THE BIOCHEMICAL EFFECTS OF ACUTE AND SUB-CHRONIC ADMINISTRATION OF ASUNTOL[R] (COUMAPHOS) ON THE LIVER OF RATS.

BY

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A THESIS SUBMITTED TO THE POST GRADUATE SCHOOL AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN BIOCHEMISTRY.

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DECLARATION

I hereby declare that the work in this thesis was carried out by me under the joint supervisions of Professor G. H. Ogbadu, Department of Biochemistry, and Dr. T. T. Iyaniwura, Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University Zaria.

No previous submission of this thesis has been made for higher degree. The referred work of other researchers are fully acknowledged and cited in the references accordingly.

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JANUARY, 1995

CERTIFICATION

We certify that the thesis entitled "BIOCHEMICAL EFFECTS OF ACUTE AND SUB-CHRONIC ADMINISTRATION OF ASUNTOL^{R} (COUMAPHOS) ON THE LIVER OF RATS" presented by TARFA, Y. PETER meets the regulations governing the award of higher degree of Master of Science (MSc) Biochemistry of the Ahmadu Bello University, Zaria and is approved for its contributions to knowledge and literary presentation.

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DEDICATION

This research work is dedicated to all persons who pursue excellence in their respective endeavours so as to make this environment a better place to live in.

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ABBREVIATIONS AND SYMBOLS

AST	Aspartate Transaminase
ALT	Alanine Transaminase
ALP	Alkaline Phosphatase
DDT	Dichloro - diphenyl trichloro ethane
WHO	World Health Organisation
LD_{50}	Median Lethal Dose
FAO	Food and Agricultural Organisation
USEPA	United States Environmental Protection Agency
AChE	Acetylcholinesterase enzyme
Ach	Acetylcholine
OP	Organophosphorus
EC	Enzyme Commission
ICD	Isocitrate dehydrogenase
HBD	alpha - hydroxybutyrate dehydrogenase
LDH	Lactate dehydrogenase
OAA	Oxaloacetic acid
DNPH	Dinitro phenylhydrazine
IU	International Unit
BSA	Bovine Serum Albumin
BCG	Bromo Cresol Green

ABSTRACT

The acute and sub-chronic toxicity of Asuntol^{R} (Coumaphos) on the liver of rats were studied using biochemical methods. Seventy eight albino rats of both sexes aged between 1 - 2 months old, weighing between 78g to 108g constituted the study population.

The rats were fed ad-libitum with local layer marsh bounded with wheat flour for both acute and sub-chronic studies for a period of 8 weeks. Pre-determined levels of coumaphos were added to the feeds for the sub-chronic study, while no coumaphos was added the feeds for acute study.

Results of the acute toxicity test showed a moderate toxicity in rats (139.6mg/kg). For the sub-chronic studies, highest dose of 200mg/kg feed caused a significant ($P < 0.05$) increase in the levels of AST in the serum after the first four weeks of feeding trial, while it caused a significant ($P < 0.05$) decrease in ALT levels after the eight weeks. Lower doses of lOmg and lOOmg/kg feed showed no significant decrease in the levels of AST throughout the duration of the study. There was a significant decrease in the level of serum ALT with the lOOmg dose throughout the duration of the study. A significant ($P < 0.05$) decrease in the serum levels of ALP was observed with all three doses at one stage or the other during the study.

No significant change was observed throughout the study in the serum total protein and albumin levels which indicates normal protein turnover in the animals.

CHAPTER ONE

INTRODUCTION

1.1.0 Introduction:

Environmental pollution has continued to be a major source of concern to individuals and governments at all levels including international communities because of the obvious health problems arising from exposure to various environmetal agents like pesticides, industrial effluents, agrochemicals to mention but a few. (Hollum 1977, Iyaniwura 1991). These agents could pollute the air, land, surface and underground waters with great threat to human health if not effectively managed. This development has increasingly made it necessary to subject various chemical substances to toxicity test and their effects on health (USEPA Federal Register, 1987).

Man in his struggle for survival has established the need to chemically control pests and diseases that might affect his livestock or plants. In this regard, pesticides are quite useful and occupy a rather unique position among the many chemicals that man encounters daily in that they are deliberately put into use in the environment for the purpose of controlling insect vectors of disease (Murphy, 1975).

Pesticides are defined as refering to insecticides, herbicides, fungicides, nematicides, rodenticides and defoliants (Beat, 1970). The term pesticide is therefore a general classification of a variety of chemicals with different uses, and they have been so called because the target organisms to which they are directed are undesirable

and pose a threat to the economy and health of the society. Pesticides are therefore valuable chemicals which are used to maximise food production, protect very valuable house materials and control insect vectors that affect man (Iyaniwura, 1991). The use of pesticides for controlling these pest(s) has a favourable cost benefit effect (Murphy, 1980). For example cocoa production rose in Ghana by 110% in 1957 when pesticides were introduced to protect cocoa pods from infestation with fungus that was responsible for the black pod disease. Similarly, Japan in 1960 recorded an increase in rice production when insecticides were introduced to protect rice from the stem borer - Pricularia orizae (Fest and Schmidt, 1982).

The need to use pesticides use extends beyond their role in crop protection. Substantial livestock and poultry losses have been recorded as a result of disease epidemic, insect infestation and internal parasites. Pesticides are valuable 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 of similium flies.

Eventhough pesticides are useful and important, They are very potent poisons despite the fact that they are designed to be selectively toxic under careful and moderate application. At high doses and indiscriminate application, they could result in severe food, water and environmental pollution with consequent hazards to man and his domestic animals (Iyaniwura, 1990). Since each of these agents act biochemically in a

specific manner, their impact upon man and the ecosystem is not uniform. For instance, the organophosphates and carbamates are less persistent in nature and provoke acute episodes of human toxicity, while the organochlorines pose serious tissue and ecological problems due to the fact that they are less biodegradable (Edward, 1986). The concern over the hazards of pesticides to man has grown over the last few years. It has been estimated that each year about 500,000 cases of pesticide poisoning are reported with a mortality rate of 1% (WHO, 1982). Various human toxicosis resulting from pesticide use has been reported in literature (Beat, 1970; Hatch, 1982).

Generally, insecticides represent a group of pesticides which are used mainly and essentially in the elimination of insect pests. In 1939, dichloro-diphenyl trichloroethane (DDT), a remarkable insecticidal compound that had been synthesised in Switzerland in 1874, was demonstrated as an insecticide by Geigy Company. Since then, there has been great improvement on insect control (Symes, 1962). DDT was used for public health purposes such as control of bed bug and body lice during world war II (Symes , 1962).

The most common insecticides being used nowadays are the organophosphorus compounds such as coumaphos. It is used throughout the world in crop protection, control of animal infestation in domestic domain and for the suppression of the vectors of diseases. Coumaphos is a pesticide developed and marketed specially for use in animal hygiene. It has however, been unsuitable for use on crops on account of its high

phytotoxicity. Accidental exposure to coumaphos could therefore pose some hazards to man and domestic animals. It would therefore be necessary to evaluate coumophos for mammalian safety before use. This study was therefore designed to evaluate the toxicity of coumaphos on laboratory rats and to particularly study the changes that take place in living tissues such as the liver. Both acute and sub-chronic investigations would be conducted in the experimental animals to determine the effect of a high level one-time exposure to this chemical (acute effect) as well as a moderate but prolonged exposure to the compound as would be the case in diets (sub-chronic effect).

In this study therefore, the following parameters were to be specifically measured as ways of determining the health effects of exposure to coumaphos:

i. the LD_{50} of coumaphos in rats using the intra-peritoneal route.

ii. liver function tests after four and eight weeks of feeding trials which include assays for:

   (a) aspartate transaminase activity (EC 2.6.1.1).

   (b) alanine transaminase activity (EC 2.6.1.2).

   (c) alkaline phosphatase activity (EC 3.1.3.1).

   (d) total protein concentration.

   (e) serum albumin concentration.

iii effect of the test chemical on organ body weight ratio.

CHAPTER TWO

LITERATURE REVIEW

2.1.0 Pesticides: Definition, Classification And Formulation

Pesticides are valuable chemicals defined by the Food and Agricultural Organisation (FAO) 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 plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing food, agricultural commodities, wood and wood-products or animal feedstuff, or which may be administered to animals for the control of insects Archnids or other pests in or on their bodies (WHO, 1990).

Pesticides are biologically active compounds. The principal classes of pesticides are classified according to their roles as first outlined by (Newman, 1978) as follows:

(i) rodenticides, designed to kill rats, mice, moles etc. i.e mammals.

(ii) insecticides, designed to kill insects and other related species that prey on crops farm stock etc.

(iii) fungicides, which are agents intended to control moulds, fungi etc.on crops,stored foods,timber etc.

(iv) herbicides designed to kill weeds.

(v) plant growth controls which are substances designed to modify the growth and form of the plants,usually hormonally without actually killing them.

Pesticides occupy a rather unique position among the many chemicals that man encounters daily in that they are deliberately added to the environment for the purpose of killing or injuring some forms of life (Casarett and Doull, 1975). Thus all pesticides are toxicants. A pesticide is said to be selective in toxicity if it is highly toxic only to a few related organisms, or broad-spectrum in toxicity if it has high toxicity to a wide range of organisms (Matsumura, 1975). Ideally, the injurious action of pesticides would be highly specific for undersirable target organisms and non-injurious to desirable non-target organism. However, most of the chemicals that are used as pesticides are not highly selective but are generally toxic to many non-target species including man and other desirable forms of life that co-inhabit the environment (Okonkwo, 1991).

The preparation of an 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. Most active ingredients are biologically very active and it would be difficult to spread them evenly over a field at a rate of less than about 1kg/ha if they were not diluted in some way (Hassall, 1982). In addition, many of the compounds are greasy, organic substances insolubles 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 (3) major formulations for this purpose are the aqueous 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).

2.1.1 Development And Uses Of Pesticide

The history of pesticide discovery dates back to the period of world war II, when the chemicals were used in chemical warfare, hence their description as nerve poison. During the second world war, German Scientists under the direction of Gerhard Schrader were engaged on the task of developing highly toxic nerve gases for use in warfare. As a spin-off, several organophosphorus compounds structurally related to the nerve gases tubun and sarin were found to be effective insecticides. (Hassall, 1982).

By the nineteenth century, altogether some 100,000 organophosphorus compounds have been screened for their possible insecticidal action and 100 have been marketed for this purpose. An important feature of the group is that different members posses very different physico-chemical properties, in particular, they have different vapour pressure at room temperature and different solubilities in water. They also exhibit considerable variability in their chemical stability and their toxicity to mammals.

In the years between the first and second world wars, both the number and the complexity of chemicals for crop

protection increased. Tar oil was and is still being used to control the eggs of aphids on darmant 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 U.S.A. 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 also 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 carbamate herbicides were discovered by workers in United Kingdom and the organochlorine insecticide chlordane was introduced in the U.S.A. and in Germany. Shortly afterwards, the insecticidal carbamates were developed in Switzerland. The urea derivatives were developed as herbicides in the U.S.A. between the period from 1950 to 1955. The fungicides, capton and glyodin appeared, and malathion was introduced. Between 1955 and 1960, other new products including herbicidal triazines and quaternary ammonium herbicides. Dichlorobenil, trifluralin, and bromoxynil were discovered between 1960 and 1965 while the systemic fungicide benomyl was discovered in 1968, and the leaf-acting herbicide glyphosate was introduced soon afterwards.

In the 1970's and 1980's, many new pesticides were introduced on the basis of more thorough understanding of the biological/biochemical mechanisms of their action and are often more effective at lower doses. As a result of better

understanding of host-pest interactions, a new approach to the design of pesticides is now developed, 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 (WHO, 1986; Kagan, 1977).

Pesticides are termed "economic poison". This implies that these toxic agents needed in our daily life is at same time a hazard to health (WHO, 1986).

In agriculture, pesticides are widely used as anthelmintics (Clarke and Clarke, 1975, Bartik and Piskal, 1981) and as chemical defoliants (Abou-Donia et al, 1980a). 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; Dauterman, 1971). 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, plague, Onchocerciasis, chaga's disease, African trypanosomiasis and yellow fever. With such a wide diversity of applications, it is that the potential for animal and human exposure is relatively great (Taylor, 1980).

.2 **Modes of Exposure and Toxicity of Pesticide**

Evaluations of toxicological effects or hazards

associated with handling and use of pesticides have for many years focused primarily on preventing injury to man. These hazards should however not underate the benefits e.g. in cases where mass starvation due to destruction of food crops by insects and noxious weed is incomparable to the injury caused to a few members of the population (Casarett and Doull, 1975).

Greater hazards or damage is brought about by large scale exposure to pesticides through spray of buildings, greenhouses and factories, particularly when many individuals are exposed over extended areas. Approximately 20% of all pesticides are used in private homes and gardens and by public officials in buildings, parks and street; thus exposure to risk is quite high (Hayes, 1982).

The hazards of pesticides use arises usually as a result of occupational exposure or of careless use, or mis-handling of pesticides. Mortality rate has been attributed to poisoning by pesticides at 0.65 per million population in the United States (Hayes, 1969). Man and his animals can be exposed to pesticide by accidental encounter or as residues in food, water and air resulting often in acute poisoning (Matsumura, 1975). Hazardous environmental pollution with pesticides may arise from

i. application of chemicals to control agricultural pest.
ii. application of pesticides to pests causing public health problems.
iii. industrial waste; and
iv. misplacement and leakage during the transportation,

distribution or storage processes as well as during the manufacturing processes (Matsumura, 1975).

These chemical agents in the environment are usually picked up by the biological systems and are passed onto man and domestic animals via the food chain, sometimes with disasterous effects. Pesticides may cause ecological inbalance and man now suffers an indirect effect of the pesticide (White-Stevens, 1973). A typical example was the use of DDT in 1942 for the protection of agricultural products and public health against vector-borne diseases. The protection was reported by the authors to be effective for about two decades without injury, until in the third decade, the effects on the environment began to raise serious doubts, hence its application was restricted (Casarett and Doull, 1975). Hazards of pesticides used in agriculture have been attributed to their intake through foods that have been treated with pesticides before and or after harvest (Davis and Lewis, 1956). In a health survey of 300 workers in a plant manufacturing toxic organochlorine pesticide over a nine year period, no fatalities or permanent injury were recorded, but 17 of the workers had convulsive intoxication and 5 of the 17 had more than one convulsion (Hoogendam et al, 1965).

To eliminate a pest in agriculture, spraying, dusting, baiting, drenching, dipping and painting are being employed Pesticides are being added even to such consumer products as paint, household cleaning fluids, floor waxes, deodarizers and shelf and drawer paper used in kitchen, food storage areas. They vaporize through furnaces and ventilating equipment.

For a toxicant to exert its characteristic effects in vivo, it must arrive at the site(s) of action, that is the target points (Brooks 1976). The most immediate toxic effect of a pesticide in poisoned person is usually related to its mode of action on the target organ. Different pesticides produce different acute disturbance. Some affect the nervous system, which if severe may interfere with the respiratory system with possible fatality (Brooks, 1976). Because so many applications of pesticides are by way of sprays, it is widely believed that the most significant route of exposure is the respiratory tract, however, analysis of residues on masks or on pad placed on exposed skin surfaces of workers involved in pesticide applications revealed that the dermal route offers the greatest potential for occupational exposure (Wolfe et al, 1967). Thus, the only pesticides that are of potential danger by inhalation are those few preparations which are released as smokes or vapour and against which special protection by respirators can be provided (Brooks, 1976).

A study on health effects among workers involved in the manufacture of certain chemicals revealed also that exposure was mainly through the skin (Chattopadhyay, 1988). The author's findings revealed that the neurological symptoms were due to recent exposure to the chemical and were related to the duration/intensity of exposure. An increase in enzyme levels among the exposed workers was suggestive of some degree of liver damage. Therefore the knowledge that the skin is the principal route of exposure for pesticides means that attention should be focused on protection of the exposed areas

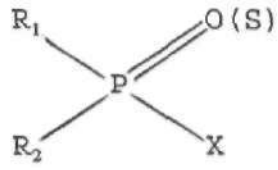
and an insistence on regular washing during any prolonged period of exposure.

2.1.3 Development And Uses of Organophosphorus Insecticide

Organophosphorus are complex organic molecules which are generally regarded as esters of phosphoric acid or its derivatives (Health, 1961). The pesticide group includes such insecticides and herbicides as parathion, malathion, azodin, diazinon, dicaphton, ronnel, dichlorophenton, leptophos and phosdrin. These poisons are related to the nerve gas diisopropyl fluorophosphate, which was developed in Nazi Germany, during world war II. They attack a vulnerable part of the nervous system. Acetylcholine, an acetyl derivative of choline, having blood pressure lowering properties, is responsible for transmitting impulses from one nerve fibre to another and from nerves to muscles.The enzyme acetylcholinesterase (AChE) breaks down this messenger chemical after it has completed its task (Green, 1976). The organophosphate pesticides and nerve gases disrupt this delicate mechanism by preventing the AChE enzyme from breaking down acetylcholine. As a result, acetylcholine builds up in the body and the nerve impulses remain unchecked. Experimental animals succumb to muscular twitching and convulsions, caused by excess stimulation of the nervous system (Wasserman and Wiles, 1985).

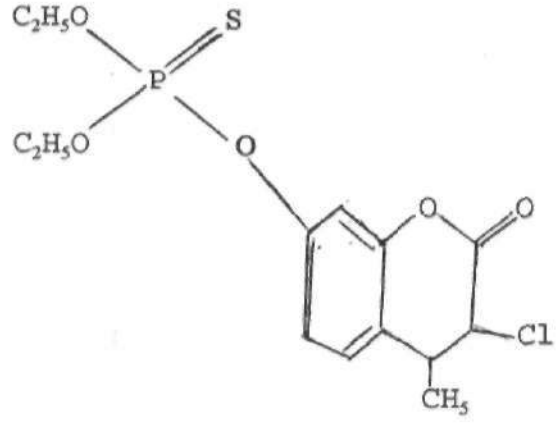
Over 50,000 organophosphorus (OP) compounds are known (Koller and Klawans, 1979) and at least 100 OP insecticides have been reviewed by WHO for consideration as agents for the control of disease vectors (WHO, 1986).

The general formular for organophosphorus pesticide compound is (Health, 1961)



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

In Coumaphos the active ingredient present is O,O-diethyl-O-(3-chloro-4-methyl-7-coumarinyl) phosphorothioate. The trade names by which coumaphos is known includes; asuntol[R50] agridip, Co-ral, Meldane, Muscatox, Resistor, and Suntol. The chemical structure (Health, 1961) is



O,O-Diethyl-O-(3-chloro-4-methyl-7-coumarinyl) phosphorothioate (COUMAPHOS)

Coumaphos is a pesticide developed and marketed especially for use in animal hygiene, being unsuitable for use on crops on account of their high phytotoxicity. Coumaphos is rather toxic when given by mouth, having an acute oral LD_{50} in rats of 90-110mg/kg. However its dermal toxicity is low and is relatively safe when applied to the skin. The dermal LD_{50} for rats is approximately 900mg/kg.

Coumaphos is a fine crystalline solid with a melting point of 90°-92°C. It is soluble in organic solvents. It is almost insoluble in water (1.5 ppm) and is hydrolized in alkaline media. It is used to treat cattle, poultry, horses, sheep and dogs for control of cattle grups, lice, horn flies, screw worms, mites and ticks.

2.2.0 Evaluation Of Health Risk For Man And Effects On Environment

The usual symptoms of organophosphate poisoning in mammals are defecation, urination, lacrimation, muscular twitching and fabrilation, and convulsions, generally clonic (i.e. repetitive movements) and less often tonic (i.e. limbs stretches and rigid). Usually death follows a clonic convulsion. Smaller doses lead to corresponding but less severe symptoms and occasionally produces protracted and milder forms of these symptoms. Salivation, diarrhea, and muscular weakness are common. Animals can become adapted to chronic poisoning. These insecticides can be absorbed through the skin as well as the respiratory system and gastro intestinal tract, although dermal absorption tends to be slow. However these compounds are difficult to remove, so

dermal absorption may be prolonged. High temperature increases dermal absorption as does the presence of dermatitis (O'Brien, 1967).

Generally, the manifestation of signs and symptoms of OP exposure occurs more rapidily following inhalation rather than gastro-intestinal 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, its metabolism and individual variations in responses (Rosenstock, 1989). The quality of signs and symptoms as well as their durations may differ markedly for different compounds by virtue of differences in rate of biotransformation, distribution and affinities for acetylcholinesterase (Murphy, 1975).

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 metabolised and excreted. Sub-acute or chronic poisoning by virtue of accumulation of the compounds in the body does not occur (Murphy, 1975).

2.2.1 Clinical Diagnosis Of Organophosphorus Intoxication

The clinical diagnosis of organophosphorus intoxication can be determined through the medical history and circumstances of exposure, and presence of several of the symptoms characteristic of organophosphate poisoning particularly broncho constriction and pin-point pupils not reactive to the light, pulse rate is not of diagnostic value, because AChE effects on the heart reflects the complex

innervation of this organ (Iyaniwura,1990).On the other hand, since changes in the conduction and excitability of the heart might be life-threatening,monitoring should be performed. Confirmation of diagnosis is made by measurement of AchE in red blood cells (RBC) or plasma-Pseudocholinesterase and, also of the dibucaine number. Measurement of blood cholinesterase during theraphy are also useful in assessing the treatment with oximes; though there might not be a correlation between the severity of symptoms and the degree of cholinesterase inhibition. Comparison should be made with pre-exposure levels, whenever possible (Iyaniwura, 1990).

Chemical analysis of body fluids (Urine, blood astric lavage) can identify the compounds that caused the poisoning. (Iyaniwura, 1991).

2.2.2 General Treatment And Precautions Protocol In Pesticides Intoxication

The treatment of acute human poisoning with pesticides is directed along the following three main lines (Iyaniwura, 1990):

i. Prevention of prolonged absorption of unabsorbed poison involves removal of orally ingested poison by induction of emesis by using Ipecacuanha syrup and possibly gastric lavage. Ipecacuanha syrup is however contra-indicated in poisoning with corrosive agents or petroleum distillate. Gastric lavage requires specialised clinical skills and is best arranged in the hospital or a health centre. Eye contamination should be washed with plenty of clean water.

ii. Start supportive and symptomatic treatments especially of vital organs like respiration and cardiovascular functions so as to counter the systemic effects of the absorbed poison. The air passages are freed of mucus by placing the poisoned victim in a prone position with head down and to one side. The jaw should be opened wide and the tongue pulled forward. Mouth to mouth recurcitation should be avoided because patients vomitus may contain highly toxic material. Instead the mouth and pharynx should be cleaned with a cloth or by suction. An oropharyngeal, nasopharyngeal or endotracheal intubation may be administered if there is obstruction of air passages. Artificial respiration may also be needed.

iii. Institution of appropriate antidotal therapy to neutralise the effects of the poison once the offending pesticide has been identified.

All cases of organophosphorus poisoning should be dealt with as an emergency and the patient sent to hospital as quickly as possible. Although symptoms may develop rapidly, delay in onset or a steady increase in severity may be seen upto 48 hours after ingestion of some formulated OP insecticides.

To avoid or minimise the risk associated with insecticides exposure, the following precautions should be observed while handling insecticides (Fernald and Shepard, 1955):

i. insecticides should not be handled carelessly;

ii. some insecticides are particularly dangerous and special

label precautions should be followed;

iii. containers should not be opened or insecticides mixed in a closed room;

iv. one should avoid breathing particles and fumes, especially those from concentrated sprays and dust;

v. an approved type of respirator and rubber gloves should be used when label precaution so direct;

vi. after prolonged exposure to insecticides, one should bathe, using soap freely;

vii. if liquid concentrates are spilled on the skin or clothing,the clothing should be removed immediately and the person bathed with soap and water;

vii. insecticides should be stored where they are not accessible to children and animals;

ix. empty containers should be destroyed by burning in the open or burying by the proper licensed bodies;

x. insecticides should not be allowed to drift over pastures, feed or food crops that may remain contaminated until consumed;

xi. those insecticides mixtures that contain considerable proportions of oil and other inflammable ingredients should be used and stored with the fire hazard in mind;

xii. pesticide factories should not be located near high settlement areas and precautions should be taken to prevent contamination of rivers and water bodies;

xiii. eating, smoking and drinking in pesticide working areas must be disallowed, and workers must wash their hands thoroughly after work and before meals;

xiv. pesticides must not be loaded on the same vehicle as foodstuff and drivers should have a basic knowledge of the toxicity of pesticides being carried. Vehicles should also be equipped with simple protective equipment such as impervious gloves, eye protector etc. Accidental spillages should be reported to the appropriate authorities immediately;

xv. instruction on manufacturer's leaflets in packaged or re-packed pesticide products must be clear and unambiguous.

2.2.3 First-Aid In Cases Of Pesticide Poisoning.

The following first-aid measures are considered relevant when the patient is out of the reach of a clinic, hospital, or even human habitation:

i. remove the person from any further contact with the chemical, i.e.from spraying area and other polluted environment;

ii. relax the person and keep him at rest;

iii. devest the person of any contaminated clothing and wash any area of affected skin with soap and water or with water alone, the first-aider taking care himself to avoid contact with any noxious material;

iv. in the event of respiratory arrest, make sure that the air way is cleaned immediately and institute assisted ventilation by whatever method feasible. Note that mouth to mouth technique is not suitable if poison has been taken by mouth;

v. for poisoning by ingestion, the stomach should be emptied

as soon as possible. In the field, gastric aspiration and lavage are seldom practicable and induced emesis has to be considered. This should of course be abjured if the person is unconscious. Manual simulation of the back of the throat is seldom successful,and salt, mustard, etc. are not recommended. Syrup of ipecasuanha is probably the agent of choice, if it is at hand;

vi. apart from instances of negligible or insignificant exposure, it is prudent to transfer the patient to hospital as soon as possible. The usual rules should be observed in transit that is the left lateral and headdown position, with the tongue drawn forward and assisted ventilation if there is any indication that the spontaneous activity is inadequate;

vii. to assist the hospital medical staff towards effective treatment, a note should accompany the patient stating clearly the identity of the suspect agent(s) or better still, a lable or container.

2.3.0 The Liver Function Tests

Although most, if not all organs of the body are involved in the detoxification of poisonous substances, the liver plays a foremost role because it is especially rich in enzyme systems that metabolize chemicals of all kinds (Kachmar and Hoss, 1976).

The functions of the liver have been categorized into five major parts (Mathenheimer, 1971):

i. storage of nutrients and their release into the blood, for example, storage of carbohydrates in the form of

glycogen, release of glucose into the blood, and storage of fat, iron, copper, and vitamin B_{12}.

ii. synthesis e.g. conversion of glucose into fat, amino acids (proteins) into glucose; galactose and other sugars into glucose, and synthesis of protein (albumin, some globulins, clotting factors), urea, cholesterol, purines and pyrimidines.

iii. detoxication: conjugation of toxic substances with glucoronate and sulphate, and metabolic conversion of drugs.

iv. excretion: excretion of bile pigments, bile acids and cholesterol.

Histopathologically, the determination of the effects of toxic substances on an organ is assayed from a change in the weight of the organ or from structural abnormalities. But this assay has been criticized for their low sensitivity and failure to reveal much about the mode of action of a toxic substance (Barnes and Denz, 1954). Consequently organ function tests are now employed for the assessment of organ damage in addition to the tests previously being done (Paget 1970; Cutler, 1974). The results of liver function tests are however not usually correlated with the results of the histopathological examination (Cutler, 1974). Earlier studies had shown that the measurement of activity changes of certain enzymes in blood serum was a very reliable indicator of liver functions (Matthenheimer, 1971).

All serum enzymes have their origin in the cells

(Ringler and Dabich, 1979). The clinically useful enzymes are normally found in serum in relatively low concentration, thus increased serum concentration of a particular enzyme is an indicator of damage to the cells from which the enzyme was released. In the rat, diagnostic serum enzymology has been mainly directed towards early and reliable identification of liver injury due to toxicants. Serum enzyme responses usually vary with the rate and magnitude of absorption of the toxicant and hence may serve as an indication of severity of exposures (Ringler and Dabich, 1979). On this basis, the authors concluded that there is a correlation between increases in serum enzyme activity and severity of toxic damage to a particular tissue (Okonkwo, 1991).The principal reason for an elevated serum enzyme activity in response to pathophysiological conditions was the liberation of enzymes from the damaged organ into the general circulation (Wolf, 1972). A decrease in serum enzyme activity may arise occasionally in the case of specific enzyme deficiency for example as a result of blockage of de novo enzyme synthesis because of liver damage from prolonged exposure to the toxicant (De Bruin, 1976).

Assays of the activities of serum enzymes have proved a very useful approach to detecting and diagnosing toxic effects leading to damage of organs, establishing the nature of the disease process and locating the cellular and intracellular sites of hepatic pathology (Wilkinson, 1976; Gowenlock, 1988). Therefore, the genuine search for useful liver function test is basically a study of the activity of

a number of enzymes in the serum.

2.3.1 Enzymes In Clinical Diagnosis Of Liver Diseases

Liver diseases were among the first disorders to which serum enzyme tests were applied and has proved to be useful in diagnosing disease of the liver (Ikediobi and Ukoha, 1985). Enzymes are usually compartmentalised in various organ cells of the body. For example in rat liver cells, the enzymes of glycolysis are located in the cytoplasm, whereas enzymes of the citric acid cycle are in the mictochondrion (Wilkinson, 1976). Although there are exceptions, the amount of enzyme released is related to its activity in a particular tissue (Bernirschke et al, 1978). Many of these enzymes are involved in intermediary metabolism and are present in the liver cells in high concentrations. Hence when the cells are injured or disrupted as occurs in acute liver disease, these enzymes are released into the serum and their increased level is often of diagnostic significance (Williams, 1959).

The enzymes related to liver function include the transaminases AST and ALT, ALP, ICD ,HBD and LDH (Mattenheimer, 1971). However, in the majority of clinical laboratories, the results of both the transaminases and ALP measurements are usually reported in response to a clinical request for liver function tests (Wilkinson, 1976; Ikediobi and Ukoha, 1985).

CHAPTER THREE

MATERIALS AND METHODS

3.1.0 Test Animals

The test animals were adult albino rats of both sexes with ages ranging between 1 to 2 months weighing between 78g to 108g and bred locally in the animal house of the faculty of pharmaceutical sciences, Ahmadu Bello University Zaria. The animals were housed in plastic cages and kept under close observation for two weeks before commencement of experiment. Any animal that showed signs of ill health was excluded from the experiment.

3.1.1 Animal Feed

The animals were fed local layer mash (sanders^{R}) bound with wheat flour in a ratio of 1 part wheat flour to four parts layer mash for both acute and sub-chronic toxicity studies. In the sub-chronic toxicity study, pre-determined levels of Asuntol^{R} were incorporated into the feeds as solution, then moulded into large balls and sun-dried, these were fed to the relevant groups ad-libitum. In the acute toxicity study, coumaphos was not incorporated into the animal feed.

Water in drinkers was similarly fed ad-libitum. The water was changed every other day.

3.1.2 Test Chemical

Asuntol^{R}, (manufactured by Bayer Leverkusen, Germany) a fine crystalline organophosphorus insecticide served as the chemical under investigation. Various reagents (See

Experimental Methods) were used for the enzyme assays.

3.1.3 Apparatus

The following specialized apparatus were used in addition to routine laboratory instruments.

i. Plain/non-heparinized centrifuge tubes were used for collecting whole blood samples while Boujior bottles were used for collecting serum.

ii. Avery balance (W. and T. Avery Limited Birmingham, England) was used in weighing the experimental animals and their feed.

iii. Centrifuge Machine (International centrifuge, model K. No 20130 P) for centrifuging the whole blood in order to collect serum.

iv. Colorimeter (Arning 253) for determination of the levels of parameters in the serum.

3.2.0 Intraperitoneal Determination Of LD_{50}

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

Pilot Study

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 was used as a guide in the subsequent experiments to determine the LD_{50}.

In the initial pilot study, each of the five dose levels

were selected at 0.6mg/kg b.w interval and administered to two rats. In the second test, dose levels selected at 0.15mg/kg b.w intervals were administered to five groups of two rats each.

In the final test, thirty normal healthy rats (average weight 105g) were used. They were divided into 5 groups of 6 rats (3 males, 3 females). The control group received 0.5ml each of distilled water. The other four were administered dose of the test chemical at 0.3mg/kg b.w. interval as determined from the pilot studies. The number of animals that died within 24 hours in each group was noted. The LD_{50} was determined by substitution in the formula

$$LD_{50} = \text{Min. dose that killed all animals in a group} - \frac{\text{sum(dose difference x mean dead)}}{\text{Total number of animals per group}}$$

Acute toxicity study in experimental animals was aimed at detecting the short term effects of high doses of coumaphos, and in this way provide fundamental data for the design of more complex animal studies in order to estimate the safety level for humans. Therefore for safety of any poisonous chemical, it is important to determine the LD_{50}. For convenience, chemicals can be classified according to their LD_{50} values (Matsumura, 1975) as follows:

i. "Extremely toxic" (LD_{50} in order of 1mg/kg or less)

ii. "Highly toxic" (LD_{50} of 1 - 50mg/kg)

iii. "Moderately toxic" (LD_{50} of 50 - 500mg/kg)

iv. "Slightly toxic " (LD_{50} of 0.5 - 5g/kg)

v. "Practically non-toxic" (LD_{50} of 5 - 15g/kg)

vi. "Relatively harmless" (LD_{50} of more than 15g/kg)

Though the classification is convenient it is only a crude approximation.

3.2.1 Sub-chronic toxicity study (Oral)

The sub-chronic toxicity study was designed to last for a period of eight weeks. Administration of drug was by oral route (through inclusion in feed). The 48 normal healthy animals were divided into four groups of twelve animals each with each group housed in a separate labelled plastic cage. The animals were weighed at the start of experiment and on the day of sacrifice. The animals were grouped into four groups fed with the moulded layer mash treated with the following doses of the test chemical respectively, 0.0 mg, 10mg, 100mg and 200mg/kg feed.

Treatment was continued for 8 weeks, six rats from each group were removed and sacrificed by decapitation for organ function tests at 4 weekly intervals. Blood samples were collected from the neck region directly into plain centrifuge tube; sera were collected after clotting and centrifugation. These were immediately stored in a deep freezer (-20°C) for analysis.

3.2.2 Determination Of Serum Aspartate And Alanine Transaminases

The estimation of the transaminases enzymes was by the colorimetric method of Reitman and Frankel, 1957.

Aspartate and alanine transaminases catalyse the transfer of the amino group from aspartate and alanine respectively onto

oxoglutarate. Aspartate is converted to oxaloacetic acid (OAA) and alanine to pyruvate. The OAA formed being relatively unstable subsequently decarboxylates into pyruvate which is measured by its colour reaction with 2,4 dinitrophenylhydrazine to form the corresponding phenylhydrazone, a coloured compound whose intensity reflects the concentration of oxaacids present. These reactions were carried out at alkaline pH 7.4 and the brown colour measured colorimetrically at 510nm.

(a) Preparation Of Reagents

1. Substrates (200mmol/L)

   For AST; 6.6g L-Aspartic acid was dissolved in 90ml of 1N NaOH; 0.146g of alphaketoglutaric acid was added and dissolved by adding NaOH. The pH was adjusted to 7.4 and the solution made up to 500ml with phosphate buffer.

   For ALT; 4.5g L-alanine was dissolved in 90ml of H_2O with the addition of 2.5ml of 1N NaOH to adjust the pH to 7.4: 0.146g of alphaketoglutaric acid was added and dissolved by adding NaOH. The pH was further adjusted to 7.4 and the solution made up to 500ml with phosphate buffer.

2. Stock Pyruvate Standard (20mmol/L)

   220mg of sodium pyruvate was dissolved in 100ml of phosphate buffer. The solution was stored at -15°C.

3. Working Pyruvate Standard (4mmol/L) Stock pyruvate standard was diluted 1 in 5 with phosphate buffer and stored at -15°C.

4. 2,4 - Dinitrophenyl Hydrazine (1mM)

19.8mg of DNPH was dissolved in 10ml of concentrated HCl and made upto 100ml with water solution stored in brown bottle at room temperature.

5. Sodium Hydroxide (NaOH 0.4N)

16g of NaOH was dissolved in 1 litre of distilled water to give a 0.4N solution.

3.2.3. **Determination Of Serum Alkaline Phosphatase**

Phosphatases are enzymes which catalyse the hydrolysis of monophosphate esters. In King-Amstrong method, disodium phenyl phosphate is hydrolysed by phosphatase under standard conditions (pH 10 and incubation temperature of 37°C for alkaline phosphatase). The hydrolysis produce phenol which is condensed with 4 - amino phenazone and then oxidized with alkaline potassium ferricyanide to give a reddish brown colour, the intensity of which is proportional to the enzymatic activity.

The king - Armstrong unit of phosphatase is the concentration of enzyme which will set free 1mg of phenol in 15 minutes at pH 10 and temperature of incubation of 37°C for the alkaline phosphatase.

3.3.0 **Determination Of Serum Total Protein (Biuret Method)**

The serum total protein was determined by the Biuret Method (Mokrash and McGilvery, 1956). This method is based on reactions of copper ion in alkaline solutions with the protein peptide bonds. The reaction produces a violet colour which is proportional to the amount of protein peptide bonds present which is correspondingly proportional to the protein

concentration.

The biuret reaction is positive for proteins and peptides bearing at least two peptide bonds (-C-NH). The reaction takes its name from the fact that the simple substance, biuret ($NH_2.CO.NH.CO.NH_2$), gives the same kind of colour with cupric ions. Each copper atom complexes with four molecules of biuret, the linkages being to the central nitrogen atom. The violet colour is due to the formation of a complex with copper ions.

Total serum protein is determined by comparing the intensity of a coloured solution of dilute protein with that of a known protein concentration (standard solution).

3.3.1 Determination Of Serum Albumin Using Dye-Binding

Albumin reacts with the dye, Bromo Cresol green which has specific affinity for it in acidic solution to produce a complex which is measured colorimetrically. The intensity of the colour is proportional to the concentration of albumin in the sample (Gowenlock, 1988).

(a) **Preparation Of Reagents.**

1. Stock BCG Dye solution (0.6M)

   419mg Bromocresol green was dissolved in 10ml of 0.1M NaOH and diluted to 1 litre. Solution was stored at 4°C

2. Stock Brij 35 (250g/L).

   25g solid Brij 35 was warmed in water to dissolve and made up to 100ml with distilled water.

3. Working BCG Dye Solution

   Every volume of stock BCG solution was mixed with 3

volumes of succinate buffer. 4ml of 30% Brij 35 solution was added per litre and the pH adjusted to 4.2 ±0.05 by the addition of 5ml of 5N NaOH and stored at 4°C.

4. Standard Solution

Bovine serum Albumin (1:10) was diluted with normal saline to give a 30g/L solution.

(b) **Method For Determination Of Serum Albumin**

In this experiment, 20ul (0.02ml) serum was added to 4ml working dye solution, mixed and incubated at 37°C for 10 minutes. The absorbance was then read at 628nm against a blank of working dye solution.

(c) **Calculation**

$$\text{Serum albumin (gm/l)} = \frac{\text{Test reading}}{\text{standard reading}} \times \text{concetration of reading}$$

3.3.2 **Statistical Analysis**

Analysis of the biochemical data in this study was carried out using the student t - test. This test deals with an aspect of statistical inference. From this test, the levels of significance for the different biochemical parameters at the various dose levels were deduced by using P-value of 0.05.

CHAPTER FOUR

RESULTS, DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

4.1.0 Results and Discussions

4.1.1 Acute Toxicity Tests of Coumaphos

The intraperitoneal median lethal dose (LD_{50}) of coumaphos in rats was determined using standard procedures. The results are shown in table 4.1. The table indicates the various doses of coumaphos administered per group of the rats and the effects recorded within 24 hours of dosing. The animals in group 5 (control) were given water only.

Table 4.1: **Data on Intraperitoneal LD_{50} of Coumaphos in rats.**

EXPTL GRP	DOSE ADMIN. (mg/kg b.w.)	DOSE DIFF.	NUMBER DEAD	MEAN DEAD	DOSE DIFF. X MEAN DEAD
1	50		0		
		50		0.5	25
2	100		1		
		65		2.5	162.5
3	165		4		
		35		5	175
4	200		6		
5	-		-		
				TOTAL	262.5

From calculation thus, LD_{50} of coumaphos = 139.6mg/kg. The simplest acute toxicity study usually employs determination of the median lethal dose of the compound. The result of the acute toxicity test with coumaphos via the intraperitoneal

route of administration, showed a moderate (139.6mg/kg) acute toxicity in rats. There is a wide variation in the reported LD_{50} value for coumaphos in rats, ranging from 15mg/kg (Clarke and Clarke, 1975) to 100mg/kg (Green , 1977).

Matsumara, 1975 also reported on acute oral LD_{50} of coumaphos in rats to be between 90 - 110mg/kg. The intraperitoneal LD_{50} of coumaphos reported in mice is 205mg/kg (Iyaniwura: 1990). The dermal toxicity of coumaphos is however low and is relatively safe when applied to the skin (Matsumura, 1975). The dermal LD_{50} for rats is approximately 900mg/kg. Thus it can be said that the discrepancies in toxicity of coumaphos is dependent upon the animal species, and the route of administration (Santolucito and Morrison,1971).

4.1.2 Sub-chronic Oral Toxicity Study.

4.1.2.1 Effects of Coumaphos on Enzyme Levels in the Serum

Oral administration of coumaphos to rats via the feed at a dose of 200mg/kg feed caused significant ($P < 0.05$) increases in the levels of AST in the serum in the first month of feeding trial (Table 4.2) with no further increase observed at the end of the second month, while it caused a significant ($P < 0.05$) decrease in ALT levels in the second month of study. Rats fed lower doses of 10mg/kg feed dose and 100mg/kg feed dose showed no significant difference in the levels of AST throughout the duration of the study. The 100mg/kg dose however caused a significant ($P < 0.05$) decrease in the level of serum ALT in the first month of the work. All three (3)

doses used in this study caused significant ($P < 0.05$) decreases in the serum levels of Alkaline Phosphatase (ALP) at one stage or the other during the cause of the study (Table 4.2).

Sub-chronic toxicity studies are usually designed to provide data on the toxic effect of a chemical and to determine the dose level and time course required for these effects to manifest. The most widely used criteria for evaluation of toxic effects of substance on animal are reduction in rate of body weight gain, the detection of gross and histopathological abnormalities in the organ, the changes in organ weights and increase in motality rate (Cutler, 1974). The toxic effect of a chemical on the liver may cause similar effect as diseases that cause liver damage such as toxic hepatitis, and cancer that involves the liver. Aminotransferases are among groups of enzymes whose serum activities are drastically altered in many different disease states most of which involve liver damage (Schwar'tz, 1971).

The degree of alteration in the activities of these enzymes is most often related to the nature of the disease and the extent of damage caused. The increase in the serum AST observed at the end of the first month in the group fed the highest dose of 200mg/kg feed may be an indication of tissue damage in the heart muscle, liver, skeletal muscle or kidney which led to leakage into peripheral circulation (Kaneko and Cornelius, 1971, Wilkinson, 1976). Additionally, evaluation of chemical toxicity in live animals involves observation of physical clinical symptoms and the biochemical determination

of changes in body constituents that may give an insight into the nature of the intoxication.

The liver is the primary site of detoxification of foreign compounds in the body, and thus may be the organ of interest in studying the subchronic toxicity of a chemical in the systemic circulation. The toxic effect of a chemical on the Liver may cause similar changes in the ultra-structure and metabolism of the liver as do diseases that cause liver damage such as toxic hepatitis and hepatoma. Aminotransferases are among a group of enzymes and metabolites used in evaluating the structural and functional integrity of the liver, and whose levels in the plasma are drastically altered in many different disease states, most of which involve liver damage (Schwar'tz, 1971). The value of liver function test depend on their specificity for damage as well as their sensitivity (Cutler, 1974).

Serum levels of AST and ALT are elevated when disease processes affect the hepatocyte integrity (Kachmar and Hoss, 1976). Wolfe et al, 1967, have shown that the extent of rise in the activities of these two enzymes in the serum usually is a reflection of severity of the hepatic damage, and could be attributed to the release of enzymes from the damage tissue into the general circulation.

In the present investigation, only the highest dose (200mg/kg feed) of the three (3) doses used caused a significant ($P < 0.05$) increase in the serum levels of AST in the first 4 weeks of feeding trial. This effect was however abolished by the end of the 8th week as the AST level in the

serum normalised. AST is found in high concentration in several tissues including heart muscles, liver, skeletal muscles and kidney (Kaneko and Cornelius, 1971, Wilkinson, 1976). An earlier report demonstrated pathological lesions in the liver, intestine, brain, heart and spleen of rats fed 100mg/kg feed dose of coumaphos, while rats fed lower doses (10mg/kg) showed lesions in the heart, liver and kidney (Wilkinson,1976). The observation in the present work may well be attributed to damage in the liver or any of the tissues above leading to leakage into the systemic circulation. The normalization of the serum levels of AST in the second month may either be due to repair processes in the body which may cause healing of lesions and thus reduce extent of leakage (in which case the initial damage must have being mild); suggesting that the liver damage at this dose is reversible.

ALT levels in the serum are raised when there is damage to the liver. Infact, the level of ALT in the serum is a more specific indication of liver damage than AST (Wilkinson, 1976) because the richest source of ALT in the body is the liver. The present study however shows a significant ($P < 0.05$) fall in the level of ALT specific activity in the serum of rats fed 100mg/kg feed in the first month of study, an effect that was not observed in the second month. The higher dose of 200mg/kg feed dose showed a delayed effect that manifested in the significant fall in ALT activity in the serum observed in the second month. The explanation for these observations are obscure at present, but it seems to negate any suggestion of liver damage that leads to enzyme leakages. Perhaps in some

unknown way, coumaphos may be causing inhibition of de-novo synthesis of the enzyme or cause in-situ inhibition of the already existing enzyme activity.

The ALP activity in the sera of rats fed coumaphos showed a dose related decrease from normal levels. The lowest dose of 10mg/kg feed showed significant effect only in the second month while the highest dose manifested its effect in the first month but the effect was nullified in the second month. The 100mg/kg feed caused a persistent effect throughout the study. Again the enzyme activity in the serum is expected to be raised in hepatic damage. The present observation seemingly ruled out irreversible liver damage. It is instructive to note that phosphatases are enzymes which hydrolyse phosphate moiety from various substrates and coumaphos is an organophosphorus compound.

Although an earlier report had shown necrotic lesions in the liver, heart, kidney and spleen of rats fed coumaphos for period of 10 weeks, Cutler, (1974) had also reported that the result of histopathologic examinations particularly of the liver do not always correlate with the result of liver function test. Thus while damage to the ultra-structure of hepatocytes may be detected by histopathological studies, the expected increase in the levels of certain serum marker enzymes may not manifest. The overall view of this present work does not suggest liver damage; however microscopic examination of liver tissues may show some pathological lesions. Histopathological examinations of organ was however not done in this work.

4.1.2.2: Effect of Coumaphos on Serum Total Protein and Albumin Levels.

The dietary intake of coumaphos at the different dose levels used in this study didn't cause any significant ($P > 0.05$) change in serum level of total protein and albumin throughout the duration of the study. (Table 4.2). This indicates normal protein turnover in the animals despite the presence of coumaphos in the system. The significant ($P < 0.05$) increase observed in group III at the end of the first month may be an abberration.

4.1.2.3: Effect of Coumaphos on Body Weights and Organ/Body Weights Ratios.

The animals in group II gained weights at a significantly ($P < 0.05$) faster rate than those in group I throughout the duration of the work (Table 4.15). The 100mg/kg feed dose caused an increase in body weight which was significantly ($P < 0.05$) higher than the control group but significantly ($P < 0.05$) lower than that observed in group II throughout the 2 months period of the study. The highest dose caused an initial gain of weight in the first one month but a loss of weight was recorded at the end of the second month. The above may be an indication of delayed toxicity, possibly an annexigluric action of coumaphos at 200mg/kg feed dose.

At the end of the first one month, the first two doses (i.e 10mg/kg feed and 100mg/kg feed) caused equivalent increases in liver/body weight ratio that were significant ($P < 0.05$) when compared with the levels in the control animals. The 200mg/kg feed dose however caused a significant

($P < 0.05$) decrease from the normal levels. These effects were not observed at the end of the second month. Only the 200mg/kg feed dose caused a significant ($P < 0.05$) decrease in the kidney/body weight ratio during the first month of study. In the second month however, groups II & III showed significant ($P < 0.05$) increases in these ratios (Table 4.14). The two higher doses (i.e 100mg/kg feed and 200mg/kg feed) caused significant increases in the spleen/body weights ratio in the animals at the first stage of the study; these ratios were however significantly lower than that of the control animals in the second month of the study. The lowest dose although had no effect in the first one month, significantly ($P < 0.05$) increase the ratio at the end of the second month.

As earlier stated one of the criteria for determining toxicity in animals is the reduction in the rate of body weight and organ weight gain (Cutler, 1974). In the present study, coumaphos appeared to have caused an increase in the rate of body weight gain in the first two doses. The highest dose while it affects the body weight gain in the first month, caused a decrease in the rate of body weight gain in the second month. This may be an indication of delayed toxicity (possibly an annexigluric action) of coumaphos at 200mg/kg feed dose.

During the first month of study, coumaphos caused significant increase in the liver/body weight ratios which may indicate a greater rate of increase in the mass of liver in the coumaphos fed group. This effect was however not observed in the second month of study, this may be due to initial

sensitization of the liver by the coumaphos, thus causing proliferation of certain component of the liver which caused an increase in mass relative to the body weight. In the second month, it is possible that the liver has already adapted to the sensitization, thus no further increase was observed. The effect on the kidney/body weight ratio was not as dramatic in the first month, but a significant reduction was observed in this ratio in the groups fed the highest doses of 100mg/kg feed and 200mg/kg feed coumaphos respectively. This may be an indication of delayed response since the major site of detoxication is the liver, so the effect is seen much more earlier in the liver. What appeared to be spleenomegaly was observed in the first one month in groups fed 100mg/kg and 200mg/kg coumaphos. This effect was delayed in the group fed 10mg/kg coumaphos till the second month. The spleen however reduced in mass in the last two groups during the second month. The spleen is one of the major sites of producing erythro-endothelial cells that clear the system of foreign invaders. It is possible that coumaphos sensitises the spleen to produce more of these cells and this could lead to the increase in the mass of the spleen observed in the first month. By the end of the second month however, the spleen may have adapted to this continuous sensitization.

Table 4.2: Data from liver function test, mean values of specific enzyme activity, total protein and serum albumin, + standard error of mean.

Month of Study	Dose Level (mg/kg feed)	AST (u/g)	ALT (u/g)	ALP (u/g)	Total Protein (gm/l)	Serum Albumin (gm/l)
1st Month (n = 6)	0.00	1.36 ± 0.35	0.51 ± 0.16	2.28 ± 0.21	79.67 ± 14.69	34.33 ± 5.39
	10	1.03 ± 0.33	0.49 ± 0.13	2.04 ± 0.43	80.17 ± 4.88	35.17 ± 1.60
	100	1.22 ± 0.24	0.28 ± 0.06	1.26 ± 0.39	107.50 ± 12.06	30.33 ± 6.92
	200	2.18 ± 0.28	0.37 ± 0.07	0.80 ± 0.18	82.17 ± 8.40	35.00 ± 7.16
2nd Month (n = 6)	0.00	2.38 ± 0.56	0.74 ± 0.22	1.56 ± 0.13	71.67 ± 7.56	35.83 ± 5.74
	10	2.11 ± 0.54	0.70 ± 0.13	1.07 ± 0.28	71.33 ± 12.14	35.33 ± 2.42
	100	2.63 ± 0.66	0.83 ± 0.27	0.80 ± 0.20	71.67 ± 17.59	41.50 ± 11.15
	200	2.43 ± 0.54	0.45 ± 0.17	1.27 ± 0.45	75.83 ± 5.04	35.83 ± 4.07

4.2.0 Conclusions

The analyses of the laboratory experimental investigations on the animals studied and discussions revealed the following conclusions and the subsequent recommendations:

i. That the acute and sub-chronic studies which however did not attempt to establish the degree of histopathological conditions or degenerations involved, jointly revealed that coumaphos compounds are hepatotoxic to animals and probably humans.

ii. That the reduction of alanine transaminase in the serum level studied at the highest dose of 200mg/kg feed dose could serve as a vital signal that some tissue damages might have been involved, and that the damages could take

a progressive pattern and vary according to the amount of coumaphos compounds intake.

iii. The normal recovery of the liver recorded in the experimental animals was only in the sub-chronic study, this implies that at moderate and chronic intake of coumaphos compounds, the physiological detoxification and excretion ability of the liver and kidney respectively had taken place, and hence no permanent damage or serious ill-health conditions were recorded. This has established a base for the need of setting guidelines and standards as to what quantity of this compound be present in aquatic, terrestial and aerial environment.

The primary objective of this work was to study the biochemical effects of coumaphos compounds on the experimental animals with particular emphasis on enzymatic properties of liver and its ability to detoxify the chemical at various dose levels of intake. The findings from the study were intended to determine the safe dose levels which could subsequently serve as a guide in policy formulation, guidelines and regulations on the use of pesticides of organophosphate group.

In view of the findings established from the study, the following recommendations were made:

1. It is advisable that further studies should be conducted to establish histopathological effects of coumaphos compounds on livers of experimental animals to allow such findings guide in policy formulation, setting of guidelines, standards and regulations on application of pesticides.

2. The users of the chemical in agricultural sector of the economy should be adequately educated on their safe storage, transportation and application to avoid possible danger of chemical poisoning.

3. Its application for purpose of disinfestation on animals should be moderate within safe range to prevent intensive bioaccumulation and subsequent intake by humans through food chain.

4. It is also recommended that further research be conducted to see to the possibility of detoxifying residual total pesticides after they might have fulfilled their desired purpose to prevent their likely damage to aquatic creatures, and soil bacteria that have been found to be of immense importance to food production.

5. That guidelines and regulations relating to manufacturing, exportation, importation and application of pesticides should be adequately enforced world-wide as already advocated by the United Nation Environment Programme (UNEP).

6. While it has been recommended that the use of pesticides be encouraged to preserve food items vulnerable to pest infestation with a view to combat the world-wide food shortage, the need to conduct studies to identify other pesticides that are relatively biodegradable, less toxic, and non-carcinogenic to replace the banned ones has been suggested.

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TABLE 4.3: COMPARISON OF MEAN VALUES OF SERUM CONCENTRATION OF AST, ALT, ALP, TOTAL PROTEIN AND SERUM ALBUMIN AFTER THE FIRST AND SECOND MONTHS OF STUDY

MONTH OF STUDY	GROUP COMPARED	10 t0.05	AST		ALT		ALP		TOTAL PROTEIN		SERUM ALBUMIN	
			tcal.	P-Value	tcal.	P-Value	tcal.	P-Value	tcal.	P-Value	tcal.	P-Value
1st MONTH OF STUDY	GI Vs GII	2.228	1.6814	P>0.05	0.2378	P>0.05	1.2292	P>0.05	0.0792	P>0.05	0.3662	P>0.05
	GI Vs GIII	"	0.8086	P>0.05	3.2990	P<0.05	5.6440	P<0.05	3.5888	P<0.05	1.1177	P>0.05
	GI Vs GIV	"	4.4840	P<0.05	1.9648	P>0.05	13.1151	P<0.05	0.3620	P>0.05	0.1832	P>0.05
	GII Vs GIII	"	1.1413	P>0.05	3.5949	P<0.05	3.2932	P<0.05	5.1487	P<0.05	1.6702	P>0.05
	GII Vs GIV	"	6.5128	P<0.05	1.9920	P>0.05	6.5198	P<0.05	0.5046	P>0.05	0.0568	P>0.05
	GIII Vs GIV	"	6.3803	P>0.05	2.3926	P<0.05	2.6248	P<0.05	4.2241	P<0.05	1.1492	P>0.05
2nd MONTH OF STUDY	GI Vs GII	2.228	0.8507	P>0.05	0.3837	P>0.05	3.8900	P<0.05	0.0582	P>0.05	0.1967	P>0.05
	GI Vs GIII	"	0.7079	P>0.05	0.6334	P>0.05	7.8090	P<0.05	-	-	1.1082	P>0.05
	GI Vs GIV	"	0.1575	P>0.05	2.5565	P<0.05	1.5175	P>0.05	1.1222	P>0.05	-	-
	GII Vs GIII	"	1.4946	P>0.05	1.0633	P>0.05	1.9232	P>0.05	0.0389	P>0.05	1.3254	P>0.05
	GII Vs GIV	"	1.0270	P>0.05	2.8632	P<0.05	0.9249	P>0.05	0.8390	P>0.05	0.2588	P>0.05
	GIII Vs GIV	"	0.5748	P>0.05	2.9191	P<0.05	2.3393	P<0.05	0.5572	P>0.05	1.1708	P>0.05

APPENDIX B

TABLE 4.4: RESULTS OF LIVER FUNCTION TEST ON GROUP 1 (CONTROL, 0.00Mg/Kg FEED DOSE) AFTER 1ST MONTH OF STUDY

SAMPLE NO.	ENZY. ACT. AST. (U/L)	SP. ENZY. ACT. (AST)	ENZY. ACT. ALT (U/L)	SP. ENZY. ACT. (ALT)	ENZY. ACT. ALP (U/L)	SP. ENZY. ACT. (ALP)	TOTAL PROTEIN (gm/L)	SERUM ALBUMIN (gm/L)
G1 R1	104	1.65	27	0.43	155	2.46	63.0	31.0
G1 R2	156	1.58	72	0.76	188	1.90	99.0	28.0
G1 R3	104	1.16	52	0.58	205	2.28	90.0	43.0
G1 R4	72	0.83	25	0.29	211	2.43	87.0	38.0
G1 R5	91	1.21	38	0.51	180	2.40	75.0	32.0
G1 R6	112	1.75	30	0.47	140	2.19	64.0	34.0
MEAN		1.36		0.51		2.28	79.67	34.33
± S. D.		±0.35		±0.16		±0.21	±14.69	± 5.39

APPENDIX C

TABLE 4.5: RESULTS OF LIVER FUNCTION TEST ON GROUP II (10.0 mg/kg FEED DOSE) AFTER 1ST MONTH OF STUDY

SAMPLE NO.	ENZY. ACT. AST. (U/L)	SP. ENZY. ACT. (AST)	ENZY. ACT. ALT (U/L)	SP. ENZY. ACT. (ALT)	ENZY. ACT. ALP (U/L)	SP. ENZY. ACT. (ALP)	TOTAL PROTEIN (gm/L)	SERUM ALBUMIN (gm/L)
G2 R1	72	0.86	38	0.45	164	1.95	84.0	38.0
G2 R2	118	1.53	45	0.58	168	2.18	77.0	34.0
G2 R3	56	0.72	38	0.49	106	1.36	78.0	34.0
G2 R4	59	0.75	34	0.43	212	2.68	79.0	35.0
G2 R5	89	1.01	27	0.31	173	1.97	88.0	34.0
G2 R6	100	1.33	51	0.68	156	2.08	75.0	36.0
MEAN		1.03		0.49		2.04	80.17	35.17
± S.D.		±0.33		±0.13		±0.43	± 4.88	± 1.60

APPENDIX D

TABLE 4.6: RESULTS OF LIVER FUNCTION TEST ON GROUP III (100 mg/kg FEED DOSE) AFTER 1ST MONTH OF STUDY

SAMPLE NO.	ENZY. ACT. AST. (U/L)	SP. ENZY. ACT. (AST)	ENZY. ACT. ALT (U/L)	SP. ENZY. ACT. (ALT)	ENZY. ACT. ALP (U/L)	SP. ENZY. ACT. (ALP)	TOTAL PROTEIN (gm/L)	SERUM ALBUMIN (gm/L)
G3 R1	118	1.02	29	0.25	122	1.05	116.0	41.0
G3 R2	198	1.62	25	0.21	124	1.02	122.0	22.0
G3 R3	118	1.28	35	0.38	188	2.04	92.0	25.0
G3 R4	152	1.31	29	0.25	122	1.05	116.0	31.0
G3 R5	105	1.05	33	0.33	114	1.13	101.0	35.0
G3 R6	101	1.03	26	0.27	125	1.28	98.0	28.0
MEAN		1.22		0.28		1.26	107.50	30.33
± S.D.		±0.24		±0.06		±0.39	±12.06	±6.92

APPENDIX E

TABLE 4.7: RESULTS OF LIVER FUNCTION TEST ON GROUP IV (200 mg/kg FEED DOSE) AFTER 1ST MONTH OF STUDY

SAMPLE NO.	ENZY. ACT. AST. (U/L)	SP. ENZY. ACT. (AST)	ENZY. ACT. ALT (U/L)	SP. ENZY. ACT. (ALT)	ENZY. ACT. ALP (U/L)	SP. ENZY. ACT. (ALP)	TOTAL PROTEIN (gm/L)	SERUM ALBUMIN (gm/L)
G4 R1	204	2.29	38	0.43	69	0.76	89.0	33.0
G4 R2	152	2.03	25	0.33	73	0.97	75.0	29.0
G4 R3	134	1.68	21	0.26	40	0.50	80.0	49.0
G4 R4	230	2.47	34	0.37	78	0.84	93.0	31.0
G4 R5	200	2.35	38	0,45	63	0.74	85.0	35.0
G4 R6	161	2.27	27	0.38	69	0.97	71.0	33.0
MEAN		2.18		0.37		0.80	82.17	35.0
± S.D.		±0.28		±0.07		±0.18	±8.40	±7.16

APPENDIX F

TABLE 4.8: RESULTS OF LIVER FUNCTION TEST ON GROUP 1 (CONTROL, 0.00mg/kg FEED DOSE) AFTER 2ND MONTH OF STUDY

SAMPLE NO.	ENZY. ACT. AST. (U/L)	SP. ENZY ACT. (AST)	ENZY. ACT. ALT (U/L)	SP. ENZY. ACT. (ALT)	ENZY. ACT. ALP (U/L)	SP. ENZY. ACT. (ALP)	TOTAL PROTEIN (gm/L)	SERUM ALBUMIN (gm/L)
G1 R1	198	3.09	68	1.06	106	1.66	64.0	29.0
G1 R2	118	1.84	42	0.66	106	1.66	64.0	28.0
G1 R3	150	2.00	52	0.69	122	1.63	75.0	40.0
G1 R4	206	2.82	68	0.93	104	1.43	73.0	40.0
G1 R5	154	1.83	36	0.43	113	1.35	84.0	38.0
G1 R6	190	2.71	48	0.69	113	1.61	70.0	40.0
MEAN		2.38		0.74		1.56	71.67	35.83
± S.D.		±0.56		±0.22		±0.13	±7.56	±5.74

APPENDIX G

TABLE 4.9: RESULTS OF LIVER FUNCTION TEST ON GROUP II (10mg/kg FEED DOSE) AFTER 2ND MONTH OF STUDY

SAMPLE NO.	ENZY. ACT. (AST. (U/L)	SP. ENZY ACT. (AST)	ENZY. ACT. ALT (U/L)	SP. ENZY. ACT. (ALT)	ENZY. ACT. ALP (U/L)	SP. ENZY. ACT. (ALP)	TOTAL PROTEIN (gm/L)	SERUM ALBUMIN (gm/L)
G2 R1	164	2.34	50	0.71	66	0.94	70.0	33.0
G2 R2	134	1.62	52	0.63	78	0.94	83.0	37.0
G2 R3	118	1.33	44	0.49	58	0.65	89.0	39.0
G2 R4	170	2.70	50	0.79	84	1.33	63.0	33.0
G2 R5	122	2.10	44	0.76	78	1.35	58.0	34.0.
G2 R6	168	2.59	54	0.83	80	1.23	65.0	36.0
MEAN		2.11		0.70		1.07	71.33	35.33
± S.D.		±0.54		±0.13		±0.28	±12.14	±2.42

APPENDIX B

TABLE 4.10: RESULTS OF LIVER FUNCTION TEST ON GROUP III (100mg/kg FEED DOSE) AFTER 2ND MONTH OF STUDY

SAMPLE NO.	ENZY. ACT. (AST. (U/L)	SP. ENZY ACT. (AST)	ENZY. ACT. ALT (U/L)	SP. ENZY. ACT. (ALT)	ENZY. ACT. ALP (U/L)	SP. ENZY. ACT. (ALP)	TOTAL PROTEIN (gm/L)	SERUM ALBUMIN (gm/L)
G3 R1	178	3.00	52	0.87	60	1.00	60.0	28.0
G3 R2	192	3.20	78	1.30	58	0.97	60.0	45.0
G3 R3	193	2.12	50	0.55	60	0.66	91.0	36.0
G3 R4	193	2.38	68	0.84	78	0.96	81.0	45.0
G3 R5	152	1.71	50	0.56	47	0.53	89.0	60.0
G3 R6	164	3.35	42	0.86	33	0.67	49.0	35.0
MEAN		2.63		0.83		0.80	71.67	41.5
± S.D.		±0.66		±0.27		±0.20	±17.59	±11.15

APPENDIX F

TABLE 4.11: RESULTS OF LIVER FUNCTION TEST ON GROUP IV (200mg/kg FEED DOSE) AFTER 2ND MONTH OF STUDY

SAMPLE NO.	ENZY. ACT. (AST. (U/L)	SP. ENZY ACT. (AST)	ENZY. ACT. ALT (U/L)	SP. ENZY. ACT. (ALT)	ENZY. ACT. ALP (U/L)	SP. ENZY. ACT. (ALP)	TOTAL PROTEIN (gm/L)	SERUM ALBUMIN (gm/L)
G4 R1	134	1.74	26	0.34	62	0.81	77.0	34.0
G4 R2	166	2.31	28	0.39	115	1.60	72.0	36.0
G4 R3	288	3,39	68	0.80	85	0.97	85.0	33.0
G4 R4	176	2.32	34	0.45	151	1.99	76.0	42.0
G4 R5	187	2.53	30	0.41	96	1.23	74.0	39.0
G4 R6	161	2.27	23	0.32	72	1.01	71.0	31.0
MEAN		2.43		0.45		1.27	75.83	35.83
± S. D.		±0.54		±0.17		±0.45	±5.04	±4.07

APPENDIX J

TABLE 4.12: DATA ON ORGANS AND BODY WEIGHTS OF INDIVIDUAL GROUPS AFTER 1ST MONTH OF STUDY

SAMPLE NO	BODY WEIGHT (GM)	LIVER WEIGHT (GM)	KIDNEY WEIGHT (GM)	SPLEEN WEIGHT (GM)	TOTAL PROTEIN (GM/L)	ALBUMIN (GM/L)
G1R1	98	3.84	0.98	0.28	63	31
G1R2	144	3.49	0.92	0.41	99	28
G1R3	119	4.73	0.93	0.27	90	43
G1R4	138	3.98	0.92	0.30	87	38
G1R5	118	1.53	0.78	0.22	75	32
G1R6	120	3.71	0.94	0.25	64	34
MEAN	122.83	3.88	0.91	0.29	79.67	34.33
± S.D.	± 16.38	±0.46	±0.07	±0.07	±14.69	± 5.39
G2R1	155	5.14	1.13	0.38	84	38
G2R2	160	6.13	1.11	0.42	77	34
G2R3	169	5.21	1.21	0.36	78	34
G2R4	181	6.06	1.10	0.50	79	35
G2R5	171	6.00	1.25	0.41	88	34
G2R6	165	5.19	1.08	0.38	75	36
MEAN	166.83	5.62	1.15	0.41	80.17	35.17
± S.D.	± 9.09	±0.49	±0.07	±0.05	± 4.88	± 1.60
G3R1	120	4.46	0.88	0.49	116	41
G3R2	137	4.30	0.86	0.37	122	22
G3R3	126	4.28	0.92	0.34	92	25
G3R4	125	5.24	0.89	0.49	116	31
G3R5	127	4.35	0.86	0.40	101	35
G3R6	130	4.60	0.90	0.42	98	28
MEAN	127.50	4.54	0.89	0.42	107.50	30.33
± S.D.	5.68	±0.36	±0.02	±0.06	± 12.06	± 6.92
G4R1	140	4.92	0.96	0.48	89	33
G4R2	189	5.88	1.22	0.39	75	29
G4R3	177	4.85	0.90	0.32	80	49
G4R4	153	4.01	0.96	0.32	93	31
G4R5	167	4.78	0.91	0.36	85	35
G4R6	150	4.36	0.98	0.34	71	33
MEAN	162.67	4.80	0.99	0.37	82.17	35.0
± S.D.	± 18.36	±0.63	±0.12	±0.06	± 8.40	± 7.16

APPENDIX K

TABLE 4.1: DATA ON ORGANS AND BODY WEIGHTS OF INDIVIDUAL GROUPS AFTER 2ND MONTH OF STUDY

SAMPLE NO	BODY WEIGHT (GM)	LIVER WEIGHT (GM)	KIDNEY WEIGHT (GM)	SPLEEN WEIGHT (GM)	TOTAL PROTEIN GM/L)	ALBUMIN (GM/L)
G1 R1	[illegible]	4.73	0.93	0.56	64	29
G1 R2	[illegible]0	5.45	1.11	0.42	64	28
G1 R3	[illegible]	5.85	0.98	0.50	75	40
G1 R4	[illegible]0	6.00	0.99	0.49	73	40
G1 R5	[illegible]3	5.51	0.91	0.45	84	38
G1 R6	[illegible]4	4.96	0.95	0.51	70	40
MEAN	[illegible]5.17	5.42	0.98	0.49	71.67	35.83
S. D.	± [illegible].68	±0.49	±0.07	±0.05	±7.56	±5.74
G2 R1	[illegible]67	6.98	1.11	0.43	70	33
G2 R2	135	9.16	1.46	0.33	83	37
G2 R3	[illegible]55	9.46	1.46	0.46	89	39
G2 R4	182	5.47	1.01	0.32	63	33
G2 R5	[illegible]63	5.98	1.07	0.42	58	34
G2 R6	[illegible]87	6.33	1.10	0.39	65	36
MEAN	[illegible]04.66	7.23	1.20	0.39	71.33	35.33
S.D.	±40.29	±1.69	±0.20	±0.06	±12.14	±2.42
G3 R1	161	5.22	1.19	0.41	60	28
G3 R2	[illegible]91	5.74	1.16	0.57	60	45
G3 R3	152	5.28	0.93	0.40	91	36
G3 R4	[illegible]65	7.34	1.00	0.38	81	45
G3 R5	[illegible]72	4.98	1.01	0.39	89	60
G3 R6	[illegible]65	5.31	0.96	0.40	49	35
MEAN	[illegible]67	5.65	1.04	0.43	71.67	41.5
S.D.	[illegible]13.17	±0.87	±0.11	±0.07	±17.59	±11.15
C1 R1	[illegible]57	4.24	0.82	0.29	77	34
C1 R2	141	4.69	0.92	0.27	72	36
C1 R3	[illegible]33	4.83	0.95	0.30	85	33
[illegible]	[illegible]46	4.75	0.95	0.42	76	42
[illegible]	[illegible]57	5.27	0.94	0.25	74	39
C1 R6	[illegible]50	5.10	0.96	0.30	71	31
MEAN	[illegible]47.3	4.81	0.92	0.31	75.83	35.83
S. D.	[illegible]5.40	±0.36	±0.05	±0.06	±5.04	±4.07

APPENDIX L

TABLE 4.14: DATA ON ORGAN/BODY WEIGHTS RATIO AFTER 1ST AND 2ND MONTHS OF STUDY

MONTH OF STUDY	GROUP NO	LIVER/BODY WT RATIO	KIDNEY/BODY WT RATIO	SPLEEN/BODY WT RATIO
1st MONTH OF STUDY	GROUP I	0.032	0.0074	0.0024
	GROUP II	0.034	0.0069	0.0025
	GROUP III	0.036	0.007	0.0033
	GROUP IV	0.0300	0.0060	0.0033
2nd MONTH OF STUDY	GROUP I	0.0330	0.0059	0.0030
	GROUP II	0.0353	0.0059	0.0037
	GROUP III	0.0340	0.0062	0.0026
	GROUP IV	0.033	0.0063	0.0021

APPENDIX M

TABLE 4.15: DATA ON PERCENTAGE INCREASE IN BODY WEIGHTS FOR THE 1ST AND 2ND MONTH OF STUDY

GRP NO	INITIAL WTS (GM)	1ST MONTH WTS	% INCREASE	2ND MONTH WTS	% INCREASE
1	86.70 ± 17.7	122.83 ± 16.38	43.81 ± 14.81	165.17 ± 7.68	96.91 ± 44.32
2	87.40 ± 9.41	166.83 ± 9.09	91.99 ± 14.53	204.66 ±40.29	131.89 ± 29.88
3	77.80 ± 8.80	127.50 ± 5.68	65.17 ± 16,09	167.67 ±13.17	116.37 ± 10.67
4	107.40 ± 24.51	162.67 ± 18.36	55.67 ± 26.06	147.33 ± 9.40	42.61 ± 33.64