TOXICOLOGICAL INTERACTION OF ORGANOCHLORINE AND ORGANOPHOSPHATE INSECTICIDES IN VIVO

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DECLARATION

I hereby declare that this thesis has been written by me and that it is a record of my own research work. It has not been presented in any previous application for a higher degree. All sources of informations are specifically acknowledged by means of references.

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DEDICATION

I dedicate this thesis to my beloved wife, RAHAB, our child, RIMAMTSWAB and ones yet unborn, whose understanding, sacrifices and love inspired me to complete this work.

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ABSTRACT

Acute symptoms of toxicity and median lethal dose (LD_{50}) values by the intraperitoneal route over 24 hours for commercial formulations of dimethoate (Rogor® and Perfekthion®), aldrin and lindane were determined in mice. The difference found in LD_{50} values for Rogor® (272. 1mg/kg) and Perfekthion® (165mg/kg) formulations of dimethoate showed that differences in formulations could alter the toxicity of technical grade compounds. Aldrin (LD_{50}, 40mg/kg) was found to be more toxic than lindane (LD_{50}, 249 0mg/kg).

Interactions between aldrin, and lindane individually with dimethoate were investigated *in vivo* in mice. Decreased dimethoate survival time was observed in mice pretreated for four days with a single dose of 16mg/kg aldrin. The data (Table IX) on the effect of aldrin on mice weight and **feed** intake appear to suggest that this decrease (in survival time) could be due to anorexic effects on the mice which interferred with their feeding habits, rather than induction of hepatic microsomal enzymes produced by aldrin. Two days lindane pretreatiuenc of mice with a single dose of 98mg/kg, increased dimethoate survival time. By

reducing the pentobarbitone sleeping time, exposure to lindane appears to offer potection to dimethoate toxicity by a mechanism in the animals that appears to involve an induction of the hepatic microsomal enzymes; since the same dose of lindane shortened the pentobarbitone sleeping time in mice.

Subchronic daily intraperitoneal exposure to 25, 50 and lOOmg/kg of dimethoate over thirty days produced histologic lesions in the lungs, livar, and kidneys of some of the animals. These most probably were due to secondary effects of the pesticides on the tissues of the animals.

It was concluded that exposure to moderate doses of organochlorires especially those with a high inductive effect on the liver enzyme may decrease the mammalian toxicity of some organophosphates.

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GLOSSSARY OF ABBREVIATIONS

ACHE	=	Acetylcholinesterase
EPN	=	O-ethyl O-P-nitrophenyl phenyl phosphonothioate
DDT	=	2,2-bis(P-chlorophenyl) 1,1,1 trichloroethane
DEF	=	5,5,5-tributyl phosphorotri-thiolate
EPSP	=	Excitatory Postsynaptic Potential
IPSP	=	Inhibitory Postsynaptic Potential
MFO	=	Mixed Function Oxidase
SKF 525-A	=	2-Diethylaminoethyl 2,2-diphe-nylvalerate hydrochloride.
TOCP	=	Tri-o-tolyl phosphate OR Tri-o-cresyl phosphate
TOTP	=	Tri-o-tolyl phosphate

CHAPTER ONE

LITERATURE REVIEW

INTRODUCTION

Pesticide chemicals are of great value in increasing crop yield and in the prevention of crop losses before and after harvest. Cocoa production rose in Ghana by 110% in 1957 when pesticides were introduced to protect cocoa pods from the black pod disease. Japan in 1960 also recorded increase in rice production when pesticides were introduced to protect rice from the stem borer, *Pricularia oryzae* (Fest *et al.*, 1982).

Pesticides are also useful in public health programmes to control vector-borne diseases. For instance, the use of pesticide has been described as the best way to control *Anopheles* mosquito as part of malaria control. This was proved over forty years by the World Health Organisation (W.H.O., 1985). It was also shown that onchocerciasis control depends entirely on the destruction of vector simuliids since no drug is availabe at present that may be directly used in mass control programmes of the disease. Regular insecticide (DDT) spraying of river rapids where larvae breed were successful against *S. naevi* and *S. damnosum* in Kenya and Uganda in the 1950's.

In Nigeria today there is a growing awareness and demand for the use of pesticide in agriculture. Two reasons could be said to be responsible. Firstly, there is a considerable increase in extension services being freely provided to farmers by the Ministries of Agriculture of the various state governments on modern farming techniques; secondly, many retired civil and army officers have become large-scale modern farmers. In order to effectively control pests and thus decrease crop losses, the use of pesticides has to be employed.

Toxicity of pesticides is however, not limited to the target organisms. Pesticides are also toxic to man and his invaluable animals. Exposure of man to pesticide occurs in several ways. Occupational exposure occurs during the manufacture, transportation and at the site of application. These exposures could produce acute or chronic toxic effects. Accidental poisoning or consumption of crops sprayed with pesticide constitute other means of human intoxication.

Rxposure may be via inhalation, dermal or oral routes. While it may be posible to detect acute poisoning and thus provide adequate medical care, chronic exposure is not readily detected. Efforts to find ways of minimizing pesticide harzards to man has resulted in intensive scientific investigations. Toxicological evaluations of the harzard of handling and use of pesticides have for many years focused on prevention of injury to man or his animals. Laboratory animals serve as experimental models for man's biochemical, physiological and pathological responses to these chemicals.

Conventional procedures for evaluating the toxicity of pesticides in the past consisted of acute and chronic tests on laboratory animals aimed at determining their individual levels of toxicity and their general pharmacologic actions. It was then assumed that pesticides with similar pharmacologic actions would act additively on simultaneous exposure, as they would have acted independently.

However, the discovery in 1957 by Frawley *et al* that o-p-nitrophenylphenylphosphonothioate (EPN), an organophosphate, markedly potentiated the toxicity of malathion, when these were given simultaneously to laboratory animals, has called for an increased research effort on the combined toxic effects of pesticides before any reliable predictions could be made regarding their human toxicity.

In practice pesticides are used in combinations either for the purpose of controlling different pests or to achieve greater effects. These combinations could lead to interactions via various mechanisms to alter the toxic dose of the individual components. Mechanisms of pesticide interactions in vertebrates have been reviewed by various authors (Murphy, 1969; DuBois, 1969 and Iyaniwura, 1988a). The effects of interactions could be potentiation, antagonism or additive. The implications of these in relation to harzard is that the most important single factor influencing pesticide toxicity, that is dosage, is altered. Highly toxic compounds are excluded from use in vector control precisely because of the impracticability of reducing human exposure to them (W.H.O, 1979). A pesticide with previously good safety record could cause poisoning among spraymen if the toxic dosage has been altered significantly by an impurity or other pesticide in the formulation. Several cases of such poisoning were reported in Pakistan in 1976 among malaria spraymen using formulation which contained isomalathion as impurity (Aldridge *et al*, 1979).

1.12 MECHANISMS OF PESTICIDE INTERACTION

Various mechanisms have been postulated by various authors to explain the possible mechanisms of interactions that occur when pesticides are combined (DuBois, 1969; Iyaniwura, 1988b). Most of these mechanisms implicate induction or inhibition of liver microsomal enzymes. These enzymes are responsible for the metabolism of many exogenous chemicals. Other processes affecting toxicity of a chemical agent include direct chemical or physical interactions in the gastrointestinal tract, interactions in protein binding and interaction via excretory routes.

1.13 Interactions That Increase Organophosphorus Toxicity.

Metabolic interactions constitute a major mechanism by which pesticides alter their toxicities when used in combination as reviewed by Murphy (1969). Organophosphorus pesticides are principally metabolised by oxidation and hydrolysis by esterases (Plapp *et al*, 1958; Hayes, 1982). Some organophosphates require activation *in vivo* to their toxic forms. For instance, parathion has been shown to be activated to paraoxon, its oxygen analogue, which is the toxic form of parathion (Dauterman, 1971) for this group of organophosphates it would be expected that interactions that increase the activity of the enzymes responsible for the activation process would increase their toxicity. However, as would be discussed subsequently, other overiding factors exist that determine the overall toxicity of a pesticide.

The potentiation by EPN of the toxicity of malathion was explained by Murphy and DuBois (1957b) as due to an EPN inhibition of the enzymatic hydrolysis of the carboxyl-ester linkages of malathion. It may therefore be expected that any compound capable of inhibiting aliesterase in the same way as does EPN would also inhibit the detoxification of organophosphates that are normally detoxified by aliesterases thereby increasing the organophosphate toxicity.

However, in practice, this is not usually the case. For example, SKF 525A which inhibits microsomal enzymes optimally after forty minutes pretreatment in rats was shown by Murphy (1958) to decrease Guthion (azinphosmethyl) toxicity. No decrease in toxicity was observed with parathion or EPN of SKF 525A pretreatment. O'Brien (1961) found a decreased dimethoate toxicity following pretreatment with SKF 525A but obtained potentiation with most other phosphorothioates.

No general statements can therefore be made with regards to extrapolation of a mechanism of interaction for one inhibitor of enzymes on one organophosphate on the bases of experiment carried out on another organophosphate.

1.14 Interactions That Decrease Organophosphorus Toxicity

The story is different however, with regards to the metabolic mechanism that offer protection against poisoning by organophosphates, as certain predictions may be made. Protective action of pre-exposure to various chemicals against poisoning by organophosphates could be explained by one or more of the following (Murphy, 1969; Iyaniwura, 1983a, b):-

a) Increase in non-critical binding sites

b) Increase in rate of formation of aryl-esterase

c) Increase in rate of direct cleavage of the parent (P=S) as influenced by pretreatment chemical and insecticide involved.

d) Competition for binding sites by chemicals.

Mechanisms (a) and (b) have been implicated in most interactions and may be further discussed:-

a). Some chemicals induce formation of non-critical binding sites to which the phosphorothioates bind, so that less binding sites are thus available to interfere with the more vital enzyme acetylcholinesterase. The protective action of chlorcyclizine against parathion poisoning was shown by Welch and Conn (1964) to be partly due to the formation of non-critical binding sites.

b) Chemicals which induce formation and activity of enzymes responsible for the metabolism of the organophosphate protect the animals against poisoning by the pesticide. For example the influence of phenobarbitone pretreatment on the acute toxicity of several organophosphate pesticides to rats and mice was studied by DuBois (1969). It was found that induction of the enzymes did not increase the toxicity of any of the compounds even though most of them depend upon microsomal oxidation for conversion to anticholinesterase agents. It was also noted that the rate of microsomal detoxification is more responsive to enzyme induction than the microsomal activation to produce the toxic metabolites.

Organochlorine pesticides are also known to induce formation and activity of liver microsomal enzymes; this will be discussed in further details later.

1.15 ORGANOPHOSPHORUS PESTICIDES

These are systemic and contact pesticides used to control soft-bodied insects. The biochemical basis by which organophosphorus compounds produce their toxicity is by inhibition of acetylcholinesterase activity in nerve tissues which results in accumulation of acetylcholine postjunctionally (O'Brien, 1967; Bowman, 1984). This results in signs and symptoms characteristic of muscarinic, nicotinic and central nervous system acetylcholine receptor stimulation.

1.16 Clinical Signs And Symptoms of Organophosphorus Poisoning

A number of authors have given detail descriptions of signs and symptoms arising from organophosphorus toxicity (O'Brien, 1960 Radeleff, 1964). Signs and symptoms arising from muscarinic receptor stimulation include increased salivation, lacrimation, wheezing expiration due to bronchoconstriction, increased gastro-intestinal motility causing diarhoea, nausea, vomiting involuntary defaecation or micturition, and constriction of the pupils of the eye. Those arising from excessive stimulation of nicotinic receptors include muscular twitching, fatigue and weakness, difficulty in breathing, and cyanosis. The central nervous system signs are characterized by restlessness, anxiety and tension, depression of respiratory and circulatory centers, with death usually resulting from respiratory failure.

1.17 Mechanism Of Toxic Action of Organophosphates

As mentioned previously, Organophosphates produce their toxicity via the nervous system functional disturbances. The neuron is the basic structural unit of the nervous system which comprises the central nervous system and peripheral nervous system (Bowman, 1984; Weiner *et al*, 1985; O'Brien, 1967). Contact exists between one nerve cell and another via a gap, the synaptic gap, a distance of about 100-500 Angstroms. Neurohumoral information is transmitted from the presynaptic neurone to the postsynaptic neurone via the release of chemical transmitter subtances from vessicles in the presynaptic nerve terminals. Post-synaptic neurones may closely approach effector cells to release chemical transmitters (upon stimulation) at autonomic neuroeffector junction or at the skeletal neuromuscular junction.

Acetylcholine is the chemical transmitter at the following sites:-

1) All autonomic ganglionic synapses,
2) Skeletal neuromuscular junctions, and
3) some of the autonomic neuro-effecor junctions- specifically post-ganglionic parasympathetic fibres, and sympathetic fibres to sweat glands.
4) Certain nerve endings within the central nervous system.

Noradrenaline is the chemical transmitter at some of the other autonomic neuro-effector junctions constituting the Sympathetic Nervous system. Figure I shows a schematic representation of the nervous system.

In the normal resting state, the axon is polarised electrically with its outside more positive than its inside. This state of polarization is maintained by metabolic pumps involving the ATPase system which keep the external sodium ion concentration higher than the internal sodium while the potassium ion concentration in the axon is kept higher than outside it. Following stimulation of the cholinergic nuerone, acetylcholine is released and diffuses across the synapse to combine with specialized receptors on the postjunctional membrane. This may result in an increase in permeability to all types of ions particularly the sodium ions which move down its concentration gradient resulting in a localized depolarization of the membrane, that is Excitatory Post Synaptic Potentical [EPSP], selective permeability may occur resulting in hyperpolarization of the membrane constituting an inhibitory Postsynaptic Potential (IPSP). For mormal impulses to be transmitted at high frequencies, efficient means of disposing the transmitter in order to return the neurone to its normal resting state, following each impulse, is necessary. This function is accomplished by the enzyme acetylcholinesterase (ACHE) which hydrolysis the acetylcholine to acetic acid and choline by the following mechanism:-

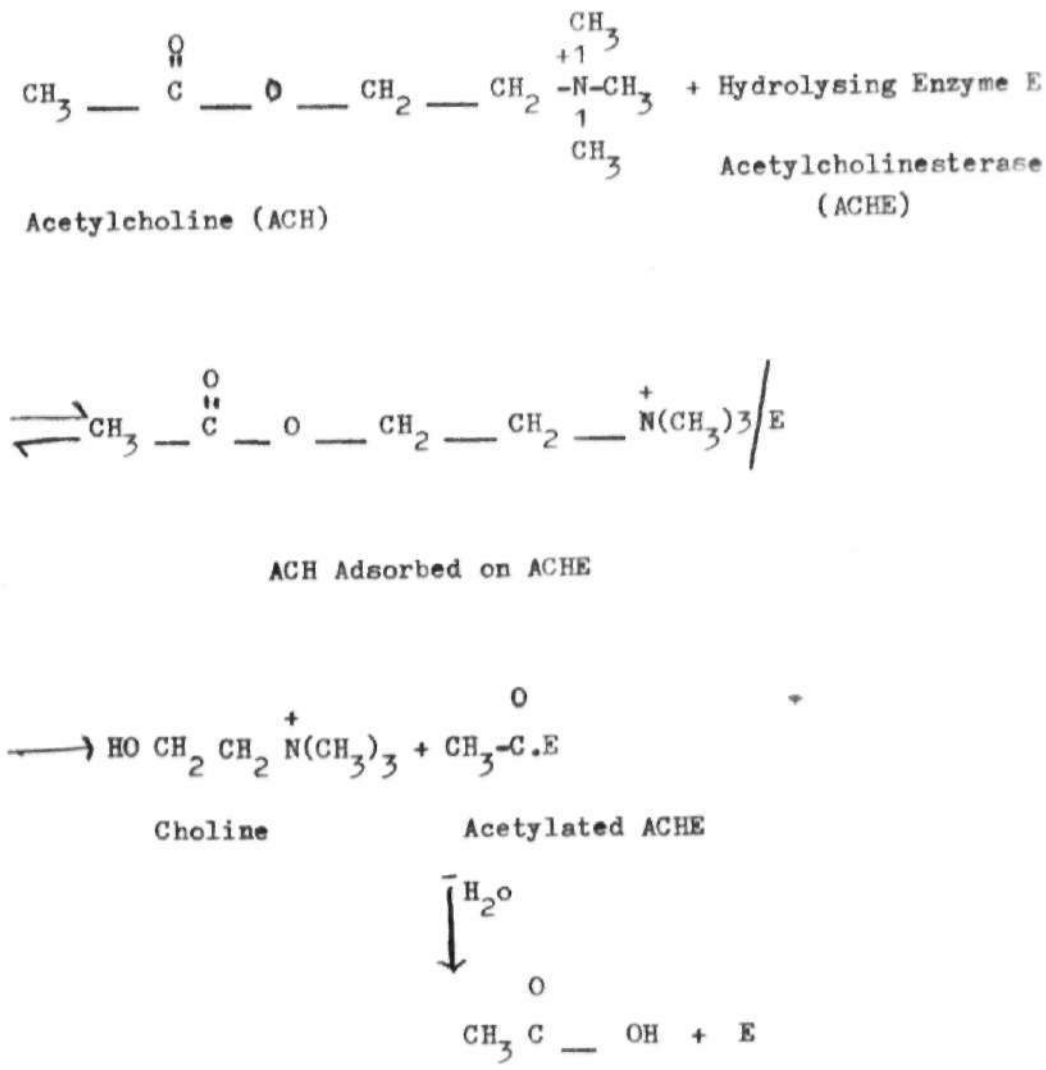

Fig. II

Interaction between acetylcholine and the hydrolysing enzyme.

The first step in the reaction involves an equilibrium between the cholinesterase enzyme, the substrate, ACH and one adsorption complex of both enzyme and substrate (Matsumura, 1975). For the formation of the complex, the acyl Carbon of the acetylcholine binds to the esteritic site on the enzyme while the cationic nitrogen moiety binds to the anionic site on the enzyme:

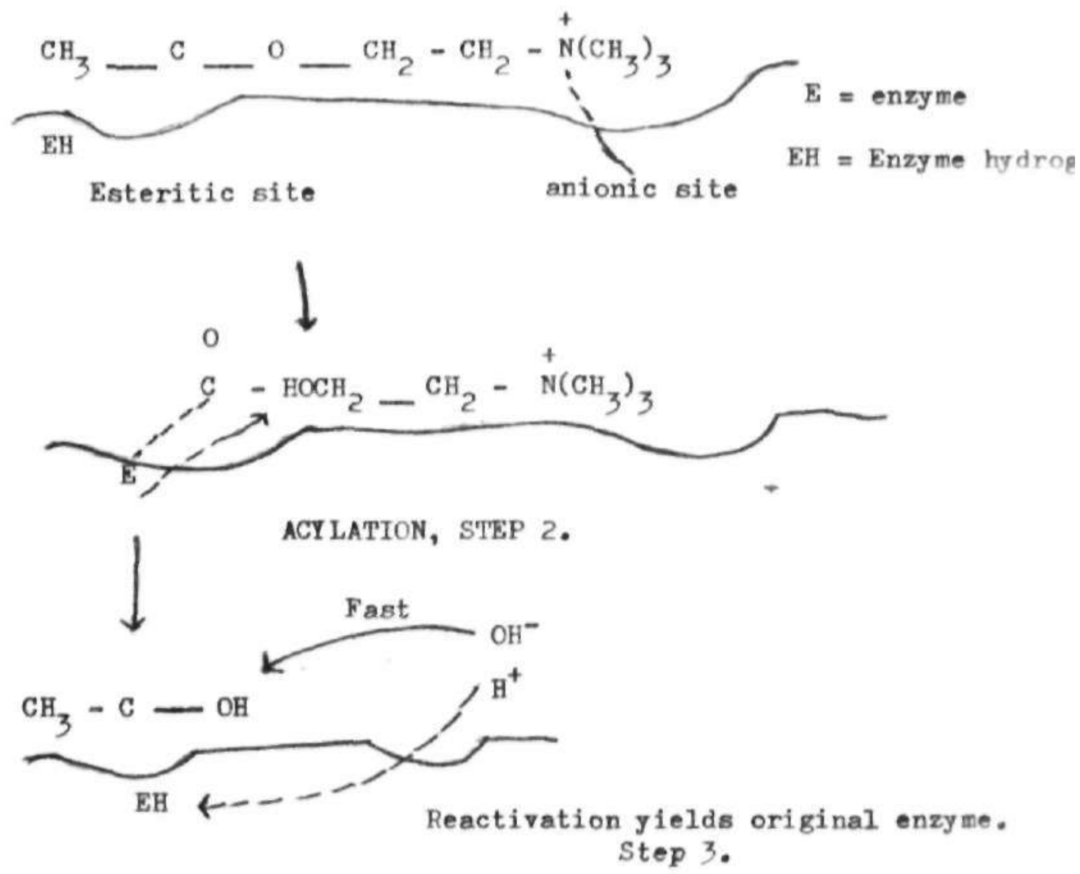

Figure III

Stages of Acetylcholine Hydrolysis by Acetylcholinesterase.

The second step involves the release of choline and the acetylated enzyme. The third step involves hydrolysis of the acetylated enzyme in the presence of water to acetic acid and free enzyme. Extensive studies have been conducted on the kinetics of the enzymic hydrolysis of acetylcholine (Fest and Schmidt, 1982; Hayes, 1982).

The acetylcholinesterase enzyme has greater affinity for organophosphates than for acetylcholine. In the presence of organophosphorus compound, an enzyme-organophosphate complex is perferentially formed. The process of organophosphate inhibition of cholinesterase follows a similar pattern to the early stage of acetylcholine hydrolysis described above. Acetylcholine is thus spared from destruction and accumulates at the synapses into which they are released. This leads to a preponderance of the actions that are due to acetylcholine at various junction sites.

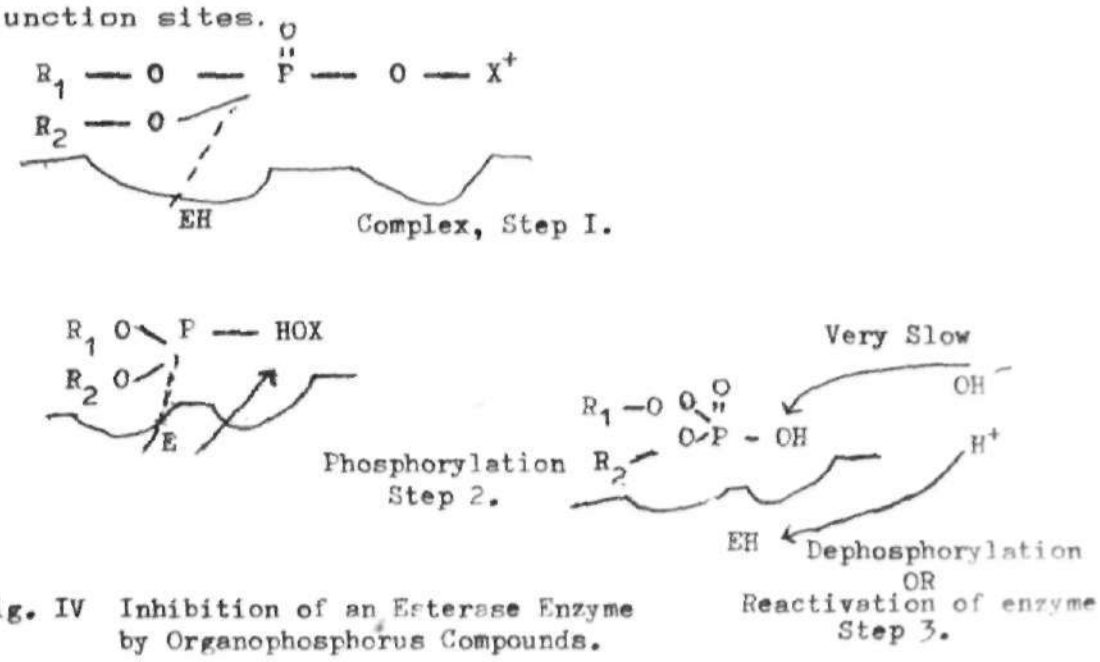

Fig. IV Inhibition of an Esterase Enzyme by Organophosphorus Compounds.

The process of organophosphate interaction with the enzyme however differs from that of acetylcholine at the final stage of enzyme recovery. While in the case of acetylcholine deacylation of the enzyme occurs rapidly, with organophosphates the process of dephosphorylation occurs rather extremely slowly. Although the reaction eventually yields the original enzyme, in practice it is considered irreversible. This is because once phosphorylated, recovery of the cholinesterase enzyme depends on partial reversal of inhibition, aging and synthesis of new enzymes (Hayes 1982). The slow process of dephosphorylation is considered as being responsible for the powerful cholinesterase inhibition by organophosphorus compounds (Matsumura, 1975).

1.18 Metabolism Of Organophosphorus Compounds

Most of the organophosphorus pesticides in current use are dialkyl, aryl esters of thiophosphoric acid with the structure described in the figure below. The metabolic pathways described occur *in vivo* in mammals (Murphy, 1969).

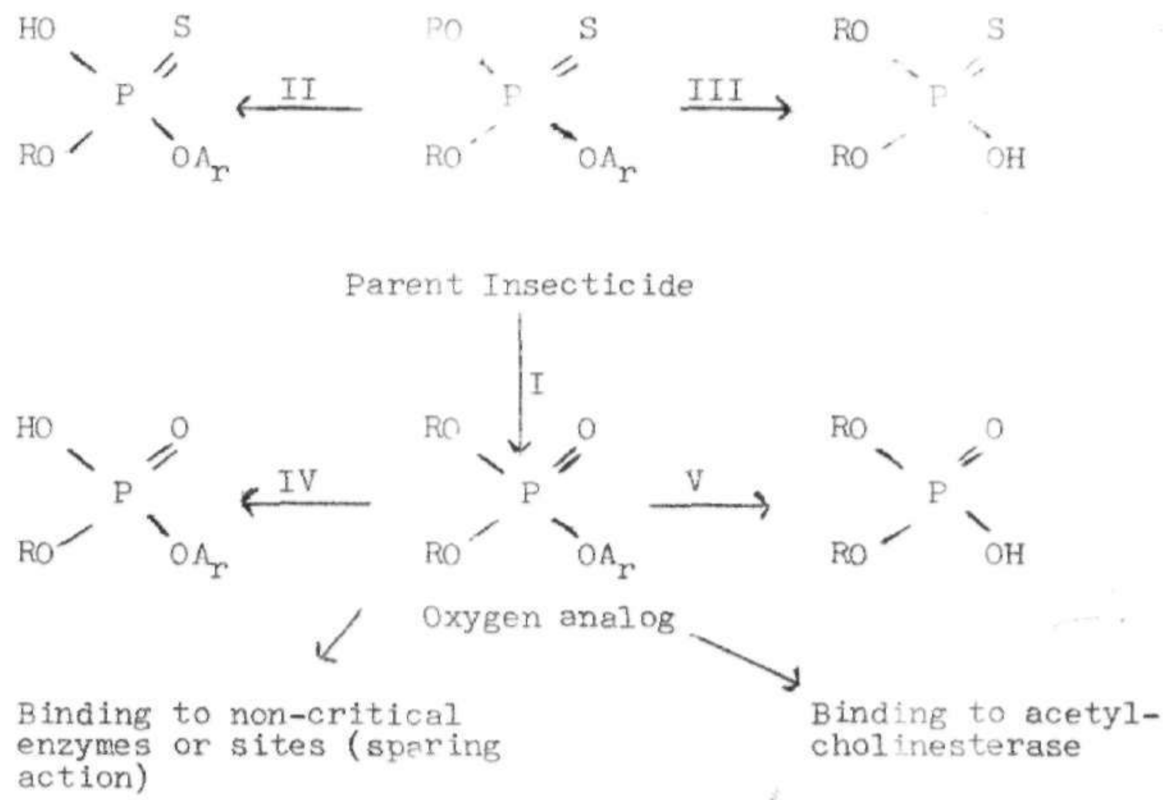

Figure V(a)

Metabolic Pathway of Organophosphates in Mammals

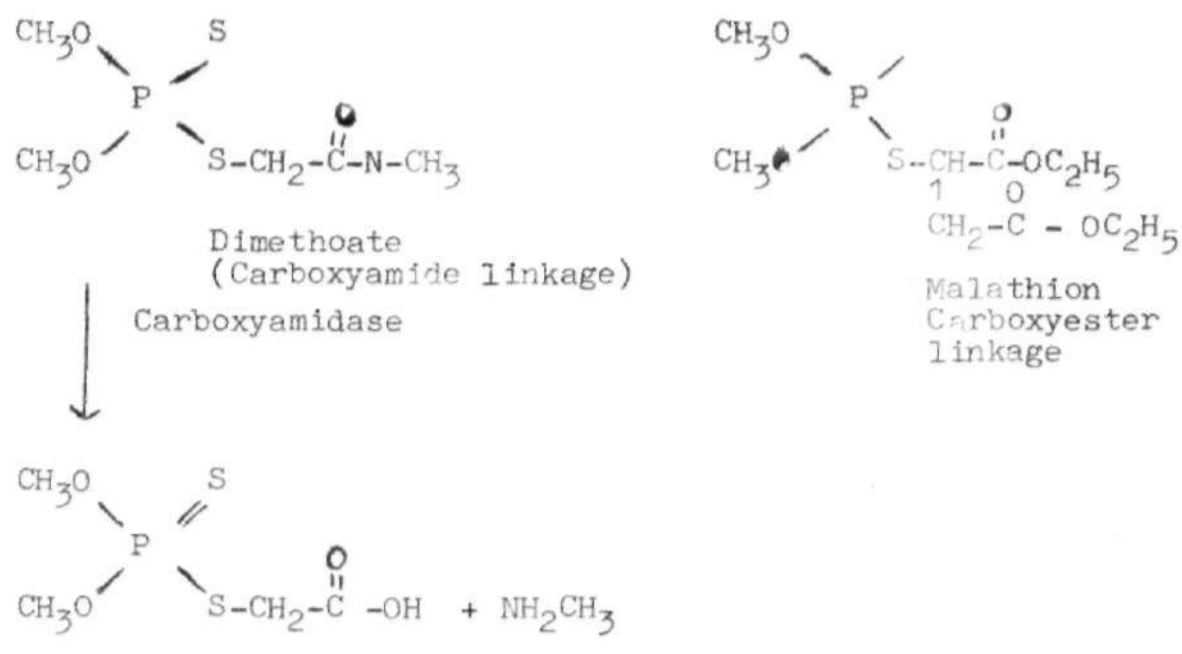

Figure V(b)

Reaction I involving substitution of =O for =S is a prerequisite for toxicity, while reactions II, III, IV and V are detoxication reactions yielding products which do not inhibit acetylcholinesterase. Reaction V is usually accountable for major detoxication of the pesticide. Organophosphates containing the thio (=S) group are normally non-toxic and have to be activated *in vivo*, to the oxygen analogue (=O) to be toxic (O'Brien, 1967). This activation is normally accomplished by liver microsomal enzymes. Parathion, for example, is activated by the liver enzymes to paraoxon, the toxic form (Dauterman, 1971). Modification of the alkyl and aryl groups also influence toxicity.

There are however, other groups of organophosphates that contain, instead of the aryl substituent, a carboxyester linkage (e.g. Malathion) or a carboxyamide linkage (e.g Dimethoate), with structures shown in the figure (Vb). The major path of dimethoate metabolism is illustrated in the figure (Vb) for man. Other metabolic pathways have been suggested in guinea Pigs and insects.

1.19 Histopathologic Effects of Dimethoate

Acute data alone may not be sufficient in evaluating the potential hazards posed by a chemical agent. Studies have been conducted on the potential effects of the administration of several pesticides. Ivanova reported in 1972 that DDT and polychlorinated pinene administered at the daily doses of 0.01, 3.5 and 4.25mg/kg to rats caused histologic changes in the myocardium. Uzoukwu *et al.* (1972) reported that dieldrin at a dose of 15mg/kg caused histopathologic damages in the lungs of guinea pigs. Studies on organophosphates have revealed other symptoms which are apparently unrelated to cholinesterase inhibition. Delayed neuromyelopathy which could occur even from exposure to a single toxic dose has been associated with triorthocresyl phosphate (TOCP), an organophosphates (Murphy, 1975). The toxic manifestations which may present after two weeks of exposure is characterised by flaccid paralysis - particularly of the legs in man. Microscopic examination of the neurons in exposed organisms reveal damages to myelin sheaths which surround most neurons. The distal fibres are more damaged than the proximal fibres (Cavanaugh, 1969; Stuart *et al.*, 1982).

Not all organophosphates give rise to this condition. Gaines (1969) screened in hens the effect of 30 organophosphates out of which some 20 induced peripheral neuropathy. Some carbamate pesticides were also associated with a similar toxic condition. In the 1930's, in the United States, it was reported that several thousands of people who drank ginger liquor contaminated with TOCP developed the condition then popularly called "ginger jake paralysis" (Smith *et al.*, 1930). A similar accidental poisoning occurred in Morocco in the 1950's when vegetable oil, used for cooking, was contaminated with lubricating oil that contained TOCP (Smith *et al.*, 1959).

Studies have shown that organosphate induced neuromyelopathy occurred only in some species of animals. Chickens and man have shown unusual sensitivity in this regard, with 40.000 recorded cases in man alone (Davies, 1963). Monkey and dog are reportedly insensitive while other sensitive species include cat, rabbit and lamb (Davison, 1954). Other than the observation of axonal degeneration and demyelination, the mechanism by which this group of organophosphates effect this condition has so far not been adequately explained (Stuart *et al.*, 1982).

Several investigations of acute, subacute and chronic toxicities of organophosphorus pesticides have been reported in the literature. In a comparative study, Iyaniwura (1985) investigated seven organophosphates for their toxicity *in vitro* by monitoring erythrocyte cholinesterase inhibition. Dimethoate which inhibited cholinesterase by less than 10% at micromolar concentration was shown to have the least toxicity of all the pesticides tested. Azinphoosethyl which inhibited cholinesterase by 90% at the same concentration was shown to be the most toxic of the seven compounds. Thus it would appear that dimethoate is a relatively safe pesticide, especially with reference to cholinesterase inhibition. In this present study, histopathologic examinations of organs of mice following 30 days daily exposure to various dosages of dimethoate at specifically 25mg/kg, 50mg/kg and 100mg/kg (i.p.) were conducted with a view to ascertaining other potential damages that may be encountered on prolonged exposure to dimethoate.

1.20 ORGANOCHLORINE PESTICIDES

This is one of the most important group of pesticides in use today. Organochlorines however, are known to persist in the environment and in biological systems. This has discouraged their extensive use in the developed countries (Murphy, 1975).

Toxic signs and symptoms of poisoning including paresthesia, irritability, tonic and clonic convulsions. Chronic intoxication could result from the release of compounds that have been gradually accumulated in the tissues in small doses.

1.21 Mechanism Of Toxic Action Of Organochlorines.

The mechanism by which organochlorines effect their toxicity has not been clearly established. However several studies have been conducted in an attempt to elucidate the mechanism of toxicity that might be involved at the molecular level. It is generally accepted that the primary target of organochlorine toxicity is the nervous system. There are however, differences of opinion as to exactly how the interference with the nervous system is brought about. A concensus of opinion indicated that DDT, often considered as a *prototype of the organochlorine*, acts by prolonging neuronal recovery time by increasing the negative after-potential. This is the time interval after a spike potential during which the neuron slowly returns to its resting state (Narahashi *et al*, 1968) DDT acts by keeping the sodium "gate" on the post-synaptic neurone partially open while completely blocking membrane permeability to potassium. This leads to a delay in the restoration of the axon to its resting state after a single impulse, as the membrane remains partially depolarised. The axon becomes easily excitable with the tendency for a stimulus which would in a normal animal provide only a single impulse giving rise to a series of impulses. The hyperexcitability is seen in the whole animal and is often referred to as the "DDT jitters" (O'Brien, 1975). Other membrane properties such as conductance, resistance etc are also altered from those of the normal membrane and these in addition account for the mechanism of DDT toxicity.

1.22 Metabolism of Organochlorines

Chlorinated hydrocarbons consist of compounds which include DDT and its various analogues, chlorinated cyclodienes and hexachlorocyclohexanes. Unlike the organophosphates, the organochlorines generally do not yield easily to rapid and complete biotransformation due to their lipophilic character. This results in their accumulation in biological tissues and the envirnoment.

Several studies conducted to elucidate metabolic pathways of DDT related compounds in mammals (Peterson *et al.* 1964; O'Brien, 1967) identified main DDT metabolites as DDD, DDE and DDA, which may be excreted as such or further degraded into other chemicals. For example, DDE was found to be further metabolised not only to excretable phenols but also m-methylsulfone-p,p'-DDE. Five principal routes of DDT metabolism are described in the figure (VI)

As a result of the double bond that exists in the cyclodienes, epoxidation is the main route for their metabolism (Murphy, 1975). Both the parent compound and the epoxidation product are toxic in their own right.

The hexachlorocyclohexanes, represented by lindane are metabolized by a process that could envolve dehydrochlorination, glutathione conjugation and aromatic hydroxylation (Murphy, 1975).

The posibility of metabolic interactions occuring to enhance organochlorine acute toxicity in mammals is low due to their low mammalian toxicity. However, their ability to induce hepatic enzymes and interaction affecting their storage could significantly influence their toxicity and those of other chemicals.

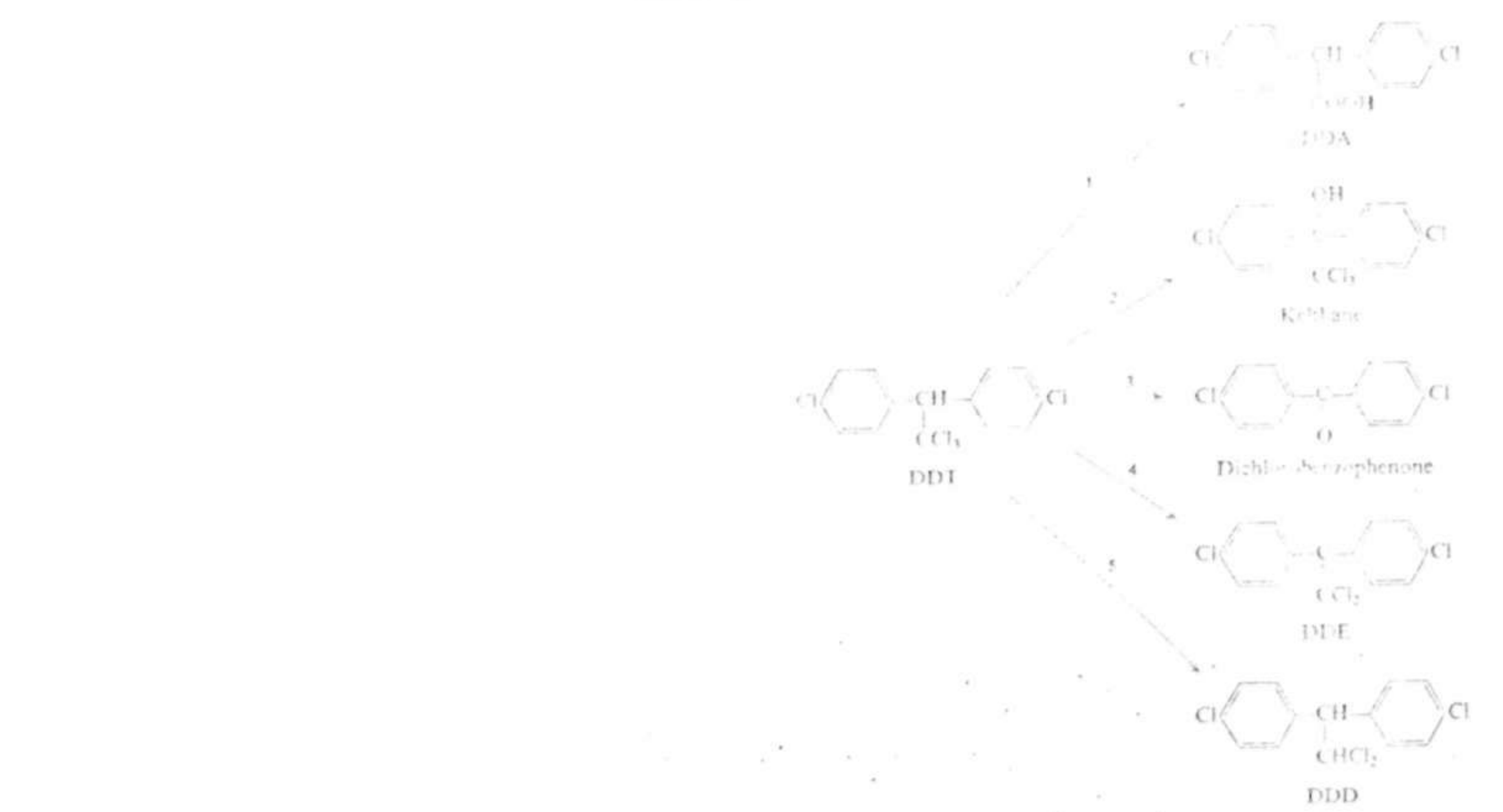

Fig. vi Metabolic Pathways of DDT.

1.23 Enzyme Induction By Organochlorines

The organochlorine are well documented for their ability to stimulate liver microsomal enzymes. Induction produces an increase in the levels and activity of the liver microsomal enzymes resulting in an increase in the rate of metabolism of drugs and chemicals that are catalysed by these enzymes. Induction could produce an increase or a decrease in toxicity of chemicals depending on whether the compound requires activation *in vivo* or not; or, whether the component of the microsomal enzyme that is induced is the activation or the detoxifying enzyme

Organochlorines have been shown to reduce the toxicity of some organophosphates, (Ball *et al*, 1954). Conney (1965 and 1971) reviewed several examples of these interactions as well as the biochemical mechanisms involved. In most cases, organochlorine insecticides were shown to increase the concentration as well as activity of liver microsomal enzymes that catalyse the metabolism of the chemicals studied. Studies conducted on DDT and toxaphene showed that maximal enzyme induction occured after these pesticides were administered for one week, with a tendency to reverse these effects in subsequent weeks (Kinoshita, *et al*, 1966). The studies emphasize the significance of pretreatment time. It is apparent therefore, that the simultaneous administration of the two compounds may not reveal the capacity of one compound to potentiate the toxicity of the other. Hagan *et al* (1961) also demonstrated the importance of pretreatment time using Delnav and malathion. Aldrin has been shown to increase liver aryl-esterase and to protect mice against parathion toxicity (Ball *et al*, 1954; Triolo and Coon, 1966b).

In a study conducted on the influence of phenobarbitone pretreatment on the acute toxicity of several organophosphate pesticides to rats and mice, DuBois (1969) found that the induction of hepatic microsomal enzymes did not increase the toxicity of any of the compounds, even though most of them depend upon microsomal oxidation for conversion to anticholinesterase agents. It was further noted that the rate of microsomal detoxification is more responsive to enzyme induction than the microsomal activation producing the toxic metabolites.

1.24 METHODOLOGY FOR TOXICOLOGICAL STUDIES

Various criteria have been used to measure toxicity of a chemical agent. For chemicals whose mechanism of toxic action is known, the obvious parameter would be one that is most sensitive to its effects, with a sharp end point.

As earlier shown, toxicity of organophosphates is well correlated to their inhibition of acetylcholinesterase enzymes. Various studies have been conducted to develop a quantitative procedure for measuring cholinesterase activity in relation to various levels of organophosphates. One of such studies was conducted by DuBois *et al* (1968). In evaluating the toxicity of organophosphates, quantitative measure of aliesterase inhibition would be the obvious parameter to use. In this study, availability of facilities presents limitations to the use of these methods. Lethality as in the determination of maximal tolerated dose, median lethal dose, minimal dose lethal to a whole animal population or survival time has been widely used in the literature, though with undue emphasis. These methods which could provide a guide to relative toxicities have limitations when toxic effects of small doses of chemicals have to be extrapolated to humans.

Enzyme induction, as previously noted, is an important cause of interactions between various combinations of pesticides and chemicals. DuBois (1969) developed a procedure for predicting the influence of enzyme-inducing agents on pesticide. The procedure consisted of determining the acute toxicity of several organophosphates on mice pretreated with phenobarbitone 50mg/kg for five days compared to acute toxicity on mice not treated. He showed that treatment of rats and mice with phenobarbitone for a period long enough to cause enzyme induction, decreased their susceptibilty to the acute toxicity of several organophosphates. It would be expected therefore, that any chemical agent capable of producing enzyme induction would influence the toxicity of organophosphates the same way as phenobarbitone. For instance, aldrin which is capable of enzyme induction would be expected to alter the toxicity of other pesticides that are metabolised by the induced enzymes. Dimethoate is metabolized by the liver enzyme carboxyamidase and which could be induced by aldrin, thus affecting its toxicity. In the current work the potential interaction between aldrin-dimethoate and Lindane-dimethoate would be investigated *in vivo* using mice.

1.25 Estimation Of Rate Of metabolism By Liver Enzyme

Alteration in the duration of drug action has been used to provide a measure of the rate of drug metabolism *in vivo* under a set of conditions. Estimation of the rate of metabolism of drugs by liver microsomal enzymes based on the duration of drug action could be achieved by any of the following methods (Fouts, 1971):-

a) Determination of pentobarbitone sleeping time as indicated by loss of righting reflex in mice

b) Determination of recovery from zoxazolamine induced paralysis time.

c) Determination of drug levels in blood or whole blood.

Procedures (a) and (b) are both based on similar principles and depend on loss of the righting reflex which both pentobarbitone and zoxazolamine produce. Correlation exists between the duration of loss of the righting reflex after administration of pentobarbitone and the rate at which it is metabolised by liver microsomal enzymes. Chemicals that induce liver enzymes that are responsible for pentobarbitone metabolism would be expected to reduce the duration of the loss of the righting reflex. This principle would be used to determine whether aldrin or lindane pretreatment brought about enzyme induction and, which could provide a possible explanation of the mechanism of interaction (if any).

1.26 AIM AND SCOPE OF STUDY

The aim of this study is to evaluate the potential interactions betwen aldrin-dimethoate, and lindane-dimethoate, *in vivo*. An attempt would be made to define the possible mechanism of interaction. The toxicological implications of any such interaction would also be defined. Very briefly the project would attempt to determine if pretreating mice with aldrin, or lindane, both microsomal enzyme inducers, would protect or reduce dimethoate toxicity. The following general experimental protocol would be used.

1. Determination of

    (i) Median lethal dose LD_{50} for Rogor® and Perfkthion® formulations of dimethoate

    (ii) Clinical Symptoms following acute poisoning.

2. Determination of

    (i) Median lethal dose, LD_{50}, for

        (a) aldrin,

        (b) lindane

    (ii) Clinical symptoms of poisoning from each pesticide.

3. Determination of survival time following lethal challenge dose of dimethoate in

    (i) mice pretreated with aldrin

    (ii) mice pretreated with lindane

    (iii) controls

4. Determination of pentobarbitone sleeping time for mice pretreated with

    (i) aldrin

    (ii) lindane

    (iii) controls.

5. Histopathology: of mice exposed to various dose of dimethoate for 30 days.

CHAPTER TWO

MATERIALS AND METHODS

2.10 MATERIALS

2.11 **Perfekthion®** pesticide formulation containing 400mg/millilitre of dimethoate, manufactured by BASF Aktiengesellschaft D-6700 Ludwigshafen, Federal Republic of Germany, was obtained from a local Agro-allied products shop in Samaru, Kaduna State.

2.12 Rogor®, also a commercial formulation of dimethoate, containing 400mg/ml of the pesticide and was manufactured by Fisons Agrochemical Division, Cambridge, England.

2.13 Aldrin dust, containing 5% aldrin was obtained from the Institute for Agricultural Research, Zaria.

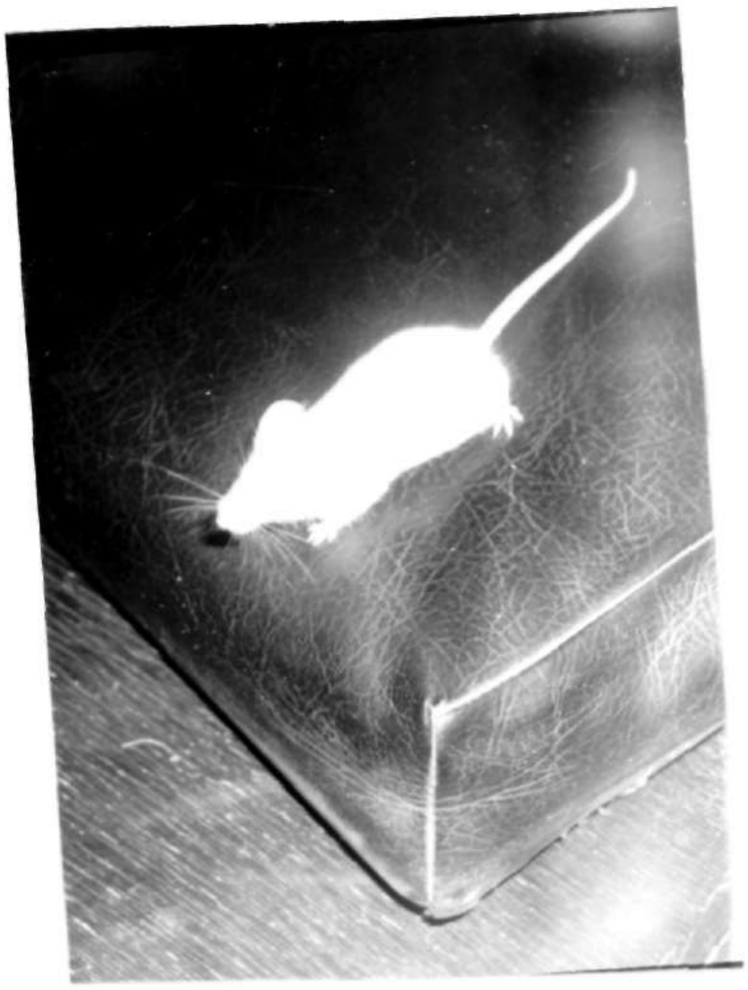

Figure 3.1 The albino mouse

2.14 Test Animals:

Adult Albino Mice (wistar strain), males and females were bought from the Animal House, faculty of pharmaceutical sciences, Ahmadu Bello University, Zaria. They were housed in well-ventilated rooms in cages with free access to water and feed. only healthy animals were used for the study.

2.15 Apparatus

Some of the apparatus used are universal bottles (20ml), test-tubes, pippetes 100ml measuring cylinder, 1ml, syringes and needles.

The balance used for weighing the mice was the Avery balance manufactured by W and T Avery limited, Birmingham, England. The Metler balance (P. 162 Gallenkamp U.K.) was used for weighing aldrin powder and organs of the mice. A Vortex Mixer 220/250V AC (Stuart U.K.) was used to enhance mixing of solutions.

2.16 Corn oil, bought from a local shop, Distilled sterile water, and Pfizer animal Feed Pellets obtained from the pfizer (Nig. Ltd.) shop at Kaduua.

2.17 Solutions:

Rogor® is an oily formulation of dimethoate. The solutions were made by diluting the calculated volume of the stock concentrate with corn oil to the strength of solution required. The vortex mixer was used to aid dispersion.

Perfekthion® is an aqueous formulation. The strengths of the solutions required were made by diluting the stock concentration with appropriate volume of distilled water.

Aldrin suspensions were made by weighing the required amounts of powder into the universal bottles and making the suspensions up to the required volumes with water.

The vortex mixer was used in all cases to aid dispersion. Fresh solutions were used for each experiment to avoid the possibility of deterioration on storage.

2.20 EXPERIMENTAL METHODS

2.21 Determination Of Median Lethal Dose (LD_{50})

An initial pilot study was conducted to determine the dose-range of the test pesticide likely to produce 10-90% death in the animal group. Based on this initial test, a range of doses were selected for each test pesticide consisting of the highest dose that did not cause death to the lowest dose that caused death in all mice in a group (Aliu *et al* 1932). This range was further divided into dose levels at proportionate intervals. To each dose level selected was asigned a group of five mice, selected randomly but ensuring near equal sex representation. Another group of five mice was used as the control group. Animals in each group were given the corresponding dose of pesticide for the group. The control animals received only the vehicles of the solutions.

Route of administration was intraperitoneal. The number of animals in each group dying within 24 hours of pesticide administration were noted. Test pesticides used were Rogor®, Perfekthion® formulations of dimethoate; aldrin and lindane.

2.22 Gross Examination.

Examination of the gross signs and symptoms were made visually following administration of acute doses of each pesticide studied.

2.23 Interaction Studies

An initial pilot study was conducted to determine suitable pesticide doses for pretreating the animals. Pesticide doses were selected such that minimal symptoms would be produced for each animal exposed to them. Two studies were conducted. The first study was conducted using aldrin pretreatment. In this study two groups of five mice selected were pretreated with 10mg/kg and 16mg/kg respectively. The control group also of five mice were treated with equivalent volumes, with respect to their body weight, of the vehicle used for the solutions. The animals were allowed free access to feed and water for the period of four days. On the fourth day, they were exposed to 288mg/kg of dimethoate and the survival time was noted for each animal. The mean survival time for the treated group was compared to that of the control.

The second part of the experiment was conducted using lindane for pretreatment. 98mg/kg of lindane was used for the pretreatment of the animals for 48 hours. The control group received only the equivalent volumes of the vehicle used for making the solution, in this case, distilled sterile water. The animals were treated to a dose of 300mg/kg of dimethoate at the end of the period and their survival times were noted and compared with those of the control.

2.24 Determination Of Pentobarbitone Sleeping Time

Two groups of mice were used for the first part of this study. Group A mice were pretreated with aldrin 16mg/kg single dose. The control, group B, received distilled sterile water. Four days later pentobarbitone 40mg/kg was administered. i.p to both the treated and control animals. The onset and duration of sleep produced (here taken as the loss of righting reflex) on each mouse were recorded. For the second part of the experiment, mice were pretreated with lindane 98mg/kg for two days, while the control group received sterile distilled water only. The onset and duration of sleep produced when 40mg/kg of pentobarbitone was given, were noted

2.25 Arithmetic Method of Karber

This method for calculation of LD_{50} for chemicals utilizes the following formula:-

$$LD50 = \text{Minimal dose that Killed all animal population} - \frac{\text{(Sum [Dose difference x Mean dead])}}{\text{Total number of animals per group}}$$

where LD50 = the dose of the chemical that kills 50% of the animal population.

Using this method, the LD_{50} for aldrin was calculated as follows

Dose	Dose Diff.	Dead	Mean Dead	Dose Diff. x Mean Dead
20mg/kg		0		
	10		0.5	5
30mg/kg		2		
	10		2.5	25
40mg/kg		3		
	10		3.0	30
50mg/kg		3		
	10		4.0	40
60mg/kg		5		
			Total	100

It may be noted from the above table that minimal dose that Killed all animal population = 60mg/kg
Total number of animals per group =5
The sum of (dose difference x mean dead) = 100

Using the formula; LD50 given above,
LD50 = 60 - 100/5 = 60 - 20 = 40mg/Kg for aldrin.

2.26 Statistical Method

The Students 't' test (pooled) was used for comparing differences in survival time, pentobarbitone sleeping time and organ/body weight ratios. An Amstrad PCW 8256 microcomputer running the Basic program "F&T' Test" was used to calculate the 't' values.

2.27 Materials and Methods for Histologic Studies

Young adult mice (about four to five weeks old) of the wistar strain were randomly assigned to four groups each consisting of 10 mice. They were provided free access to food and drinking water. Groups I, II and III mice received 25, 50, and 100mg/kg of dimethoate (i.p.) daily for thirty days. These doses were selected on the basis of an initial pilot study. Group IV mice, the control, received distilled water only. During this period, feed consumption, weight loss or gain, toxic manifestations, including death were recorded. At the end of the treatment period, surviving animals were weighed, sacrificed and the ratio of organ/body weights determined. Both dead and sacrificed mice were taken to the Veterinary Teaching Hospital for gross postmortem examinations and histopathologic studies. Some organs : brain, liver, heart, kidney, lungs and spleen were removed from the posted animals and weight and portions of these organs were fixed in a 10% formol-saline. These tissues were then sliced (3-4mm thick) for further processing.

The tissues were fixed in a 10% buffered formaline for two hours and then dehydrated in varying concentrations of alcohol in an ascending order of 70% , 90% and 100% v/v. They were later transferred into xylene for 2 hours (for cleaning) after which they were impregnated with molten paraffin and allowed to cool. The microtome was set at 5 microns and used to section the tissues. Tissues were trimmed, attached to slides and then allowed to float on a warm water bath. They were later dried on a hot plate and stained with haematoxylin and eosin.

The staining procedure consisted of dewaxing the sectioned tissues in xylene and then rehydrating them in varying concentrations of alcohol in a descending series of 100% 90% and 70% alcohol. Haematoxylin was used to stain the slides for 10 minutes, before transfering them to a 1% acid alcohol for differentiation and then rinsing with scotts tap water. This was followed by staining in eosin for 3 minutes. slides were rinsed again and dehydrated in alcohol in an ascending order of 70%, 90% and 100%. The slides were finally cleansed in xylene and then mounted. The slides were examined microscopically for lessions, with the assistance of a Veterinary Pathologist at the Veterinary Teaching Hospital, of Ahmadu Bello University, Zaria.

CHAPTER THREE

RESULTS

3.00 ACUTE TOXICITY STUDIES

3.10 MEDIAN LETHAL DOSES, LD_{50}

3.11 ROGOR® AND PERFEKTHION® FORMULATIONS

Tables I and II show acute toxicity for dimethoate from Rogor® and Perfekthion® formulations respectively. The results show that from the Rogor® formulation, a dose of 120mg/kg produced no death in the group of mice, while the dose of 491.5mg/kg killed all the animals in the group. At doses in between these two doses (ie 120.0-491.5mg/kg) animals that died, died within 24 hours of pesticide treatment. Although the surviving mice were not entirely devoid of the symptoms of toxicity, these symptoms were prominent in those that died. The LD_{50} for Rogor® was calculated from the data to be 272.1mg/kg using the Arithmetic Method of Karber.

The Perfekthion® formulation acute toxicity results showed (Table II) that 100mg/kg did not kill any mouse while beyond 200mg/kg none of the animals in the group survived. All deaths resulting from pesticide exposure occurred within a 24-hour period. Toxic symptoms were evident in surviving animals but these subsided within 24 hours of pesticide administration.

Results from acute toxicity of the two formulations showed that the maximal dose that produced no death was 120mg/kg for Rogor® while a lower dose of 100mg/kg was observed for the Perfekthion® formulation. 60% of mice that received 192.2mg/kg Rogor® survived whereas all those that received 200mg/kg Perfekthion® died. The data would then appear to suggest that the Perfekthion® formulation produced toxic effects at much lower doses than the Rogor® formulation. This is further supported by the LD_{50}* values for these two formulations: Perfekthion®, 165mg/kg and Rogor®, 272.1mg/kg.

*N.B. Lower LD_{50} value indicates greater toxicity for a compound.

3.12 ALDRIN AND LINDANE (LD_{50}s).

The result for acute toxicity of aldrin (Table III) showed that 20mg/kg produced no death in the mice group whereas 60mg/kg produced 100% death in the mice group. Toxic symptoms were observed (section 3.22) in mice that survived exposure to aldrin within this dose range. All recorded deaths occurred within 24 hours of aldrin administration, the LD_{50} for aldrin was calculated from the data to be 40mg/kg.

Lindane dose of 192.8mg/kg did not kill any of the mice and a dose of 331.7mg/kg killed all animals in the group (Table IV). Deaths recorded occurred with a 24-hour period of exposure to lindane. The LD_{50} obtained from the data for lindane was 249.0mg/kg.

As compared with the LD_{50} for aldrin (40mg/kg) this data suggests that the toxicity to mice of aldrin is much greater than that of lindane . Infact previous studies in rats showed that aldrin (LD_{50}, 39mg/kg) is more toxic than lindane (LD_{50}, 88mg/kg) Gauines, 1969).

3.20 GROSS SYMPTOMS

3.21 DIMETHOATE

At doses of dimethoate shown in Tables I and II, the following symptoms were observed, with the onset of action occuring within thirty minutes: Initial restlessness in the exposed mice followed by decreased activity, salivation, lachrymation, watery stool and micturition; the eyes were bulged out and pin-point pupils of the eyes were observed. The breathing pattern became irregular and shallow, and then death followed, apparently from respiratory failure. All recorded deaths occurred within 24 hours of pesticide administration.

3.22 ALDRIN AND LINDANE

Symptoms of toxicity following acute exposure of mice to aldrin were similar to those exhibited on exposure to lindane. Symptoms to aldrin however developed earlier and were more severe. These were: hyperexcitability, tremor throughout the body, frequent prostration, tonic convulsions and death which occurred within 24 hours for most of the animals. Some died at a much later day.

3.30 Interaction Studies.

The results obtained in this study (Table V) show that dimethoate mean survival times of mice prexposed to 10mg/kg aldrin was not significantly different from the control, using the pooled t statistics (at $P < 0.05$). while the survival time for mice pretreated with 16mg/kg was significantly shorter ($P < 0.05$) than in the controls. Although mice exposed to this formulation showed considerable decrease in weight, as they fed less. Aldrin appears to have caused anorexia in these animals.

The effect of pretreating mice with lindane on the survival time of dimethoate challenge dose (Table VI) shows that the mean survival time of lindane pretreated mice is significantly longer ($P < 0.05$) than that of the control group.

3.40 Pentobarbitone Sleeping Time

Tables VII and VIII show effects of pretreating mice with aldrin and lindane on pentoborbitone sleeping time. In each case, the mean sleeping time for the treated group was significantly ($P < 0.05$) shorter than for the control. The pooled t statistics was used for the test.

3.41 Histopathologic Findings

Symptoms characteristic of organophosphorus toxicity were noticed within thirty minutes of administration of the pesticide and toxic signs persisted for several hours. These subsided after about 6 hours. When the subsequent doses were to be administered the following day the animals appeared to have recovered from symptoms of the previous day's dose. However, recovery from these symptoms for the group receiving 100mg/kg (group III) were much slower and less complete as the animals appeared less active than those of the other groups and control. The organ/body weight ratio treatment is shown in Table 5.1. The mean organ/body weight ratios for each of the treated groups are not significantly different (P <0.05) from the control mean using the student 't' test.

Histopathologic studies indicate that some organs had lesions while others were apparently normal. The lung was the most affected. in most of the treated animals the lung was severely congested and thickened with interstitial pneumonia. The degree of severity of damage in the lungs did not appear to be dose related. The liver was the next organ in order of severity of damage although this was observed in only a small proportion of the animals. The observed lesion was characteristically a mild to severe congestion, and intestitial edema of the hepatic tissues. The kidney did not appear to have been affected in most of the animals. A few however had mild to severe congestion. No lesions were observed in the heart, brain and spleen of any of the treated and control group animals.

Serial No	Dose Group	Number of dead mice	Number of mice survived
1	Group		
2	(A)	0	5
3			
4	120mg/kg		
5			
6	Group		
7	(B)	2	3
8			
9	192,2mg/kg		
10			
11	Group		
12	(C)	3	2
13			
14	307,2mg/kg		
15			
16	Group		
17	(D)	5	0
18			
19	491,5mg/kg		
20			

Table I

Acute Toxicity of dimethoate in Rogor® commercial formulation.

Serial No	Dose Group	Number of dead mice	Number of mice survived
1	Group		
2	(A)	0	5
3	100mg/kg		
4			
5			
6	Group		
7	(B)	1	4
8	150mg/kg		
9			
10			
11	Group		
12	(C)	5	0
13			
14	200mg/kg		
15			
16	Group		
17	(D)	5	0
18	288mg/kg		
19			
20			
21	Group	5	0
22	(E)		
23	345mg/kg		
24			
25			

TABLE II

Acute toxicity of perfekthion®

Serial No	Dose group	Number of Dead mice	Number of mice survived
1	Group		
2	(A)		
3	20mg/kg	0	5
4			
5			
6	30mg/kg	2	3
7	Group		
8	(B)		
9			
10			
11			
12	40mg/kg	3	2
13	Group		
14	(C)		
15			
16			
17	50mg/kg	3	2
18	Group		
19	(D)		
20			
21			
22	60mg/kg	5	0
23	Group		
24	(E)		
25			

TABLE III

Acute toxicity of aldrin.

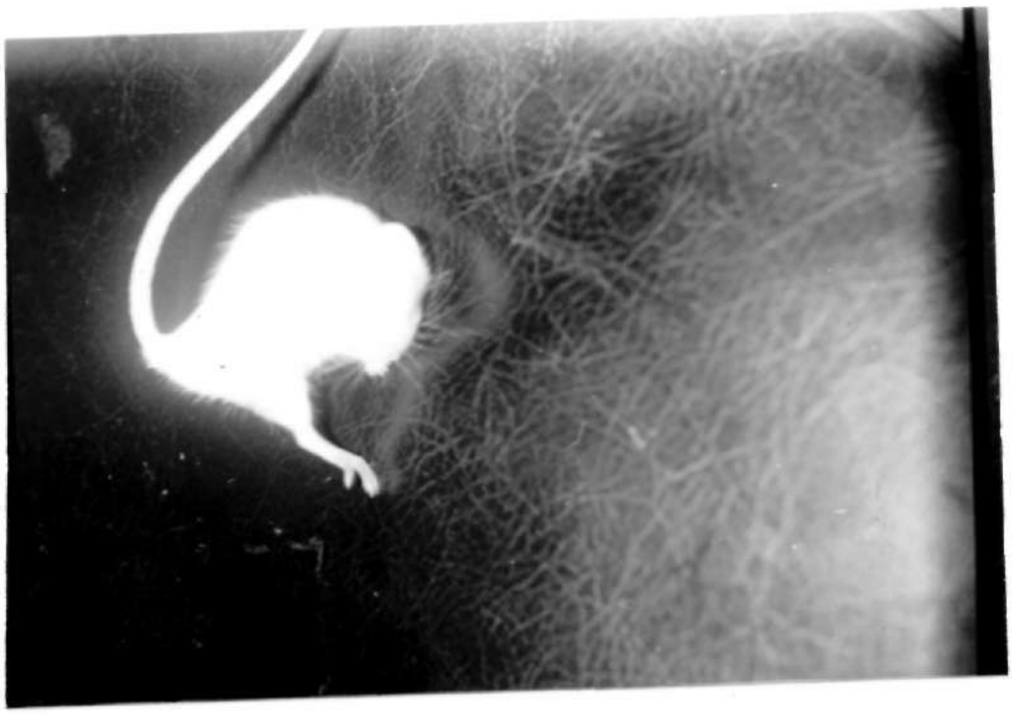

Figure 3.2 Convulsions produced in mouse following acute exposure to aldrin

Serial No	Dose Group	Number of Dead mice	Number of mice survived
1			
2	Groun		
3	(A)	0	5
4	192,8mg/kg		
5			
6	Group		
7	(B)	3	2
8			
9	230mg/kg		
10			
11	Group		
12	(C)	3	2
13			
14	276mg/kg		
15			
16	Group		
17	(D)		
18		4	1
19	307,3mg/kg		
20			
21	Group		
22	(E)		
23			
24	331,77mg/kg	5	0
25			

TABLE IV

Acute Toxicity of lindane.

Dose Group	Serial No	Survival Time (mins)	Mean Survival Time (mins)
I Received	1	57	
10mg/kg	2	40	54,8
	3	116	
	4	40	
	5	31	
	6	23	
II Received	7	29	29,6
16mg/kg	8	37	
	9	29	
	10	30	
	11	78	
III Contl Recd	12	50	93,6
Only	13	47	
Vehicle	14	144	
	15	149	

Table V

Effect of aldrin pretreatment on the survival time of mice challenged with 288mg/kg of dimethoate

Serial Nº.	Survival Time (minutes	
	Lindane-Pretreated group	Control group
1	110	65
2	192	70
3	128	64
4	130	62
5	170	67
6	149	69
7	152	66
8	215	61
Mean	155.75	65.5

Table VI

Effect of lindane pretreatment on the survival time of mice challenged with 300mg/kg of dimethoate.

Group	Serial No.	Onset Time (minutes)	Sleep Duration (mins)	Mean
	1	2	48	
	2	4	52	
	3	9	47	
	4	7	39	47,28
Control	5	irregular		
	6	13	58	
	7	5	45	
	8	5	42	
Aldrin	9	3	11	
16mg/kg	10	7	11	
Treated	11	No sleep	-	
group	12	9	8	12,14
	13	5	12	
	14	4	14	
	15	5	15	
	16	6	14	

Table VII

Effect of aldrin pretreatment on

pentobarbitone sleeping time in mice

Group	Serial No	Sleep onset Time (mins)	Sleep Duration (mins)	Mean
	1	4	7	
Lindane	2	No sleep		
98mg/kg	3	4	3	
Pretrd group	4	6	5	5,33
	5	No sleep		
	6	8	6	
	7	5	5	
	8	7	6	
control	9	6	25	
group	10	8	15	
	11	10	11	
	12	7	14	16,5
	13	5	18	
	14	5	15	
	15	4	18	
	16	5	16	

Table VIII

Effect of lindane pretreatment on

pentobarbitone sleeping Time in mice

Group	Mouse serial No.	Initial Weight (g)	Wt after 4 days (g)	Group initial Wt, of feed	Group Feed Wt after 4 days	Feed intake per group
I	1	19	17			
Received	2	17	16			
16mg/kg	3	16	15	150g	132	18
aldrin	4	18	15			
	5	17	14			
II	1	15	16			
Control	2	20	20			
Received	3	17	17	150g	104	46
distilled	4	21	21			
water	5	17	18			

Table IX

Effect of Aldrin pretreatment on weight and feed intake of mice.

	WEIGHT OF ORGAN±S.D.					
Group	Heart	Kidney	Lungs	Liver	Brain	Spleen
1	0,005 ±0,0007	0,016±0,0012	0,0108±,0049	0,0504±0,0040	0,0176±0,0046	0,0046±0,0013
2	0,00046±0,0011	0,0192±0,0021	0,013±0,0036	0,0528±0,0094	0,0210±0,0027	0,0068±0,0023
3	0,007	0,015	0,008	0,04	0,021	0,002
4#	0,00400±0,0005	0,015±0,002	0,0106±0017	0,0466±0,0048	0,0195±0,0032	0,007±0,0030

= control

Table X

Mean organ/body weight ratio of mice daily exposed to dimethoate at 25, 50 and 100mg/kg in groups I, II and III respectively over thirty days.

CHAPTER FOUR

DISCUSSION

4.11 ACUTE TOXICITY OF DIMETHOATE

Since 1941 when Adrian *et al* demonstrated the inhibition of cholinesterase enzyme by organophosphates, the observed symptoms (see section 3.21) of toxicity following organophosphate poisoning have been generally attributed to their ability to inhibit cholinesterase enzymes. Although doubts were raised as to the validity of this mechanism of toxicity to the organophosphates (Chadwick, 1963) evidence for its acceptance is overwhelming (O'Brien, 1960, 1967): the observed symptoms of poisoning are similar to those expected from cholinesterase inhibition, the muscarinic symptoms were blocked by atropine, an established cholinergic antagonist; nicotinic symptoms were relieved by oximes, well known cholinesterase reactivators, and there is correlation between the level of cholinesterase inhibitiion, symptoms of toxicity and dose of the pesticide administered, in the animals. Most analytical methods for determining organophosphate poisoning in animals or humans utilize cholinesterase inhbition analysis (Ancher, 1963; Gage, 1961; Iyaniwura, 1985), although other methods have also been employed for safety evaluations in humans, with greater precision. These methods test for insecticide level in the blood and residues or metabolites in the urine. For example, Mattson *et al* (1960) conducted controlled feeding tests on a human volunteer in which the man was fed with malathion 0.16mg/kg and 0.84mg/kg. Tests showed no blood cholinesterase inhibition, but revealed a total of 19-23% of the original dose in the urine in each case.

Dimethoate, like other organophosphates, produce cholinesterase inhibition via an activation product (the phosphorus-oxygen, P(O) analogue) dimethoxon. This observation was demonstrated in houseflies, cockroaches and mice (Krueger *et al*, 1960). Dimethoate toxicity depends upon the net availability of active metabolite dimethoxon at vital target sites. The activation and degradation of dimethoate is almost entirely restricted to the biotransforming enzymes of the liver (Uchida *et al*, 1964, 1967).

The degradation of dimethoate in the liver is catalysed by carboxyamidase (amidase, for short) and phosphatase, with amidase deactivation being the principal route in most mammals and plants. The degradation route varies with species (O'Brien, 1967). Degradation may be either through phosphatase in some species, amidase in others or both in some species. The relative lack of carboxyamidase enzymes in insects has made them to be selectively susceptible to the toxicity of dimethoate as compared to mammals (Krueger et al 1960, Uchida et al 1964). For example, the LD_{50} for dimethoate in the locust *E. plorans* is 40ug/g (MacCraig, 1961). The housefly is especially noted for its sensitivity to dimethoate - with the LD_{50} being 0.53ug/g (Uchida *et al*, 1965). It was found that the ability to block degradation of dimethoate was an excellent index of ability to synergize. This was demonstrated by the ability of EPN to block degradation and thus synergize toxicity of dimethoate in mice, less in guinea pigs and absent in houseflies, or milk weed bugs (O'Brien, 1967). The Mammalian Selectivity Ratio (MSR), which is the ratio of oral LD_{50} to the topical LD_{50} in female houseflies in mg/kg for both species, for dimthoate is 390 (Metcalf, 1972). This indicates that mammals with much higher amidase, are much less susceptible to dimthoate than houseflies.

This metabolic selectivity to dimethoate is confered by the presence of the hydrolysable carboxyamides group, $-C(O)NHCH_3$ in dimethoate described by O'Brien (1967) as its selectophore: a chemical grouping which confers selective properties on a toxicant. The ideal pesticide is one that has high degree of selective toxicity to target organism. The selectophore concept led to the development of some highly selective organophosphates, such as acethion. In practice, however, some other factors, in addition to the seletophore have been demonstrated to influence selectivity and toxicity in general. For example sensitivity of dimethoate carboxyamidase has been shown to differ in different organisms (O'Brien, 1967).

In this preliminary study, the median lethal dose of dimethoate from two commercial formulations Rogor®, and Perfekthion® were investigated (see section 3.11). The median lethal dose obtained in this study for Rogor® (272.1mg/kg) compares favourably with the literature value, 250mg/kg in mice (Panshina, 1963). Rogor® and Perfekthion® are two different formulations of the same compound - dimethoate.

The median lethal dose shows that the Perfekthion® formulation (LD_{50}, 165mg/kg) is more toxic than Rogor® (LD_{50} 272.1mg/kg). This difference signifies that types of formulations can influence toxicity of a compound. There are several literature reports on effect of various formulations on the toxicity of technical products. Aldridge *et al* (1979) reported an instance in Pakistan where a major poisoning incident occurred because of a shift from the use of one formulation of malathion to another, during a malaria control programme. About 3,000 out of the 7,500 sprayers were intoxicated and 5 of them died, from the use of the toxic formulation. Investigations revealed that isomalathion, present in the toxic formulation as one of the impurities was mainly responsible for the potentiation of malathion toxicity

The difference in toxicty of the Rogor® and the Perfekthion® formulations used in the current study is most probably due to the fact that Rogor® is an oily preparation and tends to release the active ingredient, dimethoate, more slowly as compared to that released from the aqueous formulation of Perfekthion®. The presence of an impurity in the formulations is also likely although this was not investigated. Dimethoate toxicity has also been demonstrated to increase with shelf-life (Meleny and Peterson, 1964). These findings would imply that safety evaluations should be conducted on finished products of pesticides before they are allowed to be distributed for use in pest control programmes.

The LD_{50} gives an idea of the magnitude of the acute toxic dose and pattern of toxicity of a chemical (Frazer and Sharratt, 1969). Perfekthion® formulation was used in subsequent studies since this formulation is easier to inject and appears to give a more consistent response.

4.12 ACUTE TOXICITY OF ALDRIN

The symptoms of poisoning by aldrin are described in section 3.22. Similar symptoms have been described in the literatures (O'Brien, 1967). In addition to these symptoms Gowdey (1952-55) noted slowing of the heart and salivation, which are normally peculiar to peripheral parasympathetic symptoms and attempted to implicate cholinesterase inhibition as mechanism for aldrin toxicity. This was disproved by Smallman (1955) who demonstrated that aldrin does not inhibit cholinesterase enzymes *in vitro*.

Lalonde *et al* (1954) further demonstrated that the cyclodienes, represented by aldrin, were acting in a mode similar to DDT (see section 1.20) and suggested that the target site upon which aldrin was acting to produce toxicity was the nerves. This view has not been disproved.

Adrin is metabolised in animals, plants and soil to dieldrin, its epoxide, which is particularly noted for being one of the most stable chemicals ever known to man (Matsumura, 1975). Both aldrin and dieldrin are toxic. Brooks *et al* (1963) described the biotransformation as toxification process because dieldrin, the metabolite, is faster-acting and more persistent than the parent compound, aldrin. The stability of dieldrin was demonstrated in housefly by Earle (1963), who showed that dieldrin absorbed by housefly larvae persisted through metamorphosis and into the adult housefly. The dieldrin excreted from the adult fly remained unchanged.

Studies on human volunteers showed that daily doses of dieldrin up to 210 μg for 18 months did not cause any haematological changes (Hunter *et al*, 1967) nor any other clinical abnormalities. Cases of occupational intoxication in workers who handled aldrin were reported by Jager (1970) the symptoms consisted of headache, lassitude, fatigue, loss of appetite, weight loss, insomnia, frequent nightmares, inability to concentrate, loss of memory and hyperirritability. In an attempt to find the critical level at which dieldrin cause poisoning in humans, a number of studies were conducted in both humans and animals. In one of such studies, Keane and Zavon (1969) fed dogs with 2.0mg/kg/day up to 63 days for acute poisoning, and 0.2-2.0mg/kg/day for 150 days for the subacute group. They analysed blood samples for animals showing symptoms of toxicity and found a direct relationship between concentration of dieldrin and symptoms. However, like DDT, establishing correlation between its critical level and signs of poisoning is made difficult by its tendency to accumulate in adipose tissue. For example, in an experiment with rats, Dale *et al* (1962) fed rats chronically with DDT and achieved a high level in their adipose tissue without symptoms of toxicity. However, when they subjected the rats to a severe fast, the animals exhibited symptoms typical of acute poisoning. They explained that during fast depletion of the adipose tissue by catabolic process led to the release and distribution of the stored DDT to critical nerve sites the released compound also caused an induction of microsomal enzymes in the liver (Dale *et al*, 1962).

4.13 ACUTE TOXICITY OF LINDANE

The symptoms of lindane poisoning (see section 3.22) are similar to those of DDT poisoning (see section 1.19). This similarity of symptoms have led to the feeling that lindane was acting via the same mechanism as DDT to produce toxicity (O'Brien, 1967). Various views have been expressed as to the exact mode of action of lindane. For example Slade (1954) had suggested that lindane was having similar configuration with myoinositol an essential dietary requirement of mammals and fungi and competitively interfered with uptake of myoinositol in the animals to produce toxicity. O'Brien (1967) reviewed some of these arguemente and concluded that lindane was not acting as an antimetabolite, but as a neurotoxicant, acting in a mode similar to DDT. Large differences exist in toxicity among the closely related isomers of Hexachlorocyclohexane (HCH) of which lindane is a member. These differences were thought (Mullins, 1955) to arise from the "varying ability to 'fit' into a hyopthetical lattice in the axonic membrane" by the HCH isomers. A rigid spatial arrangement of the molecule was a prerequisite for insecticidal activity and molecules that were not fitting in exactly would have no effect. This model of HCH fitting into a membrane lattice is however, still speculative and has not yet been comfirmed.

Lindane is degraded into non-toxic derivatives within 2 weeks of application on crops. (Lichtenstein *et al*, 1959). Lindane poisoning in humans have been associated with serious blood dyscrasias, priamarily aplastic anaemia, and allergic reactions (West, 1967). Lodge (1965) associated lindane with bone marrow damage.

4.14 ALDRIN AND LINDANE

In this study the gross symptoms following aldrin and lindane poisoning (see Section 3.22) are similar for either pesticide. These have also been reported in several literature. O'Brien (1967), for example listed symptoms which occur from poisoning with lindane as follows: (a) tremor, ataxia, convulsion and prostration; (b) stimulated activity in respiration; (c) excessive electrical activity in poisoned axon (d) acetycholine levels in nerves of poisoned insects substantially increased. It was suggested that the two compounds, being organochlorines, exert their activity via the same mechanism and in a mode similar to DDT.

Athough both aldrin and lindane appear to act by the same mechanism, aldrin (LD_{50} 40mg/kg) appears in the present data to be more toxic than lindane (LD_{50} 249.0mg/kg). Infact this observation is suported by previous studies which reported LD_{50} of 39mg/kg for aldrin and 88mg/kg for lindane (Gaines, 1969).

4.20 INTERACTION STUDIES

Tetraethyl pyrophosphate, the first potent organophosphate insecticide (Murphy, 1975) did not find wide application because of its extremely toxic effects in humans. Parathion, the once very popular organophosphate soon found disfavour for the same reason. Human safety is the primary consideration of the acceptability of a compound for pest control programmes. The ideal pesticide should satisfy the dual purposes of being selectively toxic to target organisms while retaining low toxicity to humans.

Following the work of Frawley *et al* in 1957 it became apparent that a compound with a previously good record of human safety when used alone could be very toxic when used in combination with safe doses of other chemicals. Consequently, the United States Food and Drug Administration introduced the requirement that chemicals to be employed on food crops for the control of pests should be tested in combinations - in an attempt to detect potential interactions between the pesticides. Impetus was thus given for intense research in combination toxicity of pesticides .

Of various pairs of organophosphate pesticides tested for acute toxicity several additive, less than additive and synergism have been reported. For example DuBois (1961) found the following pairs synergistic in female rats: Malathion-trichlorform, Malathion-EPN and azinphosmethyl-trichlorform. Each of the pesticides that formed synergistic pairs with malathions was found to selectively inhibit carboxylesterase enzymes reponsible for detoxification of malathion, and this was suggested to be the mechanism reponsible for the synergistic action. Several other interactions between organophosphates in mice were also reported by Casida (1961) and Casida *et al* (1963). They also attributed observed synergistic actions to inhibition of detoxifying carboxylesterases. Su *et al* (1971) in a similar work noted that organophosphates with anticholinesterase effects that compared closely with their aliesterase inhibition were not likely to potentiate malathion toxicity at permitted dietary levels; but organophosphates which inhibit aliesterase at much lower dose levels than those required for cholinesterase inhibition were more likely to potentiate malathion toxicity. Other pairs of organophosphates found to be synergistic in various species include: Fenchlorphos-EPN, parathion-Fenchlorphos, DEF(s,s,s,-tributylphosphorotrithiolate)-EPN, DEF-Dimethoate, EPN - Dimethoate (Bull *et al*, 1965; McCollister *et al*, 1959).

Carboxylesterase inhibition was inadequate an explanation for some of the synergism encountered and other mechanisms were suggested, some of which have been elaborated upon in the introductory section of this report.

Various combinations of organochlorines studied (Keplinger *et al*, 1967) showed that endrine-chlordane endrin-aldrin, methoxychlor-chlordane were more than additive when given simultaneously. Interaction with storage and metabolism were also reported with organochlorine combinations. The simultaneous administration of dieldrin and DDT resulted in marked reduction of dieldrin storage in adipose tissues (Street *et al*, 1969). It was suggested that this effect was mediated by induction of microsomal enzymes by DDT which accelerated the rate of conversion of dieldrin to hydrophilic products subsequently excreted in faeces and urine.

Simultaneous administration of two compounds however, may not reveal the capacity of one agent to potentiate the toxicity of the other compound (Hagan *et al*, 1961). The organochlorines have been well known for their ability to induce hepatic microsomal enzymes. This ability enables them to influence toxicity of other compounds via enhanced biotransformation process for which these enzymes are catalysts (Conney, 1971; Sher, 1971; Remmer, 1972; Iyaniwura, 1988a). The importance of mixed function oxidases (MFOs) in the biotransformation of insecticides is well documented (Conney *et al*, 1972). The induction of these enzymes would be expected to increase toxicity of compounds that are primarily activated by these enzymes. Thus the toxicity of schradan was reportedly (Kato, 1961) increased following four days pretreatment of rats with thiopental, a well known enzyme inducer. DDT administration produced in rats an increased ability in their liver microsomes to epoxidize aldrin and heptachlor (Gillett *et al*, 1966). The epoxides being more toxic than parent compounds. Whereas, for compounds that are primarily detoxified by these enzymes, an induction would be expected to decrease their toxicity. Several examples have been reported in the literature. Black *et al* (1975) reported that 24 to 240 hours ovex pretreatment of rats provided protection against parathion and paraoxon. Egaas *et al*, (1988a) showed that aldrin and lindane induced MFO system in the yellow mealworm, *Tenebrio molitor*. They also demonstrated that aldrin pretreatment of the insect larvae with sample mean body weight of 0.23g resulted in a decrease lindane toxicity and attributed this to the induction of MFO by aldrin (Egaas *et al*, 1988b).

In the present study the influence of pretreating mice with aldrin or lindane on the toxicity of dimethoate was investigated. Previous studies (Seume *et al*, 1960) demonstrated that simultaneous administration of EPN or TOTP with dimethoate potentiated dimethoate toxicity in mice, possibly by the ability of these compounds to inhibit carboxylamidase, which metabolised dimethoate (Seume *et al*, 1960). Since dimthoate is mainly biotransformed in the liver (Uchida *et al*, 1964, 1967), it would be expected that the effect of pretreating animals with liver enzyme inducers should decrease dimethoate toxicity, particularly as it has been suggested for organophosphates that the rate of microsomal detoxification is more responsive to enzyme induction than the microsomal activation to produce the toxic oxygen analogues (DuBois, 1969). It was reported however (O'Brien, 1967b) that mice pretreated with the hepatic enzyme inducer, phenobarbital, increased their susceptibility to poisoning by dimethoate contrary to expectations.

4.21 ALDRIN VERSUS DIMETHOATE

Aldrin at a dose which was clearly capable of inducing liver microsomal enzymes as indicated by the effects on pentobarbitone sleeping time (Table VII), was not giving protection to dimethoate toxicity (Table V). Previous reports suggested that the inducers of hepatic microsomal enzymes produced protection (Murphy, 1969). Organochlorines are well known inducers and pre-exposure to aldrin would be expected to provide protection to the mice. Aldrin was also observed at the same dose to suppress appetite (anorexic effects) in the exposed mice. These animals were found to be weak, less active and had reduced weight compared with controls. It is possible that administration of a lethal dose of dimethoate after aldrin-pretreatment constituted more stress for the animals than in the controls. Apparently these anorexic effects outweighed any protective benefits that would have arisen from induction of the liver microsomal enzymes. Thus rather than give protection, aldrin has potentiated the toxicity of this compound (section 5.30).

Ball *et al* (1954) however, reported that four days pretreatment of female rats with aldrin did provide protection to parathion (a well known organophosphate pesticide similar to dimethoate). Failure to obtain similar results in this study with mice could also be attributed to the formulation of aldrin used and possibly the presence of impurities (Aldrige, 1979). This is supported by the suggestion made by Clarke (1975) that the toxicity of aldrin differs to some extent according to the method of preparation, possibly resulting from the presence of a variable quantity of a more toxic impurity. Since the commercial formulation of aldrin used in this study contains 5% aldrin in the total weight of powder, it is possible that the large proportion of the diluent in the formulation may constitute impurities which are capable of biological activities which could influence the toxicity of the active compound. Further studies would, however, be needed to elucidate this fact.

4.22 LINDANE VERSUS DIMETHOATE

At the same dose which lindane reduced toxicity of dimethoate (Table VI and section 3.30) it also reduced pentobarbitone sleeping time Table VIII). Pentobarbitone sleeping time correlates well with the period of time for which there is an effective concentration of the drug in the animal body. Chemicals which induce activity of the hepatic metabolizing enzymes for this drug would therefore shorten the sleeping time as the drug would be cleared from circulation faster. It would appear the reduction in dimethoate toxicity following lindane pretreatment is via the same mechanism of enzyme induction, as lindane at the same dose reduced pentobarbitone sleeping time. By increasing the activity and amount of microsomal enzymes, lindane is causing an increase in the rate at which dimethoate is cleared from the animal body thus reducing its toxicity in mice. The overall dynamics of enzyme induction has been shown to favour detoxification (DuBois, 1969). In a study with a similar compound, an organophoshate, parathion, Ball *et al* (1954) showed that pretreatment with aldrin decreased the toxicity of parathion to female rats. Alary and Brodeur (1969) suggested that both activation and detoxification routes for parathion were stimulated at induction of the enzymes but that the overall process was in favour of direct degradation of parathion than its activation. They however did not make direct measurements of paraoxon levels to buttress their point. Other studies showed that paraoxon formed from parathion provides a readier substrate for detoxification (Black *et al*, 1975) than parathion. Dimethoate is similarly activated *in vivo* to dimethoxon the phosphorus-oxygen (P=O) analogue assumed to be the actual toxicant (Krueger *et al*, 1960) which in turn is detoxified by liver microsomal enzymes (Uchida, 1964). Induction of microsomal enzymes appears to favour the rate of clearance of dimethoxon *in vivo* much faster than the rate of activation of dimethoate and thus the net effect is a reduction in dimethoate toxicity.

The effect of lindane pretreatment is thus protection from the acute toxic effects of dimethoate. It would appear that lindane provides this protection by increaseing overall rate, of breakdown of dimethoate in the liver. Although further studies would be required to completely define the mechanism of interaction between lindane and dimethoate, the present data however would appear to suggest that induction of liver microsomal enzymes by lindane plays a prominent role in the interaction process.

4.23 Histopathology

Durham (1963) reviewed the literature on toxicity and inhibition of cholinesterase by various organophosphorus insecticides. He noted that the degradation of these pesticides was so rapid that they do not accumulate in mammalian tissues. Tsumuki *et al.* (1970) also confirmed this finding but noted that there was cummulative physiologic effectiveness in which toxic signs persisted for long after the chemical has been excreted from the animal. Observations in this study is consistent with these findings, as animals appeared to have recovered from previous exposures before subsequent re-administration for the lower dosage groups. Animals exposed to the highest dose group, that is 100mg/kg recovered from the toxic symptoms much more slowly, and some of the symptoms were still evident after 24 hours post exposure. No determination of metabolic residues of the toxicant were made, however, in the present study.

Organ/body weight ratios were neither increased nor decreased in this study and there were no indications of cancerous lesions. Tsumuki *et al.* (1970) reported a slight reduction in liver/body weight ratio of mice treated with 100ppm dietary dimethoate for 45 days.

The histopathologic examinations revealed the presence of lesions in the lungs, liver and kidney of some these mice. It would be recalled (General introduction) that organophosphates produce their toxic signs by inhibition of the cholinesterase enzymes, thereby causing accumulation of the acetylcholine and subsquent overstimulation of the cholinergic systems. The cholinergic systems innervate the respiratory center in the medulla-oblongata of the central nervous system. The liver and kidneys are similarly innervated by cholinergic system. The observed lesions could therefore result from the physiopathologic manifestations of the nerve endings at the affected organs. The data suggest that chronic exposure to high doses of dimethoate could cause histopathologic changes in the lungs, liver and kidney. However these lesions appear to be secondary to the clinical signs rather than any direct effect of the agent as evidenced by the lack of cellular infiltration. It also does appear from the data that histopathologic effects of a dose as high as 25mg/kg dimethoate subchronically administered shows no considerable tissue damage in mice. Such high doses are unlikely to be encountered in human exposure. Allowed Daily Intake (A.D.I) for dimethoate is 0.2mg/kg (W.H.O., 1973) which is considerably well below doses that have been found from the current study to cause histopathological damage.

These observed histopathologic effects were studied using dimethoate alone. The pattern of lesions may be altered when dimethoate is used in combination with other compounds. The interaction of dimethoate with other pesticidal compounds as related to histopathologic effects were not examined in the current study and this could be an area of future investigations.

4.24 CONCLUSION

Although pesticide combinations sprayed on plants may accumulate as residues, pesticide poisoning from food residues may not constitute a major problem. This is because compliance with suitable formulation, timing and procedure of crop spraying, and permitted dietary levels set by the

World Health Organisation (WHO) and the Food and Agricultural Organisation (FAO) of the United Nations would permit only extremely small doses of pesticides as residues whose interactions may not yield any significant effects in human. However, individuals with a history of exposure to pesticides such as may occur in occupational exposure, could respond unpredictably to other drugs or toxicants applied subsequently.

Furthermore, pesticide combinations for household use could be selected such that combinations with abvious potential hazard to man could be avoided. These interaction findings raise the hope that pesticide combinations could be designed with the aim of achieving a higher level of selective toxicity sparing non-target organisms and the environment.

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