STUDIES ON IN VIVO AND IN VITRO EFFECTS OF PRE-EXPOSURE TO LINDANE ON COUMAPHOS TOXICITY IN RATS AND ISOLATED RABBIT ILEUM.

BY

RAMON-YUSUF, SULEIMAN BABATUNDE (DVM).

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FACULTY OF VETERINARY MEDICINE
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ZARIA, NIGERIA.

JANUARY, 1993.

DECLARATION

I hereby declare that the work presented in this thesis was carried out by me. No previous submission of it has been made elsewhere for a higher degree. The works of other researchers are fully acknowledged and referenced.

DIGITIZED



JAN. 8, 1993

RAMON-YUSUF, SULEIMAN BABATUNDE (DVM)
DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY
FACULTY OF VETERINARY MEDICINE
AHMADU BELLO UNIVERSITY
ZARIA, NIGERIA

DATE

CERTIFICATION

This thesis titled STUDIES IN VIVO and IN VITRO EFFECTS OF PRE-EXPOSURE TO LINDANE ON COUMAPHOS TOXICITY IN RATS AND ISOLATED RABBIT ILEUM submitted by RAMON-YUSUF, SULEIMAN BABATUNDE meets the regulations governing the award of the degree of MASTER OF SCIENCE (ENVIRONMENTAL TOXICOLOGY) of Ahmadu Bello University, and is approved for its contribution to science and Literary presentation.

Internal Examiners

Dr. T. T. Iyaniwura, B.Sc., Ph.D. — Date: 8/1/93
Department of Pharmacology and Clinical Pharmacy
Faculty of Pharmaceutical Sciences
Ahmadu Bello University, Zaria

Professor Y. O. Aliu, DVM, Ph.D. — Date: 8-1-93
Department of Physiology and Pharmacology
Faculty of Veterinary Medicine
Ahmadu Bello University, Zaria

Dr. I. M. Hussaini B.(Pharm.) Ph.D. (Lond.) — Date
Department of Pharmacology and Clinical Pharmacy
Faculty of Pharmaceutical Sciences
Ahmadu Bello University, Zaria

External Examiner

Professor E. N. Sokomba B.Sc.(Pharm), Ph.D. — Date: 8/1/93
Dean, Faculty of Pharmaceutical Sciences
University of Jos,

Postgraduate School

Professor D. Mohammed, B.A., Dip.(Lings. & Phons), Ph.D.
Dean, Postgraduate School,
Ahmadu Bello University, Zaria. — Date: 17/1/96

DEDICATION

This thesis is dedicated to my lovely daughter Habiba Ifedolapo for the joy she brings to my life daily and to my wife Halima Kimberly for her love, and unparalleled belief in my ability.

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GLOSSARY OF ABBREVIATIONS

EPN - O,ethyl-o-p-nitrophenylphosphonothioate

ACH - Acetylcholine

ACHE - Acetylcholinesterase

MFOs - Mixed Function Oxidases

OP - Organophosphorus

AliE - Aliesterase

TOCP - Triorthocresyl phosphate

OPIDN- Organophosphorus-induced Delayed Neurotoxicity

CaM Kinnase II - Ca2+-Calmodulin-dependent ptotein

NTE - Neurotoxic Esterase

DPF - Di-isopropylphosphorofluoridate

ABSTRACT

Investigations were conducted into the in vitro and in vivo effects of pre-exposure to lindane on coumaphos toxicity.

In vitro Lindane was found to attenuate the response of the cholinergic receptor to coumaphos. When the calcium ion concentration of the medium was reduced by use of the calcium antagonist nifedipine, an augmentation of the effect of Lindane was observed.

This effect became more profound as indicated by weaker muscular contractions in a calcium - free medium.

A 10-week subchronic toxicity study in rats suggests that pre-exposure to Lindane offers some protection against coumaphos toxicity as indicated by the mildness of the histopathological lesions in groups of animals pretreated with Lindane vis-a-vis those that did not receive lindane pretreatment prior to exposure to the same dose levels of coumaphos.

The intraperitoneal median lethal dose (LD_{50}) of coumaphos in rats was found to be 139.6 mg/kg.

In Lindane pre-teated and non pre-treated animals, coumaphos did not affect the organ; body weight ratio.

CHAPTER ONE

1.1.0 Introduction

In most parts of the world, there is at one time or the other, the need to chemically control pests and diseases either of livestock or plants. In this regards, pesticides are quite useful and occupy a rather unique position among the many chemicals that man encounter daily in that they are deliberately added to the environment for the purpose of killing or injuring some form of life (Murphy, 1980).

Excessive loss of food crops to insects and other destructive pests contribute to an obvious health problem - starvation. In countries faced with this problem, the use of chemicals for controlling these pests has a favourable cost-benefit relationship (Murphy, 1980). For example, cocoa production rose in Ghana by 110% in 1957, when pesticides were introduced to protect cocoa pods from infestation with Phytophthora palmivora, the cause of the blackpod disease. Japan in 1960 also recorded an increase in rice production when insecticides were introduced to protect rice from the stemborer Pricularia orizae (Fest *et al.*, 1982).

It has been estimated by the Food and Agricultural Organization (FAO) of the United Nations that cessation of all use of crop protection chemicals in the USA would reduce total

output of crops and livestock by 30% and would increase the price of farm products to the consumer by between 50% and 70% (Green, 1976).

The need for pesticides extends beyond their role in crop protection. Substantial livestock and poultry losses occur as a result of diseases, insects and internal parasites. Pesticides are invaluable in the control of vector-borne diseases and have been found to be the most effective means of controlling some diseases such as onchocerciasis where pesticides have to be entirely depended upon for the destruction of vector simuliids. Presently no drug is available that may be used in mass control programmes of the disease (WHO, 1985).

Overall pesticides use in agriculture, in terms of amounts applied per hectare has been very much greater in Japan, Europe and the United States than in the rest of the world. Although China is a major user, the fastest growing market however is Africa with an increase in sales of 18.2% between 1980 and 1984 (Edwards, 1986).

In Nigeria, although there is a dearth of statistics on pesticide production and usage, there is evidence of a rise in usage from 1975 to date judging by the increased agricultural activity in the country especially occasioned by such government programmes as the "Operation Feed the Nation" and "Green

Revolution" as well as the advent of large scale farms. The economic inclemency in the wake of the Structural Adjustment Programme (SAP) has also turned virtually everyone into at least a part-time farmer. This increased agricultural activity is associated with a rise in pesticide usage and the attendant hazard to man and his livestock. Since pesticide toxicity is not restricted to the target organisms and some of these pests tend to share the environment rather intimately with humans, their chemical control without concurrently injuring the well-being of both humans and other living things has become of great importance.

The ideal pesticide would be highly toxic to the pest, but quite innocuous to other forms of life with which it comes into contact. However, the ideal pesticide does not exist (Turner, 1982).

The widespread use of preventive crop protection treatments results in large amounts of pesticides being applied when they may not actually be needed. This increases the possibility of some risk to the environment leading to increased attention being focused upon possible biologic effects of chronic, low-level exposure to these agents (Green, 1976); Santodicito and Morrison, 1971).

In practice, however, pesticides are often used in combination in plant protection and public health programmes to overcome resistance, as well as to increase the efficacy of the compound. The phenomenon of the interaction of several insecticides gains importance therefore, particularly as it could influence the toxicological profile of the individual insecticide (Dikshith et al., 1987). Many reports of pesticide interactions exist in the literature (Frawley, 1957; Murphy, 1969; Dikshith et al., 1987; Iyaniwura, 1989; Iyaniwura and Gubio, 1990; Iyaniwura, 1990a; Iyaniwura, 1990b).

In Nigeria, the potential interaction of pesticide combination is real, since farm workers are exposed to many of these chemicals during each farming season while livestock are not immune from such combined exposures. The insufficient information on combined toxicity of pesticides in Nigeria necessitates this study which is an attempt at assessing the interaction of organophosphates and organochlorines *in vivo* and at the receptor level *in vitro*.

The main objectives of the study are:

1. Determination of the effect of pre-exposure to Lindane on the response to coumaphos *in vitro*

2. Evaluation of the effect of Lindane on cholinergic receptor activation by Acetylcholine and response coupling.
3. Effect of calcium ion concentration on the response to Lindane at the Postsynaptic membrane.
4. Effect of calcium antagonist (Nifedipine) on response to Lindane at the Postsynaptic membrane.
5. In vivo determination of the effect of pre-treatment with Lindane on subchronic toxicity of coumaphos in rats.
6 Determination of the LD_{50} of coumaphos in rats.

CHAPTER TWO

2.1.0 LITERATURE REVIEW

The Food and Agricultural Organization (FAO) (1986a) defined a pesticide as any substance or mixture of substances intended for preventing, destroying or controlling any pest including vectors of human or animal disease, unwanted species of plant or animals causing harm during, or otherwise interfering with production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products, or animal feedstuff, or which may be administered to animals for the control of insects, Arachnids or other pests in or on their bodies.

Systemic pesticides are usually first absorbed by the host and then by its parasites and are particularly suitable for the control of those livestock pests that spend part of their life-cycle in the body of the host where they are inaccessible to topical treatment. Cattles grubs, the larval stages of the warble flies Hypoderma bovis and H. Lineatum are striking examples of this type of economically important parasites (Khan, 1973). Contact pesticides are usually applied on the surface of livestock and plants either as dusts or sprays whereby they destroy pests as they infest the host.

For a very long time the pesticides in use were primarily compounds of heavy metals and those of plant origin, but since

the second half of the 1940s there has been a marked increase in total pesticide usage and a rapid proliferation of sythetic organic compounds. In 1969, there were approximately 900 chemicals registered for sale as pesticides against 2000 pest species in the United States of America (Secretary's Commission on Pesticide, 1969). World production and use of pesticides continue to rise with a 10-fold increase in production between 1955 and 1985 (Rosenstock *et al.*, 1991).

Acute pesticide poisoning are an important cause of morbidity and mortality world wide (W.H.O., 1986, 1990). Much of this burden is borne by developing countries where 99% of fatal pesticide poisonings occur and where 25 million episodes of intoxication occur annually among agricultural workers alone (Jeyaratnam, 1985, 1990).

The problem is however not restricted to this area of the world, for example, the estimated incidence of pesticide - related disease in the United States of America is between 150,000 and 300,000 a year (Coye, 1985).

Man becomes exposed to pesticides in several ways Dermal, respiratory and gastrointestinal absorption are all potential routes of intoxication, although absorption through the skin is the most significant and usual route of occupational intoxication (Rosenstock, 1987). Occupational exposure occurs

during the manufacture, transportation and application. These exposure could produce acute or chronic toxic effects (Rosenstock, 1987). Accidental poisoning by consumption of crops sprayed with pesticides is another means of human intoxication and is also a veritable means by which livestock becomes poisoned. Organophosphorous compounds are usually responsible for the majority of such cases due to their extensive use in agriculture (Abdelsalam, 1987). Occasionally animals may become intoxicated when their premises are dusted or sprayed or animals may be directly dusted or painted with excessive amounts of pesticide (Hatch, 1982). The opportunities for exposure of humans and other non-target species to pesticides are considerable: it is also apparent that exposure to pesticides far remote from the source of application are possible. This results from the translocation of these chemicals from their site of application through the various media of the environment (Murphy, 1980). Increased control and regulation of pesticide use should prevent exposure to toxicologically significant quantities. Government must aim at enacting legislation which will minimise these risks without seriously detracting from the benefits (Green, 1976).

In evaluating the potential health-related effects of all classes of pesticides, it is important to think not only of the active ingredients but also of the carriers, which are often added to enhance delivery; carriers include talcs, oils, solvents and binding agents. These so-called inert ingredients

may themselves have toxic properties explaining some of the acute and persistent health effects, acting as irritants, and less commonly sensitizing agents in exposed individuals (Rosenstock, 1987).

2.1.2 Pesticide Formulation

The preparation of the active material in a form which is convenient for its intended use is known as formulation (Hassall, 1982). Pesticides are rarely applied in their original form (Speight, 1980). Most active ingredients are very biologically active and it would be difficult to spread them evenly over a field at a rate of less than about 1 kg/ha if they were not diluted in some way (Hassall, 1982). In addition, many are greasy, organic substances insoluble in water, so simply mixing substances with water in spray tank would be very inefficient. A common maxim is that no active ingredient can perform more adequately in the field than its method of formulation will allow.

An active ingredient may be applied in the dry state, in which case it may be formulated and applied as a dust or as granules. It may also be applied as, or in the presence of a liquid. The three major formulations for this purpose are the aqueous concentrate, the wettable powder and emulsifiable concentrate, each of which is diluted by addition of water in

the spray tank. Miscellaneous formulations of occasional importance are smoke generators, aerosols, emulsified liquid, poison bait and microcapsules (Hassall, 1982). Some of the formulations most widely used in agricultural outlets have been tabulated by Speight, 1980 (Table 1).

Table 1 Some of the formulations most widely used in agricultural outlets (Speight, 1980)

Formulation Type	Physical State	How Applied
Emulsifiable concentrate	Liquid	Diluted with water
Water-miscible concentrate	"	"
Aqueous solution	"	"
Suspension concentrate	"	"
Mayonnaise Emulsion	"	"
Ultra low vol. concentrate	"	without further dilution
Spreading oil	"	"
Fogging concentrate	"	(Usually) diluted with solvent
Dust	solid	Without further dilution
Granule	"	"
Wettable powder	"	Diluted with water.

In the preparation of pesticidal dusts or powder, a suitable sorptive material is impregnated with a concentrated solution of the pesticide. The solvent is then evaporated and the powder diluted with a large bulk of the same sorptive substance. The minerals whose particles are impregnated with pesticides are of two kinds: the clays which include kaolinite and attapulgite (fuller's earth) and the silicas which include talc, diatomite and pumice (Polon, 1973). The use of synthetic silica is considered less desirable due to its association with silicosis (Speight, 1980).

Most dusts are so prepared that almost all particles are less than 50um diameter and the majority normally lies within the range of 3 to 30um. Although small particles are often more efficient than larger ones, they are also a greater inhalation hazard (Hassall, 1982).

Wettable powders are solids so formulated that they disperse when stirred into water in the spray tank. They are thus quite different from dusts which are applied dry. Wettable powders consist of an active ingredient which may be solid or liquid, one or more spreading and dispersing agents, and a solid, inert diluent or filler.

Effects of Formulation on the Hazards of Pesticides.

Virtually all pesticide formulation posses a toxicological hazard and some have an additional hazard-flammability. The toxicological hazard of a formulation is a combination of the inherent toxicity of the pesticide and other ingredients together with exposure (Speight, 1980). The most important characteristics of pesticide formulations that affect toxicological hazard are probably physical properties and composition.

A dust cloud from a wettable powder formulation is likely to contain a relatively high pesticide content and remain suspended in the atmosphere for much longer than dust formulation thus presenting a potential toxic hazard. The pesticide concentration of liquid formulation varies considerably from relatively low (100g/litre) to very high (>800g/litre) and since they exist as bulk liquids, prior to use the inhalation exposure is not as great as for wettable powders and dust. However, because of their low viscosity they may readily spread over the skin and give rise to dermal exposure. Gross spillage of liquid formulation can quickly lead to contamination of large floor areas, drains and other channels. Mayonnaise emulsions and certain suspension concentrate formulations are much more viscous and do not spread so rapidly on surfaces thus presenting a lesser dermal exposure (Speight, 1980).

Organic solvents are often used as "carrier" in pesticide formulation; xylene and closely related materials are good solvents for organophosphate and organochlorine insecticides. Solvents influence the percutaneous toxicity of formulations altering the dermal penetration. Speight (1980) reported, after a single dose acute toxicity study of dicrotophos, that solutions in cotton seed oil and xylene (LD50 approximately 50mg/kg to male rats) were more toxic than solutions in isopropanol or water (LD50 190 and 140mg/kg respectively).

Brown and Muir (1971) indicated that liquid formulations of chlorfenvinphos and monocrotophos were more toxic via the percutaneous route than solid formulations.

Carriers used in solid formulations are often innocuous and are therefore less likely to constitute a hazard than are solvents used for emulsifiable concentrates, etc. (Speight, 1980).

2.1.3 History of Pesticide Use

The use of inorganic chemicals to control insects possibly dates back to classical Greece and Rome.

Homer as quoted by Hassall (1982) mentioned the fumigant value of burning sulfur, and pliny cited by Hassall (1982)

advocated the insecticidal use of arsenic and referred to the use of soda and Olive oil for the seed treatment of legumes. The Chinese were employing moderate amounts of arsenicals as insecticides by the sixteenth century and not long afterwards nicotine was used, in the form of tobacco extracts.

By the nineteenth century both pyrethrum and soap had been used for insect control and also a combined wash of tobacco, sulfur, and lime to combat insects and fungi.

The middle of the nineteenth century marked the beginning of the first systematic scientific studies into the use of chemicals for crop protection. Work on arsenic compounds led to the introduction in 1867 of Paris green an impure form of copper arsenite. It was used in the USA to check the spread of the Colorado beetle and by 1900 its use was so widespread that it led to the introduction of what was probably the first pesticide legislation in the world.

In 1896 a french grape grower, applying Bordeaux mixture (Copper sulphate and calcium hydroxide) to his vines, observed that the leaves of yellow charlock growing nearby turned black. This chance observation demonstrated the possiblity of chemical weed control, and shortly afterwards it was found that iron sulphate, when sprayed un-to a mixture of cereal and weeds, killed the weeds without damaging the crop. Within a decade,

several other inorganic substances had been shown to act selectively at appropriate concentrations. Another important landmark was the introduction of the first organomercury seed dressings in 1913 in Germany.

In the years between the first and second world Wars, both the number and the complexity of chemicals for crop protection increased. Tar oil is used to control the eggs of aphids on dormant trees. Dinitro-orthocresol was patented in France in 1932 for the control of weeds in cereals and in 1934 thiram, the first of several dithiocarbamate fungicides was patented in the USA.

During the second World War, the insecticidal potential of DDT was discovered in Switzerland and insecticidal organophosphorus compounds were developed in Germany. At about the same time work was in progress in the United Kingdom that was to lead to the commercial production of herbicides of the phenoxyalkanoic acid group. In 1945 the first soil-acting carbonate herbicides were discovered by workers in the United Kingdom and the organochlorine insecticide chlordane was introduced in the USA and in Germany. Shortly afterwards the insecticidal carbonates were developed in Switzerland.

In the period from 1950 to 1955 Urea derivatives were developed as herbicides in the USA. The fungicides capton and

glyodin appeared, and malathion was introduced about the same period. Between 1955 and 1960 other new products included herbicidal triazines and quaternary ammonium herbicides. Dichlobenil, trifluralin, and bromoxynil were described between 1960 and 1965 and the systemic fungicide benomyl in 1968; shortly after the leaf-acting herbicide glyphosate was introduced.

In the 1970s and 1980s many new pesticides were introduced based on a more thorough understanding of biological/biochemical mechanisms are often more effective at lower doses than the older pesticides. Example of these new generation pesticides include the herbicidal sylfonylureas and the synthetic light-stable pyrethroid insecticides developed from the naturally occurring pyre-thrins (Hassall, 1982).

As a result of a better knowledge of host-pest interactions, a new approach to the design of pesticides is now being developed (for example, dieldirns is now being used in place of the more persistent ·DDT for aerial spraying) as well as new strategies for formulation and new methods of application. These developments provide an opportunity to reduce risk of pesticide poisoning.

The potential usefulness of microbial and other biological pest control agents is at present being studied by several institutions throughout the world (W.H.O., 1990).

General Uses of Pesticides

Apart from their potential use as agents of chemical warfare, i.e. the so-called 'Nerve gases' (McCombie and Saunders, 1946), the organophosphorus esters are generally beneficial to mankind, being widely used in both industry and agriculture. They are invaluable as plasticizers, gasoline additives, stabilizers in lubricating and hydraulic oils, and as flame retardants for various fabrics (Hamilton and Hardy, 1974). In agriculture, they are widely used as pesticides, anthelmintics (Clarke and Clarke, 1975; Bartik and Piskac, 1981) and as chemical defoliants (Abou-Donia *et al.*, 1980b).

The organochlorine pesticides, especially, DDT, have attracted the most attention in their wide applications in the area of public health and veterinary medicine (Brooks, 1974). One of the most important applications of DDT in disease vector control is against the malaria-carrying mosquito. Other important human diseases where halogenated hydrocarbon pesticides have been effective in controlling the relevant insect vectors include typhus, plagues, onchocerciasis, 'chaga' disease, African trypanosomiasis, yellow fever and leishmaniasis

(Busvine and Pal, 1968). With such a wide diversity of applications, it is clear that the potential for human and animal exposure is relatively great (Stuart, 1982).

2.1.4 Exposure to Pesticide Combinations

Combinations of pesticides are used in plant protection and public health programmes to overcome resistance, as well as to increase the efficacy of the compounds. The phenomenon of interaction of several insecticides gains importance, therefore, particularly as it could influence the toxicological profile of the individual insecticide (Dikshith *et al.*, 1987). Food crops at various stages of growth may be exposed to different combinations of pesticides: insecticides, fungicides herbicides and postharvest to pesticides used in crop preservation (Iyaniwura, 1990). The phenomenon whereby the action of a given compound may increase the toxicity of another compound is called potentiation and the first compound is said to potentiate the toxicity of the second. Where the toxicity of two compounds given together is greater than the additive effects of the two compounds given separately, this is called synergism (O'Brien, 1967).

The synergistic effects of pesticides on various pests have been widely used in agriculture, synerphos for instance is a synergistic combination of several organophosphates, (Iyaniwura,

1990). A mixture of malathion and EPN (O'ethyl-o-p-nitrophenyl phenylphosphonothioate) was found to be more toxic to dogs than the total effect of the two insecticides would have been if they had been given separately (Frawley *et al.*, 1957). Malathion is one of the insecticides that have been observed most frequently as one constituent of potentiating pair of organophosphate insecticides. This normally relatively safe insecticide is detoxified by carboxylesterases that are inhibited by other organophosphorus (OP) insecticides (Murphy, 1980). The toxicity or the duration and intensity of pharmacological actions of several drugs have been shown to be altered by pre-exposure of animals to organochlorine insecticides (Murphy, 1969). Iyaniwura and Gubio (1990) also reported that pre-treatment with lindane offered some protection to carbaryl toxicity in vivo. Similarly, pre-treatment with drugs such as chlorcyclizine and phenobarbital has been known to reduce susceptibility of mice to parathion and malathion (Welch and Coon, 1964).

When pesticides in combination are acting additively, prediction of toxicity of mixtures may be a simple numerical problem once the individual toxicity and fractional composition are known. However, in vivo interaction of one pesticide with the mechanisms of detoxification or activation of another may result in significant potentiation or inhibition of toxicity (Iyaniwura, 1990). Pesticide interactions in vitro have also

been reported, for instance the potentiation of aldicarb by carbofuran and oxamyl (Iyaniwura, 1989).

Formulation impurities on interacting with active ingredients may elicit pesticide toxicity in man, since pesticides are usually formulated as a mixture of technical substances with solvents and additives some of which may be toxic (Iyaniwura, 1990; Rosenstock, 1987).

Pellegrini and Santi (1972) demonstrated that impurities present in technical grade samples of malathion and phenthoate potentiate the toxicity of these compounds, thus accounting for the greater toxicity of technical samples as compared to highly purified samples. These impurities may also account for the apparent "self-potentiation" reported by Murphy (1967). The presence of isomalathion as an impurity played a significant role in the reported cases of human poisoning and fatalities among malaria control workers in Pakistan (Baker *et al.*, 1978).

Two major sources of contamination of pesticide formulations have been identified (Rosival and Batora, 1980). Formulation ingredients may contain impurities, for instance, Toxaphene formulated by chlorinating camphene of technical grade may contain impurities. The manufacturing process itself may be by routes which lead to the formation of biologically active by-products, for instance, ethylene thiorea is formed in ethylene

bis-dithiocarbamate. Polychlorinated dioxins and furans are formed as by-products in the synthesis of pentachlorophenol and hexachlorophene (Rosival and Batora, 1980). Unsuitable formulation of technical grade active ingredients is a secondary source of contamination; such as the isomerization of organophosphorous thioates and dithioates.

2.1.5 Mechanisms of Pesticide Interaction

The mechanisms underlying the interaction (Synergism) among organophosphorus insecticides (Dubois, 1969), Murphy, 1969) and insecticides in general (Street, 1969), Diechman *et al.*, 1971; Iyaniwura, 1990) have been reviewed.

Two major mechanisms are believed to be involved in the synergism among organophosphorus insecticides viz: inhibition of detoxification by tissue carboxylesterase (aliesterase) and amidases, and competition for nonvital binding sites that normally act as a buffer system to spare the vital acetylcholinesterase enzyme (Murphy, 1969). Thus compounds that have a high potency as inhibitors of carboxylesterase relative to their anti-cholinesterase potency are likely to potentiate other organophosphorus insecticides or to alter the toxicity of other drugs and chemicals containing carboxylester or amide linkages (Murphy, 1980). Testing for carboxylesterase inhibiting potency of organophosphates has been advocated by Su *et al*, (1971) as useful tests in acute studies and subacute

feeding experiments aimed at screening for potentiating organophosphorus compounds.

Pesticide interaction also occurs at the various stages of *absorption, distribution, biotransformation and excretion* (Dubois, 1969; Murphy, 1969; Iyaniwura, 1990). During the distribution phase for instance, one pesticide may alter the distribution of another thus altering its toxicity. This is examplified by the prevention of paroxon from binding to various tissue proteins by triorthocresyl phosphate when used in pretreatment of laboratory animals prior to paraoxon exposure. This resulted in an increase in the degree of acetlcholinesterase inhibition and consequent toxicity (W.H.O., 1981). Organochlorines have also been shown to increase the non-catalytic binding sites of organophosphates (Dubois, 1969; Murphy, 1969).

Certain chemicals exert some modifying effects upon the liver microsomal enzymes responsible for the metabolism of toxic agents therefore altering their toxicity. These effects on the microsomal enzymes could be induction or inhibition. The chlorinated hydrocarbons are prominent among compounds exerting the former (induction) effect on the drug metabolizing enzymes (Conney *et al.*, 1972). Pretreatment of animals with the organochlorine pesticide aldrin resulted in increased rate of detoxification of parathion thus causing a significant decreases

in parathion toxicity (Ball *et al*., 1954; Mark, 1967). Similar effects of another organochlorine (Ovex) on parathion toxicity has also been reported by Black *et al*., (1973). Potentiation of organophosphate toxicity by organochlorines can also be cited, for instance dieldrin enhances the acute toxicity of dimethoate (WHO, 1981) while chlordane potentiates parathion and malathion toxicity in mice.

Organochlorine combinations have been shown to interfere with the storage and metabolic excretion of one another. Storage of dieldrin and DDT were reduced in adipose tissues when they were fed in combinations and this effect has been attributed to accelerated rate of metabolism and excretion possibly by induction of microsomal enzymes (Street *et al*., 1969)

Kaloyanova (1983) suggested that resistance and cross resistance in insects after they have been sprayed with various pesticide formulations may be due to the ability of pesticides particularly organochlorines to stimulate their own metabolism and biotransformation of other pesticides.

Combination of Lindane, thiuram and heptachlor have been found to exhibit potentiation in rats and cats (Kagan, 1981). Heptachlor is metabolized to the toxic epoxide, the potentiation of heptachlor toxicity by lindane is possibly due to its

induction of the mixed-function oxidases, with the microsomal liver fraction producing the toxic epoxide (Kaloyanova, 1983).

Unlike their chlorinated hydrocarbon counterparts, organophosphates have been shown to be inhibitors of hepatic microsomal enzyme (WHO, 1981). The consequence of this is a decrease in the rate of metabolism of exogenous compounds and an increase in their effects and/or toxicity through a variety of mechanisms such as depression of enzyme synthesis. The organophosphates are hydrolysed by aliesterases; such that compounds capable of inhibiting the aliesterases are most likely to potentiate organophosphate toxicity (Dubois *et al.*, 1969).

Pesticide interactions have also been known to occur when concentration of toxic agents for instance exceed the capacity of renal tubular transport which is an active energy requiring process (WHO, 1981). Diechman *et al.*, (1971) reported that DDT fed in combination with aldrin resulted in greater accumulation of these compounds in fat and blood. The interaction between these compounds seems to have caused a decrease in their rate of elimination.

Certain interactions lead to increased or decreased toxicity of organophosphorus compounds. For instance, three to four days after administration of the organochlorine insecticide, aldrin, the toxicity of parathion to female rats was found to decrease

by about seven-fold (Ball *et al.*, 1954), while Main in 1956 showed that oral but not the intravenous toxicity of paraoxon was decreased by aldrin pretreatment with the same effect being exerted by aldrin on both oral and intravenous toxicity of parathion. The same author also reported that aldrin pretreatment doubled liver A-esterase activity and decreased serum A-esterase by 50 per cent. He therefore concluded that the mechanism by which aldrin reduced parathion toxicity was by stimulation of the hydrolysis of paraoxon in the liver after it was formed from parathion within this organ.

Triolo and Coon (1966a) confirmed the protective action of aldrin against parathion in mice and extended these studies to include several other organophosphorus insecticides and some anticholinesterase drugs. Their studies showed that Dieldrin and chlordane also protected against parathion and that ethionine prevented both the aldrin-induced elevation of liver A-esterase and the protection against parathion, which suggested that aldrin acts to stimulate synthesis of the liver A-esterase. They speculated that, since mice were not protected against compounds such as octamethylpyrophosphoramide (OMPA) and neostigmine, which do not readily penetrate the blood-brain barrier, aldrin might also act to alter the penetration of the insecticides into the brain or decrease the susceptibility of the brain cholinesterase inhibition.

Sequel to in vitro enzyme studies, about four plausible metabolic mechanisms have evolved to attempt to elucidate the protective action of pre-exposure to the various drugs and chemicals against poisoning by phosphorothioates viz: (i) increased liver A-esterase (ii) increased rate of formation of oxygen analogs to provide a substrate for A-esterase (iii) increased non-critical binding sites (iv) increased rate of direct cleavage of the parent (P = S) compounds.

The effectiveness of each of these mechanisms is determined by the pre-tratment chemical and the insecticide in question (Murphy, 1969).

Interactions of organophosphorus compounds with certain chemicals have also been shown to increase their toxicity. O'Brein (1967) reported that pretreatment with pentobarbital which can induce microsomal enzymes, increased the susceptibility of mice to piosoning by dimethoate and, to a small extent, by Guthion(R) (azinphosmethyl). Also susceptibility of female rats to Guthion has been found to be increased by pretreatment with methylcholanthrene (Murphy, 1969)

The potentiation of aldicarb toxicity by carbofuran and oxamyl in vitro was suggested to be due to competition for binding sites on proteins by the pesticides (Iyaniwura, 1989).

2.2.0 ORGANOPHOSPHORUS (OP) INSECTICIDES

2.2.1 General Introduction

The organophosphorus compounds are complex organic molecules which are generally regarded as esters of phosphoric acid or its derivatives (Heath, 1961). They are normally esters, amides or thiol derivatives of phosphoric, phosphonic, phosphorothioic or phosphoniothioic acid (WHO, 1986).

Over 50,000 organophosphorus compounds are known, (Koller and Klawans, 1979) and at least 100 OP insecticides have been reviewed by WHO for consideration as agents for the control of disease vectors (WHO, 1986). OP pesticides are used as contact insecticides and acaricides; animal systemic or topical insecticides and parasiticides (Hatch, 1982, Khan, 1973).

Because of the attractiveness of these agents in terms of their shortlived residues and minimal persistence in the environment, these chemicals have become of increasing commercial importance (Rosenstock, 1987). Concomitant with their more prominent role as pesticides is an increase in intoxications with OP compounds (Wasserman and Wiles, 1985; Midtling *et al.*, 1985; Murphy, 1980) such that acute organophosphate pesticide poisonings cause substantial morbidity and mortality world-wide (Rosenstock, 1991).

Ronnel (O, *o-dimethyl* O-2, 4, 5,-*trichlorophenyl* phosphorothiate) was the first OP compound to be used as a systemic insecticide for livestock. It was effective for systemic control of cattle grubs (the larval stages of the warble flies Hypoderma bovis and was used in the form of a bolus at a dose of 100mg per kg (McGregor and Bushland, 1957; Roth and Eddy, 1957). Ronnel killed nearly all the grubs in the host's body but was less effective in the warbles (Roth and Eddy, 1957).

Coumaphos soon succeeded Ronnel. It was used as a spray (Smith and Richards, 1954; Brundrett *et al.*, 1957; Graham, 1958; Khan *et al.*, 1959) and was effective for the control of grubs developing in the animals body as well as in the warbles (Smith and Richards, 1954; Roth and Eddy, 1955). After coumaphos, came crufomate which was used as an emulsifiable concentrate for low-volume application along the spine of cattle for the systemic control of cattle grubs (Rogoff and Kohler, 1960). This method of application commonly known as "pour-on" eliminated the risk of chilling and the use of heavy equipment required for spraying cattle at a high pressure.

General Characteristics of Organophosphorus Esters

The general formula for these compounds is:

$$\begin{matrix} R_1 \\ \\ R_2 \end{matrix} \quad P \quad \begin{matrix} O \\ \\ X \end{matrix} \quad (\text{or } S)$$

Where R_1 and R_2 are usually simple alkyl; aryl amino; 0-alky; s-alkyl groups, both of which may be bonded directly to phosphorus (in phosphinates) or linked via 0-, or - S (in phosphates), or R_1 may be bonded directly and R_2, bonded via one of the above groups (phosphonates).

In phosphoramidates, carbon is linked to phosphorus through an -NH group. The group X can be any one of a wide variety of substituted and branched aliphatic, aromatic or heterocyclic groups linked to phosphorus via a bond of some liability (usually 0 - or - S -) and is referred to as the leaving group. The double-bonded atom may be oxygen or sulphur and related compounds would be phosphates or phosphorothioates (WHO, 1986).

Following the general formula, Koller and Klawans (1979) divided the Op compounds into two main groups, the aryl phosphates, having three benzenes rings and various substituents attached to the phosphate group, and the alkyl phosphates, possessing one or more alkyl radicals connected to phosphorus

through an oxygen oramide linkage. WHO (1986) divides the organic phosphates into 3 main groups: phosphate (without a sulphur atom), phosphorothioate (with one sulphur atom) and phosphorodithioates (with 2 sulphur atoms).

Further details of classification and structure have been highlighted in previous reports. (Koller and Klawans, 1979; Holmstedt, 1959 and WHO, 1986).

Ultraviolet radiation and sunlight have been shown to increase the in vitro anticholinesterase activity of some OP compounds notably parathion. However, the acute toxicity of parathion decreased under ultraviolet radiation, although the in vitro anticholinesterase activity increased as a result of the formation of more polar products. The metabolites were identified as paraoxon and the S-ethyl and S-phenyl isomers of parathion, together within unknown products (Dauterman, 1971).

The hydrolysis of OP compounds is influenced by solutes, e.g. some amino acids, Hydroxyl ammonium derivatives and metal ions such as copper ions act as catalysts. Solvents used in formulating organophosphorus compounds to obtain properties that will increase the chances of contact between the insecticide and the target organism influence their stability (WHO, 1986). Dimethoate in certain hydroxylic solvents, particularly 2-alcoxyethanols, exhibits increased toxicity on storage (Casida

and Sanderson, 1963). The acute oral LD50 for rats decreased from 150 - 250mg/kg body weight to 30-40mg/kg. after 7 months storage at normal temperatures. Another type of reaction occurs when OP compounds containing a sulphide group (R-S-R) are stored undiluted or in an aqueous solution. Heath and Vandekar (1957) observed that a 1% solution of demeton-S-methyl increase in toxicity spontaneously at 35°C during the course of one day. This increase was found to be due to the formation of a trans-alkylated sulfonium derivative, the toxicity of which was more than 1000 times that of the parent compound.

2.2.2 Biotransformation of OP pesticides

Basically, the metabolism of these compounds may be categorized as either activation or detoxification (Fukuto and Metcalf, 1969), involving reactions with both hydrolytic and oxidative enzymes. Many of these enzyme systems are located in the liver and are catalysed by the microsomal mixed function oxidases (MFOs) that require $NADPH_2$ and oxygen (Murphy, 1980). Other enzymes involved in mammals are the carboxyesterases and carboxyamidases (Derache, 1977) along with various phosphoryl-phosphatases (Koller and Klawans, 1979). Generally, the alkyl phosphates do not require activation to be toxic (Cavanagh and Koller, 1979). The aryl phosphates are not active *in vitro* (Aldridge, 1954) but must be activated by the liver microsomes in a process involving hydroxylation and cyclization (Eto *et*

al., 1962), to become active in vivo. In other compounds the oxidation reactions are of prime importance in activation (and detoxification) Stuart, 1982).

Biotransformation reactions can be divided into three distinct classes (WHO, 1986), (a) mixed-function oxidases (b) hydrolases and (c) transferases. Binding of organophosphorus insecticide oxons to tissues is also a significant biotransformation reaction

Mixed - Function Oxidases (MFOs)

Many apparently unrelated substrates can be oxidized by mixed-function oxidases (MFOs) systems associated typically with liver endoplasmic reticulum, but present also in some other tissues such as the intestine, lung, and kidney (WHO, 1986). Within the liver there appears to be a family of MFOs possibly with some enzymes in common, but utilizing slightly different cytochromes of which cytochrome P-450 is the best known. The MFO activity in the liver can vary greatly according to the nutritional and hormonal state of the animal and also according to stimuli arising from the ingestion of some foreign compounds. The potentiation of pesticide toxicity due to some of these foreign compounds as reported by Pellegrini and Santi, (1972) has been discussed in section 2.1.5.

Some of the reactions catalysed by the MFOs include oxidative desulfuration, N-dealkylation, O-dealkylation, de-arylation, thioether and side-chain oxidation (W. H. O., 1986).

HYDROLASES

Hydrolysis of the acid anhydride type ester bond of the leaving group in pesticidal triesters is well known. The monobasic diesters and their derivatives are the major urinary metabolites of OP insecticides (W. H. O., 1986). The enzymes commonly known as A-esterases or phosphoryl phosphatases are widespread in mammalian tissues, such as liver, plasma, intestine, etc., though they are less abundant in many birds and may be present in some insects (Brealy *et al*., 1980). Although plasma contains enzymes that can distinguish between closely related structures such as paraoxon and 4 - nitrophenyl ethyl or propylphosphonate, the enzymes are not totally specific (Becker and Barbaro, 1964); the same is probably true of A-esterases in other tissues.

Hydrolysis of carboxylic acid ester bonds and carboxylamide bonds in OP insecticides may be catalysed by carboxylesterases (or B-esterases) which again occur widely in mamalian tissues.

Malathion, which contains 2 carboxylic ester bonds is the best known OP pesticide that is hyrolysed in this way (Dauterman, 1971). The importance of this metabolic route is

shown by the fact that the rat Oral LD50 for pure malation can be reduced from 10,000 to 100mg/kg, body weight, when the tissue carboxyesterases are inhibited (W. H. O 1986).

Transferases

The only tranferase reaction that is known to deal with the intact pesticidal OP triesters involves glutathione, which is required as a substrate for a number of transferase enzymes present in the liver and some other tissues. The enzymes have limited but overlapping specificity so that the glutathione transferase responsible for demethylating methyl paraoxon is distinct from that which conjugates the p - nitrophenol group in parathion.

Activity in the liver is greatest with methyl ester, but no evidence has been found of methyl phosphonates undergoing this reaction (Dauterman, 1971).

Non-Catalytic Detoxification of Acetylcholinesterase Inhibitors by Liver and Plasma Proteins.

Non-catalytic detoxication is exemplified by reactions involving the organophosphorus (OP) and cholinesterases. It is well established that the acute lethality of most OP's can be attributed to the inhibition of acetylcholinesterase (AchE) the enzyme responsible for termination of synaptic impulse

transmission in cholinergic nerve synapses and neuromuscular junctions.

OP's, however, are not selective for AchE, and are known to inhibit a variety of serine - containing carboxylesterases. The inhibition of non-neural esterases is therefore a potential mechanisms of non-catalytic detoxication (Chambers and Chamber, 1989). Strong evidence for the significance of this mechanism was first reported by Lauwerys and Murphy (1969a,b).

AchE of erythrocytes, butyrylcholinesterase of plasma and aliesterase (AliE) of liver, plasma and other tissues represent a large pool of non-target sites available for removal of available OP.

The reversible binding of OP's to serum albumin is an important phenomenon even though this is not a detoxication process in the conventional sense, a decrease in the effective concentration of OP available to penetrate into target tissue is achieved (Chambers and Chambers, 1989). While the reversibility of the interaction would limit the efficacy of the process, this binding may well temporarily sequester sufficient OP to reduce toxicant concentrations while detoxication and elimination of other OP molecule is occurring and this could alter the overall level of toxicity.

Further evidence of the protective role of non-target esterases has been obtained in insects. In the aphid Myzus persicae, OP resistance is associated with high levels of an esterase designated E4. Even though E4 is capable of measurable rates of hydrolysis of dimethyl phosphates, diethyl phosphates inhibit the esterase with only slow recovery. Yet, the insects exhibit resistance to both types of OPs.

Resistance to diethyl phosphates was attributed to the increased capacity, as much as a 60 - fold increase in one strain, of E4 to sequester the OP by acting as an alternate target. (Devonshire and Moores, 1982).

2.2.3.0 Route of Absorption of OP Insecticides.

Dermal, respiratory and gastrointestinal tract absorption are all potential routes of intoxication, although absorption via the skin is the most significant route of occupational intoxication (Wolfe *et al.*, 1967; Rosenstock, 1989). The type of pesticide formulation used is a factor in the relative contribution of the respiratory route of exposure. When aerosols were used, an average of 2.87 per cent of the total (dermal and respiratory) exposure was by the respiratory route, compared with 0.23% for dilute sprays and 0.94% for dusts (Wolfe, 1967).

In experimental animals however, Gaines (1960, 1969) found that 41 out of 44 OP compounds were more toxic to rats by the oral than by the dermal route and only three compounds, fenitrothione, merphos and mevinphos, were more toxic by the dermal route. Fenthion was more toxic to rats and guinea-pigs by the oral than by the intraperitoneal route and sulfotep was toxic to cattle only by the dermal route (Chemagro, 1962).

Individual differences in the rates of dermal absorption of pesticides also exist. In rabbits for instance, 10-fold differences were found between individuals in the dermal absorption of sarin (Nabb *et al.*, 1966).

The route of absorption has also been found to have an effect on recovery of chEs in the blood the recovery was slower in cattle treated dermally than in those treated intramuscularly with an OP insecticide (Khan, 1973). This was probably due to the persistence on the hair and skin of dermally applied insecticide which was then absorbed in the animal system for several weeks. In cattle, after a dermal application of 56mg/kg crufomate, the chE in the erythrocyte was inhibited to a maximum of 25% and returned to pretreatment level 13 weeks after treatment (Anderson and Machin, 1969) But after an intramuscular injection of 30mg/kg crufomate, the whole-blood chEs were inhibited to a maximum of 67% and returned to pretreatment level 6 weeks after treatment (Khan, 1961).

Similarly, after a dermal application of 35mg/kg, the chE in the erythrocyte was inhibited by 24% and was restored to 95% of pretreatment level 9 weeks after treatment (Anderson and Machin, 1969).

Source of OP Insecticide Exposure

Apart from occupationally exposed workers or populations exposed as a result of disease-vector control programmes, marked exposure of the general population is not expected (W.H.O., 1986). Animal poisoning however, occurs through a variety of ways; feeds may be contaminated at the processing plant or during shipping, storage or handling on the farm. Feed crops may be dusted with insecticides or the harvested crop may be accidentally contaminated. Animal premises may be sprayed or dusted or animals may be directly dusted or painted with excessive amounts of pesticides.

Systemic antiparasitic compounds may be used in amounts exceeding the recommended dosages, or they may have additive or synergistic effects with other pesticides to which animals have been exposed (Hatch, 1982). Drinking water may be contaminated by spillage or by rinsing out of spray tanks and hoses. Empty pesticide containers are never really empty; animals can be poisoned if they are fed or watered with these containers or if

they have access to dumps where chemicals containers are discarded (Hatch, 1982).

2.2.4 *TOXICITY OF OP INSECTICIDES*

Acute Toxicity

The acute toxicity of OP pesticides involves the inhibition of AchE, accumulation of Ach, and over stimulation of some central and peripheral cholinergic neurons and of the symphathetic and para-sympathetic nervous systems.

In animals and man, the muscarinic cholinergic manifestations include profuse salivation lacrimation, serous or seromucous nasal discharge, increased bronchial secretion pinpoint pupils, bronchoconstriction, pronounced GI sounds, colic, diarrhoea bradycardia, vomiting and frequent urination (Hatch, 1982; Namba *et al.*, 1971; Kagan, [illegible] Taylor, 1980).

Nicotinic cholinergic overstimulation is manifested as fasciculation of fine muscule (include the diaphragm in severe cases), tremors, twitching, spasms and hypertonicity causing a stiff gait or rigid stance and tachycardia.

Central nervous system manifestations in animals include apparent anxiety, restlessness, hyperactivity that may be

proceed to clonic or clinic-tonic convulsive activity (Hatch, 1982). In man, the CNS manifestations include headache, altered sleep patterns, impaired cognition, dizziness, coma and depression of the respiratory centre (WHO, 1986; Rosenstock, 1989; Taylor, 1980; Plestina, 1984).

All these symptoms/sings can occur in different combinations and can vary in time of onset, sequence, and duration depending on the chemical, dose, and route of exposure. Mild poisoning might include muscarinic and nicotinic signs only. Severe cases always show central nervous system involvement, the clinical picture is dominated by respiratory failure, sometimes leading to pulmonary edema. Overstimulation of nicotinic cholinergic receptors may cause depolarizing-type neuromuscular blockade with resulting muscular paralysis (Hatch, 1982).

In general, the onset of symptoms and signs from OP intoxication occurs more rapidly following inhalation rather than gastrointestinal or skin absorption. Onset of effects may range from minutes to hours but there is significant variation in the onset of symptoms depending on the nature of the chemical and its metabolism as well as variations in individual responses (Ronsenstock, 1989). The quality of signs and symptoms, their durations may differ markedly for different compounds by virtue of differences in rate of biotransformation, distribution and affinities for acetylcholinesterase (Murphy, 1980).

The duration of symptoms is generally from one to five days. In fatal untreated cases, death usually occurs within 24 hours. Op insecticides in common use are rapidly metabolized and excreted and subacute or chronic poisoning, by virtue of accumulation of the compounds in the body does not occur (Murphy, 1980).

Delayed Neurological Effects of Acute Op Intoxication

Independent of effects of cholinesterase inhibition is the recognized axonopathy that occurs from a few OP compounds (Rosenstock, 1989). Although this can result from a single (toxic dose, the neuropathology is generally delayed in onset (Murphy, 1980). the prototype of this process occurs with overexposure to triorthocresyl phosphate (TOCP), although certain OPs are also well known to be associated with a delayed peripheral neurological syndrome (also known as organophosphate - induced delayed neuropathy) which may occur 2 - 3 weeks later (Murphy, 1986), a paralytic process (the so - called intermediate syndrome) occurring 24 - 96 hours after acute poisoning has been described in a series of patients (Senanayake and Karallieade, 1987). The functional disturbances associated with phosphate triester neuropathy begin in the distal parts of the lower limbs in both man and other sensitive animals. Mild sensory disturbances and motor weakness with ataxia occur, progressing

in severity and extent to increased weakness and flaccidity of the legs and varying amounts of sensory disturbance. Upper limbs may also become involved. After several days to a few weeks the peak of the process is reached and thereafter improvement in the functional disturbance begins. Recovery is slow and not always complete (Murphy, 1975).

Chronic central nervous system effects of acute organophosphate pesticide intoxication have been reported by Rosenstock *et al.*, (1991) and Savage *et al.*, (1988). Rosesnstocke and co-wokers did a retrospective cohort study of agricultural workers in Nicaragua who are admitted to hospital for occupation-related OP poisoning and found that the poisoned group did significantly less well in tests assessing a wide array of neuropsychological functions (including auditory, attention, visual, memory, visuomotor, speed, sequencing and problem solving, and motor steadiness, reaction and dexterity) than matched control group.

The poisoned group was also significantly more likely to report symptoms consistent with central nervous system involvement, a finding similar to that described for solvent workers, which is another neutrotoxic-exposed group (Hogstedt *et al.*, 1984). The consistent pattern of decreased neuropsychological function exhibited by the poisoned group could not be explained by potential confounders such as exposure

to other occupational or environmental neurotoxicants, or, by differences in socio-economic status including education (Rosenstock *et al.*, 1991). Savage *et al.*, (1989) also conducted a cohort follow-up study of OP poisoned heterogenous group of individuals with primarily occupationally related poisoning and found abnormalities in a wide range of neuropsychological variables, including visuomotor, attention, and language function.

Electroencephalographic (EEG) abnormalities due to OP intoxication have been reported (Metcalf and Holmes, 1969). EEG disturbances, manifested mostly as an increase in beta activity have been reported in human (Dutt *et al.*, 1979) and animal (Burchfield *et al.*, 1976) studies.

Effects of cholinergic agents on the visual system have been studied by Revzin (1980), in urethane anaesthesized pigeons with implanted electrodes, in which he observed various changes in the response of specific neurons of the optic tectum and of the hippocampus after doses of both mevinphos and atropine. The lowest effective dose of mevinphos was only one-third of that (0.15mg/kg body weight) which produced parasympathetic signs that would certainly be associated with susbstantial inhibition of AchE. Some possible effects of anticholinesterases on non-cholinesterase targets have been considered by O'Neil (1981) who found that no clear effects seem definable at concentrations

lower than those that inhibit AchE and are probably secondary to stimulation of non-cholinergic nerves with cholinergic innervation. However, an endopeptidase that can hydrolyse putative transmitter peptides in the nervous system is known to be inhibited by di-isopropyl phosphorofluoridate at a concentration similar to that which inhibits AchE (Kato *et al*., 1980). Thus some involvement of non-cholinergic pathways in the CNS effects resulting from organophosphate poisoning cannot be excluded (WHO, 1986).

Even single episodes of clinically significant OP intoxication are believed to be associated with persistent decline in neuropsychological functioning (Rosenstock, 1991).

Post Mortem and Histopathological Lesions

There are no pathognomonic post mortem lesions in cases of poisoning by organophosphorus compounds. Lesions such as hemorrhagic gastroenteritis, pulmonary edema, degenerative changes in liver and kidney are usually secondary to the symptoms (Clarke and Clarke, 1975).

In the delayed neurotoxic effect, histopathological studies of the peripheral nerves show that distal fibers are affected earlier and more severely than proximal fibers. There is also a tendency of large diameter fibers to be affected more than

smaller-diameter fibers. The lesion has been described as a "dying-back" process where the ends of the long nerves, distal to the nerve cell body, are affected first. Axonal degeneration followed by myelin degeneration is observed. These effects are believed to be due to a disturbance in the metabolism of the nerve soma in spinal tracts with the consequence that nutrients are not synthesized and transported at a significant rate to maintain the long axons of the peripheral nerves (Cavanagh, 1969).

Although the conventional view has been that the axonal degeneration primarily affects the distal ends of the long large nerve fibers, Bouldin and Cavanagh (1979a) using a teased-fiber technique on nerve fibres from cats poisoned with di-isopropyl fluorophosphate (DFP) have revealed that the initial axonal degeneration is not in the extreme terminus of these fibers, but is focal, in the distal region of the fibers. And that, preceding the axonal degeneration, nerve fibre varicosities and paranodal demyelination occur.

Subsequent to this, axonal degeneration was shown to proceed somatofugally to involve the entire, distal axon. These workers thus proposed that the organophosphorus esters cause a "chemical transection" of the axon, which precipitates the degeneration of the more distal axon. Bouldin and Cavanagh (1979a) further reasoned that even though demyelination was observed before

actual axonal degeneration, the random distribution of the demyelination was an indication of a disturbances of the axon, rather than being primary to the schwann cell.

Ultrastructural studies (using the electron microscope), of the nerve fiber varicosities and paranodal demyelination conducted by Bouldin and Cavanagh, 1979b), have demonstrated that the nerve fiber varicosities were always associated with either intra-axonal or intra-myelinic vacuoles (within the inner myelin sheath), or both. The vacuoles were further described as being electron - lucent, unilocular and at least partially membrane-bound. These intra-axonal vacuoles appeared to displace relatively large amounts of the axoplasm, the intramyelinic vacuoles were described as occurring most frequently between the inner loop of schwann cell cytoplasm and inner-most dense line of the myelin sheath. In most cases, however, the periaxonal space was not distended or collapsed.

In relation to axons themselves, the varicosities associated with intra-axonal vacuoles, whether intramyelinic vacuoles were present or not, always contained a normal (nondegradable) axon. In contrast, the varicosities associated with mainly intramyelinic vacuoles contained degenerate axons (Bouldin and Cavanagh, 1979b).

Factors Influencing OP Insecticide Toxicity

A number of physicochemical factors affect toxicity of organophosphate insecticides. Toxicity of these compounds decreases as they are degraded by sun, water, microbes, alkali or metal ions such as iron, or copper (Hatch, 1982). Increase in toxicity may occur by storage activation, in which highly toxic isomers of certain pesticides are formed spontaneously in polar solvents or water. The phosphorothionates and phosphorothiolothionates are notable in this regard and can partly isomerize to the corresponding thiolates and dithiolates, which are more toxic than the original compounds. For instance, the mammalian toxicity of dimethoate increased on storage in 2--alkoxyethanols often used as solvent. (Casida and Sanderson, 1963) for OP compounds.

The toxicity of Meta-Systox and related compounds increased on storage or in water (Heath and VandeKar, 1957).

Another kind of storage activation is chemical alteration to a more toxic pesticide. Thus trichlorfon can change to dichlorvos in solutions of pH 7 or 8 the storage activation phenomena are good reasons to use only freshly prepared suspensions, emulsions or solutions (Hatch, 1982).

Ambient temperature affects the toxicity of OPs. High temperature increases toxicity of parthion in mice, low temperature increases toxicity of malathion in rats, volatility of dichlorvos is greater at higher temperatures, thus increasing the inhalation hazard. The type of vehicle in which the pesticide is dispersed is another physicochemical factor that affects the toxicity of pesticides, for instance organic solvents or oils can carry pesticides through the skin and cause toxicosis.

The species of animal also affect toxicity of OPs e.g. cattle are more sensitive than sheep to dimethoate, ronnel, crufomate and phosmet, more sensitive than swine to phorate but more resistant than any other species to parathion. Swine horses are less sensitive than cattle to crufomate. Dogs are very resistant to Chlorfenvinphos and fairly resistant to crufomate and Ronnel. Mice are more sensitive than rats or guine-pig to fenthion. Many of these species differences are due to differences in enzymatic activation or degradation of various pesticides (Hatch, 1982).

Age and sex are other biologic factors that influence OP toxicity. It is well known that the microsomal MFOs and other drug-metabolizing enzymes are present at comparatively low levels in neonatal animals, but activity develops to approximately the adult level early in maturation.

Since microsomal MFOs are involved in both the activation and degradation of many OP pesticides, the likely net result in terms of LD50 is hard to predict (WHO, 1986). One-day-old rats were 9 times more susceptible to malathion than 17-day-old animals (Mendoza, 1976). The toxicity of methyl parathion and of parathion for rats decreased from birth through the developmental period; the decrease was best correlated with the increasing capacity of the animals to metabolize the oxygen analogues by both oxidative and hydrolytic pathways (Benke and Murphy, 1975).

Liver MFO activity fluctuates according to the hormonal status of female animals (WHO, 1986). LD50 values quoted for males and females often differ, but these values generally arise from different laboratory animals subjected to many variable factors (including the purity of the test sample). Only parathion exhibits a marked and apparently real difference in LD50 between the sexes, the oral LD50 in male rats being 5 - 30mg/kg body weight depending on the solvent, compared with 1.8 - 5mg/kg in females (FAO/WHO, 1964).

Nutrition is another factor that affects OP toxicity, although no clear-cut effects have been reported (WHO, 1986). Thus the acute toxicity of diazinon is greater (up to 2-fold) in rats maintained on a diet, either very low (4%) or very high

(81%) in protein compared with a standard (29%) protein diet (FAO/WHO, 1971b). A similar increase in toxicity is seen with naled (0-0-dimethyl 0-1, 2-dibromo - 2, 2-dichloroethyl posphate (Kaloyanova and Tasheva, 1983).

Drugs that have neuromuscular blocking or acetylecholinestrase-inhibitting properties may enhance OP toxicity or vice versa. Such drugs include inhalant anaesthetics, magnesium ion, aminoglycoside antibiotics (Streptomycin, dihydrostreptomycin, neomycin, kanamycin gentamicin, polymyxin-B, etc) and the depolarizing and non-depolirizing, neuromuscular blocking agents (Hatch, 1982).

Potentiation of the toxicity of organophosphorus pesticides for insects by inhibition of MFO activity is well known, and many potent synergists are used in agriculture for this purpose (Wilkinson, 1971).

2.2.5 Mechanism of Action of OP Insecticides

The primary biochemical effect associated with toxicity caused by organophosphorus pesticides is inhibition of AchE. The normal function of AchE is to terminate neurotransmission due to AchE that has been liberated at cholinergic nerve endings in response to nervous stimuli (WHO, 12986). the effect of AchE inhibition is accumulation of AchE in neuromuscular junctions,

parasympathetic postganglionic terminals in smooth muscle, cardiac muscle and glands, in all autonomic ganglia, and in cholinergic synapses within the CNS. Thus all muscarinic and nicotinic cholinergic receptors are over-stimulated by AchE, which would normally be destroyed if AchE were not inhibited. If this overstimulation is intense enough, Ach receptors may become blocked, just as if a depolarizing type of neuromuscular or ganglion blocking agent had been administered. Usually, complete blockade of all cholinergic receptors is not produced, and clinical signs (at least early ones) reflect hyperfunction of these receptors (Hatch, 1982).

The chemistry of inhibition of AchE and of many other esterases by OP compounds is presented schematically in figure 1.

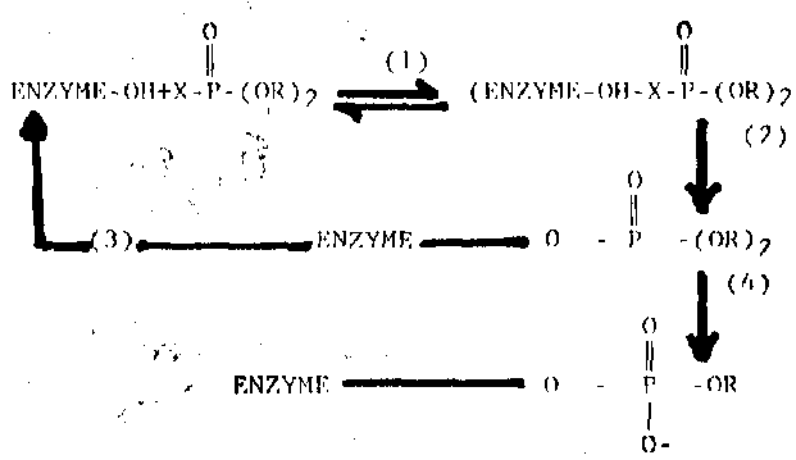


Fig. 1: Inhibition of an esterase enzyme by organophosphorus compounds (W.H.O., 1986).

(1) Formation of Michaelis complex

(2) Phosphorylation of the enzyme

(3) Reactivation reaction

(4) "Ageing"

Following the formation of a Michaelis complex (reaction 1), a specific serine residue in the protein is phosphorylated with loss of the leaving group x (reaction 2). Two further reactions are possible: reaction 3 (reactivation may occur spontaneously at a rate that is dependent on the nature of the attached group and on the enzyme protein and is also dependent on the influence of pH and of added nucleophilic reagents, such as oximes, which may catalyse reactivation. Reaction 4 ("Ageing)" involves cleavage of an R-O-O- bond with the loss of R and the formation of a charged monosubstituted phosphoric acid residue still attached to protein.

Molecular Mechanisms of Organophosphorus Induced Delayed Neurotoxicity (OPIDN)

Although the immediate hazard associated with organophosphorus compounds seems to be related to their ability

to inhibit acetylcholinesterase, several of these compounds also are capable of producing delayed neurotoxicity (Abou-Donia, 1985). Organophosphorus compound induced delayed neurotoxicity (OPIDN) is characterized by a delay of 6 -14 days before on set of ataxia and paralysis (Abou-Donia, 1981). Histopathologic lesions are seen as Wallerian-type degeneration of the axon and myelin in the distal parts of the longest tracts in both central and peripheral nervous system (Cavanagh, 1964).

Occupational exposure to several OP compounds has caused delayed neurotoxicity in workers. Some of these compounds include mipafox (Bidstrup *et al.*, 1953), metamidophos (Senanyake *et al.*, 1982), Trichlorphan (Shiraishi *et al.*, 1977), trichlornate (Jedrezjowska *et al.*, 1980). Other organophosphorus compounds shown to produce OPIDN in experimental animals are S, S, S-tributylphosporo-trithioate (DEF) (Abou-Donia, *et al.*, 1979a), (Abou-Donia *et al.*, 1980b), S-Seven (Abou-Donia *et al.*, 1979b) Haloxon (Malone, 1964); Coumaphos (Abou-Donia *et al.*, 1982), Cyanofenphos (Abou-Donia and Graham, 1979), Leptophos (Abou-Donia *et al.*, 1974) and desbromoleptophos (Abou-Donia *et al.*, 1980a).

Many workers (Casida *et al.*, 1961; Aldridge and Barnes, 1961 and 1966a; Eto *et al.*, 1962) have espoused the esterase theory, suggesting an esterase other than cholinesterase in the neurotoxicity of OP compounds. The work of Baron and Casida

(1962) with various cholinersterase reactivators further implicated an esterase other than acetylcholinesterase (AchE).

Johnson (1969a), experimenting with DFP in hens, identified a phosphorylation site present in thebrain and spinal cord which was bound by the known neurotoxic organophosphorus esters, but not by the known non-neurotoxic compounds. Johnson (1969b) further demonstrated that the phosphorylation site was an esterase (different from AchE) which was inhibited in vivo and in vitro by known neurotoxic esters, while remaining uninhibited in vivo and only slightly inhibited in vitro by non-neurotoxic esters. The proof that the "neurotoxic esterase" (NTE) (Johnson, 1970b) was indeed the site of initial neurotoxic activity came when it was shown that certain protective compounds (some carbamates and sulphonyl fluorides) (Johnson and Lauwreys, 1969c) bind to the neurotoxic esterase in vivo, thus preventing the neurotoxic effects of known neurotoxic phosphate esters. The protective action persisted until about 70% of the enzyme site was again available for phosphorylation. It was therefore concluded that the nature of the group binding at the active site (i.e. phosphorylation), and not the binding (inactivation) itself, was the determinant of the toxic response.

Johnson (1974) went further to demonstrate that the binding of NTE by neurotoxic compounds was followed by hydrolysis of an

ester or amide bond, leaving an ionized (charged) acidic group on the phosphorus. This process was shown to be time dependent and thus termed "ageing". He went on to hypothesize that the charged group on NTE, which was known to be attached to cellular membranes, might alter membrane functions such that it could be the initial biochemical lesion at the cellular level in delayed neurotoxicity. The 'ageing' process also fits in nicely with the time interval between exposure and effect observed in delayed neurotoxicity (Stuart, 1987).

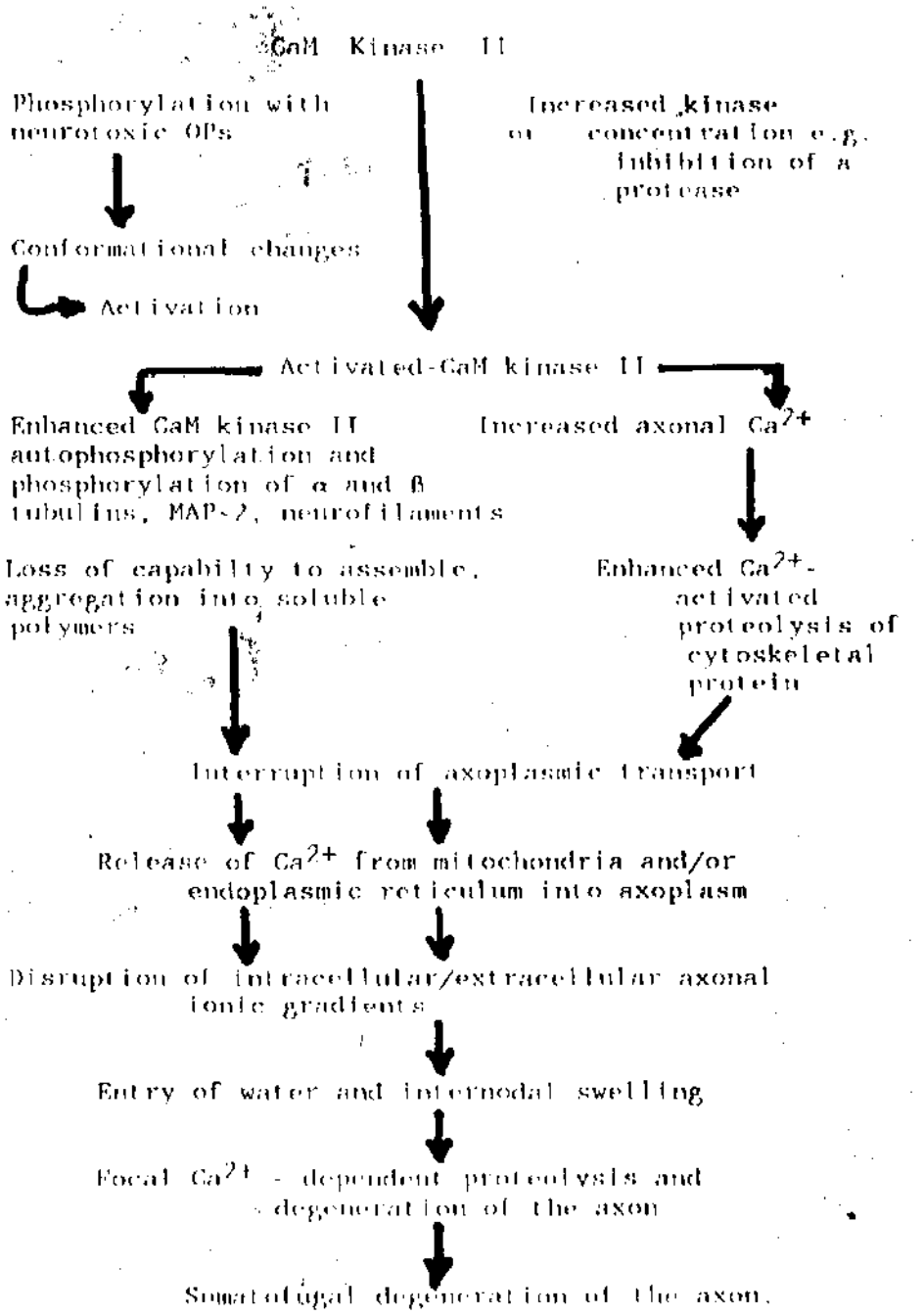
For an organophosphorus ester to have the potential to produce OPIDN, it must cause at least 70% inhibition of hen brain NTE 24 hours after administration of an LD50 dose. NTE has the following characteristics: (1) it is a membranebound protein (Carrington and Abou-Donia, 1985a) (2) it has a molecular weight of 155-178 KDa (Carrington and Abou-Donia, 1985a) (3) it has a target size, as determined by radiation inactivation of 105 KDa (Carrington *et al.*, 1985) (4) it is transported in the sciatic nerve of hens at a fast axonal transport rate of about 300mm/day (Carrington and Abou-Donia, 1985b) (5) it is reversibly inhibited by paraoxon (Carrington and Abou-Donia, 1985c) (6) studies on the kinetics of substrate hydrolysis and inhibition by mipafox of NTE indicated that the higher apparent Ki values that occur with low concentrations of Mepafox are attributed to the formation of a Michaelis complex at higher concentrations (Carrington and Abou-Donia, 1986)

rather than the formation of the two NTE isoenzymes as has been suggested (Chemnitus *et al.*, 1983, Abou-Donia *et al.*, 1984).

To elucidate the molecular mechanism of OPIDN, Abou-Donia and Lapadula (1989) investigated the hypothesis that delayed neurotoxic organophosphorus compounds may interfere with endogenous kinase-mediated protein, thereby adversely affecting the regulation of normal neuronal processes and resulting in axonal degeneration. This investigation involved the use of triorthorcresyl phosphate (TOCP) and they suggested that Ca^{2+}/calmodulin-dependent protein kinase type II (CaM Kinase II) may be the enzyme involved in the increased protein phosphorylation with TOCP. CaM Kinase II normally mediates ATP phosphorylation of neuronal cytoskelal proteins including α and ß tubulin, Microtuble associated proteins (MAP-2), neurofilament triplet proteins, and synapsin I as tryosine hydroxylase, tryptophan hydroxylase, and myelin basic protein (Lin *et al.*, 1987).

This and a series of studies (Abou-Donia *et al.*, 1988; Suwita *et al.*, 1986a; Abou-Donia and Lapadula, 1989) led Abou-Donia and co-workers to propose a molecular mechanism of organophosphophorus induced delayed neurotoxicity (OPIDN) which is presented below (Figure 2)

Figure 2: A PROPOSED SCHEME OF THE MOLECULAR EVENTS LEADING TO OPIDN.



2.3.0 Organochlorine Insecticides

2.3.1 General Introduction

The spectrum of pesticidal activity shown by the halogenated hydrocarbon pesticides is remarkably broad.

This group of pesticides has a wide range of practical application against both agricultural pests and pests affecting man and animals (Turner, 1982). There is no doubt that their introduction as agricultural insecticides made a remarkable contribution to the increase in world grain and other crop production. The application of the halogenated hydrocarbon pesticides, especially DDT in the areas of public health and veterinary medicine have attracted the most attention (Brooks, 1974). No insecticide has received so much attention as DDT (Winteringham and Barnes, 1955.

It is also believed that no chemical made by man not even penicillin, streptomycin and the sulphonamides - has saved so many lives as has DDT. In fact for a time, DDT was outstandingly successful in controlling the vectors of organisms responsible for such mortal and debilitating diseases as malaria, river blindness and yellow fever (Hassall, 1982). The cost effectiveness of these pesticides has remained unequaled since their introduction some four decades ago. Indeed, the very hallmark of these compounds, which characterized them originally

as wonder chemicals, was the small amount needed over a given area to effect complete control of insects (Brooks, 1974). The effective user of these compounds has not been without their attendant complications. In certain instances, depending on the type of halogenated hydrocarbon used and the plant involved, phytotoxicity may occur when used as an insecticides. In addition, the use of these pesticides can undoubtedly influence the ecology of beneficial (non target) insects such as pest predators and aquatic insects (Brooks, 1974).

In the wake of the early spectacular successes of these compounds in insect control, came a gradual failure of many applications due to the development of resistance to them in target species. The emergence of insect resistance has been a major set back in the use of halogenated hydrocarbon pesticides (Brook, 1974; Busvine and Pal, 1968; Matsumura *et al.*, 1972).

This group of insecticides has also come into disfavour because they are very persistent in the environment and tend to accumulate in biologic as well as nonbiologic media (Murphy, 1980). the interactions between the halogenated hydrocarbon pesticides and the biotic and abiotic elements of the environment, especially as they relate to the possible effects of the compounds on wild life, are highly controversial and of grave concern to environmentalists (Brooks, 1974; Turner, 1982). At an earlier stage of its development, it was discovered that

DDT tends to be stored in body fat and is excreted in cow and human milk (Abbot *et al*., 1977; Brooks, 1974; Morgan and Roan, 1971).

As a class, the organochlorine insecticides are often considered less acutely toxic, but of greater potential for chronic toxicity than the organophosphate and carbamate insecticides. There is however a wide range of acute toxicities of individual organochlorine compounds, from extremely toxic e.g. endrin to slightly toxic e.g. Methoxychlor. The organochlorine insecticides can also be classed as neuropoisons (Murphy, 1980).

Organochlorine insecticides are divided into three groups based on chemical structure. These are the Dicophane group, cyclodiene group and a group of heterogenous compounds that can be called a miscellaneous group (Hatch, 1982). Members of the different families do, however, have important physicochemical characteristics in common. The chemical stability of many members of the group (or of their immediate and often toxic metabolities) is high because their molecules are constructed, entirely or largely from C-C, C-H and C-Cl bonds all of which tend to be chemically rather inactive under normal environmental conditions (Hassall, 1982)..

In addition to the above physicochemical properties is their low solubility in water. This coupled with their strongly

lipophilic character, means that they possess partition coefficients which strongly favour accumulation in biolipids, such that unless the organism's defence mechanisms can degrade them to excretable products at a rate that is sufficiently rapid to outmatch the rate of entry, accumulation in body lipids will take place (Kenaga, 1972). Most organochlorine insecticides are waxy solids at room temperature. Their vapour pressures are low but not negligible, their volatilisation leads to atmospheric contamination, while the volatility of such compounds as aldrin and Y-BHC enable them to be used as soil fumigants. Y-BHC and most members of the DDT family undergo dehydrohalogenation in the presence of ethanolic KOH but they are otherwise inactive. Some organochlorine compounds are slowly destroyed by ultraviolet radiation but only when they are in a finely divided state (Hassall, 1982).

With some exceptions (e.g. endosulfan and perthane, which are biodegradable) organochlorine insecticides are very persistent in the environment because they resist chemical or microbial decomposition especially when protected by layers of soil (Brooks, 1974).

Their solubility in oils and organic solvents makes it possible for organochlorine com[pounds to be used as dusts, wettable powders, emulsions or suspensions. An oily vehicle or an organic solvent vehicle facilitates penetration of

insecticide through intact skin; poisoning has occurred by this mechanism. The volatility of this group of insecticides is also responsible for some loss of pesticide from exposed surfaces, uptake of pesticide by foliage and exposure of insects and animals to the pesticide by inhalation (Hatch, 1982).

2.3.2 Toxicity of Organochlorine Pesticides

The more toxic compounds (BHC, aldrin, dieldrin, endrin, isodrin, chlordane, heptachlor and camphechlor) are diffuse stimulants of the central nervous system. Expressions of diffuse stimulation are of extreme variety but are predominantly neuromuscular. The onset of symptoms may occur from a few minutes to days depending on the chemical and on the dose applied or ingested (Radeleff, 1970). An affected animal may first become apprehensive and hyersensitive or it may become belligerent. Soon blepharo spasms and fasciculations of facial and cervical muscles will appear, followed by clonic spasms of cervical muscles, then those of the forequarters and finally of the hindquarters. These spasms may be rapidly repeated or may appear intermittently at regular or irregular intervals. Poisoned animals have been known to become comatose, and to remain so for several hours before death. These various symptoms may progress to convulsion which are clonic-tonic in nature accompanied by periods of paddling movements, nystagmus, grinding of the teeth, groaning and grunting. Convulsive

seizures may be repeated at regular or irregular intervals or, once begun may persist until death.

Convulsive seizure if prolonged for more than a few minutes , cause the body temperature to rise sharply and to remain as high as 45.6°C to 46.7°C. Much of this increase in temperature is due to the muscular activity involved in the seizures. It is also believed to be due to a concomitant interference with the heat regulating centres and mechanism distinct from the factor of work (Radeleff, 1970). Seizures following accidental exposure have been observed in man and the chlorinated hydrocarbon insecticides are a recognised human hazard (Gestaut, *et al*., 1969; Joy, 1976). Detailed description of the acute toxicity of organochlorine insecticides may be found in Radeleff (1970) and Clarke and Clarke (1975).

The sign of chronic toxicity are similar in general outline to those of acute poisoning, but usually first appear as tremors in the muscles of the neck and head. These gradually extend so as to involve most of the muscles of the body and increase in intensity so that any purposeful movement becomes difficult or impossible. The tremors give way to convulsions which gradually become more frequent and more severe. Terminally, depression appears with respiratory failure and death intervening (Clarke and Clarke, 1975).

Unlike most of the orhganophosphate insecticides, DDT is poorly absorbed after dermal exposure, especially when applied

in the powder form. This poor absorption from the skin probably accounts for the rather good safety record of DDT in spite of its wide and sometimes careless use by applicators and formulators (Hayes, 1971). Although the functional injury produced by high doses of organochlorine insecticides are attributed to effects in the central nervous system there is little pathologic change in the cells and tissues of the central nervous system in acute poisonings. Inhalation of the dust results in irritation in the lungs but nonfatal doses or from subacute or chronic feeding, are observed in the liver. With large doses, centrilobular necrosis of the liver has been reported. Smaller doses result in liver enlargement, which in rodents is somewhat characteristic in that the cells and mitochondria themselves are enlarged (Hayes, 1959).

2.3.3 Mechanism of Action of Organochlorine Insecticides

Members of the three families of organochlorine insecticides are neurotoxic substances. The initial effect of DDT is upon the peripheral nervous system whereas YBHC and aldrin appear to attack the central nervous system. However, the general effect of all of them is to destabilise neural activity and this is manifested by hyperexcitability of nerves and muscles (Hassall, 1982).

DDT is capable of altering the transport of sodium and potassium ions across the membrane of nerve axons, it blocks potassium efflux across the membrane resulting in an increased negative after-potential (O'Brien, 1967). DDT also slows the turning off process of sodium conductance (Narahashi, 1979). The membrane-destabilizing affects of Y-BHC and dieldrin are similar to those of DDT although the precise mechanism by which these effects are brought about is still obscure. However, two diverse type of explanations have been profered, the first of which is that the insecticides may inhibit one or several enzymes of importance to the mechanism of membrane stability or of nerve impulse transmission. There also exist the possibility that they may initiate some physical change in the structure of the nerve membrane which rather literally causes it to misfire (e.g. by alteration of its permeability to ions or to other solutes (Hassall, 1982).

Membrane-bound enzymes exist which in vitro, appear to split ATP to give ADP and inorganic phosphate. In vivo, such splitting is probably an overall effect, the reactants and products really representing fuel of, and waste from, a reaction which drives a vital mechanical process. Thus ATPases are probably associated with muscular activities, mitochondrial processes and solute movement through tonoplast and plasmalemma as well as with the activity of nerve membranes. It appears that one sort of ATPase is dependent on sodium and potassium

ions and is actually a part of the sodium pump; another type is dependent on the presence of calcium ions and a third type requires the presence of both calcium ions and magnesium ions (Mullins and Brinley, 1975).

2.3.4 AIM AND SCOPE OF STUDY

The in vitro study is aimed at evaluating the potential interaction between the organophosphorus insecticide, coumaphos (Asuntol) (R) and the organochlorine insecticide, lindane (Gammalin 20) (R) at the receptor level i. e to attempt to answer the following questions:-

(a) What is the effect of coumaphos on the cholinergic response to Acetylcholine?

(b) What would be the effect of pre-exposure to Lindane on the response to coumaphos at the receptor level?

In addition, an attempt will be made to shed some light on the somewhat obscure mechanism of action of lindane by verifying, through calcium atagonism, some suggestions (Hassall, 1982; Dede *et al.*, 1991) of the involvement of calcium channels in the mode of action of lindane. This experiment will seek to find asnwers to the following:

(a) How will Nifedipine (a calcium antagonist) influence the response to Lindane at the receptor level?

(b) What will be the effect of nifedipine on the response to Lindane in a medium devoid of exogenous calcium?

The in vivo (sub-chronic toxicity) study also seeks to evaluate potential interaction between coumaphos and Lindane. Specifically, to determine the effect of pre-exposure of animals to a sub-lethal dose of Lindane on coumaphos toxicity.

The experimental protocols adopted for the studies are discussed in the succeeding chapter.

CHAPTER THREE

3.0 In Vitro Experiments

Introduction

The in vitro experiments, using isolated Rabbit ileum preparations sought to examine the potential interactions between organophosphate and organochlorine pesticides at the receptor level and to focus attention on the mechanism of action of the organochlorine, Lindane.

3.1.1 MATERIALS

3.1.2 Test Chemicals

Lindane (Gammalin-20)(R), 20% w/v, Imperial Chemical Industries ICI, England was the organochlorine insecticide tested; while coumaphos (Asuntol)(R), Bayer Leverkusen, (Germany) served as the test organophosphorus insecticide.

Acetylcholine (British Drug House, BDH) was used to demonstrate the typical cholinergic responses at the receptor level.

Physiological Solution

The Tyrode's solution in the organ bath for the in vitro experiment was prepared in the sequence recommended in the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria, experimental monograph, 1990 viz:

Reagent	g/10 litres
Nacl	90g
10% w/v KCl	20ml
10% w/w $NaH_2PO_4.2H_2O$	5ml
D-Glucose	10g
$NaHCO_3$	10ml
10% $CaCl_2$	20ml
$NaCl_2.6H_2O$	1ml

3.1.3 Apparatus

The apparatus consisted of microdynamometer 7050 with flat-bed recording (Ugo Basile, Milan Italy), Petri dishes, measuring cylinders, Erlammeyer's flasks, beakers, Analytical balances.

3.2.0 EXPERIMENTAL METHODS

Rabbits with average body weight of 1.4kg purchased in the local market were used for this in vitro experiment. After the preconditioning period of three days and having been deprived of food, but allowed water ad libitum on the day prior to the experiment, animals were stunned with a blow on the occiput and then sacrificed by a single neat incision across the neck severing the jugular veins.

The abdomen was then incised along the linea alba and the intestine exteriorised. The ileum was located, removed and placed in a petri dish containing oxygenated Tryode's solution, in readiness for introduction into the organ bath.

The experimental set up was in accordance with the methodology described in the experimental monograph of the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria (1990). With a chart speed of 24mm/minute, organ bath temperature of 37°C, organ bath volume of 25ml, agonist contact time of 30 seconds and antagonist contact time of 2 minutes.

Responses of Rabbit ileum to Acetylcholine (Ach) were first studied to determine a satisfactory working dose which was

1μg/ml. The working doses for coumaphos (40μg/ml) and Lindane (20μg/ml) were similarly obtained.

The effect of 40μg/ml coumaphos on the tissue response to 1μg/ml Ach was then demonstrated and repeated five times (n = 5) allowing the usual 30 seconds agonist contact time before adding Ach and then washing out after another 30 seconds.

3.2.1 Effect of Coumaphos on the Response to Acetylcholine

The effect of 40μg/ml coumaphos on the tissue response to 1μg/ml Ach was determined by first establishing the baseline effect of Ach on the rabbit ileum.

40μg/ml coumaphos was then injected into the organ bath, allowing the usual 30 seconds agonist contact time, Ach (1μg/ml) was introduced before finally washing out the organ bath after another 30 seconds.

This procedure was repeated five times (i.e. n=5) and recorded.

3.2.2 Effect of Pre-exposure to Lindane on the Response to Coumaphos.

After washing out the organ bath 3 to 4 times, 20μg/ml Lindane was infused into the organ bath, allowing an antagonist contact time of 2 minutes, but without washing out, 40μg/ml was then infused into the organ bath, after the 30 seconds agonist time, 1μg/ml of Ach was infused. The organ bath was finally washed out after the agonist contact time for Ach.

This procedure was also repeated five times.

3.2.3 Effect of Nifedipine (a Calcium Antagonist) on the Response to Lindane.

A pilot dose-response experiment was conducted to ascertain the working dose of Nifedipine to be used in the above experiment.

After obtaining a suitable working dose (5μg/ml) for Nifedipine, the study was conducted as follows:

The first step was to determine the effect of Nifedipine in normal (0.2% Calcium) Tyrode's solution on the response to Lindane.

1μg/ml of Acetylcholine was infused into the organ bath, allowing the normal agonist contact time, 20μg/ml Lindane was infused. After the antagonist time of 2 minutes, the organ bath

was washed out. This was replicated five time (n - = 5). 1μg/ml Ach was then infused into the organ bath followed, after the agonist time, by an infusion of 5μg/ml Nifedipine which was then washed out after the antagonist contact time.

The tissue was exposed to 1μg/ml Ach, 2μg/ml Lindane and 5μg/ml Nifedipine following the protocol described above. The procedures were also repeated five times and recorded appropriately.

3.2.4 Effect of Nifedipine on the Response to Lindane in a Calcium-free Medium.

For this study, the Tyrode's solution was prepared as described earlier (section 3.11.2) except that it was devoid of Calcium. The same protocol outlined in section 3.12.3, above was followed.

3.3.0 In Vivo Experiments.

The same organochlorine (Lindane) and organophosphorus (coumaphos) insecticides as in the in vitro studies were used in this study.

3.3.1 Test Animals

The test animals were adult male and female albino rats weighing between 185 and 190g, bred locally in the Animal house of the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. All animals were under close observation during the two-week pre-conditioning period before commencement of the sub-chronic toxicity studies.

Animal Husbandry

The rats were held in plastic cages (53cm x 38cm x 20cm, E. K. Brown Ltd., London, U. K.). Five (5) Animals were kept in each cage to avoid overcrowding, giving each animal enough room.

Animals were fed with Sander$^{(R)}$ chick mash, SEEPC Nigeria Ltd. The mash was molded into large, sundried boli.

Drinking water in 250ml and 500ml plastic drinkers was available to all animals ad libitum. The water in the drinkers was changed every other day.

3.4.0 EXPERIMENTAL METHODS

3.4.1 Determination of Median Lethal Dose (LD_{50}) of Coumaphos in Rats

The intraperitoneal median lethal dose (LD_{50}) of coumaphos in rats was determined using standard procedures (Aliu and Nwude, 1982).

Two pilot studies were first conducted in which five increasing doses of coumaphos were injected into small groups of rats of equal sex distribution. The doses were logarithmically selected and the least dose that killed all animals in a group in both pilot tests were used as a guide in the subsequent experiment to determine the LD_{50} of coumaphos.

In the initial pilot study each of the five dose levels selected at 0.6log intervals (factor of 4) was given to two rats and in the second test, dose levels selected at 0.15log intervals (factor of 1.4) were administered to five groups of two rats each.

The final tests were conducted using a factor of 2 (log. 0.3). Thirty normal healthy rats (average weight 178g) were used. The animals were divided into five groups of six (6) rats

(3 males and 3 females). The control group received 0.5ml each of distilled water.

The number of animals that died within 24 hours in each group was noted. The survivors were observed daily for 14 days for signs of delayed toxicity. The LD_{50} was calculated by the Arithmetic method of Karber (Aliu and Nwude, 1982).

3.4.2 Effect of Pre-Exposure to Lindane on the Sub-chronic Toxicity of Coumaphos.

Experimental animals were divided into six (6) groups of five rats in addition to a control group which was not exposed to any of the test insecticides.

Pre-treatment was by injecting an aqueous solution of 1:100 dilution of lindane intraperitoneally into rats. The first pre-treatment was done a day before commencement of the ten-week sub-chronic toxicity study. Three other pre-treatments were administered at 2-week interval during the study.

Sub-chronic toxicity of coumaphos was then determined in pre-treated and non-pre-treated groups of rats, by incorporating varying levels of coumaphos into the feed for a period of ten weeks according to the following experimental protocol:

Group 1A Pre-treated with Lindane, and fed 1mg/kg Coumaphos

Group 1B Not pre-treated with Lindane, but fed1mg/kg Coumaphos

Group 2A Pre-treated with Lindane, and fed 10mg/kg Coumaphos

Group 2B Not pre-treated with Lindane, but fed 10mg/kg Coumaphos

Group 3A Pre-treated with Lindane, and fed 100mg/kg Coumaphos

Group 3B Not pre-treated with Lindane but fed 100mg/kg Coumaphos

Control Not pre-treated with Lindane but injected with distilled water intraperitoneally. Not fed Coumaphos.

At the end of the ten-week period, all animals were sacrificed by decapitation and the visceral organs (kidneys, liver, spleen, heart, lungs, intestines) and brain were removed, weighed examined for gross pathological lesions and fixed in 10% formalin preparatory to tissue sectioning, processing and histopathological examination.

3.4.3 STATISTICAL METHOD

The *students 't' test (Snedecor and Cochran, 1967)* was used in the comparison of organ/body weight ratios in control and treated groups of animals as well as for 't' value calculations in vitro studies.

CHAPTER FOUR

RESULTS

4.0 IN VITRO STUDIES

4.1.0 EFFECT OF COUMAPHOS ON THE CHOLINERGIC RECEPTOR RESPONSE TO ACETYLCHOLINE

This study showed a dose-related potentiation by coumaphos of cholinergic receptor response to acetylcholine since the force of ileum contraction increased from an average baseline value of 3.0cm to 5.0cm (10μg/ml coumaphos) 3.0 cm to 6.0 cm (20μg/ml coumaphos) 3.0cm to 5.67cm (40μg/ml coumaphos and 3.0cm to 5.7 (50μg/ml coumaphos) Table 2, Figure 3A, 3B).

4.1.1 EFFECT OF PRE-EXPOSURE TO LINDANE ON THE RESPONSE TO COUMAPHOS

Injection of 20μg lindane into the organ bath prior to the injection of 40μg coumaphos attenuated the response of the cholinergic receptor to coumaphos from 6.4cm to 4.2cm (Table 3, Figure 4B). This indicates that lindane pre-exposure reduces the in vitro toxic effect of coumaphos.

4.1.2 EFFECT OF CALCIUM ANTAGONIST (NIFEDIPINE) ON THE CHOLINERGIC RECEPTOR RESPONSE TO LINDANE

Calcium antagonism (using 5μg/ml Nifedipine), further increased the attenuation of the cholinergic response to acetylcholine due to lindane from 2.58cm to 0.92cm (Table 5). Indicating that reduction of the calcium concentration (due to calcium antagonist) augmented the in vitro toxic effect of lindane.

4.1.3 EFFECT OF CALCIUM ANTAGONIST (NIFEDIPINE) ON THE CHOLINERGIC RECEPTOR RESPONSE TO LINDANE IN A CALCIUM-FREE MEDIUM

In Tyrode's solution devoid of calcium ions, Lindane attenuated the muscarinic cholinergic receptor response to acetylcholine decreasing force of ileum contraction from 4.66cm to 0.82cm. In the presence of Nifedipine, the cholinergic receptor response was less (0.82cm vs 0.08cm). When the effect of Nifedipine on the response to Lindane in normal Tyrode's solution was compared with the effect in a calcium-free medium, it was observed that in a calcium-free medium Nifedipine caused a further reduction in the cholinergic receptor response (0.92cm vs 0.08cm) Tables 5 and 6; Figures 6A, 6B and 7.

The overall cholinergic receptor responses were found to be significantly ($P<0.001$) less in a calcium-free medium than in a medium containing 0.2% calcium.

Table 2: DOSE-RELATED EFFECT OF COUMAPHOS ON THE RESPONSE TO ACETYLCHOLINE

Drug	Force of ileum contraction (n = 5) (cm)	
	$\bar{x}$	SEM
1μg Ach (Control)	3.0	+ 0.24
10μg Cou. + 1μg Ach	5.0	+ 0.50
20μg cou. + 1μg Ach	6.0	+ 0.33
40μg cou. + 1μg Ach	5.67	+ 0.34
50μg cou. + 1μg Ach	5.7	+ 0.42

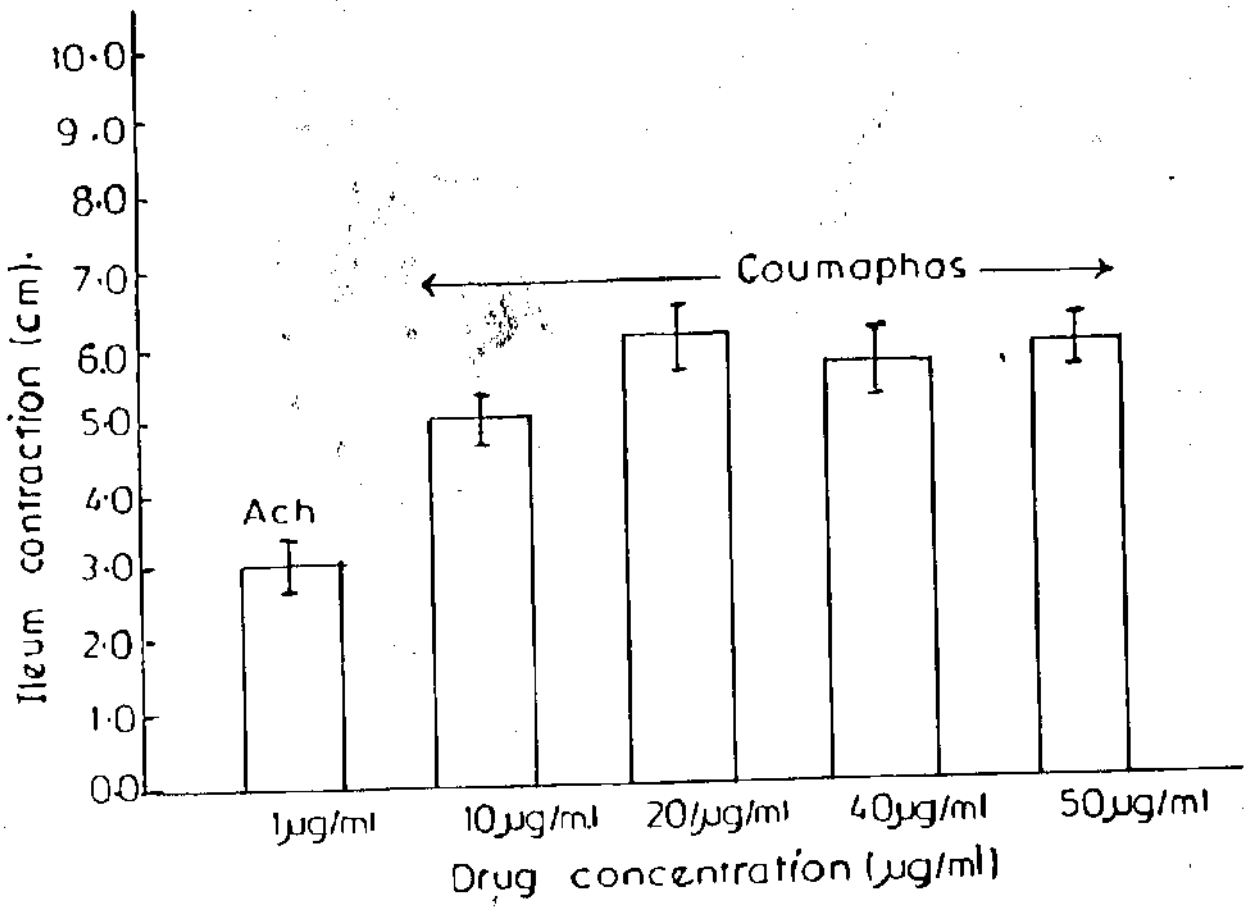


Figure 3A
Effect of graded doses of coumaphos on Ach pre-treated rabbit ileum

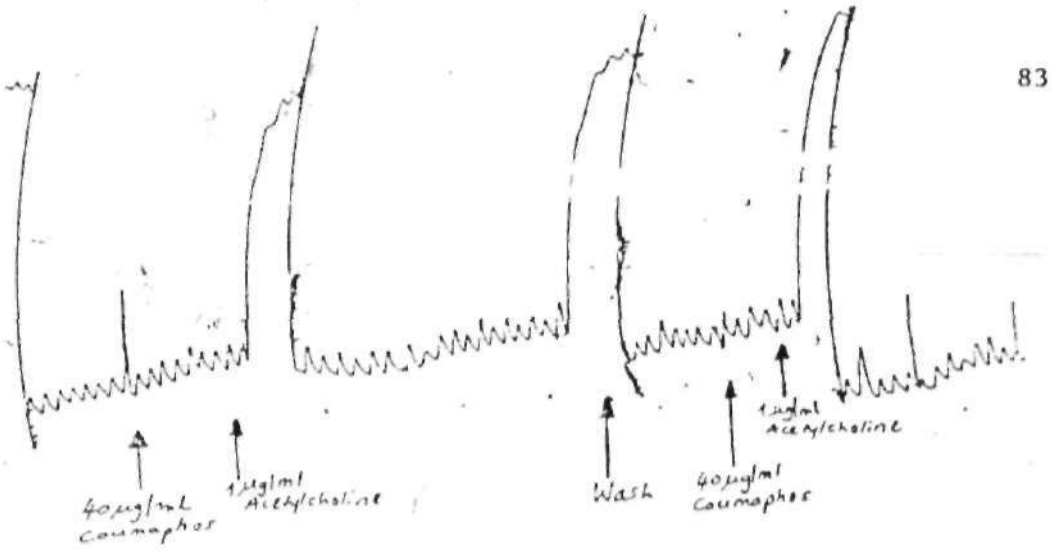


Figure 3B
Effect of coumaphos on the cholinergic receptor response to Acetylcholine

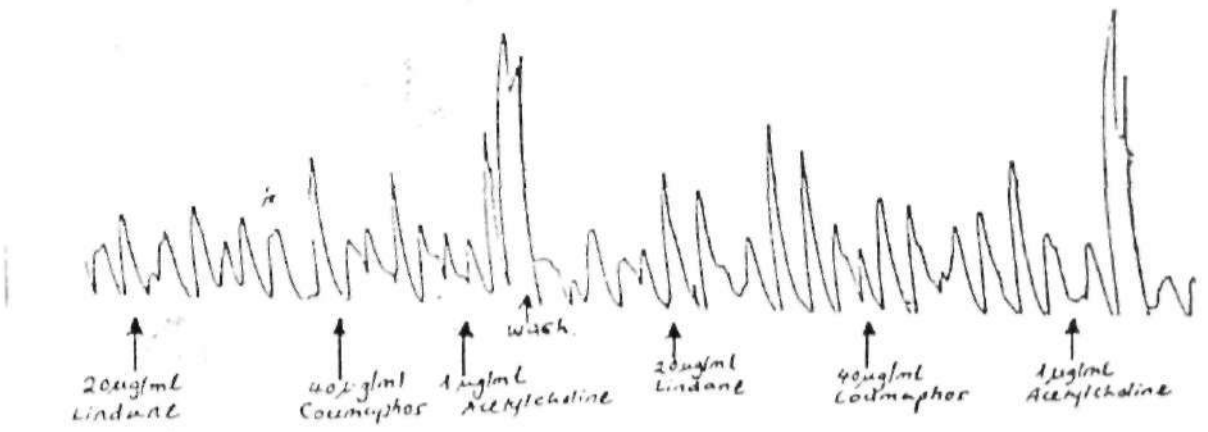


Figure 4A

Interaction of Lindane, coumaphos and Acetylcholine at the cholinergic receptor

4.2 EFFECT OF PRE-EXPOSURE TO LINDANE ON THE RESPONSE TO COUMAPHOS

Table 3: EFFECT OF LINDANE ON THE RESPONSE OF THE RABBIT ILEUM (CHOLINERGIC RECEPTOR) TO ACH

Drug	Mean Force of ileum contraction (cm)	
	$\bar{x}$	SEM
1ug Ach	4.9	+ 0.27
20ug Lindane + 1ug Ach	3.6	+ 0.54
40ug Lindane + 1ug Ach	2.5	+ 0.35

Table 4: EFFECT OF COUMAPHOS, LINDANE, AND LINDANE AND COUMAPHOS ON THE RESPONSE OF RABBIT ILEUM TO ACETYLCHOLINE

Drug	Mean Force of ileum contraction (cm)	
	$\bar{x}$	SEM
1μg Ach	4.9	+0.21
40μg Coum. + 1μg	6.4	+0.29
20μg Lindane + 1μg Ach	3.6	+0.55
20μg Lindane + 1μg Coum		
1μg Ach	4.2	+0.40

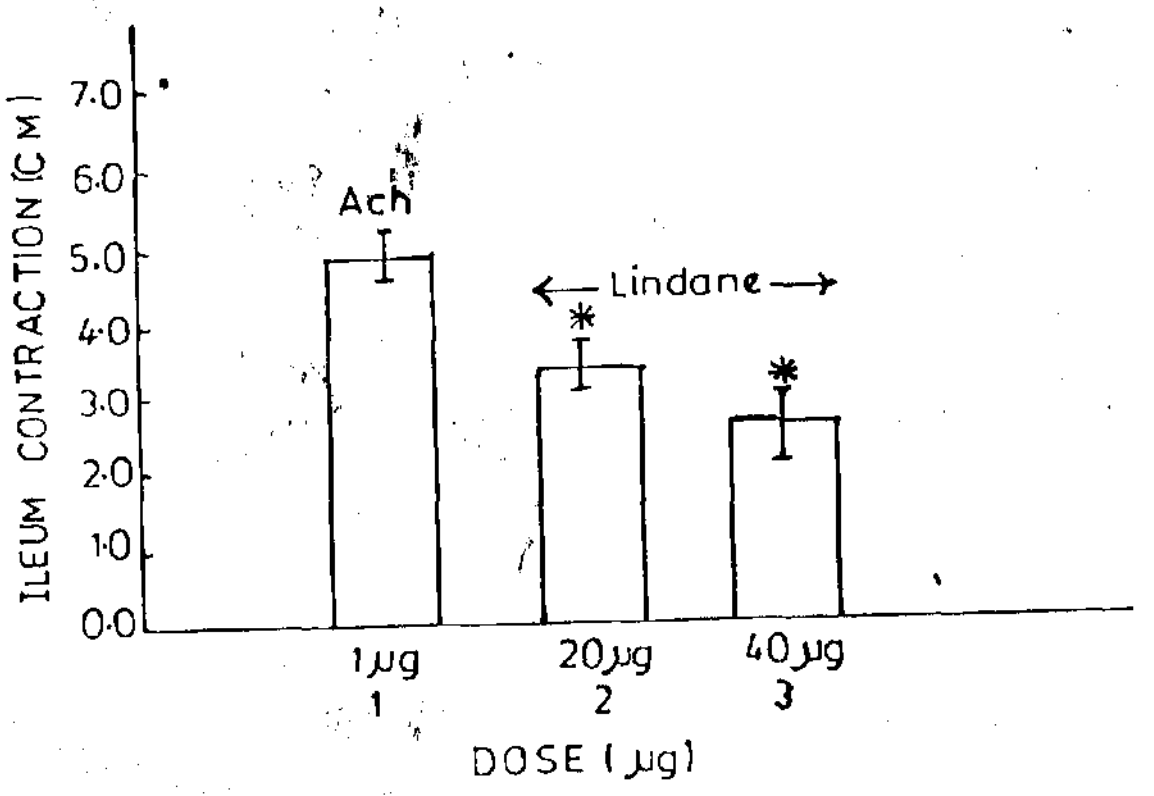


Figure 48:

Effect of Lindane on the response of the rabbit Ileum to Acetylcholine.

* = Significantly different from Ach ($P < 0.001$)

Table 5: EFFECT OF CALCIUM ANTAGONIST (NIFEDIPINE) ON THE CHOLINERGIC RECEPTOR TO LINDANE IN TYRODE'S SOLUTION

Drugs	Mean Ileum contraction (cm)	
	$\bar{x}$	SEM
1μg/ml Ach	6.76	+ 0.3
1μg/ml Ach + 2μg/ml Lindane	2.58	+ 0.8
1μg/ml Ach + 5μg/ml Nifedipine	3.66	+ 0.5
1μg/ml Ach + 2μg/ml Lindane + 5μg/ml Nifedipine	0.72	+ 0.4

Table 6 EFFECT OF CALCIUM ANTAGONIST (NIFEDIPINE) ON THE CHOLINERGIC RECEPTOR RESPONSE TO LINDANE IN A CALCIUM FREE MEDIUM

Drugs	Mean Ileum contraction (cm)	
	$\bar{x}$	SEM
1μg/ml Ach	4.66	± 0.9
1μg/ml Ach + 2μg/ml Lindane	0.82*	+ 0.2
1μg/ml Ach + 5μg/ml + Nifedipine	0.74*	+ 0.1
1μg/ml Ach + 2μg/ml + Lindane + 5μg/ml Nifedipine	0.08*	+ 0.2

Statistically significant at $P<0.001$ (student's 't' test)

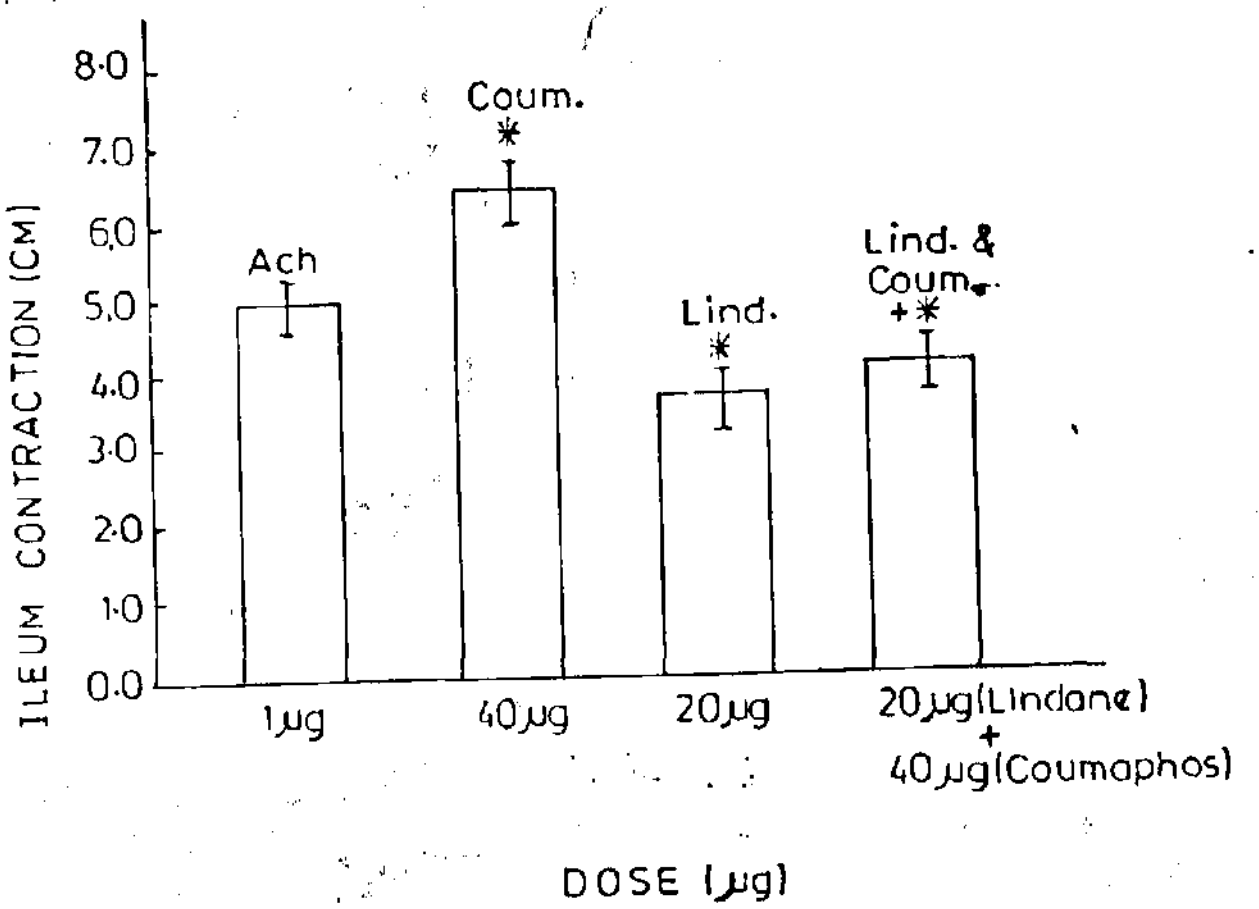


Figure 5:

EFFECT OF COUMAOPHOS, LINDANE, LINDANE AND COUMAPHOS ON THE RABBIT ILEUM RESSPONSE TO ACH

* = Significantly different from Ach (P <0.01)

** = Significantly different from Ach and Coumaphos (P <0.001)

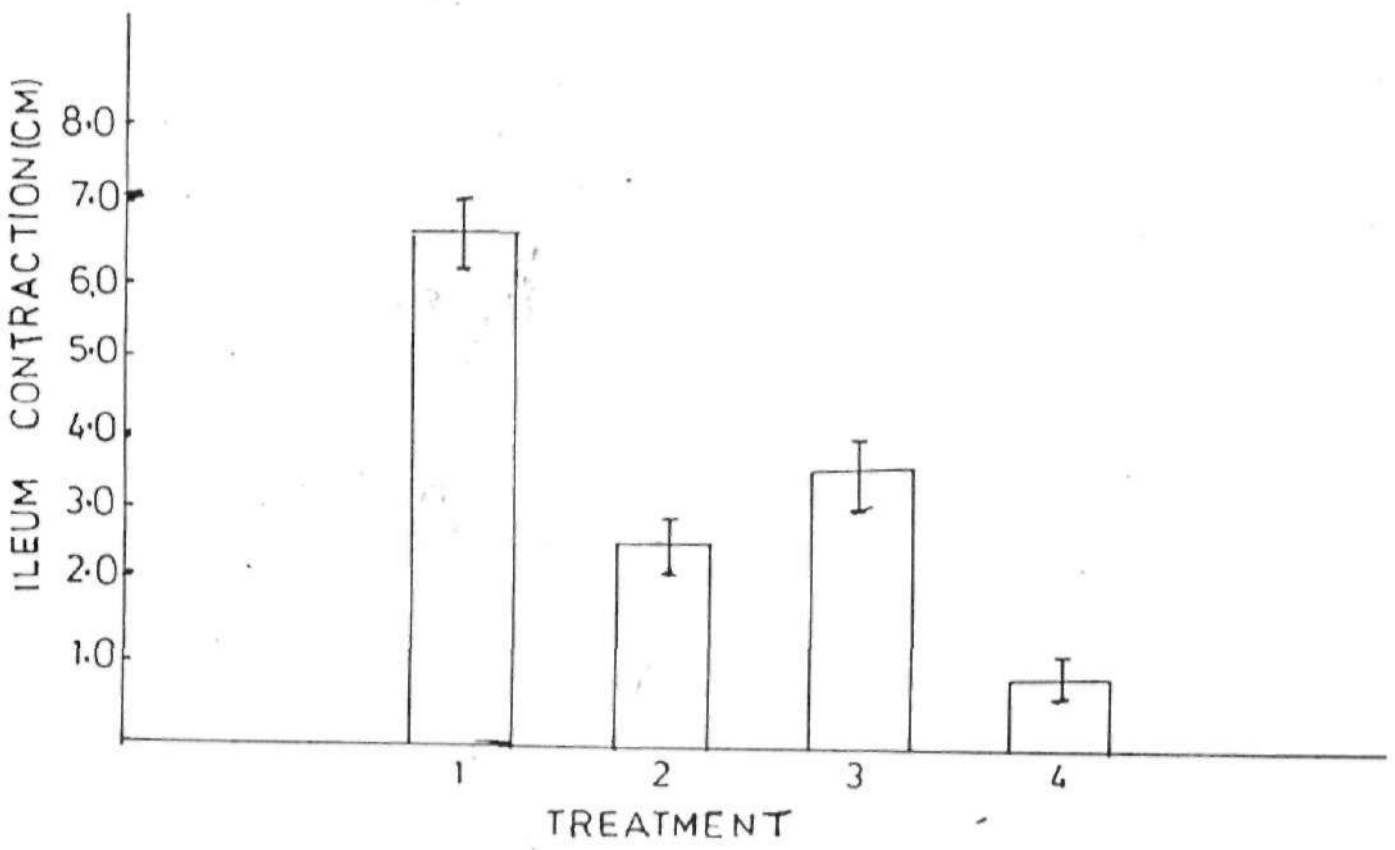


Figure 6A

EFFECT OF CALCIUM ANTAGONIST (NIFEDIPINE) ON THE RESPONSE OF THE RABBIT ILEUM TO LINDANE

Key
1 = 1μg Ach (control)
2 = 1μg Ach + 2μg Lindane
3 = 1μg Ach + 5μg Nifedipine
4 = 1μg Ach + 2μg Lindane + 5μg Nifedipine

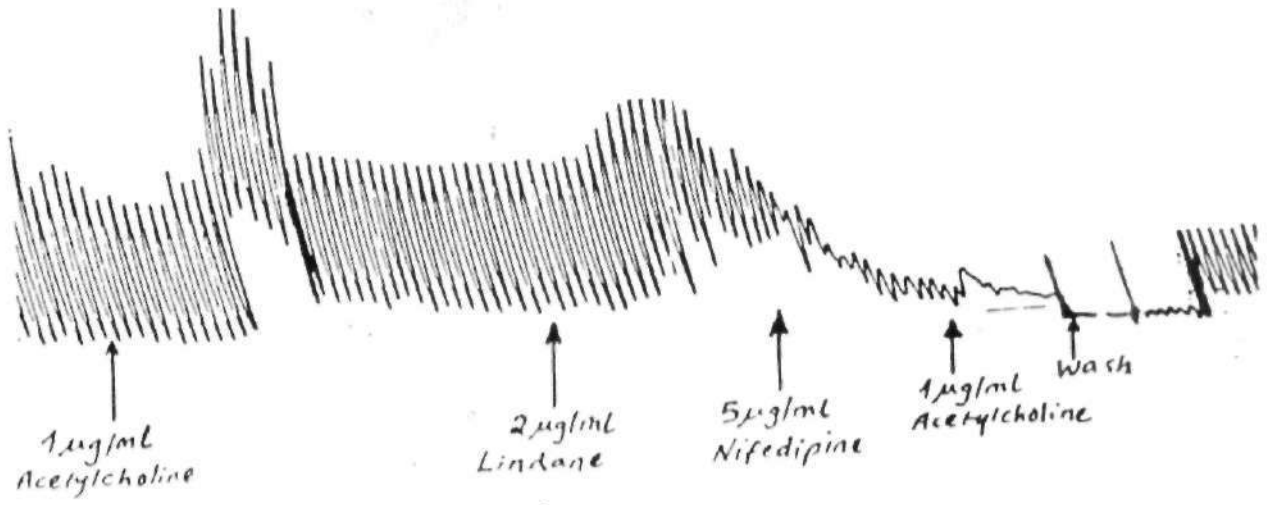


Figure 6B

Effect of lindane plus Nifedipine on ileum contraction

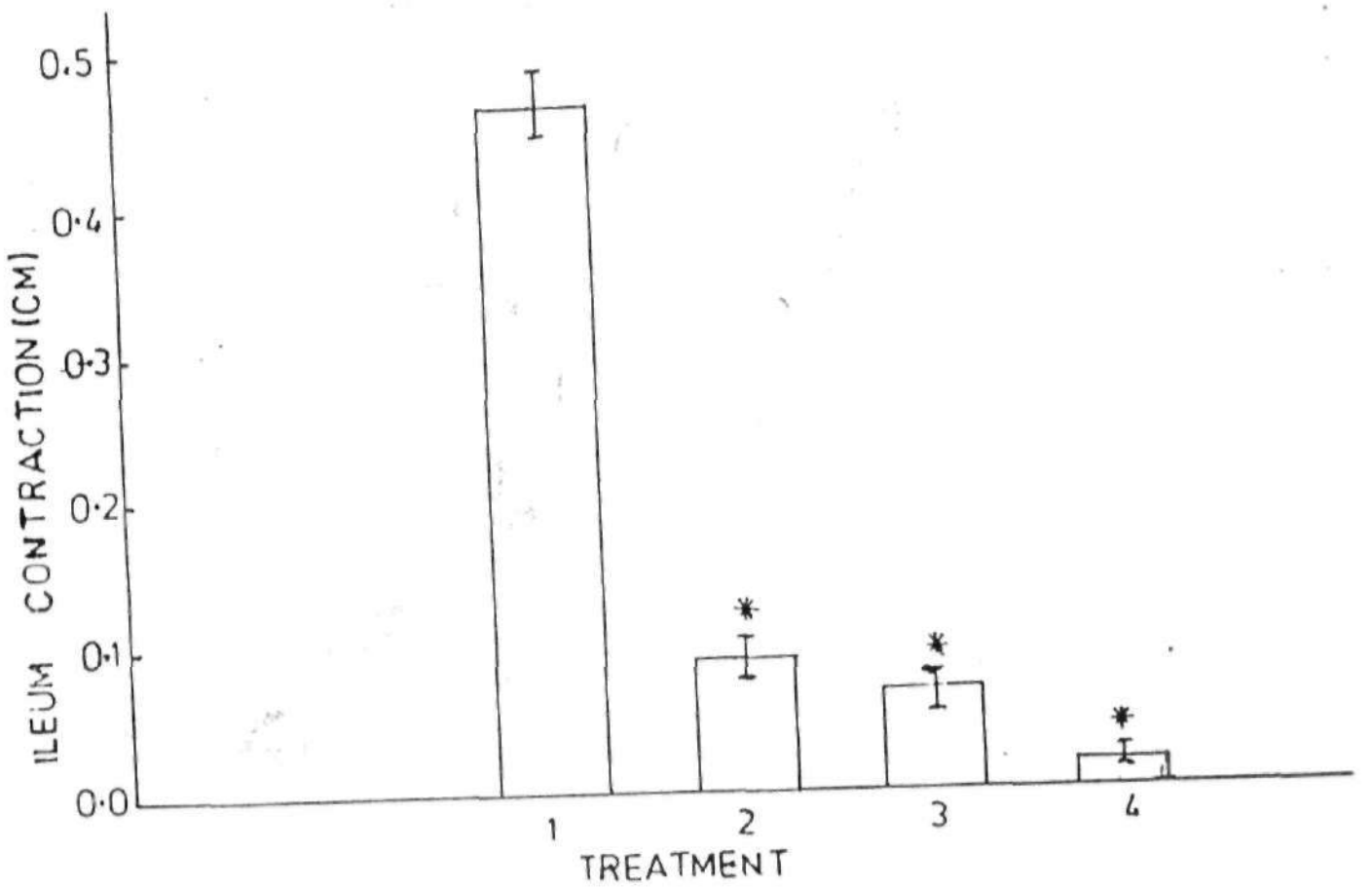


Figure 1

EFFECT OF CALCIUM ANTAGONIST ON THE CHOLINERGIC RESPONSE OF THE RABBIT ILEUM TO LINDANE IN A CALCIUM FREE MEDIUM

Key
1 – 1μg Ach (control)
2 – 1μg Ach + 2μg Lindane
3 – 1μg Ach + 5μg Nifedipine
4 – 1μg Ach + 2μg Lindane + 5μg Nifedipine

4.2.0 IN VIVO STUDIES

4.2.1 ACUTE TOXICITY

4.2.2.1 MEDIAN LETHAL DOSE (LD50) OF COUMAPHOS IN RATS

The intraperitoneal LD_{50} of coumaphos in male and female rats was determined. The results are presented in Table 7. Using the arithmetic method of Karber, (Aliu and Nwude, 1982) 139.6mg/kg was obtained as the dose that killed 50% of a group of rats.

The results showed that a dose of 50mg/kg produced no death in the group of rats; while at 300mg/kg and above all animals in the group were killed.

At doses between 50mg/kg and 200mg/kg, animals that died did so between 24 and 48 hours of coumaphos injection. Animals that eventually died and some of the survivors exhibited signs of organophosphate poisoning with a preponderance of manifestation of signs of nictotinic cholinergic stimulation mainly fasciculation of muscles, tremors, spasms and stiff gait sequel to hypertonicity. In severe cases, Table especially at 200mg/kg and higher doses, central nervous system manifestations were evident, chiefly hyperactivity with clonic convulsion.

Muscarinic signs such as wet eyes (lacrimation) nasal discharges with wetness of the muzzle were also observed in some of the poisoned rats

Table 7: INTRAPERITONEAL MEDIAN LETHAL DOSE (LD_{50}) OF COUMAPHOS IN RATS (USING THE ARITHMETIC METHOD OF KARBER)

Dose	Dose Difference	Dead	Mean Dead	Dose Difference X Mean Dead
50mg/kg		0		
	50		0.5	25
100mg/kg		1		
	65		2.5	162.5
165mg/kg		4		
	35		5.0	175
200mg/kg		6		
			Total =	362.5

NB: Minimum dose that killed all animals in a group = 200mg/kg

Total number of animals per group = 6

The sum of (dose difference x mean dead = 362.5

Substituting into the formula:

$$LD_{50} = \text{Minimal dose that killed all animal population} - \frac{(sum[Dose\ difference \times mean\ dead])}{\text{Total number of animals per group}}$$

Where LD_{50} = dose of the chemical that kills 50% of the animal population

LD_{50} of coumaphos = $200 - \frac{362.5}{6} = 200 - 60.42$

LD_{50} of coumaphos = 139.6mg/kg.

Table 8: ORGAN/BODY WEIGHT RATIO OF RATS PRETREATED AND NON-PRETREATED WITH 1MG LINDANE AND FED VARYING LEVELS OF COUMAPHOS FOR 10 WEEKS

Group	Coumaphos Level (mg/kg feed)	Organ/Body Weight Ratio ± SD.					
		Kidneys	Lungs	Livers	Brain	Heart	Spleen
1. Control	None	0.86±0.18	1.89±0.36	3.71±0.52	1.26±0.38	0.34±0.67	0.39±0.24
2A. Non-Lindane pretreated	1	0.83±0.33	0.94±0.95	3.57±0.74	0.95±0.19	0.36±0.10	0.39±0.15
2B. Lindane pretreated	1	0.64±1.29	0.90±0.10	3.12±0.20	1.83±0.14	0.37±0.01	0.31±0.00
3A. Non Lindane pretreated	10	0.68±0.02	0.80±0.20	3.79±0.22	0.97±0.12	0.29±0.00	0.33±0.00
3B. Lindane pretreated	10	0.69±0.12	0.76±0.13	3.68±0.26	0.86±0.07	0.29±0.00	0.32±0.07
4A. Non-Lindane pretreated	100	0.77±0.08	0.85±0.81	3.54±0.75	0.96±0.07	0.29±0.02	0.18±0.00
4B. Lindane pretreated	100	0.68±0.06	0.69±0.11	3.27±0.28	0.78±0.00	0.26±0.00	0.19±0.02

4.2.3 SUB-CHRONIC TOXICITY

4.2.4 EFFECT OF SUB-CHRONIC TOXICITY OF COUMAPHOS ON MEAN ORGAN/BODY WEIGHT RATIO OF RATS

This study indicates that coumaphos does not significantly affect the mean organ/body weight ratio of poisoned animals. Both Lindane-pre-treated and non-pre-treated groups of rats fed varying levels of coumaphos over a period of 10 weeks did not exhibit any statistically significant difference in their organ/body weight ratios (Table 8).

4.2.5 HISTOPATHOLOGIC STUDIES OF THE EFFECT OF PRE-EXPOSURE TO LINDANE ON THE SUB-CHRONIC TOXICITY OF COUMAPHOS

In the group of rats fed 1mg/kg coumaphos, no significant pathological lesions were found in most organs, except in the kidneys of three animals. Particularly in those not pre-exposed to lindane there was non-suppurative necrosis of proximal and distal convoluted tubules involving some glomeruli as well as focal areas of edema in the cortex (Figure 8).

The kidneys of animals pretreated with lindane exhibited congestion of renal blood vessels.

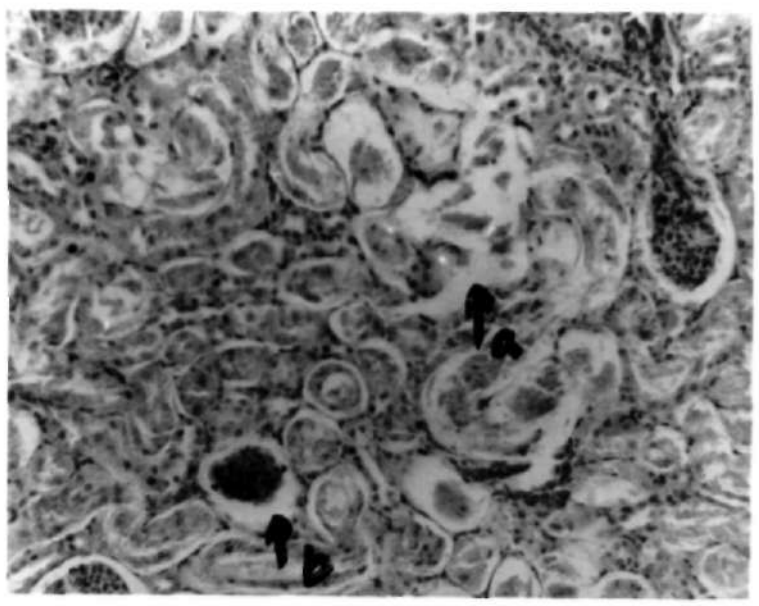


Figure 8:
Photomicrograph of kidney of rat fed 1mg/kg coumaphos without pre-exposure to lindane. Arrows indicate, (a) non-suppurative necrosis of proximal and distal convoluted tubules and (b) necrotic glomerulus.

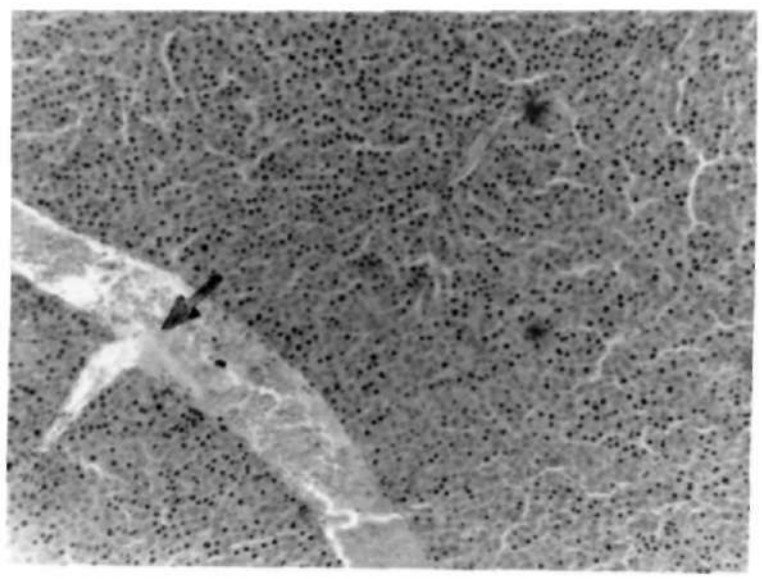


Figure 9:

Photomicrograph of liver of rat pretreated with lindane and fed 10mg/kg coumaphos showing congested hapatic blood vessel. (Note the otherwise normal hepatic architecture).

Pathological lesions were found in the heart, liver and kidneys of animals fed 10mg/kg coumaphos. While those pretreated with lindane exhibited congested hepatic blood vessels (Figure 9) the group of non-pre-treated rats had congestion of renal blood vessels accompanied by mononuclear cellular infiltration of the cortico-medullary junction and glomerular atrophy (Figure 10).

In the groups of rats fed 100mg/kg coumaphos, the liver, intestine, brain, heart and spleen exhibited pathological lesions. Among those exposed to coumaphos alone, there was mono-nuclear infiltration of the lamina propria of the gut, necrosis and desquamation of the epithelial lining of the tip of the intestinal villi. The liver manifested areas of focal non-suppurative necrosis. The cerebral cortex was vacuolated. (Figure II), while myocardial blood vessels were congested.

Some dark-greenish pigment akin to hemosiderin were diffusely deposited in the spleen along with non-suppurative necrosis of the red pulp.

In animals pre-treated with lindane, lesions were mild, confined to the kidneys and manifested as congestion of blood vessels with atrophy of a few glomeruli.

The above findings indicate that pre-exposure to lindane offers some protection against the sub-chronic toxicity of coumpahos.

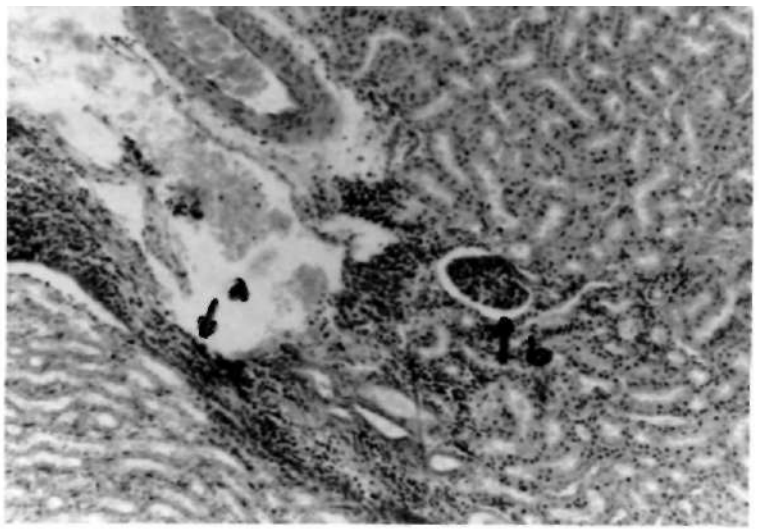


Figure 10:
Photomicrograph of kidney of rat fed 10mg/kg coumaphos without prior exposure to lindane

Note: Monocuclear cellular infiltration of cortico -medullary junction (a) and glomerular atrophy (b)

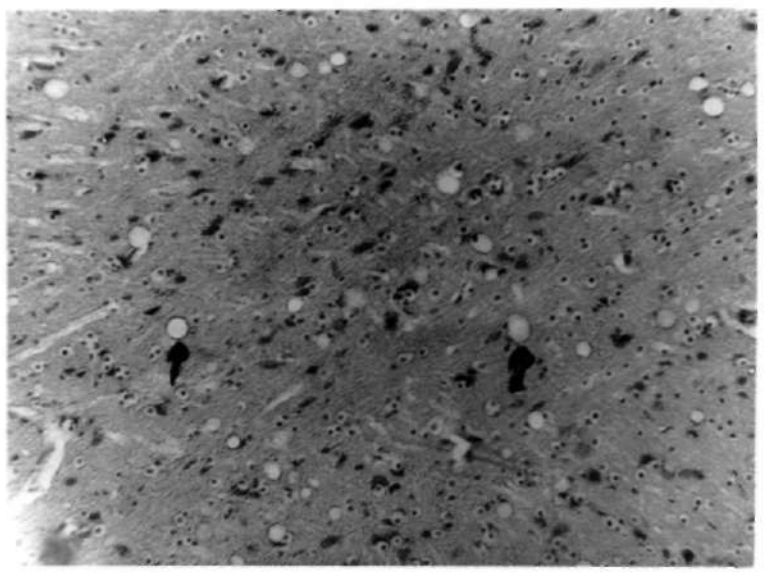


Figure 11:

Photomicrograph showing cerebral cortex of rat fed 100mg/kg coumaphos without prior exposure to Lindane

Note: Vacuoles in the cortex (arrows).

World Health Organization (1981). Health effects of combined exposures in the work environment. Technical Report Series No. 662, Geneva.

World Health Organization (1990). Public Health impact of pesticides used in Agriculture. W.H.O., Geneva.

CHAPTER FIVE

5.0 DISCUSSION

5.1.0 IN VITRO STUDIES

Organophosphorus compounds exert their toxic action by competitive inhibition of cholinesterase (ChE) with consequent accumulation of acetylcholine (Ach) at the neuromuscular junctions, autonomic ganglia and effector cells (O'Brien, 1960). With the accumulation of acetylcholine, complex nervous system functions are disrupted, causing disturbances in glands, muscles, in parts of the brain and all regions where acetylcholine mediates transmission of impulses (Plestina, 1984).

The observed potentiation by coumaphos of the cholinergic receptor response to Ach in this study can be attributed to the build up of Ach at the parasympathetic postganglionic terminals occasioned by an inhibition of ACHE by the organophosphate coumaphos. Since the normal function of AChE is to hydrolyse Ach that has been liberated at cholinergic nerve endings in response to nervous stimulation and terminate neurotransmission (WHO, 1986); the accumulated Ach then caused an over-stimulation of the cholinergic receptor of the rabbit ileum manifested as a stronger contraction of the isolated smooth muscle than that

observed in the control, eventually leading to spastic paralysis of the muscle from a depolarizing blockade (Table 3, Figure 3).

In vivo, potentiation of Ach by coumaphos is responsible for the toxicity of the pesticide (Iyaniwura, 1990). The muscarinic and nicotinic effects of excessive acetylcholine at central and peripheral sites and the attendant signs and symptoms have been discussed in section 1.26.1.

When the isolated rabbit ileum was pre-exposed to the organochlorine lindane, it was observed that lindane significantly ($P<0.001$) decreased the in vitro toxic effect of coumaphos by attenuating the initial coumaphos-induced potentiation of the cholinergic receptor response to Ach.

This, by extension, is consistent with the observation made in in vivo studies by other workers. For instance, Ball *et al.* (1954) reported that three to four days after administration of the organochlorine insecticide, aldrin, the toxicity of parathion to female rats decreased by about seven-fold. Main (1956) also showed that the oral but not the intravenous toxicity of paraoxon was decreased by aldrin pretreatment, while both the oral and the intravenous toxicity of parathion were decreased. Following the observation by Main (1956) that aldrin pretreatment doubled liver A-esterase activity and decreased serum A-esterase by 50 percent, he concluded that the mechanism

by which aldrin reduced parathion's toxicity was by stimulation of the hydrolysis of paraoxon in the liver after it was formed from parathion in this organ.

Other instances of the protective action of organochlorine insecticides have been reported in the literature, for instance protection by aldrin against parathion toxicty in mice (Triolo and Coon, 1966a and b). Lindane has also been shown to protect mice against the toxic effects of the carbamate insecticide carbaryl (Iyaniwura and Gubio, 1990). It was espoused that the protection afforded by lindane pretreatment may be due to an induction of the hepatic microsomal enzymes (Iyaniwura & Gubio, 1990).

The hepatic microsomal enzymes catalyze the detoxification of drugs and other xenobiotics in the body and the chlorinated hydrocarbons are especially known for their ability to stimulate the drug metabolizing enzymes (Dubois, 1969, Conney and Burns, 1972). The protection afforded by organochlorine pesticides against other chemical compounds via the induction of hepatic microsomal enzymes is believed to be limited by the amount of such chemicals to which the animal is exposed, because above a certain dose, the animal can no longer adapt by increasing microsomal metabolism, and the compound interferes with essential cellular processes (Hoffman *et al.*, 1970). The biphasic responses observed in the liver after dieldrin

administration (Klion, 1966) is in line with this belief. Hutterer *et al*. (1969) noted that in small doses dieldrin induces drug-metabolizing enzymes, while lager amounts result in depression of induction and simultaneous mitochondrial injury.

From the point of view of pesticide interactions, organochlorine pesticides apart from protecting animals against organophosphorus toxicity have also been shown to potentiate the toxicities of both organophosphorus and organochlorine pesticides (WHO, 1981, Street *et al*., 1969).

The various mechanisms underlying pesticide interactions have been discussed in section 1.17. Further elaboration may be found in the work of Murphy (1969) and Iyaniwura (1990).

Although the mechanism of action of the organochlorine insecticides is somewhat obscure, (Hassall, 1982, Plestina, 1984) the major toxic action of this group of chemicals is on the central and peripheral nervous systems while systemic action in acute overdosage is confined to the central nervous system and the symptoms are those of CNS stimulation such as headache, apprehension and excitement accompanied by dizziness disorientation, vomiting, weakness of the skeletal muscles, tremor and finally epileptiform convulsions (Plestina, 1984). Y-BHC (lindane) and dieldrin exert a destabilizing effect on the nerve membrane somewhat similar to that of DDT. As a result of

this effect, a single electrical stimulus leads to multiple uncontrolled responses of poisoned nerves with consequent muscular spasms (Hassall, 1982).

Different reasons have been advanced for these observed effects, the first of which is that the insecticides may inhibit one or several enzymes of importance to the mechanism of membrane stability or of nerve impulse transmission. It may also be due to initiation of some physical change in the structure of the nerve membrane which rather literally causes it to misfire (e.g. by alteration of its permeability to ions or to other solutes).

It is believed that ATPases are probably associated with muscular activity, mitochondrial processes and solute movement through tonoplast and plasmalemma as well as with the activities of nerve membranes (Hassall, 1982). There appears to be one sort of ATPase which is dependent on sodium and potassium ions and is actually a pat of the sodium pump; another type is dependent on the presence of calcium ions while a third type requires both calcium and magnesium ions.

Mullins and Brinley (1975) have shown that the calcium-dependent ATPase located on the outer surface of axons (Ecto-ATPase) is much more sensitive to DDT than the ATPase associated with the workings of the sodium pump.

EctoATPase appears to regulate the binding of calcium ions to the surface by the membranes. In the face of a shortage of calcium ions, axonic excitation occurs which closely resembles DDT poisoning. It has also been shown that the neurotoxic symptoms of DDT poisoning are enhanced if the calcium ion concentration is reduced below 20 mM and greatly diminished if calcium ion concentration is elevated above 100 mM (Matsumura and Narahashi, 1971).

In consideration of the above observation by Matsumura & Narahashi (1971) and the suggestion of a seeming similarity of the membrane-destabilizing effects of DDT and Y-BHC (Lindane); a study was conducted to determine the effects of a reduction of the calcium concentration in the medium (by calcium antagonism using nifedipine) on the in vitro toxic effect of lindane.

The result of the study indicates that lindane attenuates the response of the cholinergic receptor to Ach (Table 6, fig. 6) and a reduction in the calcium concentrations in the medium significantly ($P<0.001$) exacerbated the lindane-induced attenuation of cholinergic receptor response to acetylcholine (Fig.6). In a medium completely devoid of calcium, cholinergic receptor response was significantly ($P<0.001$) reduced, as manifested by weaker contraction of the smooth muscle of the rabbit ileum (Table 7) as compared with the contraction in normal Tyrode's solution containing 0.2% calcium (Table 6,

fig.6). This finding is in agreement with the suggestion that lindane may be exerting its toxic effect through a calcium-linked mechanism (Iyamiwura *et al.*, 1991; Dede *et al.*, 1991). It is also consistent with the observation by Matsumura and Narahashi (1976) that the neurotoxic symptoms of DDT poisoning are enhanced if the calcium ion concentration is reduced, since the effects of DDT and Y-BHC (lindane) are similar (Hassall, 1982). It may be deduced that lindane toxicity is augmented by low calcium ion concentration. It would appear that lindane, like DDT, through its effect on the Ecto-ATPase which regulates the binding of calcium ions to the surface of the neurolemma, interferes with the mobilization of calcium at the nerve terminal. The effect of inhibition/reduction in the influx of Ca^{++} ions into the nerve terminal is an interference with the quantal release of Ach which is an impediment to neuromuscular transmission. This may account for the weak muscular contractions observed in this study.

5.2.0 IN VIVO STUDIES

5.2.1 ACUTE TOXICITY OF COUMAPHOS

For safety of any poisonous chemical, it is important to determine the oral median lethal dose (LD_{50}). Chemicals can be classified for convenience according to their LD_{50} values (Matsumura, 1975).

5.2.2 PRE-EXPOSURE TO LINDANE AND THE SUB-CHRONIC TOXICITY OF COUMAPHOS

From the result of the sub-chronic toxicity study, it may be deduced that pre-exposure to Lindane affords some form of protection to animals poisoned with coumaphos, considering the mildness of pathologic lesions in rats pre-exposed to lindane vis-a-vis those without prior exposure to lindane but receiving similar doses of coumaphos.

This observation corroborates the findings in the in vitro experiments of the present study where it was evident that lindane significantly decreased the in vitro toxic effect of coumaphos at the cholinergic receptor and re-affirms previous reports of the protective action of organochlorine insecticides reported in the literature (Dubois, 1969; Conney and Burns 1972; Iyaniwura and Gubio 1990).

Whereas the in vitro attenuation by lindane of coumaphos toxicity is supposed to be via a calcium-linked mechanism (Iyaniwura *et al.*, 1991, Dede *et al.*, 1991), the amelioration of toxic effects due to coumaphos and other chemical compounds is believed to be by way of the induction of hepatic microsomal enzymes which metabolically detoxify or inactivate environmental chemicals especially the cytrochrome P-450-dependent mixed

function oxidases of the liver endoplasmic reticulum (Parke, 1975; Iyaniwura and Gubio 1990).

The major requirement for the production of enzyme induction being that the inducing agent be a substrate of the mixed function oxidases and that it be only slowly metabolized, thereby being bound to the active site of the enzyme for sufficiently long periods to cause a momentary decrease in its availability (Parke, 1975). Thus the early stage of enzyme induction involves a process of enzyme inhibition which may last for some 1-12 hours, this provokes an increased de novo enzyme synthesis with a subsequent increase in enzyme activity, this type of induction is often described as the "substrate type" and is characteristic of the drug metabolising enzymes of the liver, gastrointestinal tract and other tissues (Parke, 1975).

This induction of cytochrome P-450 or the "substrate or phenobarbital type" induction may generally be expected to enhance the detoxification of ingested chemicals, with consequent reduction in toxicity.

5.2.3 CONCLUSION

Since pesticides have become a ubiquitous part of the environment, increased attention should be focused on possible biologic effects of chronic, low-level exposure to these agents.

Although the organophosphorus insecticides are non-residual, there is dire need for caution in their application against the backdrop of the organophosphorus-induced delayed neuro-toxicity which may be triggered by a single episode of toxicity.

The common practice of combined use of pesticides for enhanced efficacy and control of resistance especially involving the persistent organochlorine pesticides must take cognisance of the potentially deleterious effect of the interaction between these chemicals in an environment that is so intimately shared by pests and non-target human and livestock.

The protection offered by organochlorine pesticides against the organophosphates does not necessarily invalidate the untoward effects of acutely toxic quantities of the organophosphorus pesticides.

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