective management.

(2) Sub-acute – This type of exposure is due to repeated exposure to moderate doses and symptoms of toxicity are delayed for a week to one year.

(3) Chronic – In this type of exposure, a person is exposed to small doses over a long period of time and manifestation of symptoms is delayed for one year or longer (Igbedioh, 1991).

There are three main routes of entry of pesticides into the human body: oral (by mouth), dermal (through the skin) and respiratory (by inhalation) (WHO/UNEP, 1990$_c$; Hetzel, 1996). The type and route of exposure for any individual or group of persons depends on the source by which they come into contact with the chemicals.

2.5.1 Sources of Human Exposure to Pesticides

Sources of human exposure to pesticides may be classified as unintentional and intentional.

A. Unintentional Exposure

This is the more common type of exposure and it involves occupational, non-occupational and accidental sources of exposure.

    1) Occupational Exposure – This applies to workers in the industry who are

involved in the manufacturing, formulation and packaging of pesticides. Also, farmers and farm workers responsible for application of pesticides to crops are at high risk of exposure. This risk is especially high if leaking or inappropriate application equipment is used or protective clothing is not used during application.

Public health workers directly involved in spraying these chemicals in residential or industrial premises are also exposed through this source. The route of exposure is usually via contact with the skin and/or by inhalation. This type of exposure may be acute, sub-acute or chronic depending on the degree and length of exposure.

2) Non-occupational Exposure – This usually involves the general public who take in small doses of pesticides via consumption of agricultural products which have been treated or are contaminated with these chemicals. Vegetables and fruits in Egypt have been reported to contain organochlorine and organophosphorus pesticides, with some at levels above maximum residue limits (Abou-Arab, 1998). Processed foods may also contain residues of pesticides arising from residues in raw materials or water or due to contamination during processing.

Sources of drinking water supply may be contaminated by run-offs from agricultural fields due to over-application of pesticides or adverse weather conditions (Croll B.T. 1995). Exposure may also occur when pesticide containers are ignorantly used for storing food or water meant for human consumption. The route of entry is oral and exposure is usually chronic as low doses are consumed over a long period of time.

The public may also be exposed through inhalation of contaminated air when pesticides are used in homes and public health programmes against disease vectors. In this case, the route of exposure is respiratory or dermal.

3) Accidental Exposure - This refers to unintended (occupational or non-occupational) exposure to high doses of pesticides. Such exposure may occur when pesticides are stored in containers without labels or within reach of children and consequently consumed as medicines or food. Seeds dressed for planting may be erroneously released for consumption leading to unacceptable levels of exposure. Accidental exposure may also occur due to spillage of improperly packaged pesticide products, lack of protective clothing during application and use of leaking or inappropriate application equipment. The three routes of entry into the body may be involved and exposure may be acute or sub-acute.

B.   Intentional Exposure

Although most human exposure to pesticides is unintentional, intentional use occasionally occurs mainly for the purposes of suicides or homicides. The route of entry is usually oral but sometimes by inhalation. This type of exposure results in acute poisoning or death. Occasionally, the route of exposure may be by dermal contact as occurs when the chemical is poured on the victim to cause chemical burns on the skin.

Figure 2.12 shows a summary of sources of human exposure to pesticides.
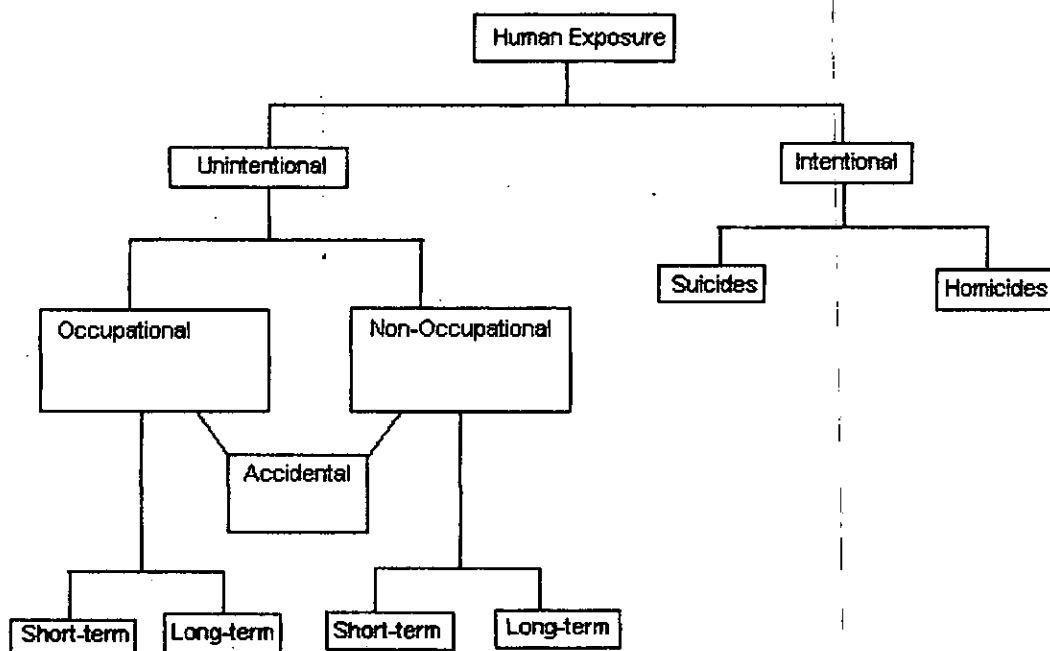
Figure 2.12: Summary of Sources of Human Exposure to Pesticides.

2.5.2 Assessment of Human Exposure to Pesticides

Human exposure to pesticides may be potential or actual. Potential exposure may be estimated by environmental monitoring. This involves measurement of levels of pesticide residues in all environmental sources of human exposure, such as air, food, water and soil (WHO/UNEP, 1990c). The values are then compared with guideline values established by WHO. How much of the pesticide residues found in the environment will eventually get into the human body depends on several factors such as:

1. Chemical nature of the pesticide – Chemically stable pesticides are not easily degradable in the environment and are therefore more likely to be found in human tissues at higher levels than the less stable compounds.

2. Climatic Conditions – High temperatures and humidity may aid degradation of some pesticides and reduce their chances of getting into the human body.

3. Level of education and awareness of users – This will help in the choice of safer pesticides and observance of Good Agricultural Practice such as observing waiting periods between time of application of pesticides to crops/food and time of harvest or consumption. This will greatly reduce the concentration of pesticide residues present in food at the time of consumption.

4. Processing – Washing removes some surface residues especially the water-soluble ones while cooking could cause breakdown of others especially the thermally unstable pesticides. Thus processing could lead to lower residue levels in food at the point of consumption (Handa *et al.*, 1999; Soliman, 2001; Rasmussen *et al.*, 2003). Some pesticides are however resistant to commonly used processing methods.

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It is therefore important that environmental agents of exposure (especially food) be analyzed regularly to ensure that maximum residue limits (MRL) set by regulatory bodies are not exceeded. MRL is the maximum concentration of a pesticide residue expected on a crop or food commodity resulting from the use of the pesticide according to Good Agricultural Practice. It is expressed in milligrams of pesticide residue per kilogram of commodity. In other words, MRL is the highest amount of residue to be found on a commodity after a pesticide has been used on it and is toxicologically acceptable. The capacity for regular analyses is however lacking in most third-world countries (WHO, 2001; Mansour, 2004).

Actual human exposure to pesticides, on the other hand, can only be measured by biological monitoring of human tissues and body fluids. This involves analyses of such samples as fat, serum, urine, blood, breast milk, skin or hair for pesticide residues. Measurement of specific effects such as cholinesterase activity (for organophosphorus and carbamate pesticides) may also be used as a method of assessment of actual exposure (Petersen, 2000; Guilhermino et al., 2004). Residue values are used to calculate daily intakes which are then compared with acceptable daily intakes (ADI) established by FAO/WHO. ADI of a pesticide is the daily intake of the pesticide which during a life time appears to be without appreciable risk to the health of the consumer (Handa et al., 1999). It is expressed in milligrams of pesticide per kilogram of body weight.

2.5.3 Ways to Reduce Human Exposure to Pesticides

Human exposure to pesticides can be minimized if attention is paid to the different sources of exposure and adequate precautions taken. Areas requiring such attention include:

1. Packaging, storage and transportation of pesticides

Pesticides should be packaged in appropriate containers and adequately labeled for safe and effective use. Inappropriate packaging may lead to leakages and spillage while inadequate labeling or use of foreign languages on labels would encourage misuse or abuse. Premature loss of labels may lead to accidental consumption. All these contribute to increased human exposure. Manufacturers are therefore required to provide information about the use, storage, handling and disposal of their products on the label. Information to be provided on the label of a pesticide product includes the following:

- i. Name of product
- ii. Name of active ingredient (as well as other ingredients
- iii. What it is to be used for
- iv. How to mix it
- v. How it should be used
- vi. Rate of application
- vii. How toxic it is
- viii. Precautions to take
- ix. Re-entry times
- x. Kind of clothing and personal protective equipment needed
- xi. Symptoms of poisoning
- xii. What the antidote is (if any)

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Pesticides should be stored out of reach of children and away from food stuffs and other items for human consumption. They should be transported in separate vehicles and handled with care during the process of transportation to avoid spillage and accidental contamination of consumable goods.

2. Education of Users

The end-users must be adequately educated and trained on all aspects of pesticide use including:

a. Use of appropriate application methods and equipment to reduce overall exposure.

b. Use of protective clothing and equipment during mixing and application to avoid skin contact and excessive inhalation. Pesticides can be absorbed through the skin and eyes and can also cause permanent damage to these organs. Certain personal protective equipment must therefore be used when working with pesticides to protect the user from drifts and splashes. Some are designed to prevent inhalation of toxic fumes and mists. Examples of protective clothing and equipment are long sleeve shirts, long pants, overalls, shoes and socks, rubber gloves, goggles/face shields, rubber boots, waterproof head wears and respirators.

The protective items selected for use are determined by the toxicity level of the pesticide being used. Use of protective clothing may however constitute a problem in the tropics due to difficulty in wearing such clothing in hot climates.

c. Appropriate disposal of pesticide containers and unused stock. For example, empty pesticide containers should not be used to transport or store drinking water or food.

d. Observance of good agricultural practice (GAP): This involves use of the right pesticide in appropriate concentrations with the aim of avoiding high residues in crops/food. Farmers should also be trained to observe waiting periods between the time pesticides are applied to crops/food and the time the crops/food are harvested or released for consumption. Re-entry times after application should also be observed.

Education and training is especially important in developing countries where end-users are mostly illiterate farmers who often lack awareness of the potential hazards of pesticides to human health and the environment (PAN, 2001).

3. Use of Alternative Methods of Pest Control

The use of alternative methods of pest control will help to reduce the amount of pesticides in food and environment thereby reducing human exposure. Such methods include biological control, use of natural predators and pheromones and cultivation of pest-resistant crops as presented in section 2.10.

2.6   BENEFITS OF PESTICIDES TO MAN

Pesticides are of immense benefit to human health and economy in a number of ways. These benefits out-weigh their potential hazards if the chemicals are used in quantities necessary to control pests without contaminating the environment. Benefits of pesticide use include:

1. Increased Agricultural Food Supply: – Pesticides help to increase food production for the teeming world population by protecting crops against destruction by pests before and after harvest.

2. Control of Human Diseases: – Pesticides destroy disease vectors, thereby reducing human morbidity and mortality from vector borne diseases. They therefore contribute to good human health. Examples of disease vectors controlled with pesticides are mosquitoes, tsetse flies, houseflies, sand flies, ticks, lice, fleas and rodents.

3. Control of Veterinary Diseases: - Ectoparasites of pets and farm animals can be controlled by the use of pesticides. This increases the quality and quantity of animal products available for human use.

4. Reduction of Human Labour: – Herbicides control weeds in farms, gardens, industrial premises, etc. thereby saving time and manual or mechanical labour. Use of herbicides is a more economical way of weeding which contributes to increase in quality and yield of agricultural crops.

5. Decongestion of waterways: - Herbicides are used to destroy seaweeds which clog waterways and reduce the speed of ships.

6. Preservation of materials such as wood and cotton.

7. Preservation of lawns, garden flowers, trees and ornamental plants.

2.7   HAZARDS OF PESTICIDE USE

Inspite of their benefits to man, pesticides are poisons and are known to be potentially hazardous to human health as well as the environment (Purdom and Anderson, 1983; Ezugwu and Okonkwo, 1999; Osibanjo, 2001; Turgut, 2007). These hazards multiply and out-weigh benefits if the chemicals are not properly used or are carelessly handled. Potential hazards associated with pesticides include effects on human health and the environment.

2.7.1   Damage to Human Health

Pesticides can cause harm to humans and other animals because they are designed to kill or otherwise adversely affect living organisms. Toxic effects of pesticides vary with chemical class of the agent and its mechanism of pesticidal action. For example, organophosphorus and carbamate compounds are known to act on the insect nervous system by inhibiting the enzyme acetylcholinesterase (AChE). This leads to accumulation of acetylcholine (ACh) at neuromuscular junctions with consequent cholinergic effects such as muscle twitching and eventual paralysis (Hetzel, 1996; Koprucu et al., 2006). These classes of pesticides are capable of producing acute and chronic neurotoxic effects in mammals because of the basic similarities between mammalian and insect nervous systems. Organochlorines are also known to be neurotoxic by disrupting the delicate balance of sodium and potassium ions within the neurons thereby preventing normal transmission of nerve impulses. Chronic exposure of humans to these groups of pesticides can therefore lead to adverse neurological effects and subtle behavioural changes (WHO/UNEP, 1990c).

Pesticides, being a class of xenobiotics, affect the functions of major organs such as the liver. Liver enzymes such as the cytochrome P-450 family of oxidases are ready targets (Renwick, 2004). For example, organochlorines induce mixed-function oxidases of liver microsomes while dithiocarbamates inhibit them. This may lead to changes in the metabolism of compounds handled by these enzymes. Accumulation of pesticides in the body may also affect biochemical tests and interact with food and drugs. Pesticides that contain a secondary amine group can interact with dietary nitrite to form nitrosamines which may be mutagenic or carcinogenic. Two or more pesticides may also interact in the human body and become more toxic (synergism or potentiation) or sometimes less toxic (antagonism). Many pesticides have been identified as endocrine distrupting chemicals (Jobling et al., 1995; Mansour, 2004; How-Ran et al., 2006). This means that they can interfere with human growth and reproduction. Human exposure to pesticides must therefore be minimized to reduce these health risks.

Effects of pesticides on human health may be acute or chronic.

Acute Poisoning – This involves the immediate manifestation (within 24 hours) of adverse effects after exposure to high doses, usually resulting from occupational, accidental or intentional exposure. Acute poisoning may arise from consumption of highly contaminated food such as grains treated directly with high doses of pesticides or animals that had fed on contaminated food. Poisoning may also be due to excipients in the pesticide formulation e.g. solvents, carriers, emulsifiers, synergists, or due to bye-products or metabolites.

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Acute poisoning may be fatal or non-fatal. An annual estimate of 1 million cases and a fatality rate of 0.4 – 1.9% has been reported (WHO/UNEP, 1990$_b$). In Egypt, 14% of all cases of acute poisoning recorded at a poison centre in 1994 were due to pesticides (Mansour, 2004). The effects of acute poisoning may be systemic or local and symptoms include dizziness, headaches, sweating, fatigue, numbness, vomiting, cramps, coma, chemical burns of the eye and skin, neurological effects, respiratory tract irritation, liver and kidney damage. The effects manifested and the reaction time depend on the type of pesticide, the level of toxicity of the pesticide, the amount of the pesticide received, length of exposure time, the mechanism of action and the route of entry into the body. Fortunately, only a small proportion of the population is exposed to pesticide levels high enough to cause severe acute effects. The comparative toxicity of organophosphates, carbamates and pyrethroids measured in terms of their oral and dermal lethal doses are shown in Table 2.6.

Certain factors may lead to increased incidence of acute poisoning. These include:

i. Unsafe or improper packaging causing leakage of pesticides during storage or transportation. This may lead to accidental contamination of food or spillage on human skin.

ii. Premature loss or damage to labels on pesticide containers leading to accidental consumption or misuse.

iii. Carelessness and/or ignorance in which grains dressed with pesticides for planting are released for human consumption.

iv. Failure to observe the waiting period (time between pesticide application and crop harvest) which may lead to freshly sprayed produce with high residues getting to the consumer.

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Table 2.6 - Comparative Toxicity of Organophosphates, Carbamates and Pyrethroids (Perrin, 1995).

Chemical Group	Typical Range for Technical Material	
	Oral LD_{50} (mg/kg)	Dermal LD_{50} (mg/kg)
Organophosphates	10 – 500	50 – 3000
Carbamates	20 – 100	1000 – 5000
Pyrethroids	100 - 5000	1000 – 5000

Chronic Poisoning – This occurs due to exposure of humans to low doses of pesticides over a long period of time, usually through residues in food, water and air. This means that no segment of the general population is completely protected against potential health effects due to chronic exposure to pesticides. As in the case of acute poisoning, additives in pesticide preparations may also be responsible for some chronic toxicity effects. Many organic and metal-based pesticides can pass from mother to unborn child through the placenta, potentially causing birth defects, abnormal development of the immune system or fetal death (Sesline and Jackson, 1994; Chao *et al.*, 2006).

Chronic poisoning may affect the function of individual organs to produce effects such as cancinogenicity, mutagenicity, teratogenicity, infertility, impotence, immunological disorders, blood dyscrasias, neurotoxic disorders, liver damage, kidney damage, skin alterations and worsening of existing health conditions. These effects can be produced even by compounds with low acute toxicity. For instance, malathion which is considered a very low toxicity pesticide (oral LD_{50} =2100mg/kg body weight) can depress humoral immune responses after exposure to low doses over prolonged periods (Mansour, 2004).

Examples of confirmed or suspected health hazards arising from chronic exposure to specific pesticides include the following:

1. High birth defects were recorded (in Vietnam, 1960) in areas where U.S.A forces applied 2,4,5-trichlorophenoxyacetic acid (2,4,5–T) herbicide for defoliation of forests during the Vietnam war. (PAN, 2001).

2. Carbendazim, used as a fungicide on yam has been found to disrupt growth and affect sperm count in humans (Kelce *et al.*, 1995).

3. Exposure to pesticides and solvents has been found to be associated with measurable immunosuppression in several species (Kerkvliet, 1994).

4. DDT has been linked to breast cancer (due to estrogenic activity) and male infertility as it blocks the action of male hormones, that is, anti-androgenic action (Krieger *et al.*, 1994).

5. Lindane has been identified as an endocrine disruptor as it mimics the female hormone, oestrogen and is linked with fertility problems, breast and other cancers. (Jobling *et al.*, 1995).

6. Cataract formation has been associated with exposure to diaquat (WHO/UNEP, 1990c).

7. Non-Hodgkin's Lymphoma (a rare type of cancer) was found six times as often in farmers who had been spraying herbicides especially 2,4-Dichlorophenoxyacetic acid (2,4–D) for more than 20years than in non-farmers. (PAN, 2001).

2.7.2 Effects on Environment

Effects of pesticide use on the environment include the following:

A. **Environmental Contamination** - Pesticides contaminate air, soil, food and water when used either for agricultural or other purposes. This is because after use, some of these chemicals are bound in soils, taken up into plants, drain into rivers and lakes or carried by the wind through long distances. They can affect microflora in soil and cause changes in soil productivity. From environmental media, pesticides find their way into living tissues where they accumulate up the food chain from prey to predator. Some pesticides are consistently present in food and environmental media either because they are very persistent or because of their continuous output into the environment. The highly persistent and lipophilic compounds such as the organochlorines accumulate in fatty tissues of organisms and biomagnify.

Some chemicals used as pesticides (eg. methyl bromide) contribute to depletion of ozone layer thereby aiding global warming.

B. Toxicity to aquatic and non-target animals - Pesticides are toxic to aquatic animals such as fish, crab, etc. (Koprucu *et al.*, 2006). High levels of persistent organochlorines have been found in fish and other marine animals (Mansour, 1998; Borrell and Aguilar, 2006). A positive correlation has also been established between concentrations of organochlorine pesticides in human breast milk and seafood consumption by mothers in Taiwan (Chao *et al.*, 2006).

Pesticides may destroy non-target animals some of which are useful to humans. Such useful animals include bees, earthworms, birds and natural predators which feed on some pests, thereby reducing their population. Poisoning of bees for instance, affects bee keepers' income from honey and also results in reduced pollination with adverse consequences on agriculture and natural vegetation.

C. Appearance of new pests - Use of pesticides may upset the natural balance between different organisms living in the same ecosystem. The destruction of natural enemies of some organisms may cause the population of such organisms to increase to pest level, that is, a level at which they begin to cause significant health or economic damage.

2.7.3 Development of Resistance to Pesticides

Resistance to pesticides is the ability of a pest population to survive a toxic dose that was initially toxic to majority of the individual pests. One of the mechanisms for the development of resistance to pesticides is the development and spread of genetic material for the detoxification of the pesticide. Inappropriate or excessive use of pesticides may lead to complete eradication of susceptible species, giving room for rapid multiplication of resistant strains. Emergence of resistant pest strains may lead to increased use of chemicals leading to further damage to environment and human health.

Pests that are already resistant to one pesticide can easily develop resistance to other products, from the same or different chemical class (PAN, 2001). This is due to the fact that the detoxification mechanism of the pest responds faster and more effectively after one mechanism had been developed. This leads to the phenomena of multiple and cross resistance.

2.8 SYMPTOMS AND MANAGEMENT OF PESTICIDE POISONING

2.8.1 Symptoms of Pesticide Poisoning

Pesticides are used in agriculture and public health for the benefit of man but their use poses danger to both the environment and human health. Man is inadvertently exposed to these chemicals and may manifest acute or chronic symptoms. Symptoms of pesticide poisoning vary and are affected by factors such as nature of chemical, quantity, duration of exposure, physical or health condition of the victim and ambient weather conditions. Health conditions that may affect the toxicity of pesticides in the human body include: nutritional state of the victim, presence of infections, physical and psychological stress, presence of other poisons and existing organ damage.

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Symptoms associated with acute pesticide poisoning include dizziness, vomiting, sweating, cramps and coma. In chronic pesticide poisoning, the following symptoms may occur: nausea, headache, chest pain, restlessness, liver damage, kidney damage, infertility, impotence, congenital malformations, allergies, cancers, changes in blood count, skin alterations, neurotoxic disorders and worsening of existing health conditions.

2.8.2 Management of Pesticide Poisoning

Dangers posed by pesticides to human health can be managed using both preventive and treatment measures. Chronic poisoning is better avoided by adoption of preventive measures.

A. Preventive Measures

1. Education and training of pesticide users on appropriate and safe use of pesticides - This includes information on the use of protective clothing during application, disposal of remnants and empty containers and compliance with instructions on labels and good agricultural practice (GAP) guidelines.

2. Use of alternative methods of pest control to reduce the amount of pesticides in food and environment - Such methods include biological control (of pest reproduction), use of natural predators and pheromones and cultivation of pest-resistant crops. Use of combinations of these methods constitutes what is generally referred to as integrated pest management (IPM).

B. Treatment of Acute Poisoning

The following actions should be taken in cases of acute poisoning:

1. Remove victim from source of contamination.

2. Remove contaminated clothing and wash skin with water.

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3. Induce vomiting within one hour if the pesticide had been swallowed.

4. Give antidote if the pesticide is known, e.g. atropine or pralidoxime for organophosphorus and carbamate agents. Atropine acts by binding to acetyl choline (ACh) receptors thus preventing the excess ACh from binding. Pralidoxime helps regenerate the phosphorylated or carbamylated enzyme by cleaving the covalent bond between the poison and the enzyme. The enzyme is thus made available to remove the excess ACh.

5. Give general supportive treatments e.g. give intravenous fluids, assist respiration, etc.

2.9 REGULATORY CONTROL OF PESTICIDE USE

The use of pesticides is controlled all over the world because of the huge number of compounds in use as pesticides and their potential to cause harm to people and environment. A pesticide is registered in any country if comprehensive tests reveal that it is safe and that benefits from its use out-weigh the risks. Many pesticides are registered for agricultural use while others are registered only for public health and other uses. In addition, a registered compound may become banned or its use restricted if a review of its use finds that it poses unreasonable risks to human health and environment. The assessment of health risks of a pesticide compound in order to determine its status (registered, banned or severely restricted) is therefore a continuous process.

Decisions to ban or severely restrict the importation and use of any compound are usually taken by national regulatory authorities, guided by information from international advisory/reference authorities. Two of such bodies are Codex Alimentarius Commision which deals mainly with the control of chemicals used in food and the Prior Informed

Consent (PIC) Procedure which controls the import and export of pesticides and industrial chemicals. National decisions are also based on such factors as:

i. Toxicity profile of the pesticide

ii. Use pattern

iii. Local production capacity

iv. Trade benefits

v. Available alternatives

vi. Local monitoring and supervisory capacity

vii. Local research findings

viii. Other local peculiarities

In the United States of America, the Environmental Protection Agency (EPA) issues registration for marketing of pesticide products. EPA also conducts enforcement activities in conjunction with the Food and Drug Administration (FDA) and the Department of Agriculture, to ensure compliance with registration conditions or decisions. Enforcement activities involve inspection of product packaging and labeling, plant operations and actual use of products by farmers and other end users. The activities also include monitoring of residues in food and environment.

In Nigeria, pesticide products are registered by NAFDAC which is the body given the mandate to regulate and control the importation, manufacture, exportation, distribution, advertisement, sales and use of chemicals including pesticides (Ugbeye, 2004). NAFDAC should also carry out enforcement activities in conjunction with the Federal Ministry of Environment and the various state ministries of agriculture. NAFDAC regulation is to ensure that undesirable, toxic and hazardous pesticides do not get into the country. Table 2.7 shows a list of pesticides that have been either banned or severely restricted in

Nigeria. In order to ensure that pesticide residues in food do not pose high risks to human health, safety levels have been established for individual pesticide active ingredient. Such safety levels include maximum residue limits (MRLs) and acceptable daily intakes (ADIs).

Maximum Residue Limits – These are statutory limits set on active ingredient and commodity combinations. An MRL is the maximum acceptable concentration of a pesticide residue likely to occur in or on a crop or food commodity resulting from approved use of the pesticide or after the pesticide has been used according to Good Agricultural Practice (GAP) (Handa et al., 1999). It is expressed in milligrams of pesticide residue per kilogram of commodity. In other words, MRL is the highest amount of residue to be found on a commodity after a pesticide has been used on it and is legally acceptable.

Maximum residue limits are determined based on field trials, that is, the crop is treated with the pesticide and samples of the crop are analyzed to determine residue levels. However, in the absence of data from field trials or when approved use is not expected to result in residues, MRLs are set at limits of determination (LOD). Such levels are called LOD MRLs.

MRLs provide a quantifiable means of ensuring that pesticides are not mis-used and may be used for enforcement purposes to ensure that the pesticide is only being used in accordance with GAP. It is therefore an offence to trade in commodities with residue levels that exceed the relevant MRL.

Table 2.7 - Banned and Severely Restricted Pesticides in Nigeria (NAFDAC, 2003).

Banned	Severely Restricted
Aldrin, Binapacryl, Captafol,Chlordane, Chlordimeform, Chlorobenzilate, Cyhexatin, Delta HCH, 1,2-Dibromoethane, Dichlorodiphenyl trichloroethane (DDT), Dieldrin, Dinoseb, Endrin, Fluoroacetamide, HCH (mixed isomer), Gama-HCH (Lindane), Heptachlor, Hexachlorobenzene, Mercury Compounds, Methamidophos, Methyl bromide, Methyl parathion, Parathion, Pentachlorophenol, 2,4,5-T, Toxaphene.	Aldicarb, Arsenic and its compounds, Ethylene oxide, Ethylene dichloride, Malathion, Monochrotophos, Phosphamidone.

Acceptable Daily Intakes – The ADI of a pesticide is the daily intake of the pesticide which during a life time appears to be without appreciable risk to the health of the consumer (Handa et al., 1999). It is expressed in milligrams of pesticide per kilogram of body weight. ADIs are derived from the results of long term feeding studies in laboratory animals. Maximum residue limits (MRLs) and acceptable daily intakes (ADIs) of some pesticides are presented in Table 2.8.

2.10   OTHER METHODS OF PEST CONTROL

There are several methods of managing pests and the damage they cause. These methods fall into three categories: Cultural/mechanical, biological and chemical. Chemical methods use mainly pesticides. To reduce the environmental load of pesticides with consequent decrease in potential human health hazards, alternative methods of pest control without the use of chemicals have been developed. The methods include:

2.10.1   Biological Control

These are control methods that involve the use of biological agents such as parasites, predators and pathogens to keep a pest population below the level at which it causes economic damage (i.e. below economic threshold). Predators or natural enemies can exist in a region or foreign ones can be introduced. For example, the Ladybird beetle is good for controlling aphids and the praying mantis can be used to control a variety of garden pests (Purdom and Anderson, 1983).

Safe as biological control may be, it requires many years of research and thorough understanding of pest population dynamics to find a suitable control organism. A suitable control organism should be able to grow in the pest habitat and not cause ecological side effects such as destruction of useful organisms. It should also not be a vector of human or

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Table 2.8 - Maximum Residue Limits (MRLs) and Acceptable Daily Intakes (ADIs) of Some Pesticides.

Pesticide	MRL (Maize) (mg/kg)	ADI (mg/kg/day)
Aldicarb	0.05	0.005
Captafol	0.05	0.1
Carbofuran	0.1	0.003
Cypermethrin	0.05	0.05
Deltamethrin	1	0.01
Diazinon	0.02	0.002
2,4-D (2,4-Dichlorophenoxy-acetic acid)	0.05	0.3
Dichlorvos	2	0.004
Dieldrin	0.01	0.0001
Endosulfan	0.05	0.0075
Fenvalerate	0.02	0.007
Iprodione	0.02	0.3
Lindane (γ-HCH)	0.01	0.01
Malathion	8	0.02
Parathion methyl	0.02	0.001
Permethrin	0.05	0.05
Phorate	0.05	0.0002
Phosphamidon	0.05	0.001
Pirimiphos-methyl	5	0.01
Trichlorfon	0.1	0.005

animal diseases. Biological control is therefore not a suitable solution to an immediate pest problem since it is easier and faster to use pesticides than to develop biological control methods. This explains why pesticides are still being used widely for pest control. Biological control agents include birds, insects, bacteria and viruses.

2.10.2 Agricultural Modification

Certain agricultural practices can help maintain pest population at a manageable level. Such practices include:

1. Mixed Cropping – This can be used to obtain higher plant diversity which increases natural control of pests. Diverse or natural vegetation maintains the natural balance of a system and keeps pests at a low level. On the other hand, removal of natural vegetation or planting of monocultures result in areas dominated by a few species without natural enemies. This increases pest population.

2. Timing – Proper timing of planting and harvesting can also be used as a pest control mechanism. Some pests migrate during certain seasons while others increase in population at certain times of the year. Planting and harvesting outside these problem times can help reduce the destructive activity of pests.

3. Weeding – Removal of weeds along the edges of a field or tillage of soil around a plant can reduce the number of pests entering the field or eliminate some of the pests in the soil.

4. Trap Cropping – This practice involves planting of crops preferred by a particular pest in fields bordering the cash crop. This serves to attract the pest and divert it

from the crop of interest. For example, corn has been used to attract melon flies away from fields of melon.

5. Companion Planting – Some plants produce substances that are toxic to certain pests. Such plants can protect other plants in the same field against the pests. Also, some flowers produce substances that repel certain insects thereby protecting other plants against attack by such insects.

2.10.3 Genetic Modification

The populations of some insects can be kept at low levels by genetic manipulation. One form of this technique is the sterile insect program in which the males of the insect are made sterile either by gamma radiation or by chemicals and released into the infested area. The normal females mate with these sterile males but are unable to reproduce leading to a drop in population. This technique is suitable if the larvae form of the insect is the nuisance and if the program will have no negative effect on other species in the ecosystem. It has been used against screwworm flies and fruit flies.

Another form of genetic control is the development of crop varieties that are resistant to damage by several pest species. This has been done for some crops like corn, oat, wheat and barley while sources of resistance have not been found for others. Resistant crops may also lead to more virulent strains of pathogens like the fungi.

2.10.4 Attractants

These are chemicals such as pheromones used for communication in some animals. They may be food or sex attractants but when isolated and combined with pesticides, they can be used to attract pests for destruction. Some plants attract beneficial insects which help to destroy other pests in the area.

2.10.5 Pest Control Devices

These are physical or mechanical means of getting rid of pests. They are used mainly for large pests such as rodents, birds and some insects. Examples are snap traps, glue traps and repellent sound devices.

Repellent sound devices are electronic devices which generate sounds that annoy pests but at a frequency that is not heard by humans. The irritating sounds distress the pests and cause them to leave. A repellent unit can be plugged into a building's existing wiring through an electrical outlet.

2.10.6 Integrated Pest Management

Pest management is any action or combination of actions taken to reduce pest numbers and minimize their damage (Shetlar, 2007). The term management is used in recognition of the fact that one can never totally rid any environment of pests and that any single control method will eventually fail. Control of pests using pesticides may be fast and easy but it is potentially hazardous to human health and environment. Pesticides also need to be re-applied periodically. Alternative methods of pest control are expensive to develop and require extensive long-term research. It is therefore necessary to develop an integrated approach which involves the use of a combination of different methods (with

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the use of pesticides) to reduce pest population. This involves such actions as identifying and altering factors that allow pests to thrive in an environment.

In integrated pest management programmes, pesticides are used as a last resort and in reduced quantities. This increases effectiveness and reduces environmental load of chemicals as well as total impact on the natural ecosystem. The combination of different pest control methods is referred to as integrated pest management (IPM). IPM has however not been accepted widely among pesticide users because of difficulties in developing appropriate IPM programmes and in convincing farmers about the advantages of the system (Mengech et al., 1995).

2.11 OTHER ENVIRONMENTAL CONTAMINANTS

Environmental pollution has continued to increase with growing world population and rising industrialization/urbanization. Soil, air, water and food are contaminated. Pollutants, even in trace quantities can have detrimental effects on human health and stability of the environment. Apart from pesticides, there are other contaminants in the environment arising from activities of production factories, oil refineries and automobile industry. Major environmental pollutants and their sources are presented in Table 2.9.

Table 2.9 - Major Environmental Pollutants and their Sources.

Pollutant Group	Examples	Sources
Polychlorinated Biphenyls (PCBs)	Compounds used in electrical capacitors and transformers, paints plastics and other industrial products.	Municipal and industrial waste discharge.
Heavy Metals	Arsenic, copper, lead, mercury, nickel, selenium, silver, thallium and zinc.	Industrial discharges, mining activity, urban runoff, erosion of metal-rich soil, dental amalgams (mercury).
Other Inorganics	Asbestos and cyanide	Asbestos – waste from industrial and roofing use. Cyanide – waste from industrial uses.
Solvents and Vapours	Halogenated aliphatics, ethers, esters, alcohols, phenols, monocyclic aromatics, polycyclic aromatic hydrocarbons.	Manufacturing/Production activities, industrial wastes, chlorination of water, fossil fuels, oil refineries.
Gases	Carbon monoxide, sulfur oxides, hydrocarbons, nitrogen oxides.	Fumes from industries.
Nitrosamines	Compounds in organic chemicals and rubber.	Food cooking operations.
Radioactive Isotopes	Strontium-90, plutonium, iodine-131, calcium-45, cesium-137, radioactive carbon.	Fallouts from nuclear reactors and weapon testing.
Pesticides	Organochlorines (such as aldrin, dieldrin, endrin, DDT, heptachlor, lindane) and organophosphates.	Direct application to food, farms, forests, lawns, and gardens; runoffs from application sites; discharge in industrial waste water.

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2.12 ANALYTICAL METHODS FOR DETERMINATION OF PESTICIDE RESIDUES IN FOOD

There are several methods for determination of pesticide residues in food. These are single residue methods (SRM), single class multiresidue methods (sMRMs) and multiclass multiresidue methods (mMRMs). Choice of method is based on targeted compounds or class of compounds and the type of sample matrix. If the pesticide treatment history of the sample is unknown, in which case no compound or class is targeted, a multiclass multiresidue method is used (Sandra et al., 2003). Basically, determination of pesticide residues in food consists of a chain of steps as shown in Figure 2.13. (Pesticide Analytical Manual, 1999).

2.12.1 Sample Collection and Preparation

This first step is usually done with great care to avoid errors which are capable of making the other steps meaningless. Sample collection involves taking a representative sample of the material to be analyzed. Handling of sample during and after collection should be such that it is protected against damage and contamination. Storage containers should be clean and inert. Plastic containers for instance are not recommended in pesticide residue analysis because they are a potential source of contamination with phthalate esters. Storage conditions vary with the type of sample material. While some materials need to be kept frozen, others can simply be kept cool. Samples should be correctly and adequately labeled to provide such information as name, nature, origin, date and place of sampling.

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┌─────────────────────────────────────────────────┐
│        Sample Collection and Preparation        │
└─────────────────────────────────────────────────┘
                        │
                        ▼
┌─────────────────────────────────────────────────┐
│  Extraction of Pesticide Residues from Sample Matrix │
└─────────────────────────────────────────────────┘
                        │
                        ▼
┌─────────────────────────────────────────────────┐
│              Clean-up of Extracts               │
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                        ▼
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│   Identification and Quantification of Residues │
└─────────────────────────────────────────────────┘
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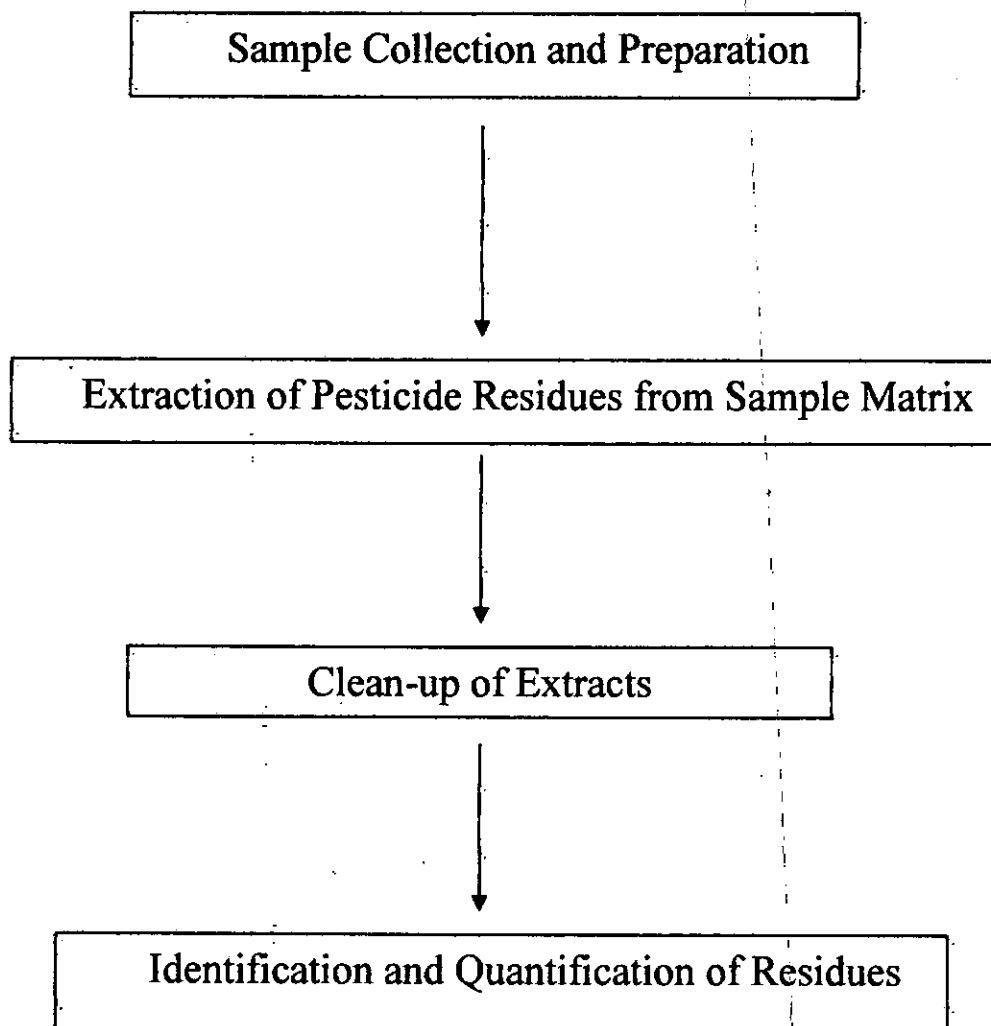
Figure 2.13: Steps in Pesticide Residue Analysis

Samples for analysis usually require some form of preparation to remove extraneous materials and to permit efficient extraction of the residues from the sample matrix. Preparation may include size reduction (by chopping, grinding or blending) or mixing. Once prepared, a representative sub sample can then be taken for the extraction process.

2.12.2   Extraction of Residues from Samples

Pesticide residues need to be extracted from the sample matrices for them to be determined. Several procedures (such as shaking with solvent, maceration with solvent, solid phase extraction (SPE), supercritical fluid extraction (SFE), microwave extraction (ME) and soxhlet extraction) are available for the extraction process. The method chosen depends on the nature of the sample, the target pesticides and facilities available. A schematic diagram of a soxhlet extractor is shown in Figure 2.14.

A variety of solvents are available for extraction of pesticide residues from samples. Solvents or their combinations are chosen such that the target compounds are selectively extracted with minimum co-extractives. The choice is guided by the nature of the sample material and the target pesticides. Generally, polar solvents are used for extraction of polar compounds while non-polar solvents are used for extraction of non-polar compounds. Solvent mixtures such as acetone-hexane or methanol-dichloromethane may be used to widen the scope of residues that can be extracted. Solvents for extraction in pesticide residue analysis include: methanol, acetone, acetonitrile, dichloromethane, diethyl ether, ethyl acetate, hexane and petroleum ether.

Once the residues have been extracted from the sample matrix into a suitable solvent, the solvent is evaporated to reduce the volume of the extract for the cleanup stage.
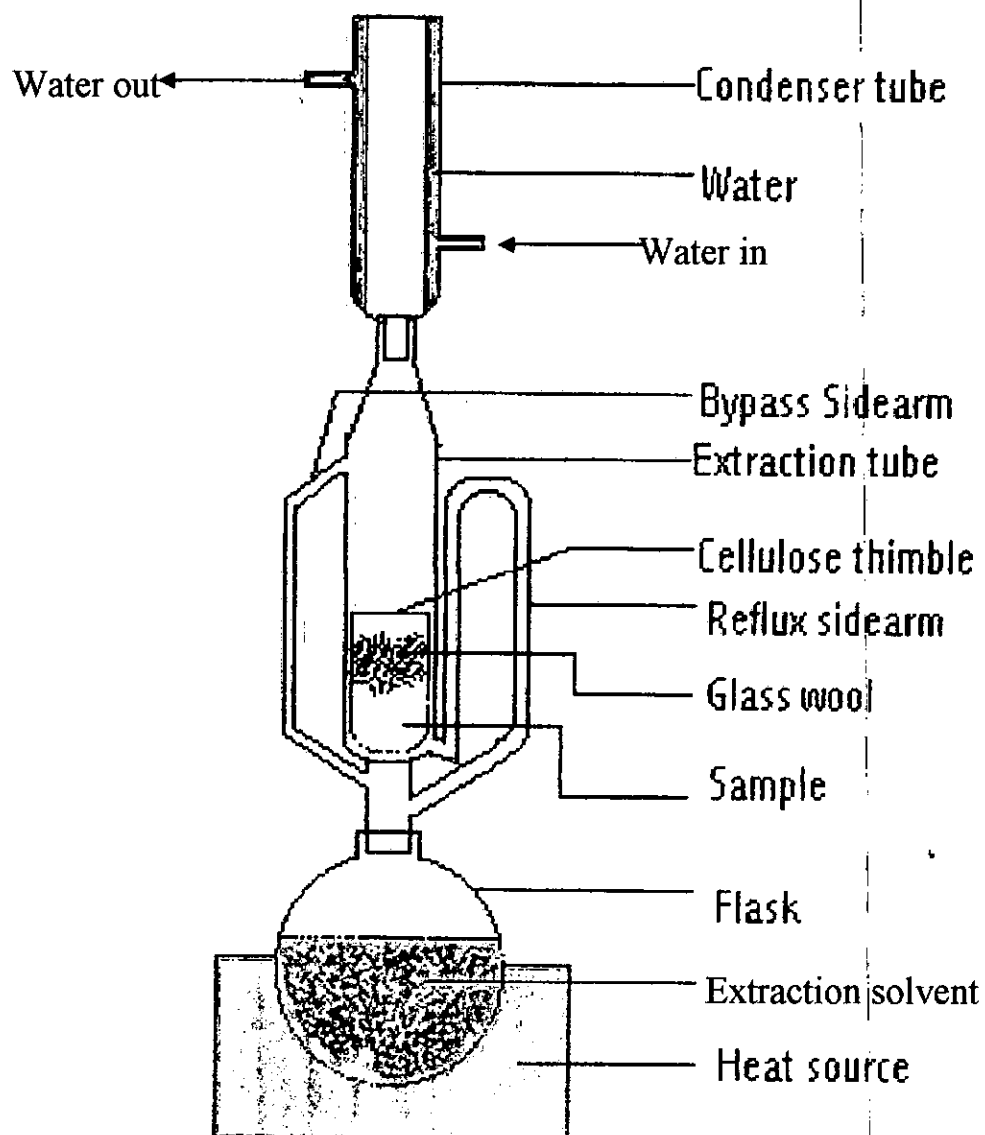
Figure 2.14: Schematic Representation of a Soxhlet Extractor.

2.12.3   Clean-up of Extract

During the extraction process, some non-target compounds are inadvertently extracted along with target residues. These, if not removed, will interfere with identification and quantification of the analytes and adversely affect determinative step instrumentation. Cleanup is therefore necessary to further select the residues of interest from the co-extractives or to selectively remove the co-extractives.

There are a variety of clean-up procedures which use differences in solubility, molecular weight and affinity for adsorbents (such as silica gel, florisil, activated charcoal and alumina) for separation of analytes from interfering substances. The adsorbents are usually in columns or cartridges. Clean-up procedures involve the use of liquid-liquid partitioning, adsorption column, thin layer chromatography and size exclusion or gel permeation chromatography (SEC/GPC). Cleaned extracts are then concentrated for the final determinative step.

Clean-up of extracts is very important as it serves the following purposes:

i.   Protects instrument detectors used for analysis. This leads to more consistent detector response and better detector baseline.

ii. Protects analytical columns used in GC and HPLC analysis.

iii. Minimizes the risk of false positive or false negative results (masking of true results)

2.12.4   Identification and Quantification of Residues

Clean sample extracts can be analyzed using a variety of techniques. This involves separation of each residue and its selective detection and quantitative measurement. Thus several pesticides can be determined simultaneously. The method chosen depends on the physical and chemical properties of the analytes as well as the equipment available.

Chromatography is the most commonly used determinative technique in pesticide residue analysis, although spectrophotometry may be used for some pesticides like dithiocarbamates and paraquat. Three main chromatographic techniques commonly used in determination of pesticide residues are:

1) **Gas Chromatography (GC)** – This is one of the most widely used techniques in pesticide residue analysis in food and environmental samples (Handa *et al.*, 1999). GC separates the components of a sample based on their differential distribution between a stationary phase and a mobile gas phase. It is generally used for analysis of volatile compounds but non volatile compounds can be made volatile by derivatisation (replacing hydrogen bonds with alkyl or silyl bonds) or pyrolysis (fragmenting the molecule to produce volatile fragments).

The sample to be analyzed is injected onto the head of the column via an injection port. It is vaporized, carried through the column (containing the stationary phase) by a carrier gas and separated by adsorption or partition, depending on whether the stationary phase is a solid or a liquid. That is, sample components are separated based on their distribution between the stationary phase and the mobile gas phase. Separated components are detected and recorded as they elute from the end of the column at different times. A record of the detector response against time is called a chromatogram. Each component

appears as a peak on the chromatogram and is identified by its retention time (t_R), that is, the time taken for it to elute from the column after injection. Peak area or height is used to determine the quantity of analyte. The quality of separation by GC is affected by the nature of the stationary phase, gas flow rate, column temperature and other factors.

The stationary phase (SP) material is generally of low vapour pressure and high thermal stability so that it can withstand high temperatures that are often used in GC analysis. The SP is contained in a packed or capillary column. In a packed column, the SP (partition liquids) is coated on solid materials such as methyl silicones. Capillary columns are open tubular columns the inside wall of which is coated with the partition liquid. They are basically fused silica columns. For pesticide residue analysis, non-polar columns are commonly used. Column varieties permit the separation of large number of compounds in a single analysis. Thus GC is well suited for multiresidue analysis. Capillary columns are more efficient than packed columns (Willett, 1987). The column is kept in an oven for temperature control. For analysis of complex mixtures, the temperature may be programmed to accommodate compounds of a wide range of boiling points.

The mobile phase or carrier gas is an inert gas and must be free from impurities to avoid a noisy and fluctuating baseline. Examples of carrier gases in use are nitrogen, helium, argon, hydrogen and carbon dioxide. Nitrogen and helium are the most commonly used.

Detectors used in GC analysis are of a wide variety. They include:

   i. Electron capture detector (ECD)

   ii. Electrolytic conductivity detector (ELCD)

   iii. Flame ionization detector (FID)

   iv. Flame photometric detector (FPD)

v. Flame photometric phosphorus detector (FPPD),

vi. Nitrogen phosphorus detector (NPD)

vii. Photo ionization detector (PID)

viii. Thermionic detector (TID)

ix. Thermionic phosphorus detector (TPD).

In the analysis of complex mixtures such as pesticide residues in food, detectors selective for the target compounds are used. For example, FPD is widely used for organophosphorus compounds while ECD is detector of choice for analysis of organohalogen compounds. GC may also be combined with molecular spectrometry to identify separated components as in gas chromatograph with mass spectrometry (GC/MS). A block diagram showing the major components of a gas chromatograph is shown in Figure 2.15.

2) **High Performance Liquid Chromatography (HPLC)** – This is also a powerful technique in pesticide residue analysis. It is suitable for non- volatile and thermally unstable compounds. In HPLC, analytes are separated using a solid stationary phase tightly packed in stainless steel column and a liquid mobile phase. A solvent similar to the mobile phase is used to dissolve the mixture to be analyzed. The solution is then introduced by means of an injection device onto the top/head of the column (stationary phase). The sample is carried through the column by a continuous flow of mobile phase delivered from the solvent reservoir by means of a pump. Components are detected as they elute from the column and recorded in a chromatogram. A chromatogram gives both qualitative and quantitative information about the sample.
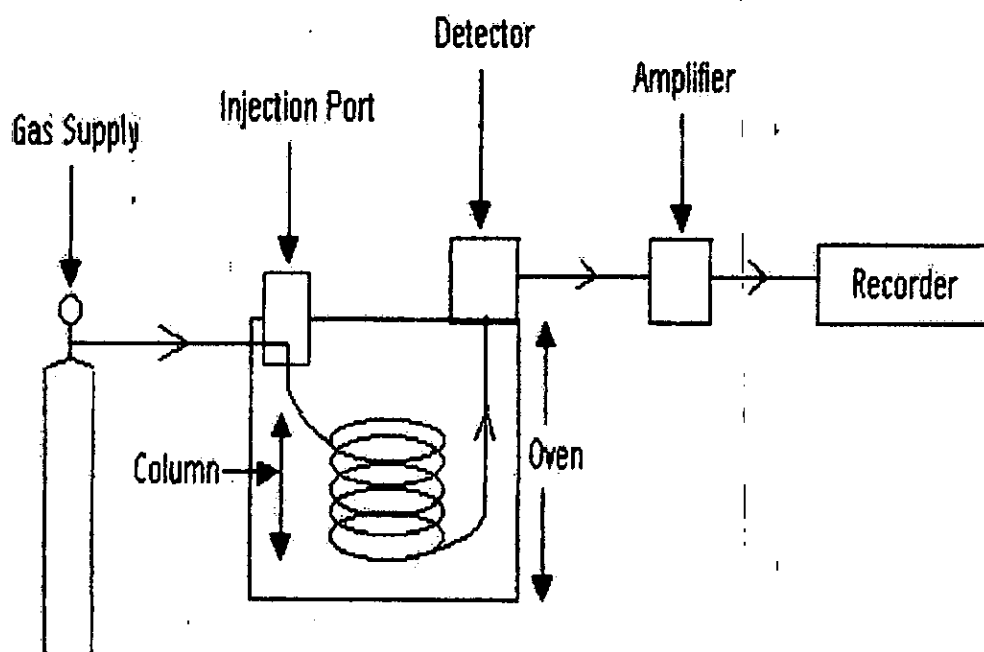
Figure 2.15: Block Diagram of a Gas Chromatograph

Depending on the polarity of the compounds to be analyzed, normal, reverse-phase or ion exchange columns may be used.

a. Normal Phase Column – This uses a polar stationary phase to separate molecules based on polarity, the least polar eluting first. An example is Silica Gel column.

b. Reverse Phase Column – The stationary phase is non-polar and separation is based on solubility parameters, partition coefficients and polarity. The most polar compounds elute first from this column. An example is Octadecyl Silane (C_{18}) column.

c. Ion Exchange Column – Here the stationary phase is ionic and components are separated on the basis of molecular charge.

Detectors available for use with HPLC include ultraviolet absorption, electrochemical and fluorescence detectors. Figure 2.16 is a block diagram of an HPLC.
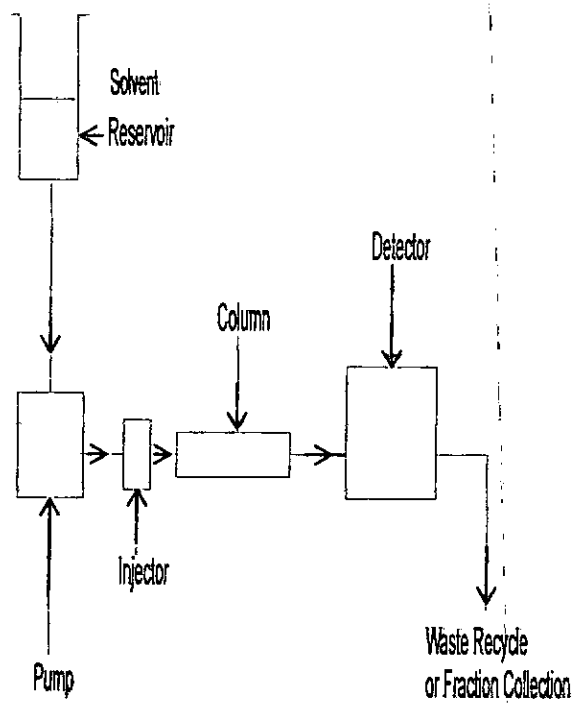
Figure 2.16: Block Diagram of HPLC

3) **Thin Layer Chromatography (TLC)** – This technique is used mainly for separation and identification of pesticides. It is simple, reliable and cheap. In TLC, a mixture of the pesticides to be analyzed is applied to a plate coated with a thin layer of finely divided stationary phase adsorbent. The plate is then placed in a mobile phase solvent which then moves up the stationary phase by capillary action. Components of the mixture are thus separated as spots on the basis of their differences in solubilities, pK values and capability to form hydrogen bonds. Separated spots are detected through the use of location methods such as visual location, use of ultraviolet light, iodine vapour, corrosive reagents such as sulphuric acid, etc.

Stationary phase materials used in TLC include silica gel, alumina, kieselguhr (diatomaceous earth), florisil (magnesium silicate), cellulose and ion exchange resins. Mobile phase solvents may be single or mixture of solvents. Choice of solvent generally depends on the compounds to be separated and the stationary phase to be used. Ideally, the solvent should be cheap, stable, non-toxic and easy to remove from the plate after a chromatographic run. The solvent should also not react with the compounds to be separated. Examples of mobile phase solvents used in TLC are acetic acid, acetone, acetonitrile, benzene, chloroform, cyclohexane, hexane, methanol and toluene.

The basic measurement in TLC is retardation factor (R$_f$) value which is defined as

$$R_f \quad = \quad \frac{\text{Distance moved by compound from the origin}}{\text{Distance moved by solvent front from the origin}}$$

R_f values of separated components are compared with standard values for identification. The performance of TLC can be enhanced by the use of optimized plate coating material, improved sample application and plate development techniques. Under such optimized conditions, the TLC is then called high performance thin layer chromatography (HPTLC).

CHAPTER THREE

EXPERIMENTAL

3.1 INSTRUMENTS AND EQUIPMENT

The following instruments and equipment were used in this research work:

i. Gas chromatograph (Trace 2000 Series, ThermoQuest, Italy).

(Courtesy of Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, UK)

ii. Mass-selective detector (Fisons MD 800, Italy).

iii. Autosampler (AS 2000, Italy).

iv. Nitrogen sample concentrator (TECHNE, Switzerland).

v. Rotary evaporator (Rotavapor BUCHI 461, Switzerland).

vi. Centrifuge (Labofuge 400, Germany).

vii. Vortex mixer (TECHNE,UK).

viii. Weighing balance (Mettler H8, UK).

ix. Refrigerator (Snaige 224, UK).

x. Grinding machine (Christy, Chelmsford, England).

xi. Fume cupboard (Nordia, Walthamstow, England).

xii. Solid phase extraction vacuum manifold (International Sorbent Technology, UK).

To minimize contamination from apparatus, all glasswares were washed thoroughly with liquid detergent and water, rinsed with acetone and left to dry. Each was then rinsed with the appropriate solvent just before use.

3.2 MATERIALS, CHEMICALS AND REAGENTS

3.2.1 Materials

The food stuffs analyzed for pesticide residues were as follows:

a. Maize samples – Four types of maize were purchased from seven different wholesale markets across Lagos State.

b. Beans samples – Four types of beans were purchased from seven different wholesale markets across Lagos State.

The names, description and codes for the types of maize and beans studied are shown in Tables 3.1 and 3.2 while markets and sources of origin of the samples are presented in the Appendix. Figures 3.1 and 3.2 are photographs of the different types of maize and beans studied.

Table 3.1= Names, Description and Codes for the Types of Maize Studied.

Botanical Name	Local Names	Common Names	Types (Description of Grain)	Codes
Zea mays L. *(Family Gramineae)*	Agbado (Yoruba) Masara (Hausa) Oka (Igbo)	Maize, Corn, Indian corn, etc.	Yellow, big, flat	MYa
			Yellow, small, round	MYb
			White, big, flat	MWa
			White, small, round	MWb

Table 3.2 - Names, Description and Codes for the Types of Beans Studied.

Botanical Name	Local Names	Common Names	Types (Description of Seed)	Codes
Phaseolus vulgaris L. *(Family Leguminosae)*	Ewa (Yoruba) Wake (Hausa) Agwa (Igbo)	Common bean, Kidney bean, French bean, Runner bean, Salad bean, etc.	Brown, big	BBa
			Deep brown, small	BBb
			White, big	BWa
			Light brown, long	BWb
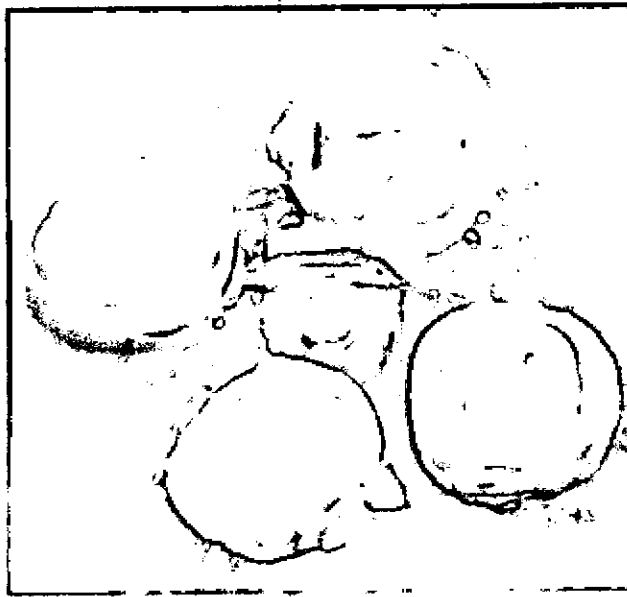
MYa

MYb
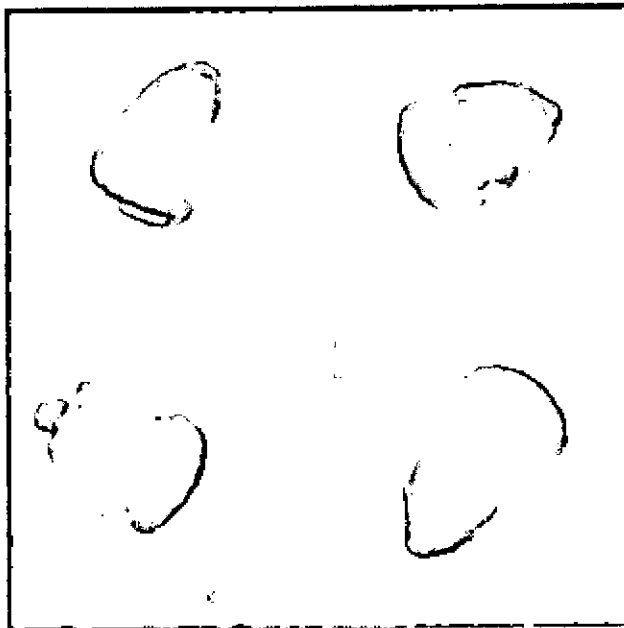
MWa

MWb

Figure 3.1: Photograph of Types of Maize Studied.
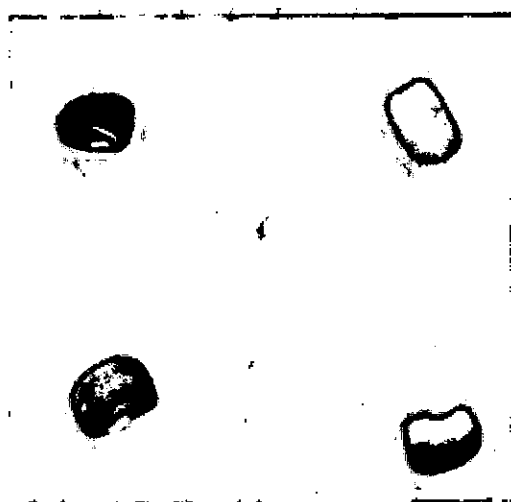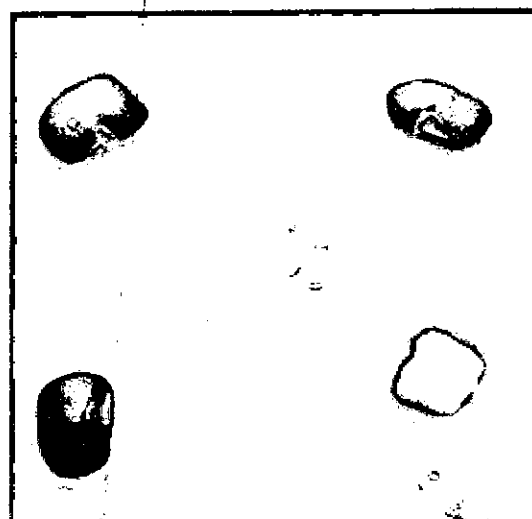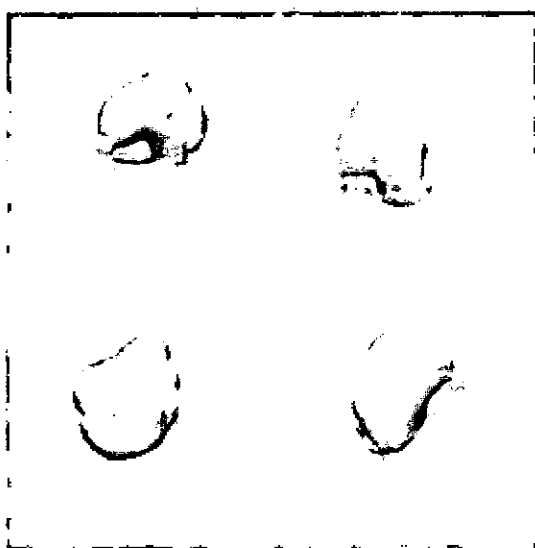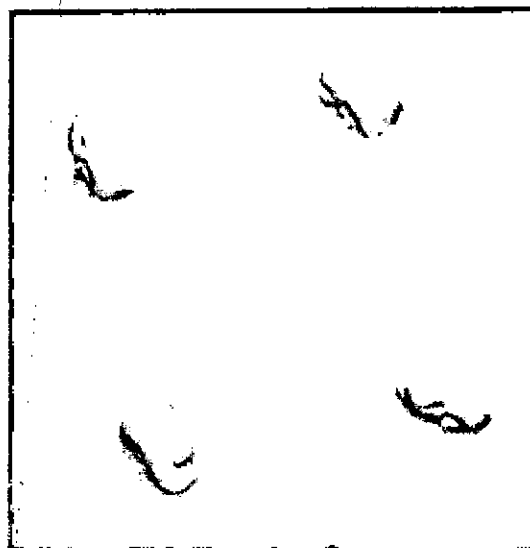
Figure 3.2: Photograph of Types of Beans Studied.

3.2.2 Chemicals and Reagents

A. Pure Standards:

The following pesticide standards were purchased from Sigma-Aldrich, Germany and all were above 99% purity.

    i. Organochlorine compounds – aldrin, dieldrin, dichlorodiphenyltrichloroethane (DDT), endosulfan and endrin.

    ii. Standard mixtures of organophosphate and carbamate compounds containing chlorpyrifos, diazinon, dichlorvos, fenitrothion, malathion, parathion, pirimiphos-methyl, carbaryl and carbofuran.

    iii. Internal standard (2-chloroanthracene).

B. Reagents and Solvents

The following reagents and solvents were used in the analysis:

    i. Ethyl acetate - Fisher Scientific, UK.

    ii. Hexane - Rathburn Chemicals, Scotland.

    iii. Sodium sulphate - Sigma-Aldrich, Germany.

    iv. SPE cartridges (florisil 500mg/6ml) – Applied Separations, Allentown, UK.

All reagents were of analytical grade and above 99% purity. The solvents were distilled in all-glass apparatus before use.

3.3 PROCEDURES

3.3.1 Collection of Samples

Samples were obtained directly from local sellers in seven wholesale markets across Lagos State. Names and codes of the markets of collection are presented in the Appendix. Two types of white maize and two types of yellow maize were collected. Samples of each type of maize were purchased from three different shops and stored in glass bottles with tight covers to protect samples from moisture and contamination. Plastic containers were avoided to prevent contamination with phthalate esters which are common interferences in pesticide residue analysis.

The same procedure was used for collection of beans samples. Two types of white beans and two types of brown beans were collected. All samples were code-named. The sources of the samples (i.e. places from where they were brought into Lagos State) were sought and recorded as shown in the Appendix.

3.3.2 Preparation of Samples

The samples were cleaned by picking out stones and other extraneous materials. Each sample was thoroughly mixed and a 200.0g portion was taken and milled (using a Christy grinding machine) to 20 mesh particle size to produce a good homogenate. The grinding machine was previously cleaned with acetone. The milled samples were then stored in glass bottles with appropriate labels in a refrigerator at 4^0C. Duplicate portions (200.0g) of the samples were stored as whole grains in labeled glass bottles in the refrigerator until ready for use.

3.3.3 Preparation of Calibration Curves

Individual stock solutions of organochlorine pesticide standards were prepared by accurately weighing 10.0mg of each into a 10.0ml volumetric flask. This was dissolved and made up to volume with ethyl acetate. The stock solutions were then serially diluted by means of automatic pipettes to produce different concentrations of individual pesticides. The stock solutions were also used to prepare mixed standard solutions of concentrations 0.05, 0.50, 1.00, 2.00, 4.00 and 8.00µg/ml, each containing 5µg/ml of the internal standard. The internal standard, 2-chloroanthracene was chosen based on its relative stability, chemical similarity to the pesticides and medium retention time. Stock standard solutions were stored in amber coloured bottles at 4^{0}C in a refrigerator and working standard solutions were prepared fresh before use.

Individual standard solutions of the pesticides were run in GC/MS to obtain their retention times under the set chromatographic conditions. Mixed standard solutions were also run under the same conditions and mean peak areas were plotted against concentrations to obtain calibration curves of individual pesticides.

The concentrations of various organophosphate and carbamate compounds in three mixtures are shown in Table 3.3.

Table 3.3: Organophosphate and Carbamate Pesticides and their Concentrations in the Mixed Standard Solutions.

Pesticide	Concentration (µg/ml)		
	Solution 1	Solution 2	Solution 3
Carbaryl	0.75	1.5	3.0
Carbofuran	1.75	3.5	7.0
Chlorpyrifos	2.3	4.6	9.2
Diazinon	2.1	4.2	8.4
Dichlorvos	2.6	5.2	10.4
Fenitrothion	2.5	5.0	10.0
Malathion	1.1	2.2	4.4
Parathion	0.6	1.2	2.4
Pirimiphos-methyl	2.0	4.0	8.0

3.3.4 Determination of Limits of Detection (LOD)

To determine the limit of detection of the equipment for each pesticide, an air blank sample was run under the experimental conditions to obtain the detector baseline noise. The baseline noise is the deference between the maximum and the minimum noise signal in the chromatogram of the air blank at the elution time of the analyte. A detectable ion should produce a signal that is at least three times the baseline noise, that is, signal- to-noise (S/N) ratio = 3 (Maštovská et al., 2004; Wei-Guo et al., 2006; Chrysoula et al., 2006). The LOD of each pesticide was determined by running serially diluted solutions of the pesticide at the set chromatographic conditions and finding the concentration at which S/N = 3.

3.3.5 Extraction of Samples

Extraction of samples for pesticide residues was according to the methods of Wei-Guo et al., 2006 and Zawiyah et al., 2006 with slight modifications.

The milled sample was properly mixed and 2.0g was weighed into a 20.0ml sample vial. Anhydrous sodium sulphate (1.0g) was added and mixed with the sample to absorb any moisture present. The sodium sulphate was previously heated at 650^0C for one hour and stored in a desiccator. Ethyl acetate (10.0ml) was added to the vial. (Ethyl acetate was used as extraction solvent because all the target pesticides were found to be soluble in it). The mixture was vortex mixed for 5min and then allowed to stand for 45min. It was mixed again and centrifuged for 5min. at 2500rpm. The supernatant was transferred into a flask as carefully as possible. The extraction procedure as described above was repeated twice using 10.0ml ethyl acetate each time. The supernatants were combined and reduced to about 5ml using a rotary evaporator at 35°C. The solution was then transferred to a

sample tube and reduced to about 1ml under a gentle stream of nitrogen gas using a nitrogen evaporator at 36°C. This was then taken for florisil cleanup.

3.3.6 Sample Clean-up

Each solid phase extraction cartridge (florisil, 500mg/6ml) was conditioned with 5.0ml of the eluting solvent mixture (hexane/ethyl acetate 50:50), ensuring that the solvent level does not go below the florisil surface. The 1ml sample extract was loaded on the florisil. The sample tube was rinsed three times with 1.0ml eluting solvent, and the rinses added to the florisil column. The sample was then eluted with 5.0ml of the same solvent mixture into a receiving glass tube. The florisil column was rinsed with another 3.0ml of the eluting solvent mixture into the same receiving glass tube. The eluant was then evaporated to dryness under a gentle stream of nitrogen gas and the residue reconstituted in 1.0ml ethyl acetate for GC-MS analysis.

3.3.7 Determination of Residue Content

All compounds were determined and quantified with the aid of a gas chromatograph with a mass-selective detector (GC-MS).

3.3.7.1 GC-MS Conditions

The GC was equipped with an autosampler, a split-splitless injector and a DB-5 fused silica capillary column of 30m x 0.25μm i.d. x 0.25μm film thickness, coated with cross-linked 5% phenyl dimethyl polysiloxane; the carrier gas was helium (99.999% purity) at a flow rate of 1.0ml/min; Oven temperature was maintained initially at 70°C for 1min, increased at 15°C/min to 175°C, then at 2°C/min to 215°C, at 10°C/min to 265°C and

finally at 20°C/min to 290°C and held for 8min. Injection volume was 1µL, injected in splitless mode at injection temperature of 250°C.

The mass spectrometer was operated in electron impact (EI) ionization mode with a detector voltage of 700V, ion source temperature of 200°C, GC interface temperature of 320°C and emission current of 150µV. Acquisition mode was selected ion monitoring (SIM).

3.3.7.2 Selection of Monitored Ions

All compounds were determined by monitoring characteristic ions. Each monitored ion was selected based on its relative abundance. The pesticide standards and a blank sample were first run in full-scan MS mode (scan range m/z 50-450) and the mass spectrum of each pesticide compared with that of the blank sample at the same retention time. The base peak and other high abundant ions were selected except when there were interfering ions at the same m/z values from the blank sample. In such cases, lower abundant ions were selected to eliminate interference.

3.3.7.3 Identification and Quantification

Pesticides were identified and confirmed if the monitored ions appeared, the retention times matched those of the standards and the relative abundances were within 10% of those of the standards. Identified pesticides were quantified using the external standard method of comparing sample peak areas with those of the pesticide standards under the same conditions. Each sample was analyzed three times and the mean values obtained. The pesticide content of each sample was calculated as:

$$\text{Pesticide Content} = \frac{As \times Vf}{Wt_s \times CF}$$

Where   As =   peak area of sample

Vf =   final volume of clean extract

Wt_s =   weight of sample extracted

CF =   calibration factor.

The CF of each pesticide was calculated as

$$\frac{\text{Peak Area of Standard}}{\text{Total Amount of Standard Injected}}$$

3.3.8   Recovery Studies

Recovery experiments were carried out using blank samples which were selected for spiking. For organochlorines, six levels of mixed standard solutions used for preparation of calibration curves were used for spiking blank samples. Each standard solution (1.0ml) was added to 2.0g of ground sample to give fortification levels of 0.025, 0.25, 0.5, 1.0, 2.0 and 4.0μg/g respectively. Each spiked sample was allowed to stand for six hours and passed through the same analytical procedure as the samples. Each sample was spiked and analyzed three times and mean values obtained. Peak area ratios of pesticides to those of internal standard were calculated for both standard solutions and spiked samples. The percent recovery of each pesticide was then calculated as follows:

$$\frac{\text{Peak Area ratio of pesticide in spiked sample}}{\text{Peak Area ratio of pesticide in standard solution}} \times \frac{100}{1}$$

3.3.9 Effect of Heat

To determine the effect of heat on pesticide residues, 200g of whole-grain beans (two samples of brown type (a) from each market) were boiled in water at moderate heat (about 150°C) for 1hr. They were then meshed into a smooth paste using a porcelain mortar and pestle; 2g of each meshed sample was taken for analysis using the exact procedure described for the milled uncooked samples.

3.3.10 Estimation of Daily Intakes

The pesticide residue levels determined were used to estimate daily intakes of the various residues in beans using the methods of Handa *et al.*, 1999. Estimated daily intakes (EDIs) were calculated based on an average daily consumption rate of 60g of beans per person and a correction factor of 0.5 for processing. The consumption rate assumes an average 200g meal of beans eaten twice a week. Therefore, EDIs were calculated as follows:

$$EDI = Rm \times Cr \times 0.5$$

Where Rm = Mean residue concentration in beans samples in µg/kg

Cr = Consumption rate in kg/person/day

If a total diet of 1.5kg per person per day is assumed (Handa *et al.*, 1999), then beans is contributing only 4% of a person's diet. An estimated total diet intake (ETDI) of residues can therefore be calculated as:

$$ETDI = EDI \times 25$$

Values obtained were compared with maximum permissible intakes (MPIs). The MPIs were calculated from acceptable daily intakes (ADIs) fixed by FAO/WHO and average body weight (Wt) of 60kg as follows:

$$MPI = ADI \times Wt$$

CHAPTER FOUR

RESULTS

4.1 Sample Codes and Sources

Samples were coded based on market of purchase, grain type and variety. Market codes and sources of the maize and beans samples are shown in the Appendix.

4.2 Calibration Curves and Limits of Detection

Analyses of individual and mixed standard solutions of the pesticides were carried out to obtain their retention times and calibration curves. The retention times, limits of detection, coefficients of determination and selected monitored ions of the pesticides are presented in Table 4.1. Chromatograms of individual and mixed standard pesticides are presented in Figures 4.1 – 4.13 while Figures 4.14 – 4.27 show calibration curves for some of the pesticides.
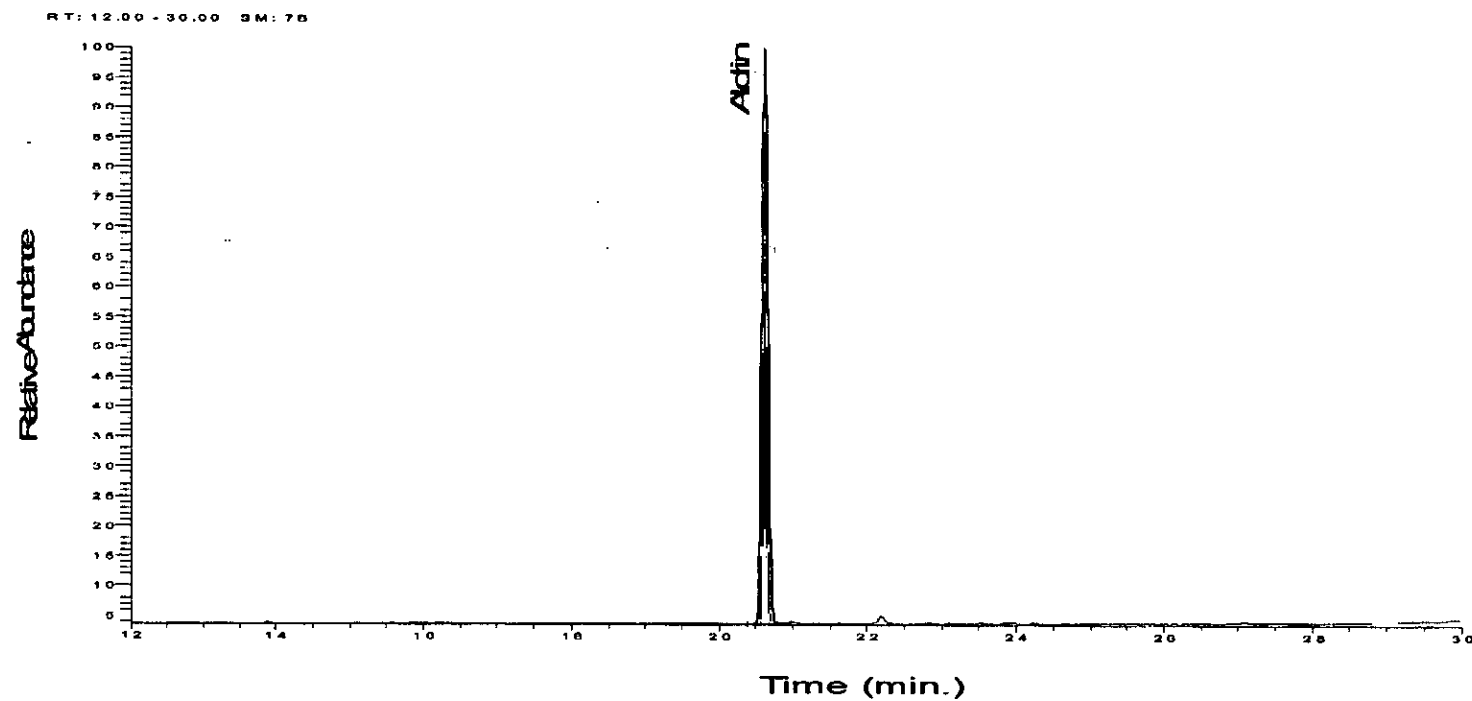
Figure 4.1: Total Ion Chromatogram of Standard Aldrin at 4μg/ml.
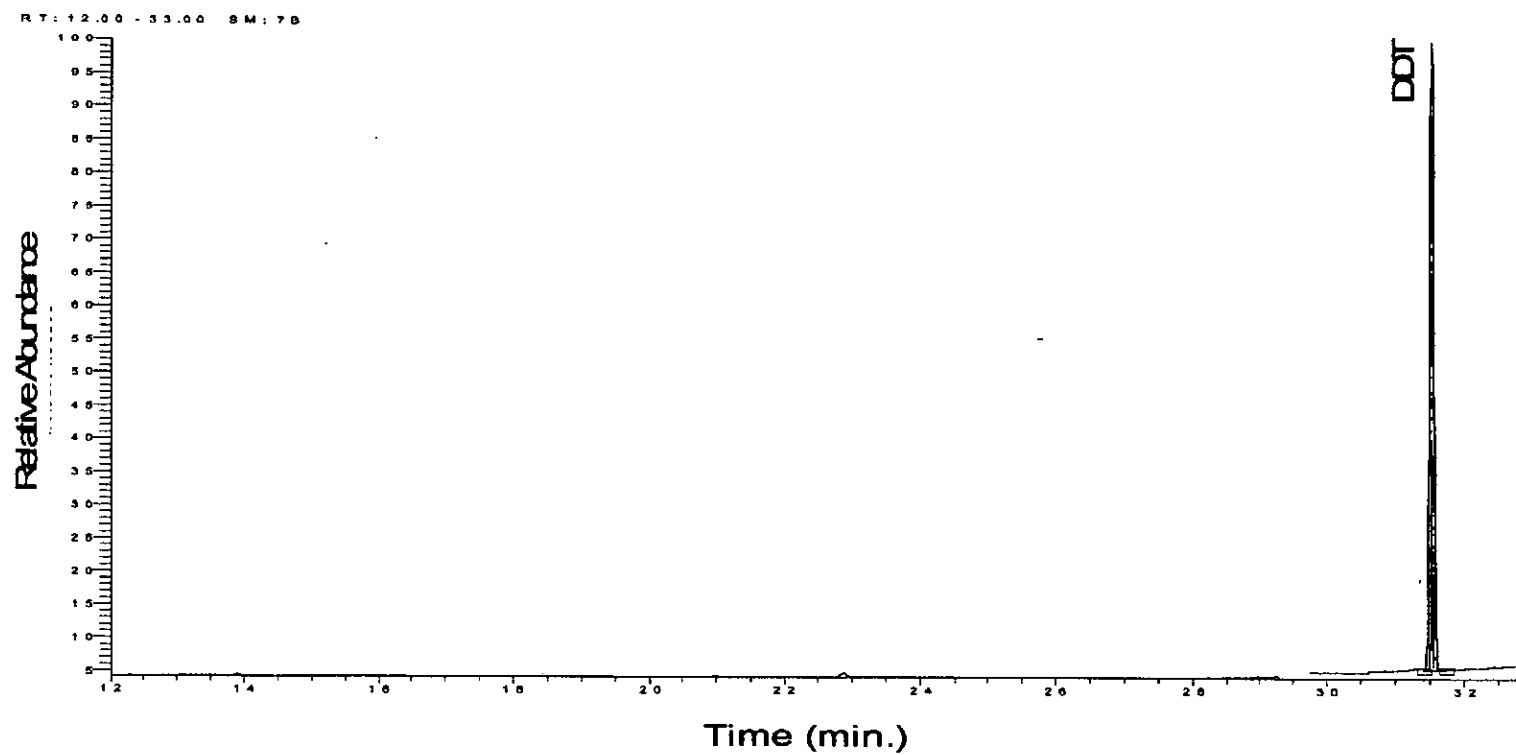
Figure 4.2: Total Ion Chromatogram of Standard DDT at 4μg/ml.
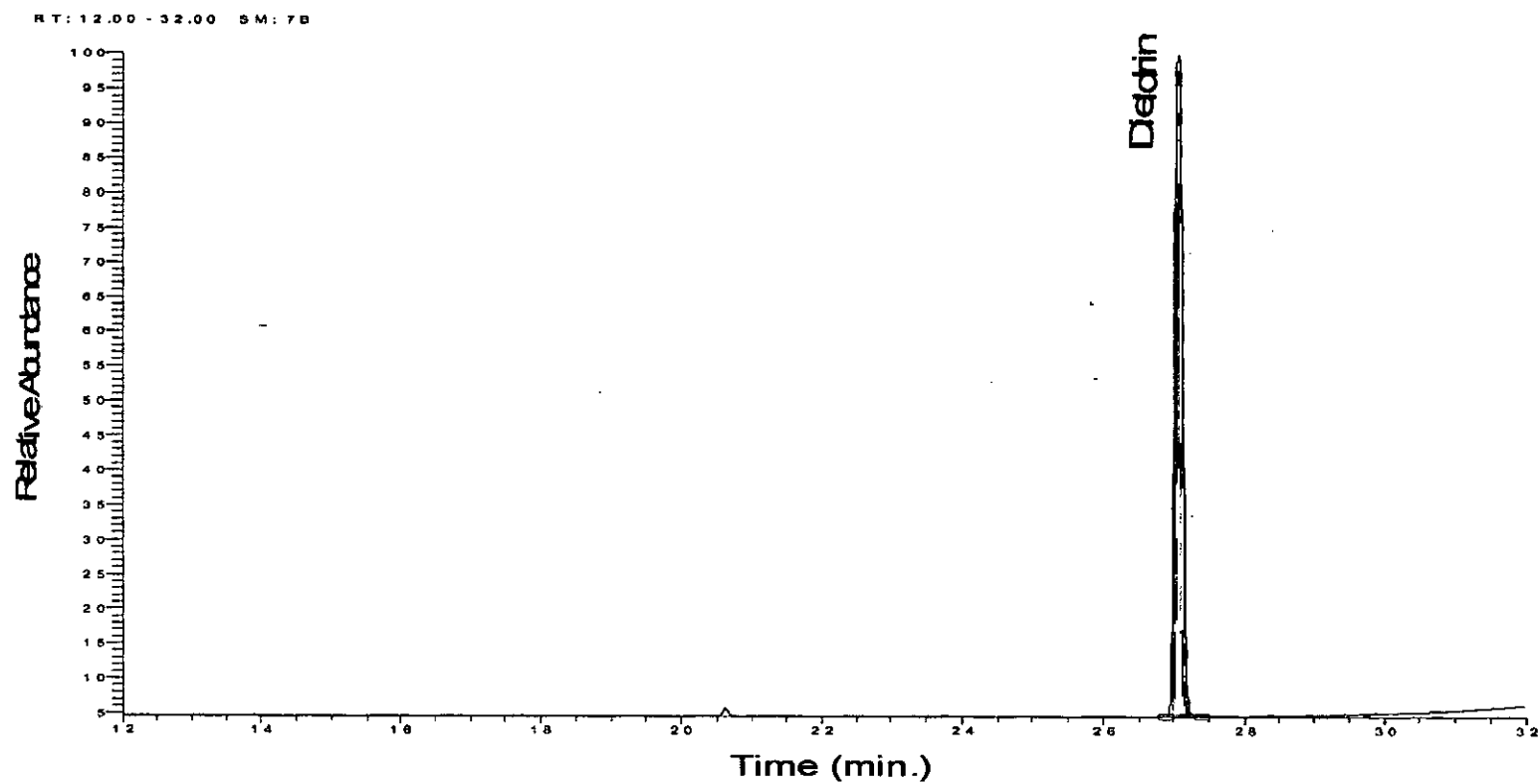
Figure 4.3: Total Ion Chromatogram of Standard Dieldrin at 4µg/ml.
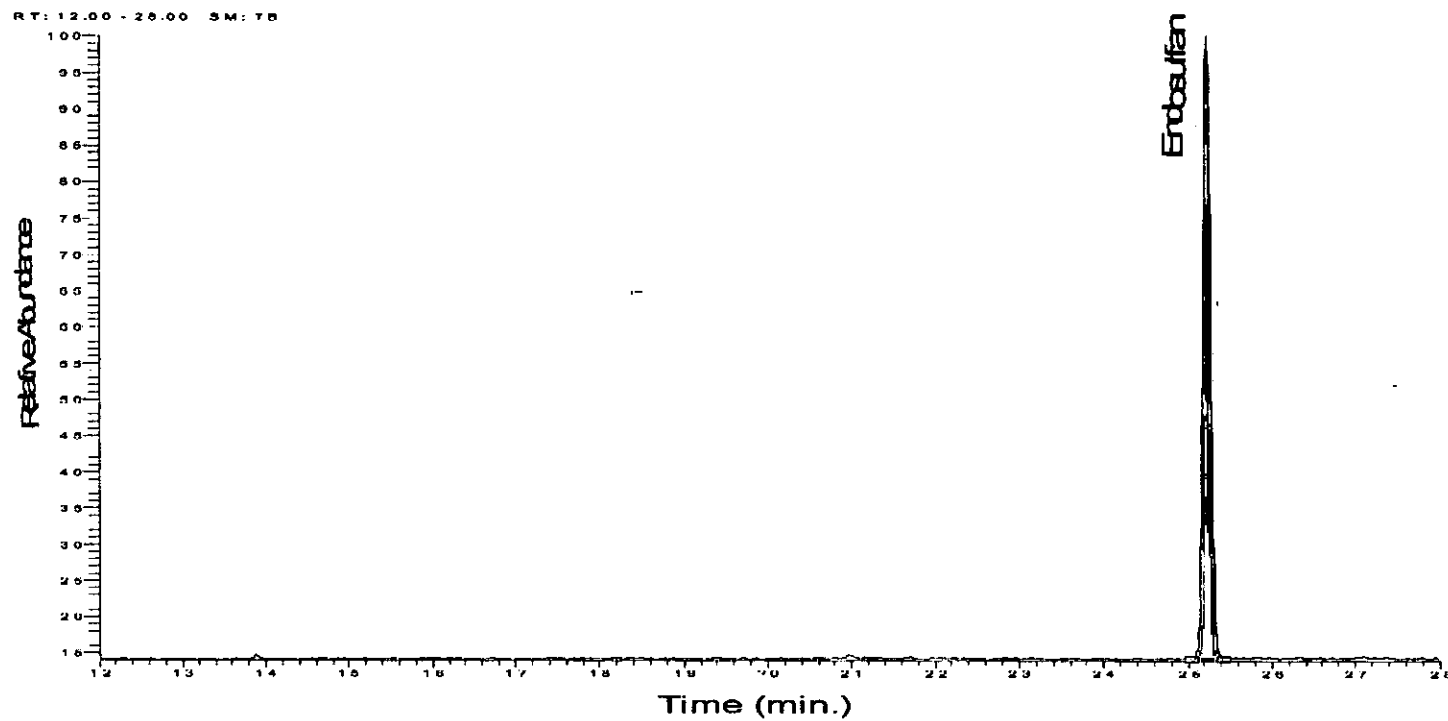
Figure 4.4: Total Ion Chromatogram of Standard Endosulfan at 4µg/ml.
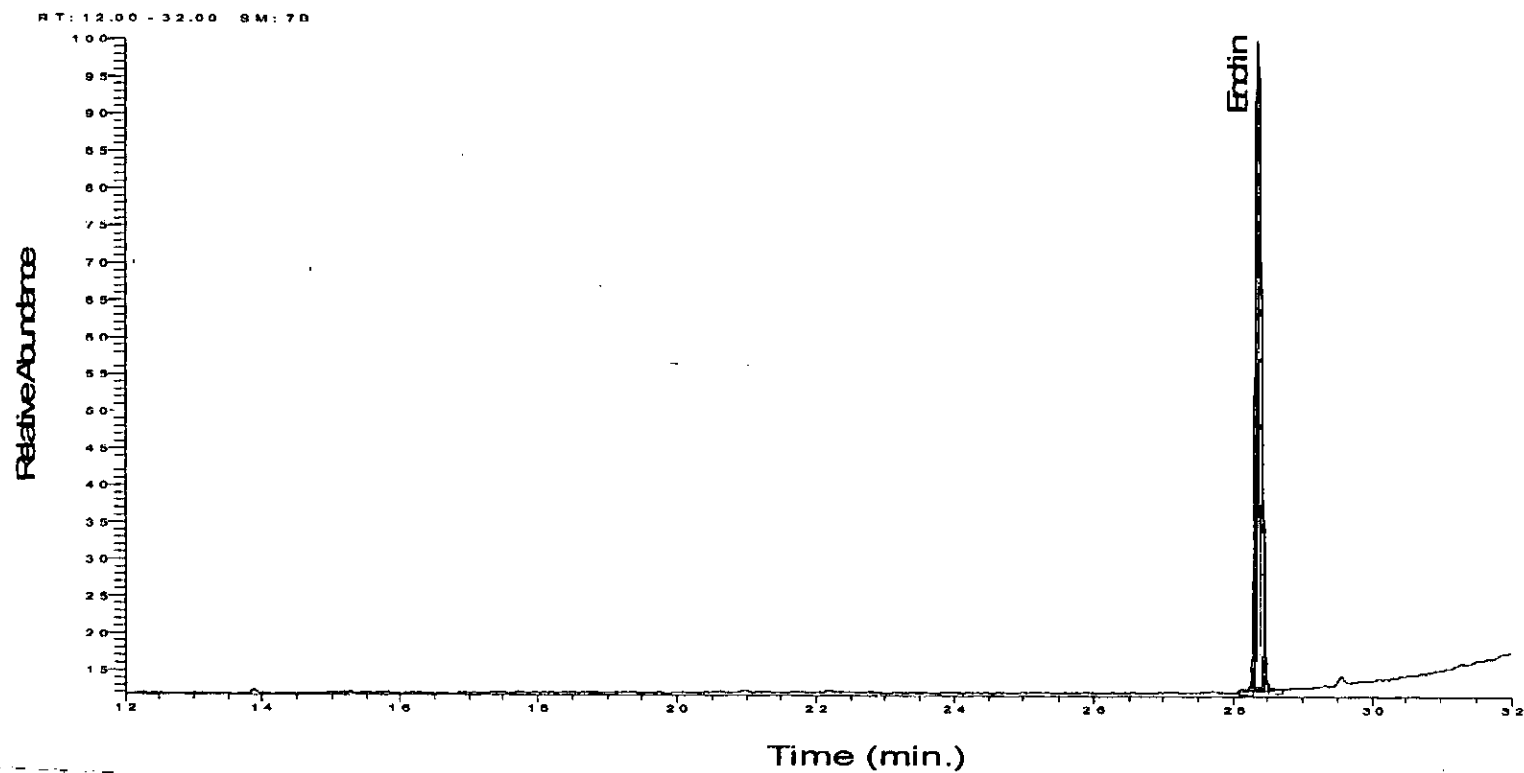
Figure 4.5: Total Ion Chromatogram of Standard Endrin at 4µg/ml.

123
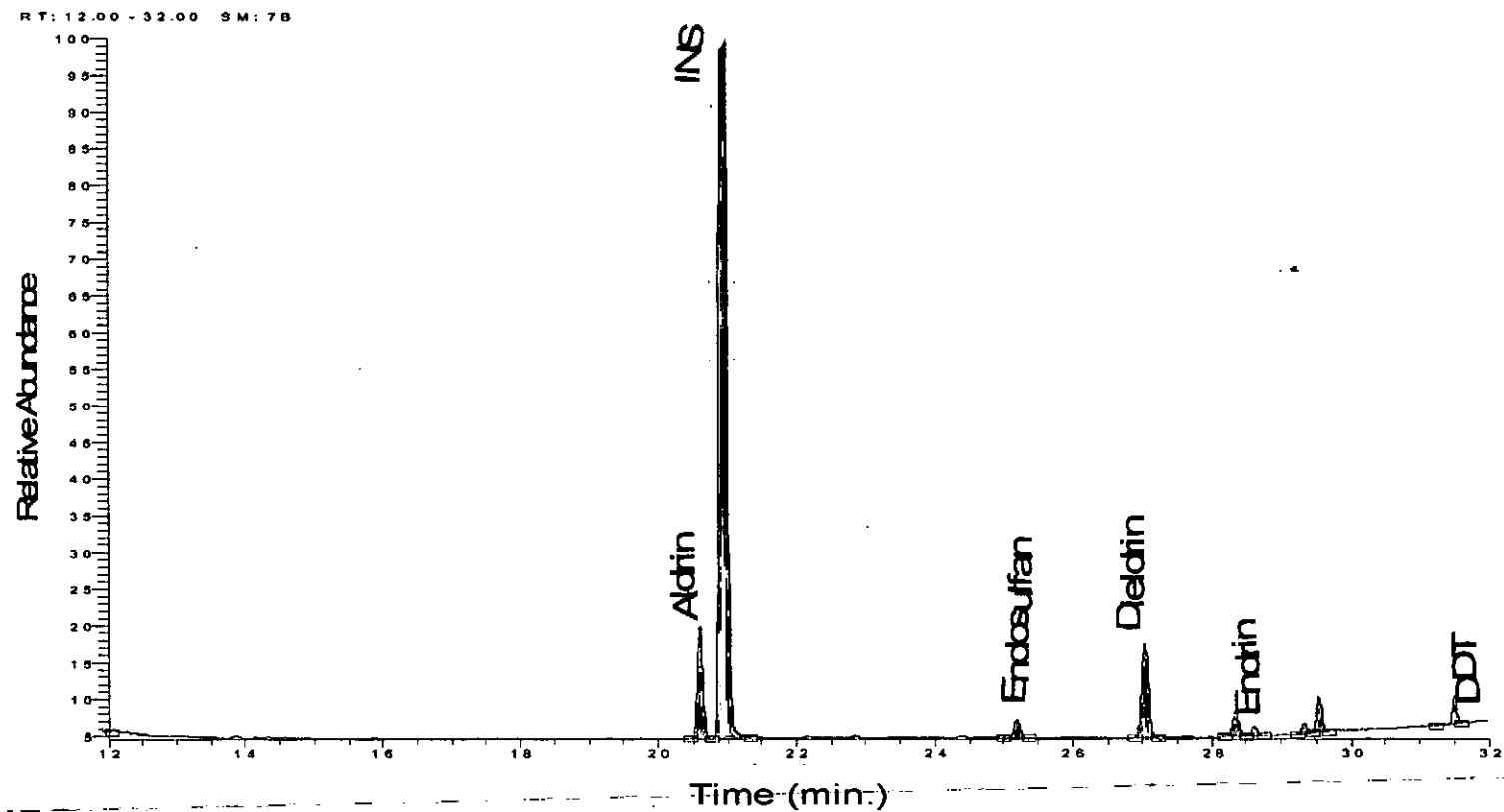
Figure 4.6: Total Ion Chromatogram of Mixed Organochlorine Pesticide Standards (Aldrin, DDT, Dieldrin, Endosulfan and Endrin) at 0.5µg/ml. INS = Internal Standard at 5µg/ml.
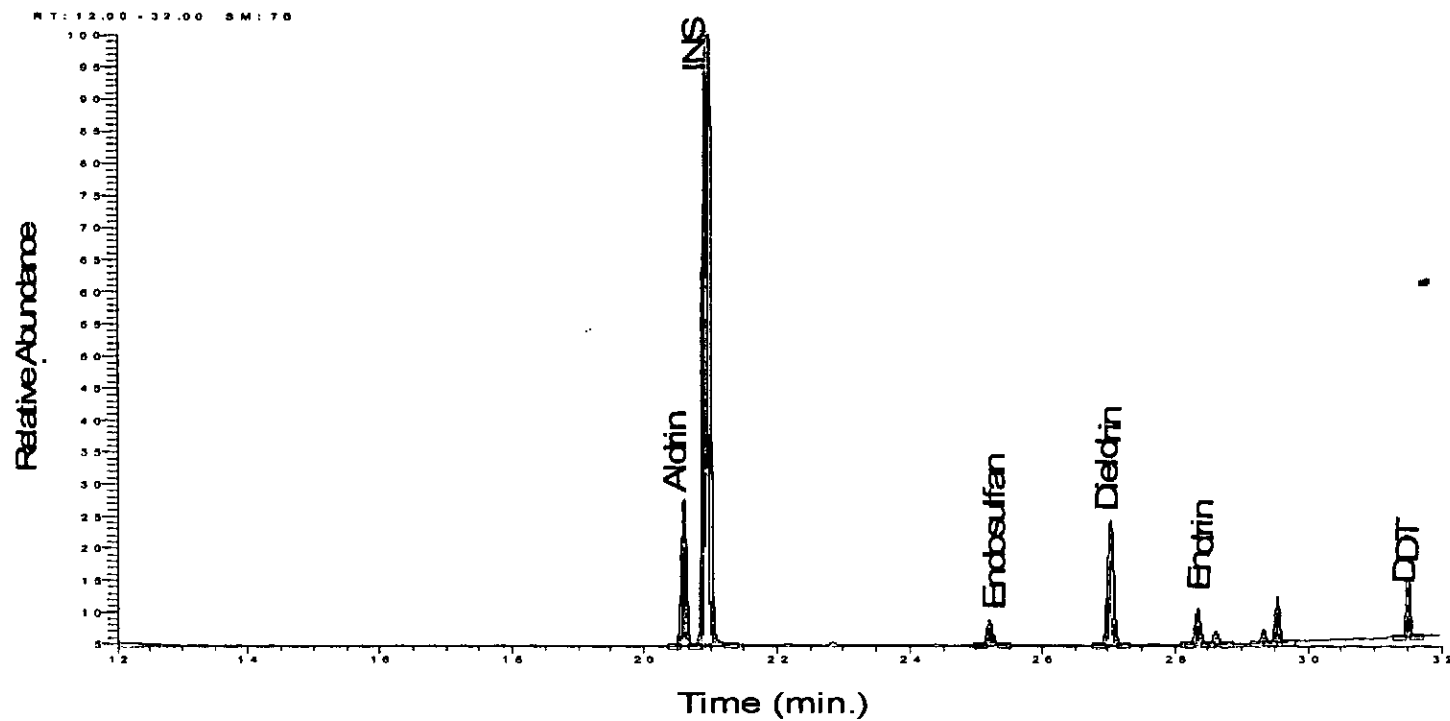
Figure 4.7: Total Ion Chromatogram of Mixed Organochlorine Pesticide Standards (Aldrin, DDT, Dieldrin, Endosulfan and Endrin) at 1.0µg/ml (B). INS = Internal Standard at 5µg/ml.
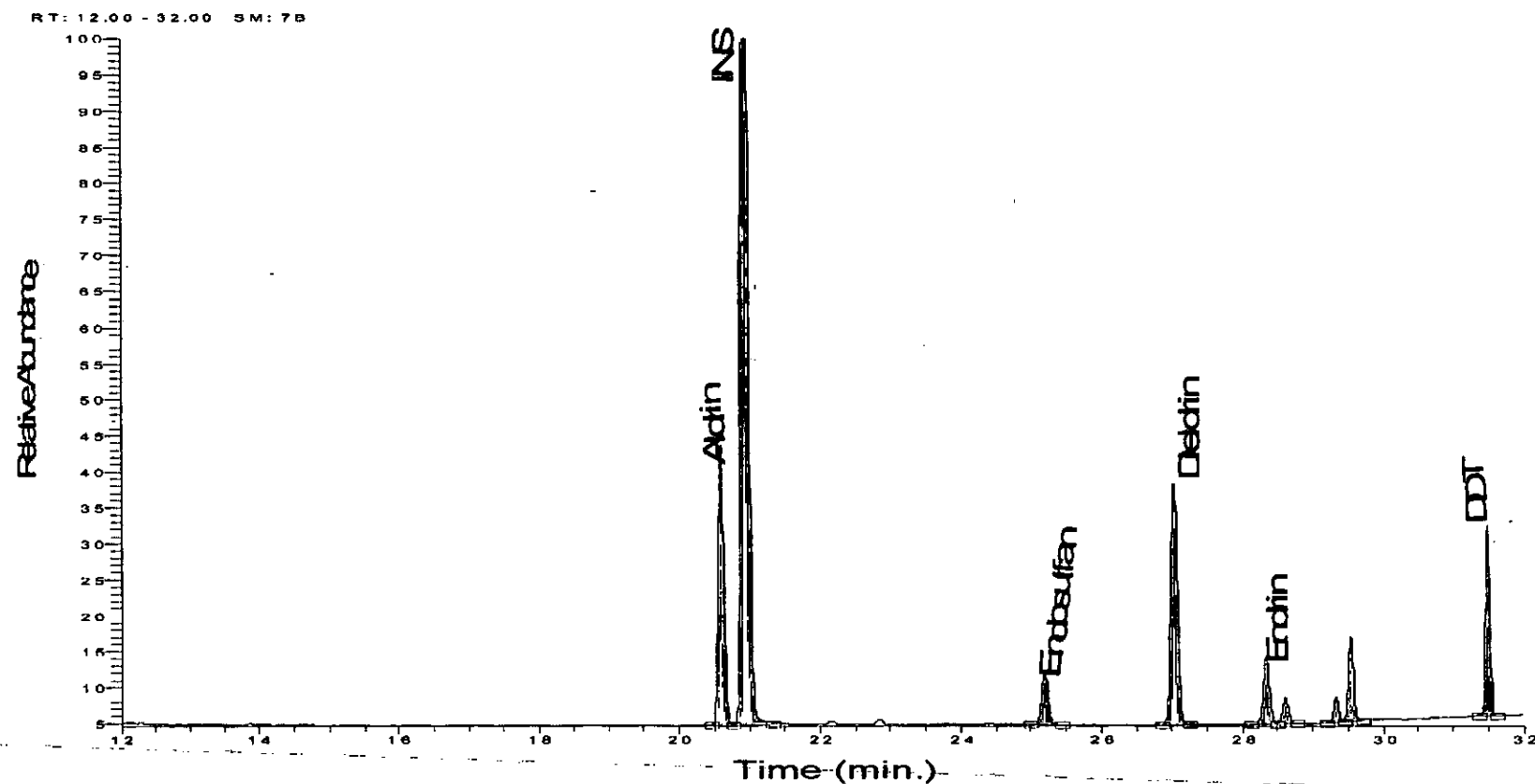
Figure 4.8: Total Ion Chromatogram of Mixed Organochlorine Pesticide Standards (Aldrin, DDT, Dieldrin, Endosulfan and Endrin) at 2.0µg/ml. INS = Internal Standard at 5µg/ml.

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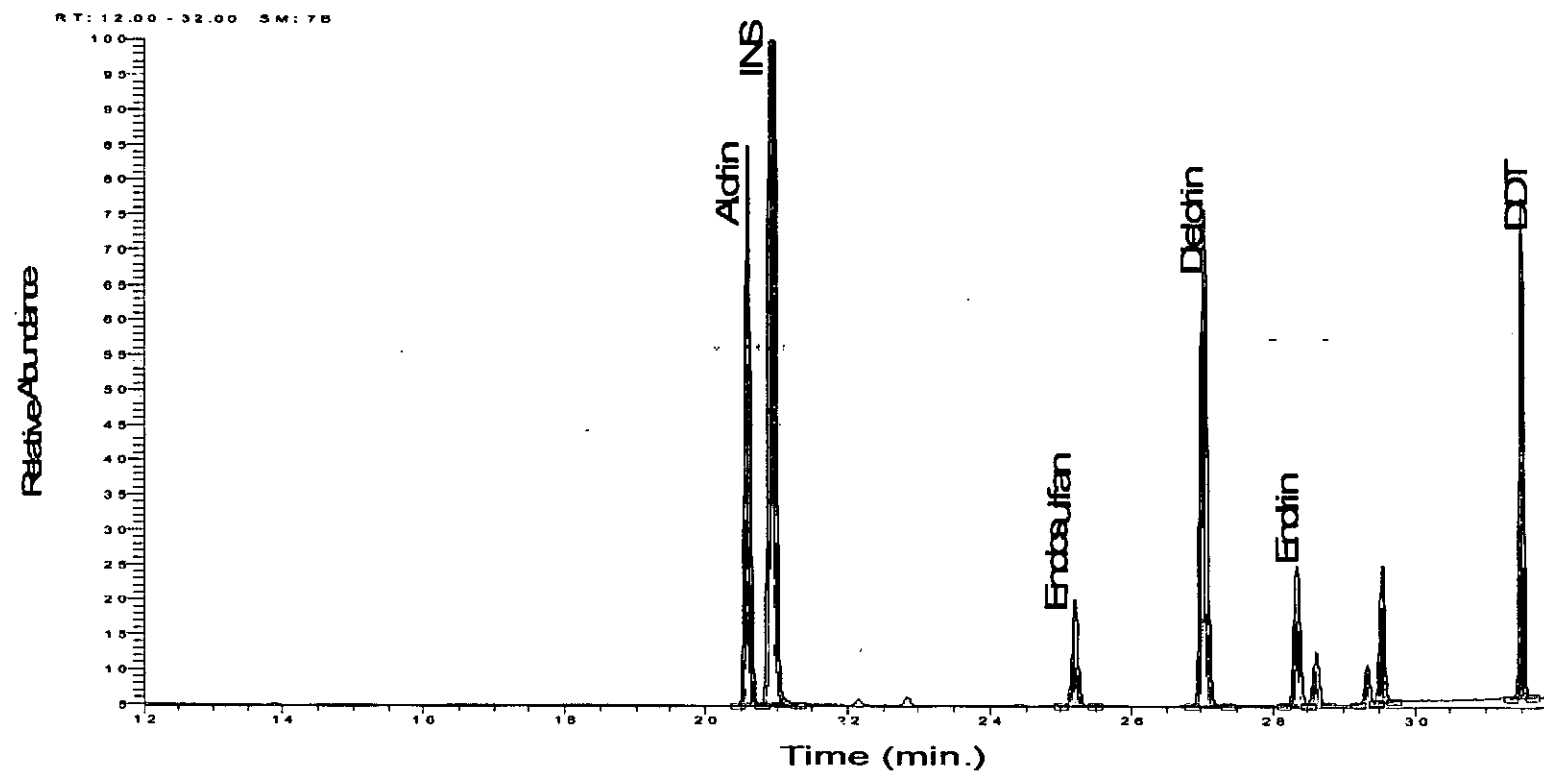
Figure 4.9: Total Ion Chromatogram of Mixed Organochlorine Pesticide Standards (Aldrin, DDT, Dieldrin, Endosulfan and Endrin) at 4.0µg/ml. INS = Internal Standard at 5µg/ml.
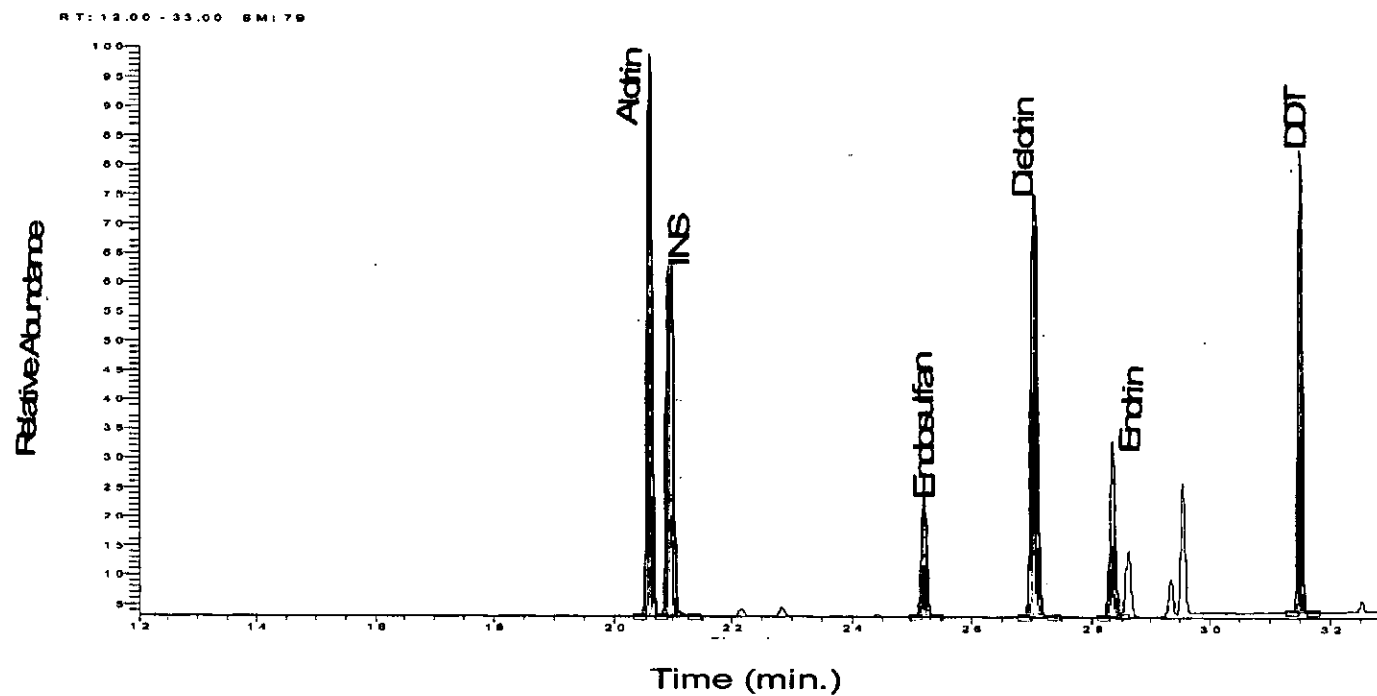
Figure 4.10: Total Ion Chromatogram of Mixed Organochlorine Pesticide Standards (Aldrin, DDT, Dieldrin, Endosulfan and Endrin) at 8.0µg/ml. INS = Internal Standard at 5µg/ml.
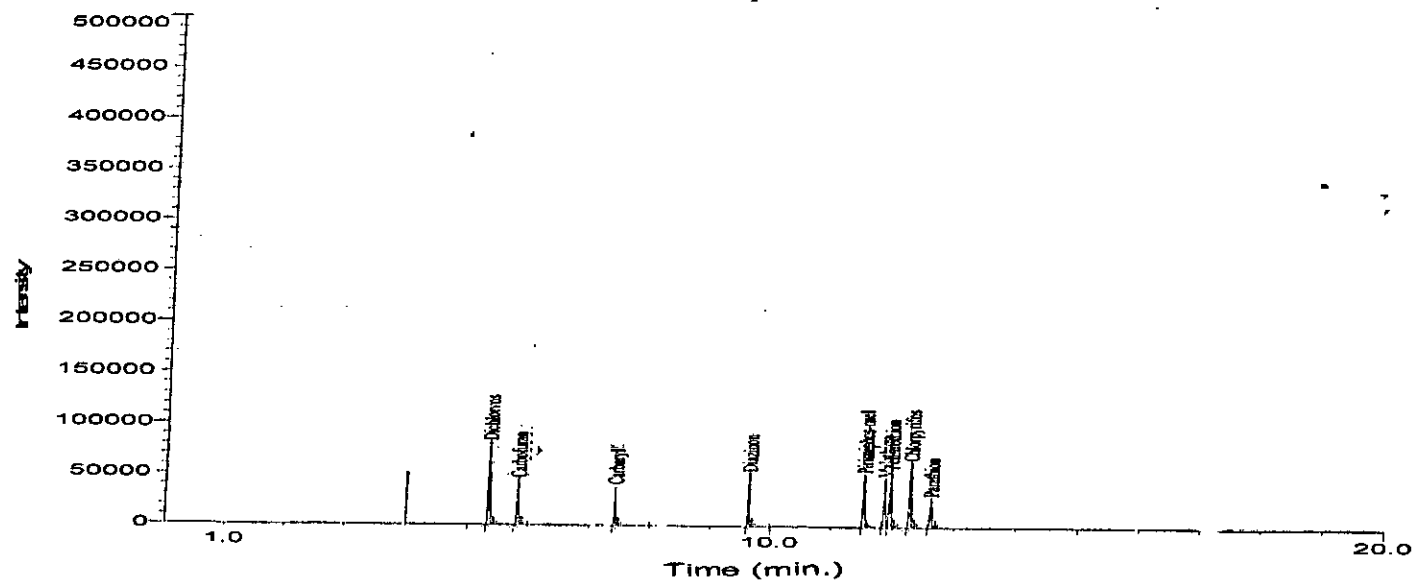
Figure 4.11: Total Ion Chromatogram of Mixed Organophosphate and Carbamate Pesticide Standards (Dichlorvos,

Carbofuran, Carbaryl, Diazinon, Pirimiphos-methyl, Malathion, Fenitrothion, Chlorpyrifos, and Parathion)

in solution 1.
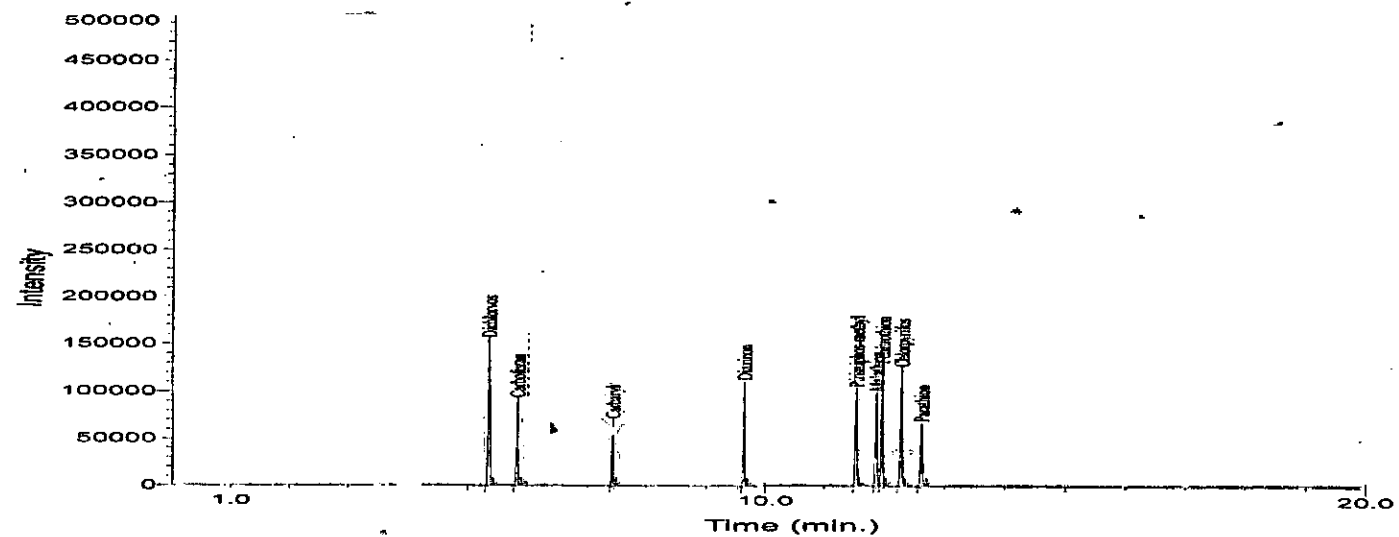
Figure 4.12: Total Ion Chromatogram of Mixed Organophosphate and Carbamate Pesticide Standards (Dichlorvos, Carbofuran, Carbaryl, Diazinon, Pirimiphos-methyl, Malathion, Fenitrothion, Chlorpyrifos, and Parathion) in solution 2.
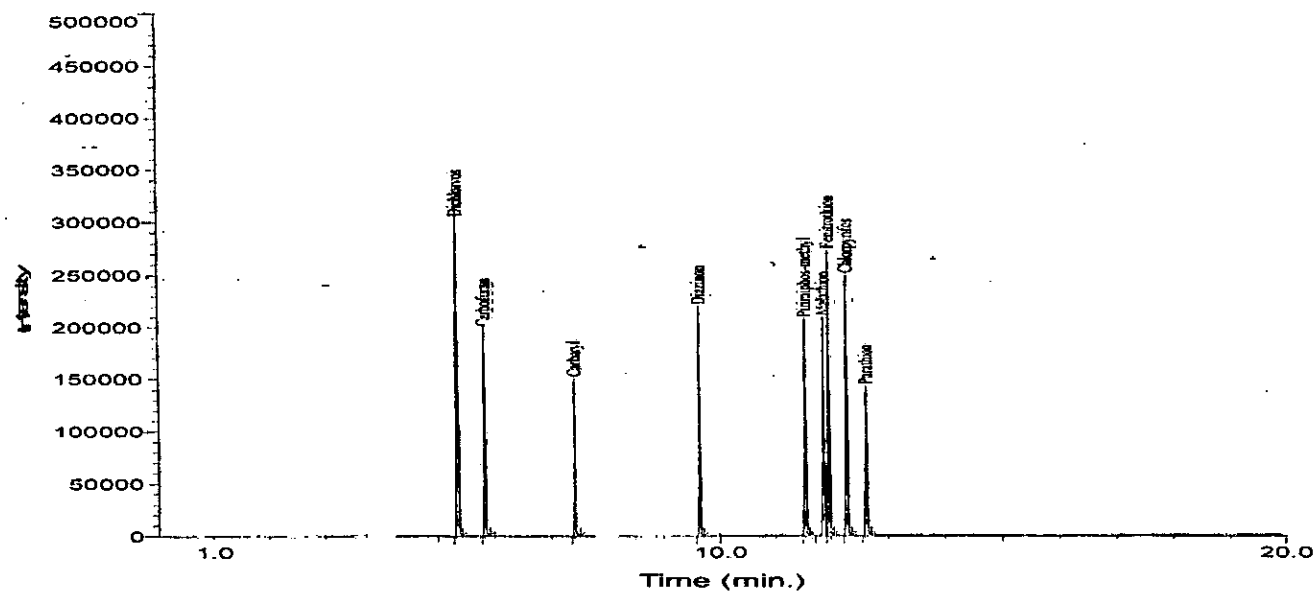
Figure 4.13: Total Ion Chromatogram of Mixed Organophosphate and Carbamate Pesticide Standards (Dichlorvos, Carbofuran, Carbaryl, Diazinon, Pirimiphos-methyl, Malathion, Fenitrothion, Chlorpyrifos, and Parathion) in solution 3.

$R^2 = 0.9992$
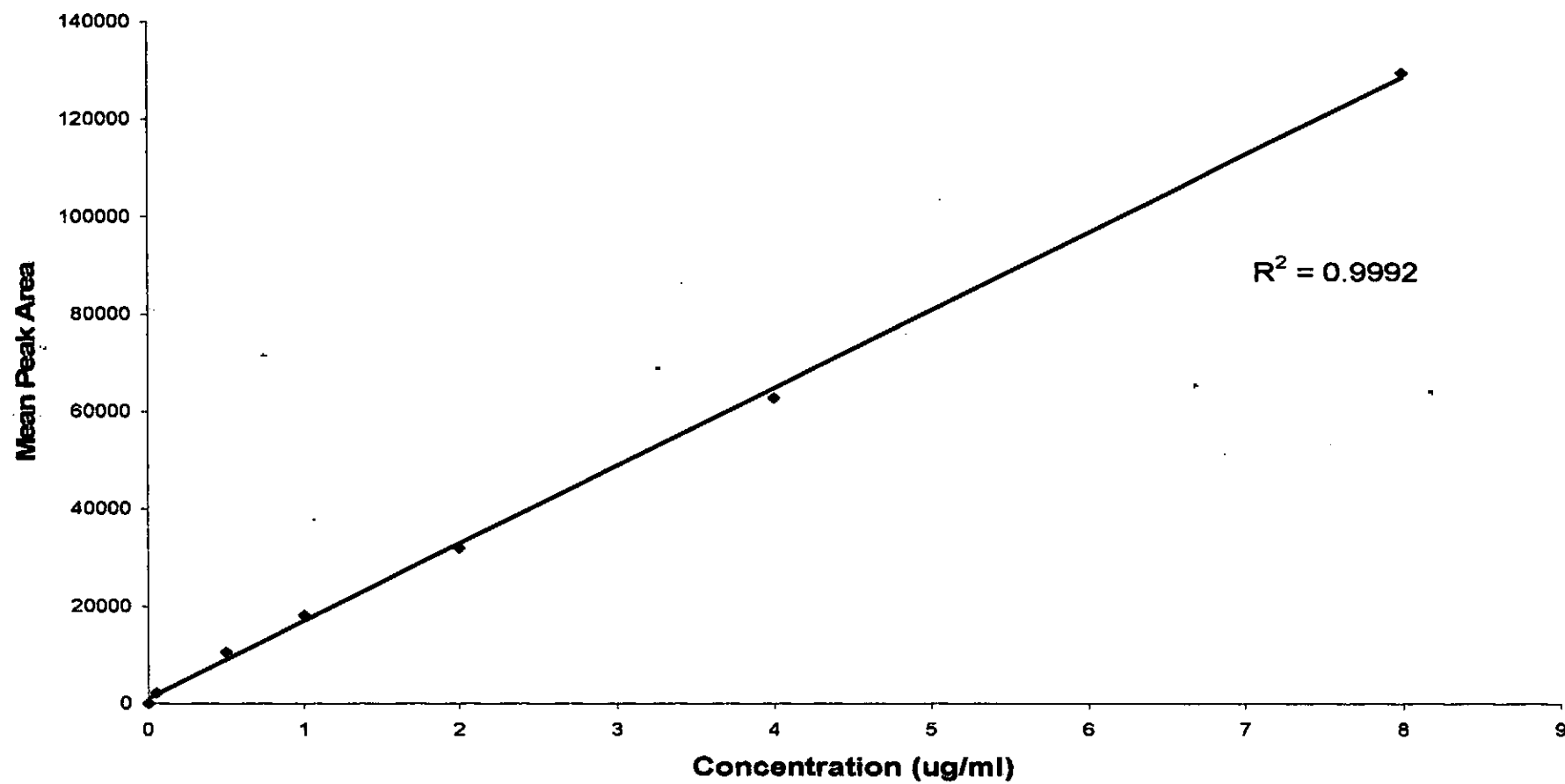
Figure 4.14: Standard Calibration Curve for Aldrin.
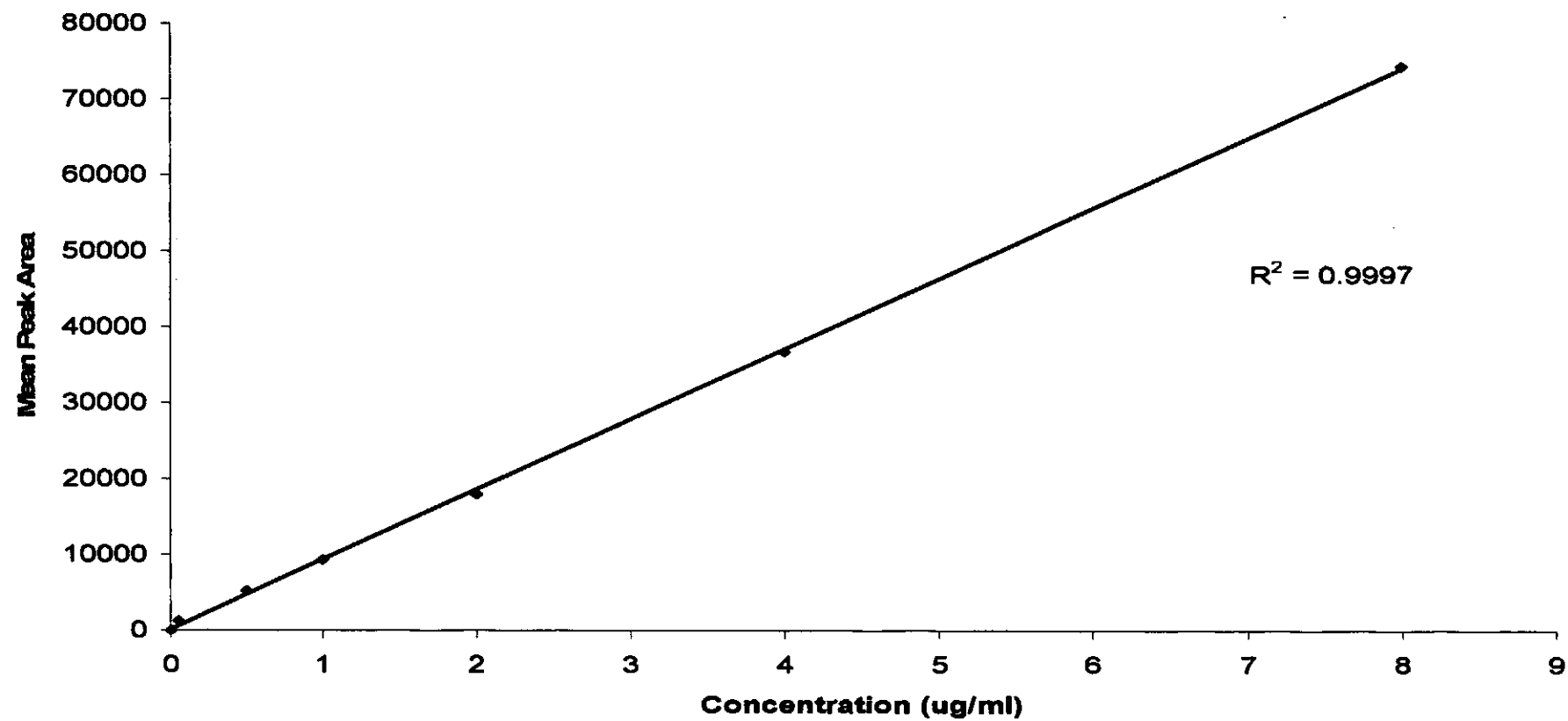
Figure 4.15: Standard Calibration Curve for DDT.

$R^2 = 0.9994$
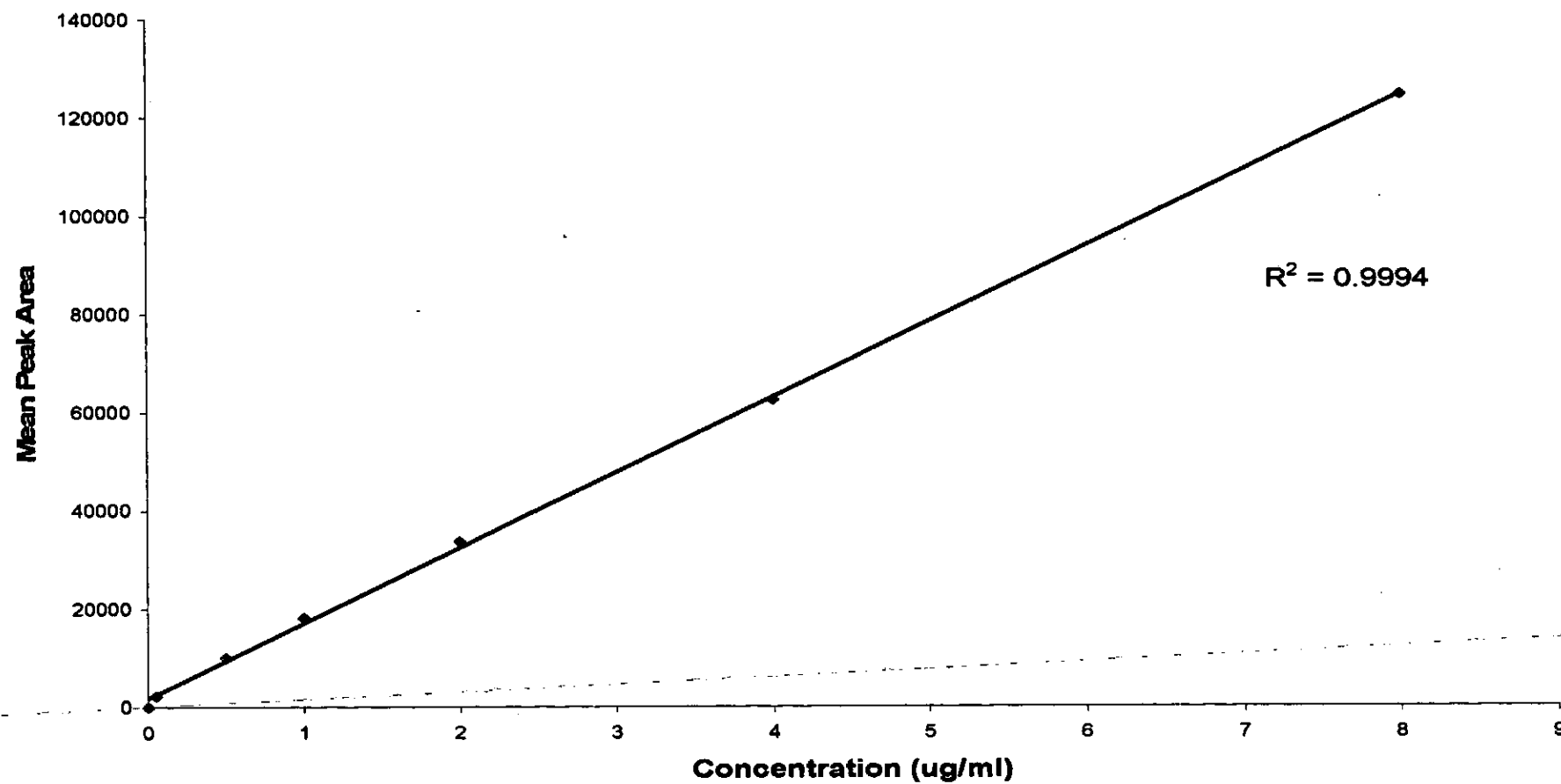
Figure 4.16: Standard Calibration Curve for Dieldrin.
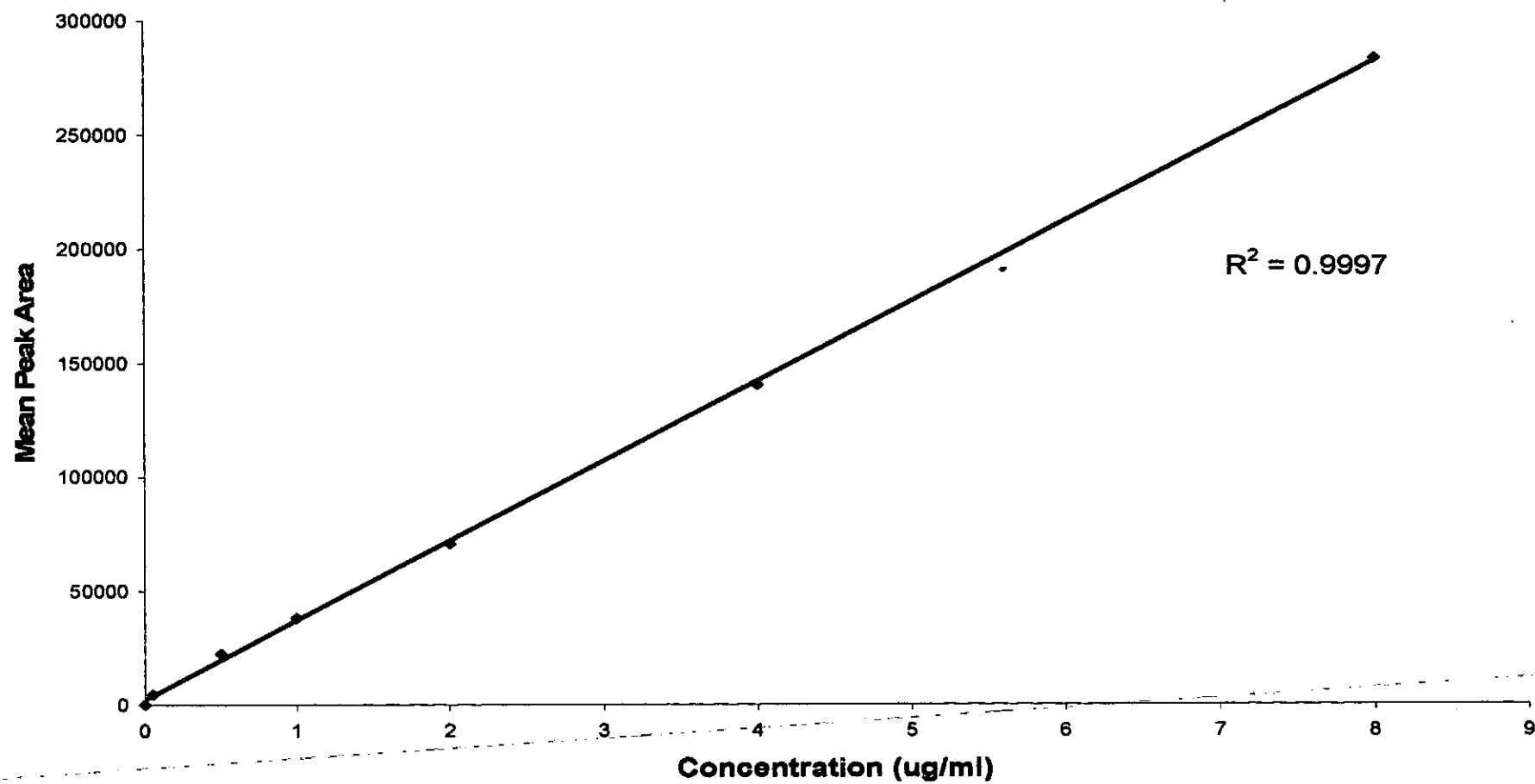
Figure 4.17: Standard Calibration Curve for Endosulfan.

$R^2 = 0.9994$
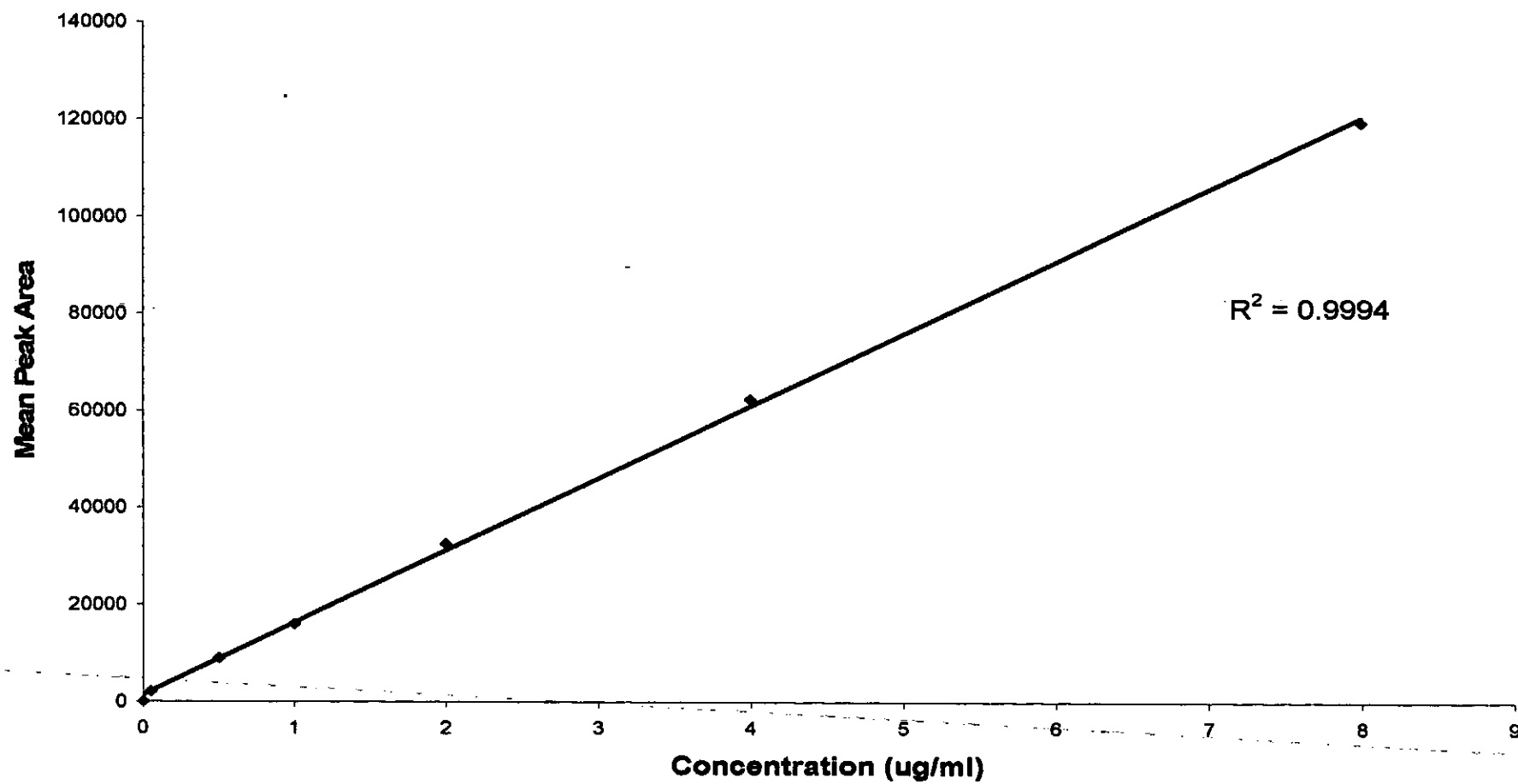
Figure 4.18: Standard Calibration Curve for Endrin.

$R^2 = 0.9999$
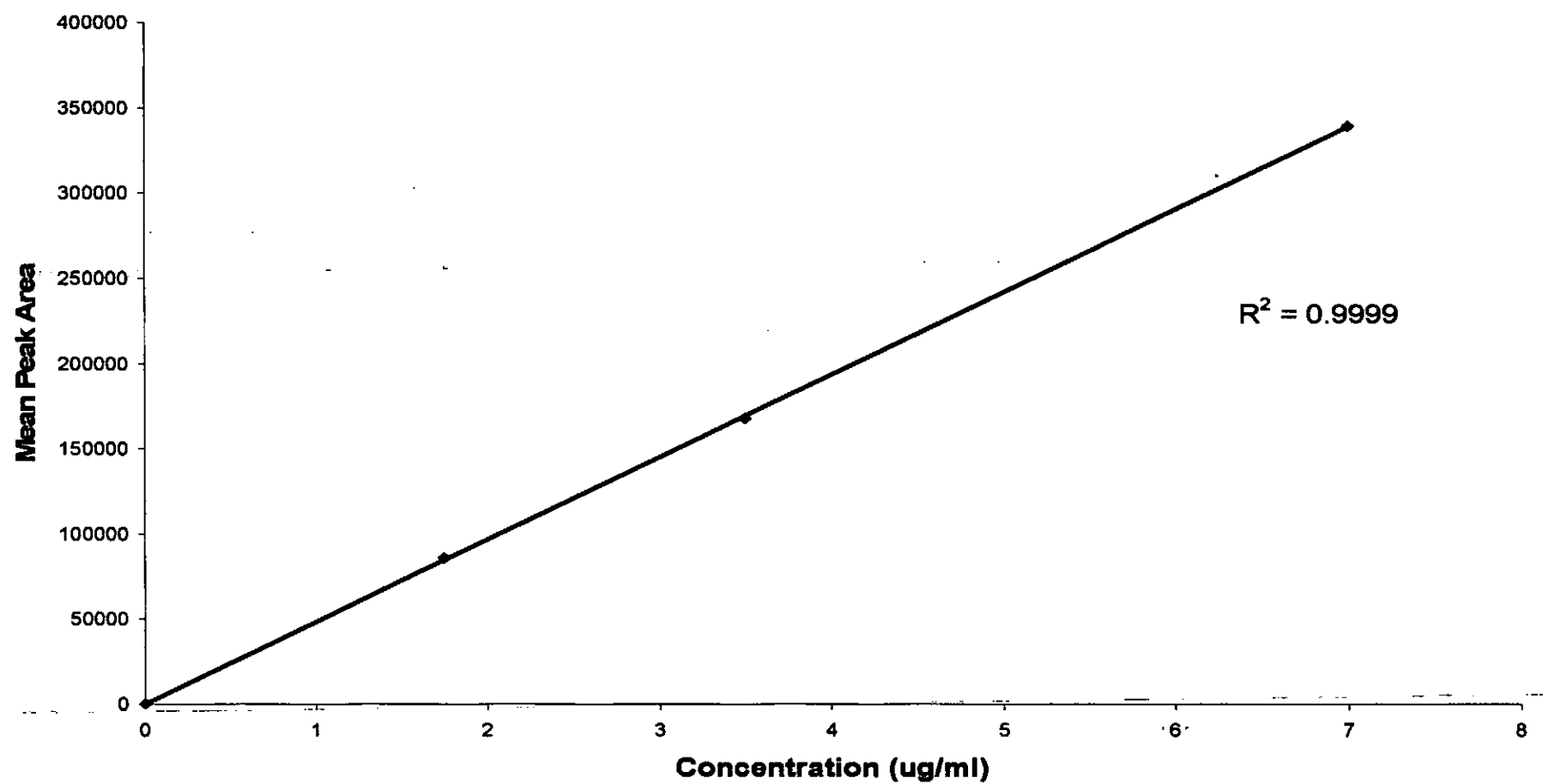
Figure 4.19: Standard Calibration Curve for Carbofuran.
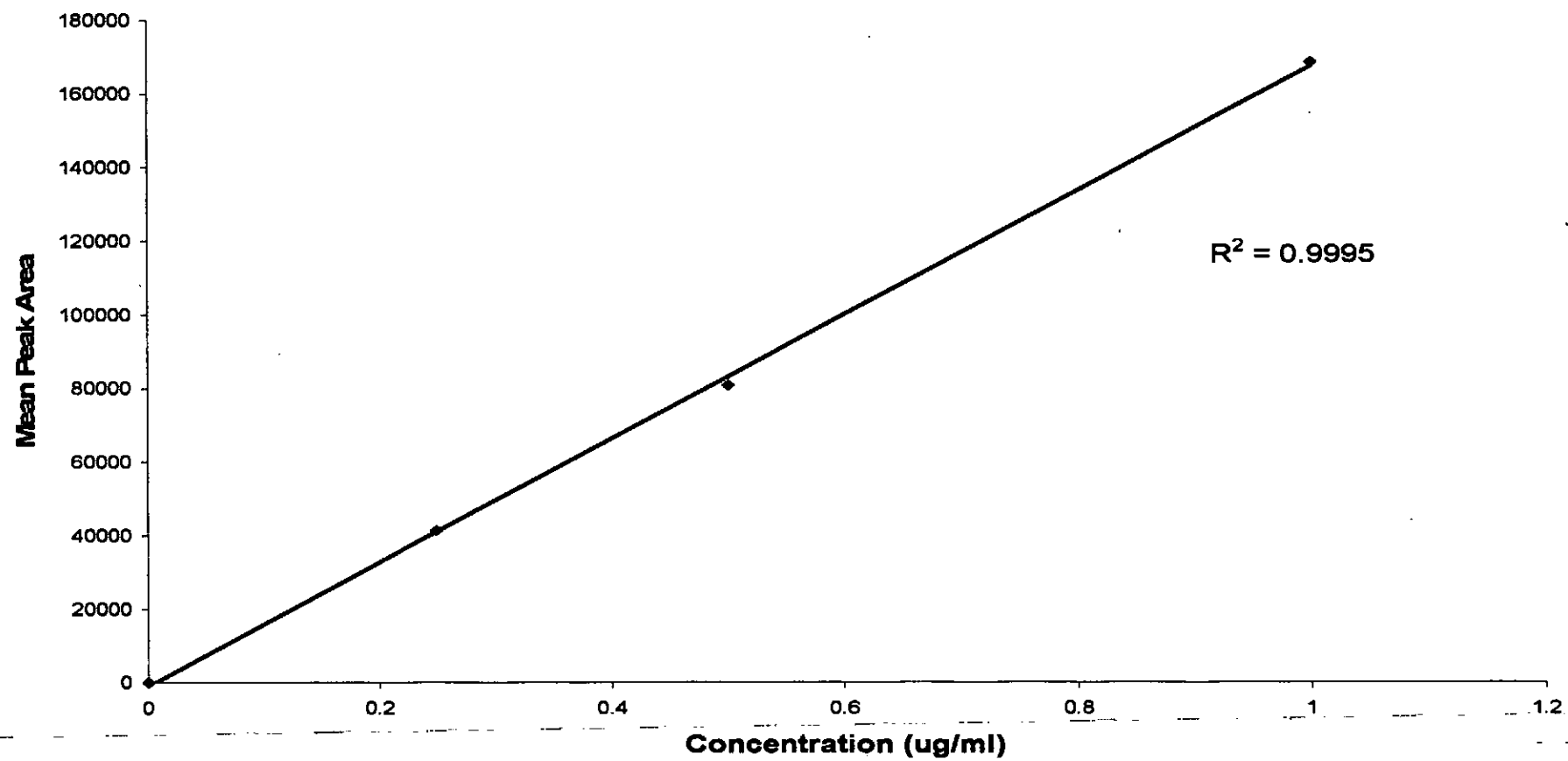
Figure 4.20: Standard Calibration Curve for Carbaryl.
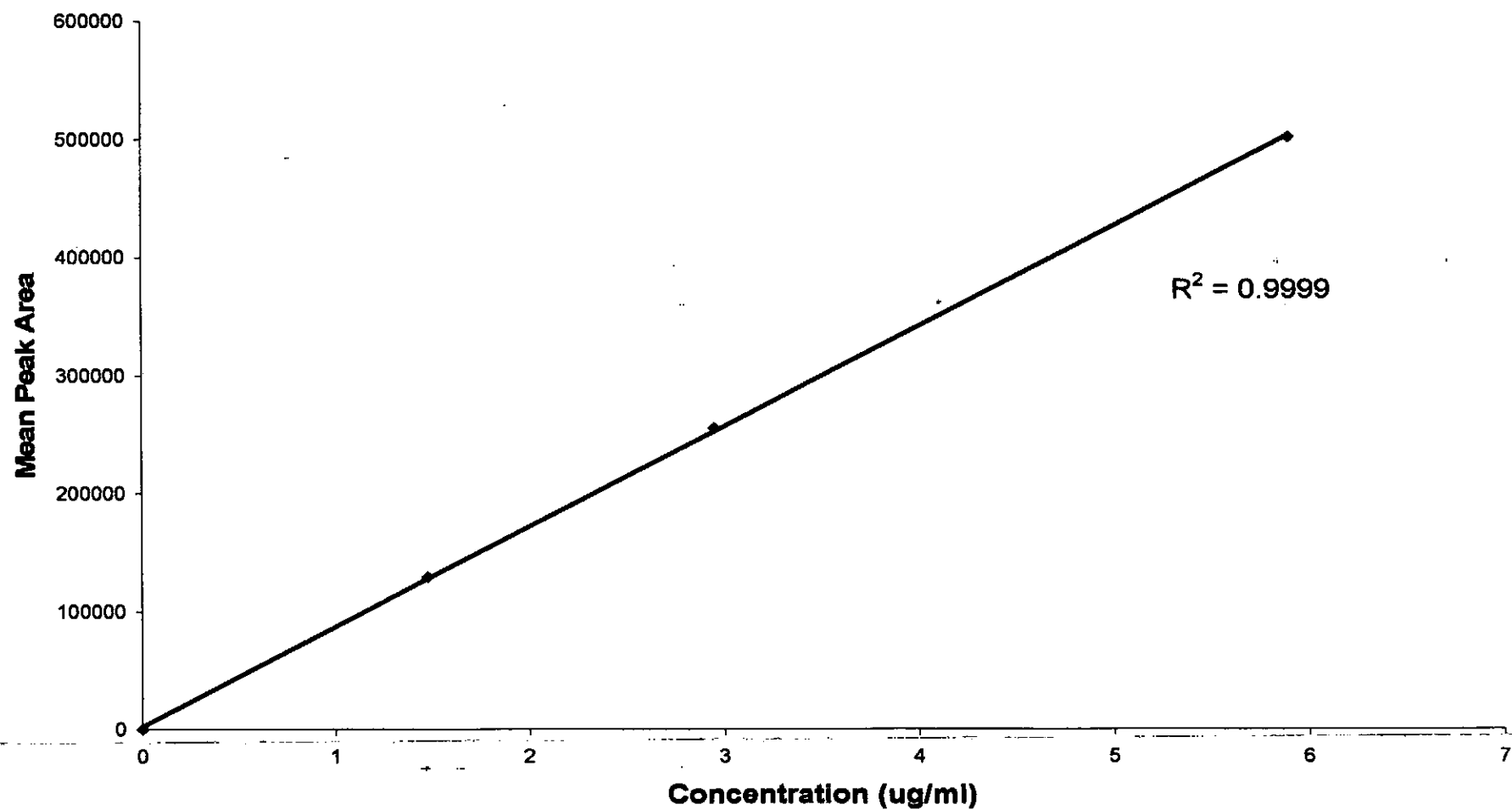
Figure 4.21: Standard Calibration Curve for Chlorpyrifos.
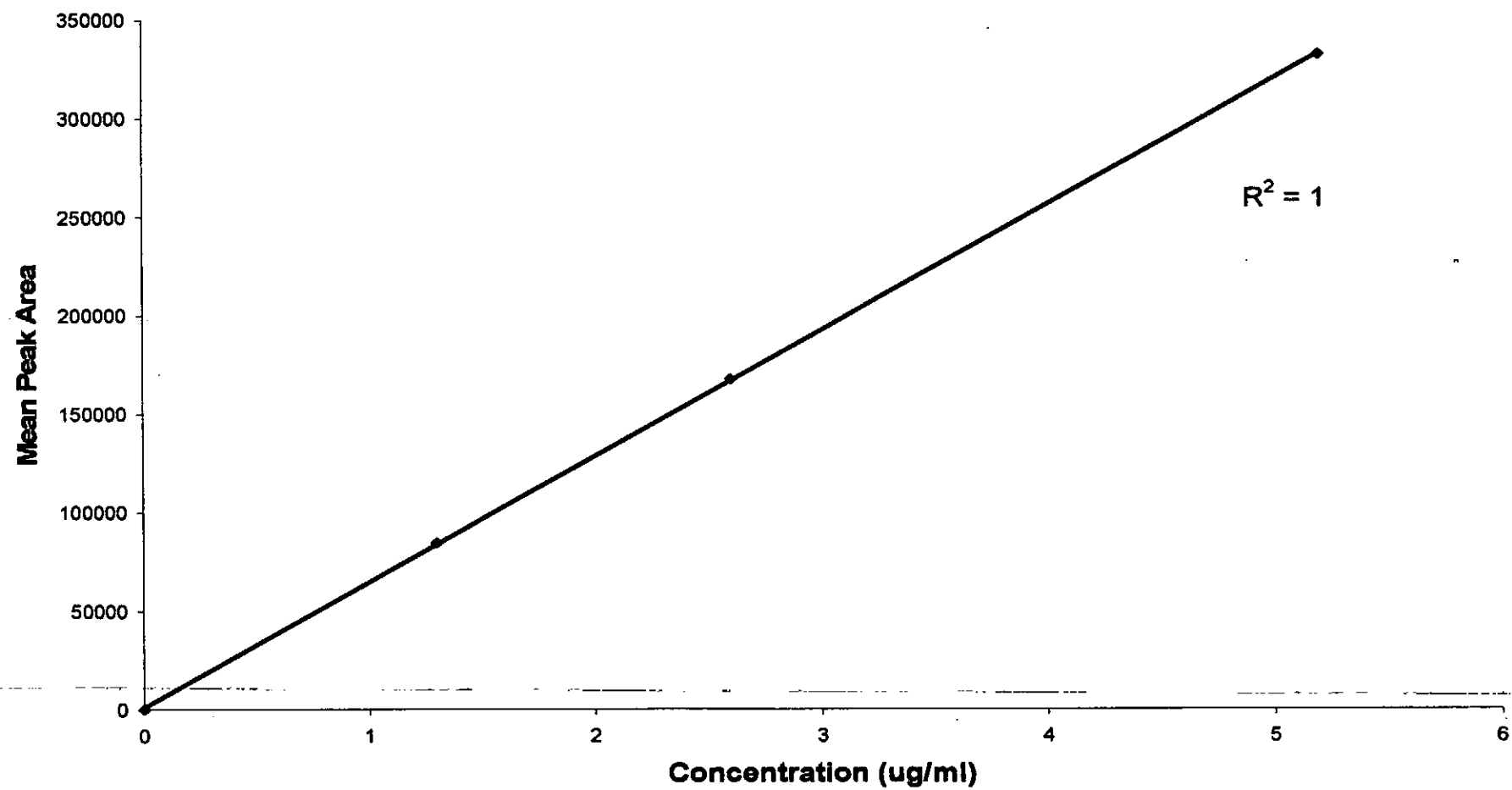
Figure 4.22: Standard Calibration Curve for Diazinon.
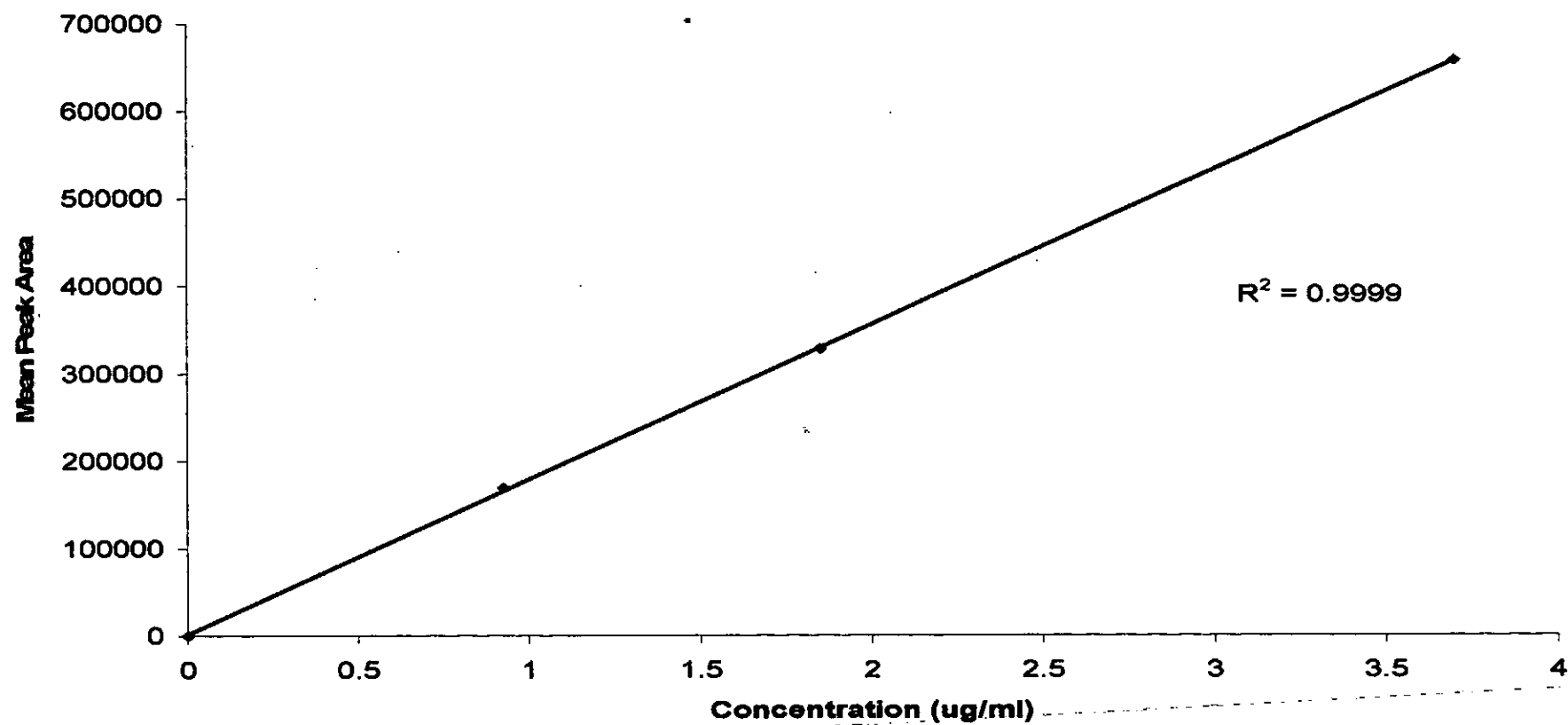
Figure 4.23: Standard Calibration Curve for Dichlorvos.
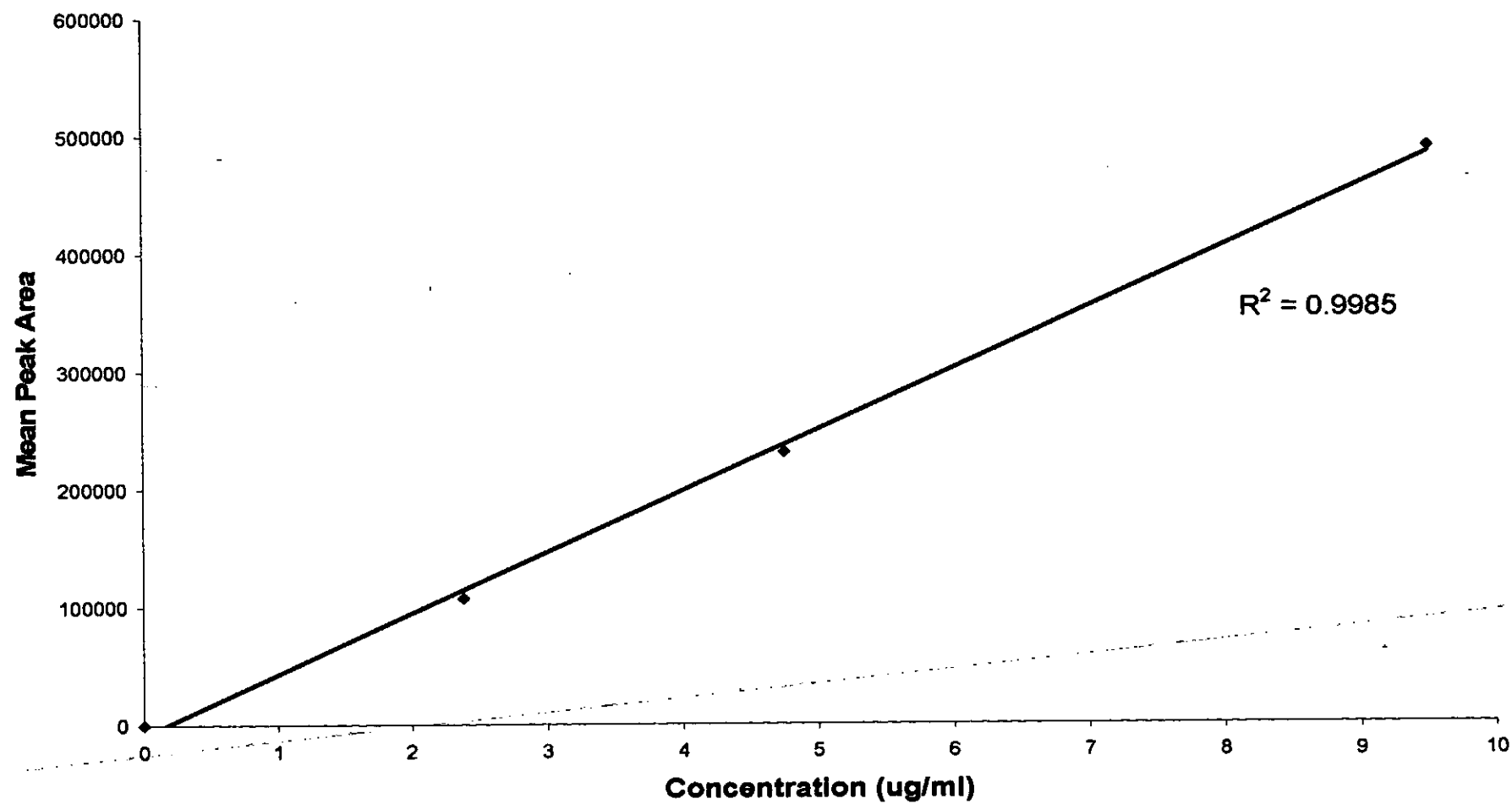
Figure 4.24: Standard Calibration Curve for Fenitrothion.
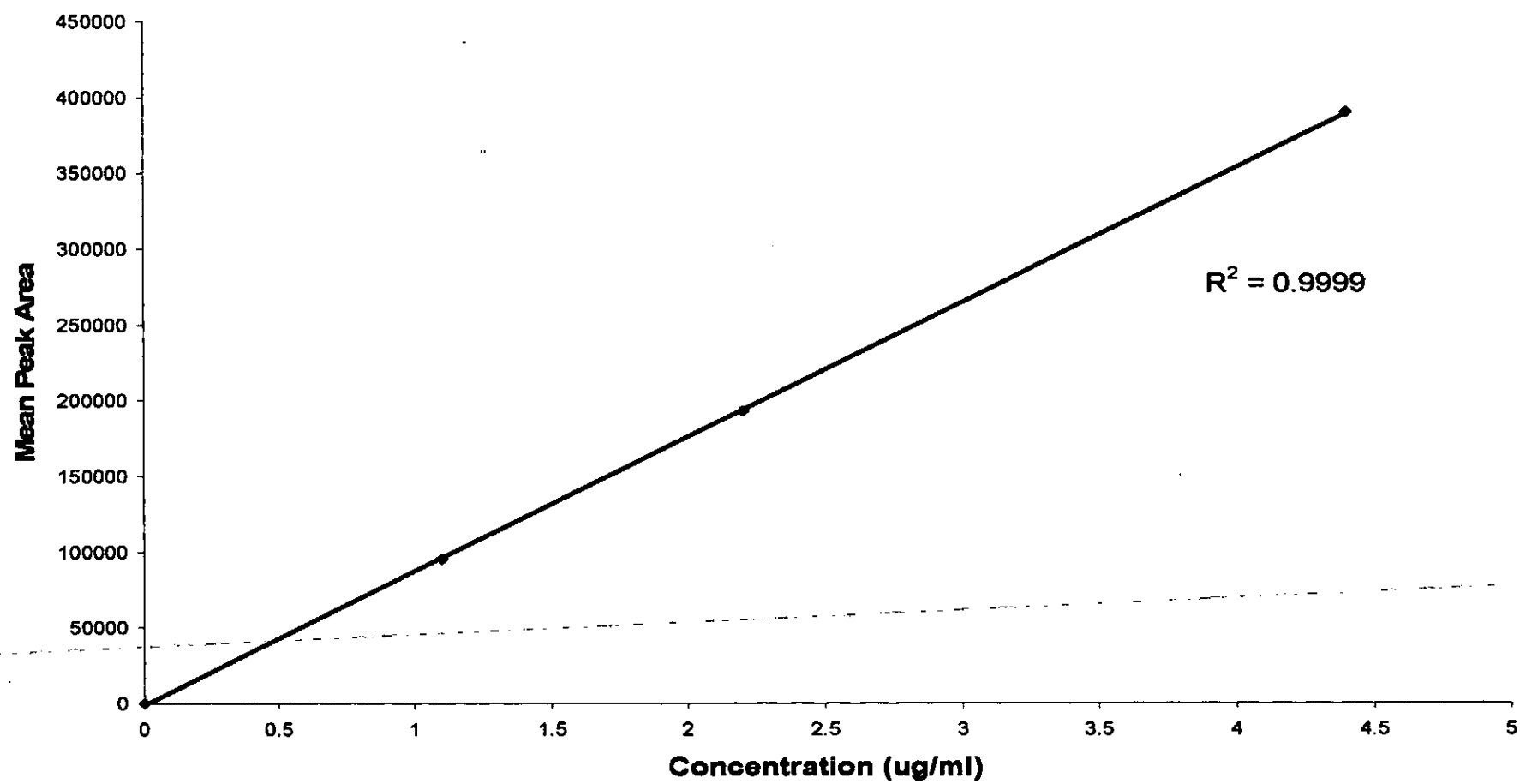
Figure 4.25: Standard Calibration Curve for Malathion.
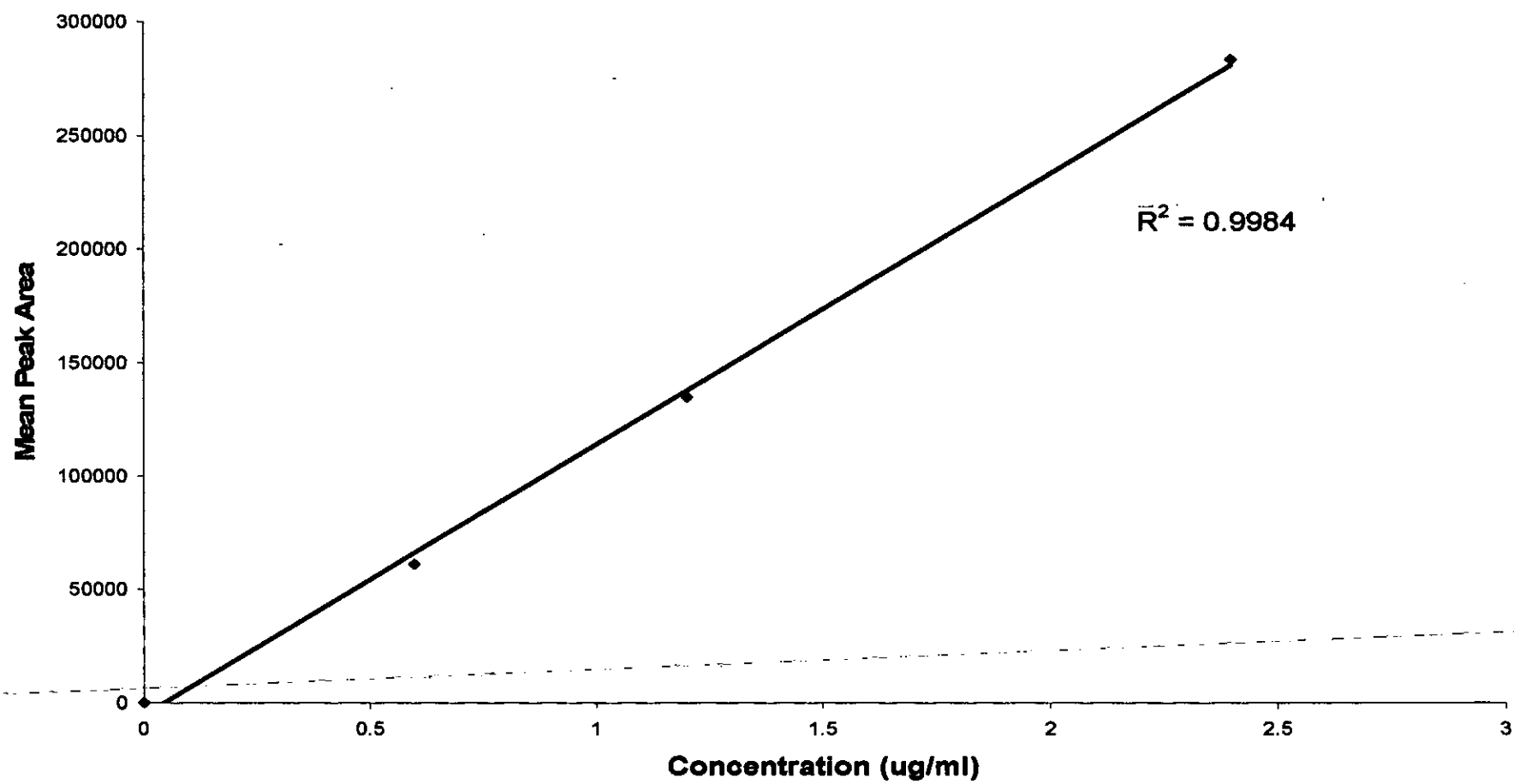
Figure 4.26: Standard Calibration Curve for Parathion.
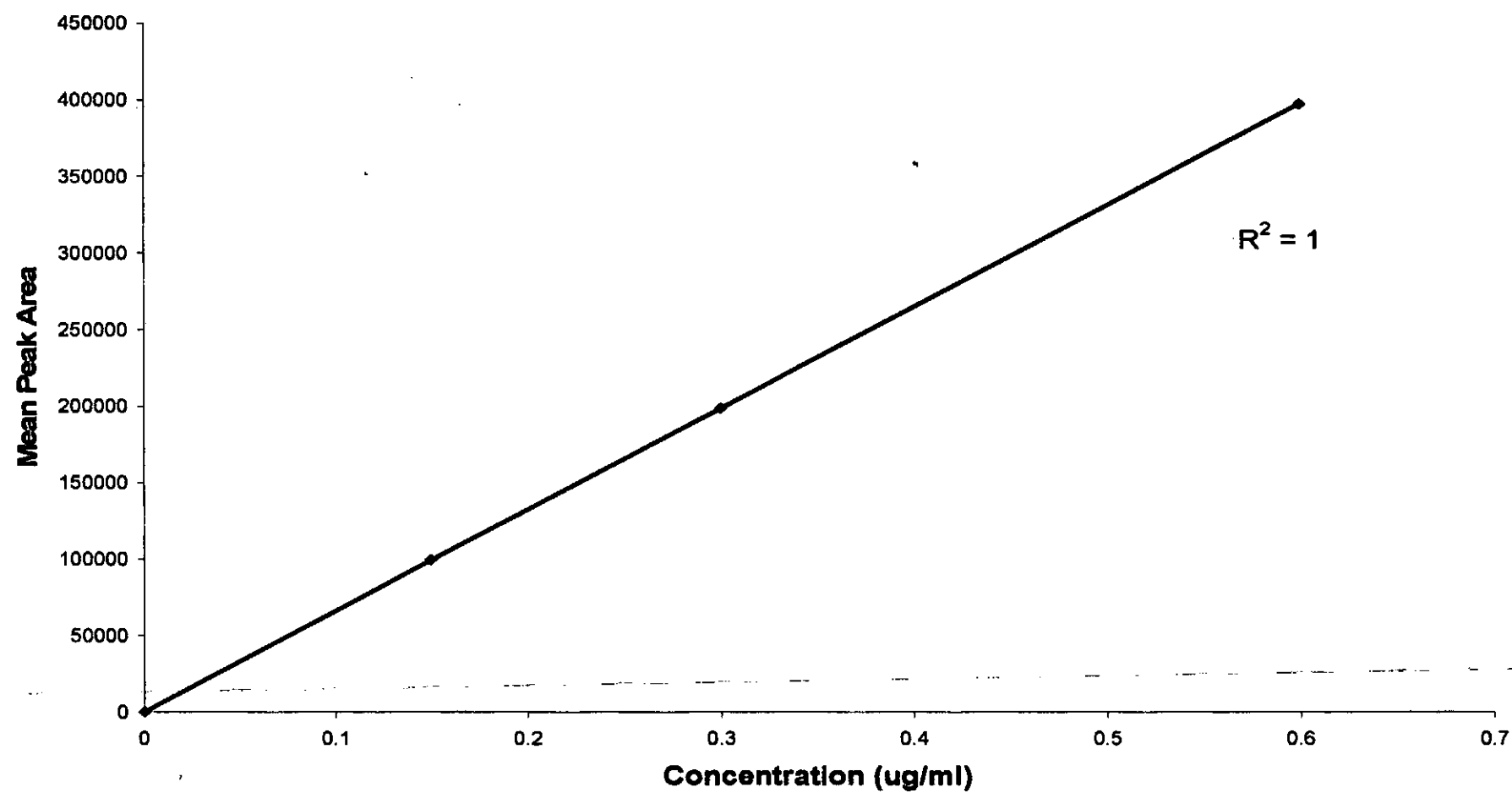
Figure 4.27: Standard Calibration Curve for Pirimiphos-methyl.

Table 4.1 – Names, Retention Times (RT), Limits of Detection (LOD), Coefficients of Determination and Selected Monitored ions of the Pesticides.

Pesticide Name	RT (min.)	LOD (µg/ml)	Coefficient of Determination (r^2)	Monitored Ions
Aldrin	20.63	0.0009	0.9992	263, 265, 293
Carbaryl	7.42	0.0075	0.9995	144, 115, 116
Carbofuran	5.83	0.0066	0.9999	164, 131, 221
Chlorpyrifos	12.28	0.0025	0.9999	314, 197, 318
Diazinon	9.65	0.0009	1	304, 179, 137
4,4'-DDT	31.54	0.0008	0.9997	235, 237, 199
Dichlorvos	5.35	0.0168	0.9999	185, 109, 220
Dieldrin	27.08	0.0017	0.9994	79, 263, 277
Endosulfan	25.25	0.0022	0.9997	239, 241, 243
Endrin	28.38	0.0006	0.9994	263, 265, 261
Fenitrothion	11.96	0.0007	0.9985	277, 125, 214
Malathion	11.87	0.0082	0.9999	173, 158, 254
Parathion	12.62	0.0062	0.9984	291, 235, 263
Pirimiphos-methyl	11.53	0.0014	1	290, 276, 305
2-chloroanthracene (Internal standard)	20.98	0.0025	---------	266, 264, 268

4.3 Recovery Studies

Recovery studies were undertaken to investigate matrix effects on the analytical process. The results are presented in Tables 4.2 - 4.3 and in Figure 4.28.

Table 4.2 – Percent Recoveries of Organochlorine Pesticides at Different Concentrations.

Pesticide	Concentrations (μg/g) / % Recoveries					
	0.025	0.25	0.5	1.0	2.0	4.0
Aldrin	90.0 (1.4)*	100.7 (3.6)	88.9 (2.2)	92.9 (5.1)	91.9 (1.3)	89.5 (2.7)
DDT	70.3 (4.5)	73.5 (6.2)	98.1 (6.5)	72.4 (3.3)	89.6 (4.8)	85.6 (5.7)
Dieldrin	97.1 (1.8)	92.4 (3.5)	80.2 (3.1)	88.4 (1.6)	85.0 (4.8)	85.4 (2.4)
Endosulfan	73.9 (6.9)	70.2 (8.5)	78.5 (6.1)	75.1 (9.7)	77.5 (4.4)	78.6 (9.3)
Endrin	76.5 (9.5)	68.2 (7.1)	71.1 (5.6)	70.2 (8.2)	72.3 (6.9)	70.8 (5.8)

*Standard deviations in brackets.

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Table 4.3 – Mean Recoveries and Concentrations of Organophosphate and Carbamate Pesticides.

Pesticide	Concentration (μg/g)	Recovery (%)
Carbaryl	0.125	85.8 (6.8)*
Carbofuran	0.875	80.2 (4.3)
Chlorpyrifos	0.74	79.5 (2.7)
Diazinon	0.65	92.3 (7.4)
Dichlorvos	0.463	95.1 (3.5)
Fenitrothion	1.19	70.6 (9.1)
Malathion	0.55	89.2 (5.2)
Parathion	0.3	90.4 (8.6)
Pirimiphos-methyl	0.075	94.7 (3.9)

*Standard deviations in brackets
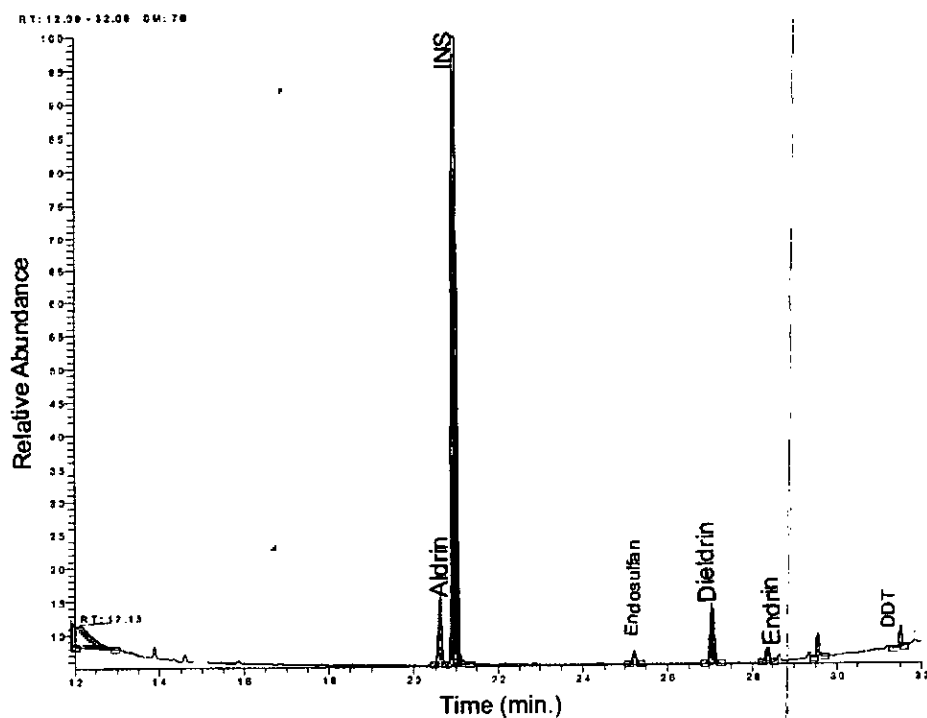
Figure 4.28: Total Ion Chromatogram of a Maize Sample Spiked with a Mixture of Organochlorine Pesticide Standards (Aldrin, DDT, Dieldrin, Endosulfan and Edrin) at 0.5μg/ml and Internal Standard (INS) at 5 μg/ml.

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## 4.4 Pesticide Residue Content of Samples

The results of analyses of samples for pesticide residue content are presented in Tables 4.4 – 4.14 and in Figures 4.29 – 4.39.

Table 4.4 – Pesticide Residue Content of Maize Samples.

Pesticide	Sample Codes / Pesticide Concentrations (µg/kg)										
	MYa(21)*			MYb(21)			MWa(21)			MWb(21)		
	M	Range	NP	M	Range	NP	M	Range	NP	M	Range	NP
Aldrin	9.6	3.8-12.3	8	8.5	2.2-18.1	6	10.3	4.5-15.6	5	7.4	4.2-16.0	8
Carbaryl	30.1	9.1-52.9	7	28.0	8.5-60.2	4	38.3	8.3-73.5	8	32.1	9.5-70.6	7
Carbofuran	60.2	33.3-120.4	5	69.5	46.1-150.2	7	84.1	51.1-182.2	7	78.3	56.2-180.5	9
Chlorpyrifos	41.7	28.1-61.5	8	38.6	29.1-52.7	6	48.7	30.8-55.6	9	44.5	35.5-62.1	10
Diazinon	18.5	10.8-20.6	4	15.0	11.3-18.1	3	21.2	12.2-30.3	8	19.5	10.5-29.5	6
DDT	38.2	17.0-78.3	6	36.4	15.8-60.9	7	52.0	19.5-91.3	8	41.3	14.4-80.5	5
Dichlorvos	685.5	242.3-850.1	8	602.5	230-780.2	5	1127.2	482.1-2367.7	10	805.5	260.1-972.2	8
Dieldrin	8.1	5.2-9.5	5	6.9	5.6-8.7	4	12.1	4.9-16.5	9	9.6	5.7-14.3	6
Endosulfan	20.4	7.5-38.6	7	17.8	7.6-29.1	6	40.4	8.5-73.5	8	31.5	6.2-51.3	4
Endrin	8.5	6.9-12.7	8	7.9	6.3-10.2	9	10.1	5.5-18.5	5	8.6	3.1-10.4	5
Fenitrothion	Nd			Nd			12.0	9.5-15.8	4	10.1	8.9-12.3	2
Malathion	1285.6	142.5-2235.1	6	972.5	163.7-1728.3	4	1565.2	150.6-3951.4	6	1558.8	626.9-2814.4	7
Parathion	Nd			Nd			42.6	33.5-48.2	5	32.0	27.2-41.9	4
Pirimiphos-methyl	885.2	54.1-1575.4	9	848.5	48.3-1265.2	7	1455.4	91.7-3185.1	11	1045.6	75.1-2263.5	9

* number of samples in brackets   M = mean values

NP = number of positive samples   Nd = not detected at or above detection limit

MYa and MYb = Yellow maize varieties a and b   MWa and MWb = White maize varieties a and b

Table 4.5 – Pesticide Residue Content of Beans Samples.

Pesticide	Sample Codes / Pesticide Concentrations (μg/kg)											
	BBa(21)*			BBb(21)			BWa(21)			BWb(21)		
	M	Range	NP	M	Range	NP	M	Range	NP	M	Range	NP
Aldrin	8.6	5.6-13.3	4	9.8	4.1-15.5	3	Nd			Nd		
Carbaryl	38.0	8.5-61.3	6	25.4	7.2-57.4	6	42.9	10.2-73.5	5	40.1	7.5-60.4	7
Carbofuran	81.4	32.6-123.5	7	53.6	4.8-95.2	5	98.5	36.1-135.3	8	93.3	28.4-132.6	9
Chlorpyrifos	50.4	32.9-96.0	12	48.2	21.5-68.4	8	50.1	29.5-98.2	10	39.5	20.6-75.4	7
Diazinon	16.3	10.9-26.5	9	Nd			19.4	12.2-27.8	10	14.0	8.5-22.2	5
DDT	25.8	7.6-52.1	8	22.4	8.1-45.0	10	35.1	12.5-78.5	11	31.8	10.4-66.5	8
Dichlorvos	942.7	523.6-1480.5	9	820.5	430.2-1225.1	4	911.4	581.2-1150.8	12	809.2	452.3-950.4	10
Dieldrin	6.0	3.5-9.5	5	5.8	2.3-9.4	6	Nd			Nd		
Endosulfan	29.6	8.9-52.4	8	22.5	10.6-47.5	5	39.7	12.5-65.2	9	35.1	10.257.3	7
Endrin	5.5	3.2-8.5	4	4.8	3.8-7.5	2	7.8	4.5-12.3	6	Nd		
Fenitrothion	Nd			Nd			10.1	8.9-12.2	4	8.4	7.5-9.2	5
Malathion	212.0	35.4-628.1	9	185.6	31.5-595.2	7	237.6	41.5-852.5	9	209.3	38.1-680.4	8
Parathion	Nd			Nd			26.4	20.6-41.1	7	31.8	17.5-44.9	6
Pirimiphos-methyl	43.5	14.8-92.5	12	41.2	10.8-88.5	9	40.8	15.6-86.5	14	36.4	13.1-72.5	10

* number of samples in brackets   M = mean values

NP = number of positive samples   Nd = not detected at or above detection limit

BBa and BBb = Brown beans varieties a and b   BWa and BWb = White beans varieties a and b
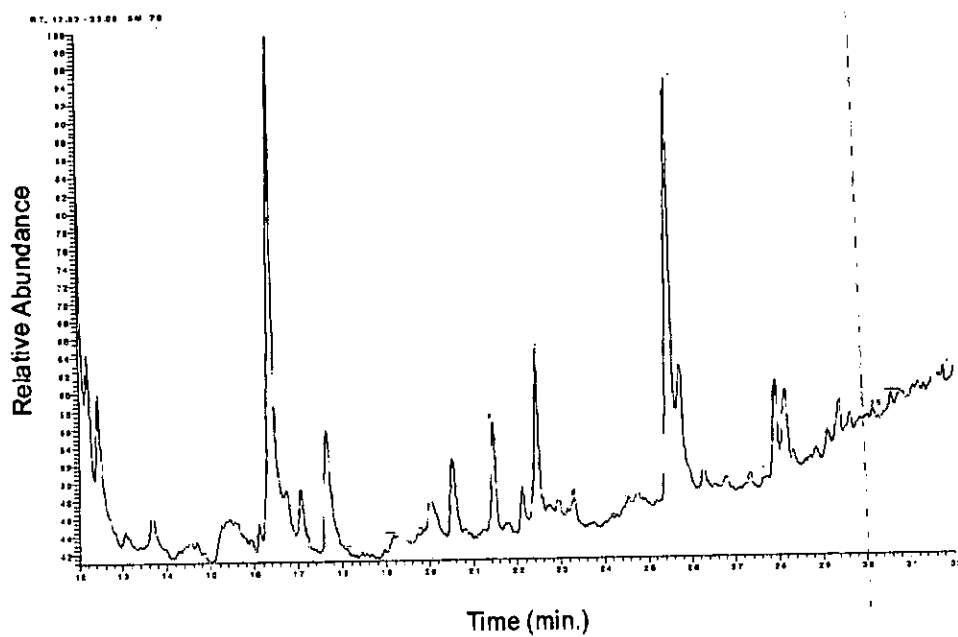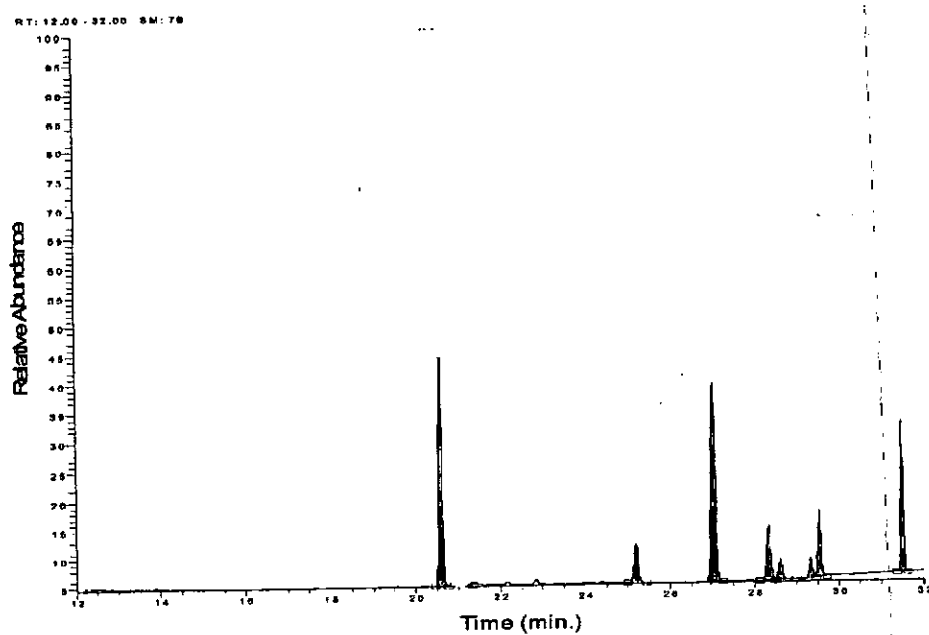
Figure 4.29: Typical Total Ion Chromatograms of a Sample before (A) and after (B) Cleanup.

Table 4.6 – Incidence of Pesticide Residues in Maize and Beans Samples.

Pesticide	Maize (84)*		Beans (84)*	
	No of Positive Samples	% Occurrence	No of Positive Samples	% Occurrence
Aldrin	27	32	7	8
Carbaryl	26	31	24	29
Carbofuran	28	33	29	35
Chlorpyrifos	33	39	37	44
Diazinon	21	25	24	29
DDT	26	31	37	44
Dichlorvos	31	37	35	42
Dieldrin	24	29	11	13
Endosulfan	25	30	29	35
Endrin	27	32	12	14
Fenitrothion	6	7	9	11
Malathion	23	27	33	39
Parathion	9	11	13	16
Pirimiphos-methyl	36	43	45	54

* Number of samples in brackets.

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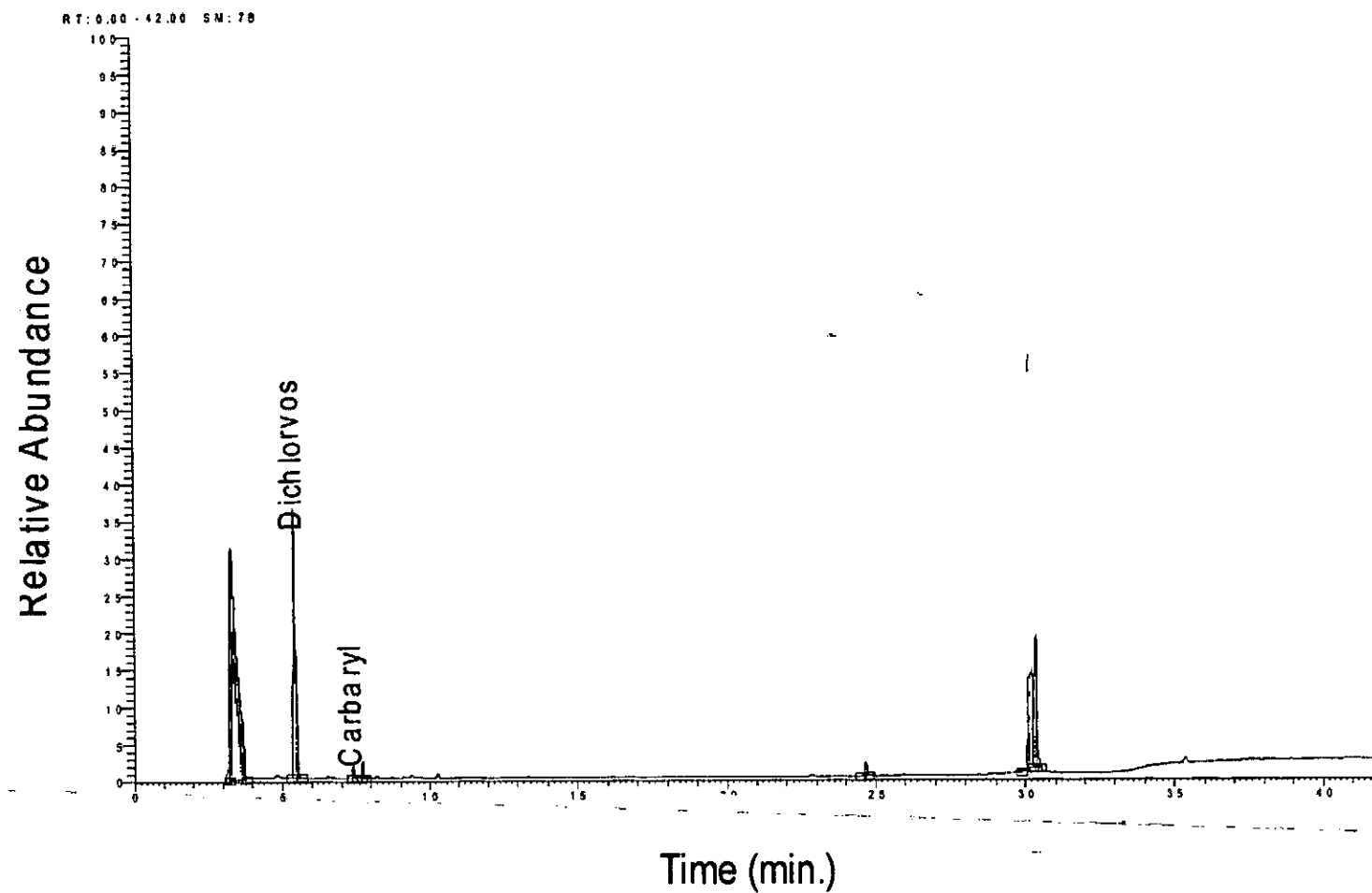
Figure 4.30: Total Ion Chromatogram of a Maize Sample.

155
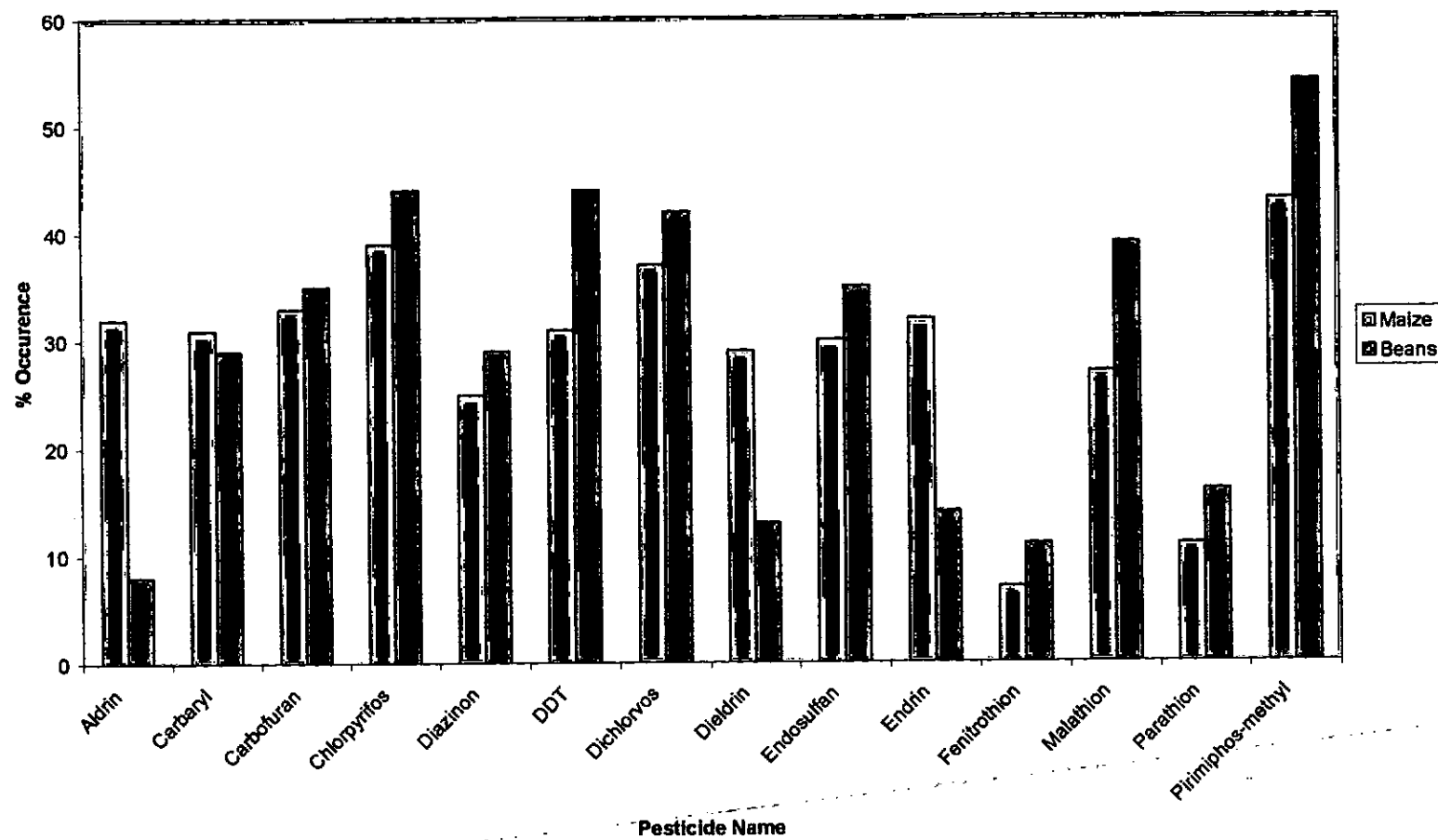
Figure 4.31: Chart Showing Incidence of the Various Pesticides in Samples of Maize and Beans.

Table 4.7: Mean Concentrations of Organochlorine Pesticides in Maize Samples.

Pesticide	Sample Codes/Mean Residues Concentrations (µg/kg)			
	MYa	MYb	MWa	MWb
Aldrin	9.6	8.5	10.3	7.4
DDT	38.2	36.4	52.0	41.3
Dieldrin	8.1	6.9	12.1	9.6
Endosulfan	20.4	17.8	40.4	31.5
Endrin	8.5	7.9	10.1	8.6

Table 4.8: Mean Concentrations of Organophosphate and Carbamate Pesticides in Maize Samples.

Pesticide	Sample Codes/Mean Residue Concentrations (µg/kg)			
	MYa	MYb	MWa	MWb
Carbaryl	30.1	28.0	38.3	32.1
Carbofuran	60.2	69.5	84.1	78.3
Chlorpyrifos	41.7	38.6	48.7	44.5
Diazinon	18.5	15.0	21.2	19.5
Dichlorvos	685.5	602.5	1127.2	805.5
Fenitrothion	Nd	Nd	12.0	10.1
Malathion	1285.6	972.5	1565.2	1558.8
Parathion	Nd	Nd	42.6	32.0
Pirimiphos-methyl	885.2	848.5	1455.4	1045.6

Nd = not detected at or above detection limit.

Table 4.9: Mean Concentrations of Organochlorine Pesticides in Beans Samples.

Pesticide	Sample Codes/Mean Residue Concentrations (µg/kg)			
	BBa	BBb	BWa	BWb
Aldrin	8.6	9.8	Nd	Nd
DDT	25.8	22.4	35.1	31.8
Dieldrin	6.0	5.8	Nd	Nd
Endosulfan	29.6	22.5	39.7	35.1
Endrin	5.5	4.8	7.8	Nd

Nd = not detected at or above detection limit.

Table 4.10: Mean Concentrations of Organophosphate and Carbamate Pesticides in Beans Samples.

Pesticide	Sample Codes/Mean Residue Concentrations (μg/kg)			
	BBa	BBb	BWa	BWb
Carbaryl	38.0	25.4	42.9	40.1
Carbofuran	81.4	53.6	98.5	93.3
Chlorpyrifos	50.4	48.2	50.1	39.5
Diazinon	16.3	Nd	19.4	14.0
Dichlorvos	942.7	820.5	911.4	809.2
Fenitrithion	Nd	Nd	10.1	8.4
Malathion	212.0	185.6	237.6	209.3
Parathion	Nd	Nd	26.4	31.8
Pirimiphos-methyl	43.5	41.2	40.8	36.4

Nd = not detected at or above detection limit.
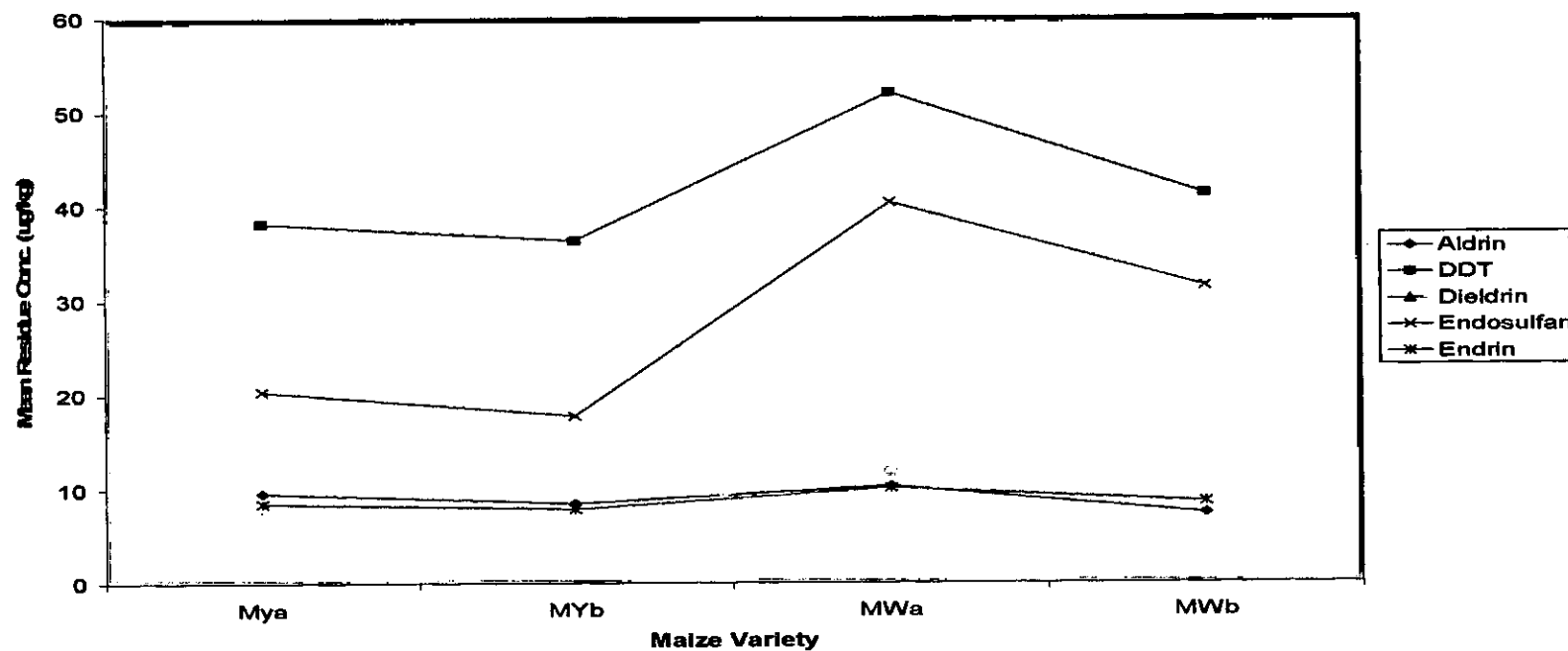
Figure 4.32: Mean Residue Concentrations of Organochlorine Pesticides in Different Types of Maize.

MYa and MYb = Yellow Maize Types (a) and (b). MWa and MWb = White Maize Types (a) and (b).
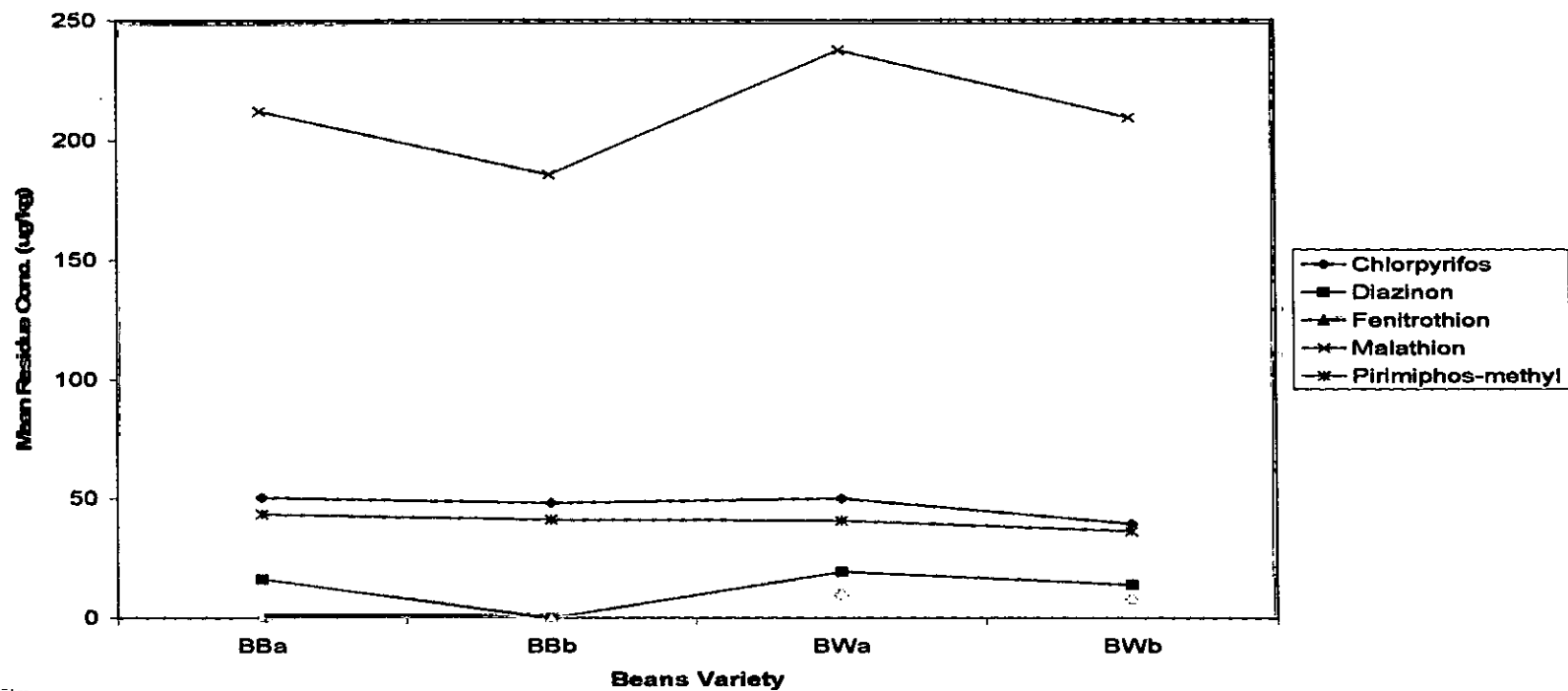
Figure 4.33: Mean Residue Concentrations of Organophosphate Pesticides in Different Types of Beans.

BBa and BBb = Brown Beans Types (a) and (b). BWa and BWb = White Beans Types (a) and (b).
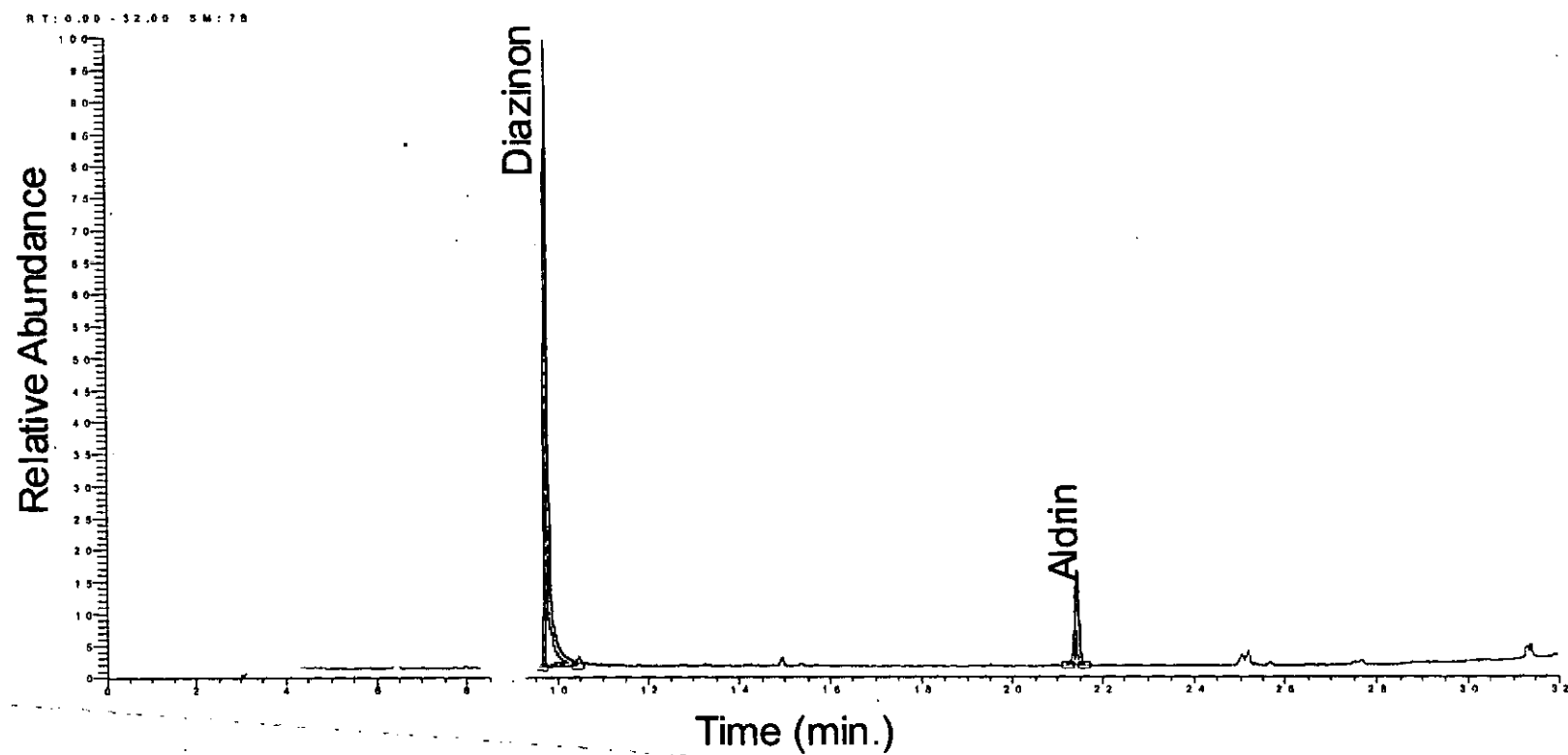
Figure 4.34: Total Ion Chromatogram of a Beans Sample.
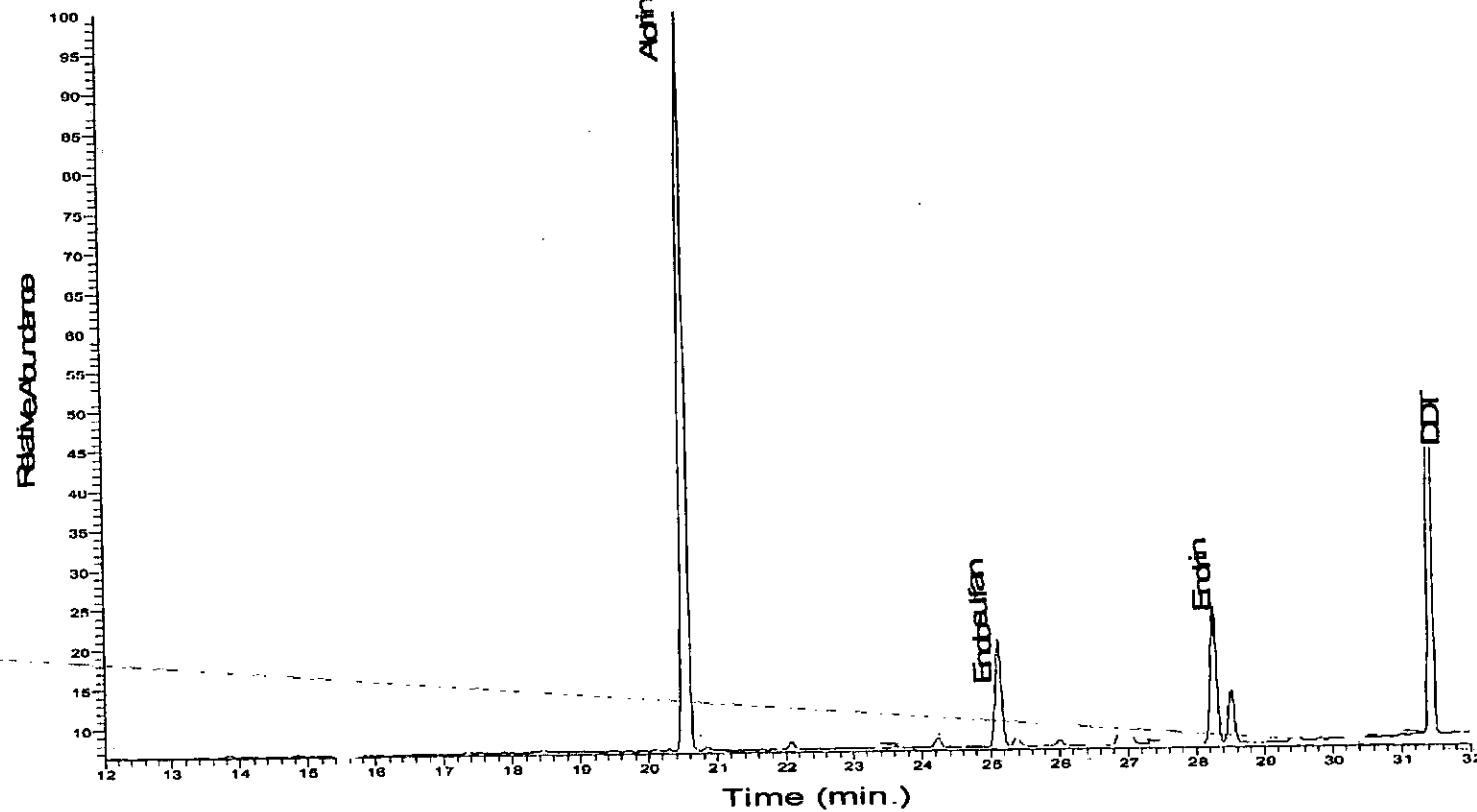
Figure 4.35: Total Ion Chromatogram of another Beans Sample.
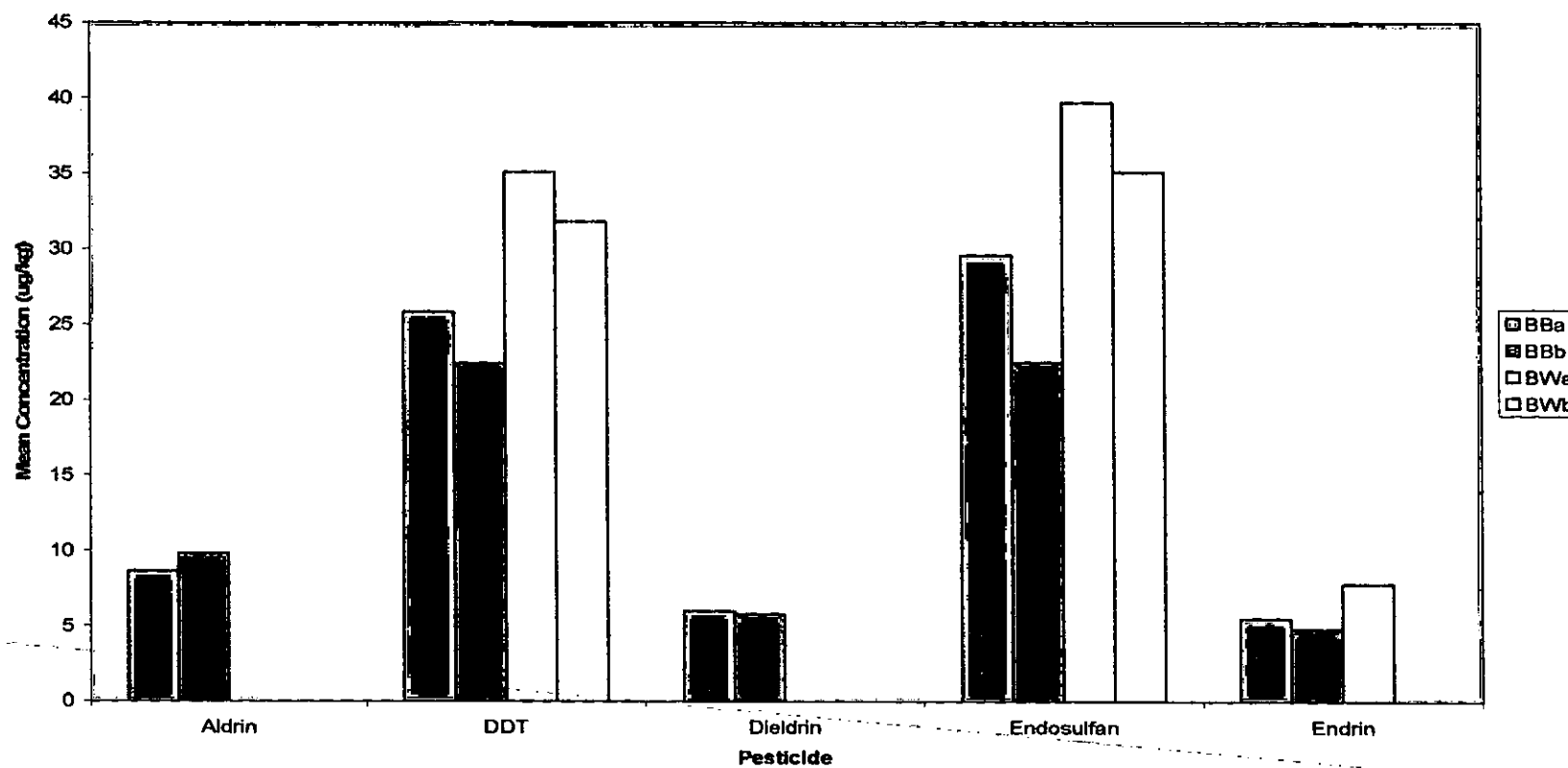
Figure 4.36: Mean Residue Concentrations of Organochlorine Pesticides in Different Types of Beans.

BBa and BBb = Brown Beans Types (a) and (b). BWa and BWb = White Beans Types (a) and (b).
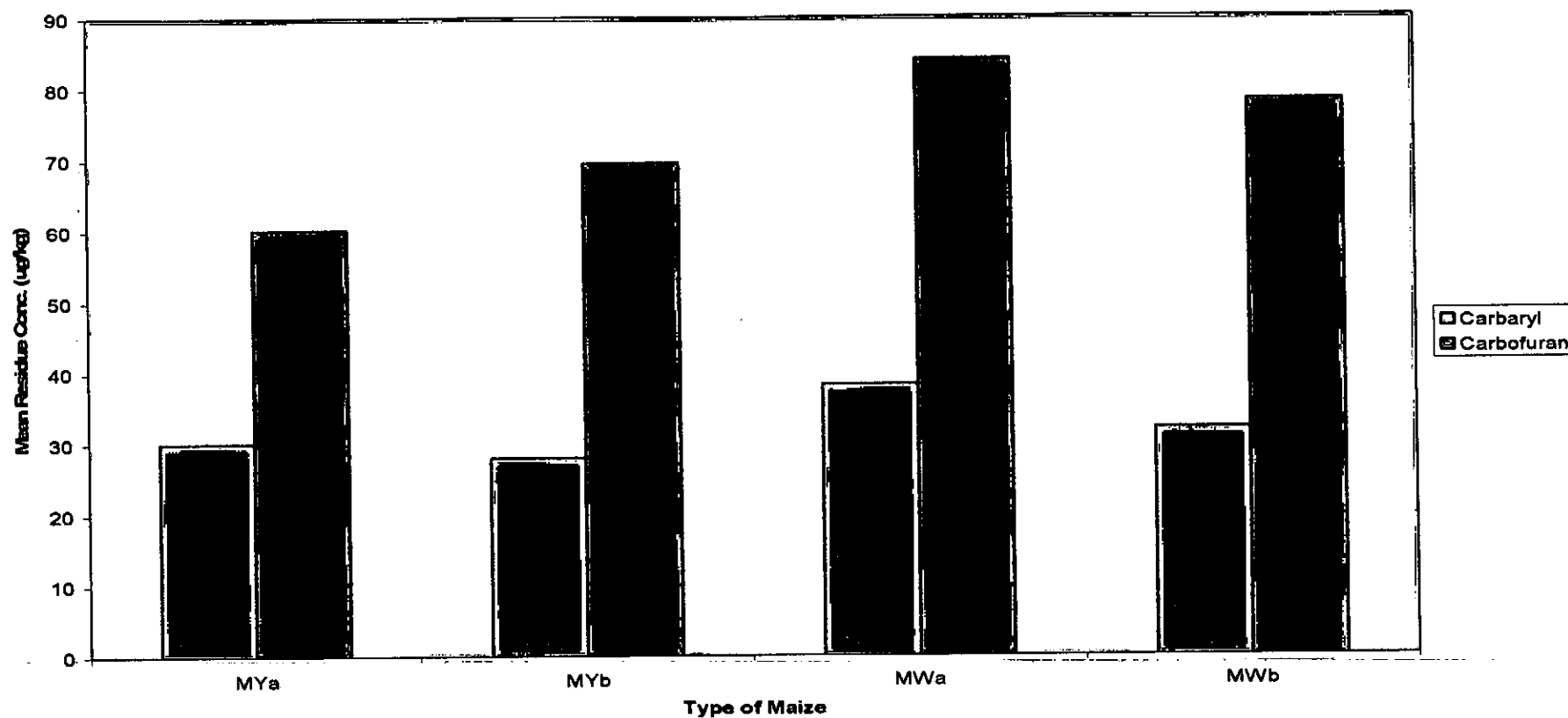
Figure 4.37: Mean Residue Concentrations of Carbamate Pesticides in Different Types of Maize.

MYa and MYb = Yellow Maize Types (a) and (b). MWa and MWb = White Maize Types (a) and (b).

Table 4.11 – Pesticide Residues above Maximum Residue Limits in Maize Samples

Pesticide	MRL (μg/kg)	Number of Samples Above MRL	Maximum Concentration (μg/kg)	% Difference From MRL
Aldrin	10	5 (6)*	18.1	81
Carbaryl	--	--	73.5	--
Carbofuran	100	4 (5)	182.2	82
Chlorpyrifos	50	8 (10)	62.1	24
Diazinon	20	5 (6)	30.3	52
DDT	50	6 (7)	91.3	83
Dichlorvos	2000	3 (4)	2367.7	18
Dieldrin	10	2 (2)	16.5	65
Endosulfan	50	4 (5)	73.5	47
Endrin	10	5 (6)	18.5	85
Fenitrothion	--	--	15.8	--
Malathion	8000	Nil	3951.4	NA
Parathion	50	Nil	48.2	NA
Pirimiphos-methyl	5000	Nil	3185.1	NA

Total number of maize samples = 84

* Percentage of samples above MRL in brackets

NA = Not applicable

Table 4.12 – Pesticide Residues above Maximum Residue Limits in Beans Samples

Pesticide	MRL (µg/kg)	Number of Samples Above MRL	Maximum Concentration (µg/kg)	% Difference From MRL
Aldrin	10	2 (2)*	15.5	55
Carbaryl	50	5 (6)	73.5	47
Carbofuran	100	6 (7)	135.3	35
Chlorpyrifos	50	8 (10)	98.2	96
Diazinon	20	5 (6)	27.8	39
DDT	50	7 (8)	78.5	57
Dichlorvos	--	--	1480.5	--
Dieldrin	10	Nil	9.5	NA
Endosulfan	50	4 (5)	65.2	30
Endrin	10	2 (2)	12.3	23
Fenitrothion	10	1 (1)	12.2	22
Malathion	--	--	852.5	--
Parathion	50	Nil	44.9	NA
Pirimiphos-methyl	50	8 (10)	92.5	85

Total number of maize samples = 84

* Percentage of samples above MRL in brackets

NA = Not applicable

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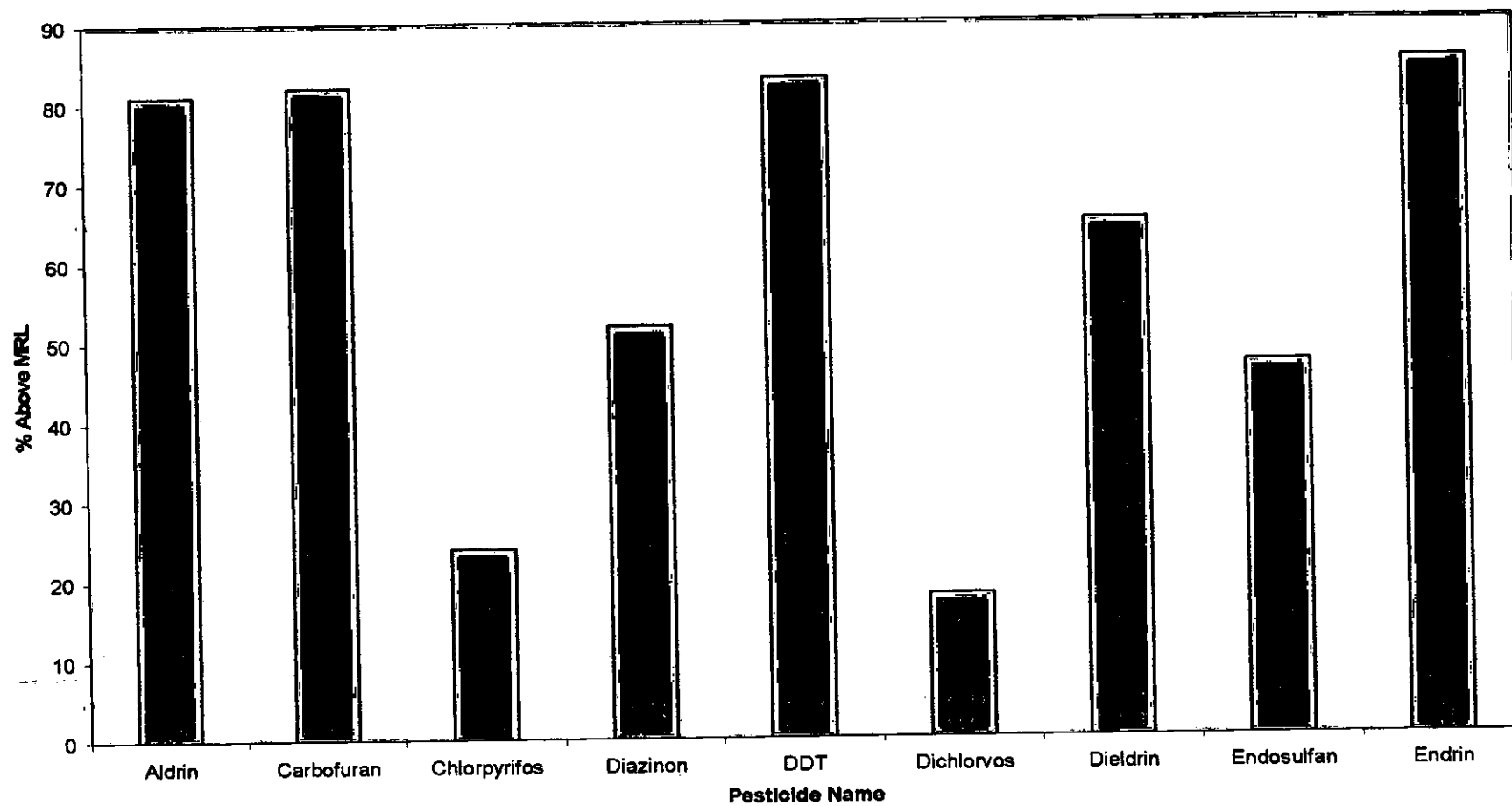
Figure 4.38: The Various Pesticides and their Percentage above Maximum Residue Limits in Maize.
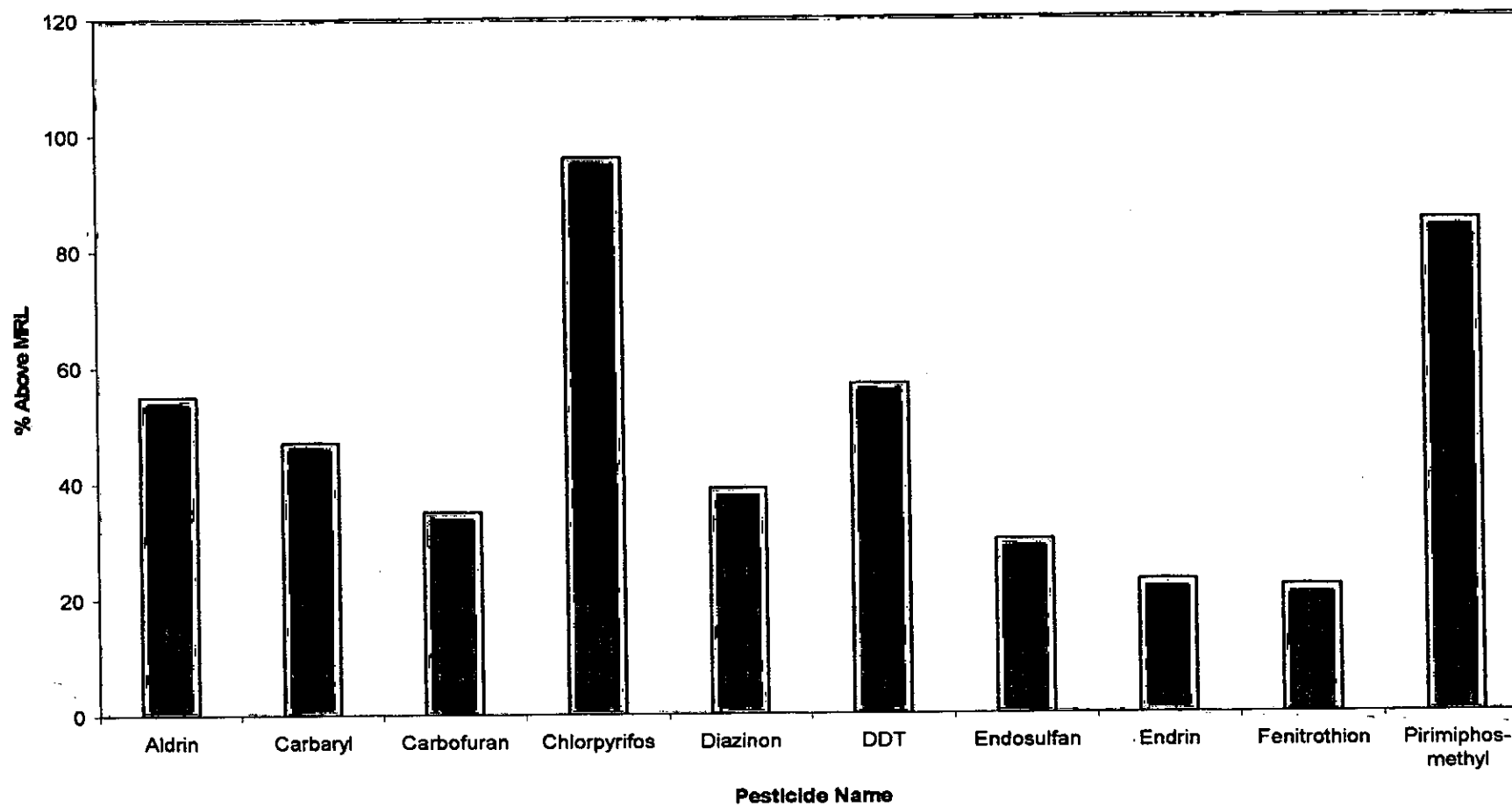
Figure 4.39: The Various Pesticides and their Percentage above Maximum Residue Limits in Beans.

Table 4.13 - Incidence of Pesticide Residues and Markets of Collection of Maize Samples

Pesticide	Number of Positive Samples							Total
	AG	BA	EP	ID	IK	MT	MU	
Aldrin	4	1	3	5	1	6	7	27
Carbaryl	2	5	2	3	5	5	4	26
Carbofuran	2	3	3	4	2	8	6	28
Chlorpyrifos	3	2	1	9	3	7	8	33
Diazinon	0	6	4	2	0	4	5	21
DDT	5	1	3	6	2	6	3	26
Dichlorvos	6	3	1	6	4	5	6	31
Dieldrin	2	2	5	4	5	3	3	24
Endosulfan	4	2	6	3	6	2	2	25
Endrin	2	2	3	5	7	4	4	27
Fenitrothion	2	0	0	1	1	2	0	6
Malathion	3	2	4	0	5	9	0	23
Parathion	0	2	1	1	0	3	2	9
Pirimiphos-methyl	3	1	2	10	3	8	9	36

AG = Agege, BA = Badagry, EP = Epe, ID = Ido, IK = Ikorodu, MT = Mile Twelve and MU = Mushin.

Table 4.14 - Incidence of Pesticide Residues and Markets of Collection of Beans Samples

Pesticide	Number of Positive Samples							Total
	AG	BA	EP	ID	IK	MT	MU	
Aldrin	2	0	1	1	2	1	0	7
Carbaryl	4	5	2	3	2	5	3	24
Carbofuran	3	1	3	8	2	5	7	29
Chlorpyrifos	3	0	5	9	4	6	10	37
Diazinon	5	0	0	2	5	8	4	24
DDT	8	3	7	5	8	3	3	37
Dichlorvos	7	4	4	10	1	3	6	35
Dieldrin	2	2	0	1	3	0	3	11
Endosulfan	4	1	2	3	6	5	8	29
Endrin	0	3	1	2	2	3	1	12
Fenitrothion	2	0	0	3	1	1	2	9
Malathion	10	1	1	6	3	7	5	33
Parathion	3	2	3	1	0	4	0	13
Pirimiphos-methyl	8	1	2	9	6	10	9	45

AG = Agege, BA = Badagry, EP = Epe, ID = Ido, IK = Ikorodu, MT = Mile Twelve and MU = Mushin.

4.5   Effect of Heat on Pesticide Residue Content of Samples.

The pesticide residue contents of beans samples after boiling are presented in Table 4.15.

Figure 4.40 is a bar chart of percentage reductions in the concentrations of the various pesticides in beans after boiling while chromatograms of a sample before and after boiling are shown in Figure 4.41.

Table 4.15 - Pesticide Residue Content of Beans Samples before and after Boiling.

Sample Code	Pesticides Detected	Residue Content Of Uncooked Sample (μg/kg)	Residue Content of Cooked Sample (μg/kg)	% Reduction
AGB1	Carbaryl	41.2	32.5	21
	DDT	12.5	10.2	18
	Dieldrin	3.5	4.2	20*
	Endosulfan	27.2	18.5	32
	Pirimiphos-methyl	16.3	5.5	66
AGB2	Carbofuran	75.2	60.1	20
	Malathion	525.6	321.3	39
	Pirimiphos-methyl	42.5	19.9	53
BAB1	Aldrin	13.3	9.5	29
	Diazinon	21.9	14.8	32
BAB2	Carbofuran	106.5	75.4	29
	Dichlorvos	1055.5	656.2	38
	Pirimiphos-Methyl	75.2	26.3	65
EPB1	Chlorpyrifos	34.2	18.5	46
	Malathion	68.3	30.8	55
	Pirimiphos-Methyl	31.0	00	100

Table 4.15 (Continued).

Sample Code	Pesticides Detected	Residue Content Of Uncooked Sample (µg/kg)	Residue Content of Cooked Sample (µg/kg)	% Reduction
EPB2	Diazinon	11.5	7.8	32
	Dichlorvos	985.0	419.5	57
	Malathion	41.5	20.2	51
	Pirimiphos-methyl	56.5	27.5	51
IDB1	Dichlorvos	1.1508	824.0	28
IDB2	DDT	50.5	39.5	22
	Endosulfan	45.2	34.6	24
	Endrin	7.1	5.5	23
	Pirimiphos-methyl	29.1	13.2	55
IKB1	Chlorpyrifos	58.6	19.9	66
	Diazinon	11.2	12.6	13*
	Dichlorvos	1201.5	911.3	24
IKB2	Aldrin	8.2	7.5	9
	Endosulfan	7.2	6.3	13
	Endrin	8.5	5.9	31
	DDT	22.5	19.4	14
MTB1	Aldrin	5.6	4.5	20
	DDT	51.6	37.2	28
	Pirimiphos-Methyl	38.4	16.5	57

Table 4.15 (Continued).

Sample Code	Pesticides Detected	Residue Content Of Uncooked Sample (μg/kg)	Residue Content of Cooked Sample (μg/kg)	% Reduction
MTB2	Carbofuran	88.5	55.2	38
	DDT	27.5	18.6	32
	Endosulfan	51.5	40.9	21
MUB1	Dichlorvos	752.5	489.5	35
	Malathion	148.1	79.1	47
	Pirimiphos-Methyl	15.6	3.0	81
MUB2	Carbofuran	48.1	00	100
	Chlorpyrifos	96.0	43.6	55
	Malathion	129.8	68.1	48
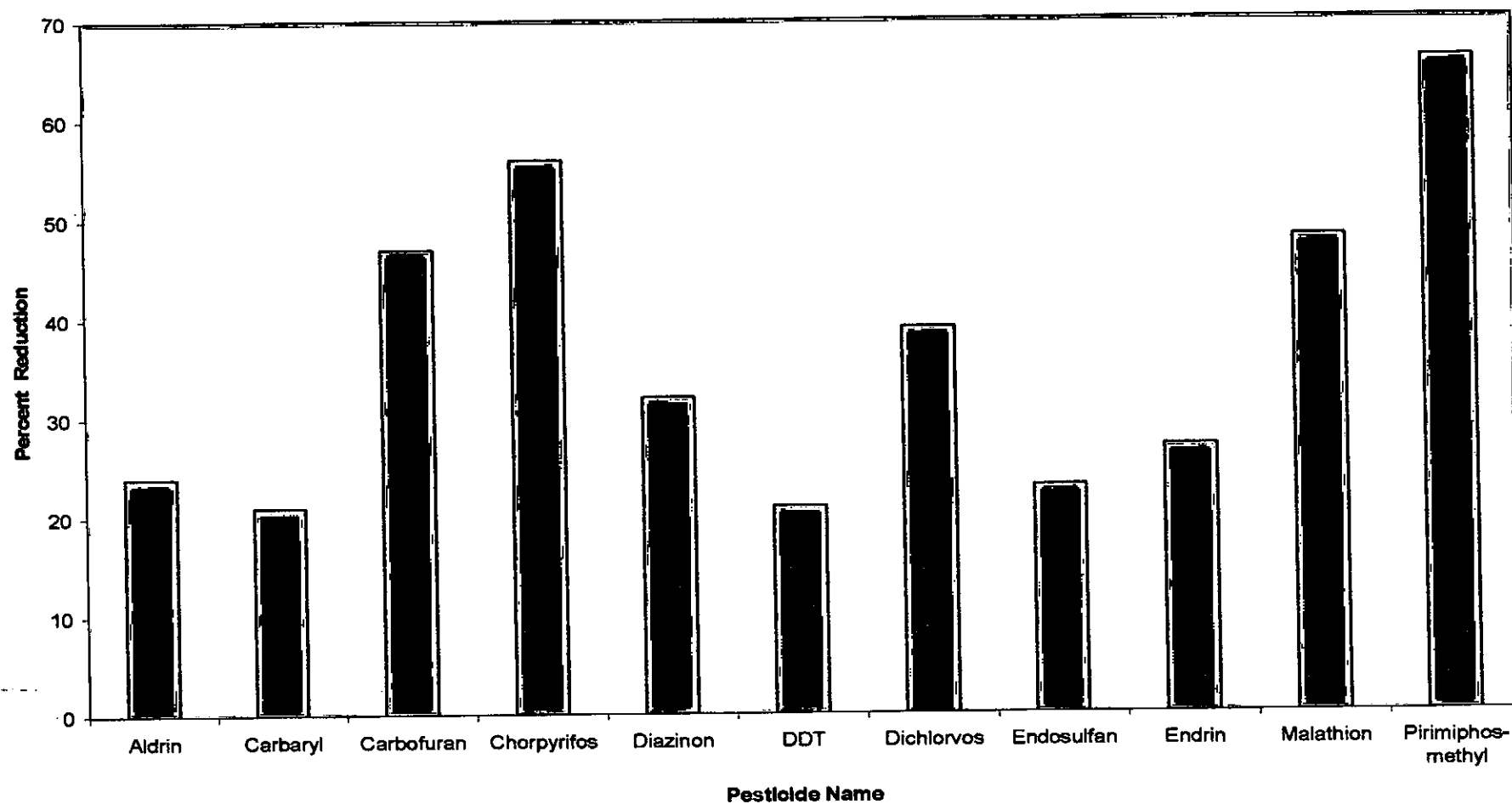
Figure 4.40: Mean Percent Reductions in the Concentrations of the Various Pesticides in Beans after Boiling.
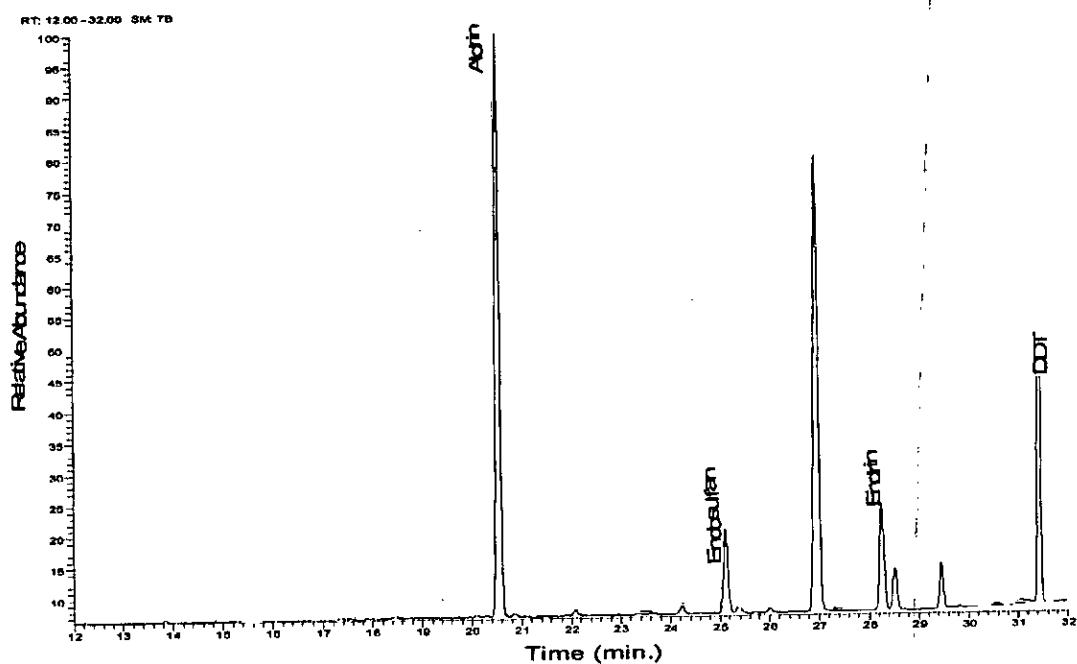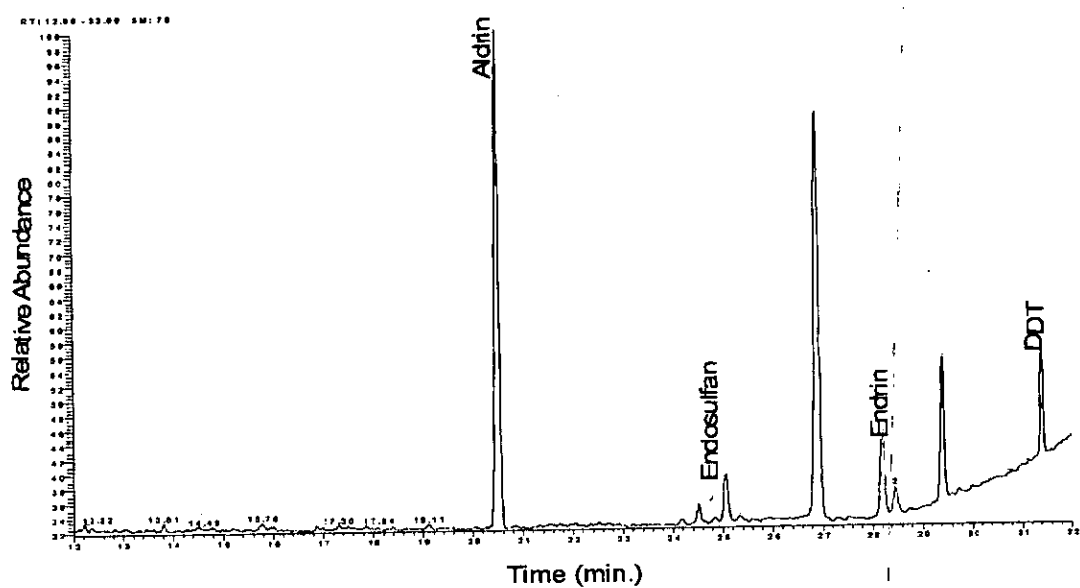
Figure 4.41: Total Ion Chromatogram of a Sample of Beans before (A) and after (B) Boiling.

4.6 Estimation of Daily Intakes

Values of estimated daily and total diet intakes of the pesticides calculated as described in section 3.3.10 are presented in Table 4.16. Figure 4.42 is a bar chart comparing estimated total diet intakes (ETDIs) of the pesticides with their maximum permissible intakes (MPIs).

Table 4.16: Acceptable Daily Intakes (ADIs), Maximum Permissible Intakes (MPIs), Estimated Daily Intakes (EDIs) and Estimated Total Diet Intakes (ETDIs) of the Various Pesticides.

Pesticide	ADI (µg/kg)	MPI (µg/person/day)	Maximum Residue Concentration (µg/kg)	EDI (µg/person/day)	ETDI (µg/person/day)
Aldrin	0.1	6	15.5	0.465	12*
Carbaryl	10	600	73.5	2.205	55
Carbofuran	3	180	135.3	4.059	102
Chlorpyrifos	10	600	98.2	2.946	74
Diazinon	2	120	27.8	0.834	21
DDT	5	300	78.5	2.355	59
Dichlorvos	4	240	1480.5	44.415	1110*
Dieldrin	0.1	6	9.5	0.285	7*
Endosulfan	7.5	450	65.2	1.956	49
Endrin	0.2	12	12.3	0.369	9
Fenitrothion	5	300	12.2	0.366	9
Malathion	20	1200	852.5	25.575	639
Parathion	1	60	44.9	1.347	34
Pirimiphos-Methyl	10	600	92.5	2.775	69

*ETDIs that are above the MPIs.
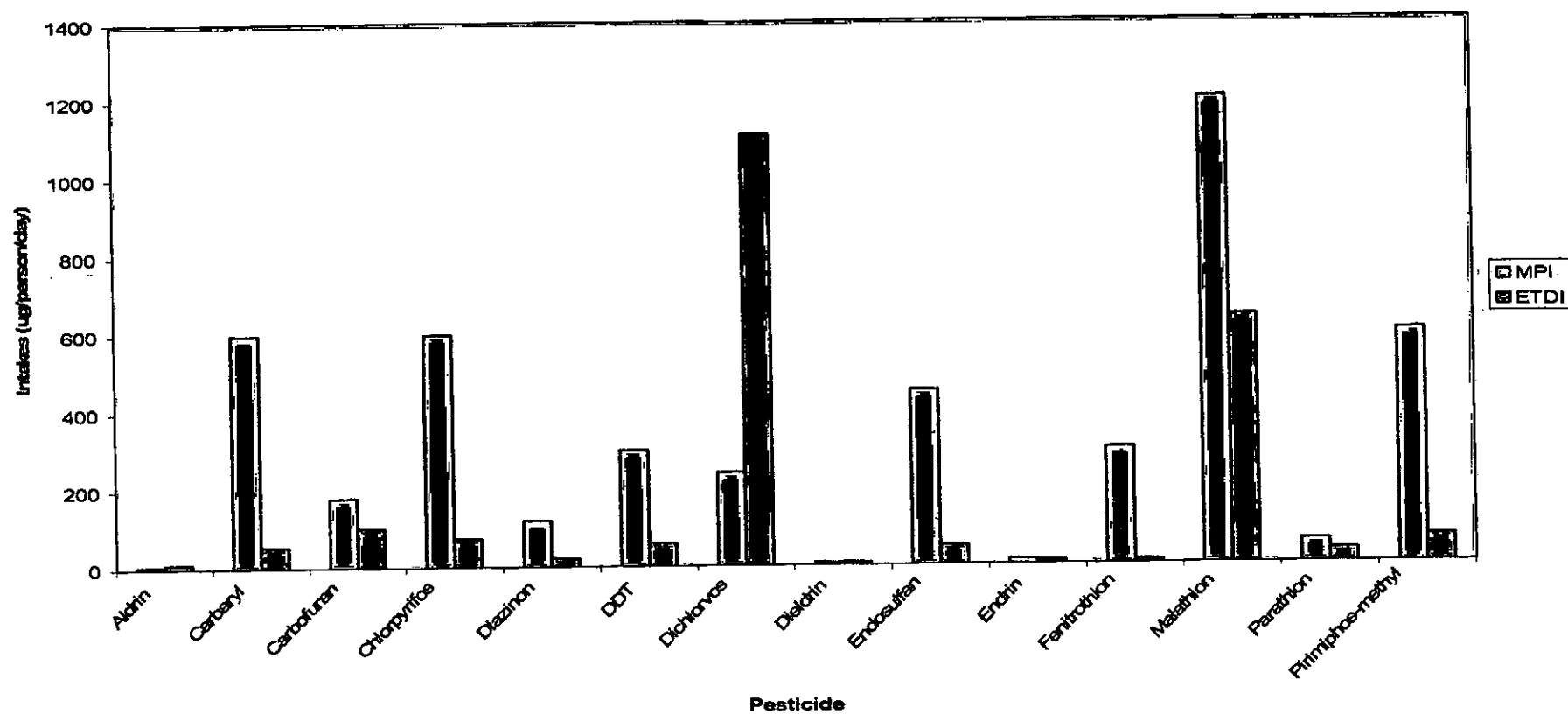
Figure 4.42: Estimated Total Diet Intakes (ETDIs) of the various Pesticides Compared with their Maximum Permissible Intakes (MPIs).

CHAPTER FIVE

DISCUSSION AND CONCLUSIONS

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5.1 DISCUSSION

5.1.1 General

Pesticides are used widely in agriculture to control destructive pests and hence increase food supply. Their use inadvertently leads to residues in harvested food crops. The amount of pesticide residues found in market-ready food items is closely related to compliance with laid down procedures for safe use of these chemicals. Pesticides also contaminate air, soil and water leading to high human exposure with adverse consequences on human health and environment, if unchecked. There is therefore the need to control the use of pesticides in agriculture but effectiveness of control measures cannot be assessed without pesticide residue analysis in food and other environmental media. Evaluation of pesticide residues in food is therefore of public health importance and the need for pesticide residue data base cannot be over emphasized.

Pesticide residue analysis involves four major steps: collection and preparation of samples, extraction of analytes from sample matrix, separation of analytes from co-extractives and determination of analytes in clean extracts with the aid of sensitive analytical equipment such as the gas chromatograph with mass selective detector (GC/MS). All four steps were involved in this study and since the pesticide history of the samples was unknown, a multiclass multiresidue method of analysis was used. This permitted the determination of residues of different classes of pesticides in a single extract (Pesticide Analytical Manual, 1999; Consuelo et al., 2004). Specific method options in each step were selected bearing in mind the commodity involved (maize and beans) and the target residues which were organochlorines, organophosphates and carbamates.

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5.1.2 Extraction and Clean-up

Selection of solvent for extraction was one of the major decisions made. For the extraction of non-polar residues from non-fatty foods, non-polar solvents are recommended (Miller, 1995). Ethyl acetate was selected from among other non-polar solvents based on the high solubility of all the target pesticides in it. Ethyl acetate is also relatively nontoxic. The weight of sample (2g) for extraction and subsequent analysis was chosen to minimize volumes of solvents used and to avoid undue extraction of fats and other contaminants which would increase the need for a more elaborate clean-up. Minimum extraction of contaminants is also beneficial to column longevity as less of these non-target chemicals are introduced into the column.

Clean-up of extracts to separate the analytes from co-extractives was achieved by the use of florisil in solid phase extraction cartridges. This helped to remove polar substances and any remaining particulate matter using small volumes of solvents. Interferences from sample co-extractives reduce equipment sensitivity by masking the detector response to the residue or by preventing injection of the specified sample equivalent. The effect of sample clean-up is demonstrated in Figure 4.29. The extract, before clean-up gave a chromatogram full of interfering peaks and a rising baseline. This is indicative of the high level of noise. The chromatogram of the cleaned extract, on the other hand showed distinct peaks.

5.1.3 Determinative Technique

Several instrumental determinative techniques are available for pesticide residue analysis. Gas chromatograph with mass-selective detector (GC-MS) was used because of its high

specificity which is due to its ability to differentiate between analytes and interferences based on differences in fragmentation patterns and masses of selected ions (Lefkovitz, 2001; Jiankai and Na, 2006). It was operated in the selected ion monitoring (SIM) mode and three ions were monitored for each pesticide. This aided the elimination of interferences and increased specificity. It also enhanced the suitability of the technique for simultaneous detection, quantification and confirmation of residues (Jiankai and Na, 2006). Analytical conditions for both the gas chromatograph and the mass spectrometer were selected for optimum detection and quantification of target residues. Availability of an auto sampler with the GC-MS automated the procedure and permitted easy analysis of large numbers of samples per day. An injection volume of $1\mu L$ was used for optimum detection of analytes and minimum introduction of interferences (Wei-Guo *et al.*, 2006).

Pesticide standards, being hazardous chemicals were handled with utmost care and all solvents and chemicals were disposed according to laid down safety regulations (University of Strathclyde, 2006).

5.1.4   Validation of Methods

Validation is a process of demonstrating that a method is fit for its purpose. To test the suitability of the methods for the samples at hand and the target pesticides, some validation procedures were carried out before application of methods to sample analysis. Such procedures which were also to assure the accuracy, reliability and reproducibility of analytical data included development of calibration curves, determination of limits of detection and recovery studies.

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Individual standards of organochlorine pesticides as well as different concentrations of standard mixtures were run in full scan for acquisition of retention data and preparation of calibration curves. The organophosphates and carbamates which were obtained only as standard mixtures were also run under the same chromatographic conditions. All pesticides gave linear calibration curves with determination coefficients >0.99 as shown in Figures 4.14 – 4.27. This was an indication of reproducible instrument response and reliability of analytical data (Edgerley, 1998; Consuelo et al., 2004). The peak heights and retention times were also reproducible as reflected in Figures 4.1 – 4.13.

Limit of detection (LOD) is the lowest concentration of residue that can be detected by a given method with acceptable degree of certainty. It is also called the minimum detectable amount (MDA). It is the concentration at which signal-to-noise ratio =3 and it is an indication of the sensitivity of the equipment to the particular residue. The LOD for each pesticide was determined and all were found to be in the range 0.0006 – 0.0168 µg/ml. These values are reliable as they agree with LODs determined by other workers using GC/MS (Wei-Guo et al., 2006; Maštovská et al., 2004).

Recovery studies were carried out to evaluate the efficiency of the method for the analysis of the target residues in the food matrix right from the extraction stage. This is to determine matrix effect. Maize samples which were found to contain none of the target pesticides at detectable concentrations were chosen as the blank samples for fortification. Known concentrations of the pesticide standards were added to known weight of sample (2g) and taken through the entire analytical procedure for sample analysis. An internal standard, 2-chloroanthracene, was also added to compensate for possible losses during the different stages of the analytical process. Percent recoveries were then calculated

from peak areas of pesticide standards relative to peak area of internal standard as described in section 3.3.8.

All the pesticides studied gave average recoveries of more than 70% which is the minimum recovery for an analytical procedure to be valid. However, endrin at concentration of $0.25\mu g/g$ gave a percent recovery of 68.2 which was below limits. The relatively low recovery rates for endosulfan, endrin and fenitrothion may be ascribed to their low stability compared to other compounds in their chemical groups (Lefkovitz, 2001). This may have led to faster degradation of these compounds during the analytical process. Aldrin at $0.25\mu g/g$ recorded 100.7% recovery, which might be an indication that the compound may have been present in undetectable amounts before fortification of the sample. Percent recoveries for organochlorine pesticides ranged from 68.2 to 100.7% and from 70.6 to 95.1% for organophosphate and carbamate pesticides. Precision of recovery data was good as standard deviation was less than 10% for all the pesticides.

5.1.5 Pesticide Residue Contents

The level of pesticide residues present in any food item is dependent on the amount of pesticides used on it and this in turn depends on the susceptibility of the food item to pest infestation. Residues may also occur in agricultural products of crops grown in soil previously treated or contaminated with pesticides. Most crops are treated with pesticides on the fields to avoid attack by destructive pests which could reduce the quality and quantity of crop harvests. In addition to field treatment, cereal grains and pulses are often subjected to post-harvest treatment with pesticides to prevent pest infestation during storage. Food commodities so often treated in the Nigerian environment are maize and

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beans. This is because they are highly susceptible to attack by insects such as weevils, mites, beetles, ants and other insects. Different classes of pesticides are available for use on agricultural commodities but the older organochlorine compounds have been either banned or severely restricted because of their persistence in the environment with consequent health hazards. Other classes approved for use are regulated to minimize the amount of residues remaining on food at the time it is made available to the consumer. Most (98%) of the samples analyzed in this study were found to contain one or more pesticides.

5.1.5.1   Pesticide Residues in Maize

Four different types of maize (two of yellow and two of white) were studied in this survey. The results of pesticide residue content of maize presented in Table 4.4 show that, on the average, the white maize contained higher concentrations of pesticides than the yellow maize, both in terms of mean values and maximum concentrations found for the pesticides detected. This may be an indication that the white variety is more susceptible to insect infestation than the yellow maize. It could also be that certain nutritional constituents of the white maize retain pesticide compounds more than yellow maize. However, the highest concentration of Aldrin (18.1µg/kg) was found in yellow type (b) although the highest mean value (10.3µg/kg) was in white type (a). Between the two types of white maize, mean and maximum values of residues were higher in type (a) than in (b). This was also the case with the two types of yellow maize. This may be as a result of differences in the nutritional constituents of the different types of maize. Fenitrothion and parathion were not detected in either of the two types of yellow maize.

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All the three classes of pesticides (organochlorines, organophosphates and carbamates) investigated in this study were detected in maize samples collected from the various markets across Lagos State. The mean concentrations of organochlorine pesticides (7.4 - 52.0μg/kg) were lower than the mean concentrations of organophosphate and carbamate pesticides (10.1 -1558.8μg/kg) as shown in Tables 4.7 and 4.8. Table 4.6 shows that Pirimiphos-methyl, Chlorpyrifos and Dichorvos had the highest incidence in maize samples with percent occurrence of 43, 39 and 37 respectively. On the other hand, Fenitrothion and Parathion had the lowest incidence occurring in 7% and 11% of samples respectively.

The number of residues per sample ranged from 0 – 5 while total amount of residues per sample was from 575 – 4182 μg/kg. Only three maize samples (4%) did not contain any pesticide residue at detectable levels.

5.1.5.2   Pesticide Residues in Beans

Beans samples studied were of four types (two of brown and two of white). Mean residue concentrations and maximum values detected were higher in the white beans than in the brown samples except for Pirimiphos-methyl, Dichlorvos and Chlorpyrifos for which higher values were found in the brown beans. As in the case of maize samples, the type (a) of both brown and white had higher concentrations of residues than the type (b). These results are reflected in Table 4.5. Brown beans samples studied did not contain Fenitrothion and Parathion at detectable levels while Aldrin and Dieldrin were not detected in the two types of white beans.

Organochlorines, organophosphates and carbamates were detected in beans samples. Like in maize samples, organochlorines were present in lower mean concentrations (4.8-35.1 μg/kg) than organophosphates and carbamates (8.4-942.7 μg/kg). These are reflected in Tables 4.9 and 4.10. Results in Table 4.8 reveal that Pirimiphos-methyl had the highest incidence of 54%, followed by DDT and chlorpyrifos (44%) each, then Malathion (39%). Incidence of Aldrin was lowest at 8% occurrence, followed by Fenitrothion which occurred in 11% of the samples.

On the whole, all the beans samples analyzed contained at least one pesticide residue (i.e. 100% incidence). Number of residues per sample ranged from 1- 5 while total amount of residue per sample ranged from 535 - 2255μg/kg.

5.1.5.3   Comparison between Residue Contents of Maize and Beans.

Maize and beans are food commodities to which pesticides are often applied during storage to prevent infestation by weevils. This is apart from the pesticides that may be applied to them in the fields. They are usually sold in open markets in bags or bowls. Among the samples studied, incidence of pesticide residues was higher among the beans samples than the maize samples, except for Aldrin, Carbaryl, Dieldrin and Endrin. The highest percent occurrence was in beans samples (54% for Pirimiphos-methyl); these results are reflected in Table 4.6. Maximum concentrations of individual pesticides were however, higher among the maize samples (15.8-3951.4μg/kg) than in the beans samples (12.2-1480.5μg/kg) as shown in Tables 4.11 and 4.12. Also, total amount of pesticides per sample was higher in maize (575 - 4182μg/kg) than in beans (535 -2255μg/kg).

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These high total levels of pesticide residues may accumulate in humans over time and become hazardous to health even though some of the samples did not exceed the MRL for the individual pesticides. Health hazards that may result from accumulation of pesticide residues in the body include neurotoxic disorders, cancers, infertility, immunological disorders, liver damage, kidney damage and skin disorders.

This study shows a decline in the mean levels of organochlorine pesticides in both maize (6.9-41.3µg/kg) and beans (4.8-39.7µg/kg) compared to the results of a similar study (10-93µg/kg for maize and 25-303µg-kg for beans) previously carried out in Nigeria (Adeyeye and Osibanjo, 1999).

Pirimiphos-methyl was the pesticide with the highest percent occurrence in both the maize and the beans samples. This may be due to storage of the grains with this pesticide. Pirimiphos-methyl is often applied as dust to grains during storage to prevent pest infestation. A popular brand of this pesticide in Nigeria is Actellic dust.

5.1.5.4   Incidence of Pesticide Residues in Samples from Different Markets

The relative occurence of pesticide residues in maize samples from the different markets sampled for this study is presented in Figure 4.13. Mile Twelve market (MT) had the highest number of samples (72) containing one or more residues, followed by Mushin (MU) and Ido(ID) markets with 59 samples each. The least number of samples (35) containing at least one residue came from Badagry market. Diazinon and Parathion were not detected in any maize sample from Agege and Ikorodu; Fenitrothion was not found in samples from Badagry, Epe and Mushin markets while Malathion was absent in samples collected from Ido and Mushin markets.

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Market incidence of pesticide residues in beans samples is as presented in Table 4.14. Ido Market had the highest incidence of samples (70) containing one or more residues, followed by Mile Twelve and Agege Markets with incidence of 62 and 58 respectively. Beans samples from Epe did not contain residues of Chlorpyrifos, Dieldrin and Fenitrothion while Aldrin and Fenitrothion were not detected in samples from Badagry. Samples from Agege Market did not contain Diazinon and Endrin just as Diazinon and Parathion were not detected in samples from Ikorodu. Dieldrin was not present at detectable levels in samples from Mile Twelve while Aldrin and Parathion were not detected in beans samples from Mushin Market.

Since most of the maize and beans in the various markets come from the same northern states of the country, the difference in the incidence of residues in these markets may be attributed to the use of pesticides on the grains during storage at the various markets. Traders, especially wholesalers often apply pesticides on maize and beans in their stores to prevent pest infestation which may cause them economic damage. Under such circumstances, banned or substandard chemicals may be used in concentrations not recommended for foodstuffs.

5.1.5.5 Comparison of Residue Contents with Maximum Residue Limits

A maximum residue limit (MRL) is the maximum concentration of a pesticide residue that is legally permitted to remain in any food. MRL of a pesticide in any food is not expected to be exceeded if the pesticide was applied in accordance with directions for its safe use. An MRL is therefore a means of ensuring that a pesticide was properly used. If

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a pesticide residue is found to exceed the MRL in any food, the food commodity is said to be adulterated because it contains an unsafe or illegal amount of pesticide residue.

In this study, maximum residue limits for some pesticides in maize were exceeded by up to 10% of samples. The highest violating concentration was that of endrin which was higher than its MRL by 85%. Chlorpyrifos exceeded its MRL in the highest number of samples (10%). The maximum residue limits for malathion and pirimiphos-methyl were not exceeded in any of the maize samples even though their mean concentrations (972.5-1565.2µg/kg for malathion and 848.5-1455.4µg/kg for pirimiphos-methyl) were high in these samples. The MRL of Parathion was also not exceeded in any of the maize samples studied. Violation rates for carbaryl and fenitrothion could not be determined because their MRL values in maize could not be obtained.

Among the beans samples, chlorpyrifos had the highest violating concentration which was 96% higher than its MRL of 50µg/kg. Chlorpyrifos is a broad-spectrum pesticide used for control of stored grain insects. Next was pirimiphos-methyl, the concentration of which was 85% above its maximum residue limit. Both pesticides exceeded their limits in 10% of samples, which was the highest among the pesticides studied. The maximum residue limits of dieldrin and parathion were not exceeded in any of the beans samples. Violation levels for dichlorvos and malathion were not determined because their MRL values in beans were not available.

The occurrence of pesticides above MRLs in up to 10% of samples of both maize and beans is an indication of some form of misuse of these chemicals. Non-compliance with MRLs can impact negatively on international trade in agricultural produce as each

commodity must meet international standards or standards of the receiving country. Also, the detection of organochlorines in both commodities suggests that these persistent pesticides may still be in use even though they have been banned in Nigeria and most countries of the world. The goal of monitoring of pesticide use in agriculture should therefore be directed at ensuring appropriate use of recommended products and that banned pesticides are no longer manufactured or imported into the country.

5.1.6   Effect of Heat

Raw agricultural commodities are often processed in one way or the other before consumption and this affects the pesticide residue content. Processing may involve washing, peeling, soaking, boiling, baking, frying, etc. So heat is a common means of food processing. Washing and peeling have been found to reduce pesticide residue contents of fruits by 18% and 85% respectively (Fytianos *et al.*, 2006). In this study, the effect of boiling on the pesticide residue content of beans samples was examined. Samples of the brown beans variety (a) from the seven markets were boiled and their residue contents compared with the residue contents of the raw portions of same samples.

It was found that in most of the samples (86%), pesticide residue contents were decreased by boiling. This agrees with the results of similar studies (Soliman, 2001; Rasmussen *et al.*, 2003). Percent reduction ranged from 9 to 100 as presented in Table 4.15. This shows that heat has a significant effect on pesticide residues. The extent of reduction was higher in the organophosphates (24-100%) and Carbamates (20-100%) than in the Organochlorines (9-32%). This may be due to the higher stability of organochlorine compounds to heat treatment. Only carbofuran (sample MUB2) and

pirimiphos-methyl (EPB1) disappeared completely after boiling, that is, 100% reduction. The residue levels of diazinon in sample IKB1 and dieldrin (sample AGB1) were found to have increased by 13 and 20% respectively after boiling. This may have been due to errors in the analytical process.

5.1.7   Toxicological Implication

Most pesticides are known to be neurotoxic while others have been found to be carcinogenic, teratogenic and to depress immune responses (Mansour, 2004; Koprucu *et al.*, 2006). Some pesticides have also been identified as endocrine disruptors, meaning that they can affect human growth and reproduction (Jobling *et al.*, 1995). In Nigeria, unfortunately, symptoms of such health effects, if they occur, may not be linked to pesticides due to inefficiency of the health system and sometimes due to cultural/religious beliefs.

The toxicological importance of pesticide residue data depends, not only on the residue content of food but also on the quantity of contaminated food consumed and the length of time over which the consumption occurs (Petersen, 2000). Therefore, more studies on food consumption patterns of Nigerians and residue contents of other food varieties are required to determine the exact total intake of pesticides and the actual toxicological importance of these residue levels.

The residue levels in beans determined in this study were used to estimate daily intakes and total diet intakes for each pesticide. Maximum permissible intakes (MPIs) were also calculated from acceptable daily intakes (ADIs) established by FAO/WHO. The ADI of a

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pesticide is the daily intake of its residue, which during the life time of the consumer appears to be without appreciable risk to health.

Results of this study reveal that estimated total diet intakes (ETDIs) for most of the pesticides were well below their MPIs. Such pesticides are not likely to cause significant health hazards even on a long term basis. It must be noted, however, that these results are based on estimated values for beans consumption rate and total diet per person per day. In addition, total diet intakes were made for individual compounds and the potential "cocktail effect" of the various pesticides when accumulated in the body was not considered. The combined effects may be additive or synergistic. This means that even pesticides that were detected at 'safe levels' may eventually pose health hazards to humans due to combined effects of other pesticides also accumulated in the body. There is therefore the need to investigate the potential cocktail effect of pesticide residues in the human body.

On the other hand, the ETDIs for aldrin, dichlorvos and dieldrin exceeded their MPIs by 100%, 363% and 17% respectively as shown in Table 4.16. This indicates excessive or inappropriate use of these chemicals. Aldrin and dieldrin are organochlorines which have been banned in Nigeria and in many other countries due to their persistence in the environment and potential hazards to human health. They however continue to be used in many developing countries because they are cheaper and have a broader spectrum of activity than their substitutes. The regulatory and monitoring agencies (NAFDAC and the Federal Ministry of Environment) should therefore step up efforts to ensure compliance with the ban on these chemicals. Farmers and other pesticide users also need to be educated on dangers of using banned products.

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Dichlorvos is not yet on the list of pesticides banned in Nigeria but being an organophosphate, it causes inhibition of blood acetylchlolinesterase (AChE) activity. It is known to cause delayed neurotoxic effects, dermatitis and skin sensitization. Its use in the country must therefore be more strictly controlled.

Maize and beans are staple foods in Nigeria. Consumers of these commodities should endeavour to wash them thoroughly before any form of processing. Processing of these foodstuffs before consumption should include heating or boiling as the result of this study has shown that the concentrations these chemicals are decreased by heat.

5.2 CONCLUSIONS

Residues of organochlorine, organophosphate and carbamate pesticides were successfully determined using gas chromatograph with mass spectrometric detector (GC-MS). The results of this study show that there is a high incidence (98%) of pesticide residues in maize and beans sold in Lagos markets. Incidence of residues was on the average higher among the beans samples than in the maize samples. Pesticide residues occurred more often among the white varieties of maize than in the yellow varieties. The white varieties of beans also had a higher incidence of residues than the brown varieties.

Higher concentrations of residues were found in maize (6.9-1565.2μg/kg) than in beans (4.8-942.7μg/kg) samples. However, the percentages of samples above MRL were low for both maize and beans samples. In maize samples, the percentages ranged from 2% for Dieldrin to10% for Chlorpyrifos while in beans samples, the range was 1% for Fenitrothion to 10% each for Chlorpyrifos and Pirimiphos-methyl. Some residues in beans samples were found to be as much as 96% (Chlorpyrifos) above MRL. This level

of violation was followed by Pirimiphos-methyl which was 85% above the MRL. The pesticide with the lowest percent above MRL in beans samples was Fenitrothion (22%). In maize samples, the maximum concentration of Endrin was 85% above MRL. The percentages above MRL for DDT (83%) and Carbofuran (82%) were also high. The lowest percent above MRL in maize samples was 18% (Dichlorvos).

Organochlorine pesticides may still be in use for crop protection in Nigeria as they were found in appreciable concentrations in the samples studied, though they were on the decline compared to concentrations found in previous studies. There is therefore a need for the relevant agency to strictly control the importation, sale, use and disposal of these persistent compounds.

This study also reveals that the concentration of pesticide residues is decreased by boiling. The percent reduction was found to range from 9 to 100. Effect of heat was more pronounced on organophosphate (24-100%) and carbamate (20-100%) pesticides than on organochlorine compounds (9-32%). This means that organochlorines are more likely to accumulate in the human body and cause chronic poisoning than organophosphates and carbamates.

It can be concluded from this study that most of the maize and beans in Lagos markets are brought in from the northern states of Borno, Sokoto, Kano, Niger, Kaduna and Nasarawa. The incidence of pesticide residues was higher in maize and beans from Mile Twelve, Mushin and Ido markets than other markets in the study.

The results of this study show that there is a need for more stringent monitoring of the use of pesticides in agriculture and food storage in Nigeria. The residue concentrations

found for three of the pesticides (aldrin, dichlorvos and dieldrin) were above safety levels. They may pose serious threat to human health due to chronic toxicity. The combined effect of various pesticides when contaminated food is consumed over a long period of time is also of serious concern. More work however, needs to be done on a wide range of foodstuffs and the food consumption patterns of Nigerians in order to determine the actual daily intake of pesticide residues by Nigerians and hence the toxicological importance of this intake.

5.3 RECOMMENDATIONS

Pesticides are useful in food production and eradication of disease vectors but they are also poisons and constitute a group of environmental contaminants. Their use is therefore controlled and monitored by governments all over the world. For effective and efficient monitoring in Nigeria, it is recommended that a national pesticide monitoring programme be put in place by the National Agency for Food and Drugs Administration and Control (NAFDAC) which is the regulatory body, in conjunction with the Federal Ministry of Environment. This programme should then be used to carry out the following activities:

a. Education and Training – Farmers and other users of pesticides should be adequately educated and trained on the use of appropriate application methods and equipment, protective clothing, disposal of empty containers and unused stock and the observance of good agricultural practice (GAP). The general public also needs to be informed about pesticide residues in food and environment, so they can take appropriate precautions.

b. Laboratory Analyses – Pesticide analytical laboratories, equipped with necessary analytical facilities and trained personnel, should be established (at

least one in each of the six geopolitical zones). These should routinely analyze food stuffs and ensure that pesticide residue levels do not exceed FAO/WHO limits. These laboratories should also analyze imported pesticide products to prevent the influx of substandard products into the country.

c. Inspections – Regular inspection visits should be paid to pesticide distribution outlets and farms to ensure that banned products are not on sale or in use. These visits will also help to check inappropriate use of approved products.

d. Poison Centres – Poison centres should be established across the country for admission and/or treatment of victims of pesticide and other poisonings. This will provide data at the national level on incidence of pesticide poisoning.

e. Research – Intensive research should be initiated to develop national data on maximum residue limits of available pesticides on various foods and to develop integrated pest control methods for the local environment.

All these will help to minimize the risks involved in the use of pesticides while deriving optimum benefits.

5.4 CONTRIBUTIONS TO KNOWLEDGE

This study has made the following contributions to knowledge:

1. Data on the incidence and level of pesticide residues in different types of beans and maize in Lagos markets have been generated using gas chromatograph with mass selective detector (GC/MS) which is an analytical equipment known for its high sensitivity and specificity.

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2. Three classes of pesticides have been analyzed simultaneously. Previous studies in the country have analyzed single class residues, mainly organochlorines.

3. The incidence of pesticide residues in beans and maize occurring above maximum residue limits has been determined.

4. The toxicological importance of pesticide residue levels in maize and beans has been evaluated.

5. The effect of heat on pesticide residues in beans has been studied.

REFERENCES

Abou-Arab A.K., Soliman K.M. and Naguib K.H. (1998). Pesticide residue contents in Egyptian vegetables and fruits and removal by washing. *Bull. Nutr. Inst .Cairo, Egypt;* 18 (1): 117-137.

Adeyeye A. and Osibanjo O. (1999). Baseline levels of common organochlorine pesticides in some Nigerian foodstuffs. *Science and Total Environment;* 231: 227-230.

Alghali A.M. (1991). Studies on cowpea farming practice in Nigeria, with emphasis on insect pest control. *Tropical Pest Management;* 37 (1): 71-74.

Ambridge E.M. (1991). Pesticides in the tropics – benefits and hazards. In: *Chemistry, Agriculture and the Environment.* Richardson M. L. (Ed.); The Royal Society of Chemistry, Cambridge, U. K. p453-458.

Atuma S.S. and Okor D.I. (1986). A preliminary survey of organochlorine contaminants in Nigerian environment. *International Journal of Environmental Studies;* 26 (4): 321-327.

Atuma S.S. and Okor D.I. (1987). Organochlorine contaminants in human milk. *Acta Paediatrica Scandinavica,* 76 (2): 365-366.

Batora V., Vitorovic S., Their H. and Klisenko M. (1981). Development and evaluation of simplified approaches to residue analysis. *Journal of Applied Chemistry;* 53: 1039-1049.

Bishop D.D., Carter L.P., Chapman S.R. and Bennett W.F. (1983). *Crop Science and Food Production;* Academic Press, London. p194-211.

Blasco C., Fernandez M., Pena A., Lino C., Silveira M., Font G. and Pico Y (2003). Assessment of pesticide residues in honey samples from Portugal and Spain. *Journal of Agriculture and Food Chemistry;* 51 (27): 8132-8.

Bomford P. and Langley A. (2003). Farm Machinery. In: *The Agricultural Notebook ,20th Edition,* Richard J. Soffe (Ed.); Blackwell Science Ltd., Oxford, UK. p605- 655.

Borrell A. and Aguilar A. (2006). Organochlorine concentrations decline during 1987-2002 in western Mediterranean bottlenose dolphins, a coastal top predator. *Chemosphere;* 66 (2): 347-352.

Bottenberg H. (1995). Farmers' perceptions of crop pests and pest control practices in rain fed cowpea cropping systems in Kano, Nigeria. *International Journal of Pest Management;* 41 (4): 195-200.

Bottrell D.G. (1996). The research challenge for integrated pest management in developing countries: A perspective for rice in South Asia. *Journal of Agriculture and Entomology;* 13 (3): 185-193.

Chao H., Wang S., Lin T. and Chung X. (2006). Levels of organochlorine pesticides in human milk from central Taiwan. *Chemosphere;* 62 (11): 1774-1785.

Chrysoula V., Elisavet E. and Evan D. (2006). Determination of chlorinated volatile organic compounds in water and municipal wastewater using headspace-solid phase micro-extraction-gas chromatography. *Journal of Chromatography A;* 1132: 310-314.

Collins D. A. (2006). A review of alternatives to organophosphorus compounds for the control storage mites. *Journal of Stored Products Research;* 42: 395-426.

Consuelo S., Beatriz A. and Jose L. (2004). Multi-residue determination of pesticides in soil by gas chromatography-mass spectrometry. *Journal of Agriculture and Food Chemistry;* 52 (6): 1445-1451.

Croll B.T. (1995). The removal of pesticides during drinking water treatment. In: *Pesticides – Developments, Impacts and Controls.* Best G.A. and Ruthven A.D. (Ed.); Royal Society of Chemistry, Cambridge, United Kingdom. p124-134.

Crouse B. (2001). Minimizing Mouse Madness. *Pesticides and You;* 20 (4): 10-14.

Dallüge J., Beens J. and Brinkman U. (2003). Comprehensive two-dimensional gas chromatography: a powerful and versatile analytical tool. *Journal of Chromatography A;* 1000: 69-108.

Das P. and John G. (1999). Induction of sister chromatid exchanges and chromosome aberrations *in vivo* in *Etroplus suratensis* (fish) following exposure to organophosphorus pesticides. *Toxicology Letters;* 104:111-116.

Davies I.M. (1995). Pesticide usage in marine fish farming - The role of the chemist. In: *Pesticides – Developments, Impacts and Controls.* Best G.A. and Ruthven A.D. (Ed.); Royal Society of Chemistry, Cambridge, United Kingdom. p135-141.

De Brun A. (1976$_a$). Metabolism of Organic Agrochemicals. In: *Biochemical Toxicology of Environmental Agents.* Hutson D. and Roberts T. (Ed.); Biomedical Press, North Holland. p171 – 209.

De Brun A. (1976$_b$). Anticholinesterase Action – Organophosphates and Carbamates. In: *Biochemical Toxicology of Environmental Agents;* Hutson D. and Roberts T. (Ed.); Biomedical Press, North Holland. p981 – 1012.

Edgerley D.A. (1998). Techniques for improving the accuracy of calibration in the environmental laboratory. *14th Annual Waste Testing and Quality Assurance Symposium, California;* 181 – 187.

Evans W.C. (1996). Pesticides of Natural Origin. In; *Trease and Evans' Pharmacognosy, 14th Edition;* WB Saunders Company Ltd., UK. p534-537.

Ezugwu C.O and Okonkwo V.V. (1999). Impact of Pesticide Use on Health in Developing Countries. *Journal of Pharmacy Practice and Continuing Education,* 2: 6 – 15.

Fytianos K., Raikos N., Theodoridis G., Velinova Z. and Tsoukali H. (2006). Solid phase micro-extraction applied to the analysis of organophosphorus insecticides in fruits. *Chemosphere;* 65 (11): 2090-2095.

Gebefugi I. and Korte F. (1983). Pentachlorophenol contamination of human milk samples. *Chemosphere;* 12 (7/8): 1055-1060.

Guilhermino L., Soares A., Tinoco-Ojanguren R. and Osten J. (2004). Effect of pesticide exposure on acetylcholinesterase activity in subsistence farmers from Campeche, Mexico. *Archives of Environmental Health; 3: 1-30.*

Guo-Fang P., Yan-Zhong C., Jin-Jie Z., Chun-Lin F., Yong-Ming L., Xue-Min L., Guang-Qun J., Zeng-Yin L., Yu-Qiu S., Yan-Ping W. and Tong-Tong G. (2006). Validation study on 660 pesticides in animal tissues by gel permeation chromatography clean-up/gas chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry. *Journal of Chromatography A;* 1125: 1-30.

Hahn K., Heeschen W. and Bluthgen A. (1986). Occurrence and sources of persistent chlorinated hydrocarbons in human milk. *Milchwissenschaft; 41 (5):269-272.*

Hajšlová J. and Zrostlíková J. (2003). Matrix effects in (ultra) trace analysis of pesticide residues in food and biotic matrices. *Journal of Chromatography A;* 1000: 181-197.

Handa S., Agnihotri N. and Kulshrestha G. (1999). Pesticide Residues: Significance, Management and Analysis. Research Periodicals and Book Publishing House, USA. p1 – 31.

Hassall K. A. (1982). The Chemistry of Pesticides. The Macmillan Press Ltd., London, U.K. p97-261.

Heeschen W., Bluthgen A. and Nijhuis H. (1983). Feeds as a source of contamination of milk with HCH and aflatoxin. *Molkeneitechnik; 61:46-54.*

Hennion M. and Pichon V. (2003). Immuno-based sample preparation for trace analysis. *Journal of Chromatography A,* 1000: 29-52.

Hetzel G.H. (1996). Safe use of pesticides in agriculture. *Virginia Cooperative Extension,* Publication Number 442-036.

Hutson D. H. and Roberts T. R. (1987). Herbicides – Progress in Pesticide Biochemistry and Toxicology, Vol. 6. John Wiley & Sons Ltd. U.K. p105-137.

Igbedioh S.O. (1991). Effects of agricultural pesticides on humans, animals and higher plants in developing countries. *Archives of Environmental Health;* 4b (4): 218 – 227.

Jiankai Z. and Na L. (2006). Simple and environmental friendly procedure for the gas chromatographic-mass spectrometric determination of caffeine in beverages. *Journal of Chromatography A;* 1136: 106-110.

Jobling S., Reynolds T., White R., Parker M. and Sumpter J. (1995). Chemicals found to mimic human estrogens. *Environmental Health Perspectives;* 103: 582-587.

Kelce W.R., Stone C.R., Laws S.C., Gray L.E., Kemppainen J.A., Wilson E.M., (1995). Persistent DDT metabolite p,p'-DDE is a potent androgen receptor antagonist. *Nature;* 375: 581-582.

Kerkvliet N.I., (1994). Immunotoxicology of dioxins and related chemicals. In: *Dioxins and Health;* Schecter A. (Ed.) Plenum Press, New York, p199-225.

Kobayashi M., Nagayama T., Takano I., Ito M., Tamura Y., Tateishi Y., Kimura N., Kitayama K., Yasuda K. and Saito K. (2001). Survey of pesticide residues in baby (1996-1998). *Shokuhin Eiseigaku Zasshi;* 42 (4): 283-288.

Koprucu S., Koprucu K., Ural M., Ispir U. and Pala M. (2006). Acute toxicity of organophosphorus pesticide diazinon and its effects on behaviour and some haematological parameters of fingerling European catfish. *Journal of Pesticide Biochemistry and Physiology;* 86: 99-105.

Krieger N, Wolff M., Hiatt R., Rivera M., Vogelman J., Orentreich N. (1994). Breast cancer and serum organochlorines: a perspective study among White, Black and Asian women. *J. Natl. Cancer Inst.* 88 (8), 589-599.

Lefkovitz L., Neff J., Lizotte R. and Hall M. (2001). Comparison of two analytical methods for measurement of chlorinated pesticides and PCB congeners in biological tissue – Trends in Boston Harbor lobster tissue. *Boston: Massachusetts Water Resources Authority, Environmental Quality Department.* Report ENQUAD 2001-02; 51pp.

Leong K. H., Tan L. B. and Mustafa A. M. (2007). Contamination levels of selected organochlorine and organophosphate pesticides in the Selangor River, Malaysia between 2002 and 2003. *Chemosphere;* 66 (6): 1153-1159.

Leoni V., Cremisini C., Casuccio A. and Gulloti A. (1991). Separation of pesticides, related compounds, polychlorobiphenyls and other pollutants into four groups by silica-gel micro-column chromatography (Application to surface water analysis). *Pesticide. Science;* 31: 209-220.

Lever B.G (1990). The Advent of Crop Protection Chemistry. In: *Crop Protection Chemicals;* Sharp D.H. (Editor); Ellis Horwood, U.K. p44 – 56.

Mansour S.A. (1998). Perspectives on the use of daphnids in aquatic toxicology of pesticides. In: *Ecology of Wetlands and Associated Systems.* Majumdar S.K., Miller F.W., Brenner F.J. (Eds.); The Pennsylvania Academy of Science, Easton, PA, USA, p372-399.

Mansour S.A. (2004). Pesticide exposure – Egyptian scene. *Toxicology;* 198: 91-115.

Matisová E. and Dömötörová M. (2003). Fast gas chromatography and its use in trace analysis. *Journal of Chromatography A;* 1000: 199-221.

Maštovská K., Hajšlová J., Godula M., Křivánková J. and Kocourek V. (2001). Fast temperature programming in routine analysis of multiple pesticide residues in food matrices. *Journal of Chromatography A;* 907: 235-245.

Maštovská K., Hajšlová J. and Lehotay S. (2004). Ruggedness and other performance characteristics of low-pressure gas chromatography-mass spectrometry for the fast analysis of multiple pesticide residues in food crops. *Journal of Chromatography A,* 1054: 335-349.

Maštovská K. and Lehotay S. (2003). Practical approaches to fast gas chromatography-mass spectrometry. *Journal of Chromatography A*, 1000: 153-180.

Maximum Residue Limits (MRLs) Spreadsheet (2001). *http://www.pesticides.gov.uk*

Mengech A. N., Saxena K. N. and Gopalan H. N. (1995). Integrated pest management in the tropics: current status and future prospects. John Wiley & Sons Ltd., London. P152-157.

Miller G. (1995). Manuals of food quality control: Pesticide residue analysis in the food control laboratory. *FAO Food and Nutrition Paper;* 14(13): 1-98.

NAFDAC (2003). National Agency for Food and Drugs Administration and Control - Consumer *Safety Bulletin;* 2 (3): 6-17.

Naylor R.E.L. (2003). Crop health – responding to weed, diseases and pests. In: *The Agricultural Notebook, 20th Edition;* Richard J. Soffe (Ed.); Blackwell Science Ltd., Oxford, UK. Pp. 213-230.

Ndengerio-Ndossi J. and Cram G. (2005). Pesticide residues in table-ready foods in Tanzania. *International Journal of Environmental Health;* 15 (2): 143-9.

Niessen K., Ramolla J., Binder M., Brugmann G. and Hofmann U. (1984). Chlorinated hydrocarbons in adipose tissue of infants and toddlers: inventory and studies on their association with intake of mothers' milk. *European Journal of Peadiatrics;* 142 (4): 238-243.

Ochiai N., Sasamoto K., Kanda H. and Nakamura S. (2006). Fast screening of pesticide multi-residues in aqueous samples by dual stir bar sorptive extraction-thermal desorption-low thermal mass gas chromatography – mass spectrometry. *Journal of Chromatography A;* 1130: 83-90.

Ojosipe S. (2003). Pesticide residues in soft drinks. *The Quarterly Newsletter of the Institute of Public Analysts of Nigeria;* 4: 9–10.

Okereke G.U. and Nwosu V.C. (1987). Crop storage losses in Southern Nigeria caused by the activities of micro-organisms. *Journal of Applied Microbiology and Biotechnology;* 3 (3): 201-210.

Oluwole Y.A. (1980). Storage of Tropical Fruits and Vegetables: a review. *Report of the. Nigerian Stored Product Research Institute;* Technical Report No. 9: 95-107.

Osibanjo O. (2001). Overview of hazardous waste according to the Basel Convention – a paper presented at the awareness raising seminar on hazardous waste management organized by UNIDO/FAO/SBC/FMENV in Lagos, Nigeria.

Parry E.H. (1976). Food, Agriculture and Disease. In: *Principles of Medicine in Africa;* Oxford University Press, Ibadan. p58 – 59.

Pesticide Action Network (2001). The Hazards of Pesticide Use for Man and the Ecosystem. In: *Pesticides in Tropical Agriculture – Hazards and Alternatives,* Pesticide Action Network (Editor); p23 – 61.

Perrin R.M. (1995). Synthetic Pyrethroids Success Story. In: *Pesticides – Developments, Impacts and Control.* Best G. A. and Ruthven A. D. (Editors); Royal Society of Chemistry, Cambridge, UK. p19 -27.

Pesticide Analytical Manual (1999). Vol. 1: Multi-residue Methods. 3rd Edition; U. S. Department of Health and Human Services/FDA.

Pesticides (2006). http://news.bbc.co.uk

Petersen B.J. (2000). Pesticide residues in food: Problems and data needs. *Regulatory Toxicology and Pharmacology;* 31:297-299.

Podolska M. and Napierska D. (2006). Acetylcholinesterase activity in hosts (herring *Clupea harengus*) and parasites (*Anisakis simplex* larvae) from the southern Baltic. *ICES (International Council for the Exploration of the Sea) Journal of Marine Science;* 63(1): 161-168.

Poole C. F. (2003). Thin-layer chromatography: Challenges and opportunities. *Journal of Chromatography A;* 1000: 963-984.

Purdom P.W. and Anderson S.H. (1983). Identification of Toxic Pollutants. In: *Environment Science, 2nd Edition;* Charles E. Merrill (Pub.), U.K. p406 – 739.

Rasmussen R.R., Poulsen M.E. and Hansen H.C. (2003). Distribution of multiple pesticide residues in apple segments after home processing. *Food Addiives and Contaminants;* 20 (11): 1044-63.

Reece C.H. (1985). The Role of the Chemical Industry in Improving the Effectiveness of Agriculture. *Philosophical Transactions of the Royal Society of London;* B. 310: 201 – 213.

Renwick A.G. (2004). Pathways of Drug and Xenobiotic Metabolism. Elsevier, Oxford. p21 – 37.

Research and Development Report (1984). Agricultural Science Service. *Reference Book of the Ministry of Agriculture, Fisheries and Food, London.* 257 (84): 107 – 117.

Saito Y. and Jinno K. (2003). Miniaturized sample preparation combined with liquid phase separations. *Journal of Chromatography A;* 1000: 53-67.

Sanchez P.A., Casado L.E. and Jimenez L.R. (1982). Estimation of levels of contamination of milk by organochlorine residues. *Boletin del Centro Nacional de Alimentacion Nutricion;* 10:24-28.

Sandra P., Tienpont B. and David F. (2003). Multi-residue screening of pesticides in vegetables, fruits and baby food by stir bar sorptive extraction-thermal desorption-capillary gas chromatography-mass spectrometry. *Journal of Chromatography A;* 1000: 299-309.

Sesline D.H. and Jackson R.J. (1994). The effects of pre-natal exposure to pesticides. In: *Pre-natal Exposure to Toxicants: Development Consequences.* Needleman H.L. and Bellinger D. (Eds.); The John Hopkins University Press, Baltimore, p233-248.

Sheets T. and Pimentel D. (1979). Pesticides: Contemporary roles in agriculture, health and environment. Humana Press, Clifton, New Jersey. 186pp.

Shetlar D.J. (2007). Common insects and associated pests. The Ohio State University Extension Fact Sheet. http://www.ohioline.ag.ohio-state.edu

Smith R.M. (2003). Before the injection – modern methods of sample preparation for separation techniques. *Journal of Chromatography A;* 1000: 3-27.

Soliman K.M. (2001). Changes in concentration of pesticide residues in potatoes during washing and home preparation. *Food and Chemical Toxicology;* 39: 887-891.

Sowunmi O.O. and Agboola S.D. (1982). Some basic considerations in the use of chemical pesticides in the protection of agricultural crops. *NSPRI Occasion Paper Series;* p1 – 20.

Taylor R. (1990). Facts on Pesticides and Fertilizers in Farming. Aladdin Books, London. p10 – 12.

Turgut C. (2007). The impact of pesticides toward parrotfeather when applied at the predicted environmental concentration. *Chemosphere;* 66 (3): 469 – 473.

Ugbeye G. (2004). Regulatory control and monitoring of pesticides and pesticide residues. Seminar Paper presented at NAFDAC Central Laboratory, Oshodi, Lagos, Nigeria.

University of Strathclyde (2006). Safety Regulations. Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde

Valles S.M. and Koehler P.G., (2003). Insects used in the urban environment: mode of action. *University of Florida, IFAS (Institute of Food and Agricultural Sciences) Extension:* ENY282.

Venant A., Borrel S., Mallet J. and Gillon E. (1989). Changes in the contamination of foodstuffs of animal origin by pesticide and polychlorinated biphenyl residues. *Sciences des Aliments;* 9 (3): 473-489.

Wang L., Jiang X., Yan D., Wu J., Yongrong Bian Y. and Wang F. (2007). Behaviour and fate of chlorpyrifos introduced into the soil–crop systems by irrigation. *Chemosphere;* 66 (3): 391-396.

Ware G.W. and Whitacre D.M. (2004). An introduction to insecticides. In: *The Pesticide Book, 6th Edition;* MeisterPro Information Resources, Ohio, USA. p1-33.

Warren M.F. (2003). Farm business management. In: *The Agricultural Notebook, 20th Edition;* Richard J. Soffe (Ed.); Blackwell Science Ltd., Oxford, UK. p304-326.

Wei-Guo Z., Xiao-Gang C., Hui-Xia C., Juan A. and Chong-Jiu L. (2006). Simultaneous determination of 109 pesticides in unpolished rice by a combination of gel permeation chromatography and florisil column purification, and gas chromatography/mass spectrometry. *Rapid Experimental Commun. Mass Spectrom.* 20: 609-617.

WHO (1986). Carbamate pesticides: a general introduction. *Environmental Health Criteria (EHC) Series 64.*

WHO (1989). Dichlorvos. *Environmental Health Criteria Series 79.*

WHO (1991). Lindane. *Environmental Health Criteria Series 124.*

WHO (2001). Chemistry and Specification of Pesticides. *Technical Reports Series 899.* Geneva; 2001.

WHO/UNEP. (1990ₐ). Public Health Impact of Pesticides Used in Agriculture; 11 – 32.

WHO/UNEP. (1990ᵦ). Toxic Effects of Pesticides: Experimental and Clinical Data; 33 – 60.

WHO/UNEP (1990c). Sources and Indicators of Human Exposure to Pesticides; 61 – 100.

Willett J. E. (1987). Gas Chromatography – Analytical Chemistry by Open Learning (ACOL). John Wiley and Sons, London; 253pp.

Yu Z., Palkovicova L, Drobna B., Petrik J., Kocan A., Tomas Tmovec T. and Hertz-Picciotto I. (2007). Comparison of organochlorine compound concentrations in colostrum and mature milk. *Chemosphere;* 66 (6): 1012-1018.

Zahm S.H. and Ward M.H. (1998). Pesticides. *Environmental Health Perspectives;* 106 (3): 893-908.

Zawiyah S., Yaakob B., Che M., Nazimah S., Abdul H. and Chin C. (2006). Determination of organochlorine and pyrethroid pesticides in fruits and vegetables using solid phase extraction clean-up cartridges. *Journal of Chromatography A;* 1127: 254-261.

APPENDIX

Codes, Markets and Sources of Maize and Beans Types Studied

MAIZE			BEANS		
Code	Market	Source	Code	Market	Source
AGM	Agege	Zaria, Minna	AGB	Agege	Maiduguri
BAM	Badagry	Ijebu	BAB	Badagry	Sokoto
EPM	Epe	Kano, Nasarawa	EPB	Epe	Kano
IDM	Ido	Kano	IDB	Ido	Maiduguri, Potiskum
IKM	Ikorodu	Zaria	IKB	Ikorodu	Sokoto, Maiduguri
MTM	Mile 12	Kano, Kotangora, Zaria	MTB	Mile 12	Maiduguri, Minna
MUM	Mushin	Maiduguri	MUB	Mushin	Maiduguri