STUDIES ON THE TOXIC EFFECTS OF SOME PESTICIDES ON THE FISH

Etroplus maculatus (Bloch)

THESIS SUBMITTED TO THE
COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

MARINE BIOLOGY

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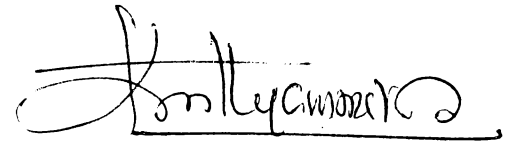
FEBRUARY 1990

TO MY PARENTS AND TEACHERS

C E R T I F I C A T E

This is to certify that this thesis is an authentic record of research work carried out by Shri K.S. GOPALAKRISHNAN, under my scientific supervision and guidance in the School of Marine Sciences, Cochin University of Science and Technology, in partial fulfilment of the requirements for the degree of Doctor of Philosophy of the Cochin University of Science and Technology and no part thereof has been presented before for the award of any other degree, diploma or associateship in any University.



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D E C L A R A T I O N

I, K.S. GOPALAKRISHNAN, do hereby declare that this thesis, entitled "STUDIES ON THE TOXIC EFFECTS OF SOME PESTICIDES ON THE FISH ETROPLUS MACULATUS" is a genuine record of the research work done by me under the scientific supervision of Dr. V.J. KUTTYAMMA, Reader, Division of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Tehnology, and has not previously formed the basis of the award of any degree, diploma or associateship in any University.

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A C K N O W L E D G E M E N T S

I wish to express my deep sense of gratitude to my supervising teacher Dr. V.J.Kuttyamma, Reader, Division of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology, for her guidance, encouragement and for scrutinising the manuscript and for suggesting improvements.

I place on record that I am greatly indebted to Prof. Dr.N.R.Menon, Head, Division of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology for initiating me to work in the field of aquatic toxicology and for his keen interest shown throughout the period.

I wish to express my sincere gratitude to Prof.Dr.P.N.K. Nambisan, Director, School of Marine Sciences, Cochin University of Science and Technology for his affection and encouragement.

I thank the authorities of Cochin University of Science and Technology for providing me all the necessary facilities for the successful completion of my research work.

I am deeply indebted to Dr.A.Mohandas, Reader, School of Environmental Studies, Cochin University of Science and Technology and to Dr.S.Radhakrishnan, Lecturer, Department of Aquatic Biology, University of Kerala for the encouragement, for critically going through the manuscript and for suggesting improvements.

I acknowledge with thanks the invaluable assistance and co-operation rendered by Dr.V.S.Jalaja Kumar, Dr.K.Santhosh, M|s.K.Sasidharan, U.K. Menon, Purandara Bekel, Asok Kumar, Philip Mathew, Miss Shiny Sreedhar and few of my friends who helped me at various stages of my research work.

The financial assistance received from the Council of Scientific and Industrial Research, New Delhi, by means of research fellowship is gratefully acknowledged.

I am thankful to Shri.S.Vancheeswaran of M|s.Associates, C.L.C. Road, Cochin-16, for getting the manuscript typed in time.

Last but not to the least, I am thankful to the non-teaching staff of the School of Marine Sciences, who, in one way or other, helped for the successful completion of my research work.

C O N T E N T S

PREFACE

PREFACE

Pollution of the aquatic environment has become one of the major concerns of the present day society. The human chemical interference of the biosphere through the induction of as many as *60,000* organic chemicals (Maugh, *1978)* for various anthropologic benefits has eventually led to the realization that these chemicals, beneficial on application, has transcended to cause serious threats to the ecosystem. It is quite unlikely that a detailed critical assessment of the specific risk can ever be accomplished for such a large number of compounds. Of these chemicals, pesticides occupy a unique place by virtue of its intended application to 'kill'. Pesticides have been defined as "economic poisons employed to regulate the impact of noxious animals and plants upon our life and economy" (Thoman and Nicolson, *1963).*

The study of the effects of pollution on organic assemblages is an important aspect of biological monitoring of pollution. Usually negative consequences of pollution manifest themselves, into detrimental deviations from the normal state of individuals. This will be reflected in populations and ecosystems which will indicate the time course of pollutional damage of an environment. At individual and populations levels, impairment of physiological functions and of body structures are the most important detectable parameters of pollution

effects. Analysis of such impairments based on laboratory investigations is the most important aspect of pollution research. Such studies could be conducted only in laboratories, where facilities are available for maintenance of animals which are sensitive to controlled conditions.

A study of toxicology based on exposure techniques has got various limitations. Factors like time course of acclimation of the concerned animals, dietary requirements, water quality and physiological status of the animal are few of the most important ones, which also reflect in the experimental data derived. A duplication of natural conditions in the laboratory is an impossibility. However, with the available facilities, earnest attempts are being made by researchers all over the world to analyse the harmful effects of the most common pollutants on aquatic organisms.

Modernisation of agricultural operations and the consequent widespread and indiscriminate permeation of the ecosystem with pesticides have resulted in biological stress at all levels of organization, not least with respect to fish populations. Intensive fish cultivation is a striking example of an activity in which stress and stress responses due to pesticide pollution are of immediate economic importance.

The species Etroplus maculatus (Family Cichlidae) is a well established species, distributed over southern peninsular India. It satisfies many of the protocols required by a laboratory test organism. Rechten *(1980)* opined it as a laboratory favourite of ethologists. However, there are difficulties in the use of fishes for pollution assessment impact. Most important of these is our limited understanding of the mechanism of toxicity. The interpretation of the significance or specificity of a measured biological response could therefore become difficult. Notwithstanding these limitations, attempts have been made to analyse the impact of pesticides, added at realistic levels to the experimental media, on the life and activity of Etroplus maculatus.

The above aspects were the most important guidelines when the present investigation was perceived. It is earnestly hoped that the information provided herein will further widen the knowledge on the toxicity of these chemicals to fishes and offers excellent background data to follow up the investigations at the organic, cellular and subcellular levels.

INTRODUCTION

I - INTRODUCTION

The birth of the modern pesticide era in the late *1940*'s was hailed as a major break-through for mankind. The philosophy that these new chemicals would stop the innumerable pests in their track thereby eradicating disease and eliminating crop reduction led to a progressive increase in their use. A belated second thought on the environmental persistance of organochlorines (new era pesticides) led to the invention of less persistent pesticides like organophosphate, carbamate and synthetic pyrethreoids.

The problem of pesticidal impact on the ecosystem has assumed considerable proportions owing to the modernisation of agricultural operations and the consequent widespread and indiscriminate permeation of the ecosystem with these pesticides. The effects of pesticides on aquatic fauna, particularly fishes, may be exhibited in a variety of ways, since the majority of them are non-selective and produce detrimental and sometimes fatal side effects on non-target species. Knowledge on pesticide toxicity levels, either by acute toxicity, residual or physiological studies, is essential to develop effective protective measures for the conservation of our already depleted freshwater fauna.

It is apparent that human chemical additions have introduced or increased environmental stress for aquatic organisms and fishes in particular. Many of the effects of pesticides to fishes are subtle and insidious. Unlike direct eradication of populations (eg fish kills), the more serious long-term decline of stocks of fish are caused by indirect factors such as predation, disease and reproductive failure. Fish which are subjected to unnatural stresses in any part of their life history may be rendered less capable of performing those functions necessary to fulfil their life cycle and if fish'es ability to defeat its natal stream is impaired by the presence of pollutants, then it may go unspawned and leave no natural means of perpetuating the species (Waldichuk, *1974*).

Studies on the sublethal effects of pesticides have gained a great deal of impetus in the last decade, partly because of their practical importance and partly owing to academic interest. Quantitative assessment of the effects of pesticides on fishes has got cardinal importance in fishery management both from the biological and ecological points of view. Moreover, a sublethal effects of pollutants are now being recognized by regulatory agencies in establishing pollution controls. Rather than applying an arbitrary "application factor", as a safety factor, to the LC 50 data obtained in acute toxicity bioassays, pollution control is now being developed by using the sublethal threshold level, derived by chronic toxicity bioassays, as

the limiting concentration. Even in administering the International Convention for the Prevention of Marine Pollution by Dumping of Wastes and Other Matter (U.K. *1972)*, the term "harmlessness" of a particular substance is being defined by application of data from sublethal toxicity studies, among others (Waldichuk, *1979)*.

The investigations of the effects of pesticides, or any other pollutants, on aquatic organisms, especially fishes, aimed at delineating the polution effects, mainly centre around two broad scientific approaches viz. ecological monitoring and laboratory investigations. Ecological monitoring and the efficacy of the approach mainly depend on the in situ effects of pollution, which in turn are controlled by the pattern of pollutant release in space and time. Negative ecological consequences of pollution manifest themselves in detrimental deviations from the normal state of individual populations or ecosystems. The second approach, namely laboratory investigations, mainly take into consideration the detrimental deviation from the normal state of individuals. Such impairments can be quantified. The philosophy of the present study holds true to the second approach.

Coastal zones are more prone to vulnerable to pollution, as this zone receives pollutants both from land and water sources. Further major industrial developments, transport and other activities causing pollution tend to take place in the vicinity of coastal zones.

Besides, coastal areas are densely populated and the coastal ecosystems are fragile by nature due to their high degree of variability in space and time. Conservation of this zone demands paramount importance as these are important areas for fisheries. Coastal area dumping grounds have much higher pollutant concentration not only because the material is being put into these shallow areas much more rapidly than it is being carried away by natural water motions, but also because of the normal structure of the oceans which tend to prevent the mixing of these inputs with the rest of the oceanic volume (Williams, *1979*).

Among the various animal groups, fishes have been identified as being very sensitive to pollutants and have been the most popular test organism because they are presumed to be the best understood organism in the aquatic environment. Fishes are one of the most important members of the aquatic food chain, and through them some toxicants may reach human beings as well. The selection of organisms for toxicity test is mainly based on certain criteria like its ecological status, position within the food chain, suitability for laboratory studies, genetically stable and uniform populations and adequate background data on the organism (Buikema et al., *1982*). The species selected for the present study viz. Etroplus maculatus satisfy most of the above protocols.

It is no longer sufficient to document aquatic pollution in terms of the chemical concentration of the contaminant. The use of bioassays as part of a comprehensive approach to pollution assessment is widely accepted nowadays. Toxicity is a biological response, which when quantified in terms of the concentration of the toxicant can constitute the basis for a bioassay procedure. Toxicity tests are defined here as estimation of the amount of biologically active substances by the level of their effect on test organism (Chapman and Long, *1983)*. The direct determination of the acute toxicity levels has been followed in the present study also in spite of limitations, as it provides the best and most practical methods of evaluatin the danger levels of pesticide contaminants commonly found in the aquatic environment and consequent risks to fish populations (Alabaster, *1969)*. Such an investigation is particularly essential with the fish E. maculatus, as no such study has hitherto been undertaken.

In general, sublethal effects cover the effect of all those concentrations which are not lethal for individuals even after prolonged exposures, but increases the population mortality, decreases its size, or changes in composition. Thus, a group of effects that affect the growth, rate, metabolism, reproductive potential behaviour or which impair the defence mechanism of an organism are referred to as

sublethal effects. In the present study sublethal effects of pesticides on a selected fish were looked into detail. Physiological responses like activation or inhibition of some selected enzymes, disturbances in haematology and histological changes are the parameters chosen for the assessment of the sublethal effects.

The present study involved investigation of the lethal and sublethal effects of three pesticides individually. The pesticides selected are the commercial formulations of DDT (organochlorine), Dimecron (organophosphate) and Gramoxone (paraquat dichloride). Synthetic pesticides, especially organochlorines and organophosphates have become increasingly important additions to chemical wastes polluting natural aquatic communities and many of these are considered hazardous because of their ability to kill or immobilize organisms even at very low concentrations. Generally the commercial formulations of pesticides are found to be more toxic to fishes than the respective active ingredient which seldom encounter with the aquatic communities.

Most toxic substances exert their effects on a basic level in the organism by reacting with enzymes or by affecting membranes and other functional components of the cells. Biochemical and physiological techniques are commonly used in laboratories to measure such effects and together with histological, histochemical and haematological studies can contribute most fruitfully to reveal the toxic

mechanism of a single or a group of substances (Bengtsson, *1979)*.

The impact of pollutants on an organism is initiated as disturbances at the subcellular and cellular levels. Since lysosomes are the subcellular units involved in the concentration, disintegration and elimination of toxicants, a knowledge on the concentration of important lysosomal marker enzymes is inevitable in monitoring the extent of pollution caused by biotic and abiotic factors. Cell membrane and the confluent endoplasmic reticulum are the first to confront pollutants. They are susceptible to the effect of pollutants as they bind to the lipoprotein layer of the membrane and induce variation in the permeability which upset the whole cellular systems. So a study of the activity of membrane bound enzymes becomes a useful index of the extent of pollution imposed (Annie, *1988)*. Investigations on the impact of pesticides on the activity of two phosphomonoesterases; Acid phosphatase, a lysosomal marker enzyme (Kendall and Hawkins, *1975)* and Alkaline phosphatase, an enzyme bound to the cell membrane and endoplasmic reticulum (Ciro et al., *1975)* is thought to be meaningful.

As stated by Meister (1955) "Transamination is a chemical reaction in which an amino group is transferred from one molecule to another without the intermediate participation of ammonia". Transamination represents one of the principal metabolic pathways for the

synthesis and deamination of amino acids. It allows an interplay between carbohydrate, fat and protein metabolism, an activity which can serve the changing demands of the organism (Cohen and Sallach, *1961)*. Transaminases are a group of enzymes that catalyze, the process of biological transamination. Of the many transaminases, the most important and widely investigated are Glutamate oxaloacetate transaminase (GOT) and Glutamate pyruvate transaminase (GPT) which play an important role in the detoxification of ammonia. Their stability and relative easiness made them subject of analysis in a variety of animals. In fishes, investigation on these has gained only limited popularity, though tissue enzyme analysis is gaining increasing importance in the field of environmental toxicology. It is felt that the study of the activity response of these two enzymes, used by fishery biologists to diagnose sublethal insult of pollutants to animal as a whole or organ-wise, to pesticide exposure will further enlighten the knowledge of stress physiology.

In physiological studies of fish, haematology is often used as an index of the effect of xenobiotic compounds to these animals. The measurement of specific physiological and biochemical changes in the blood of fish exposed to sublethal concentrations of pollutants may provide a sensitive index in predicting the effects of chronic exposure on survival of the animal. Such analysis has considerable clinical importance in mammals. But in fishes such applications are

only limited. A knowledge on the pathological effects of pesticides on the circulating blood elements and blood pigment can provide a frame work for simpler routine analysis of blood in fish toxicology. Pesticide-induced haematological changes may be of some value in assessing the impact of exposure to these chemicals and may serve as tools for biological monitoring (Murthy, *1986)*.

Several environmental contaminants have been found to induce histological changes in fish. Pesticides are no exception to this. However, histological effects of pesticides remain largely undefined and majority of the documented work are directed to acute toxicity. Most lesions have been extremely nons-specific and merely indicative of toxic insult. Since these subtle changes, that occur over long periods of exposure, are not grossly apparent, histopathological studies are necessary for the description and evaluation of potential lesions in aquatic animals exposed to various toxicants (Meyers and Hendricks, *1988)*.

The results are presented under different sections to make the presentation meaningful, and every effort was made to minimize the short comings in the design of experiments. This sort of investigation will eventually open up a very interesting aspect of toxicology, the understanding of which would help in delineating the impact of contamination by pesticides to fishes.

REVIEW OF LITERATURE

II - REVIEW OF LITERATURE

"The chemical warfare waged against pests during the last fourty years, under the euphéria generated following discovery of the highly toxic action of synthetic organic chemicals - in the false hope of eradicating all pests - has damaged the environment much more than it has eased the pest menace", wrote Murthy *(1986)* calling the attention of pollution biologists and ecologists to one of the great threats to life on earth : pesticide pollution of the biosphere. Among the pollutants pesticides rank a very important position, since pesticides and technical organic chemicals comprise the most dangerous group of pollutants. It is realized that these substances are totally alien to aquatic organisms - and obviously because of greater intimacy of aquatic organisms with their external environment, the environmental damage caused by the pesticide is perhaps most felt in aquatic ecosystem. This intimacy has made aquatic organisms more vulnerable to even minor changes in their surrounding milieu.

Aquatic ecosystem in general has no or only limited capabilities for metabolising and degrading pesticides and their derivatives and tend to accumulate and cause long term effect (Kinne,*1984*). A total of about *60,000* different organic chemicals are at present being used and many more products are being introduced every year. Although,

all of these do not find their entry into the aquatic ecosystem, quite a few reach aquatic environment in appreciably copious quantities. Fish perhaps the most important aquatic resource of man. Massive and recurrent fish kills attributable to contamination of water by pesticides reaching it by leaching from land, rainfall run off from agricultural fields or direct contamination from aerial spraying etc, are reported right from the time of introduction of pesticide application.

The review of literature on the different aspects of aquatic pollution is a hazardous task, has tremendous proliferation of printed matter in this aspect in different parts of the world. The scientific papers available can mainly be categorized under those relating to ecosystem damages, pollution by xenobiotics, radio-active pollution and thermal pollution. Kinne *(1984)* made an exhaustive review of the various categories of literature available in his volume of Ocean Management.

The present investigation has taken into consideration only a small facet of the whole problem. And more over in the present study main thrust given was to the subacute effects of pesticides and lethal toxicity study was carried out to delineate the sublethal concentrations for the acute and subacute studies. Therefore an

attempt was made to review only those scientific paper directly pertaining to the theme of the present work.

Static acute toxicity tests have been primary tool for evaluating short term effects of pesticides and other foreign compounds on aquatic organisms (Nimmo, *1985)*. In general, the LC 50 values from the static tests are greater than those from the flow-through tests. However, a well designed static tests can be useful in determining acute toxicities for comparative studies or as indicators for further acute or chronic tests (Nimmo, *1985)*.

Toxicity test will continue to be a vital part of the evaluation process, as for routine screening, but the checklist of factors (modify the toxicity) necessary for substantiation of the impact continues to grow. In toxicity testing of pesticides there appears to be a trend toward longer-term tests and toward investigation of effects (Nimmo, *1985)*. With the recognition that all pesticides are potentially harmful to fish even at relatively low concentrations, by comparison with those commonly used in spray applications, it is practice to test all new chemicals for their toxicity to fish (Holden, *1973)*. Most investigations into the toxic effects of pesticides on fish have involved the determination of the LC 50. The period of exposure is usually 24, 48 and *96* hours. The route of entry

of the chemical in such conditions is generally agreed to via gills and thus directly into the vascular system. Sprague *(1969)* has reviewed the problems of toxicity tests to fish.

The period for which the LC 50 is determined is usually of considerable importance, values normally being much lower after *96* hrs (Holden, *1973).* Katz *(1961)* determining LC 50 values for periods of 24, 48, 72 and *96* hrs in static tests using four species of fish and thirteen insecticides, in general, found only relatively small increase in susceptibility from 24 hrs to *96* hrs. Also found little difference in toxicity between 24 hr and *96* hr values for certain herbicides (Pickering, *1962).* Pickering et al.*(1962)* found that there was only a small decrease in the LC 50 value from 24 to *96* hrs while testing a number of organophosphate pesticide formulations. The occurrence of organic chemicals in the aquatic ecosystem requires evaluation on the effects that they may have directly on a species or indirectly on the ecosystem. In aquatic toxicology, the acute toxicity tests for fish enables estimation of the exposure concentration resulting in 50% mortality of test animals within 48 or *96* hrs (expressed as LC 50 value*).* The numerical value of LC 50 has assumed special importance as an index of toxicity, but with the incorporation of highly persistent substances with high concentration potentials and low water solubilities it can provide only marginal information (Ernst, *1980).*

The acute toxicity data have been used in conjunction with so-called safety factors of 0.1 to 0.01 to estimate safe concentration of chemicals for the protection of aquatic life during chronic exposure. However, these factors do not adequately consider the specific action of the individual substance (Ernst, *1980*). The concept of specific application factor define the relationship between the acute and chronic toxicity of a chemical, the accurate estimate of the specific application factor for a chemical can be derived from maximum acceptable toxicant concentrations (MATC). Numerically the application factor (AF) is the quotient of the MATC and the *96* hr LC 50. Application factors for some pesticides show that the highest concentration without any toxic effect may be more than two orders of magnitude lower than the *96* hr LC 50 (Hansen and Parrish, *1977*; Nimmo et al., *1977*). The method for determination of medium tolerance limit (TLM) was evolved long before (Hart et al., *1945*; Doudoroff et al., *1957*). Attempts were made to apply suitable factors to the TLM data for predicting long term safe concentrations (Burdick, *1957*; Henderson and Tarzwell, *1957*). Tarzwell *(1959, 1971)*, Kimura and Matsuhima *(1969)*, Masida et al., *(1970)* and Muirhead-Thomson *(1971)* suggested that the factor to determine the long term safe concentration should be derived from simple short term bioassays since static bioassay simulate most closely single or multiple applications of a pesticide to a lake or pond (Burdick, *1967*).

Johnson *(1968, 1973)*, Alabaster *(1969)* and Pimental *(1971)* gave an excellent review on the acute toxicity of pesticides to fish. No review is completed unless the reference should be made to the compilation of data on all toxicity tests conducted at the Columbia National Fisheries Research Laboratory, Missouri. Johnson and Finley *(1980)*, in an excellent compilation summed up the result of *1587* tests conducted over a period of 14 years with 271 chemicals and 28 species of fish and 30 species of invertebrates. This compilation is the single largest source of dependable information on the acute toxicity of many pesticides to fish and invertebrates, the tests having been conducted under nearly uniform conditions.

Frequently, compounds of highest purity like analytical grade or technical grade compounds are employed to test the toxicity of pesticides to the aquatic organisms but the pure compounds are seldom used in nature. The pesticides are formulated to help in dispersing the compounds better on the target sites. Various types of pesticide formulations have been extensively reviewed by Melinikov *(1971)*. Commercial formulations should be tested because it is impossible to determine the LC 50 from the concentration of the active ingredients or technical material (Alabaster, *1969)*.

Emulsifiable concentrates of organophosphates were found to be more toxic to fathead minnows and blue gills than technical grade

materials (Pickering et al., *1962*). The *96* h LC 50 of technical DDT and 25% EC to blue gills was 3.4 ug|1 and *9* ug|1 respectively (Randall et al., *1979*). A similar reduction in the toxicity of EC formulations was reported in the case of Endrin and Chlordane to fathead minnows and blue gills (Henderson et al., *1959*). Studies on the various types of formulations of Endosulfan showed that 35% EC formulation was more toxic than the technical grade material to fish. The *96* h LC 50 of technical grade Endosulfan to Labeo rohita and Channa punctatus were 1.1 and 4.8 ug|1, those of 35% EC were 1.0 and 2.5 ug|1 (Rao et al., *1980*; Devi et al., *1981*).

Decreased toxicity of an EC formulation of Parathion to fathead minnows, blue gills and goldfish in comparison with that of technical Parathion was reported by Henderson and Pickering *(1957)*. General formulations of the phenoxy herbicides 2,4-D and 2,4,5-T showed wide variations in LC 50s for blue gills depending on whether amine or an ester group was attached (Hughes and Davis, *1963*). The formulations rarely have the same toxicity as the pure compounds. When the inner ingredients themselves have no toxicity, the toxicity of a formulation depends on the amount of the active ingredient present.

The impact of pollutants on an organism is realized as perturbations at different levels of functional complexity (Moore,

1985). Although the study of changes in enzyme activity can be an important aspect of mammalian toxicology, little has been done in this respect on fish. A continuing study of metabolic and enzymatic activities of aquatic organisms is essential to provide a rational basis for anticipating and understanding the ecological effects of an accelerated input or additions of new chemicals into the freshwater or marine environment.

Jackim et al. *(1970)* studied the effects of metal poisoning on liver enzymes. Their study, apart from xanthine oxidase, catalase and RNase, included the acid and alkaline phosphatases and reported that toxin-induced changes in enzyme activity could represent the initial disorders ultimately leading to death. The effects of increased concentrations of zinc on LDH, MDH and alkaline phosphatase activities in fathead minnow cells in culture were reported by Adragna and Privitera *(1979)*. They reported that specific activity of alkaline phosphatase decreased in both the short term and long term experimental conditions compared with controls. The effects of various sublethal concentrations of phenol and pentachlorophenol on the activities of the enzymes, acid and alkaline phosphatases and succinic dehydrogenase, in the liver of the fish Notopterus notopterus exposed for 10, 20 and 30 days were reported by Dalele et al. *(1980)*.

Sastry and Sharma *(1980)* studied the effect of the insecticide Diazinon on the activities of certain enzymes of the brain of the freshwater fish Channa punctatus. They reported reduction in brain alkaline phosphatase activity after *96* hrs exposure to Diazinon. Although no alteration in enzyme action was produced following 15 day exposure, 30 day exposure was found to activate the enzyme. The activity of acid phosphatase also showed significant reduction. The impact of various sublethal concentrations of the pesticides like Thiotox, Dichlorvos and Carbofuran on the activities of acid and alkaline phosphatases and glucose-*6*-phosphatase in liver, kidney and gills of the fresh water fish Mystatus vittatus were reported by Verma et al. *(1981)*. The activity of both acid and alkaline phosphatases decreased significantly in the liver and kidneys of the freshwater fish C. punctatus following exposure to a sublethal concentration (25%) of vegetable oil-factory effluent (Saxena et al., *1982)*. They reported that the decrease in activity was to some extent greater with increase in duration of exposure time. Verma et al. *(1984)* recorded the effects of various sublethal concentrations of Thiotox, Dichlorvos and Carbofuran and their combinations on the activities of serum acid and alkaline phosphatases and glucose-*6*-phosphatase of the fish M. vittatus and reported that serum alkaline phosphatase was more sensitive than the acid phosphatase and glucose-*6*-phosphatase to stress conditions. The effect of the organophosphate pesticide Monocrotophos

on the activities of brain phosphatases of the fish Tilapia mossambica was reported by Joshi and Desai *(1983)*. Result of their study showed increase in acid phosphatase activity in the brain of T. mossambica during 2nd and 5th day of Monocrotophos treatment and observed that the increase in the activity of this enzyme could be due to increase lysosomal labilization and biochemical alteration as a result of AChE inhibition.

Lysosomal hydrolases are thought to contribute to the degradation of damaged cells and hence facilitate their replacement by normal tissue. Reddy et al. *(1984)* studied the changes in the activity of acid phosphatase in the hepatopancreas of the ricefield crab Oziotelphusa senex after exposure of the pesticide Sumithion. The increase in the acid phosphatase activity after Sumithion exposure could be attributed to lysosomal activity. Xenobiotic-induced subcellular pathology reflects perturbations of function and structure at the molecular level. In many cases, the earliest detectable changes of 'primary events' are associated with a particular type of subcellular organelles such as lysosomes, endoplasmic reticulum and mitochondria (Moore, *1985)*. Reddy et al. *(1986)* reported an increase in the activity of acid phosphatase in the hepatopancreas of the crab O. senex after Methyl parathion exposure and attributed the stimulation of acid phosphatase activity to (1) alteration in osteoblasts

resulting in more production and liberation of the enzyme (2) proliferation of smooth endoplasmic reticulum in the parenchymatous cells, that leads to more production and release of microsomal enzymes resulting in an increased activity of the enzyme (3) peroxidation of lysosomal membrane leading to membrane breakdown or increase in permeability of lysosomal membrane or both resulted in liberation of acid phosphatase thereby causing increased level and (4) degeneration and necrosis induced in tissues resulting in the high acid phosphatase activity.

Ravichandran and Ananthraj *(1984)* reported the general reduction of the activity of acid and alkaline phosphatases and Na^+K^+--dependent ATPase in liver, brain and muscle tissues of the fish Sarotherodon mossambicus exposed to phenol in different salinities. They observed the maximum reduction in acid phosphatase (about 87%) at *60%* salinity in muscle tissue while minimum reduction in alkaline phosphatase activity occured in brain at *60%* salinity. Rice and Mills *(1987)* investigated the possible hepatotoxic effects of Kepone, an organochlorine insecticide in the minnow Fundulus heteroclitus, utilizing the activities of the enzymes like acid and alkaline phosphatases and amino transferase such as aspartate amino transferase (GOT) as index. They reported that elevation of these enzymes was indicative of hepatotoxicity following Kepone exposure.

It is realized that, like 'critical species' and 'indicator organisms', 'physiological indices of stress' are specific with respect to the stressor, the organism and the physiological process. The importance of enzymes and their role in metabolism is paramount (Mahler and Cardes, *1988)*. The ability to accurately characterize enzymes with respect to their distribution and kinetics makes them attractive indices of stress (Dillon and Lynch, *1981)*. The advantage of utilizing physiological response as an index of stress lies in the fact that early detection of potential biological harm in an impacted area may be possible. Gaudet et al. *(1975)* determined the normal plasma levels of eight enzymes known to be significant in animal pathology, in trout Salmo gairdneri. Racicot et al. *(1975)* determined levels of several enzymes in blood and liver of the rainbow trout Salmo gairdneri for 10 days after CCl_4 administration and emphasised their diagnostic use. In the liver of Cunners, Tautogolabrus adspersus, exposed for 30 days to cadmium, the activity of the enzyme aspartate amino transferase was found to be significantly lower (MacInnes et al., *1976)*.

Koundinya and Ramamurthi *(1978)* reported the effect on some selected enzyme system in the fish Tilapia mossambica, exposed to the pesticide Sumithion. The simultaneous effects of salinity, temperature and food on the activities of both amino transferases in the liver and white muscle of rainbow trout Salmo gairdneri were discussed

in detail by Jurss *(1979)* and highlighted the relation between plasma amino acids and the total liver amino transferase activity. Chetty et al. *(1980)* examined the activity levels of some key enzymes (eg: LDH, SDH, GOT and GPT) of carbohydrate and nitrogen metabolism in the liver, kidney, brain and gill tissues of the fish T. mossambica exposed to sublethal concentration of ammoniacal water and reported that the GOT activity decreased by 30% in the liver and kidneys and in the brain the decrease was more pronounced. Contrarily the activity of GPT had increased in the tissues. The activity of GOT and GPT found to be stimulated in brain, liver, kidneys, heart and gill tissues of the freshwater fish Notopterus notopterus, when chronically exposed to sublethal concentrations of phenol (Gupta and Dalela, *1985*).

The aspartate (AAT or GOT) and alanine (ALAT or GPT) amino transferases are known to play strategic role in metabolising L-amino acids for gluconeogenesis and also function as links between carbohydrate and protein metabolism under altered physiological, pathological and induced environmental stress conditions (Nichol and Rosen, *1963*; Knox and Greengard, *1965*; Harper et al., *1979*). AAT and ALAT enzymes increased in the muscle, liver, gills and brain tissues of the fish Oreochromis mossambicus at different levels in sublethal concentrations of the pesticide Lindane indicating that the fish was under toxic stress and energy crisis caused by Lindane thus promoting the

utilization of amino acids for energy synthesis (Murthy et al., *1985*). Das et al. *(1986)* reported kinetic assays of catalytic activities of the two amino transferases (GOT and GPT) in different tissues of the snake head murrels Channa punctatus and revealed that the lateral red muscle of channa, in its ALAT (GPT) and AsAT (GOT) activities was more akin to the hepatic tissue than the while muscle of the fish.

The changes in the activities of amino transferases, whether induced by endogenous or exogenous factors, are often associated with changes in many other metabolic functions and may thus represent widespread alterations in the organism's physiological state. Environmental pollution appears to be one of the factors that affects amino transferase activities in aminal tissue. Dange *(1986)* investigated the effects of short term or long term exposure of the freshwater cichlid fish tilapia, Oreochromis mossambicus to naphthalene, toluene and phenol on the activities of both the amino transferases in liver and muscle tissues, along with soluble protein and free amino acid levels. Tiedge et al. *(1986)* investigated the effect of substituted phenols on the transaminase activity in the fish, Leuciscus idus melanotus and reported the increase of the activity of serum transaminases.

The measurement of specific physiological and biochemical changes in the blood of fish exposed for short periods to sublethal

environmental stressors may provide a sensitive method for predicting the effects of chronic exposure on survival, reproduction and growth. This would allow a relatively rapid evaluation of the chronic toxicity of a compound (Mckim et al., *1970*). Clinical chemical analyses used in mammalian studies are highly developed and reliable, but only limited application of the principles and methods have been extended to the realm of aquatic organisms. However, there is accumulating evidence that valid and useful analytical relations can be drawn between biochemical and physiological factors and pathology of an aquatic species. Physiological changes in the blood and tissues of fish exposed to varying degrees and types of environmental stressors have been measured by several investigators.

Mckim et al. *(1970)* studied the changes in the blood of brook trout, Salvelinus fontinalis after short term and long term exposure to copper. The parameters investigated included total erythrocyte count, haemoglobin concentration, haematocrit and serum GOT activity and opined that chemical homologous might elicit a characteristic blood and tissue response. Cameron *(1970)* investigated in detail the influence of environmental variables on the haematology of pinfish, Lagodon rhomboides and striped mullet Mugil cephalus. The earlier investigations on the physiological changes in the blood and tissues of fish exposed to varying degrees and types of environmental

stressors were made by many authors (Hall et al., *1926*; Dawson, *1935*; Abegg, *1950*; Higginbotham and Meyer, *1950*; Schiffman and Fromm, *1959*; Fujiya, *1964*; Lloyd and White, *1967*; Bouck and Ball, *1966*, *1968*; Jakim et al., *1970*).

Detailed comparative studies on the blood of man and domestic animals have given grounds for assuming that blood is a unique 'mirror' in which all vital processes taking place in the organism are reflected. This has led to the belief that haematological parameters are closely related with the activity of the animals in their environment, in general, the structure and function of their circulatory and respiratory systems in particular (Srivastava, *1968*). The blood morphology of three cyprinodontiform fishes, Fundulus heteroclitus, F. majalis and Cyprinodon variegatus were reported by Gardner and Yevich *(1969)*. Srivastava *(1968)* made a comprehensive investigation relevant to the morphological and physiological consideration of the blood of certain Indian freshwater teleosts viz. Heteropneustus fossilis, Clarius batrachus, Ophiocephalus punctatus and Amphipnous cuchia. Pickford et al. *(1971* I and II*)* investigated the stress response in the abundance of circulating leucocytes in the killi fish, Fundulus heteroclitus and the effects of cold shock sequence and hypophysectomy and the role of catecholamines were highlighted.

Many workers have stressed the need for the establishment of normal haematological values in fish with a view to the diagnosis

of diseases with those in connection with pollution and its effects. Blaxhall *(1972)* made a review of selected literature on the haematological assessment of the health of freshwater fishes and reported that such assessment by standard haematological methods might be helpful in the assessment of possible toxic effects of various environments where the toxins are in sublethal concentrations. Tandon and Joshi *(1973)* highlighted the blood glucose and lactic acid levels in the freshwater fish Heteropneustus fossilis as a possible indicator of stress. Studies of acute effects of pollutants are useful in assessing the threshold of response to a particular toxicant. McLeay *(1973)* reported quantitative measurements of specific physiological and biochemical changes in the blood and tissues of fish exposed to sublethal concentration of craft-pulp mill effluent under laboratory conditions and reported a significant decrease in red blood cell count and haematocrit values due to effluent exposure. After *60* day exposures to low levels of inorganic cadmium and mercury, Calabrese et al. *(1975)* determined the physiological and haematological damage caused by the exposure. They reported the decrease of haematocrit, haemoglobin and mean corpuscular haemoglobin (MCH) in mercury exposed winter flounder Pseudopleuronectus americanus while cadmium exposed fish showed no significant change in the gross haematological picture.

In the case of fishes the close association of the circulatory system with the external environment and with every tissue, makes

haematological parameters good indicators of sublethal effects of stress and can provide an insight into the physiological responses fishes makes to a changing external environment. The blood coagulation system has potential as a responsive system capable of serving as an indicator of environmental stress. As a part of understanding the inter-relationships between the blood and the environment, Casillas and Smith *(1977)* investigated the effects of stress on the blood coagulation system of both wild and hatchery strains of the rainbow trout Salmo gairdneri in relation to other aspects of the haematology. From their studies it was apparent that the haemostatic mechanisms of fish responded to stress and the blood coagulation system became more active in response to stress. Pandey and Pandey *(1976)* cited an increase in certain blood constituents due to asphyxiation. The constituents like serum glucose, lactic acid, cholesterol, urea and inorganic phosphorous showed significant increase during the recovery after 20 minutes of asphyxiation. Hattingh *(1976)* opined that estimations of blood sugar serves as a sensitive indicator of environmental and other stressor in the freshwater fish, Labeo capensis. The increase in blood sugar (Hyperglycemia) according to the author, was due to the stress and was probably one of 'asphyxiation hyperglycemia'. Grizzle *(1977)* reported significant increase in the erythrocyte count and haemoglobin concentrations in fingerling channel catfish exposed to sublethal concentrations of Malachite green for short period and following long term exposure, those changes

were not significant from the controls. The reported haematological changes could be attributed as a result of impairment of gas exchange by gills.

Clark et al. *(1979)*, looking into the blood profile of the largemouth bass, Micropterus salmoides reported the normal values of various haematological parameters like erythrocyte count, haemoglobin, haematocrit and derived values like mean corpuscular haemoglobin concentration, mean corpuscular haemoglobin and mean corpuscular volume and stressed the importance of the evaluation of fish haematological parameters as extremely sensitive indicators of excessive aquatic pollution due to sublethal doses of thermal effluents, toxic metals, pesticides, fertilizers and other introduced materials. Stressors and pollutants generally produce relatively rapid changes in the blood characteristics of fish. Srivastava and Mishra *(1979)* reported blood dyscrasia in a teleost Colisa fasciatus after acute exposure to sublethal concentrations of lead. The author observed significant decrease in the erythrocyte counts, haematocrit values, haemoglobin content and number of erythrocytes|1000 cells in the lead-exposed fishes. Siddiqui and Naseem *(1979)* reported the various haematological parameters of rohu, Labeo rohita. Gill and Pant *(1981)* studied the effects of sublethal concentrations of mercury on biochemical and haematological responses of a teleost Puntius conchonius. They reported hyperglycemia, depletion of liver and brain glycogen, fall in erythrocyte count and haemoglobin during initial period of mercury

exposure and subsequent increase of RBC and haemoglobin.

The changes effected by the subacute treatment of sodium lauryl sulphate for 30 and 60 days on certain haematological parameters of the fish Saccobranchus fossilis were investigated by Dalela et al. (1981). They reported that exposure to sodium lauryl sulphate caused alterations in the various haematological parameters like RBC and WBC count, haemoglobin, haematocrit, MCH, MCHC and MCV. The haematological effects of prolonged sublethal hypoxia on channel catfish, Ictalurus punctatus was reported by Scott and Rogers (1981). Their observation showed that haematocrit was not a sensitive indicator of hypoxia as no significant change from the control was noted. But the haemoglobin values, mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were found significantly elevated while RBC and WBC count showed no significant alteration. Verma et al. (1982) evaluated the effects of three pesticides Thiotox, Dichlorvos and Carbofuran and their three combinations on selected haematological parameters of the freshwater fish, Mystus vittatus. They reported that 30 day exposure to the above pesticides and their combinations produced a decrease in the prothrombin time, WBC count, haematocrit and mean corpuscular volume while clotting time, haemoglobin, RBC count, erythrocyte sedimentation rate (ESR) mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration decreased.

Limsuvan et al. *(1983)* investigated into the stress response and blood characteristics of channel catfish, Ictalurus punctatus after anaesthesia with Etomidate. Haemoconcentration, indicated by increased total erythrocyte count, haematocrit and haemoglobin resulted from anaesthesia with 1-4 ppm Etomidate for 30-180 minutes. The continuous anaesthesia of the fish with *0.6* ppm Etomidate for *96* hours caused a small but statistically significant decrease in the plasma protein concentration. Asphyxia, caused by keeping the hill-stream fish, Noemachelius rupicola, out of the water for 10 minutes and the post asphyxiation haematological changes at an intervals of *2*, *6*, *8*, *12*, *24* and *48* hours resulted in an increased total erythrocyte count (TEC) haemoglobin concentration and leucocyte count (TLC) while mean corpuscular haemoglobin (MCH) showed decline. In the asphyxic fishes, erythropoiesis and leucopoiesis appeared to be excited which subsided in more than 48 hours (Sharma and Joshi, *1985*).

The laboratory acclimatized fishes, Schizothorax plagiostomus, at 20.5°C, when transferred to low (2.4°C) and higher temperatures (30°C) exhibited marked changes in some blood parameters. Bhatt and Singh *(1985)* reported that at 2-4°C water temperature the erythrocyte count (TEC), haemoglobin (Hb) and haematocrit or packed cell volume (PCV) values of S. plagiostomus fell significantly but the total leucocyte count (TLC) as well as MCH and MCV values did not change. Haemolysis and crenulated cells were noted at 2-4°C and 30°C.

Homechaudhuri et al. *(1986)* investigated the effects of mahua oil cake (MOC) on the blood cells and blood values of the air breathing catfish Heteropneustus fossilis and the common carp, Cyprinus carpio. The authors opined that the effect of MOC was fatally critical, with gradual and complete shrinkage of erythrocytes, followed by ultima haemolysis of cells and significant reduction of the values of haematocrit, haemoglobin concentration and red cell count. The haematology of a cyprinid fish, Labeo umbratus was investigated by Van Vuren *(1986)* after exposure to four toxicants, viz. a detergent, ammonium sulphate, Metasystox (pesticide) and a fertilizer. He reported statistically significant haematological changes between experimental and control fishes and the effects of toxicants on the haematology of L. umbratus were related to the chemical nature of the specific compound.

A variety of stressors like pollutants may result in moderate to severe leucopenia. Jinde and Nimmi *(1986)* reported leucopenia accompanied by lymphopenia and granulocytosis in rainbow trout, Salmo gairdneri after acute cadmium exposure. The other change included reduction in the erythrocyte count. Bhatt and Singh *(1986)* determined the effect of acute toxicity of lead nitrate on the total erythrocyte count (TEC), total leucocyte count (TLC), haemoglobin concentration (Hb), mean corpuscular haemoglobin concentration (MCH) and differential leucocyte counts of the fish Noemacheilus montanus and following

lead exposure parameters like TEC, TLC and Hb decreased significantly while the MCH showed an increase. Lal et al. *(1986)* investigated the haematological and biochemical responses pertaining to the bioenergetics of the freshwater catfish, Heteropneustus fossilis after exposure to a sublethal concentration of 8 ppm of the pesticide Malathion and found that RBC and Hb increased after 4 days treatment but their levels returned to normal after 8 and *16* days exposure which could be attributed to the fact that certain degrees of tolerance might have been developed during Malathion exposure.

The haemodilution has been interpreted as a mechanism which reduces the concentration of an irritating factor in the circulatory system. Torres et al. *(1986)* suggested haemodilution, as evidenced by the lower values of haematocrit, haemoglobin, red cell count and leucocrit count, in the dogfish Scyliorhinus canicula following confinement stress and additional zinc treatment. Misra et al. *(1986)* studied the efficiency of mahua oil cake (MOC) and tamarind seed husk (TSH) as fish toxicant. They caused intra-vascular haemolysis and the mortality rate was correlated with the haematological values and physico chemical parameters of water. A number of haematological and biochemical variables, like erythrocyte numbers, haemoglobin, haematocrit, mean corpuscular volume, blood glucose, glycogen in liver, skeletal muscles and myocardium and cholesterol in blood, liver, ovary and testes of the freshwater fish Barbus conchonius subjected to

chronic lead poisoning were evaluated by Tewari et al. *(1987)*. The lead poisoning resulted in microcytic anaemia characterised by the reduction of erythrocyte count, haemoglobin, haematocrit and mean corpuscular volume.

Sublethal levels of a pollutant are considered to be safe as they do not produce any visible lethal effect on the organism in a given time. The evaluation of certain haematological parameters of Sarotherodon mossambicus exposed to sublethal levels of mercury, brought about physiological changes such as reduction of the number and size of RBC, haemoglobin and mean cell haemoglobin (MCH) (Aruna and Gopal, *1987*). The effect of BHC, an organochlorine pesticide on the blood serum protein profile and certain other haematological parameters like RBC count, WBC count and Hb content in an air breathing fish, Channa punctatus were investigated by Thakhur and Sahi *(1987)*. They suggested that biochemical studies like electrophoretic estimations of serum constituents like protein were very important in assessing the pesticide induced stress on the fish blood. The marked reduction in the number of blood serum protein fractions, RBC, WBC and Hb concentration is a good indicator which might help in the early detection of water pollution by pesticides. Razia Beevi and Radhakrishnan *(1987)* reported macrocytosis and hyperchromia as a result of formalin intoxication in Sarotherodon mossambicus.

The monitoring of blood parameters, both cellular and non-cellular, may have considerable diagnostic value in assessing early warning signs of pesticide poisoning in fishes. Pant et al. *(1987)* reported moderate polycythemia together with a rise in haemoglobin content and haematocrit in the freshwater fish, Barbus conchonius after chronic exposure to the commercial formulation of the pesticide Aldicarb. The polycythemia might be due to enhanced erythropoiesis.

Although major advances have been made in recent years, histology and histopathology of fish and aquatic invertebrates are still infant sciences compared with their counterparts in mammals. Regardless of the cause, there are certain fundamental changes that can occur in tissues and these changes form the basis of descriptive pathology. Many of these changes are part of or result from the inflammatory response in the living animal.

The aquatic medium is a very efficient solvent for many chemical compounds or components there of. Consequently the aquatic organisms are extremely vulnerable to toxic effects resulting from absorption or oral intake of these contaminants from the immediate environment (Meyeres and Hendricks, *1985*). Various chemical compounds have been tested to determine their potential toxicity to certain aquatic organisms, especially fish. However, many of these studies have been concerned with measuring lethality rather than the

pathological effects of contaminants on the tissue. Toxicological studies of aquatic organisms have not revealed many tissue pathologies useful in diagnosing exposure to specific compounds. Most lesions have been extremely non-specific and merely indicative of toxic insult.

Epithelial hyperplasia with lamellar fusion, epithelial hypertrophy, telangiectasia, oedema with epithelial separation from basement membranes, general necrosis and|or epithelial desquamation have occured following exposures to DDT and Malathion (Walsh and Ribelin, *1975*) and Paraquat dichloride (Hendricks, *1979*).

Hepatotoxic lesions of fatty infiltration, nuclear or general hypertrophy of hepatocytes, other degenerative changes in parenchyma (like cytoplasmic vacuolation, cellular pleomorphism, deposition of bile or ceroid pigments, hydrotic degeneration), loss of hepatic glycogen, coagulative hepatocyte necrosis, sinusoidal or vascular congestion, loss of normal muralial architecture, degeneration or necrosis of biliary epithelium and perivascular or periportal fibrosis were some of the histopathologic changes of the liver reported following exposures to DDT (King, *1962*; Mathur, *1962*; Walsh and Ribelin, *1975*), Malathion and Methyl parathion (Walsh and Ribelin, *1975*; Annes, *1976*) and Paraquat dichloride (Hendricks, *1979*). Hepatoma in DDT-fed rainbow trout was reported by Havler et al. *(1962)*, while adenomatous

changes were reported by Cope et al. *(1969)*, in livers of centrarchid fish exposed to the herbicide Dichlobenil and in cutthroat trout exposed to Endrin (Eller, *1971*). Certain industrial chemicals, such as PCBs, have been implicated as possible causes of liver neoplasia in populations of Atlantic hagfish (Falkmer et al., *1977*).

Hyperaemia hemorrhage, vascular congestion and dilation, infarction cerebral oedema, nuclear pyknosis, rupture and hemorrhage of mininx primitina and swelling of mylein sheaths around nerve fibres have been reported in fish brain following exposure to DDT and Malathion (Walsh and Ribelin, *1975*). According to Cope et al. *(1970)* marked vascular congestion of the brain appears to be the only change which can be considered to have diagnostic value in determining 2,4-D toxicity in blue gill, Lepomis macrochirus.

It is clear from the foregoing account that pesticide toxicity to fishes is a field of hectic ongoing research. As has already been mentioned, both the variety of pesticide formulation and the species of non-target organisms, especially fishes, are innumerable. A careful perusal of available relevant literature would reveal that in most, if not all, combinations of pesticide and fish tested for toxic effects on enzyme activities and haematology, the results seldom conform to a general pattern and every time a new reaction, a novel

manifestation or a peculiar alteration totally unrelated to the previous recorded ones, is observed and reported, making meaningful generalisations virtually impossible. The pesticide formulation, the experimental animal or the experimental condition individually or in combination, may be held responsible for this lack of consistency in experimental results. In these circumstances, any relevant information from any test combination would only be welcomed and any effort at collecting relevant information would not be superfluous.

MATERIAL AND METHODS

III - MATERIAL AND METHODS

3.1 INTRODUCTION

The effects of pesticides on aquatic fauna, particularly fishes, may be exhibited in a variety of ways, since the majority are non-selective and produce detrimental and sometimes fatal side effects on non-target species. Studies on the sublethal effects of pesticides have gained a great deal of impetus in the last decade, partly because of their practical importance and partly due to academic interest. Quantitative assessment of the effects of pollutants has got cardinal importance in any pollution research, both from the biological and ecological points of view.

There are various ways of investigating sublethal effects, and each technique provides an insight into the physiology or behaviour of the organism in question (Waldichuk, *1979*). Efforts were made to evaluate the lethal and sublethal effects of commercial grade pesticides individually on a selected non-target vertebrate. The animal used in the study was the Asian cichlid fish Etroplus maculatus commonly called the orange chromide and has both freshwater and estuarine distribution.

3.2 TEST ANIMAL

Etroplus maculatus (Bloch)

Etroplus maculatus is the smaller of the two species representing the cichlidae family in India and is indigenous to South India and Sri Lanka (Munro, *1955*). E. maculatus is a euryhaline fish sexually monomorphic, having an yellow ground colour with black markings and very common in the rivers, ponds, paddy fields, canals, lakes and estuaries of Kerala. This fish attains a maximum length of *6-8* cms and its small size considerably limits its utility as a food fish, however, it yields a minor fishery of economic value in certain parts of South India, particularly in the coastal areas of Kerala (Alikunhi, *1947*).

E. maculatus is a laboratory favourite of ethologists. There are studies on its behavioural ontogeny (Wyman and Ward, *1973*), courtship behaviour (Barlow, *1970*), parent-offspring communication (Cole and Ward, *1970*), aggressive behaviour (Reyer, *1975*) and reproductive colouration (Rechten, *1980*). The ecological importance of E. maculatus was highlighted by Wyman and Ward *(1972)* as a cleaning symbiosis exists between E. maculatus as cleaner and E. suratensis as the host. The young of E. maculatus actively cleans all age groups of E. suratensis which is of high economic importance and the cleaning

activity shows a daily circadian rhythm. The removal of fungus from the fins and tail appears to be an important adaptive function of this symbiosis.

Live specimens of Etroplus maculatus for the study were collected from the shallow inland water areas confluent with Cochin backwaters. The animals were collected using castnets causing minimum stress, and then transported to the laboratory in oxygen packs.

3.3 LABORATORY PROCEDURES

3.3.1 Laboratory conditioning of test animal.

The animals transported to the laboratory were maintained in large fibre glass tanks of 150 litre capacity containing well aerated water of corresponding salinity of collection areas ($7.5 \pm 7.5‰$). They were acclimated for one week and observations were made on mortality, disease symptoms or abnormal behaviour of fishes, if any. The lots showing more than 5% mortality were discarded. During the acclimation period, salinity was gradually reduced to zero and the animals were maintained at 10‰ salinity for further 48 hours before the commencement of the experiments and were fed with minced clam meat and earthworm pieces. All organisms used for any one set of experiment belonged to the same population. Only healthy and adult animals of

the same size (*6 ± 1* cms. in length group) were used for experiments, irrespective of sex.

The test medium used for the study was collected from Cochin backwaters, kept for aging in dark, filtered, diluted to zero salinity with dechlorinated tap water and aerated to full saturation before use. The pH of the experimental water was 7.5 ± 0.5. The addition of toxicants did not bring about any appreciable variation in pH. All the experiments were carried out at laboratory temperature (30 ± 1.5°C).

3.3.2 Toxicants.

The toxicants selected were the commercial formulations of three widely used pesticides, namely DDT, Dimecron and Gramoxone belonging to organochlorine, organophosphate and bipyridylium compounds, respectively. These three groups of compounds are being used extensively in agricultural and horticultural practices.

The pesticide solutions were prepared separately and added to the test media to get the desired concentrations. The DDT concentrations were prepared by mixing commercial formulation with acetone as vehicle solution in 1:1 ratio. Dimecron and Gramoxone are water soluble and the stock solutions were prepared in distilled water.

3.3.2.1 DDT^{R} 25% EC

DDT^{R} 25% EC studied is an emulsion concentrate containing 25% (w|w) DDT technical (1,1,1-Trichloro-2,2-bis-P-chlorophenyl ethane) and marketed by Premier Pesticides (P) Ltd. Though banned in agricultural operations, it is widely used in public health service for the control of mosquitoes.

3.3.2.2 Dimecron^{R}

Dimecron^{R} used for the study is the product of Hindustan Ciba-Geigy Ltd. It is a vinyl phosphate insecticide and the commercial formulation used contain 85% (w|w) of the active ingredient phosphmidon technical (1-chloro-1-N,N-diethyl carbamoyl-1-pzopenyl-2--dimethyl phosphate), and is water soluble.

3.3.2.3 Gramoxone^{R}

Gramoxone^{R} is manufactured by IEL Ltd. and is a trade mark of the Imperial Chemical Industries Ltd. PLC, London. The commercial formulation contains 24% (w|w) of the active ingredient, paraquat dichloride (1,1'-dimethyl-4,4'-bipylidium dichloride), and is water soluble. Gramoxone is mainly used as an agricultural and horticultural pesticide and is a potent inhibitor of photosynthesis in plants.

3.3.2.4 Toxicant concentration

The various concentrations of the toxicants are expressed in ppm in terms of the individual pesticide formulation. Commercial formulations were used and calculated quantity was weighed out to give the desired concentrations in the test medium.

3.3.3 Toxicity studies.

3.3.3.1 Lethal toxicity of individual toxicants

Lethal toxicity studies provide information about the relative lethality of a toxicant. This test is designed to determine the highest concentration of a pollutant that is sufficient to affect some percentage, usually 50% of a limited number of organisms. Though lethality appears to be a crude method of measurement of toxic response, its importance was highlighted by many workers (Duke, *1974*; Buikema Jr. et al.,*1982*).

The static renewal test technique, as described by the American Society for Testing and Materials (*1980*), and the Committee on Methods for Toxicity Tests with Aquatic Organisms (*1975*), was employed for the current study. Exploratory tests were conducted before performing full scale acute toxicity tests.

Experiments were carried out to assess the individual lethal toxic responses to the three pesticides, DDT, Dimecron and Gramoxone by the fish Etroplus maculatus. Laboratory conditioned fishes of uniform size (*6 ± 1* cms in length) were exposed to 50 litres of test solution that contained graded, logarithmic series of concentrations of the toxicants. Fibre glass tanks, inner coated with chemical resistant epoxy resin, were used for the toxicant exposure. Ten animals were used for each test concentration of the toxicant. The experimental tanks were kept covered to minimize external disturbances. The tests were carried out at room temperature ($30^{o}C \pm 1.5^{o}C$) and the animals were not fed during the test period. Appropriate duplicates and controls were invariably maintained for all the experiments. The test media were replenished totally every 24 hour. The animals were inspected at regular intervals, and were considered dead if it did not respond to mechanical stimulation, and the opercular movements ceased. The dead animals were removed and the cumulative percentage mortality at every 12 hour recorded. The LC 50 values and their 95% confidence limits were calculated as mentioned in section 3.4.

3.3.3.2 Short term sublethal toxicity studies

The objective of these toxicity tests was to find out the concentrations of the toxicants capable of inducing abnormal responses as well as the nature of the responses of some selected physiological and haematological indices of the test animals. The details of the selected physiological and haematological parameters are mentioned in sections 3.3.4 and 3.3.5.

The sublethal concentrations of different toxicants employed for the studies were computed in relation to the *96* h LC 50 value of the individual toxicants delineated after static bioassay studies. In the present study 1|2 of the individual *96* h LC 50 values were taken as the highest concentration along with three other concentrations fixed in the descending order. The duration of these experiments extended upto 120 hours, and the assessment of the selected parameters was carried out in animals pre-exposed to different sublethal concentrations for 24, 72 and 120 hours to the pesticides. Animals were not fed during short term studies.

3.3.3.3 Long term sublethal toxicity studies

A study of the toxicity after prolonged exposure of the test organisms to toxicants is a recent development in pollution experimentation. As in short term studies, the concentrations were selected

in relation to *96* h LC 50 value but the concentrations employed were very low. In the present study 1|10th of the individual *96* h LC 50 values were taken as the highest concentration along with three other lower concentrations fixed in the descending order. The test media were changed daily with fresh ones and the animals were fed with clam meat and pieces of earthworm, on alternate days, for 1-2 hours before the replenishment of the test media. The parameters studied were the same as in short term studies, and the evaluations were carried out in pre-exposed animals at 10, 20 and 30 days to the three pesticides.

3.3.4 Estimation of InVivo enzymatic activity

3.3.4.1 Preparation of enzyme extract

The control as well as the experimental fishes were sacrificed at the end of each test period (section 3.3.3.2 and 3.3.3.3) and the brain, gill and liver tissues were removed immediately. After rinsing in chilled glass double distilled water, accurately weighed, pooled tissues were homogenized separately in 0.25 M sucrose solution using a potter-Elvehjem type homogenizer. A 5% wet homogenate of the three tissues were prepared in the present study. The supernatent, obtained after centrifugation at 20,000 rpm at $4^{o}C$ for 15 min, was the

source of the selected enzymes and for protein estimation during the present investigation. The supernatent was kept frozen and analysed for the enzyme activity within 2-3 hours after preparation. Care was taken to maintain the tissue and extract chilled till incubation.

3.3.4.2 Assay of Alkaline phosphatase

EC 3.1.3.1 (orthophosphoric monoester phosphohydrolase, alkaline optimum)

The same procedure as described in section 3.3.4.3 was adopted to estimate the activity of alkaline phosphatase with the following changes. Instead of citrate buffer, 0.27 Glycine-sodium hydroxide buffer of pH 9.2 was used. NaCl and $MgCl_2$ were added to the buffer to get a concentration of 100 mg NaCl and 0.1 mg of $MgCl_2$ per 1 ml of the buffer. The volume of the extract used was 0.1 ml and 2 ml of 0.25 N NaCl was used to stop the reaction. The calculations and the unit of enzyme activity were the same as described in section 3.3.4.3.

3.3.4.3 Assay of Acid phosphatase activity (EC 3.1.3.2)

Acid phosphatase (orthophosphoric monoester phosphohydrolase, acid optimum) activity was determined following the methodology described in Sigma Technical Bulletin No.104 (9-82) with slight modifications. To 2 ml of 0.1 M frozen citrate buffer of pH 5.3 containing

100 mM NaCl, 0.1 ml of the enzyme extract was added. The buffer-enzyme mixture was incubated in a thermo-controlled water bath at 37 ± 0.05°C and to this reaction mixture 0.1 ml of the substrate (2 mgs of P-Nitrophenyl phosphate sodium salt [Merck] in 0.1 ml glass double distilled water) was added to initiate the reaction. After 1 hour incubation at 37°C, 4 ml of 0.25 N NaOH was added to the buffer-enzyme substrate reaction mixture to stop the activity of the enzyme. P-Nitrophenol formed during incubation by the hydrolysis of P-Nitrophenyl phosphate, catalyzed by acid phosphatase, gives an yellow colour in alkaline pH and the colour was read spectrophotometrically at 410 nm. The concentration of P-Nitrophenol formed was calculated from the standard graph. Simultaneously, the protein content of the extract was estimated by Lowry's method (1951). From this μ mol of P-Nitrophenol liberated per milligram protein per hour was calculated and the enzyme specific activity is expressed as μ mol of P-Nitrophenol liberated|mg protein|hour.

3.3.4.4 Assay of Glutamate Oxaloacetic Transaminase (GOT) or Aspartate Amino Transferase AsAT (EC 2.6.1.1)

The estimation of GOT activity was carried out by the calorimetric method of Reitman and Frankel (*1957*) as described in Methods of Enzymatic Analysis (*1974*). For estimating GOT, phosphate buffer|

substrate of pH 7.4 containing 0.1 M phosphate buffer, 0.1 M aspartic acid sodium salt, and 2 mM 2-oxoglutarate was used. The buffer|substrate mixture containing 0.1 ml of enzyme extract was incubated at 37°C for one hour. At the end of incubation the enzymatic reaction was stopped by adding 1 ml of 1 mM chromogen in HCl (2,4-dinitrophenyl hydrazine) mixed well and kept for 20 minutes at room temperature. After 20 minutes, the reaction mixture was made alkaline by adding 10 ml of 0.4 N NaOH. The colour developed by 2,4-dinitrophenyl hydrazone of the reaction product, pyruvate was determined spectrophotometrically at 546 nm. Sodium pyruvate was used to prepare the calibration curve. The estimation of protein was done by Lowry's method (*1951*).

3.3.4.5 Assay of Glutamate Pyruvate Transaminase (GPT) or Alanine Amino Transferase (AlAT) (EC 2.6.1.2)

Colorimetric method of Reitman and Frankel (*1957*) was adopted for the estimation of GPT and the procedure was the same as that for GOT with following changes. The buffer|substrate solution contained 0.1 M phosphate buffer of pH 7.4, 0.2 M DL-alanine (instead of aspartate) and 2 mM 2-oxoglutarate. The oxaloacetate formed during the reaction combined with chromogen to form 2,4-dinitrophenyl hydrazone of oxaloacetate which was read spectrophotometrically at 546 nm. Sodium pyruvate was used to prepare the calibration curve. Protein content of the extract was determined by Lowry's method (*1951*).

3.3.5 In vitro enzyme activity studies

For the in vitro enzyme activity studies only the enzyme extract (section 3.3.4.1) of the above said tissues of unexposed fishes were used. The desired concentrations of the individual pesticides were directly added to the buffer|substrate medium prior to enzyme extract addition. The procedure of the estimation of activity of the individual enzyme was the same as described for the InVivo studies (3.3.4.2).

3.3.6 Haematological Analysis

3.3.6.1 Collection of blood samples

Blood samples were collected from the caudal vein in aseptic condition by severing the caudal peduncle. With fish less than six inches in length, severance of caudal peduncle proved most feasible (Hesser, *1960*). The blood samples collected in small glass vials were treated with 3:2 mixture of ammonium oxalate and potassium oxalate at the rate of 0.5-1 mg per ml of blood to prevent coagulation. Aliquotes of pooled blood samples of 5 to 7 fishes was used for different estimations.

The different haematological analyses were carried out employing standard techniques (Hesser, *1960*; Blaxhall and Daisley, *1973*) unless specified.

3.3.6.2 Total Erythrocyte Count (TEC)

The techniques employed for the erythrocyte counts of fish blood were similar in most respects to those used in mammalian counts except a change in RBC diluting fluid. Hendrick's RBC diluting fluid was used during the present study (Hendricks, *1952*). The Hendricks fluid contained 10 gm of sodium sulphate, 2.5 gm of sodium chloride, 1.5 gm of sodium citrate and 50 ml of glacial acetic acid per 500 ml of distilled water. Neubauer type haemocytometer was used for RBC counting. Total erythrocyte count is expressed in millions of RBC per cubic mm of blood.

3.3.6.3 Estimation of Haemoglobin

Cyanomethaemoglobin method described by Ortho Diagnostic Systems (*1986*) was followed for estimating the haemoglobin content. To 0.02 ml of blood, 5 ml of Aculute reagent (modified Drabkin reagent) was added and stirred well. The potassium ferricyanide present in the reagent converts the haemoglobin iron from ferrous to ferric state to

form methaemoglobin and this in turn combines with potassium cyanide of the Aculute reagent to produce a stable pigment or the cyanomethaemoglobin which represents the sum of oxyhaemoglobin, carboxyhaemoglobin and methaemoglobin. The cyanomethaemoglobin formed was measured spectrophotometrically at 540 nm. The calibration curve was prepared by the Human Haemoglobin Standard provided with the Aculute reagent. The haemoglobin content is expressed as g% (or gm|dl).

3.3.6.4 Measurement of Haematocrit values
(or packed cell volume - Ht%)

Haematocrit value was measured by applying the method of McLeay and Gordon (*1977*). Blood was drawn into heparinised microhaematocrit tube (0.55 $\pm$ 0.05 mm diameter). One end of the tube was sealed and centrifuged in microhaematocrit centrifuge at 11500 rpm for 5 minutes. Haematocrit value was measured within 30 minutes of centrifugation and measured the red cell column using haematocrit counter provided along with the microhaematocrit centrifuge, and expressed as the percentage of whole blood.

3.3.6.5 Computation of Erythrocyte constants

From the values of Hb content (Hb%) haematocrit (Ht%) and total erythrocyte count (millions|mm^3) the following erythrocyte

constants were calculated using the respective formula (Lamberg and Rothstein, *1978*).

3.3.6.5.1 Mean Corpuscular Volume (MCV)

MC represents the average volume of individual erythrocytes in cubic microns (μ^3) and computed by the formula:

$$MCV = \frac{Ht\%}{RBC \text{ (in million/mm3)}} \times 10$$

3.3.6.5.2 Mean Corpuscular Haemoglobin (MCH)

MCH represents the average weight of haemoglobin in individual erythrocytes in picograms (pg) and calculated by the formula:

$$MCH = \frac{Hb\%}{RBC \text{ (in million/mm}^3)} \times 10$$

3.3.6.5.3 Mean Corpuscular Haemoglobin Concentration (MCHC)

MCHC is the average haemoglobin concentration per 100 ml of packed erythrocytes in percent and computed by :

$$MCHC = \frac{Hb\%}{Ht\%} \times 100$$

3.3.6.6 Calculation of Erythrocyte indices

From the values of TEC, Hb%, Ht%, MCV, MCH and MCHC the following erythrocyte indices were calculated using the respective formula. The erythrocyte indices of the control fish is taken as one and referred as unity in the text (Lamberg and Rothstein, *1978*).

3.3.6.6.1 Volume index (VI)

Volume index is the ratio of the size of the erythrocyte of the experimental fish to that of the normal or control and computed by

$$VI = \frac{\text{MCV of experimental fish}}{\text{Mean MCV of control fish}}$$

3.3.6.6.2 Colour index (CI)

Colour index represents the amount of Hb in each erythrocyte compared with normal content and calculated by

$$CI = \frac{\text{Mean RBC of control}}{\text{Mean Hb of control}} \times \frac{\text{Hb of experimental fish}}{\text{RBC of experimental fish}}$$

3.3.*6*.*6*.3 Saturation index (SI)

Saturation index is the degree of saturation of erythrocyte with Hb of experimental fish in relation to that of the normal subject and computed by the formula :

$$SI = \frac{\text{MCHC of experimental fish}}{\text{Mean MCHC of control fish}}$$

3.3.7 Histopathology

There are no standardized techniques for examining tissues in aquatic organisms. However, standard medical and veterinary techniques may be modified and used to diagnose tissue changes in fishes. The fishes were exposed to the highest sublethal concentration of individual pesticides for 20-25 days (section 3.3.3.3). The histological techniques and staining procedures to prepare the tissue sections for microscopic examination were mainly adopted from the methods described by Bucke (*1972*) and Bullock (*1978*). At the end of the test period the brain, gill and liver tissues were dissected out and fixed in Bouin's fixative for 24 hours. After fixation the tissues were graded in ascending alcohol series and cleared in methyl-benzoate for 3 to 5 hours. The gill tissue was decalcified in 8% formic acid before alcohol grading. The methyl-benzoate cleared tissues were embedded in paraffin wax after proper paraffin infiltration. The sections were cut at 7 μ thickness using the rotary microtome.

After deparaffinisation in xylene, the sections were hydrated and stained with Ehrlich's hematoxylin for 2 to 5 minutes. Stained sections were then washed in running water for 3 to 5 minutes, dehydrated in graded alcohol, and counterstained with 95% alcoholic Eosin for 2 to 5 minutes. After further dehydration (in absolute alcohol) and clearing (in xylene), the sections were mounted in DPX.

3.4 COMPUTATION AND PRESENTATION OF DATA

The median lethal concentration (LC 50) levels and their 95% confidence limits were computed using the computer software developed by the Institute for Inland Water Management and Waste Water Treatment, Netherlands, based on probit analysis (Finney, *1957*). The lethal toxicity experiments were repeated wherever necessary. The reliability of the LC 50 level was checked by the students 't' test (to see any significant variation at 5% level between the experiments). If highly significant variation was observed, the experiment was repeated again. To report, LC 50 levels with least variance and|or lowest values were selected. The LC 50 levels, ET 50 values and toxicity curves were represented graphically to demonstrate the lethal effects of individual pesticides following approved methods (Sprague, *1973*).

Graphical representation, together with Tables have been used to explain the experimental results on enzyme activity and haematological studies. These data have been subjected to statistical analysis using students 't' test to manifest the variation in comparison with the control. The variations were reported at three significant levels, viz. $p < 0.05$, 0.01 and 0.001.

All the computations involved in the work were carried out by a personnel computer (HCL model -Busybee PC|XT).

3.5 TERMINOLOGY AND ABBREVIATIONS

The terminology, related to lethal toxicity studies, used in the present work are those adopted by Sprague *(1969, 1970, 1971)*. The term median lethal concentration (LC 50) corresponds to LD 50, universally used in toxicology. Instead of the actual concentrations, abbreviations like C_1, C_2, C_3 and C_4 are used in the text to denote the four sublethal concentrations (SLC) of individual pesticides used for convenience of representation and the four SLC used in short term studies are different from that of long term studies (sections 3.3.3.2 and 3.3.3.3). The C_1 corresponds to the lowest exposed concentration and C_4 to the highest while C_2 and C_3 represents the two intermediate concentrations. The four SLC of individual pesticides used in short

term and long term studies corresponding to C_1, C_2, C_3 and C_4 are listed below.

		Short term (ppm)		Long term (ppm)
DDT				
	C_1	*0.00033*		*0.00013*
		(3.3-04)	*(1.3-04)*	*(1.3-04)*
	C_2	*0.00065*		*0.00017*
		(6.5-04)		*(1.7-04)*
	C_3	*0.0013*		*0.00026*
		(1.3-03)		*(2.6-04)*
	C_4	*0.0026*		*0.00052*
		(2.6-03)		*(5.2-04)*
Dimecron				
	C_1	*0.01*		*0.0043*
				(4.3-03)
	C_2	*0.022*		*0.0058*
				(5.8-03)
	C_3	*0.043*		*0.0087*
				(8.7-03)
	C_4	*0.086*		*0.017*

	Short term (ppm)	Long term (ppm)
Gramoxone		
C_1	*0.0034*	*0.0013*
	(3.4-03)	*(1.3-03)*
C_2	*0.0067*	*0.0018*
	(6.7-03)	*(1.8-03)*
C_3	*0.013*	*0.0027*
		(2.7-03)
C_4	*0.028*	*0.0054*
		(5.4-03)

In InVitro enzyme activity studies, uniform concentrations were selected irrespective of the pesticides and of its *96* h LC 50s and abbreviated as C_1, C_2, C_3, C_4 and C_5. The actual concentration and its corresponding abbreviations are listed below.

	Concentration	Abbreviation
	10^{-8} ppm	C_1
	10^{-7} ppm	C_2
	10^{-6} ppm	C_3
	10^{-5} ppm	C_4
and	10^{-4} ppm	C_5

EXPERIMENTAL RESULTS

IV - EXPERIMENTAL RESULTS

In this section, the results of experiments conducted on individual lethal toxicity of commercial formulations of DDT, Dimecron and Gramoxone and sublethal toxicity to different periods of exposure, exemplified by the modulation of enzyme activity of ALP, ACP, GOT and GPT in brain, gill and liver are presented. Further sublethal effects assessed with reference to the perturbations in haematology, and tissue structure of above said organs are documented. For effective representation the results are categorized and documented under different headings.

In the present investigation the experiments were designed in such a way that two sets of sublethal concentrations, both based on *96* h LC 50 of individual pesticides, were selected. In short term exposures the concentrations of each pesticides selected were comparatively higher, the highest concentration being one half of the *96* h LC 50 and three lower concentrations. In long term exposure the highest concentration was 1|10th of *96* h LC 50 and three lower concentrations. This approach, it is felt, would make the comparison of responses of the test organism (1) high dose over a short term period and (2) low dose over a long term period, to these chemically different compounds more meaningful.

4.1 LETHAL TOXICITY

Lethal toxicity of pesticides belonging to organochlorine , organophosphate and bypyridilium compounds were assessed and the results presented under this heading. The above compounds were represented by DDT, Dimecron and Gramoxone respectively and their commercial formulations were used for the present study. The lethal toxicity to individual toxicants only were assessed.

4.1.1 Behavioural responses to pesticide exposure

During the lethal toxicity study behavioural responses of the fish Etroplus maculatus to the three pesticides were more or less similar. When exposed to pesticides, fishes were initially surfaced, followed by vigorous and erratic swimming showing agitation. Quick opercular and fin movements were observed initially and gradually became feeble and often showed gulping of air. Opercular opening became wider and exhibited respiratory distress. As the exposure time passed, fishes were found to settle down to bottom and towards the final phase of exposure fishes showed barrell-rolling indicating loss of equilibrium, swimming with belly upwards and gradually became lethargic. Excess mucous of brownish hue was produced during intoxication. During initial phase of exposure fishes responded vigorously

to mechanical stimulation but later failed to respond. The yellowish body colouration of Etroplus maculatus was gradually turned to slight to moderate dark towards the final phase of exposure. Curved caudal fin was noted in some fishes, especially during DDT poisoning.

4.1.2 Lethal toxicity of DDT

The concentrations tested for the lethal toxicity of DDT ranged from 0.003 ppm to 0.01 ppm and the 96 h LC 50 value worked out was 0.0052 ppm. Mortality was observed in all concentrations (except in 0.003 ppm and ranged from 20 to 100%. Above 0.008 ppm 100% mortality was found within 12 hours of exposure. The LC 50 value showed reduction with exposure duration and between 72 and 96 hours the difference in LC 50 value noted was subtle. The ET 50 recorded were 36.0 h at 0.008 ppm and 45.0 h at 0.006 ppm. There was a sharp increase in toxicity with increase in concentration (Table 1, Fig.1.).

4.1.3 Lethal toxicity of Dimecron

After exploratory tests the concentrations selected ranged from 0.12 ppm to 0.23 ppm. The 96 h LC 50 computed was 0.17 ppm. Mortality was observed in all concentrations and more than 50% mortality was noted in concentrations beyond 0.15 ppm. Hundred percent

Table 1

Etroplus maculatus: LC50 (ppm) when exposed to DDT, Dimecron and Gramoxone over periods upto 96 hrs, alongwith 95% confidence limits*.(CL).

PESTICIDES	24HRS	48HRS	72HRS	96HRS
	(LC50 PPM) (95%CL)	(LC50PPM) (95%CL)	(LC50PPM) (95%CL)	LC50PPM) (95%CL)
DDT	0.009 (0.007-0.011)*	0.008 (0.006-0.01)*	- -	0.005 (0.004-0.006)*
DIMECRON	0.35 (0.24-0.48)*	0.22 0.19-0.23)*	0.18 0.17-0.21)*	0.17 0.16-0.18)*
GRAMOXONE	0.13 (0.1-0.18)*	0.1 (0.08-0.11)*	0.07 (0.06-0.08)*	0.05 (0.04-0.06)*

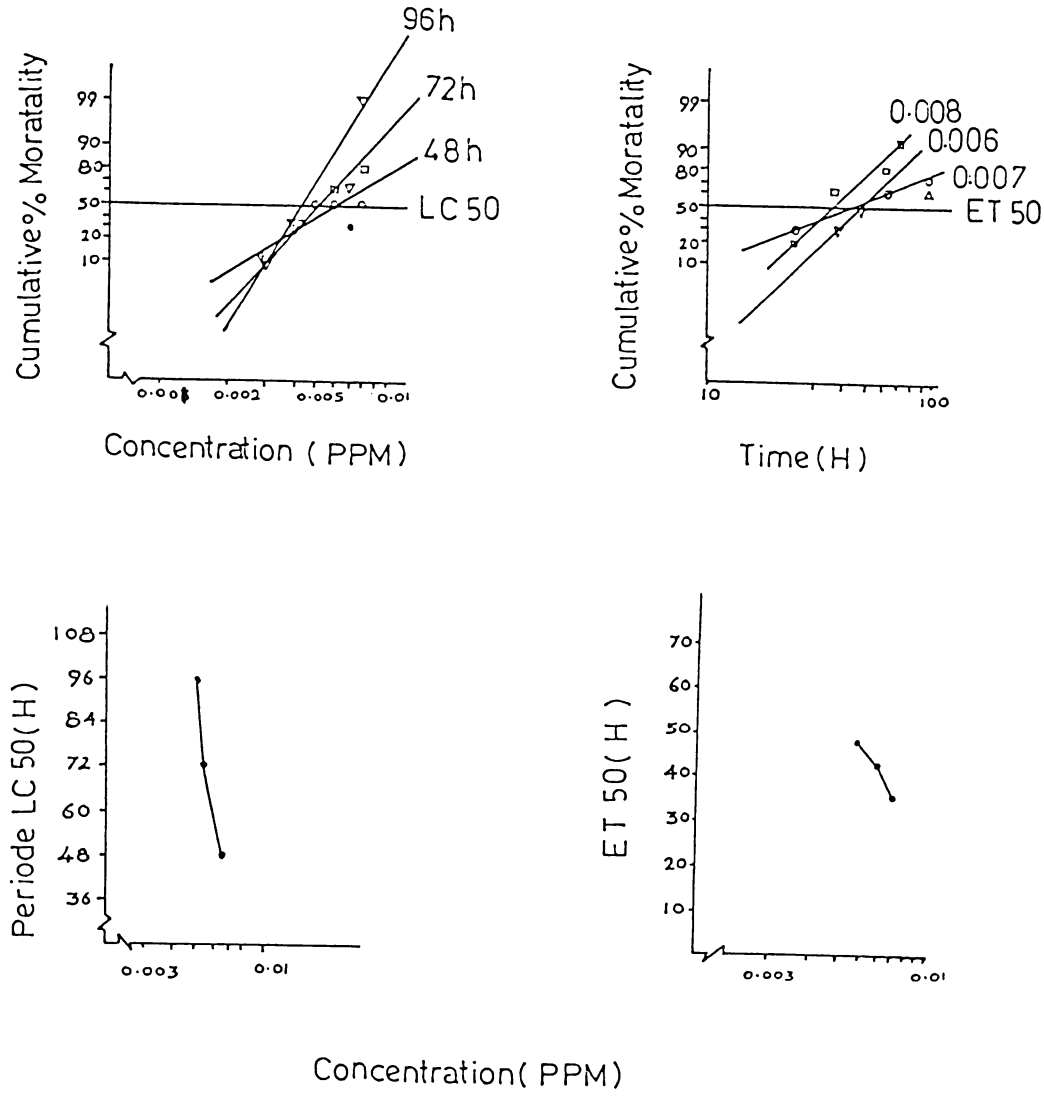

Fig.1. *Etroplus maculatus*:
Lethal effects of DDT.
(a) Progress of mortality against concentration.
(b) Progress of mortality against time.
(c) & (d) Toxicity curves.

mortality recorded in 0.23 ppm by 72 hour. In higher concentrations mortality was found to progress quickly as a function of time. In many of the concentrations maximum mortality was recorded between 72 and 96 hours. The period for LC 50 showed decrease with increase in concentration. The ET 50 recorded were 36.0 h at 0.23 ppm, 34.0 h at 0.19 ppm and 45.0 h at 0.18 ppm (Table 1, Fig.2).

4.1.4 Lethal toxicity of Gramoxone

The 96 h LC 50 computed for Gramoxone was 0.054 ppm from the range of concentrations, 0.01 ppm to 0.1 ppm. Mortality recorded in lower concentrations between 72 and 96 hours; and in 0.09 ppm and 0.1 ppm 100% mortality was recorded within the above hours. As time proceeded Gramoxone was found to become more toxic. The toxicity curve showed a sharp increase in toxicity with increase in exposure concentration and time. The ET 50 recorded were 65.0 h at 0.06 ppm, 55.0 h at 0.07 ppm and 36.0 h at 0.08 ppm (Table 1, Fig.3).

4.2 SUBLETHAL TOXIC RESPONSE.

At sublethal pesticide levels, laboratory studies have shown that there is impairment of mechanisms which are critical to survival. Regarding pesticide pollution, 'no-effects' levels rather than

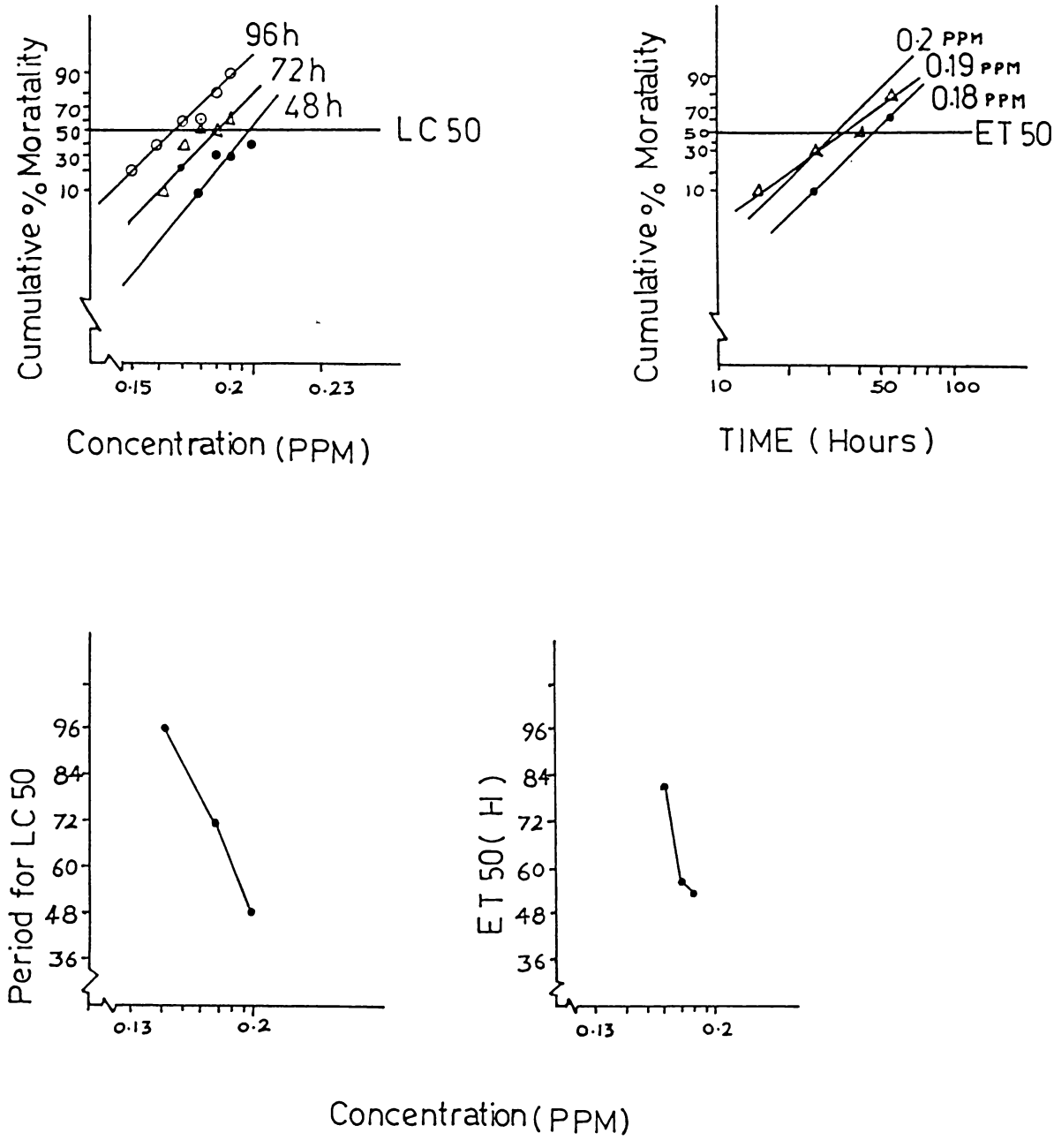

Fig.2. *Etroplus maculatus*:
Lethal effects of Dimecron.
(a) Progress of mortality against concentration.
(b) Progress of mortality against time.
(c) & (d) Toxicity curves.

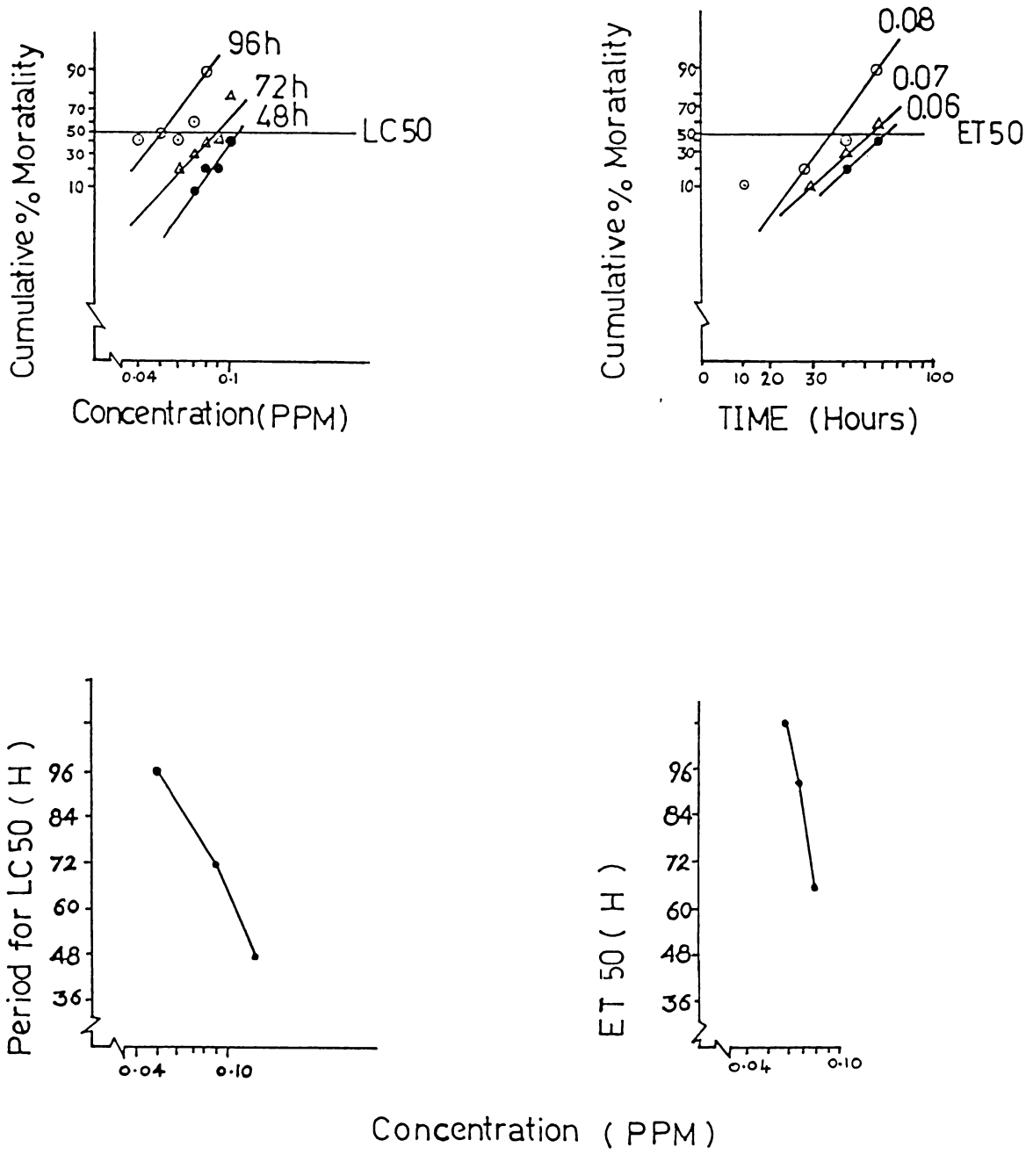

Fig.3. *Etroplus maculatus*:
Lethal effects of Gramoxone
(a) Progress of mortality against concentration
(b) Progress of mortality against time.
(c) & (d) Toxicity curves.

'no-kill' levels must serve as the basis for establishing neater quality criteria. It is only by determining the more subtle responses of fish to sublethal levels of pesticides, such 'no-effect' levels can be established (Hogan, *1968*). Among the type of observations that may eventually be used to this end are, those changes in key enzyme activity, principal haematological parameters and histopathological changes.

4.2.1 Enzyme Activity : Under individual toxicant stress

Enzymes are attractive as indicators because they are more easily quantified than other indicators, such as changes in behaviour. Useful precedents have been set in clinical medicine in the successful diagnosis of disease and evaluation of exposure to industrial chemicals or drugs by analyses of such variables as enzymes, blood chemistry and liver function. On the basis of changes in enzyme activity, biochemical assays for pollution-related alterations in fish tissues are possible (Hinton and Koenig, *1975*).

4.2.1.1 DDT

4.2.1.1.1 Brain

4.2.1.1.1.1 Alkaline phosphatase (ALP)

Short term:- The brain ALP showed significant elevation in activity from the respective control values in C_2 during 24 hours, in C_1 and C_2 during 72 hours and in C_1 during 120 hours of exposure, while significant reduction in activity was found in C_4 during 72 hour and in three higher concentrations (C_2, C_3 and C_4) during 120 hour of exposure. During 72 and 120 hour exposure, the activity of ALP showed decrease with increase in concentrations and was found significant except the activity change between C_1 and C_2 during 72 hours, and between C_2 and C_3 during 120 hours. Significant increase in activity with duration was found in C_1 while in C_2 and C_4 significant reduction of activity was observed. In C_3, except between 24 and 72 hour activity pattern, significant change in activity with duration was noted (Table 2, 3 & 4, Fig. 4).

Long term:- The brain ALP activity showed no significant change from the control in all the four exposed concentrations during 10 days exposure. Significant reduction in ALP activity from the respective controls was found in all the four concentrations during 20

TABLE 2

Etroplus maculatus: In vivo enzyme activity of ALP in brain, gill and liver of control fish and those pre-exposed to four SLC of DDT for 24 hrs.

		CONTROL	C1	C2	C3	C4
			0.00033	0.00065	0.0013	0.0026
BRAIN	a	0.3985	0.367	0.5593	0.3257	0.3808
		+/-0.034	+/-0.026	+/-0.018	+/-0.047	+/-0.033
	b		-7.90	+40.35	-18.27	-4.44
	c		NS	P<0.01	NS	NS
GILL	a	0.4301	0.3926	0.4451	0.551	0.5972
		+/-0.067	+/-0.044	+/-0.034	+/-0.029	+/-0.017
	b		-8.72	+3.49	+28.11	+38.85
	c		NS	NS	P<0.05	P<0.05
LIVER	a	0.2844	0.0346	0.0449	0.0745	0.1009
		+/-0.079	+/-0.008	+/-0.002	+/-0.002	+/-0.005
	b		-87.83	-84.21	-73.80	-64.52
	c		P<0.01	P<0.01	P<0.05	P<0.01

TABLE 3

Etroplus maculatus: In vivo enzyme activity of ALP in brain, gill and liver of control fish and those pre-exposed to four SLC of DDT for 72 hrs.

		CONTROL	C1	C2	C3	C4
			0.00033	0.00065	0.0013	0.0026
BRAIN	a	0.3646	0.4873	0.4655	0.3621	0.207
		+/-0.024	+/-0.034	+/-0.038	+/-0.057	+/-0.009
	b		+33.65	+27.69	-0.68	-44.87
	c		P<0.01	P<0.05	NS	P<0.001
GILL	a	0.4261	0.3988	0.421	0.6072	0.6651
		+/-0.099	+/-0.021	+/-0.023	+/-0.049	+/-0.016
	b		-6.41	-1.19	+42.50	+56.09
	c		NS	P<0.001	P<0.05	P<0.05
LIVER	a	0.2069	0.0988	0.1325	0.1933	0.2464
		+/-0.053	+/-0.002	+/-0.002	+/-0.005	+/-0.016
	b		-52.25	-35.96	-6.57	+19.09
	c		P<0.05	NS	NS	NS

a: Mean enzyme activity (N=3) in u mol p-nitro phenol liberated/mg pro./hr

b: % Alteration from the mean control value.

c: Level of significance/non-signifcant ns.

TABLE 4

Etroplus maculatus: In vivo enzyme activity of ALP in brain, gill and liver of control fish and those pre-exposed to four SLC of DDT for 120 hrs.

		CONTROL	C1 0.00033ppm	C2 0.00065ppm	C3 0.0013ppm	C4 0.0026ppm
BRAIN	a	0.4597 +/-0.097	0.695 +/-0.037	0.195 +/-0.032	0.2135 +/-0.04	0.1031 +/-0.007
	b		+51.19	-57.58	-53.56	-77.57
	c		P<0.05	P<0.05	P<0.05	P<0.01
GILL	a	0.3751 +/-0.076	0.3886 +/-0.054	0.3292 +/-0.082	0.2821 +/-0.058	0.2431 +/-0.019
	b		+2.27	-12.24	-24.79	-35.19
	c		NS	NS	NS	P<0.05
LIVER	a	0.2648 +/-0.082	0.1266 +/-0.003	0.1448 +/-0.003	0.3783 +/-0.018	0.557 +/-0.014
	b		-52.19	-4.53	+42.86	+110.35
	c		P<0.05	NS	NS	P<0.01

TABLE 5

Etroplus maculatus: In vivo activity of ALP in brain, gill and liver of control fish and those pre-exposed to four SLC of DDT for 10 days.

		CONTROL	C1 0.00013ppm	C2 0.00017ppm	C3 0.00026ppm	C5 0.00052ppm
BRAIN	a	0.3241 +/-0.098	0.3286 +/-0.024	0.2983 +/-0.033	0.3047 +/-0.029	0.2265 +/-0.041
	b		+1.39	-7.96	-5.99	-30.11
	c		NS	NS	NS	NS
GILL	a	0.3804 +/-0.023	0.3404 +/-0.031	0.3352 +/-0.032	0.2761 +/-0.057	0.2172 +/-0.044
	b		-10.51	-11.88	-27.41	-42.90
	c		NS	NS	P<0.05	P<0.01
LIVER	a	0.3254 +/-0.052	0.2211 +/-0.032	0.2014 +/-0.029	0.1513 +/-0.026	0.1451 +/-0.028
	b		-32.05	-38.11	-53.50	-55.41
	c		P<0.05	P<0.05	P<0.01	P<0.01

a: Mean enzyme activity (N=3) in u mol p-nitro phenol liberated/mg pro./hr.
b: % Alteration from the mean control value.
c: Level of significance/non-signifcant ns

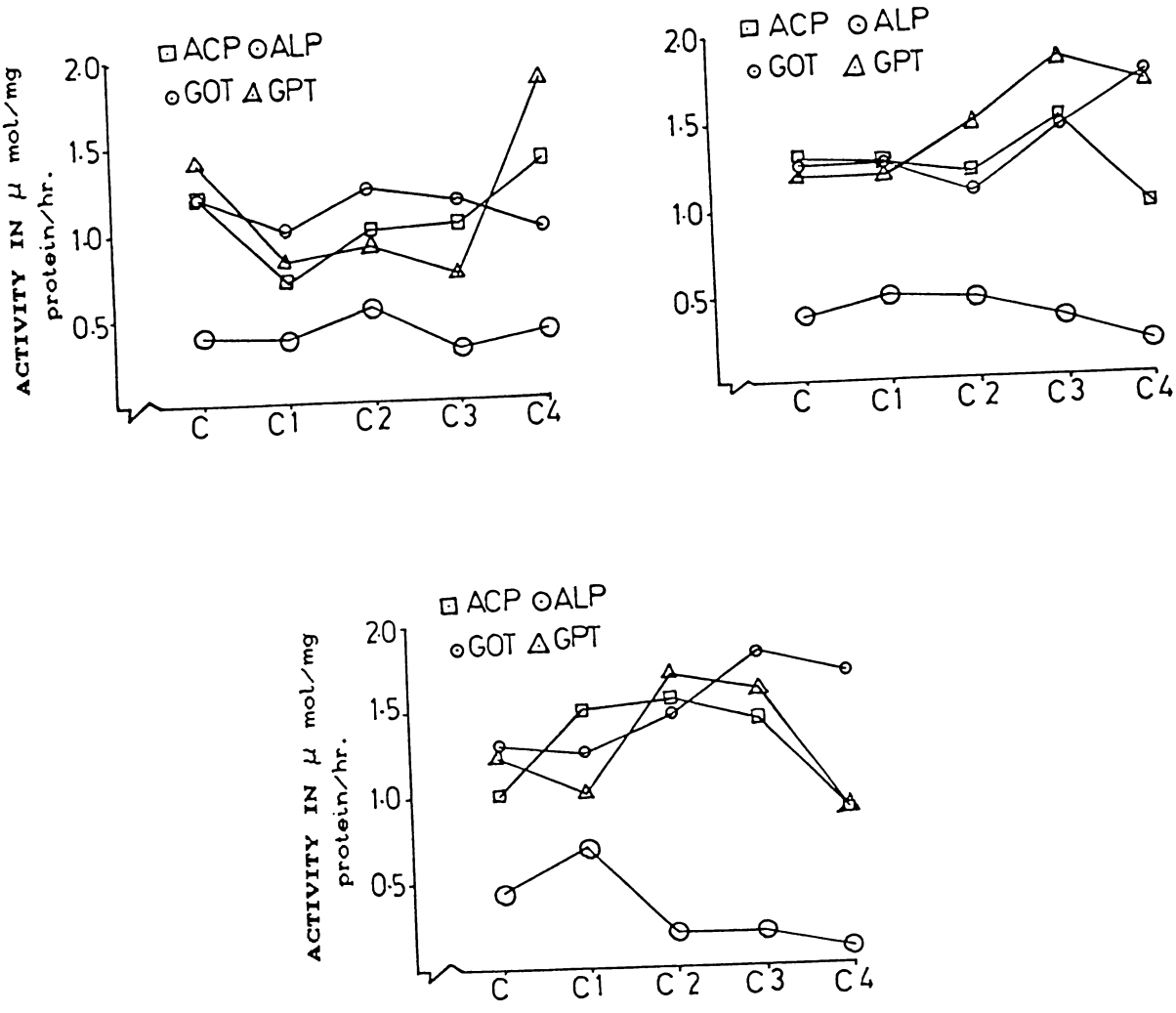

Fig.4. *Etroplus maculatus*: Mean enzyme activity of ALP, ACP, GOT and GPT in brain of control fish (C) and those pre-exposed to 4 SLC of DDT for (a)24, (b) 72and (c) 120 hrs.

and 30 days exposure to DDT. The activity of ALP showed decrease with increase in concentration and found significant during 20 and 30 days (except the change in activity between C_2 and C_3 during 20 days). The activity was also found decreasing with exposure duration in all the concentrations, and the reduction was found significant during 20 and 30 days of exposure when compared with 10th day enzyme activity, in all the concentrations (Table 5, 6 & 7, Fig.5).

4.2.1.1.1.2 Acid Phosphatase (ACP)

Short term:- Significant reduction of ACP activity, when compared to respective control values, was found in the lowest two concentrations (C_1 and C_2) during 24 hour and in the highest concentration (C_4) during 72 hour exposure while in the lowest two concentrations (C_1 and C_2) during 120 hour exposure the activity was found significantly elevated from the control. During 24 hour exposure the ACP activity showed comparatively higher values in higher concentrations while during 120 hour exposure the activity was found decreasing with increase in exposure concentration. The activity was found elevated significantly with the exposure duration in C_1, C_2 and C_4 (no significant change between 72 and 120 hour activity in C_4) and in C_3 the activity was found significantly elevated during 72 and 120

TABLE 6

Etroplus maculatus: In vivo enzyme activity of ALP in brain, gill and liver of control fish and those pre-exposed to four SLC of DDT 25% EC for 20 days

		CONTROL	C1 0.00013	C2 0.00017	C3 0.00026	C4 0.00052
BRAIN	a	0.4892 +/-0.075	0.3271 +/-0.025	0.2666 +/-0.041	0.2407 +/-0.033	0.1987 +/-0.016
	b		-33.13	-45.50	-50.78	-59.38
	c		P<0.05	P<0.01	P<0.01	P<0.01
GILL	a	0.4363 +/-0.069	0.2673 +/-0.057	0.2144 +/-0.063	0.1721 +/-0.075	0.1514 +/-0.078
	b		-38.73	-50.86	-60.55	-65.30
	c		P<0.05	P<0.05	P<0.05	P<0.05
LIVER	a	0.3843 +/-0.064	0.3503 +/-0.056	0.3106 +/-0.027	0.2636 +/-0.026	0.2418 +/-0.029
	b		-8.85	-19.18	-31.41	-37.08
	c		NS	NS	P<0.05	P<0.05

TABLE7

Etroplus maculatus: In vivo enzyme activity of ALP in brain, gill and liver of control fish and those pre-exposed to four SLC of DDT for 30 days

		CONTROL	C1 0.00013	C2 0.00017	C3 0.00026	C4 0.00052
BRAIN	a	0.4098 +/-0.09	0.1963 +/-0.016	0.119 +/-0.014	0.0859 +/-0.012	0.0243 +/-0.007
	b		-52.10	-70.96	-79.04	-94.07
	c		P<0.05	P<0.01	P<0.01	P<0.01
GILL	a	0.3925 +/-0.03	0.201 +/-0.034	0.1521 +/-0.053	0.1174 +/-0.013	0.0957 +/-0.014
	b		-48.79	-61.25	-70.09	-75.62
	c		P<0.01	P<0.01	P<0.001	P<0.001
LIVER	a	0.337 +/-0.053	0.313 +/-0.059	0.2519 +/-0.032	0.2045 +/-0.039	0.1951 +/-0.053
	b		-7.12	-25.25	-39.32	-42.11
	c		NS	NS	P<0.05	P<0.05

a: Mean enzyme activity (N=3) in u mol p-nitro phenol liberated/mg pro./hr.
b: % Alteration from the mean control value
c: Level of significance/nonsignificant ns

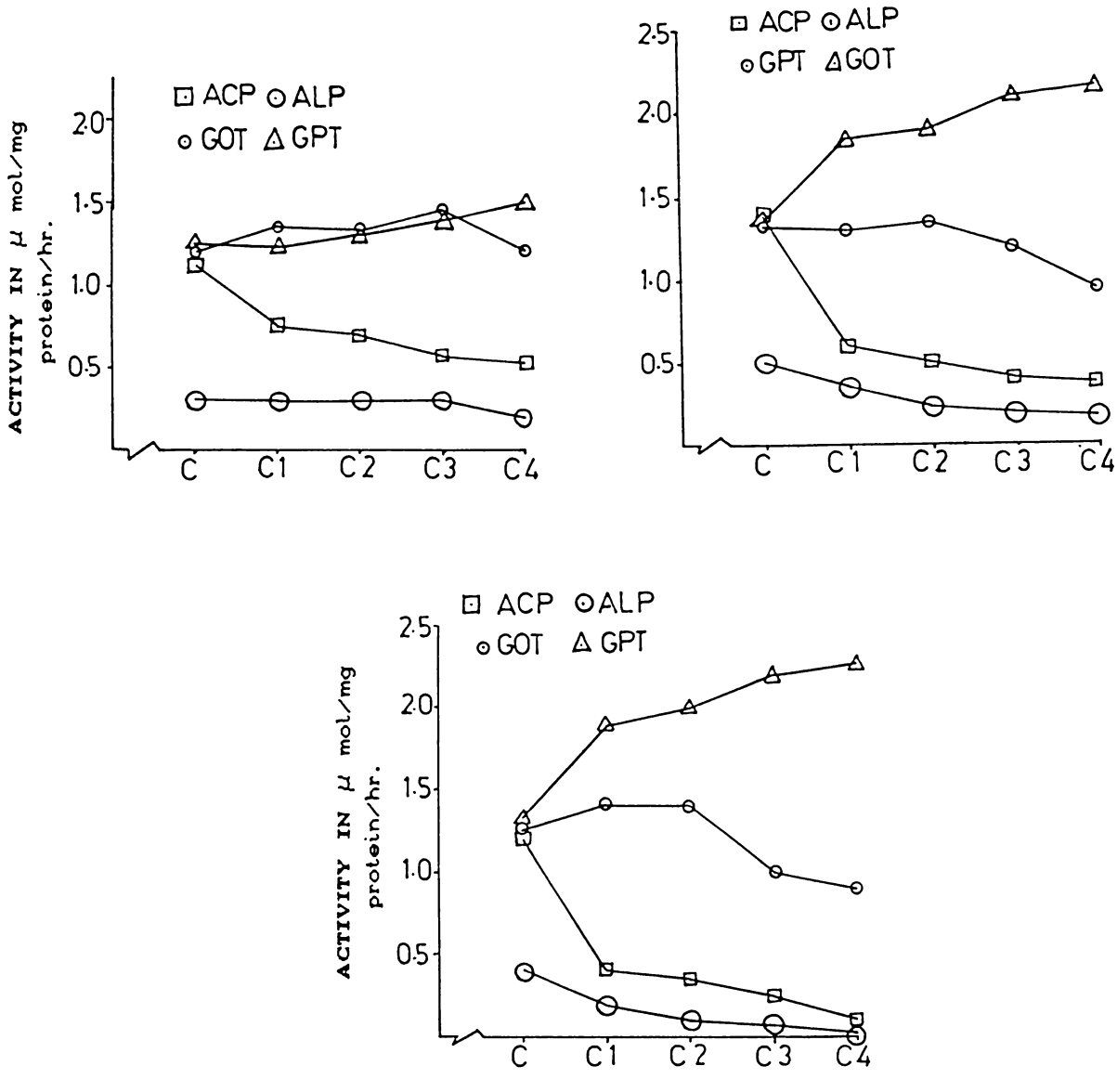

Fig.5. *Etroplus maculatus*: Mean enzyme activity of ALP, ACP, GOT and GPT in brain of control fish (C) and those pre-exposed to 4 SLC of DDT for (a)10, (b) 20 and (c) 30 days.

hours from 24 hour exposure (Table 8, 9 & 10, Fig. 4).

Long term:- During the long term exposure to DDT, the brain ACP activity showed significant reduction in all the four concentrations from the respective controls. The activity of ACP was found significantly reduced with increase in exposure concentration (the change in activity between C_1 and C_2 during 10 day, and between C_3 and C_4 during 10 and 20 days of exposure was found insignificant) as well as with the exposure duration in each concentration (Table 11, 12 & 13, Fig. 5).

4.2.1.1.1.3 Glutamate oxaloacetate transaminase (GOT)

Short term:- A significant elevation in brain GOT activity from the respective control values was found in the highest two concentrations (C_3 and C_4) during 72 and 120 hours of exposure, while the activity showed significant reduction in C_1 and C_4 during 24 hour, and in C_2 during 72 hour of exposure to DDT. Except the activity changes between C_1 and C_3, C_1 and C_4 and C_2 and C_3 during 24 hour, between C_1 and C_2 during 72 hour, and between C_3 and C_4 during 120 hour, the change of GOT activity with increase in exposed concentration was found significant. In each concentration, with increase in exposure duration, the change in GOT activity was found significant

TABLE 8

Etroplus maculatus: In vivo enzyme activity of ACP in brain, gill and liver of control fish and those pre-exposed to four SLC of DDT for 24 hrs.

		CONTROL	C1 0.00033ppm	C2 0.00065ppm	C3 0.0013ppm	C4 0.0026ppm
BRAIN	a	1.1967 +/-0.098	0.7242 +/-0.012	0.9965 +/-0.047	1.0326 +/-0.088	1.4051 +/-0.046
	b		-39.48	-16.73	-13.71	+17.41
	c		P<0.01	P<0.05	NS	P<0.05
GILL	a	1.1352 +/-0.099	1.1465 +/-0.031	1.251 +/-0.672	1.0317 +/-0.055	1.0086 +/- 0.047
	b		+0.99	+10.20	-9.12	-11.15
	c		NS	NS	NS	NS
LIVER	a	1.5882 +/-0.03	1.4772 +/-0.03	1.3067 +/-0.024	1.6796 +/-0.005	1.8791 +/-0.006
	b		-6.99	-17.72	+5.75	+18.32
	c		P<0.05	P<0.001	P<0.01	P<0.001

TABLE 9

Etropus maculatus: In vivo enzyme activity of ACP in brain, gill and liver of control fish and those pre-exposed to four SLC of DDT for 72 hrs.

		CONTROL	C1 0.00033ppm	C2 0.00065ppm	C3 0.0013ppm	C4 0.0026ppm
BRAIN	a	1.2981 +/-0.106	1.2612 +/-0.024	1.2457 +/-0.13	1.4997 +/-0.086	1.033 +/-0.118
	b		-28.43	-4.04	+15.53	-20.42
	c		NS	NS	NS	P<0.05
GILL	a	1.1274 +/-0.092	1.1914 +/-0.052	1.2525 +/-0.084	1.1421 +/-0.025	1.1052 +/-0.049
	b		+5.68	11.09	+1.30	-1.97
	c		NS	NS	NS	NS
LIVER	a	1.61 +/-0.021	1.3948 +/-0.021	1.0545 +/-0.045	1.6117 +/-0.029	1.9165 +/-0.008
	b		-13.37	-34.50	+0.11	+19.04
	c		P<0.001	P<0.001	NS	P<0.001

a: Mean enzyme activity (N=3) in u mol p-nitro phenol liberated/mg pro./hr.
b: % Alteration from the mean control value.
c: Level of significance/non-significant ns

TABLE 10

Etroplus maculatus: In vivo enzyme activity of ACP im brain, gill and liver of control fish and those pre exposed to four SLC of DDT for 120 hrs.

		CONTROL	C1 0.00033ppm	C2 0.00065ppm	C3 0.0013ppm	C4 0.0026ppm
BRAIN	a	1.1106 +/-0.111	1.5202 +/-0.061	1.5596 +/-0.65	1.2892 +/-0.063	0.935 +/-0.053
	b		+36.88	+40.43	+16.08	-15.81
	c		P<0.01	P<0.01	NS	NS
GILL	a	1.1394 +/-0.055	1.0023 +/-0.077	0.9541 +/-0.013	0.8921 +/-0.033	0.8324 +/-0.056
	b		-12.03	-16.26	-21.70	-28.94
	c		NS	P<0.01	P<0.01	P<0.01
LIVER	a	1.6152 +/-0.024	1.3151 +/-0.034	0.8887 +/-0.001	1.8176 +/-0.019	2.2506 +/-0.07
	b		-18.58	-44.98	+12.53	+39.34
	c		P<0.001	P<0.001	P<0.001	P<0.001

TABLE 11

Etroplus maculatus: In vivo enzyme activity of ACP in brain, gill and liver of control fish and those pre-exposed to four SLC of DDT for 120 hrs.

		CONTROL	C1 0.00013ppm	C2 0.00017ppm	C3 0.00026ppm	C4 0.00052ppm
BRAIN	a	1.1246 +/-0.119	0.752 +/-0.054	0.6924 +/-0.034	0.5606 +/-0.051	0.5548 +/-0.062
	b		-33.13	-38.43	-50.15	-50.67
	c		P<0.01	P<0.01	P<0.01	P<0.01
GILL	a	1.1416 +/-0.088	1.0564 +/-0.065	0.9363 +/-0.081	0.8728 +/-0.054	0.7648 +/-0.038
	b		-7.46	-17.98	-23.54	-33.01
	c		NS	P<0.05	P<0.05	P<0.05

a: Mean enzyme activity (N=3) in u mol p-nitro phenol liberated/mg pro./hr
b: % Alteration from the mean control value
c: Level of significance/non significant ns

TABLE 12

Etroplus maculatus: In vivo enzyme activity of ACP in brain, gill and liver of control fish and those pre exposed to four SLC of DDT for 20 hrs'

		CONTROL	C1 0.00013ppm	C2 0.00017ppm	C3 0.00026ppm	C4 0.00052ppm
BRAIN	a	1.3814 +/-0.117	0.5948 +/-0.02	0.4826 +/-0.03	0.4074 +/-0.024	0.3661 +/-0.036
	b		-56.94	-65.06	-70.51	-73.50
	c		P<0.001	P<0.001	P<0.001	P<0.001
GILL	a	1.1325 +/-0.1	0.9158 +/-0.066	0.8287 +/-0.027	0.7799 +/-0.083	0.6621 +/-0.052
	b		-19.13	-26.82	-31.13	-41.54
	c		P<0.05	P<0.01	P<0.01	P<0.01
LIVER	a	1.7042 +/-0.015	0.9592 +/-0.053	0.8596 +/-0.077	0.667 +/-0.005	0.6308 +/-0.078
	b		-43.71	-49.56	-60.86	-62.98
	c		P<0.001	P<0.001	P<0.00	P<0.001

TABLE 13

Etroplus maculatus: In vivo enzyme activity of ACP in brain, gill and liver of control fish and those pre exposed to four SLC of DDT for 30 days.

		CONTROL	C1 0.00013ppm	C2 0.00017ppm	C3 0.00026ppm	C4 0.00052ppm
BRAIN	a	1.1828 +/-0.099	0.4001 +/-0.044	0.3654 +/-0.052	0.2646 +/-0.052	0.1188 +/-0.022
	b		-66.17	-69.11	-77.63	-89.96
	c		P<0.001	P<0.001	P<0.001	P<0.001
GILL	a	1.1249 +/-0.084	0.8672 +/-0.062	0.7542 +/-0.0.088	0.6432 +/-0.053	0.5821 +/-0.033
	b		-22.91	-32.95	-42.82	-48.25
	c		P<0.05	P<0.01	p<0.01	P<0.001
LIVER	a	1.6656 +/-0.103	0.8898 +/-0.065	0.7801 +/-0.057	0.6073 +/-0.065	0.5493 +/-0.079
	b		-46.58	-53.16	63.54	-67.02
	c		P<0.001	P<0.001	P<0.001	P<0.001

a:Mean enzyme activity (N=3 in u mol p-nitro phenol liberated/mg pro./hr
b: % Alteration from the mean control value
c: Level of significance/nonsignificant ns

except the change in activity between 72 and 120 hours of exposure in C_1 and C_4 (Table *14*, *15* & *16*, Fig. 5).

Long term:- Significant elevation in brain GOT activity from the respective control values was found in C_2 and C_3 during 10 day, and and in C_1 and C_2 during 30 day exposure. The brain GOT activity significantly reduced in the highest two concentrations (C_3 and C_4) during 20, and in C_4 during 30 days of exposure to DDT. Comparatively higher activity was noticed in the lower two concentrations and lower activity in higher two concentrations during 20 and 30 days of exposure. In the higher two concentrations the reduction in the enzyme activity with exposure duration was found significant (except the change between 20 and 30 day activity in C_4) (Table 17, 18 & 19, Fig. 5).

4.2.1.1.1.4 Glutamate pyruvate transaminase (GPT)

Short term:- Significant stimulation of GPT activity was found when compared with the respective controls in the highest exposed concentration (C_4) during 24 hour, in the higher three concentrations (C_2, C_3 and C_4) during 72 and in C_2 and C_3 during 120 hour of exposure, while in the three lower concentrations (C_1, C_2 and C_3) the activity of brain GPT was found significantly lower than the control

TABLE 14

Etroplus maculatus: In vivo enzyme activity of GOT in brain, gill and liver of control fish and those pre exposed to four SLC of DDT 24 hrs.

		CONTROL	C1 0.00033ppm	C2 0.00065ppm	C3 0.0013ppm	C4 0.0026ppm
BRAIN	a	1.2059 +/-0.041	1.0262 +/-0.071	1.2358 +/-0.027	1.1742 +/-0.078	0.9747 +/-0.041
	b		-4.65	+2.50	-2.63	-19.17
	c		P<0.05	NS	NS	P<0.01
GILL	a	0.4579 +/-0.072	0.4571 +/-0.057	0.4088 +/-0.027	0.3301 +/-0.037	02737 +/-0.027
	b		-0.17	-10.72	-27.91	-40.21
	c		NS	NS	NS	P<0.05
LIVER	a	1.2507 +/-0.047	1.4883 +/-0.035	1.107 +/-0.032	0.954 +/-0.018	0.5448 +/-0.033
	b		+18.60	-11.50	-23.72	-56.44
	c		P<0.01	P<0.5	P<0.001	P<0.001

TABLE 15

Etroplus maculatus: In vivo enzyme activity of GOT in brain, gill and liver of control fish and those pre exposed to four SLC of DDT for 72 hrs.

		CONTROL	C1 0.00033ppm	C2 0.00065ppm	C3 0.0013ppm	C4 0.0026ppm
BRAIN	a	1.27 +/-0.063	1.2674 +/-0.094	1.0994 +/-0.048	1.4558 +/-0.078	1.7659 +/-0.137
	b		-0.20	-13.43	+14.63	+39.05
	c		P<0.05	P<0.05	P<0.05	P<0.01
GILL	a	0.4726 +/-0.079	0.485 +/-0.044	0.4579 +/-0.034	0.3383 +/-0.025	0.2769 +/-0.045
	b		+2.62	-3.11	-28.42	-41.41
	c		NS	NS	P<0.05	P<0.05
LIVER	a	1.3072 +/-0.049	1.263 +/-0.012	1.5218 +/-0.015	1.1137 +/-0.025	0.8093 +/-0.012
	b		-3.38	+16.42	-14.80	-38.09
	c		NS	P<0.01	P<0.01	P<0.001

a: Mean enzyme activity (N=3) in u mol sodiumpyruvate liberated/mg pro./hr
b: % Alteration from the mean control value
c: Level of significance/non significant ns

TABLE 16

Etroplus maculatus: In vivo enzyme activity of GOT to brain, gill anf liver of control fish and those pre exposed to four SLC od DDT for 120 hrs.

		CONTROL	C1 0.00033ppm	C2 0.00065	C3 0.0013	C4 0.0026
BRAIN	a	1.2943 +/-0.132	1.2677 +/-0.038	1.4605 +/-0.049	1.8452 +/-0.171	1.6936 +/-0.4132
	b		-2.06	+12.84	+42.56	+30.85
	c		NS	NS	P<0.05	P<0.05
GILL	a	0.4474 +/-0.061	0.4034 +/-0.019	0.3314 +/-0.032	0.3118 +/-0.041	0.2355 +/-0.015
	b		-9.83	-25.93	-30.31	-47.36
	c		NS	P<0.05	P<0.05	P<0.01
LIVER	a	1.3709 +/-0.074	0.7628 +/-0.045	1.0109 +/-0.019	0.8337 +/-0.045	0.4644 +/-0.043
	b		-44.36	-26.26	-39.19	-66.12
	c		P<0.001	P<0.01	P<0.001	P<0.001

TABLE 17

Etroplus maculatus: In vivo enzyme activity of GOT in brain,gill and liver of control fish and those pre exposed to four SLC of DDT for 10 days.

		CONTROL	C1 0.00013	C2 0.00017	C3 0.00026	C4 0.00052
BRAIN	a	1.2102 +/-0.068	1.3609 +/-0.097	1.3529 +/-0.048	1.4517 +/-0.062	1.1931 +/-0.06
	b		+12.45	+11.79	+19.96	-1.41
	c		NS	P<0.05	P<0.05	NS
GILL	a	0.3539 +/-0.047	0.529 +/-0.063	0.5031 +/-0.028	0.5539 +/-0.093	0.5521 +/-0.046
	b		+49.48	+42.16	+56.51	+56.00
	c		P<0.05	P<0.05	P<0.05	P<0.05
LIVER	a	1.1811 +/-0.095	1.302 +/-0.063	1.3153 +/-0.056	1.3065 +/-0.039	1.3687 +/-0.029
	b		+10.23	+11.36	+10.62	+15.90
	c		NS	NS	NS	P<0.05

a: Mean enzyme activty (N=3) in u mol sodium pyruvate liberated /mg pro./hr.
b: % Alteration from the mean control value
c: Level if significace/non significant ns

TABLE 18

Etroplus maculatus: In vivo enzyme activity of GOT in brain, gill and liver of control fish and those pre exposed to four SLC of DDT for 20 days

		CONTROL	C1 0.00013ppm	C2 0.00017ppm	C3 0.00026ppm	C4 0.00052ppm
BRAIN	a	1.3516 +/-0.074	1.3112 +/-0.065	1.3661 +/-0.049	1.185 +/-0.058	0.9488 +/-0.052
	b		-2.99	+1.07	-12.33	-29.80
	c		NS	NS	P<0.05	P<0.01
GILL	a	0.4189 +/-0.09	0.5367 +/-0.059	0.5916 +/-0.044	0.5989 +/-0.011	0.5854 +/-0.031
	b		+28.12	+41.23	+42.97	+39.75
	c		NS	P<0.05	P<0.05	P<0.05
LIVER	a	1.21542 +/-0.075	1.5603 +/-0.065	1.5724 +/-0.071	1.6082 +/-0.081	1.6551 +/-0.078
	b		+20.56	+21.50	+24.26	+27.89
	c		P<0.01	P<0.01	P<0.01	P<0.01

TABLE 19

Etroplus maculatus: In vivo enzyme activity of GOT in brain, gill and liver of control fish and those pre exposed to four SLC of DDT for 30 days

		CONTROL	C1 0.00013ppm	C2 0.00017ppm	C3 0.00026ppm	C4 0.00052ppm
BRAIN	a	1.2496 +/-0.044	1.3814 +/-0.061	1.3974 +/-0.03	0.9942 +/-0.083	0.9058 +/-0.061
	b		+10.55	+11.83	-20.44	-27.51
	c		P<0.05	P<0.01	P<0.01	P<0.01
GILL	a	0.4397 +/-0.091	0.6384 +/-0.092	0.62 +/-0.049	0.592 +/-0.029	0.5531 +/-0.056
	b		+45.19	+41.01	+34.64	+25.79
	c		NS	P<0.05	NS	NS
LIVER	a	1.1122 +/-0.89	1.2197 +/-0.046	1.3244 +/-0.052	1.43 +/-0.017	1.3947 +/-0.084
	b		+9.64	+19.08	+28.57	+25.4
	c		NS	P<0.05	P<0.01	P<0.01

a: Mean enzyme activity (N=3) in mol sodium pyruvate liberate/mg pro./hr
b: % Alteration from the mean control value
c: Level of significance/non significant ns

during 24 hour exposure. An elevation of activity in C_1, C_2, and C_3 was noted during 72 hour from 24 hour activity and was found significant except the change between 24 and 72 hours in C_1. During 120 hour exposure significant reduction in activity of GPT from 72 hour was observed in all except in C_2. In the highest exposed concentration (C_4) the GPT activity showed significant reduction with the exposure duration (Table 20, 21 & 22, Fig. 4).

Long term:- Significant elevation of GPT activity was observed from the respective controls in the highest two (C_3 and C_4) concentrations during 10 days, and in all the four concentrations during 20 and 30 days of exposure to DDT. The activity showed general increase with increase of exposure concentration and exposure duration, and was significant in many cases. The increase in enzymatic activity of GPT with duration was found significant except the increase in activity between 20 and 30 days in C_1, C_3 and C_4 (Table 23, 24 & 25, Fig. 5).

4.2.*1*.*1*.2 Gill

4.2.*1*.*1*.2.*1* Alkaline phosphatase (ALP)

Short term:- Significant elevation of ALP activity from the respective controls was noted in the highest two (C_3 and C_4) concentrations during 24 and 72 hour exposure, while in the highest concentration (C_4) during 120 hour exposure, a significant reduction of activity

TABLE 20

Etroplus maculatus: In vivo enzyme activity of GPT in brain, gill and liver of control fish and those pre exposed to four SLC of DDT for 24 hrs.

		CONTROL	C1 0.00033ppm	C2 0.00065ppm	C3 0.0013ppm	C4 0.0026ppm
BRAIN	a	1.4074 +/-0.107	0.8342 +/-0.075	0.8718 +/-0.093	0.7346 +/-0.052	1.8664 +/-0.066
	b		-40.73	-38.06	-47.80	+32.61
	c		P<0.01	P<0.01	P<0.01	P<0.01
GILL	a	0.9321 +/-0.096	0.7514 +/-0.072	0.8081 +/-0.041	0.8481 +/-0.051	0.9155 +/-0.032
	b		-19.39	-13.30	-9.01	-1.78
	c		NS	NS	NS	NS
LIVER	a	1.3794 +/-0.099	1.4782 +/-0.022	1.3822 +/-0.112	1.1857 +/-0.019	1.1564 +/-0.015
	b		+7.16	+0.20	-14.04	-16.17
	c		P<0.05	NS	NS	P<0.05

TABLE 21

Etroplus maculatus: In vivo enzyme activity of GPT in brain, gill and liver of control fish and those pre exposed to four SLC of DDT for 72 hrs.

		CONTROL	C1 0.00033ppm	C2 0.00065ppm	C3 0.0013ppm	C4 0.0026ppm
BRAIN	a	1.2223 +/-0.113	1.2293 +/-0.027	1.4924 +/-0.049	1.8788 +/-0.029	1.715 +/-0.047
	b		+0.56	+22.08	+53.69	+40.29
	c		NS	P<0.05	P<0.001	P<0.01
GILL	a	0.9414 +/-0.069	0.8285 +/-0.077	0.8695 +/-0.081	0.8814 +/-0.02	0.9157 +/-0.092
	b		-11.99	-7.64	-6.37	-2.73
	c		NS	NS	NS	NS
LIVER	a	1.4385 +/-0.172	1.6346 +/-0.009	1.6229 +/-0.036	1.0.9456 +/-0.008	0.7877 +/-0.035
	b		+13.63	+12.82	-34.26	-45.24
	c		NS	NS	P<0.01	P<0.01

a: Mean enzyme activity (N=3) u mol sodium pyvurate liberated/mg pro,/hr
b: % Alteration from the mean control value
c Level of significance/non-significant ns

TABLE 22

Etroplus maculatus: In vivo enzyme activity of GPT in brain, gill and liver of control fish and those pre-exposed to four slc of DDT for 120 hrs.

		CONTROL	C1 0.00033	C2 0.00065	C3 0.0013	C4 0.0026
BRAIN	a	1.2312 +/-0.109	0.9881 +/-0.119	1.7257 +/-0.045	1.645 +/-0.086	0.9332 +/-0.065
	b		-19.711	+40.12	+33.61	-24.20
	c		NS	P<0.01	P<0.01	P<0.05
GILL	a	0.9499 +/-0.089	0.7317 +/-0.059	0.7624 +/-0.045	0.7933 +/-0.066	0.8201 +/-0.056
	b		-22.9	-19.74	-16.49	-13.66
	c		P<0.05	P<0.05	NS	NS
LIVER	a	1.3937 +/-0.097	1.7054 +/-0.099	1.7221 +/-0.018	0.8601 +/-0.017	0.9175 +/-0.013
	b		+22.36	+23.56	-38.29	-34.16
	c		P<0.01	P<0,01	P<0.01	P>0.01

TABLE 23

Etroplus maculatus: In vivo enzyme activity of GPT in brain, gill liver of control fish and those pre-exposed to four SLC of DDT 25%EC for 10 days.

		CONTROL	C1 0.00013	C2 0.00017	C3 0.00026	C4 0.00052
BRAIN	a	1.2583 +/-0.069	1.2368 +/-0.032	1.2892 +/-0.061	1.4162 +/-0.061	1.4888 +/-0.047
	b		-1.71	+2.46	+12.58	+18.32
	c		NS	NS	P<0.05	P<0.01
GILL	a	0.9578 +/-0.112	0.8484 +/-0.052	0.9509 +/-0.039	0.9746 +/-0.072	1.1755 +/-0.049
	b		-11.42	-0.72	+1.75	22.73
	c		NS	NS	NS	P<0.05
LIVER	a	1.3703 +/-0.063	1.9547 +/-0.069	2.2455 +/-0.063	2.4278 +/-0.072	2.7022 +/-0.099
	b		+42.65	+63.87	+77.17	+97.20
	c		P<0.01	P<0.01	P<0.01	P<0.001

a: Mean enzyme activity (N-3) in u mol sodium pyruvate liberated/mg pro./ hour.
b: % Alteration from the mean control value
c: Level of significance/nonsignificant us

TABLE 24

Etroplus maculatus: In vivo enzyme activity of GPT in brain, gill and liver of control fish and those pre-exposed to four SLC of DDT 25%EC for 20 days.

		CONTROL	C1 0.00013	C2 0.00017	C3 0.00026	C4 0.00052
BRAIN	a	1.3682 +/-0.09	1.8569 +/-0.058	1.8701 +/-0.093	2.1154 +/-0.013	2.1425 +/-0.049
	b		+35.72	+36.68	+54.61	+56.59
	c		P<0.01	P<0.01	P<0.001	P<0.001
GILL	a	0.9993 +/-0.078	- -	- -	1.393 +/-0.026	1.5128 +/-0.72
	b	-	-	-	+39.40	+51.39
	c	-	-	-	P<0.01	P<0.01
LIVER	a	1.3479 +/-0.085	1.9676 +/-0.039	2.1731 +/-0.046	2.3604 +/-0.097	2.7292 +/-0.23
	b		+45.98	+61.22	+75.12	+102.48
	c		P<0.001	P<0.001	P<0.001	P<0.001

TABLE 25

Etroplus masculatus: In vivo enzyme activity of GPT in brain, gill and liver of control fish and those pre-exposed to four SLC of DDT 25%EC for 30 days.

		CONTROL	C1 0.00013	C2 0.00017	C3 0.00026	C4 0.00052
BRAIN	a	1.3259 +/-0.057	1.8888 +/-0.096	2.042 +/-0.047	2.1839 +/-0.075	2.2688 +/-0.089
	b		+42.45	+54.01	+64.71	+71.11
	c		P<0.001	P<0.001	P<0.001	P<0.001
GILL	a	1.1021 +/-0.089	1.2925 +/-0.066	1.3581 +/-0.073	1.4262 +/-0.079	1.4413 +/-0.095
	b		+17.28	+23.26	+29.41	+30.71
	c		P<0.05	P<0.05	P<0.01	P<0.05
LIVER	a	1.3703 +/-0/063	1.9547 +/-0.069	2.2455 +/-0.063	2.4278 +/-0.072	2.7022 +/-0.099
	b		+42.65	+63.87	+77.17	+97.20
	c		P<0.001	P<0.001	P<0.001	P<0.001

a: Mean enzyme activity (N-3) in u mol sodium pyruvate liberated/mg pro./hour.
b: % Alteration from the control value
c: Level of significance/non-sigficant us

was noted. During 24 and 72 hour exposure the activity of ALP was found increasing with exposed concentration while during 120 hour the enzymatic activity was found decreasing with increase of concentration. The activity was also found decreasing with the exposure duration (Table 2, *3* & 4, Fig. *6*).

Long term:- The gill ALP activity showed significant reduction from the respective controls in the highest two concentrations (C_3 and C_4) during 10 days, and in all the four exposed concentrations during 20 and 30 days exposure. The activity was found decreasing with increase of concentration as well as with exposure duration. Significant reduction of ALP activity was noted in higher concentrations when compared to the activity in lower concentrations. The reduction of ALP activity in all the four concentrations during 120 hour exposure was found significant when compared to the activity in the respective concentration noted during 10 day exposure (Table 5, *6* & *7*, Fig. *7*).

4.2.*1.1*.2.2 Acid phosphatase (ACP)

Short term:- The gill ACP showed significant reduction in activity, when compared to the respective controls, in the three higher concentration during 120 hour of exposure while no significant

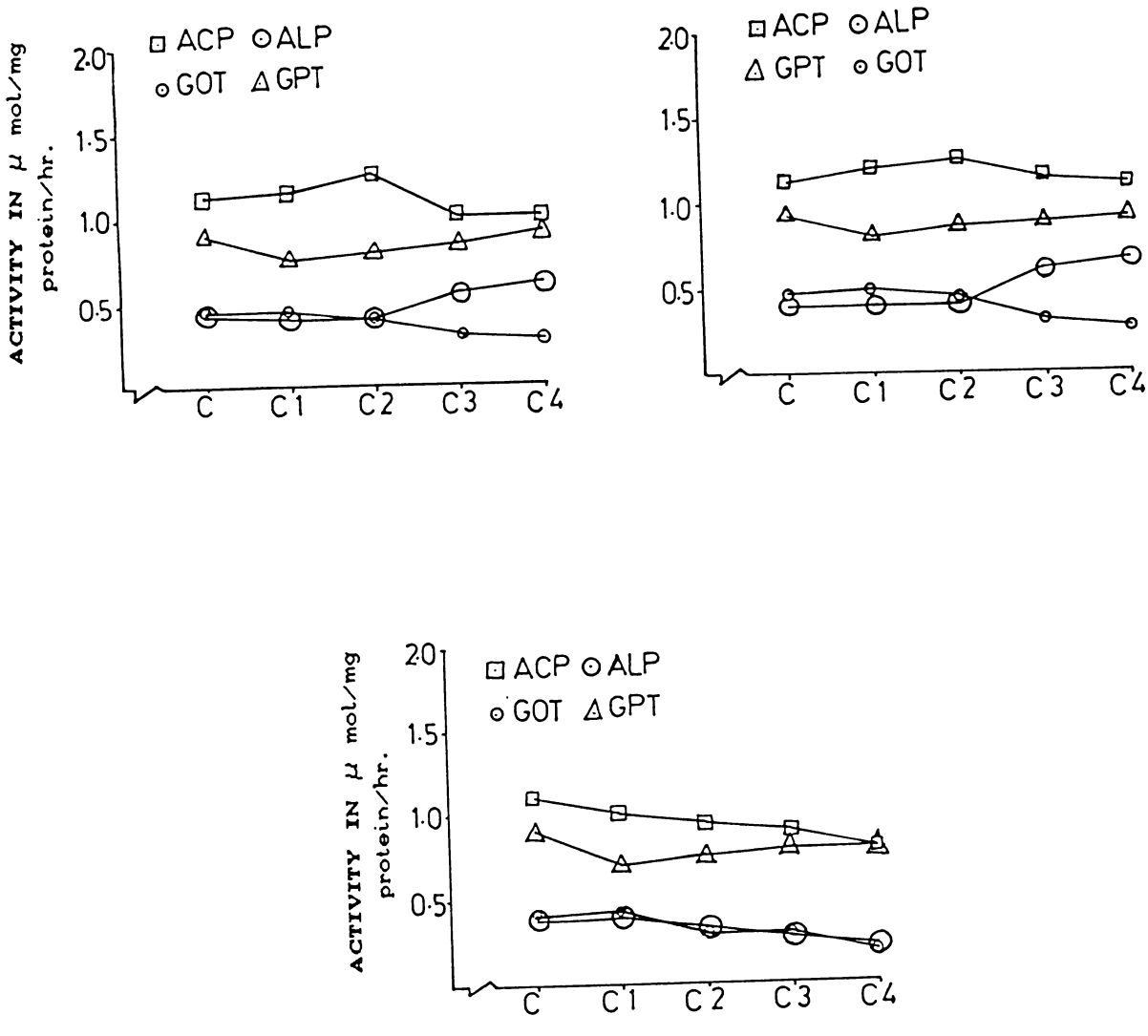

Fig.6. *Etroplus maculatus*: Mean enzyme activity of ALP, ACP, GOT and GPT in gill of control fish (C) and those pre-exposed to 4 SLC of DDT for (a) 24, (b) 72 and (c) 120 hrs.

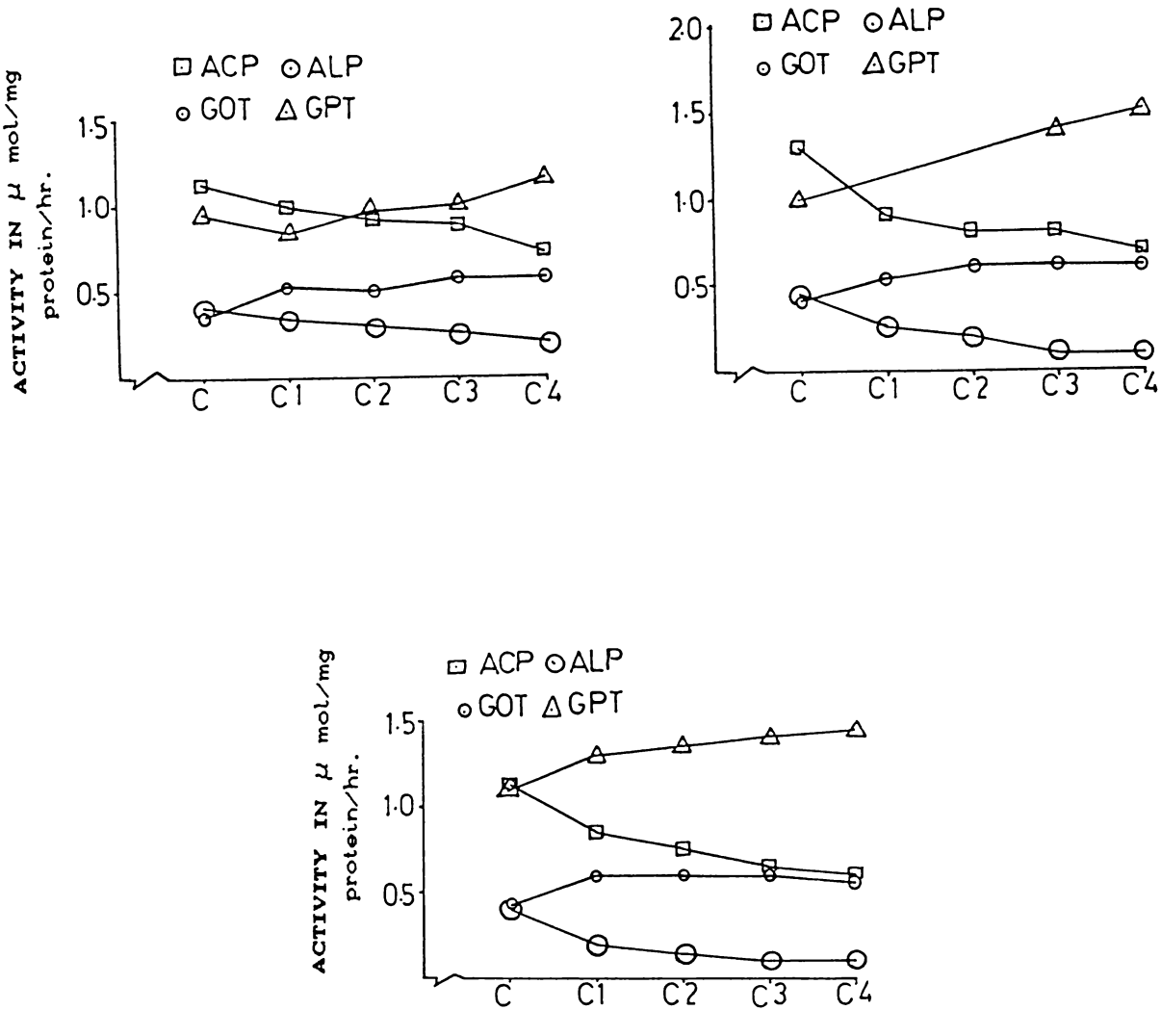

Fig.7. *Etroplus maculatus*: Mean enzyme activity of ALP, ACP, GOT and GPT in gill of control fish (C) and those pre-exposed to 4 SLC of DDT for (a) 10, (b) 20 and (c) 30 days.

change from the control was found in all the four exposed concentrations during 24 and 72 hours exposure. However, with increase in concentration a decrease in activity was observed (Table *8, 9 & 10*, Fig. *6*).

Long term:- The activity of gill ACP was found significantly reduced from the respective controls in the three highest concentrations during 10 day, and in all the four exposed concentrations during 20 and 30 days exposure. A decline in activity was noted with increase of exposed concentration as well as with the exposure duration in each concentration. Significant reduction was noted between the activities in lower and higher concentrations during the long term exposure, while reduction of activity in each concentration during 30 day was found significant from 10 day exposure, except in C_2 (Table 11, 12 & 13, Fig. 7).

4.2.1.1.2.3 Glutamate oxaloacetic transaminase (GOT)

Short term:- Significant reduction of gill GOT activity from the respective controls was noted in the highest concentration (C_4) during 24 hour, in C_3 and C_4 during 72 hour, and in the three highest concentrations (C_2, C_3 and C_4) during 120 hour exposure to DDT. The enzymatic activity was found declining with increase of exposure concentration during short term exposure. The reduction of activity

in C_1 and C_2 during 120 hour from 72 hour was found significant (Table *14, 15 & 16*, Fig. *6*).

Long term:- The activity of gill GOT showed significant elevation from the respective control values in all the four exposed concentrations during 10 day, in the three higher concentrations (C_2, C_3 and C_4) during 20 days, and in C_2 during 30 days exposure to DDT. During the 30 day exposure the activity of gill GOT found decreasing with the increase of exposed concentration, and in the two lower concentrations (C_1 and C_2) the enzymatic activity was found increasing with the exposure duration, but significant change was noted only in C_2 between 10 and 30 day activity, and 20 and 30 day activity (Table 17, 18 & 19, Fig. 7).

4.2.*1*.*1*.2.4 Glutamate pyruvate transaminase (GPT)

Short term:- The gill GPT activity showed significant reduction from the control in the two lower concentrations (C_1 and C_2) during 120 hour exposure to DDT, while in other concentrations no significant change was found (Table 20, 21 & 22, Fig. *6*).

Long term:- Significant elevation, from the respective control values, of gill GPT activity was noted in the highest concentration (C_4) during 10 day, in the two highest concentrations (C_3 and

C_4) (in the lower two concentrations activity assay could not be carried out as the sample spoiled) during 20 day, and in all the four concentrations during the 30 day exposure to DDT. Comparitive increase of activity could be noted with increase of exposure concentration as well as with exposure duration except in the highest concentration. The stimulation of enzymatic activity during 30 day exposure was found significant from the activity noted during 10 day in all the concentrations (Table 23, 24 & 25, Fig. 7).

4.2.1.1.3 Liver

4.2.1.1.3.1 Alkaline phosphatase (ALP)

Short term:- The liver ALP activity showed significant reduction from the respective controls in all the four exposed concentrations during 24 hour, in the lowest concentration (C_1) during 72 and 120 hour exposure, while in the highest concentration (C_4) during 120 hour exposure a significant elevation of ALP activity was found. The ALP activity was found significantly increasing with increase of exposed concentration and exposure duration (Table 2, 3 & 4, Fig. 8).

Long term:- Significant reduction of liver ALP activity from the respective control values was found in all the four exposed concentration during 10 day, and in the highest two concentrations

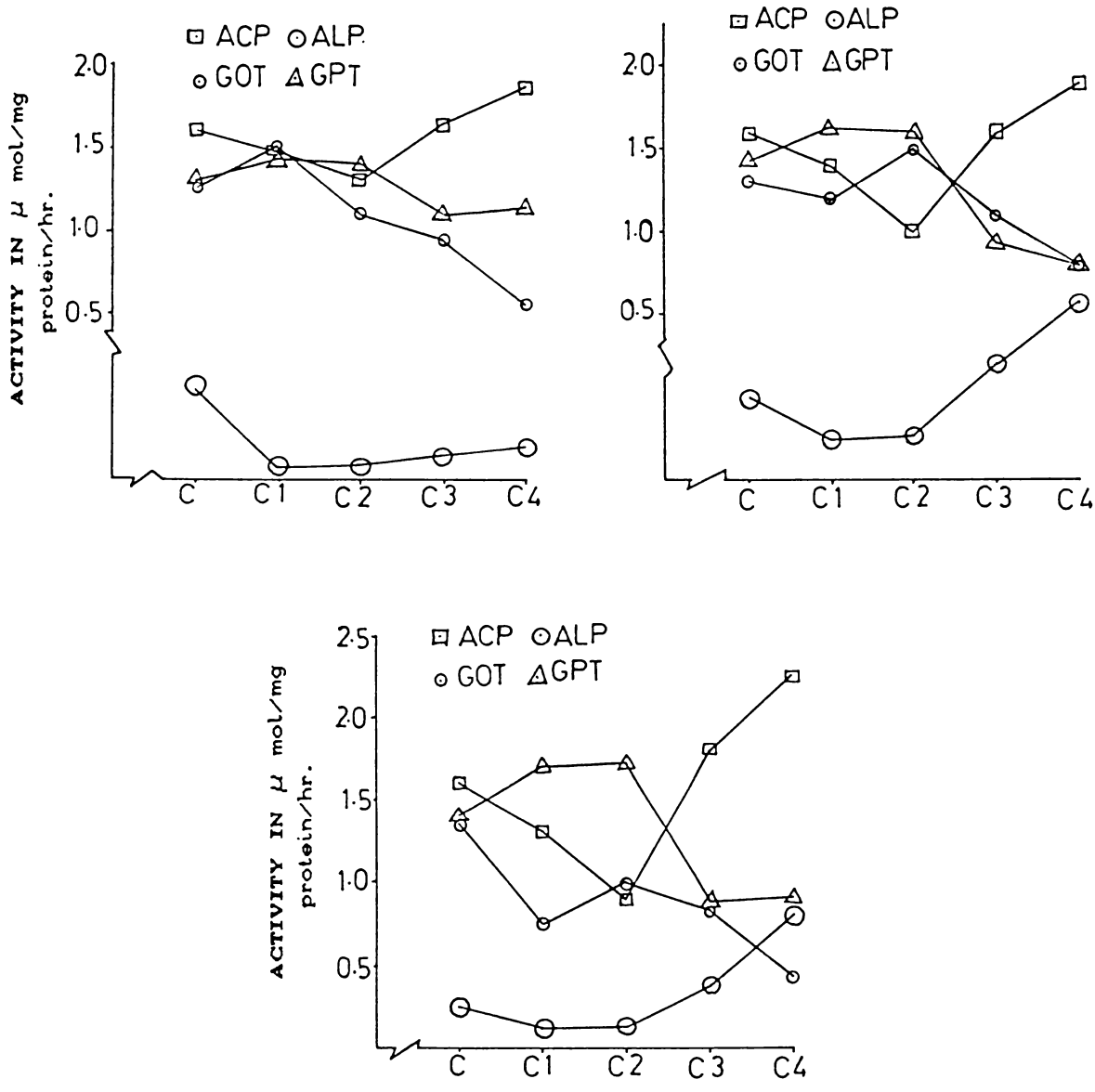

Fig.8. *Etroplus maculatus*: Mean enzyme activity of ALP, ACP, GOT and GPT in liver of control fish (C) and those pre-exposed to 4 SLC of DDT for (a) 24, (b) 72 and (c) 120 hrs.

(C_3 and C_4) during 20 and 30 days of exposure. The activity of ALP was found decreasing with the increase of concentration, and the change between the activity in lowest and the highest concentrations was found significant during the long term exposure. The elevation of ALP activity in all the exposed concentrations during 20 day exposure was found significant when compared to the activity in all these concentrations during 10 day exposure, while no significant change in activity between 10 and 30 days, and 20 and 30 days was found during the long term exposure to DDT (Table 5, *6 & 7*, Fig. *9*).

4.2.1.1.3.2. Acid phosphatase (ACP)

Short term:- Significant elevation of ACP activity from the respective controls was noted in the highest two concentrations (C_3 and C_4) during 24 hour and 120 hour, and in the highest concentration (C_4) during 72 hour exposure. Significant activity reduction from the control was found in the lowest two concentrations (C_1 and C_2) during the short term exposure. The liver ACP activity was found significantly increasing with the exposed concentration and with exposure duration during the short term exposure (Table *8, 9 & 10*, Fig. *8*).

Long term:- The liver ACP showed significant inhibition of activity from the respective control values in all the four concentrations during the long term exposure. In general, gradual reduction

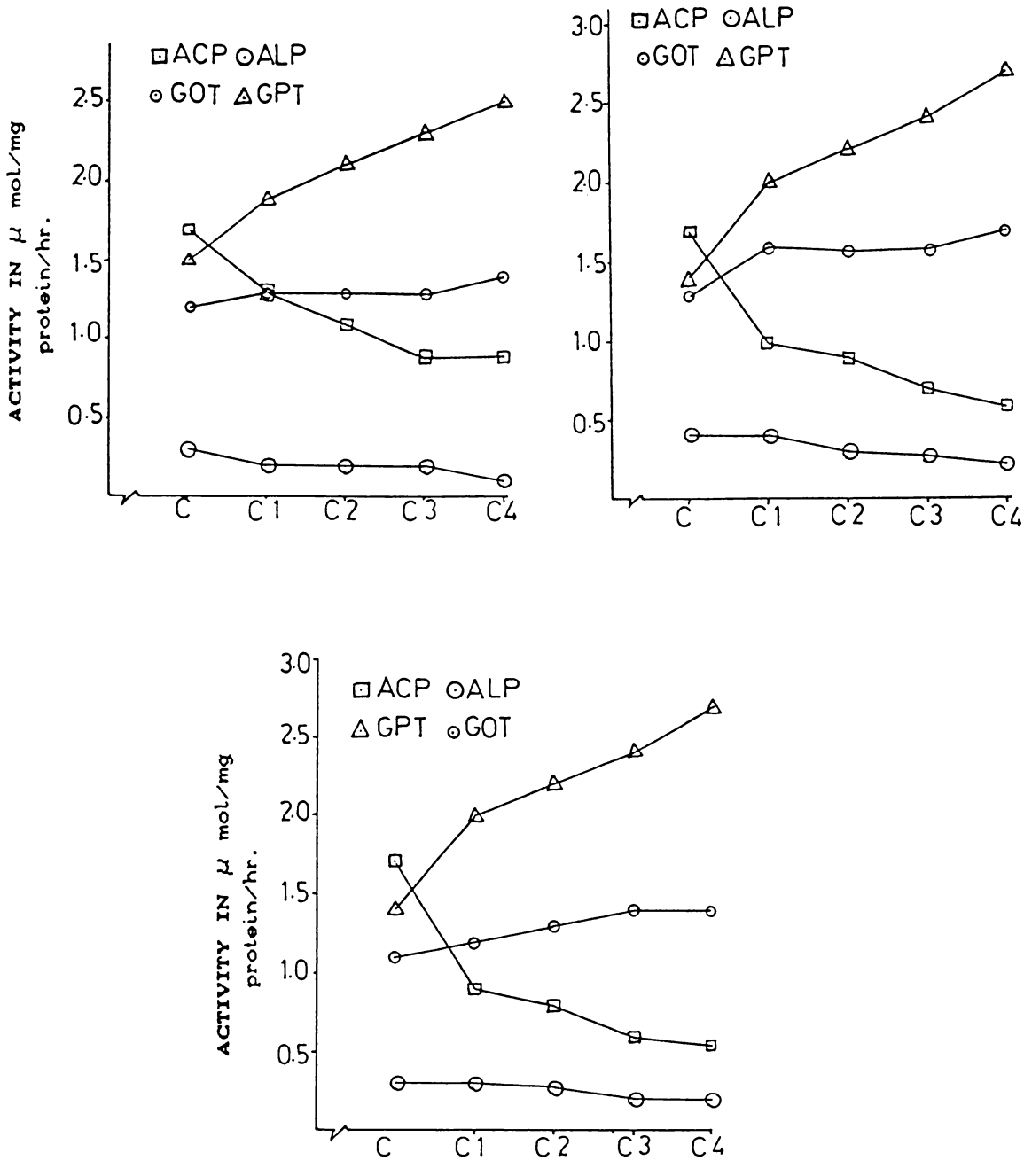

Fig.9. *Etroplus maculatus*: Mean enzyme activity of ALP, ACP, GOT and GPT in liver of control fish (C) and those pre-exposed to 4 SLC of DDT for (a) 10, (b) 20 and (c) 30 days.

in activity was noticed with increase of concentration as well as exposure of duration, and the value in many cases was significant (Table 10, 11 & 12, Fig. 9).

4.2.1.1.3.3 Glutamate oxaloacetate transaminase (GOT)

Short term:- The liver GOT activity showed significant stimulation from the respective controls in the lowest concentration (C_1) during 24 hour, and in C_2 during 72 hour exposure, while in the three higher concentrations (C_2, C_3 and C_4) during 24 hour; in C_3 and C_4 during 72 hours, and in all the four exposed concentrations during 120 hour, the liver GOT showed significant reduction. Among concentration a dose dependent reduction in activity was observed during 24 hours, and a change in activity with increase in exposed concentration was found significant during 72 hour exposure also. During 120 hour (except the activity change between C_1 and C_3) significant change in activity was found among the exposed concentrations. The liver GOT in three higher concentrations showed significant elevation in activity during 72 hour from the activity observed in 24 and 120 hours exposure (Table *14, 15 & 16,* Fig. *8*).

Long term:- Significant elevation of liver GOT activity from the respective control values was found in the highest concentration (C_4) during 10 day, in all the concentrations during 20 day and

in three higher concentrations (C_2, C_3 and C_4) during 30 day exposure. Comparatively higher activity was obtained in higher concentrations during long term exposure. The elevation of activity in all the four concentrations during 20 day exposure was found significant when compared to the activity in the respective concentrations during 10 and 30 days exposure to DDT (Table 17, 18 & 19, Fig. 9).

4.2.1.1.3.4 Glutamate pyruvate transaminase (GPT)

Short term:- Significant reduction of GPT activity in liver from the respective control values was found in the highest concentration (C_4) during 24 hour, and in the two highest concentrations (C_3 and C_4) during 72 and 120 hours exposure, while in the lower two concentrations (C_1 and C_2), the liver GPT was found elevated significantly during 120 hour from the control. The enzyme activity was found decreasing with increase in DDT concentration and the reduction in GPT activity in the higher two concentrations from the lower two concentrations was found significant during the short term exposure. The liver GPT activity in the lower two concentrations was found increasing with exposure duration and the change was found significant except in activity between 72 and 120 hour exposure in the lowest concentration (C_1). In the highest two concentrations (C_3 and C_4) the change in activity with exposure duration was found significant (Table 20, 21 & 22, Fig. 8).

Long term:- The liver GPT activity showed significant elevation from the respective controls in all the exposed concentrations during long term exposure. The activity of GPT was found increasing with increase in DDT concentration, and the increase was found significant during 20 and 30 days exposure. No significant change in GPT activity could be observed in the four exposed concentration with increase of exposure duration (Table 23, 24 & 25, Fig. 9).

4.2.1.2 Dimecron

4.2.1.2.1 Brain

4.2.1.2.1.1 Alkaline phosphatase (ALP)

Short term:- The brain ALP activity showed highly significant stimulation from the control in the three lower concentrations during 24, 72 and 120 hours exposure. Interestingly, in C_4 there was significant elevation in activity during 24 hour exposure but significant reduction in activity at 120 hours. The activity of brain ALP was found decreasing with the increase of exposure concentration, while it showed stimulation in C_1 and C_2 with the exposure duration, reduction was observed in C_3 and C_4. In general, the stimulation of activity was found more enhanced in the lower concentrations than with the higher ones, when compared with the controls (Table 26, 27 & 28, Fig. 10).

TABLE 26

Etroplus maculatus: In vivo enzyme activity of ALP in brain, gill and liver of control fish and those pre-exposed to four SLC of DIMECRON for 24 hrs.

		CONTROL	C1 0.01ppm	C2 0.022ppm	C3 0.043ppm	C4 0.086ppm
BRAIN	a	0.4879 +/-0.019	0.836 +/-0.015	0.769 +/-0.016	0.731d +/-0.009	0.6751 +/-0.029
	b		+71.35	+57.6	+49.91	+38.37
	c		P<0.001	P<0.07001	P<0.001	P<0.001
GILL	a	0.4092 +/-0.122	0,269 +/-0.0451	0.4718 +/-0.132	0.476 +/-0.071	0.5454 +/-0.055
	b		-34.26	+15.29	+16.32	+33.28
	c		NS	NS	NS	NS
LIVER	a	0.2039 +/-0.093	0.413 +/-0.018	0.5106 +/-0.025	0.551d +/-0.015	0.412 +/-0.016
	b		+102.55	+150.42	+170.43	+102.06
	c		P<0.001	P<0.001	P<0.001	P<0.001

TABLE 27

Etroplus maculatus: In vivo enzyme activity of ALP in brain, gill and liver of control fish and those pre-exposed to four SLC of DIMECRON for 72 hrs.

		CONTROL	C1 0.01ppm	C2 0.022ppm	C3 0.043ppm	C4 0.086ppm
BRAIN	a	0.522d +/-0.042	0.8848 +/-0.009	0.8087 +/-0.012	0.6941 +/-0.026	0.5202 +/-0.007
	b		+69.37	+54.80	+32.87	-0.42
	c		P<0.001	P<0.001	P<0.001	NS
GILL	a	0.481 +/-0.15	0.5271 +/-0.067	0.5822 +/-0.061	0.5361 +/-0.07d	0.5203 +/-0.058
	b		+9.58	+21.04	+11.46	+8.17
	c		NS	NS	NS	NS
LIVER	a	0.2376 +/-0.078	0.3181 +/-0.021	0.4708 +/-0.011	0.4118 +/-0.024	0.3507 +/-0.007
	b		+33.88	+98.15	+73.31	+47.35
	c		P<0.01	P<0.001	+.P<0.001	P<0.001

a: Mean enzyme activity (V=3) in u mol p-nitrophenol liberated /r pro./hr

b: % Alteration from the mean control value

TABLE 28

Etroplus maculatus: In vivo enzyme activity of ALP in brain, gill and liver of control fish and those pre-exposed to four SLC of DIMECRON for 120 hrs.

		CONTROL	C1 0.01ppm	C2 0.022ppm	C3 0.043ppm	C4 0.086ppm
BRAIN	a	0.5444 +/-0.009	0.9002 +/-0.011	0.8513 +/-0.103	0.6783 +/-0.01	0.4494 +/-0.017
	b		+65.36	+56.37	+24.59	-17.45
	c		P<0.001	P<0.001	P<0.001	P<0.05
GILL	a	0.3106 +/-0.077	0.3455 +/-0.007	0.4036 +/-0.089	0.414 +/-0.077	0.3636 +/-0.069
	b		+11.34	+29.94	+33.29	+17.06
	c		NS	NS	NS	NS
LIVER	a	0.198 +/-0.058	0.2438 +/-0.002	0.3877 +/-0.016	0.3477 +/-0.003	0.3163 +/-0.019
	b		+23.13	+95.81	+75.61	+59.75
	c		P<0.05	P<0.001	P<0.001	P<0.01

TABLE 29

Etroplus maculatus: In vivo enzyme activity of ALP in brain, gill and liver of control fish and pre-exposed to four SLC of DIMECRON for 10 days.

		CONTROL	C1 0.0043ppm	C2 0.0058ppm	C3 0.0087ppm	C4 0.017ppm
BRAIN	a	0.3733 +/-0.144	0.3636 +/-0.036	0.3167 +/-0.038	0.2786 +/-0.042	0.2563 +/-0.048
	b		-2.59	-15.16	-25.37	-31.34
	c		NS	NS	NS	NS
GILL	a	0.5198 +/-0.095	0.5816 +/-0.023	0.6016 +/-0.014	0.629 +/-0.005	0.5238 +/-0.007
	b		+11.89	+15.74	+21.00	+0.77
	c		NS	NS	NS	NS
LIVER	a	0.2207 +/-0.103	0.1727 +/-0.019	0.1554 +/-0.037	0.1317 +/-0.024	0.1069 +/-0.011
	b		-21.75	-29.59	-40.32	-51 56
	c		NS	NS	NS	NS

a: Mean enzyme activity (N=3) in u mol P-nitrophenol liberated/mg pro./hr
b: % Alteration from the mean control value
c: Level of significance/non significant ns

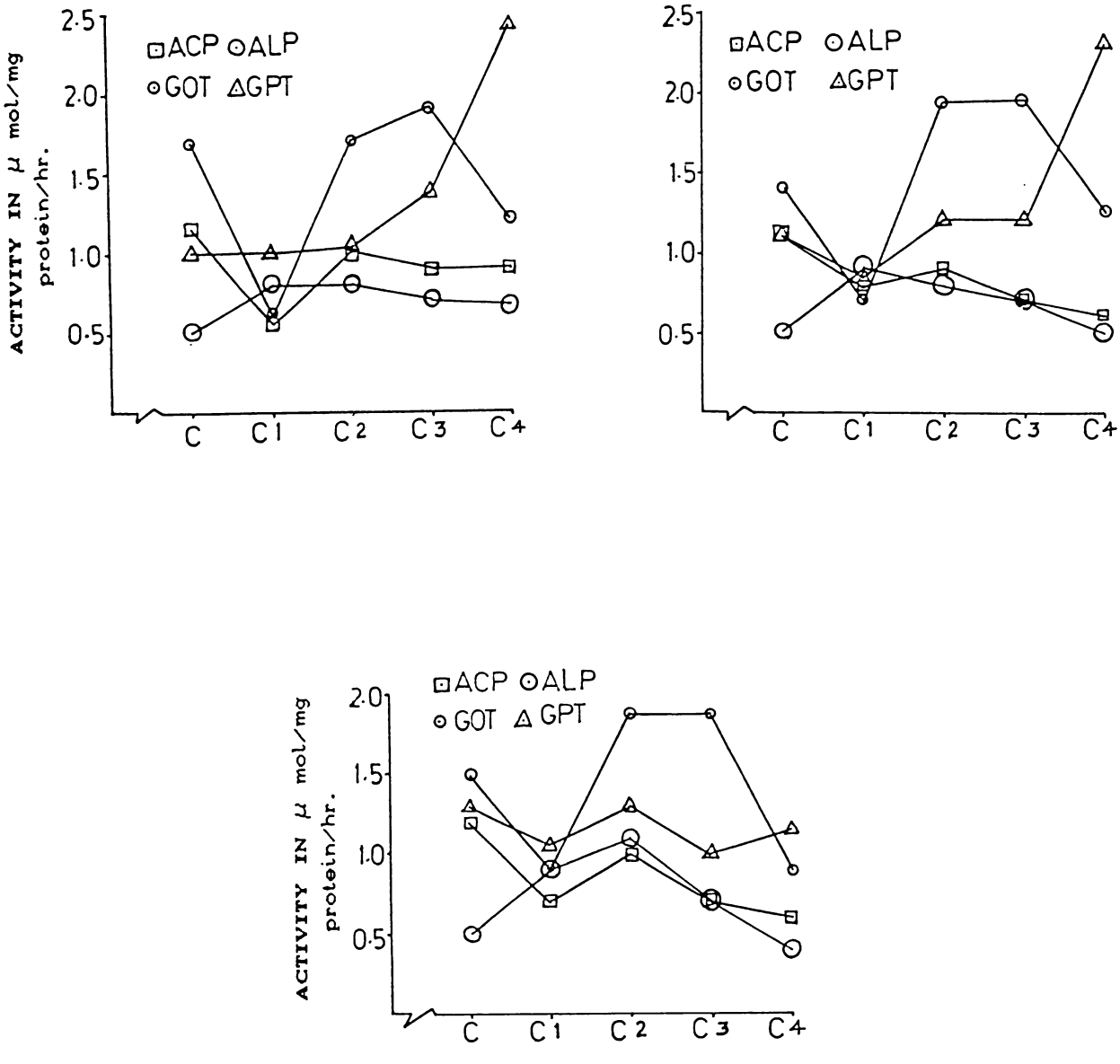

Fig. 10. *Etroplus maculatus*: Mean enzyme activity of ALP, ACP, GOT and GPT in brain of control fish (C) and those pre-exposed to 4 SLC of Dimecron for (a)24, (b) 72 and (c) 120 hrs.

Long term:- Though the brain ALP activity was found comparatively lower than control value in all the four exposed concentrations during the long term exposure, no significant alteration of activity could be observed. Generally, the ALP activity showed reduction with the increase of dose as well as exposure period (Table 29, 30 & 31, Fig. 11).

4.2.1.2.1.2 Acid phosphatase (ACP)

Short term:- The activity of brain ACP showed significant reduction from the control in all the four concentrations during all time-periods of exposure. The maximum activity was noted in C_2 among the exposed groups during the short term exposure, and the minimum activity, in general, was found in C_4. In all concentrations except C_1 the activity of ACP showed decreasing trend with increase of dose (Table 32, 33 & 34, Fig. 10).

Long term:- The brain ACP activity was found significantly reduced when compared to the control in C_4 during 10 day, in C_2, C_3 and C_4 during 20 day, and in all the four concentrations during 30 day exposure. The maximum reduction was noted in C_4 during the long term exposure. During 30 day of exposure, the activity of ACP showed decline with the increase of dose. In all concentrations except C_1,

TABLE 30

Etroplus maculatus: In vivo enzyme activity of ALP in brain, gill and liver of control fish and those pre-exposed to four SLC of DIMECRON for 20 days.

		CONTROL	C1 0.0043ppm	C2 0.0058ppm	C3 0.0087ppm	C4 0.017ppm
BRAIN	a	0.3833 +/-0.188	0.3071 +/-0.038	0.2832 +/-0.033	0.2561 +/-0.037	0.2736 +/-0.067
	b		-19.88	-26.12	-33.19	-28.62
	c		NS	NS	NS	NS
GILL	a	0.4308 +/-0.012	0.4835 +/-0.079	0.3729 +/-0..034	0.23 +/-0.051	0.2263 +/-0.071
	b		+12.23	-13.44	-46.61	-47.47
	c		NS	NS	P<0.01	P<0.01
LIVER	a	0.1339 +/-0.055	0.0925 +/-0.027	0.0767 +/-0.018	0.0795 +/-0.027	0.0655 +/-0.008
	b		-30.92	-42.72	-40.63	-51.08
	c		NS	NS	NS	NS

TABLE 31

Etroplus maculatus: In vivo enzyme activity of ALP in brain, gill and liver of control fish and those pre-exposed to four SLC of DIMECRON for 30 days.

		CONTROL	C1 0.0043ppm	C2 0.0058ppm	C3 0.0087ppm	C4 0.017ppm
BRAIN	a	0.3238 +/-0.107	0.2146 +/-0.041	0.2105 +/-0.049	0.2537 +/-0.066	0.2087 +/-0.042
	b		-33.72	-33.99	-21.65	-35.55
	c		NS	NS	NS	NS
GILL	a	0.3949 +/-0.055	0.3541 +/-0.015	0.3218 +/-0.003	0.2955 +/-0.057	0.3322 +/-0.073
	b		-10.33	-18.51	-25.17	-15.88
	c		NS	NS	NS	NS
LIVER	a	0.2294 +/-0.054	0.147 +/-0.028	0.1402 +/-0.049	0.094 +/-0.018	0.0667 +/-0.033
	b		-35.92	-38.88	-60.59	-70.92
	c		NS	NS	P<0.05	P<0.001

a: Mean enzyme activity (N=3) in u mol nitro phonol liberated/mg pro./hour
b: % Alteration from the mean control value
c: Level of significance/non-significant ns.

TABLE 32

Etroplus maculatus: In vivo enzyme activity of ACP in brain, gill and liver of control fish and those pre-exposed to four SLC of DIMECRON for 24 hrs.

		CONTROL	C1 0.01ppm	C2 0.022ppm	C3 0.043ppm	C4 0.086ppm
BRAIN	a	1.1545 +/-0.0419	0.5444 +/-0.012	0.9627 +/-0.031	0.8698 +/-0.009	0.8672 +/-0.008
	b		-52.84	-16.61	-24.66	-24.88
	c		P<0.05	P<0.01	P<0.001	P<0.001
GILL	a	0.9998 +/-0.037	1.1595 +/-0.056	1.1743 +/-0.022	1.2481 +/-0.063	1.3354 +/-0.046
	b		+15.97	+17.45	+24.83	+33.56
	c		P<0.05	P<0.01	P<0.01	P<0.001
LIVER	a	1.4098 +/-0.021	1.5205 +/-0.002	1.6999 +/-0.021	1.4346 +/-0.004	1.6937 +/-0.02
	b		+7.85	+20.57	+1.75	+20.13
	c		P<0.001	P<0.001	NS	P<0.001

TABLE 33

Etroplus maculatus: In vivo enzyme activity of ACP in brain, gill and liver of control fish and those pre-exposed to four SLC of DIMECRON 72 hrs.

		CONTROL	C1 0.01ppm	C2 0.022ppm	C3 0.073ppm	C4 0.086ppm
BRAIN	a	1.0742 +/-0.039	0.8437 +/-0.021	0.8938 +/-0.007	0.714 +/-0.006	0.6244 +/-0.014
	b		-21.45	-16.79	-33.53	-41.87
	c		P<0.001	P<0.001	P<0.001	P<0.001
GILL	a	1.0156 +/-0.072	1.1963 +/-0.059	1.2267 +/-0.034	1.2957 +/-0.062	1.3758 +/-0.078
	b		+17.79	+20.98	+27.57	+35.46
	c		P<0.05	P<0.01	P<0.01	P<0.01
LIVER	a	1.4334 +/-0.007	1.9748 +/-0.029	1.6824 +/-0.011	1.6563 +/-0.014	1.9158 +/-0.038
	b		+37.77	+17.37	+15.55	+33.65
	c		P<0.001	P<0.001	P<0.001	P<0.001

a: Mean enzyme activity (N=3) in u mol nitro phonol liberated/mg pro./ hr.
b: % Alteration from the mean control value
c: Level of significance/non-significant ns

TABLE 34

Etroplus maculatus: In vivo enzyme activity of ACP in brain, gill and liver of control fish and those pre-exposed to four SLC of Dimecron for 120 hrs.

		CONTROL	C1 0.01ppm	C2 0.022ppm	C3 0.043ppm	C4 0.086ppm
BRAIN	a	1.1692 +/-0.109	0.7364 +/-0.013	0.9623 +/-0.018	0.6983 +/-0.002	0.584 +/-0.014
	b		-37.01	-17.69	-40.27	-50.05
	c		P<0.001	P<0.001	P<0.001	P<0.001
GILL	a	1.0146 +/-0.056	1.2569 +/-0.027	1.3108 +/-0.037	1.3879 +/-0.055	1.4129 +/-0.047
	b		+23.88	+29.19	+36.79	+39.25
	c		P<0.01	P<0.01	P<0.01	P<0.001
LIVER	a	1.4621 +/-0.068	2.2797 +/-0.028	2.2798 +/-0.032	1.9111 +/-0.022	2.7673 +/-0.069
	b		+55.91	+55.92	+30.70	+89.26
	c		P<0.001	P<0.001	P<0.001	P<0.001

TABLE 35

Etroplus maculatus: In vivo enzyme activity of ACP in brain, gill and liver of control fish and those pre-exposed to four SLC of Dimecron for 10 days.

		CONTROL	C1 0.0043ppm	C2 0.0058ppm	C3 0.0087ppm	C4 0.017ppm
BRAIN	a	1.2236 +/-0.199	1.0774 +/-0.026	1.0339 +/-0.024	1.1319 +/-0.013	0.8174 +/-0.024
	b		-11.94	-15.50	-7.49	-33.19
	c		NS	NS	NS	P<0.05
GILL	a	1.0216 +/-0.051	0.8797 +/-0.057	0.7987 +/-0.054	0.674 +/-0.028	0.5555 +/-0.011
	b		-13.88	-21.81	-34.02	-45.62
	c		P<0.05	P<0.01	P<0.001	P<0.001
LIVER	a	1.5213 +/-0.207	1.5744 +/-0.139	1.4996 +/-0.149	1.169 +/-0.043	1.1131 +/-0.116
	b		+3.49	-1.42	+23.15	-26.83
	c		NS	NS	P<0.05	P<0.05

a: Mean enzyme activity (N=3) in u mol p-nitro phenol liberated/mg pro./hr.
b: % Alteration from the mean control value.
c: Level of significance/non significant ns.

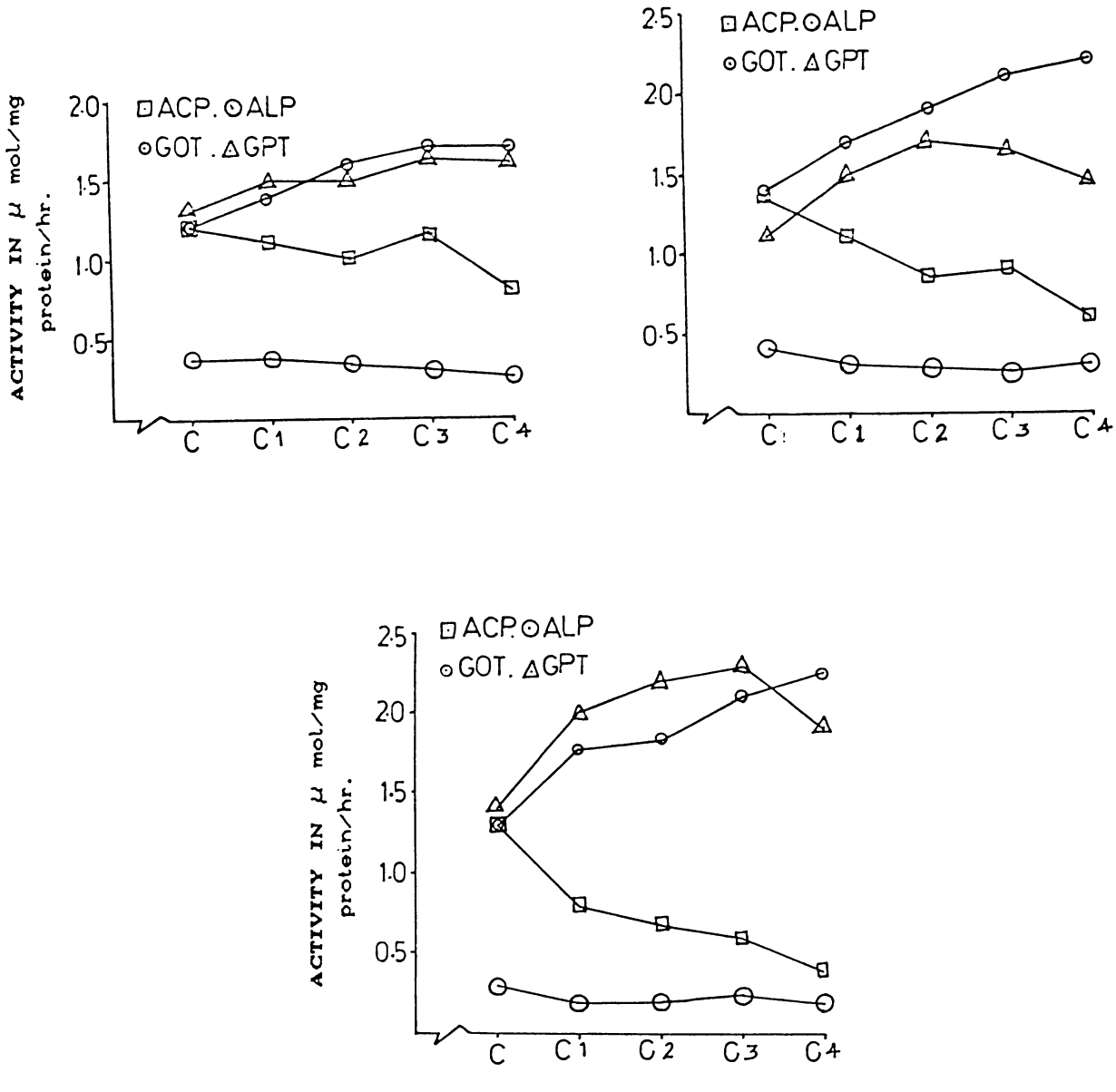

Fig.11. *Etroplus maculatus*: Mean enzyme activity of ALP, ACP, GOT and GPT in brain of control fish (C) and those pre-exposed to 4 SLC of Dimecron for (a)10, (b) 20 and (c) 30 days.

the brain ACP activity was found decreasing significantly with the exposure duration. In C_1 the reduction of activity during 30 day was found significantly altered from both 10 and 20 days exposure (Table *35*, *36 & 37*, Fig. *11*).

4.2.1.2.1.3 Glutamate oxaloacetate transaminase (GOT)

Short term:- The brain GOT value showed significant reduction from the control in C_1 and C_4 during 24, 72 and 120 hours exposure, and interestingly in C_3 showed significant stimulation during the short exposure. No definite dose dependent or duration dependent (in each concentration) responses were observed in the GOT activity during short term exposure (Table *38*, *39 & 40*, Fig. *10*).

Long term:- Significant stimulation in brain GOT activity from the control was found in the three higher concentrations (i.e. C_2, C_3 and C_4) during 10 and 20 days, and in all the four concentrations during 30 day exposure. The stimulation of GOT activity was found more pronounced in the higher concentrations during 30 day exposure. The GOT activity was found increasing with the increase in dose and significant change was observed in all the concentrations during 20 and 30 days exposure. The activity was also found increasing with the increase in exposure time in each of the exposed concentrations,

TABLE 36

Etroplus maculatus: In vivo enzyme activity of ACP in brain, gill and liver of control fish and those pre-exposed to four SLC of Dimecron for 20 days.

		CONTROL	C1 0.0043ppm	C2 0.0058ppm	C3 0.0087ppm	C4 0.017ppm
BRAIN	a:	1.3752 +/-0.233	1.1114 +/-0.058	0.8548 +/-0.044	0.9322 +/-0.048	0.6261 +/-0.082
	b:		-19.18	-37.84	-32.21	-54.47
	c:		NS	P<0.05	P<0.05	P<0.01
GILL	a:	1.0514 +/-0.069	0.8575 +/-0.057	0.6855 +/-0.0008	0.537 +/-0.092	0.3008 +/-0.065
	b:		-18.44	-34.80	-48.92	-71.39
	c:		P<0.05	P<0.001	P<0.01	P<0.001
LIVER	a:	1.3061 +/-0.136	1.0461 +/-0.068	0.959 +/-0.074	0.7951 +/-0.066	0.636 +/-0.105
	b:		-19.90	-26.57	-39.12	-51.30
	c:		P<0.05	P<0.01	P<0.01	P<0.01

TABLE 37

Etroplus maculatus: In vivo enzyme activity of ACP in brain, gill and liver of control fish and those pre-exposed to four SLC of Dimecron for 30 days.

		CONTROL	C1 0.0043ppm	C2 0.0058ppm	C3 0.0087ppm	C4 0.017ppm
BRAIN	a:	0.3414 +/-0.21	0.8248 +/-0.069	0.7216 +/-0.026	0.6459 +/-0.072	0.4274 +/-0.037
	b:		-38.51%	-46.21%	-51.85%	-68.14%
	c:		P<0.05	P<0.01	P<0.01	P<0.01
GILL	a:	1.0011 +/-0.009	0.8531 +/-0.019	0.7686 +/-0.093	0.5598 +/-0.035	0.3621 +/-0.0009
	b:		-14.78%	-23.22%	-99.44%	-63.83
	c:		P<0.001	P<0.05	P<0.001	P<0.001
LIVER	a:	1.3285 +/-0.028	0.9291 +/-0.059	0.8619 +/-0.091	0.6281 +/-0.074	0.4715 +/-0.087
	b:		-30.06%	-35.12%	-52.72	-64.50
	c:		P<0.01	P<0.001	P<0.001	P<0.001

a: Mean enzyme activity (N=3) in u mol p-nitrophenol liberated/mgpro./hr.
b: %Alteration from the mean control value.
c: Level of significance/non significant ns.

TABLE 38

Etroplus maculatus: In vivo enzyme activity of GOT in brain, gill and liver of control fish and those pre-exposed to four SLC of DIMECRON for 24 hours.

		CONTROL	C1 0.01ppm	C2 0.022	C3 0.043	C4 0.086
BRAIN	a:	1.6803 +/-0.043	0.561 +/-0.007	1.7182 +/-0.007	1.9381 +/-0.027	1.211 +/-0.013
	b:		-66.61	+2.26	+15.34	-27.93
	c:		P<0.001	NS	P<0.01	P<0.001
GILL	a:	0.4401 +/-0.105	0.5461 +/-0.086	0.5335 +/-0.029	0.6241 +/-0.032	0.6409 +/-0.071
	b:		+24.09	+21.22	+41.81	+45.63
	c:		NS	NS	P<0.05	NS
LIVER	a:	1.3304 +/-0.127	0.9625 +/-0.07	0.7574 +/-0.011	0.6914 +/-0.006	0.8225 +/-0.012
	b:		-27.65	-43.07	-48.04	-38.18
	c:		P<0.05	P<0.01	P<0.001	P<0.01

TABLE 39

Etroplus maculatus: In vivo enzyme activity of GOT in brain, gill and liver of control fish and those pre-exposed to four SLC of DIMECRON for 72 hours.

		CONTROL	C1 0.01	C2 0.022	C3 0.043	C4 0.086
BRAIN	a:	1.4334 +/-0.127	0.7128 +/-0.007	1.905 +/-0.008	1.929 +/-0.014	1.2529 +/-0.005
	b:		-50.27	+32.90	+34.58	-12.59
	c:		P<0.001	P<0.01	P<0.01	NS
GILL	a:	0.6361 +/-0.098	0.6788 +/-0.064	0.683 +/-0.059	0.7287 +/-0.093	0.7341 +/-0.035
	b:		+6.71	+7.37	+14.56	+15.41
	c:		NS	NS	NS	NS
LIVER	a:	1.2947 +/-0.127	0.8764 +/-0.071	0.8899 +/-0.021	0.763 +/-0.006	0.9735 +/-0.003
	b:		-32.31	-31.27	-41.07	-24.81
	c:		P<0.05	P<0.01	P<0.01	P<0.05

a: Mean enzyme activity (N=3) in u mol sodium pyruvate liberated/mg pro./hr.
b: % Alteration from the mean control value.
c: Level of significance/non significant ns.

TABLE 40

Etroplus maculatus: In vivo enzyme activity of GOT in brain, gill and liver of control fish and those pre-exposed to four SLC of DIMECRON for 120 hours.

		CONTROL	C1 0.01	C2 0.022	C3 0.043	C4 0.086
BRAIN	a:	1.4761 +/-0.139	0.9306 +/-0.003	1.8604 +/-0.004	1.8791 +/-0.029	0.8893 +/-0.009
	b:		-36.96	+26.03	+27.30	+39.75
	c:		P<0.01	P<0.01	P<0.01	P<0.01
GILL	a:	0.6844 +/-0.112	0.4744 +/-0.052	0.4384 +/-0.064	0.4595 +/-0.095	0.4112 +/-0.027
	b:		-30.68	-35.94	-32.86	-39.92
	c:		P<0.05	P<0.05	NS	P<0.05
LIVER	a:	1.1404 +/-0.016	0.6512 +/-0.071	0.6943 +/-0.006	0.8137 +/-0.064	1.1314 +/-0.033
	b:		-42.90	-39.12	-28.65	-0.78
	c:		P<0.001	P<0.001	P<0.01	NS

TABLE 41

Etroplus maculatus: In vivo enzyme activity of GOT in brain, gill and liver of control fish and those pre-exposed to four SLC of DIMECRON for 10 days.

		CONTROL	C1 0.0043	C2 0.0058	C3 0.0087	C4 0.017
BRAIN	a:	1.1985 +/-0.174	1.3601 +/-0.133	1.5633 +/-0.064	1.6978 +/-0.092	1.6822 +/-0.043
	b:		+13.48	+30.44	+41.66	+40.36
	c:		NS	P<0.05	P<0.05	P<0.01
GILL	a:	0.6029 +/-0.082	0.7294 +/-0.094	0.814 +/-0.08	0.8787 +/-0.026	0.8989 +/-0.081
	b:		+20.98	+35.01	+45.75	+49.10
	c:		NS	P<0.05	P<0.01	P<0.05
LIVER	a:	1.4406 +/-0.092	1.7082 +/-0.197	1.8948 +/-0.073	2.0741 +/-0.087	2.1832 +/-0.079
	b:		+18.58	+31.53	+43.97	+51.55
	c:		NS	P<0.01	P<0.001	P<0.001

a: Mean enzyme activity (N=3) in u mol sodium pyruvate liberated/mg pro./hr.

b: %Alteration from the mean control value.

c: Level of significance/non significant ns.

and the activity during 30 day was significantly elevated from both 10 and 20 day activities in C_3 and C_4 and from 10 day exposure in C_1 and C_2 (Table 41, 42 & 43, Fig. 11).

4.2.1.2.1.4 Glutamate pyruvate transaminase (GPT)

Short term:- A significant reduction in GPT activity was found when compared to the control in the lowest exposed concentration during 24, 72 and 120 hours exposure, and in C_3 during 120 hour of exposure. The brain GPT activity showed significant stimulation in C_3 and C_4 during 24 hour, and in C_4 during 72 hour of exposure. In general, the activity of GPT was found increasing with the increase in concentration of test medium. The activity of GPT in all the exposed concentrations during 120 hour of exposure was found significantly reduced from 24 hour GPT activity, while in C_1, C_3 and C_4, it significantly reduced from 72 hour of exposure also (Table *44, 45 & 46*, Fig. *10*).

Long term:- The brain GPT activity was found significantly stimulated from the respective control value in C_3 and C_4 during 10 days, and in all the four exposed concentrations during 20 and 30 days exposure. In the lower three concentrations, the GPT showed comparative increase with the dose. During 30 day exposure, in C_1, C_2 and

TABLE 42

Etroplus maculatus: In vivo enzyme activity of GOT in brain, gill and lever of control fish and those pre-exposed to four SLC of DIMECRON for 20 days.

		CONTROL	C1 0.0043	C2 0.0058	C3 0.0087	C4 0.017
BRAIN	a:	1.4113 +/-0.173	1.7023 +/-0.08	1.9086 +/-0.076	2.0753 +/-0 023	2.2047 +/-0.019
	b:		+20.62	+35.24	+47.05	+56.22
	c:		NS	P<0.05	P<0.01	P<0.01
GILL	a:	0.7404 +/-0.088	1.0464 +/-0.01	1.2695 +/-0.084	1.3155 +/-0.002	1.1496 +/-0.098
	b:		+41.33	+71.46	+77.67	+55.26
	c:		P<0.01	P<0.01	P<0.001	P<0.01
LIVER	a:	1.2154 +/-0.364	1.6057 +/-0.057	1.7692 +/-0.032	1.8753 +/-0.075	- - - - - -
	b:		+32.11	+45.57	+54.29	- - -
	c:		NS	NS	P<0.05	- - -

TABLE 43

Etroplus maculatus: In vivo enzyme activity of GOT in brain, gill and lever of control fish and those pre-exposed to four SLC of DIMECRON for 30 days.

		CONTROL	C1 0.0043	C2 0.0058	C3 0.0087	C4 0.017
BRAIN	a:	1.3204 +/-0.129	1.783 +/-0.186	1.8419 +/-0.128	2.119 +/-0.011	2.2656 +/-0.008
	b:		+35.03	+39.49	+60.48	+71.58
	c:		P<0.05	P<0.01	P<0.001	P<0.001
GILL	a:	0.7382 +/-0.042	1.0524 +/-0.065	1.2485 +/-0.038	1.3489 +/-0.008	1.3422 +/-0.033
	b:		+42.56	+69.12	+82.72	+81.82
	c:		P<0.01	P<0.001	P<0.001	P<0.001
LIVER	a:	1.5067 +/-0.215	2.3128 +/-0.023	2.57 +/-0.088	2.7735 +/-0.076	2.9329 +/-0.058
	b:		+53.50	+70.57	+84.07	+94.65
	c:		P<0.01	P<0.01	P<0.001	P<0.001

a: Mean enzyme activity (N=3) in u mol sodium pyruvate liberated/mg pro./hr.

b: %Alterationfrom the mean control value.

c: Level of significance/non significant ns.

TABLE 44

Etroplus maculatus: In vivo enzyme activity of GPT in brain, gill and lever of control fish and those pre-exposed to four SLC of DIMECRON for 24 hours.

		CONTROL	C1 0.01 ppm	C2 0.022ppm	C3 0.043ppm	C4 0.086ppm
BRAIN	a:	1.0497 +/-0.022	0.964 +/-0.018	1.0357 +/-0.003	1.3659 +/-0.019	2.4167 +/-0.019
	b:		-8.16	-8.16	+30.12	+130.23
	c:		P<0.01	NS	P<0.001	P<0.001
GILL	a:	0.9594 +/-0.038	0.9893 +/-0.096	1.1905 +/-0.022	1.235 +/-0.07	1.253 +/-0.064
	b:		+3.12	+24.09	+28.73	+30.60
	c:		NS	P<0.001	P<0.01	P<0.01
LIVER	a:	1.5154 +/-0.197	1.2089 +/-0.032	1.2776 +/-0.015	1.3783 +/-0.016	1.4574 +/-0.004
	b:		-20.23	-15.69	-9.05	-3.83
	c:		NS	NS	NS	NS

TABLE 45

Etroplus maculatus: In vivo enzyme activity of GPT in brain, gill and lever of control fish and those pre-exposed tp four SLC of DIMECRON for 72 hours.

		CONTROL	C1 0.01ppm	C2 0.022ppm	C3 0.043ppm	C4 0.086ppm
BRAIN	a:	1.0995 +/-0.036	0.8524 +/-0.003	1.222 +/-0.009	1.1927 +/-0.004	2.3337 +/-0.039
	b:		-22.47	+11.14	+8.48	+112.25
	c:		P<0.05	NS	NS	P<0.001
GILL	a:	1.0251 +/-0.048	1.3198 +/-0.021	1.2257 +/-0.096	1.5406 +/-0.039	1.445 +/-0.091
	b:		+28.75	+19.57	+50.29	+40.96
	c:		P<0.001	P<0.05	P<0.001	P<0.01
LIVER	a:	1.479d +/-0.099	1.0477 +/-0.074	1.2226 +/-0.033	1.4741 +/-0.025	1.6272 +/-0.033
	b:		-29.18	-17.36	-0.36	+9.99
	c:		P<0.01	P<0.05	NS	NS

a: Mean enzyme activity (N=3) in u mol sodium pyruvate liberated/mg pro./hr.

b: %Alteration from the mean control value.

c: Level of significance/non significant ns.

TABLE 46

Etroplus maculatus: In vivo activity of GPT in brain,gill and liver of control fish and those pre-exposed to four SLC of Dimecron for 120 hrs.

		CONTROL	C1	C2	C3	C4
BRAIN	a	1.2667 +/-0.007	1.0599 +/-0.017	1.3418 +/-0.023	0.9553 +/-0.015	1.1687 +/-1.1687
	b		-16.33	+5.93	-24.58	-7.74
	c		P<0.05	NS	P<0.01	NS
GILL	a	0.9864 +/-0.03	1.1416 +/-0.051	1.2537 +/-0.085	1.2492 +/-0.02	1.2281 +/-0.027
	b		+15.73	+27.10	+26.64	+24.50
	c		P<0.05	P<0.01	P<0.001	P<0.001
LIVER	a	1.4526 +/-0.0207	1.2159 +/-0.001	1.2563 +/-0.014	1.7895 +/-0.014	1.9758 +/-0.126
	b		-16.29	-13.51	+23.19	+36.02
	c		NS	NS	P<0.05	NS

TABLE 47

Etroplus maculatus: In vivo enzyme activity of GPT in brain, gill and liver of control fish and those pre-exposed to four SLC of Dimecron 10 days.

		CONTROL	C1 0.0043	C2 0.0058	C3 0.0087	C4 0.017
BRAIN	a	1.264 +/-0.169	1,5199 +/-0.182	1.5323 +/-0.168	1.16537 +/-0.059	1.6307 +/-0.053
	b		+20.25	21.23	+30.83	+29.01
	c		NS	NS	P<0.05	P<0.05
GILL	a	1.0309 +/-0.069	1.245 +/-0.073	1.1971 +/-0.018	1.1559 +/-0.055	1.3088 +/-0.032
	b		+20.77	+16.12	+12.13	+26.96
	c		P<0.05	P<0.05	NS	P<0.01

a: Mean enzyme activity (N=3) in u mol sodium pyruvate liberated/mg pro./hour
b: % Alteration from the mean control value
c: Level of significance.nonsignificant ns.

C_3 the GPT activity was found significantly enhanced from 10 and 20 days of exposure (Table 47, 48 & 49, Fig. 11).

4.2.1.2.2 Gill

4.2.1.2.2.1 Alkaline phosphatase (ALP)

Short term:- No significant change in gill ALP activity from the control could be noticed in all the four concentrations during the short term exposure. Nevertheless, comparatively high ALP activity could be observed in three higher concentrations during 24 hour, and in all the four concentrations during 72 and 120 hour of exposure (Table 26, 27 & 28, Fig. 12).

Long term:- In the highest two concentrations (C_3 and C_4) during 20 day exposure, the gill ALP activity was significantly reduced from the control, while in other cases no significant alteration of gill ALP could be observed. Though not significant, comparatively high ALP activity could be noted in all the concentrations during 10 day, and in C_1 during 20 day exposure while in C_2 during 20 day, and in all concentrations during 30 day exposure the gill ALP was found comparatively low (Table 29, 30,& 31, Fig. 13).

TABLE 48

Etroplus emaculatus: In vivo enzyme activity of GPT in brain, gill and liver of control fish and those pre-exposed to four SLC of Dimecron for 20 days.

		CONTROL	C1 0.0043	C2 0.0058	C3 0.0087	C4 0.017
BRAIN	a	1.1498 +/-0.159	1.5466 +/-0.087	1.748 +/-0.046	1.6504 +/-0.063	1.4542 +/-0.077
	b		+34.51	+52.03	+43.54	+26.47
	c		P<0.05	P<0.01	P<0.01	P<0.05
GILL	a	1.0547 +/-0.102	1.3597 +/-0.067	1.2017 +/-0.049	1.2444 +/-0.041	1.5839 +/-0.017
	b		+28.92	+13.94	+17.99	+50.18
	c		P<0.05	NS	P<0.05	P<0.001
LIVER	a	1.4223 +/-0.108	1.9449 +/-0.066	2.447 +/-0.244	2.2914 +/-0.278	2.7521 +/-0.125
	b		+36.74	+72.05	+61.11	+93.50
	c		P<0.01	P<0.01	P<0.01	P<0.001

TABLE 49

Etroplus maculatus: In vivo enzyme activity of GPT in brain, gill and liver of control fish and those pre-exposed to four SLC of Dimecron for 30 days.

		CONTROL	C1 0.0043	C2 0.0058	C3 0.0087	C4 0.017
BRAIN	a	1.14451 +/-0.084	2.007 +/-0.006	2.218 +/-0.049	2.2848 +/-0.037	1.9128 +/-0.045
	b		+38.88	+53.48	+58.11	+32.36
	c		P<0.001	P<0.001	P<0.001	P<0.01
GILL	a	1.0437 +/-0.076	1.3579 +/-0.022	1.1739 +/-0.02	1.2445 +/-0.059	1.9505 +/-0.027
	b		+30.10	+12.47	+19.23	+86.88
	c		P<0.01	P<0.05	P<0.05	P<0.001
LIVER	a	1.3418 +/-0.157	2.1183 +/-0.104	2.1393 +/-0.1	2.4581 +/-0.291	2.5956 +/-0.138
	b		57.87	+59.44	+83.10	+93.44
	c		P<0.01	P<0.01	P<0.01	P<0.001

a: Mean enzyme activity (N=3) in u mol sodium pyruvate liberated/mg pro./hr.
b: % Alteration from the mean control value
c: Level of significance/non sigificance ns

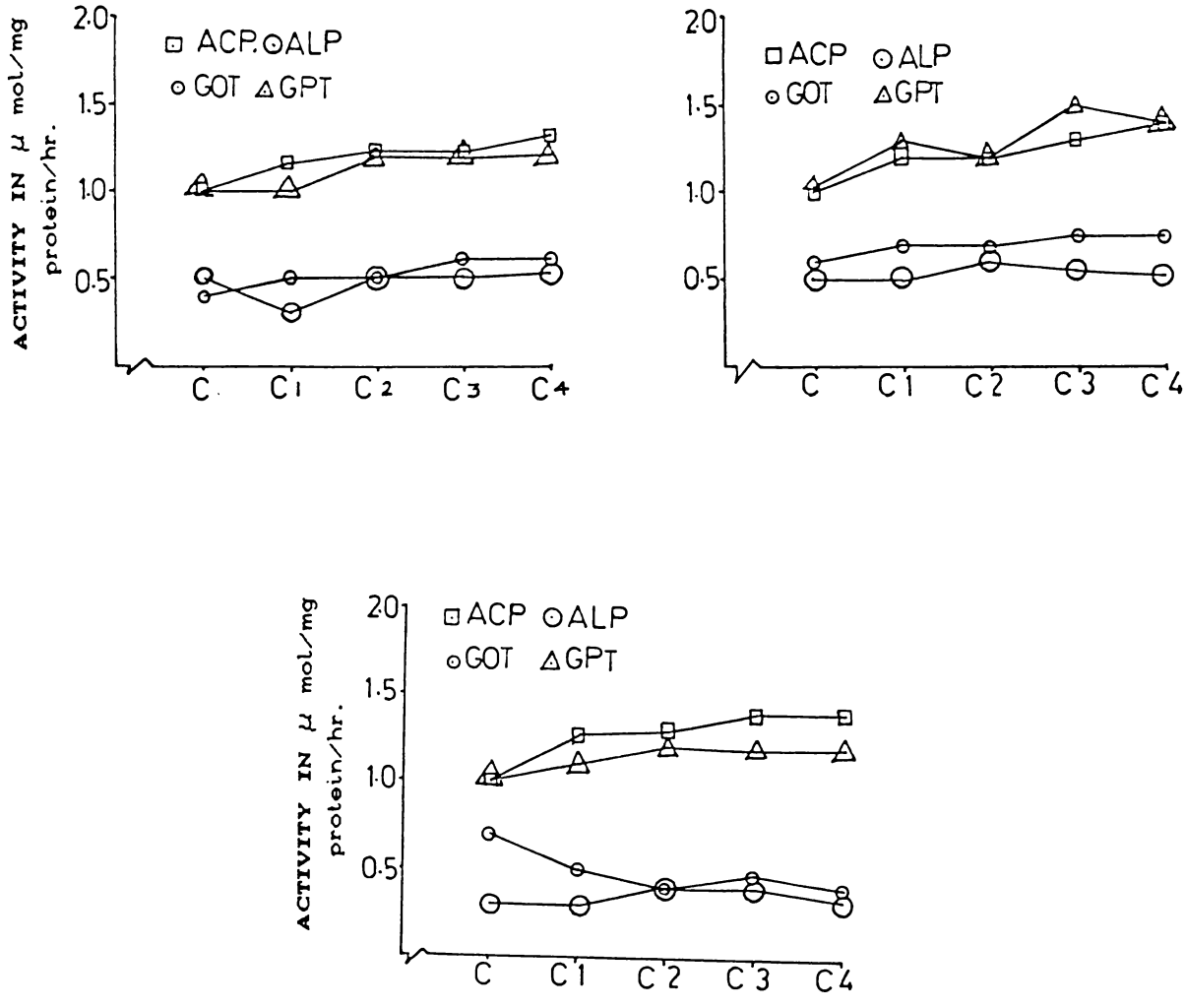

Fig.12. *Etroplus maculatus*: Mean enzyme activity of ALP, ACP, GOT and GPT in liver of control fish (C) and those pre-exposed to 4 SLC of Dimecron for (a)24, (b) 72 and (c) 120 hrs.

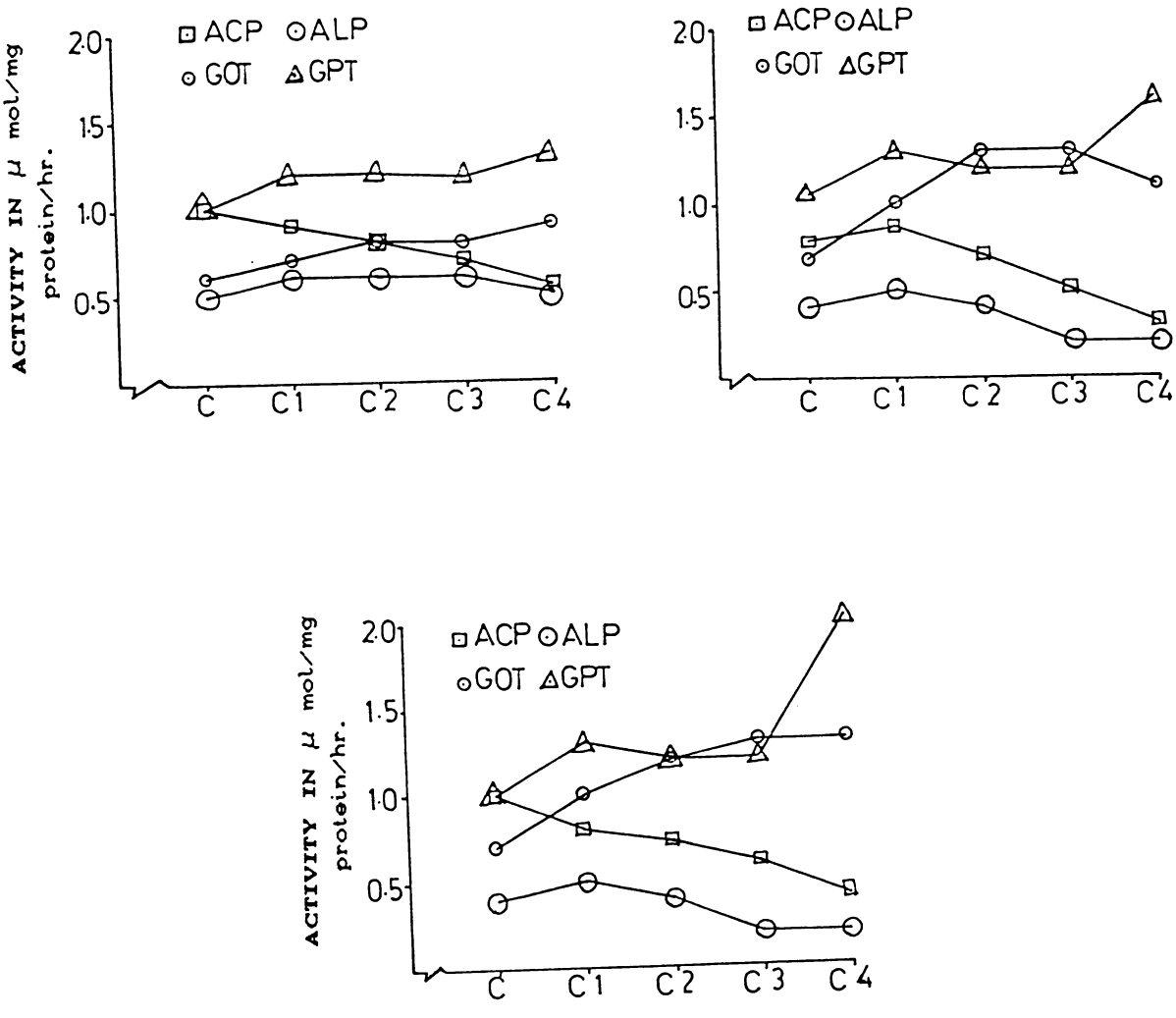

Fig.13. *Etroplus maculatus*: Mean enzyme activity of ALP, ACP, GOT and GPT in gill of control fish (C) and those pre-exposed to 4 SLC of Dimecron for (a)10, (b) 20 and (c) 30 days.

4.2.1.2.2.2 Acid phosphatase (ACP)

Short term:- The activity of gill ACP showed significant stimulation from the control in all the four concentrations during the short term exposure. The activity of gill ACP showed increased stimulation with increase in concentration in concentration as well as with increase in exposure time in each of the concentrations tested. The gill ACP activity in all the four concentrations during 120 hour exposure was found significantly high from 24 hour activity (Table *32*, *33* & *34*, Fig. *12*).

Long term:- Significant reduction in gill ACP activity was found in all the exposed concentrations from the respective controls. In general, a dose dependent and significant reduction of activity was found with increase in exposure concentration, during 10, 20 and 30 days of exposure, but in each concentration it was not generally duration dependent (Table *35*, *36* & *37*, Fig. *13*).

4.2.1.2.2.3 Glutamate oxaloacetate transaminase (GOT)

Short term:- Significant stimulation of gill GOT activity was observed in C_3 during 24 hour of exposure from the control while in C_1, C_2 and C_4 significant reduction of activity was found during

120 hour of exposure. During 24 and 72 hours of exposure the activity of GOT showed a general increasing trend with increase in dose. The activity of gill GOT in all the exposed concentrations showed significant reduction during 120 hour when compared to the activity during 24 hour (Table 38, 39 & 40, Fig. 12).

Long term:- The gill GOT showed significant stimulation of activity from the respective controls in three higher concentrations during 10 day, and in all the four concentrations during 20 and 30 days of exposure. The activity of gill GOT showed stimulation with increase in concentration (except in C_4 during 20 day exposure) as well as with exposure time. The high activity observed in all the concentrations during 20 and 30 days exposure was found significant from the activity during 10 day exposure (Table 41, 42 & 43, Fig. 13).

4.2.1.2.2.4 Glutamate pyruvate transaminase (GPT)

Short term:- The gill GPT activity was found significantly elevated from the respective controls in all the concentrations except in C_1 during 24 hour, and in all the four concentrations during 72 and 120 hours of exposure. The activity was found generally increasing with dose. During 72 hour, the activity of GPT showed comparatively higher value in all the four concentrations both from 24 and 120 hours exposure (Table 44, 45 & 46, Fig. 12).

Long term:- Significant stimulation of GPT activity could be observed in C_1, C_2 and C_4 during 10 day, and in all the concentrations during 20 (except in C_2) and 30 days of exposure when compared to the respective control values. The maximum GPT activity was observed in C_4 followed by C_1. In the highest concentration the highest activity noted during 30 day exposure was found significant when compared to the activities during 10 and 20 days while in other concentrations the change in activity with duration was found insignificant (Table *47*, *48 & 49*, Fig. *13*).

4.2.1.2.3 Liver

4.2.1.2.3.1 Alkaline phosphatase (ALP)

Short term:- Significant elevation of liver ALP activity was found in all the exposed concentrations from the respective controls. In the lower three concentrations, the activity of ALP showed increase with concentration at all time periods. With the increase in exposure time the activity in all the concentrations showed reduction and the reduction was found significant except between 24 and 72 hours in C_2, and between 72 and 120 hours in C_3. In the lowest and highest concentrations, comparatively low activity of ALP was recorded than the intermediate concentrations during short term exposure (Table *26*, *27 & 28*, Fig. *14*).

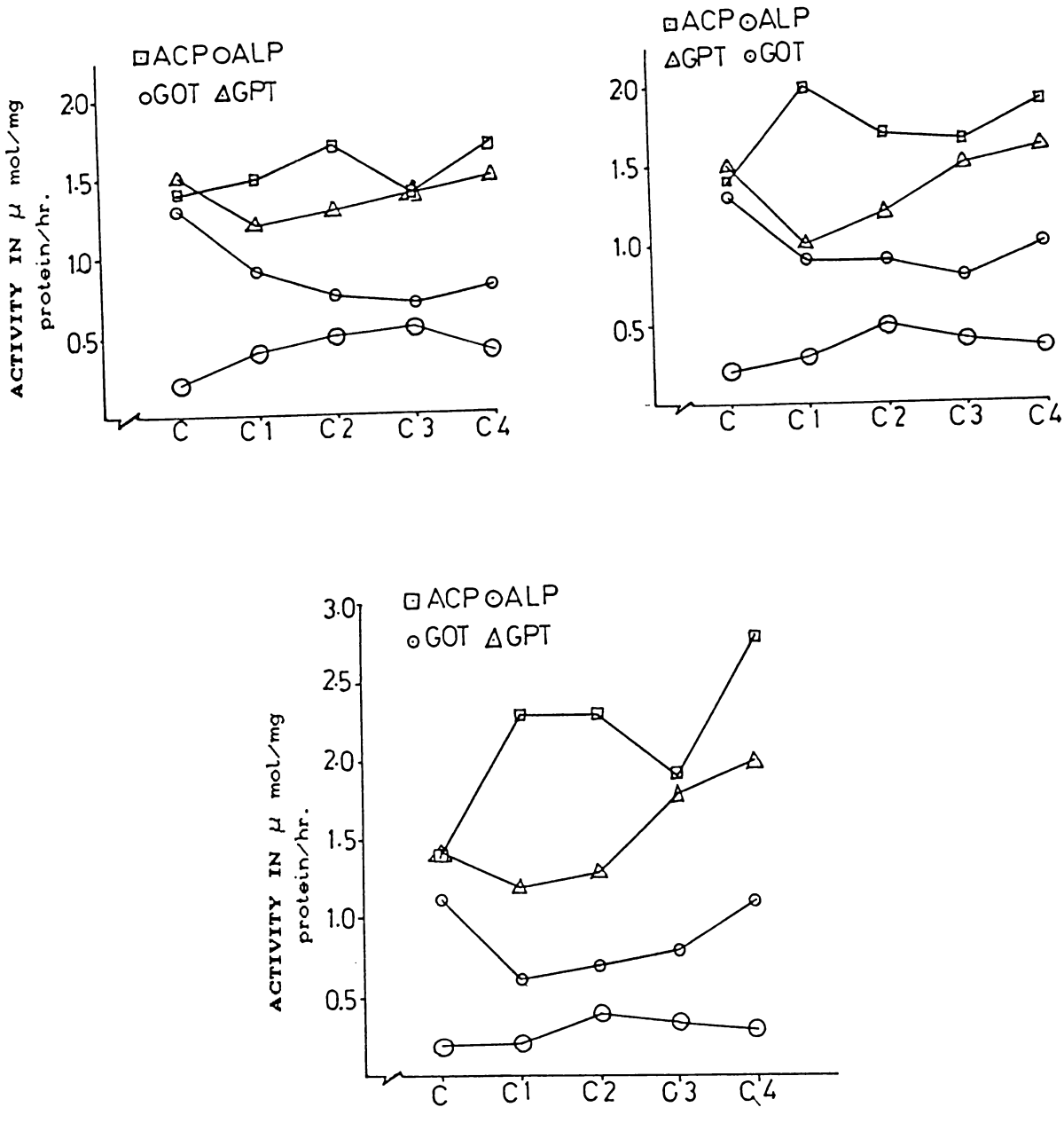

Fig.14. *Etroplus maculatus*: Mean enzyme activity of ALP, ACP, GOT and GPT in liver of control fish (C) and those pre-exposed to 4 SLC of Dimecron for (a)24, (b) 72 and (c) 120 hrs.

Long term:- Though liver ALP activity was found lower than the control in all the exposed concentrations, significant reduction was noted only in C_3 and C_4 during 30 day exposure. Generally, the activity of ALP was found decreasing with increase in concentration. The activity of ALP was found reduced during 20 day in all the three lower concentrations from the 10 and 30 day values while in C_4 reduction of activity with duration could be observed (Table *29*, *30 & 31*, Fig. *15*).

4.2.1.2.3.2 Acid phosphatase (ACP)

Short term:- The liver ACP activity showed highly significant stimulation from the respective controls in the three exposed concentrations of Dimecron (except in C_3 during 24 hours) during the short term exposure. Comparatively higher and significant activity could be observed in the lowest and the highest (i.e. C_1 and C_4) concentrations than the intermediate ones. The activity was found increasing at significant levels in each of the exposed concentration with duration (Table*32*, *33 & 34*, Fig. *14*).

Long term:- Significant reduction of liver ACP from the respective controls could be noted in C_3 and C_4 during 10 day, and in all the four concentrations during 20 and 30 days exposure. The

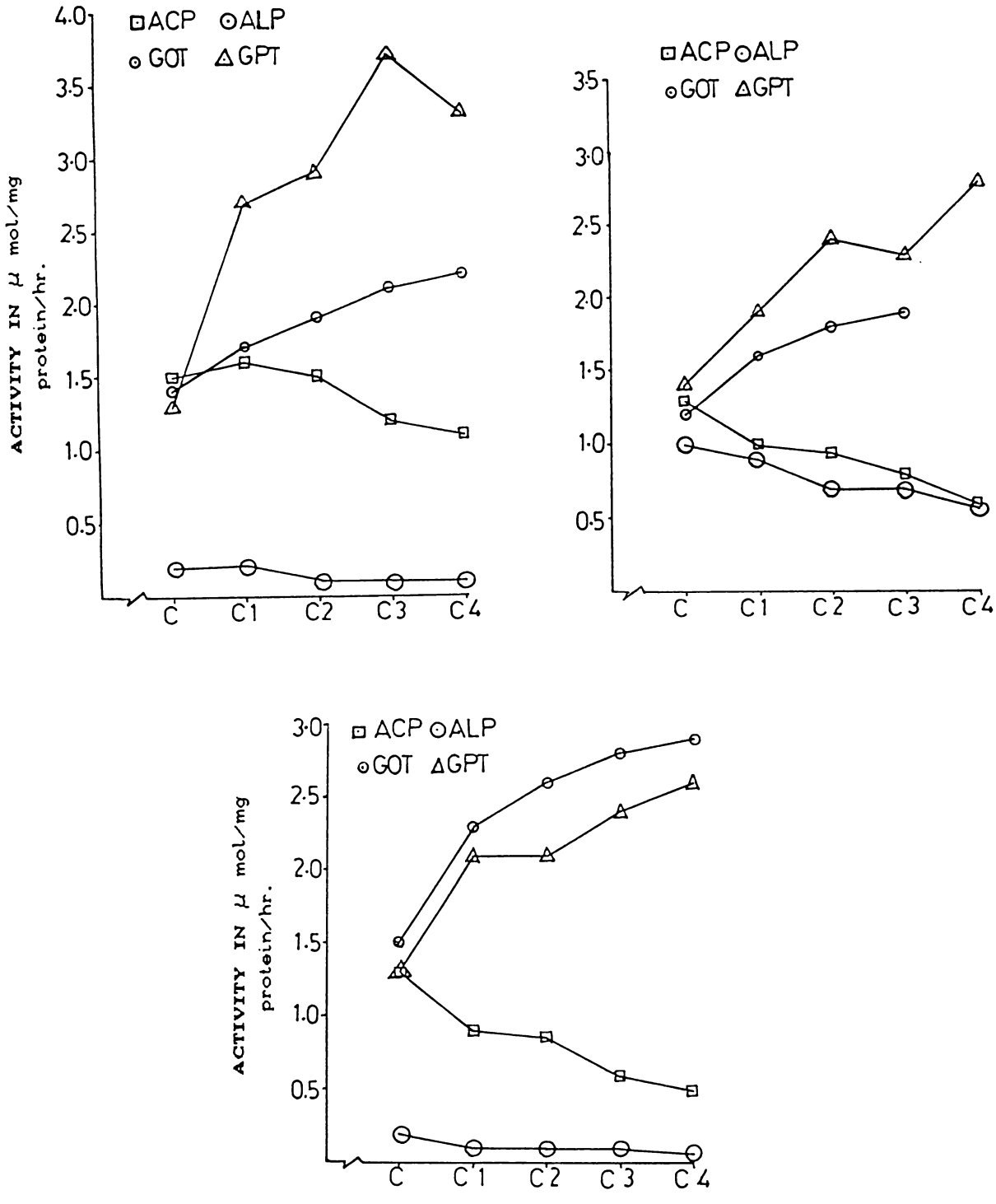

Fig.15. *Etroplus maculatus*: Mean enzyme activity of ALP, ACP, GOT and GPT in liver of control fish (C) and those pre-exposed to 4 SLC of Dimecron for (a)10, (b) 20 and (c) 30 days.

activity was found readily decreasing with increase in concentration as well as with duration in each of the exposed concentrations. The reduction of ACP activity in each concentration with duration was found significant in all the concentrations of Dimecron tested except in C_1, where the reduction of activity during 30 day from 20 day was found insignificant (Table *35, 36 & 37*, Fig. *15*).

4.2.1.2.3.3 Glutamate oxaloacetate transaminase (GOT)

Short term:- The liver GOT showed significant inhibition in activity from the respective control value in all the four exposed concentrations during 24 and 72 hour, and in three lower concentrations during 120 hour exposure. The activity was found to decrease in three lower concentrations with increase in concentration during 24 hour while the activity showed general increase with the increase of dose during 72 and 120 hour exposure. In C_1, C_2 and C_4 the change in activity of GOT during 120 hour exposure was found significant from both 24 and 72 hour activity (Table *38, 39 & 40*, *Fig. 14*).

Long term:- Significant elevation of GOT activity was observed in the three higher concentrations during 10 day, in C_3 during 20 day, and in all the four exposed concentrations during 30 day exposure when compared to the respective control values. The activity was found increasing with the increase in concentration.

TABLE 50

Etropus maculatus: In vivo enzyme activity oof ALP in brain, gill and liver of control fish and those pre-exposed to four SLC of Gramoxone for 24 hrs.

		CONTROL	C1 0.0034ppm	C2 0.0067ppm	C3 0.013ppm	C4 0.028ppm
BRAIN	a	0.2681 +/-0.056	0.3241 +/-0.046	0.5266 +/-0.07	0.6482 +/-0.034	0.6331 +/-0.055
	b		+20.89	+96.42	+141.78	+136.14
	c		NS	P<0.05	P<0.001	P<0.01
GILL	a	0.3767 +/-0.077	0.2575 +/-0.025	0.2435 +/-0.01	0.0925 +/-0.078	0.0625 +/-0.007
	b		-31.64	-35.36	-75.44	-83.41
	c		NS	P<0.05	P<0.05	P<0.01
LIVER	a	0.3863 +/-0.02	0.6623 +/-0.045	0.4854 +/-0.037	0.3925 +/-0.025	0.2895 +/-0.029
	b		+71.45	+25.65	+1.60	-25.06
	c		P<0.001	P<0.05	NS	P<0.01

TABLE 51

Etroplus maculatus: In vivo enzyme activity of ALP in brain,gill and liver of control fish and those pre-exposed to four SLC of Gamoxone for 72 hrs.

		CONTROL	C1 0.0034ppm	C2 0.0067ppm	C3 0.013ppm	C4 0.028ppm
BRAIN	a	0.2403 +/-0.042	0.3237 +/-0.061	0.6691 +/-0.045	0.5407 +/-0.03	0.3954 +/-0.058
	b		+34.71	+178.44	+125.01	+64.54
	c		NS	P<0.001	P<0.001	P<0.05
GILL	a	0.4233 +/-0.151	0.3499 +/-0.091	0.4109 +/-0.109	0.4157 +/-0.02	0.465 +/-0.043
	b		-17.34	-2.93	-1.80	+9.85
	c		NS	NS	NS	NS
LIVER	a	0.3917 +/-0.021	0.8196 +/-0.048	0.6355 +/-0.038	0.5473 +/-0.021	0.4334 +/-0.045
	b		+109.24	+62.24	39.72	+10.64
	c		P<0.001	P<0.001	P<0.001	NS

a: Mean enzyme activity (N=3) in u mol P-Nitro phenol liberated/mg pro./hr
b: % Alteration from the mean control value
c: Level of significance/non significant ns.

TABLE 52

Etroplus maculatus: In vivo enzyme activity of ALP in brain, gill and liver of control fish and those pre-exposed to four SLC of Gramoxone for 120 hrs.

		CONTROL	C1 0.0034ppm	C2 0.006/ppm	C3 0.013ppm	C4 0.028ppm
BRAIN	a	0.2503 +/-0.062	0.3178 +/-0.022	0.4535 +/-0.043	0.5271 +/-0.049	0.5525 +/-0.075
	b		+26.98	81.18	+110.59	+120.73
	c		NS	P<0.01	P<0.01	P<0.01
GILL	a	0.3364 +/-0.093	0.3871 +/-0.109	0.4056 +/-0.105	0.4421 +/-0.049	0.4251 +/-0.05
	b		+15.07	+20.57	+31.42	+26.37
	c		NS	NS	NS	NS
LIVER	a	0.3893 +/-0.026	0.6998 +/-0.046	0.5724 +/-0.59	0.442d +/-0.032	0.327d +/-0.041
	b		79,76	+47.03	+13.64	-15.90
	c		P<0.001	P<0.01	NS	NS

TABLE 53

Etroplus maculatus: In vivo enzyme activity of ALP in brain, gill and liver of control fish and those pre-exposed to four SLC of Gramoxone for 10 days.

		CONTROL	C1 0.0013ppm	C2 0.0018ppm	C3 0.0027ppm	C4 0.0054ppm
BRAIN	a	0.2601 +/-0.073	0.2763 +/-0.036	0.2498 +/-0.042	0.2126 +/-0.035	0.2186 +/-0.057
	b		+6.23	-3.96	+18.26	-15.95
	c		NS	NS	NS	NS
GILL	a	0.4771 +/-0.182	0.4339 +/-0.029	0.3987 +/-0.058	0.3507 +/-0.072	0.2397 +/-0.039
	b		-9.17	-16.54	-26.58	-49.82
	c		NS	NS	NS	NS
LIVER	a	0.3232 +/-0.123	0.297 +/-0.034	0.2743 +/-0.039	0.2641 +/-0.033	0.2425 +/-0.027
	b		-8.11	-15.13	-18.23	-24.97
	c		NS	NS	NS	NS

a: Mean enzyme activity (N=3) in u mol p-ntro phenol liberated/mg pro./hr.
b: % Alteration from the mean control value
c: Level of significance/non-significant ns.

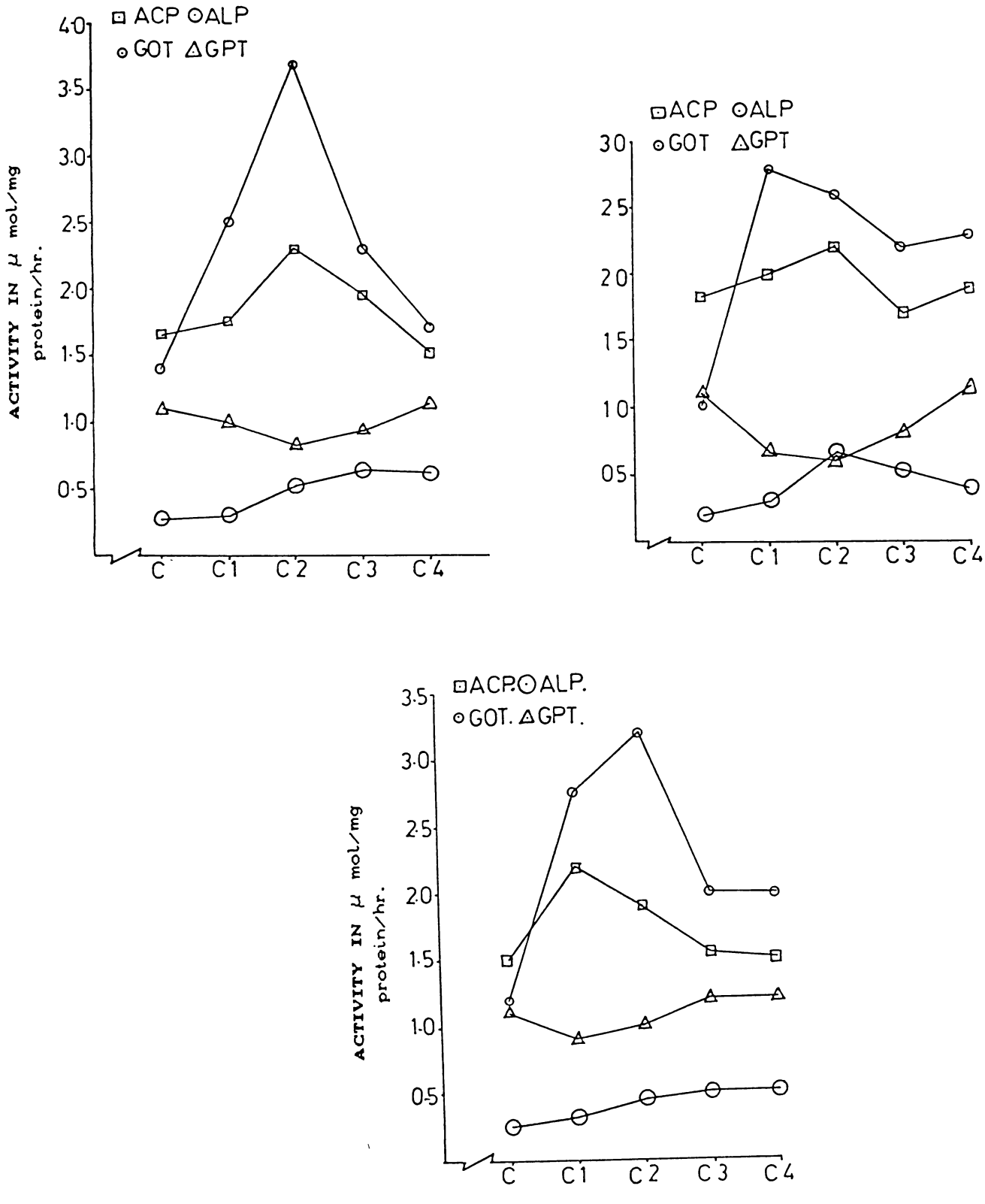

Fig.16. *Etroplus maculatus*: Mean enzyme activity of ALP, ACP, GOT and GPT in brain of control fish (C) and those pre-exposed to 4 SLC of Gramoxone for (a)24, (b) 72and (c) 120 hrs.

During 20 day, the activity showed comparatively lower value from 10 day value and found was significant in C_3, while the maximum activity in all the concentrations was obtained during 30 day, and was significantly elevated both from 10 and 20 days (Table *41, 42 & 43, Fig. 15*).

4.2.1.2.3.4 Glutamate pyruvate transaminase (GPT)

Short term:- Significant reduction of liver GPT activity was found in C_1 and C_2 during 72 hour from the control while in C_3 during 120 hour, significant elevation from the control was noted. Comparatively lower activity was noted in all the exposed groups. The Dimecron exposure elicited dual response in activity of GPT. In lower concentrations there was inhibition while in higher two concentrations stimulation of activity was noted when became pronounced with duration (Table *44, 45 & 46, Fig. 14*).

Long term:- Significant elevation of GPT activity from the respective controls was noted in all the four concentrations. The activity of GPT was found increasing with the dose. Significant reduction of activity was found in all the exposed during 30 day exposure from the 10 day activity. Reduction in activity in C_1, C_3 and C_4 during 20 day exposure was also found significant when compared to the activity during 10 day (Table *47, 48 & 49, Fig. 15*).

4.2.1.3 Gramoxone

4.2.1.3.1 Brain

4.2.1.3.1.1 Alkaline phosphatase (ALP)

Short term:- Significant elevation of brain ALP was found from the respective control values in the three higher concentrations (C_2, C_3 and C_4) during short term exposure. During 24 and 120 hours exposure comparative increase in activity with concentration increase was noted. In C_2 and C_4 the change in activity between 24 and 72 hours, and 72 and 120 hours exposure was found significant, while in C_3 the change in activity between 24 and 72 hours, and 24 and 120 hours exposure was found significant (*Table 50, 51 & 52, Fig. 16*).

Long term:- The brain ALP activity showed reduction from the control in all the four concentrations of gramoxone during 30 day exposure while during 10 and 20 days exposure no significant change in activity was noticed in the exposed groups when compared to respective control values. Generally, the ALP activity was found decreasing with increase of concentrations during the long term exposure, and in the three lower concentrations the change in activity with exposure duration was found significant except between 10 and 20 days exposure in C_3. In the highest concentration (C_4) significant reduction of

activity was found during 30 day exposure when compared to the activity during 10 day exposure to gramoxone (*Table 53, 54 & 55, Fig. 17*).

4.2.1.3.1.2 Acid phosphatase (ACP)

Short term:- Significant elevation of brain ACP activity, when compared to the respective control values, was found in the three lower concentrations (C_1, C_2 and C_3) during 24 and 120 hours, and in all concentrations except C_3 during 72 hour exposure to gramoxone while in the highest concentration (C_4) during 24 and 120 hours and in C_3 during 72 hour exposure, the brain ACP showed significant reduction. Changes in enzymatic activity of ACP with increase of concentration, was found significant during the short term exposure to DDT except the change between C_1 and C_4 during 72 hour and between C_2 and C_3 during 120 hour exposure. In the lower two concentrations (C_1 and C_2) the changes in activity with duration of exposure was found significant while in the higher two concentrations (C_3 and C_4) significant alterations of activity was noticed between 24 and 72 hours and 24 and 120 hours exposure in C_3, and between 24 and 72 hours, and 72 and 120 hours exposure in C_4((*Table 56, 57 & 58, Fig. 16*).

Long term:- The activity of brain ACP showed significant reduction from the respective control values in the lower three

TABLE 54

Etroplus maculatus: In vivo enzyme activity of ALP in brain, gill and liver of control fish and those pre-exposed to four SLC of Gramoxone for 20 days.

		CONTROL	C1 0.0013ppm	C2 0.0018ppm	C3 0.0027ppm	C4 0.0054ppm
BRAIN	a	0.3719 +/-0.156	0.4167 +/-0.03	0.3124 +/-0.025	0.2846 +/-0.057	0.1589 +/-0.109
	b		+12.05	-15.99	-23.47	-57.27
	c		NS	NS	NS	NS
GILL	a	0.4022 +/-0.092	0.3495 +/-0.028	0.2888 +/-0.079	0.3125 +/-0.078	0.2161 +/-0.094
	b		-13.10	-28.19	-22.3	46.27
	c		NS	NS	NS	NS
LIVER	a	0.322 +/-0.159	0.2376 +/-0.064	0.2359 +/-0.025	0.2895 +/-0.059	0.3064 +/-0.059
	b		-26.21	-26.74	-10.09	-4.84
	c		NS	NS	NS	NS

TABLE 55

Etroplus maculatus: In vivo enzyme activity of ALP in brain, gill and liver of control fish and those pre-exposed to four SLC of Gramoxone for 30 days.

		CONTROL	C1 0.0013ppm	C2 0.0018ppm	C3 0.0027ppm	C4 0.0054ppm
BRAIN	a	0.3674 +/-0.047	0.1882 +/-0.033	0.1052 +/-0.027	0.1169 +/-0.017	0.0788 +/-0.022
	b		-48.78	-71.37	-68.18	-78.55
	c		P<0.01	P<0.01	P<0.001	P<0.001
GILL	a	0.4444 +/-0.124	0.3227 +/-0.055	0.3536 +/-0.005	0.3082 +/-0.1	0.2002 +/-0.032
	b		-27.38	-20.43	-30.65	-54.95
	c		NS	NS	NS	P<0.05
LIVER	a	0.3749 +/-0.044	0.224 +/-0.062	0.2684 +/-0.021	0.392 +/-0.056	0.4482 +/-0.055
	b		-40.25	-28.41	+4.56	+19.55
	c		P<0.05	P<0.05	NS	NS

a: Mean enzyme activity (N=3) in u mol p-nitrophonol liberated/mg pro./hr
b: % Alteration from the mean control value
c: Level of significance/non-significant ns

TABLE 56

Etroplus emaculatus: In vivo enzyme activity of ACP in brain, gill and liver of control fish and those pre-exposed to four SLC of Gramoxone for 24 hrs.

		CONTROL	C1 0.0034ppm	C2 0.0067ppm	C3 0.013ppm	C4 0.028ppm
BRAIN	a	1.6435 +/-0.114	1.7459 +/-0.037	2.3067 +/-0.018	1.9406 +/-0.081	1.496 +/-0.014
	b		+6.23	+40.35	+18.08	-8.97
	c		P<0.001	P<0.01	P<0.001	P<0.001
GILL	a	1.1062 +/-0.182	1.2001 +/-0.043	1.2457 +/-0.102	1.2672 +/-0.119	1.0401 +/-0.041
	b		+8.49	+12.97	+14.55	-5.98
	c		NS	NS	NS	NS
LIVER	a	1.631 +/-0.029	2.2412 +/-0.06	1.8079 +/-0.042	2.0753 +/-0.19	1.9116 +/-0.056
	b		+37.41	+10.85	+27.24	+17.20
	c		P<0.001	P<0.01	P<0.05	P<0.01

TABLE 57

Etroplus maculatus: In vivo enzyme activity of ACP in brain, gill and liver of control fish and those pre-exposed to four SLC of Gramoxone for 72 hrs.

		CONTROL	C1 0.0034ppm	C2 0.0067ppm	C3 0.013ppm	C4 0.028ppm
BRAIN	a	1.8335 +/-0.191	2.0157 +/-0.078	2.1801 +/-0.063	1.7125 +/-0.029	1.9064 +/-0.072
	b		+9.94	+18.90	-6.59	+3.97
	c		P<0.01	P<0.01	P<0.01	P<0.01
GILL	a	1.0063 +/-0.014	1.1274 +/-0.109	1.2997 +/-0.136	1.0547 +/-0.048	0.945 +/-0.029
	b		+12.03	+29.15	+4.81	-6.09
	c		NS	P<0.05	NS	NS
LIVER	a	1.6653 +/-0.005	2.0444 +/-0.023	1.8189 +/-0.033	2.2501 +/-0.106	2.0481 +/-0.066
	b		+22.76	+9.22	+35.12	+22.99
	c		P<0.001	P<0.01	P<0.001	P<0.001

a: Mean enzyme activity (N=3) in u mol p- nitro phenol liberated/mg pro./hr.
b: % Alteration from the mean control value
c: Level of significance/non-significant ns.

TABLE 58

Etroplus maculatus: In vivo enzyme activity of ACP in brain, gill and liver of control fish and those pre-exposed to four SLC of Gramoxone for 120 days.

		CONTROL	C1 0.0034ppm	C2 0.0067ppm	C3 0,013ppm	C4 0.028ppm
BRAIN	a	1.509 +/-0.107	2.2185 +/-0.069	1.9759 +/-0.094	1.7996 +/-0.059	1.4782 +/-0.058
	b		+47.02	+30.94	+19.26	-2.04
	c		P<0.05	P<0.01	P<0.001	P<0.001
GILL	a	1.0243 +/-0.107	1.2302 +/-0.052	1.2047 +/-0.088	0.9664 +/-0.035	0.9225 +/-0.048
	b		+20.10	+17.61	-5.65	-9.94
	c		P<0.05	NS	NS	NS
LIVER	a	1.631 +/-0.039	1.8663 +/-0.082	2.2423 +/-0.166	2.5134 +/-0.024	2.6119 +/-0.05
	b		+14.43	+37.48	+54.12	+60.14
	c		P<0.05	P<0.01	P<0.001	P<0.001

TABLE 59

Etroplus maculatus: In vivo enzyme activity of ACP in brain, gill and liver of control fish and those pre-exposed to four SLC of Gramoxone for 10 days.

		CONTROL	C1 0.0013ppm	C2 0.0018ppm	C3 0.0027ppm	C4 0.0054ppm
BRAIN	a	1.3205 +/- 0.217	0.9503 +/-0.043	0.9184 +/-0.042	0.8268 +/-0.046	1.0334 +/-0.038
	b		-28.03	-30.45	-37.39	-21.74
	c		P<0.05	P<0.05	P<0.05	NS
GILL	a	1.1102 +/-0.097	0.9311 +/-0.042	0.8503 +/-0.093	0.5759 +/-0.06	0.449 +/-0.067
	b		-16.13	-23.41	-48.13	-59.56
	c		P<0.05	P<0.05	P<0.01	P<0.001
LIVER	a	1.5845 +/-0.155	1.491 +/-0.049	1.3389 +/-0.062	0.9682 +/-0.072	0.8887 +/-0.046
	b		-5.90	-15.50	-38.89	-43.91
	c		NS	NS	P<0.01	P<0.01

a: Mean enzyme activity (N=3) in u mol p- nitro phenol liberation/mg pro./hr.
b: % Alteration from the mean control value
c: Level of significance/non-nonsignificant ns.

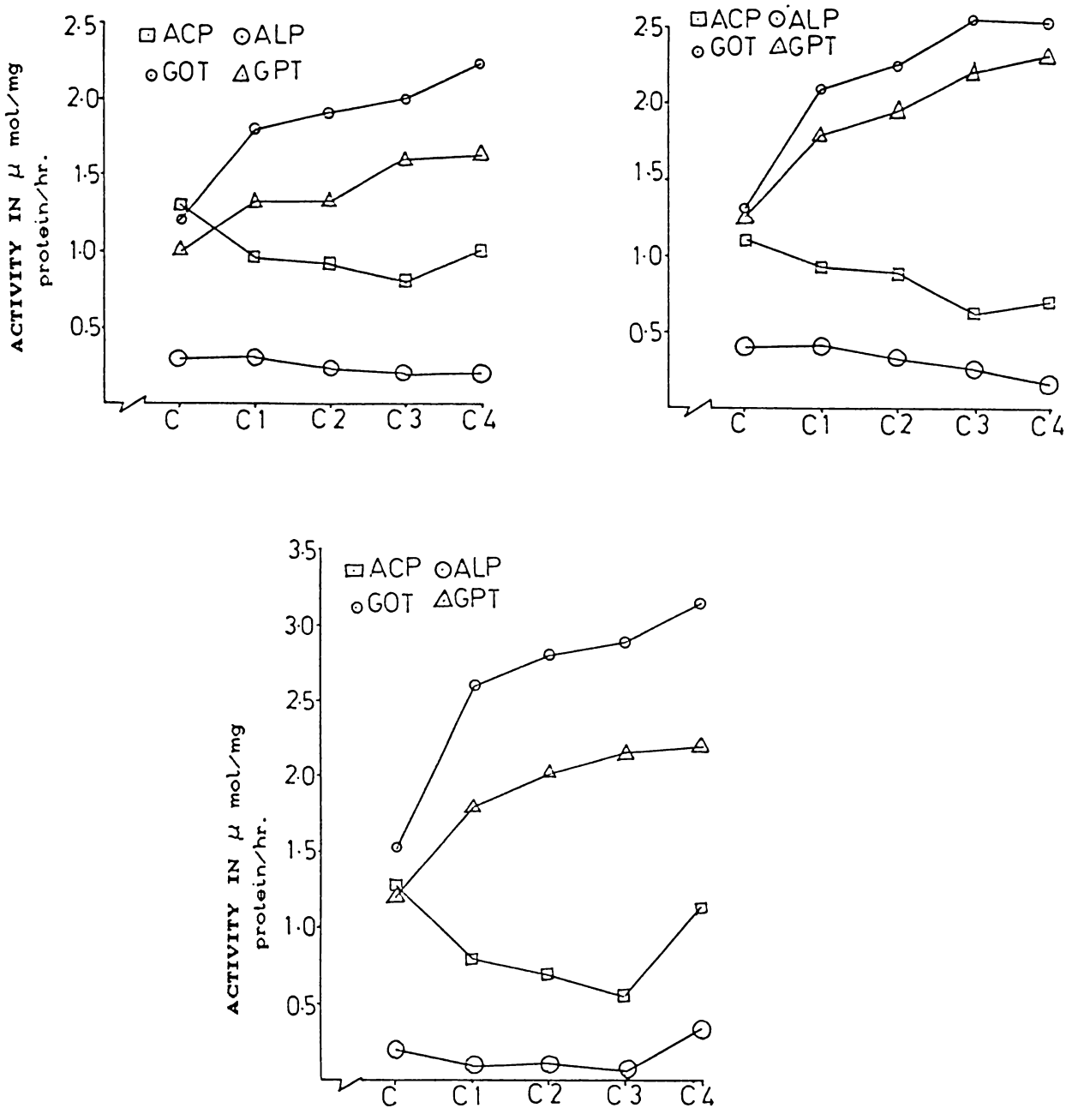

Fig.17. *Etroplus maculatus*: Mean enzyme activity of ALP, ACP, GOT and GPT in brain of control fish (C) and those pre-exposed to 4 SLC of Gramoxone for (a)10, (b) 20and (c) 30 days.

concentrations (C_1, C_2 and C_3) during 10 and 30 days, and in all the concentrations during 20 day exposure. In the lower three concentrations the activity of ACP was found to decrease with increase of concentration as well as with exposure duration. The change in activity in the lower three concentrations was found significant between 10 and 30, and 20 and 30 days of exposure, while in the highest concentration (C_4) change in activity between 10 and 20, and 20 and 30 days exposure was found significant (*Table 59, 60 & 61, Fig. 17*).

4.2.1.3.1.3 Glutamate oxaloacetate transaminase (GOT)

Short term:- Significant elevation of brain GOT was found from the respective controls in all the four exposed concentrations during short term exposure. Among exposed concentrations comparatively lower activity was noted in C_4 during 24 and 120 hours, and in C_3 during 72 hour exposure, but higher activity of GOT was encountered in C_2 during 24 and 120 hours, and in C_1 during 72 hour exposure *(Table 62, 63 & 64, Fig. 16)*.

Long term:- The brain GOT activity was found significantly elevated from the respective controls in all the four exposed concentrations of Gramoxone during the long term exposure. The activity of GOT was found to increase with increase of exposure concentration, and

TABLE 60

Etroplus maculatus: In vivo enzyme activity of ACP in brain, gill and liver of control fish and those pre-exposed to four SLC of Gramoxone for 20 days.

		CONTROL	C1 0.0013ppm	C2 0.0018ppm	C3 0,0037ppm	C4 0.0054ppm
BRAIN	a	1.1721 +/-0.217	0.19363 +/-0.039	0.8791 +/-0.048	0.7812 +/-0.044	0.9454 +/-0.044
	b		-20.12	-24.99	-33.35	-19.34
	c		P<0.05	P<0.05	P<0.01	P<0.05
GILL	a	1.099 +/-0.074	0.8518 +/-0.04	0.5792 +/-0.015	0.3865 +/-0.013	0.3695 +/-0.057
	b		-22.49	-47.29	-64.83	-66.38
	c		P<0.01	P<0.001	P<0.001	P<0.001
LIVER	a	1.4735 +/-0.068	1.0561 +/-0.051	1.164 +/-0.067	1.6576 +/-0.073	1.6463 +/-0.079
	b		-28.33	-21.00	+12.49	+11.73
	c		P<0.01	P<0.01	P<0.05	P<0.05

TABLE 61

Etroplus maculatus: In vivo enzyme activity of ACP in brain, gill and liver of control fish and those pre-exposed to four SLC of Gramoxone for 30 days.

		CONTROL	C1 0.0013ppm	C2 0.0018ppm	C3 0.0027ppm	C4 0.0054ppm
BRAIN	a	1.388 +/-0.174	0.7852 +/-0.043	0.6907 +/-0.063	0.5718 +/-0.063	1.1586 +/-0.115
	b		-43.43	-50.24	-58.80	-16.53
	c		P<0.01	P<0.01	P<0.01	NS
GILL	a	1.1112 +/-0.102	0.7338 +/-0.065	0.5677 +/-0.071	0.4497 +/-0.112	0.3541 +/-0.049
	b		-33.96	-48.91	-59.53	-68.13
	c		P<0.01	P<0.01	P<0.01	P<0.001
LIVER	a	1.5559 +/-0.086	0.9422 +/-0.057	1.3633 +/-0.036	1.3693 +/-0.063	1.8658 +/-0.052
	b		-39.29	-12.15	-11.77	+20.21
	c		P<0.001	P<0.05	P,0.05	NS

a: Mean enzyme activity (N=3) in u p-nitro phenol liberated/mg pro./hr.
b: % Alteration from the mean control value
c: Level of significance/non significant ns.

TABLE 62

Etroplus maculatus: In vivo enzyme activity of GOT in brain, gill and liver of control fish and those pre-exposed to four SLC of Gramoxone for 24 hrs.

		CONTROL	C1 0.0034ppm	C2 0.0067ppm	C3 0.013ppm	C4 0.028ppm
BRAIN	a	1.3818	2.4898	3.7049	2.3313	1.6842
		0.037	0.046	0.289	0.041	0.077
	b		+80.18	+168.12	+68.71	+21.88
	c		P<0.001	P<0.001	P<0.001	P<0.001
GILL	a	0.3694	0.358	0.4576	0.3847	0.3394
		0.123	0.108	0.101	0.024	0.031
	b		-3.09	+23.88	+4.14	-8.12
	c		NS	NS	NS	NS
LIVER	a	1.2303	1.3662	1.3353	1.5073	1.1475
		0.049	0.043	0.078	0.017	0.019
	b		+11.05	+8.53	+22.51	-6.73
	c		P<0.05	NS	P<0.001	NS

TABLE 63

Entroplus maculatus: In vivo enzyme activity of GOT in brain, gill and liver of control fish and those pre-exposed to four SLC of Gramoxone for 72 hrs.

		CONTROL	C1 0.0034ppm	C2 0.0067ppm	C3 0.013ppm	C4 0.028ppm
BRAIN	a	1.04/1	2.8159	2.6565	2.1981	2.2737
		0.021	0.123	0.046	0.089	0.209
	b		+168.92	+153.7	+109.92	+117.14
	c		P<0.05	P<0.05	P<0.001	P<0.001
GILL	a	0.3892	0.3267	0.2971	0.4282	0.4088
		0.052	0.06	0.021	0.116	0.115
	b		-16.06	-23.66	+10.02	+5.03
	c		NS	P<0.05	NS	NS
LIVER	a	1.2354	1.4818	1.5082	1.1258	1.2463
		0.024	0.061	0.056	0.006	0.039
	b		+19.94	+22.08	-8.87	+0.88
	c		P<0.01	NS	P<0.01	NS

a:Mean enzyme activity (N=3) in u mol sidium pyruvate liberated/mg pro./hr
b: % Alteration from the mean control value
c: Level of significance/non-significant ns.

TABLE 64

Etroplus maculatus: In vivo enzyme activity of GOT in brain, gill and liver of control fish and those pre-exposed to four SLC of Gramoxone for 120 hrs.

		CONTROL	C1 0.0034ppm	C2 0.0034ppm	C3 0.013ppm	C4 0.028ppm
BRAIN	a	1.2127	2.7655	3.1859	1.992	1.8989
		0.084	0.089	0.028	0.028	0.045
	b		+128.04	+162.71	+64.26	+56.58
	c		P<0.001	P<0.001	P<0.001	P<0.001
GILL	a	0.3535	0.3501	0.4069	0.4969	0.4762
		0.055	0.081	0.057	0.128	0.0126
	b		-0.96	+15.11	+40.56	+34.71
	c		NS	NS	NS	P<0.05
LIVER	a	1.3663	1.3293	1.465	1.3075	1.1043
		0.102	0.059	0.01	0.029	0.019
	b		-2.71	7.22	-4.3	-19.18
	c		NS	NS	NS	P<0.05

TABLE 65

Etroplus maculatus: In vivo enzyme activity of GOT in brain, gill and liver of control fish and those pre-exposed to four SLC of Gramoxone for 10 days.

		CONTROL	C1 0.0013ppm	C2 0.0018ppm	C3 0.0027ppm	C4 0.0054ppm
BRAIN	a	1.2227	1.8325	1.943	2.0837	2.2547
		0.179	0.07	0.065	0.074	0.252
	b		+49.87	+58.91	+70.42	+84.40
	c		P<0.01	P<0.01	P<0.01	P<0.01
GILL	a	0.6355	0.6154	0.6123	0.498	0.4605
		0.055	0.032	0.052	0.064	0.025
	b		-3.16	-3.65	-21.64	-27.54
	c		NS	NS	NS	P<0.05
LIVER	a	1.0541	1.2357	1.312	1.5429	1.6652
		0.169	0.068	0.043	0.057	0.049
	b		-19.81	-14.86	+0.123	+8.06
	c		NS	NS	P<0.01	P<0.01

a: Mean enzyme activity (N=3) in u mol sodium pyruvate liberated/mg pro./hr
b: % Alteration from the mean control value
c: Level of significance/non-significant ns.

the increase was found significant between the lowest and the highest concentrations. The increase in GOT activity with the exposure duration in each concentration was also found significant during long term exposure to gramoxone *(Table 65, 66 & 67, Fig. 17)*.

4.2.1.3.1.4 Glutamate pyruvate transaminase (GPT)

Short term:- During short term exposure to gramoxone the brain GPT showed significant reduction in activity from the respective controls in C_2 and C_3 during 24 hour, in the lower three concentrations (C_1, C_2 and C_3) during 72 hour, and in the lower two concentrations (C_1 and C_2) during 120 hour exposures. But in the highest two concentrations (C_3 and C_4) during 120 hour, a significant elevation of activity was noted. The change in activity with increase of gramoxone concentration was found significant during 72 and 120 hours exposure, while during 24 hour exposure the change in activity between lowest and highest concentrations was found significant. In lower three concentrations (C_1, C_2 and C_3) the change in enzymatic activity with exposure duration was found significant between 24 and 72 hour, and 72 and 120 hour exposure while in the highest concentration (C_4) no significant changes in activity was noted (*Table 68, 69 & 70, Fig. 16*).

TABLE 66

Etroplus maculatus: In vivo enzyme activity of GOT in brain, gill and liver of control fish and those pre-exposed to four SLC of Gramoxone for 20 days.

		CONTROL	C1 0.0013ppm	C2 0.0018ppm	C3 0.0027ppm	C4 0.0054ppm
BRAIN	a	1.3018	2.0901	2.2531	2.5575	2.5215
		0.285	0.078	0.082	0.088	0.185
	b		+60.56	+73.07	+96.45	+93.69
	c		P<0.01	P<0.01	P<0.01	P<0.01
GILL	a	0.5901	0.682	0.7256	0.7721	0.8091
		0.072	0.009	0.042	0.019	0.019
	b		+15.57	+22.96	+30.84	+37.11
	c		NS	P<0.05	P<0.05	P<0.01
LIVER	a	1.3301	1.5967	1.6869	1.9515	2.088
		0.105	0.072	0.063	0.027	0.088
	b		+20.04	+26.82	+46.72	+56.98
	c		P<0.05	P<0.01	P<0.001	P<0.001

TABLE 67

Etroplus maculatus: In vivo enzyme activity of GOT in brain, gill and liver of control fish and those pre-exposed to four SLC of Gramoxone for 30 days.

		CONTROL	C1 0.0013ppm	C2 0.0018ppm	C3 0.0027ppm	C4 0.0054ppm
BRAIN	a	1.5346	2.6405	2.8378	2.9404	3.1558
		0.099	0.124	0.17	0.13	0.76
	b		+72.06	+84.92	+91.60	+105.64
	c		P<0.001	P<0.001	P<0.001	P<0.001
GILL	a	0.5347	0.7421	0.7891	0.8324	0.8744
		0.035	0.101	0.026	0.033	00.063
	b		+38.78	+47.57	+55.67	+63.53
	c		P<0.05	P<0.001	P<0.001	P<0.01
LIVER	a	1.3692	1.831	1.9233	2.2254	2.3867
		0.134	0.061	0.064	0.06	0.101
	b		+33.72	+40.46	+62.53	+74.31
	c		P<0.01	P<0.05	P<0.001	P<0.001

a:Mean enzyme activity (N=3) in u mol sodium pyruvate liberated/mg pro./hr.
b: % Alteration from the mean control value.
c: Level of significance/non-significant ns

TABLE 68
Etroplus maculatus: In vivo enzyme activity of GPT in brain, gill and lever of control fish and those pre-exposed to four SLC of GRAMOXONE for 24 hours.

		CONTROL	C1 0.0034ppm	C2 0.0067ppm	C3 0.013ppm	C4 0.028ppm
BRAIN	a:	1.1024 +/-0.074	0.9955 +/-0.030	0.8328 +/-0.041	0.9633 +/-0.034	1.1435 +/-0.057
	b:		-9.69	-24.45	-12.61	+3.73
	c:		NS	P<0.01	P<0.05	NS
GILL	a:	0.9286 +/-0.060	0.7361 +/-0.042	0.8152 +/-0.166	0.9142 +/-0.105	0.9297 +/-0.087
	b:		-20.73	-12.21	-1.55	+0.12
	c:		P<0.05	NS	NS	NS
LIVER	a:	1.2912 +/-0.017	2.1234 +/-0.071	2.2275 +/-0.017	1.8188 +/-0.074	1.5022 +/-0.032
	b:		+64.45	+72.51	+40.86	+16.34
	c:		P<0.01	P<0.001	P<0.01	NS

TABLE 69
Etroplus maculatus: In vivo enzyme activity of GPT in brain, gill and lever of control fish and those pre-exposed to four SLC of GRAMOXONE for 72 hours.

		CONTROL	C1 0.0034ppm	C2 0.0067ppm	C3 0.013ppm	C4 0.028ppm
BRAIN	a:	1.1153 +/-0.051	0.6856 +/-0.01	0.6106 +/-0.028	0.8322 +/-0.055	1.1729 +/-0.050
	b:		-38.55	-45.28	-25.42	+5.12
	c:		P<0.001	P<0.001	P<0.01	NS
GILL	a:	0.8855 +/-0.0156	0.7876 +/-0.079	0.8323 +/-0.036	0.8616 +/-0.117	0.9353 +/-0.036
	b:		-11.05	-6.01	-2.70	+5.62
	c:		NS	NS	NS	NS
LIVER	a:	1.3033 +/-0.105	0.9398 +/-0.046	0.8734 +/-0.0622	2.1184 +/-0.0406	1.0268 +/-0.177
	b:		-27.89	-32.98	+62.54	-21.21
	c:		P<0.05	P<0.05	P<0.001	Ns

a: Mean enzyme activity (N=3) in u mol sodium pyruvate liberated/mg pro./hr.
b: %Alteration from the mean control value.
c: Level of significane/non significant ns.

TABLE 70
Etroplus maculatus: In vivo enzyme activity of GPT in brain, gill and lever of control fish and those pre-exposed to four SLC of GRAMOXONE for 120 hours.

		CONTROL	C1 0.0034ppm	C2 0.0067ppm	C3 0.013pm	C4 0.028ppm
BRAIN	a:	1.1182 +/-0.027	0.9038 +/-0.047	0.9917 +/-0.033	1.2166 +/-0.03	1.2314 +/-0.047
	b:		-19.17	-11.31	-8.80	+10.12
	c:		P<0.01	P<0.01	P<0.05	P<0.05
GILL	a:	0.8919 +/-0.1295	0.7591 +/-0.0908	0.9368 +/-0.029	0.943 +/-0.010	0.9539 +/-0.048
	b:		-14.89	+5.03	+5.73	+6.95
	c:		NS	NS	NS	NS
LIVER	a:	1.3323 +/-0.181	2.1617 +/-0.043	2.3265 +/-0.067	1.186 +/-0.040	0.6207 +/-0.068
	b:		+62.25	+/d.62	-10.98	-53.41
	c:		P<0.01	P<0.001	P<0.001	P<0.001

TABLE 71
Etroplus maculatus: In vivo enzyme activity of GPT in brain, gill and lever of control fish and those pre-exposed to four SLC of GRAMOXONE for 10 days.

		CONTROL	C1 0.0013ppm	C2 0.0018ppm	C3 0.027ppm	C4 0.0054ppm
BRAIN	a:	0.9935 +/-0.233	1.3656 +/-0.046	1.4486 +/-0.086	1.6554 +/-0.067	1.6342 +/-0.232
	b:		+37.45	+45.81	+66.62	+64.49
	c:		NS	P<0.05	P<0.001	P<0.05
GILL	a:	0.9535 +/-0.063	0.8814 +/-0.0324	0.7954 +/-0.029	0.7341 +/-0.0421	0.6647 +/-0.035
	b:		-7.56	-16.58	-23.01	-30.29
	c:		NS	P<0.05	P<0.01	P<0.01
LIVER	a:	1.2015 +/-0.099	0.8821 +/-0.072	0.723 +/-0.102	1.3264 +/-0.065	1.4101 +/-0.077
	b:		-26.58	-39.82	+10.40	+17.36
	c:		P<0.05	P<0.01	NS	P<0.05

a: Mean enzyme activity (N=3) in u mol sodium pyruvate liberated/mg pro./hr.
b: %Alteration from the mean control value.
c: Level of significance/non significant ns.

Long term:- Significant elevation of GPT activity from the respective controls was noted in the three higher concentrations (C_2, C_3 and C_4) during 10 day, and in all the four concentrations during 20 and 30 days exposures. The GPT activity was found increasing with increase of concentration and the increase was found significant during 20 and 30 days exposure. The increase in activity in each of the exposed concentrations with exposure duration was found significant between 10 and 20, and 10 and 30 days exposure (*Table 71, 72 & 73, Fig. 17*).

4.2.1.3.2 Gill

4.2.1.3.2.1 Alkaline phosphatase (ALP)

Short term:- The gill ALP activity was significantly reduced from the respective controls in the three higher concentrations (C_2, C_3 and C_4 during 24 hour, and no significant change was noted in all the concentrations during 72 and 120 hours exposure (*Table 50, 51 & 52, Fig. 18*).

Long term:- No significant change in the activity was found, from the respective controls, in all the exposed concentrations during long term exposure except in the highest concentration (C_4) during 30 day exposure where the activity recorded a significant reduction

TABLE 72
Etroplus maculatus: In vivo enzyme activity of GPT in brain, gill and lever of control fish and those pre-exposed to fouir SLC of GRAMOXONE for 20 days.

		CONTROL	C1 0.0013ppm	C2 0.0018pm	C3 0.0027pm	C4 0.0054ppm
BRAIN	a:	1.2702	1.772	1.9542	2.1956	2.2851
		+/-0.105	+/-0.016	+/-0.055	+/-0.095	+/-0.0718
	b:		+39.51	+53.85	+72.85	+79.90
	c:		P<0.01	P<0.001	P<0.001	P<0.001
GILL	a:	0.9051	0.9292	0.9395	1.0875	1.1391
		+/-0.093	+/-0.041	+/-0.031	+/-0.051	+/-0.0344
	b:		+2.66	+3.80	+20.15	+25.85
	c:		NS	NS	P<0.05	P<0.05
LIVER	a:	1.1194	0.8125	0.6287	1.5091	1.3847
		+/-0.107	+/-0.048	+/-0.059	+/-0.034	+/-0.047
	b:		-27.41	-43.83	+34.81	+23.70
	c:		P<0.05	P<0.01	P<0.01	P<0.05

TABLE 73
Etroplus maculatus: In vivo enzyme activity of GPT in brain, gill and lever of control fish and those pre-exposed to four SLC of GRAMOXONE for 30 days.

		CONTROL	C1 0.0013ppm	C2 0.0018ppm	C3 0.0027ppm	C4 0.0054ppm
BRAIN	a:	1.2107	1.7913	2.0363	2.1896	2.2111
		+/-0.211	+/-0.087	+/-0.045	+/-0.071	+/-0.014
	b:		+47.95	+68.19	+80.85	+82.63
	c:		P<0.05	P<0.01	P<0.01	P<0.01
GILL	a:	0.9757	0.9477	0.9954	1.1721	1.1954
		+/-0.093	+/-0.046	+/-0.01	+/-0.065	+/-0.073
	b:		-2.87	+2.02	+20.13	+22.52
	c:		NS	NS	P<0.05	P<0.05
LIVER	a:	1.1586	0.7599	0.5686	1.3439	2.2269
		+/-0.101	+/-0.045	+/-0.056	+/-0.051	+/-0.112
	b:		-34.41	-50.92	+15.99	+92.21

a: Mean enzyme activity (N=3) in u mol sodium pyruvate liberated/mg pro./hr.
b: %Alteration from the mean control value.
c: Level of significane/non significant ns.

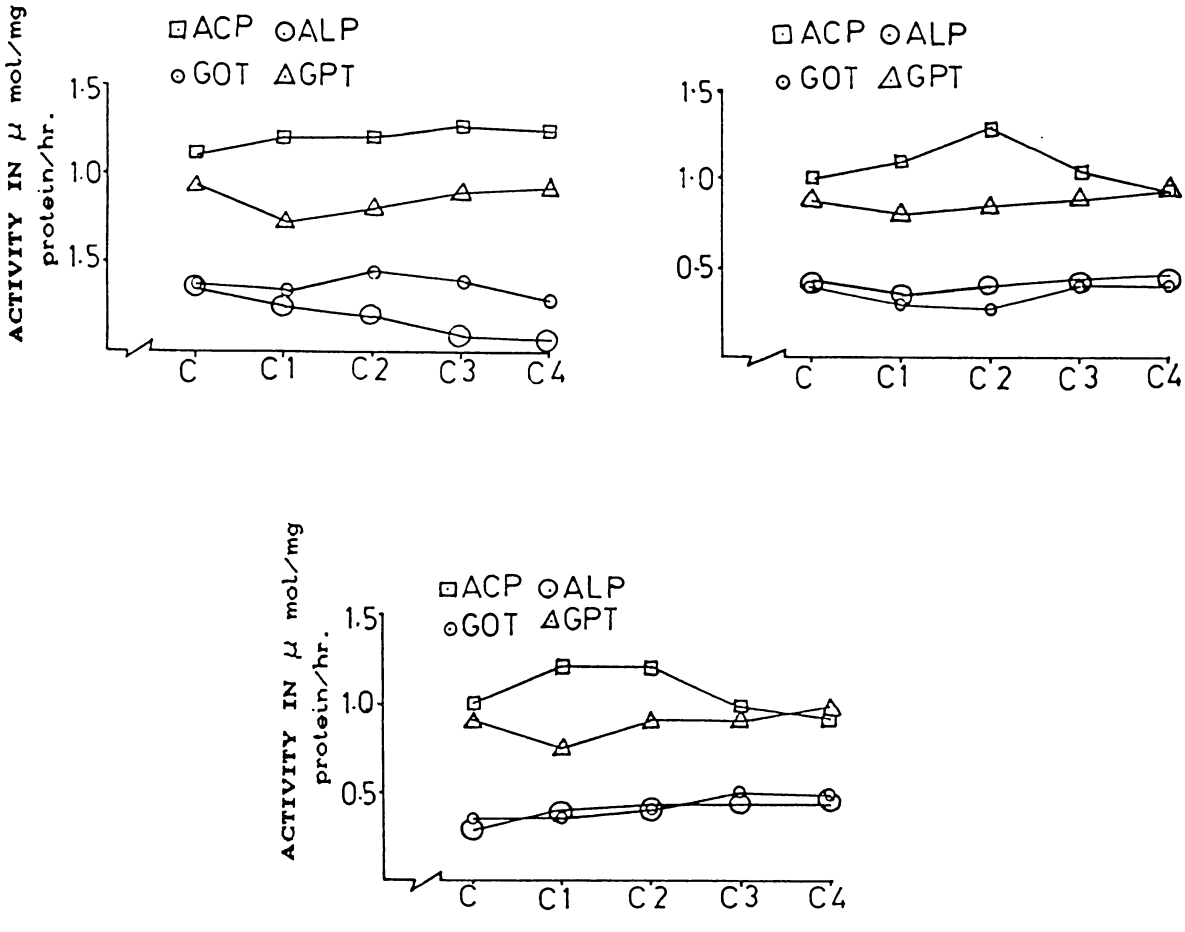

Fig.18. *Etroplus maculatus*: Mean enzyme activity of ALP, ACP, GOT and GPT in gill of control fish (C) and those pre-exposed to 4 SLC of Gramoxone for (a)24, (b) 72and (c) 120 hrs.

from the control. Except in the lowest concentration (C_1) during 10 day, the activity was found comparatively lower than the control and found decreasing with increase of concentration, and with exposure duration (*Table 53, 54 & 55, Fig. 19*).

4.2.1.3.2.2 Acid phosphatase (ACP)

Short term:- The activity of gill ACP was found significantly elevated from the respective controls in C_2 during 72 hour, and in C_1 during 120 hour exposure, while change in other concentrations during the above periods, and in all the concentrations during 24 hour exposure was found insignificant, and comparatively higher activity in lower concentrations and lower activity in higher concentrations (when compared to control values) were noted (*Table 56, 57 & 58, Fig. 18*).

Long term:- Significant reduction in activity, from the respective controls, was found in all the four exposed concentrations during long term exposure. The activity was found decreasing with increase of concentration, and the changes in activity between two lower and two higher concentrations were significant except the activity change between C_2 and C_3 during 30 day exposure. Also, the activity showed reduction in each concentration with exposure duration,

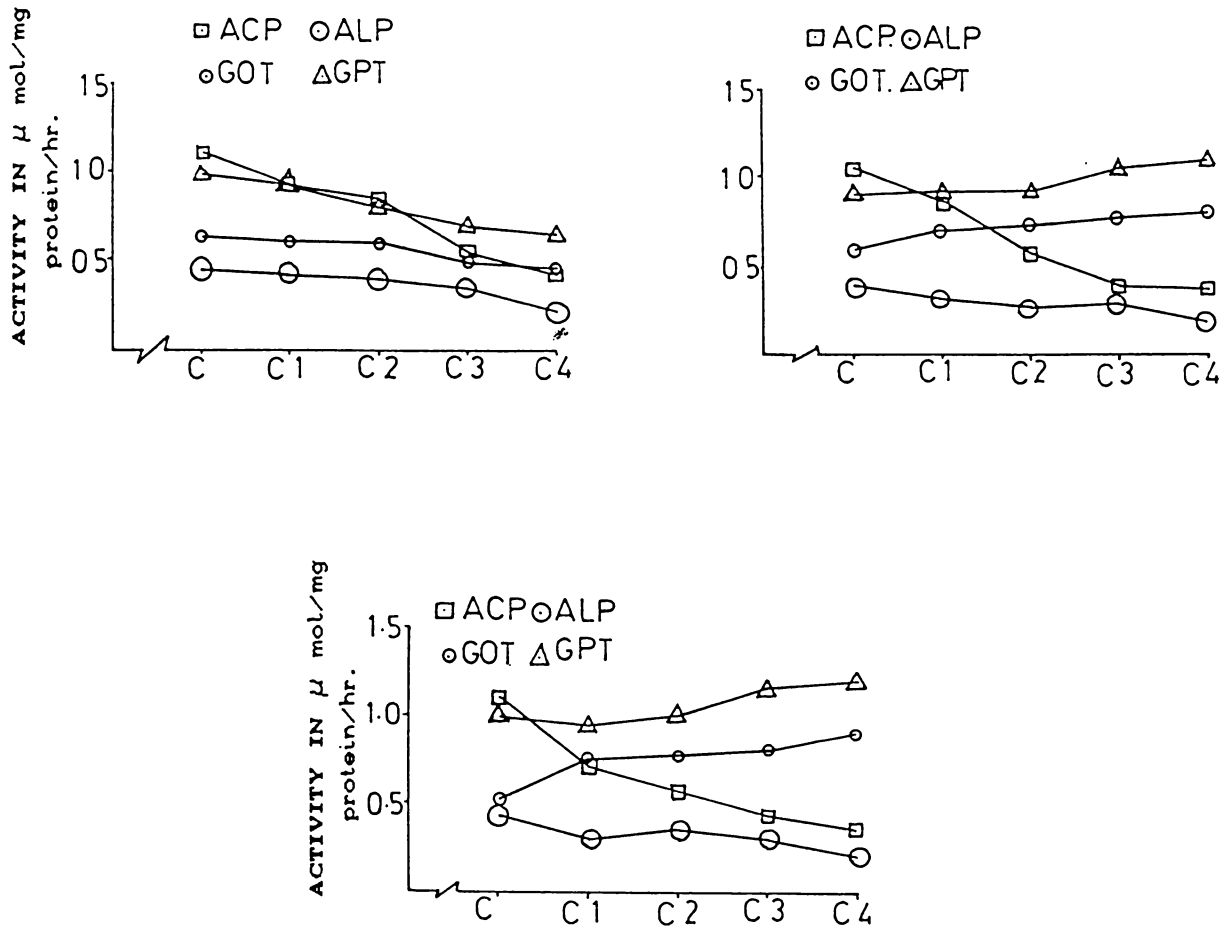

Fig.19. *Etroplus maculatus*: Mean enzyme activity of ALP, ACP, GOT and GPT in gill of control fish (C) and those pre-exposed to 4 SLC of Gramoxone for (a)10, (b) 20and (c) 30 days.

and the changes in ACP activity were found significant between 10 and 20, and 10 and 30 days exposure in the lower two concentrations (C_1 and C_2), while in C_3 the change between 10 and 20 days activity of ACP was found significant. In the highest concentration (C_4) no significant alteration of activity was noticed (*Table 59, 60 & 61, Fig. 19*).

4.2.1.3.2.3 Glutamate oxaloacetate transaminase (GOT)

Short term:- The gill GOT activity showed no significant alteration from the respective control values in all the concentrations during short term exposure except in C_2 during 72 hour where a significant reduction from the control, and in the highest concentration (C_4) where a significant stimulation of activity from the control was noted. No significant change in activity of GOT was found in the exposed concentrations with exposure duration (*Table 62, 63 & 64, Fig. 18*).

Long term:- In the highest concentration (C_4) during 10 day exposure the activity showed significant reduction from the control, while in the three higher concentrations (C_2, C_3 and C_4) during 20 day, and in all the four exposed concentrations during 30 day exposure, the activity was found elevated significantly from the control. During 10 day the enzyme activity showed a gradual decrease with increase of

concentration, while during 20 and 30 days exposure, it showed increase with concentration increase. The activity of GOT was found increasing with increase of exposure duration, and in C_2, C_3 and C_4, the changes in activity between 10 and 20, and 10 and 30 days exposure were found significant, while in C_1 the only significant change in the activity of GOT was found between 10 and 20 days exposure (*Table 65, 66 & 67, Fig. 19*).

4.2.1.3.2.4 Glutamate pyruvate transaminase (GPT)

Short term:- The activity of gill GPT showed no significant change from the respective controls during the short term exposure except in the lowest concentration (C_1) during 24 hour exposure where a significant reduction from the control was noted (*Table 68, 69 & 70, Fig. 18*).

Long term:- The gill GPT activity showed, when compared to the respective controls, significant reduction in the three higher concentrations (C_2, C_3 and C_4) during 10 day exposure while in the highest two concentrations (C_3 and C_4) during 20 and 30 days exposure, the activity was found significantly elevated. During 10 day exposure the activity was found decreasing with increase of concentration, and during 20 and 30 days exposure it showed an increasing trend with

concentration. The activity of gill GPT was found increasing with increase of exposure duration and in the higher three concentrations (C_2, C_3 and C_4) the activity changes between 10 and 20, and 10 and 30 days exposure were found significant (*Table 71, 72 & 73, Fig. 19*).

4.2.1.3.3 Liver

4.2.1.3.3.1 Alkaline phosphatase (ALP)

Short term:- The liver ALP activity showed significant elevation from the respective controls in the lowest two concentrations (C_1 and C_2) during 24 hour, and in the lower three concentrations during 72 and 120 hour exposure, while in the highest concentration during 24 hour exposure the activity was significantly reduced. The reduction in ALP activity with increase of concentration was found significant during the short term exposure. During 72 hour exposure, the liver ALP activity in all the concentrations was found comparatively high from 24 and 120 hours exposure and the elevation was found significant when compared to 24 hour activity in all the concentrations. In C_1, C_3 and C_4, the reduction of activity in all the concentrations during 120 hour from 72 hour exposure was found significant (*Table 50, 51 & 52, Fig. 20*).

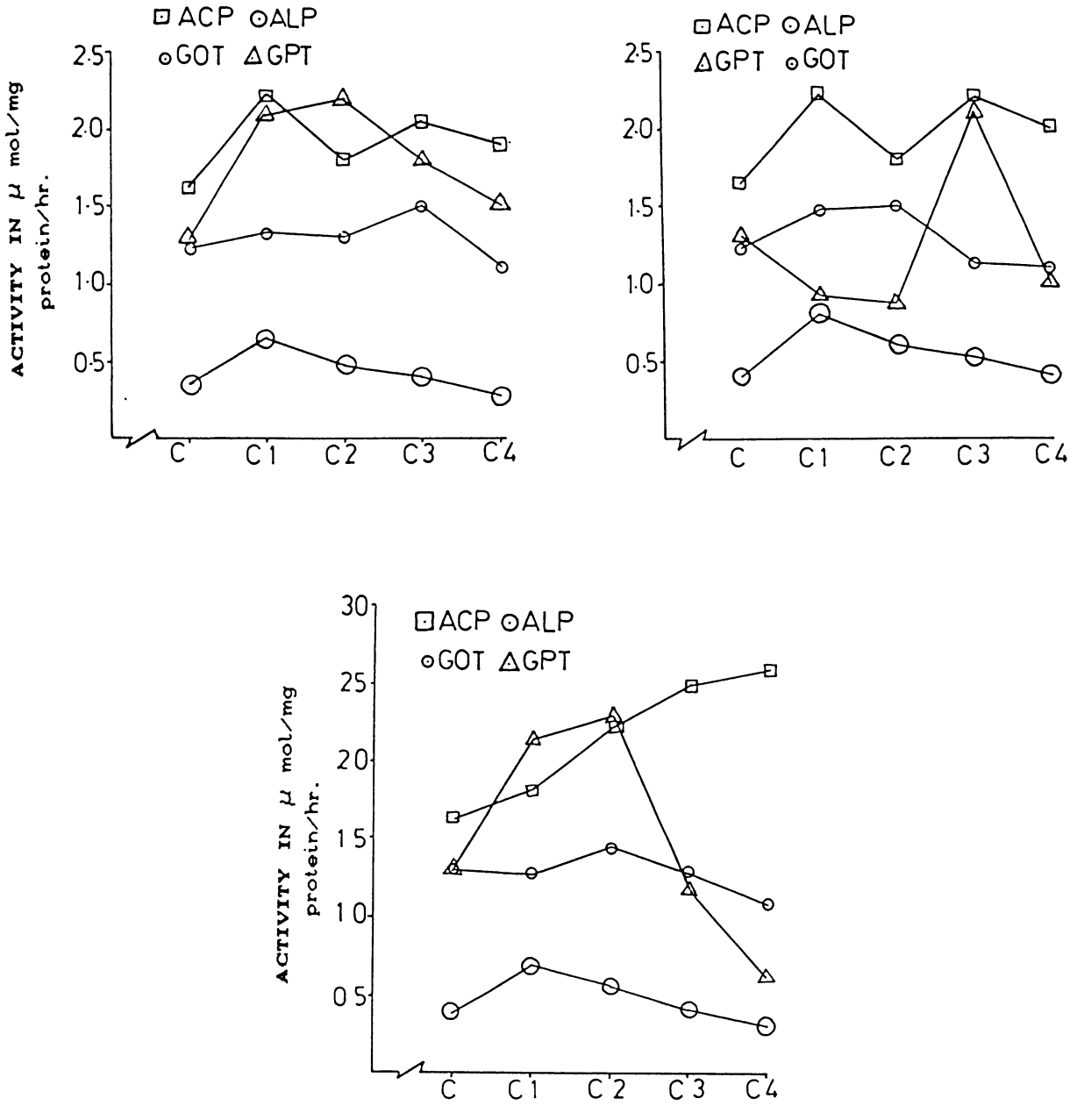

Fig.20. *Etroplus maculatus*: Mean enzyme activity of ALP, ACP, GOT and GPT in liver of control fish (C) and those pre-exposed to 4 SLC of Gramoxone for (a) 24, (b) 72 and (c) 120 hrs.

Long term:- The activity of liver ALP showed no significant change from the respective controls during the long term exposure except in the lowest two concentrations (C_1 and C_2) during 30 day exposure where a significant reduction of activity from the control was noted. Nevertheless, the activity of ALP was found comparatively lower than the control values and found decreasing with increase of concentrations and exposure duration (*Table 53, 54 & 55, Fig. 21*).

4.2.1.3.3.2 Acid phosphatase (ACP)

Short term:- The activity of liver ACP showed significant elevation from the respective control values in all the four exposed concentrations. A change in activity with concentration increase was found significant during 72 and 120 hour exposure except the activity change between C_1 and C_4 during 72 hour, and C_1 and C_2 during 120 hour exposure. The activity was found increasing with increase of gramoxone concentration. The change in activity with increase of exposure duration was found significant in higher three concentrations (C_2, C_3 and C_4) between 24 and 120 hours, and 72 and 120 hours exposure, and in C_1 the activity change of ACP was found significant with increase of exposure duration (*Table 56, 57 & 58, Fig 20*).

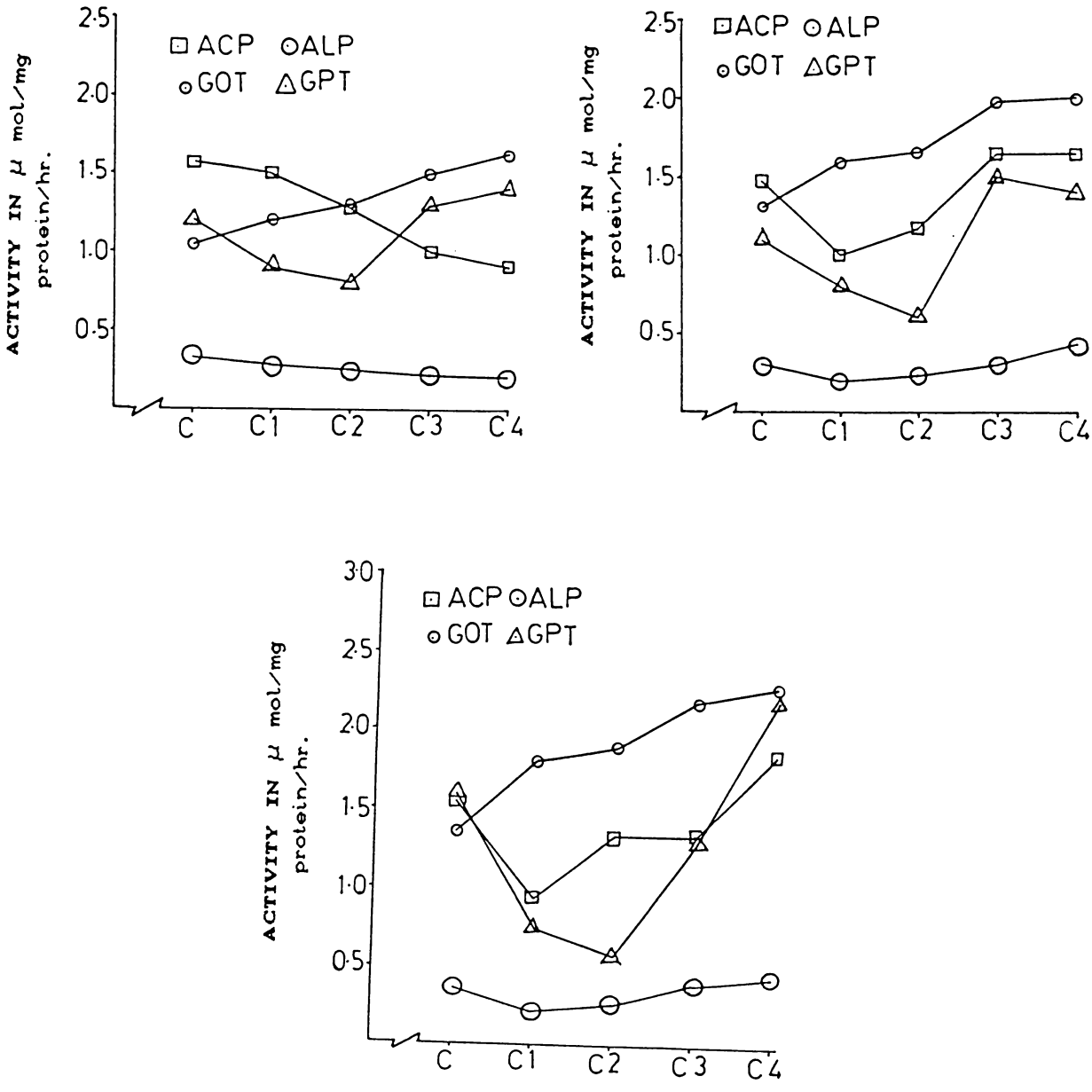

Fig.21. *Etroplus maculatus*: Mean enzyme activity of ALP, ACP, GOT and GPT in liver of control fish (C) and those pre-exposed to 4 SLC of Gramoxone for (a) 10, (b) 20 and (c) 30 days.

Long term:- Significant reduction of liver ACP was found from the respective control values in the higher two concentrations (C_3 and C_4) during 10 day, in the lowest two concentrations (C_1 and C_2) during 20 day, and in the three lower concentrations (C_1, C_2 and C_3) during 30 day exposure. During 20 day, the activity was found significantly elevated from the control in the two highest concentrations (C_3 and C_4). The activity was found decreasing with increase of concentration during 10 day, and the decrease was significant. During 20 and 30 days exposure, the ACP activity was found increasing with concentration increase, and the changes were found significant. In the highest two concentrations (C_3 and C_4) the change in ACP activity with exposure duration was found significant. The changes in activity between 10 and 20 days, and 10 and 30 days in C_1, and between 10 and 20 days, and 20 and 30 days exposure in C_2, were found significant (*Table 59, 60 & 61, Fig. 21*).

4.2.1.3.3.3 Glutamate oxaloacetate transaminase (GOT)

Short term:- Significant elevation of GOT activity from the respective controls was found in C_1 and C_3 during 24 hour and in C_1 during 72 hour of exposure, and in the highest concentration (C_4) significant reduction was noted during 120 hour exposure. The changes in activity of GOT between the lowest two concentrations (C_1 and C_2)

and the highest two concentrations (C_3 and C_4) were found significant except the changes between C_1 and C_3 during 120 hour exposure. During 72 hour exposure, the GOT activity was found comparatively higher than 24 and 120 hours of exposure in C_1, C_2 and C_4, and the elevation was found significant when compared to the 24 hour activity and the changes in activity with increase of exposure duration was found significant in C_3 (*Table 62, 63 & 64, Fig. 20*).

Long term:- The liver GOT showed significant elevation in activity from the respective control values in the highest two concentrations (C_3 and C_4) during 10 day, and in all the four concentrations during 20 and 30 days exposure to gramoxone. The GOT activity showed increase with increase of concentration, and the changes in activity between the lower two concentrations (C_1 and C_2) and the highest two concentrations (C_3 and C_4) were found significant during long term exposure. The increase in activity of ALP with the increase of exposure duration was also found significant in all the concentrations (*Table 65, 66 & 67, Fig. 21*).

4.2.1.3.3.4 Glutamate pyruvate transaminase (GPT)

Short term:- The liver GPT activity showed significant elevation from the respective control values in the three lower

concentrations (C_1, C_2 and C_3) during 24 hour, in C_3 during 72 hour, and in the two lower concentrations (C_1 and C_2) during 120 hour exposure to gramoxone, while in the higher two concentrations (C_3 and C_4), during 120 hour, and in the lower two concentrations during 72 hour, the activity was found significantly reduced. The GPT showed general decrease in activity with increase of concentration. During 72 hour in the higher two concentrations (C_3 and C_4) the GPT activity was found comparatively higher than the two lower concentrations. In the lower two concentrations (C_1 and C_2) the GPT activity was found significantly reduced during 72 hour both from 24 and 120 hours exposure. In C_3 elevation was noted in GPT activity during 72 hour, and in C_2 during 120 hour exposure and in C_4 the activity was found reducing with increase of exposure duration and the changes were found significant (*Table 68, 69 & 70, Fig. 20*).

Long term:- Significant reduction in GPT activity was found in the lower two concentrations (C_1 and C_2) during long term exposure when compared to the respective control values. But in the highest two concentrations (C_3 and C_4) the activity showed significant elevation during 20 and 30 days exposure, and in C_4 during 10 day exposure. In the highest two concentrations (C_3 and C_4), the activity of GPT was found significantly higher than the activities noted in the lower two concentrations (C_1 and C_2) during the long term exposure, and during

20 and 30 days exposure the changes in activity with increase of concentration were found significant. No significant change in activity was noted in C_1 and C_2 with exposure duration, while in C_3 the activity during 20 day altered significantly from the activity during 10 day exposure, and the 30 day from 20 day. In C_4 significant change in activity was noted during 20 and 30 days from 10 day exposure (Table *71, 72 & 73, Fig. 21*).

4.2.1.4 InVitro enzyme activity : Direct effect of individual pesticides

In this section the results of the direct effect of the three pesticides on the activity of ALP, ACP, GOT and GPT of brain, gill and liver is reported. A peripheral objective of this sort of experimentation is to gain an insight into the mechanisms of pesticide poisoning. Thus, a comparison could be made between the direct (in vitro) effects of pesticides on the enzymes and the indirect (in vivo) effects as measured in tissue preparations from fishes pre-exposed to pesticides.

Disruption of the enzyme system of different tissues was recorded following in vitro treatment of the three pesticides individually. Uniform concentrations, irrespective of pesticides, ranging from 10^{-8} ppm to 10^{-4} ppm, were added directly to the enzyme extract prepared from the brain, gills and liver. Fig. 22-24 show the effect

TABLE 74
Etroplus maculatus: Effect of increasing concentration of DDT in vitro on the activity of ALP in Brain, Gill and Liver

	CONTROL		C1 10-8ppm	C2 10-7ppm	C3 10-6ppm	C4 10-5ppm	C5 10-4ppm
		a	0.4559 +/-0.045	0.3472 +/-0.061	0.2781 +/-0.042	0.2004 +/-0.057	0.1726 +/-0.066
BRAIN	0.5479 +/-0.028	b	-16.79	-36.63	-49.24	-63.42	-68.49
		c	P<0.05	P<0.01	P<0.001	P<0.001	P<0.001
GILL		a	0.6227 +/-0.027	0.6118 +/-0.072	0.4959 +/-0.022	0.3217 +/-0.064	0.2614 +/-0.012
	0.6732 +/-0.038	b	-7.5	-9.12	-26.34	-52.21	-61.17
		c	NS	NS	P<0.01	P<0.01	P<0.001
LIVER		a	0.4722 +/-0.021	0.3608 +/-0.077	0.3136 +/-0.009	0.2916 +/-0.08	0.2463 +/-0.017
	0.5778 +/-0.095	b	-18.28	-37.56	-45.73	-49.53	-57.37
		c	NS	P<0.05	P<0.01	P<0.05	P<0.01

a: Mean enzyme activity (N=3) in u mol p-nitrophenol/mg pro./hr.
b: %Alteration from the mean control value.
c: Level of significance/non significant ns.

TABLE 75
Etroplus maculatus: Effect of increasing concentration of DDT in vitro on the activity of ACP in Brain, Gill and Liver.

	CONTROL		C1 10-8ppm	C2 10-7ppm	C3 10-6ppm	C4 10-5ppm	C5 10-4ppm
BRAIN		a	1.4678 +/-0.065	1.4063 +/-0.062	1.3122 +/-0.058	1.2488 +/-0.049	1.2452 +/-0.091
	1.5665 +/-0.068	b	-6.3	-10.23	-16.23	-20.28	-20.51
		c	NS	P<0.05	P<0.01	P<0.01	P<0.01
GILL		a	1.2074 +/-0.043	1.0721 +/-0.072	0.9321 +/-0.052	0.8127 +/-0.049	0.6974 +/-0.048
	1.2166 +/-0.062	b	-0.76	-11.88	-23.38	-34.0	-42.68
		c	NS	NS	P<0.01	P<0.001	P<0.001
LIVER		a	1.7393 +/-0.079	1.5842 +/-0.014	1.2471 +/-0.083	0.9497 +/-0.059	0.8321 +/-0.019
	1.9761 +/-0.094	b	-11.98	-19.83	-36.89	-51.94	-57.89
		c	P<0.05	P<0.001	P<0.001	P<0.001	P<0.001

a: Mean enzyme activity (M=3) in u mol p-nitrophenol/mg pro./hr.
b: %Alteration from the mean control value.
c: Level significance.

TABLE 76
Etropius maculatus: Effect of increasing concentration of DDT in vitro on the activity of GOT in Brain, Gill and Liver.

		CONTROL	C1 10-8ppm	C2 10-7ppm	C3 10-6ppm	C4 10-5ppm	C5 10-4ppm
BRAIN	a		1.2382 +/-0.054	1.2031 +/-0.037	0.8767 +/-0.027	0.5978 +/-0.022	0.4473 +/-0.056
	b	1.3729 +/-0.082	-9.81	-12.37	-36.14	-56.46	-67.42
	c		NS	P<0.05	P<0.001	P<0.001	P<0.001
GILL	a		0.5259 +/-0.07	0.4557 +/-0.044	0.4157 +/-0.01	0.3574 +/-0.028	0.2923 +/-0.103
	b	0.6914 +/-0.055	-23.94	-34.09	-39.87	-48.31	-57.72
	c		1.1483	1.0043	0.9848	0.8086	0.7376
LIVER	a		1.1483 +/-0.098	1.0043 +/-0.073	0.9848 +/-0.089	0.8086 +/-0.026	0.7376 +/-0.051
	b	1.3397 +/-0.014	-14.29	-25.04	-26.49	-39.64	-44.94
	c		P<0.05	P<0.01	P<0.01	P<0.001	P<0.001

a: Mean enzyme activity (N=3) in u mol sodium pyruvate/mg pro./hr.
b: %Alteration from the mean control value.
c: Level of significance.

TABLE 77
Etroplus maculatus: Effect of increasing concentration of DDT in vitro on th activity of GPT in Brain, Gill and Liver.

		CONTROL	C1 10-8ppm	C2 10-7ppm	C3 10-6ppm	C4 10-5ppm	C5 10-4ppm
BRAIN	a	1.3472 +/-0.059	1.2852 +/-0.078	1.208 +/-0.053	1.1043 +/-0.061	0.9943 +/-0.047	0.8321 +/-0.093
	b		-4.6	-10.33	-18.03	-26.19	-38.23
	c		NS	P<0.001	P<0.01	P<0.01	P<0.01
GILL	a	0.7346 +/-0.027	0.7085 +/-0.051	0.6324 +/-0.048	0.5241 +/-0.022	0.4908 +/-0.013	0.4073 +/-0.093
	b		-3.55	-13.91	-28.66	-33.19	-44.55
	c		NS	NS	P<0.001	P<0.001	P<0.01
LIVER	a	1.6575 +/-0.067	1.5197 +/-0.073	1.4784 +/-0.022	1.4127 +/-0.039	1.3527 +/-0.055	1.2014 +/-0.083
	b		-8.31	-10.81	-14.77	-18.39	-27.52
	c		NS	P<0.01	P<0.01	P<0.01	P<0.01

a: Mean enzyme activity (M=3) in u mol sodium pyruvate/mg pro./hr.
b: %Alteration from the mean control value.
c: Level of significance.

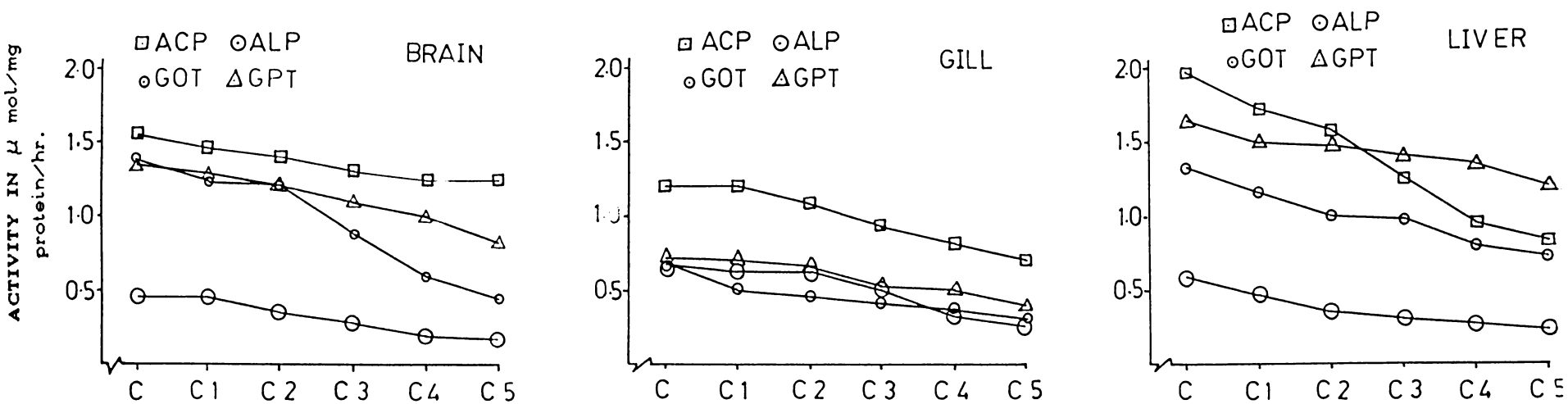

Fig.22. *Etroplus maculatus*: Effect of increasing concentration of DDT *in vitro* on the activity of ALP, ACP, GOT, GPT in (a) Brain (b) Gill and (c) Liver.

TABLE 78
Etroplus maculatus: Effect of increasing concentration of DIMECRON in vitro o the activity of ALP in Brain, Gill and Liver.

		CONTROL	C1 10-8ppm	C2 10-7ppm	C3 10-6ppm	C4 10-5ppm	C5 10-4pm
BRAIN	a		0.5327 +/-0.011	0.4576 +/-0.077	0.4107 +/-0.028	0.3621 +/-0.057	0.3099 +/-0.049
	b	0.5714 +/-0.093	-6.77	-19.91	-28.12	-36.62	-45.76
	c		NS	NS	P<0.05	P<0.05	P<0.05
GILL	a		0.5353 +/-0.028	0.4721 +/-0.015	0.4009 +/-0.072	0.3307 +/-0.052	0.2731 +/-0.046
	b	0.5779 +/-0.036	-7.37	-18.30	-30.62	-42.77	-52.74
	c		NS	P<0.01	P<0.05	P<0.05	P<0.01
LIVER	a		0.5081 +/-0.023	0.4799 +/-0.042	0.4212 +/-0.051	0.3791 +/-0.028	0.3193 +/-0.033
	b	0.5422 +/-0.028	-6.289	-11.49	-22.31	-30.08	-41.11
	c		P<0.001	P<0.01	P<0.001	P<0.001	P<0.001

a: Mean enzyme activity (N=3) in u mol p-nitrophenol/mg pro./hr.
b: %Alteration from the mean control value.
c: Level of significance.

TABLE 79
Etroplus maculatus: Effect of increasing concentration of DIMECRON in vitro on the activity of ACP in Brain, Gill and Liver.

		C1	C2	C3	C4	C5
CONTROL		10-8pm	10-7pm	10-6ppm	10-5ppm	10-4pm
BRAIN	a	1.5426	1.5071	1.4472	1.3371	1.2124
		+/-0.099	+/-0.021	+/-0.098	+/-0.052	+/-0.09
1.5866	b	-2.77	-5.01	-8.78	-15.72	-23.58
+/-0.1						
	c	NS	NS	NS	P<0.05	P<0.01
GILL	a	1.0013	0.8487	0.7533	0.6384	0.5001
		+/-0.062	+/-0.097	+/-0.019	+/-0.022	+/-0.098
1.1497	b	-12.9	-26.18	-34.47	-44.47	-56.5
+/-0.043						
	c	P<0.05	P<0.01	P<0.001	P<0.001	P<0.001
LIVER	a	1.7599	1.6329	1.4738	1.3159	1.1977

a: Mean enzyme activity (N=3) in u mol p-nitrophenol/mg pro./hr.
b: %Alteration from the mean control value.
c: Level of significance.

TABLE 80
Etroplus maculatus: Effect of increasing concentration of DIMECRON in vitro on the activity of GOT in Brain, Gill and Liver.

	CONTROL		C1 10-8ppm	C2 10-7ppm	C3 10-6ppm	C4 10-5ppm	C5 10-4ppm
BRAIN		a	1.4614 +/-0.07	1.3914 +/-0.103	1.2987 +/-0.071	1.2014 +/-0.038	1.1341 +/-0.13
	1.5064 +/-0.089	b	-2.98	-7.634	-13.78	-20.24	-24.71
		c	P<0.001	NS	P<0.05	P<0.05	P<0.01
GILL		a	0.4376 +/-0.087	0.3671 +/-0.093	0.2741 +/-0.043	0.2119 +/-0.048	0.1993 +/-0.021
	0.5934 +/-0.017	b	-26.25	-38.136	-53.8	-64.29	-66.41
		c	P<0.05	P<0.05	P<0.05	P<0.001	P<0.001
LIVER		a	1.2873 +/-0.021	1.1321 +/-0.012	0.9677 +/-0.099	0.7914 +/-0.091	0.6301 +/-0.052
	1.4763 +/-0.069	b	-12.8	-23.315	-34.45	-46.39	-57.31
		c	P<0.001	P<0.05	P<0.01	P<0.01	P<0.001

a: Mean enzyme activity (N=3) in u mol sodium pyruvate/mg pro./hr.
b: %Alteration from the mean control value.
c: Level of significance.

TABLE 81
Etroplus maculatus: Effect of increasing concentration of DIMECRON in vitro the activity of GPT in Brain, Gill and Liver.

		CONTROL	C1 10-8ppm	C2 10-7ppm	C3 10-6ppm	C4 10-5ppm	C5 10-4ppm
BRAIN	a	1.3021 +/-0.083	1.2288 +/-0.044	1.1635 +/-0.045	1.0287 +/-0.056	0.8732 +/-0.091	0.8255 +/-0.029
	b		-5.62	-10.64	-20.99	-32.93	-36.6
	c		NS	NS	P<0.01	P<0.01	P<0.001
GILL	a	0.6025 +/-0.08	0.5431 +/-0.057	0.4984 +/-0.029	0.4278 +/-0.077	0.3416 +/-0.014	0.2418 +/-0.019
	b		-9.85	-17.27	-28.99	-43.3	-59.86
	c		NS	NS	NS	P<0.01	P<0.01
LIVER	a	1.7159 +/-0.039	1.5104 +/-0.06	1.2579 +/-0.054	1.2159 +/-0.021	0.7948 +/-0.029	0.7023 +/-0.093
	b		-11.97	-26.69	-29.139	-53.68	-59.07
	c		P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

a: Mean enzyme activity (M=3) in u mol sodium puruvate/mg pro./hr.
b: %Alteration from the mean control value.
c: Level of significance.

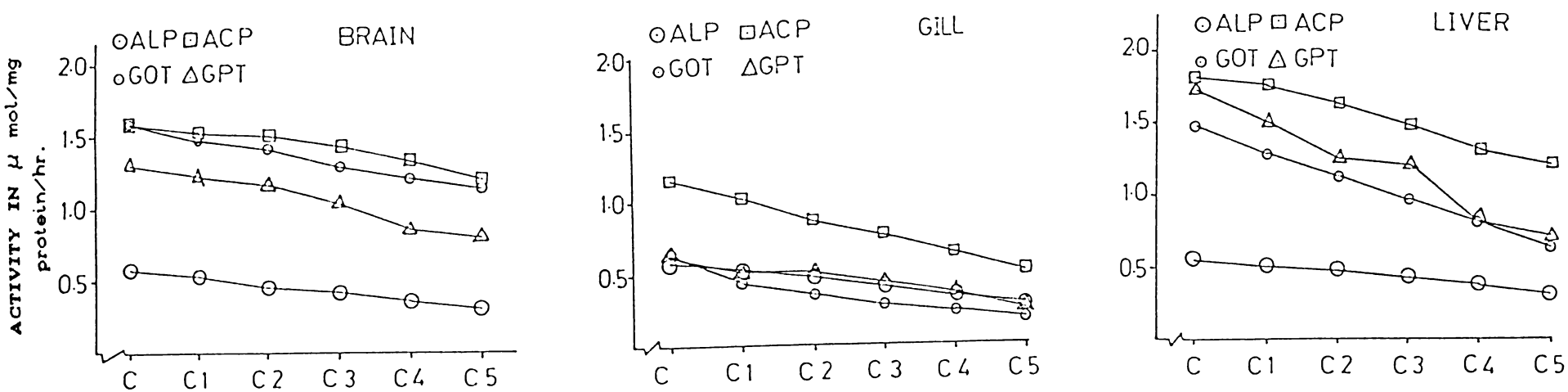

Fig.23. *Etroplus maculatus*: Effect of increasing concentration of Dimecron *in vitro* on the activity of ALP, ACP, GOT, GPT in (a) Brain (b) Gill and (c) Liver.

TABLE 82
Etroplus maculatus: Effect of increasing concentration of GRAMOXONE in vitro on the activity of ALP in Brain, Gill and Liver.

		CONTROL	C1 10^{-8} ppm	C2 10^{-7} ppm	C3 10^{-6} ppm	C4 10^{-5} ppm	C5 10^{-4} ppm
BRAIN	a	0.4324 +/-0.023	0.3728 +/-0.019	0.3017 +/-0.068	0.2516 +/-0.085	0.2174 +/-0.047	0.1984 +/-0.043
	b		-13.78	-30.22	-41.81	-49.72	-54.11
	c		P<0.05	P<0.05	P<0.05	P<0.01	P<0.01
GILL	a	0.5143 +/-0.062	0.4981 +/-0.021	0.4709 +/-0.019	0.4574 +/-0.074	0.3021 +/-0.033	0.2671 +/-0.037
	b		-3.14	-8.438	-11.06	-41.25	-48.06
	c		NS	NS	NS	P<0.01	P<0.01
LIVER	a	0.4317 +/-0.043	0.4057 +/-0.039	0.3991 +/-0.03	0.1909 +/-0.042	0.1844 +/-0.022	0.1259 +/-0.012
	b		-6.02	-7.55	-55.77	-57.28	70.83
	c		NS	NS	P<0.01	P<0.001	P<0.001

a: Mean enzyme activity (M=3) in u mol p-nitrophenol/mg pro./hr.
b: %Alteration from the mean control value.
c: Level of significance.

TABLE 83
Etroplus maculatus: Effect of increasing concentration of GRAMOXONE in vitro on the activity of ACP in Brain, Gill, and Liver.

		CONTROL	C1 -8 10 ppm	C2 -7 10 ppm	C3 -6 10 ppm	C4 -5 10 ppm	C5 -4 10 ppm
BRAIN	a		1.2953 +/-0.039	1.1494 +/-0.074	0.8399 +/-0.098	0.5567 +/-0.093	0.2247 +/-0.061
	b	1.5427 (0.07)	-16.03	-25.49	-45.55	-63.91	-85.43
	c		P<0.01	P<0.01	P<0.001	P<0.001	P<0.001
GILL	a		1.0724 +/-0.012	1.0126 +/-0.074	0.8744 +/-0.051	0.6471 +/-0.096	0.4424 +/-0.099
	b	1.1974 +/-0.027	10.43	-15.43	-26.97	-45.95	-63.05
	c		P<0.01	P<0.05	P<0.001	P<0.001	P<0.001
LIVER	a		1.5349 +/-0.099	1.0148 +/-0.098	0.7437 +/-0.054	0.5963 +/-0.073	0.3271 +/-0.044
	b	1.7214 +/-0.101	-10.83	-41.04	-56.79	-65.35	-80.99
	c		P<0.05	P<0.001	P<0.001	P<0.001	P<0.001

a: Mean enzyme activity (M=3) in u mol p-nitrophenol/mg pro./hr.
b: %Alteration from the mean value.
c: Level of significance.

TABLE 84

Etroplus maculatus: Effect of increasing concentration of GRAMOXONE in vitro on the activity of GOT in Brain, Gill and Liver.

		CONTROL	C1 -8 10 ppm	C2 -7 10 ppm	C3 -6 10 ppm	C4 -5 10 ppm	C5 -4 10 ppm
BRAIN	a		1.2485 +/-0.028	1.1197 +/-0.098	0.9876 +/-0.077	0.7657 +/-0.045	0.5533 +/-0.099
	b	1.3744 +/-0.103	-9.16	-18.53	-28.14	-44.28	-59.74
	c		NS	P<0.05	P<0.01	P<0.001	P<0.001
GILL	a		0.4376 +/-0.023	0.3465 +/-0.033	0.2995 +/-0.029	0.1454 +/-0.051	0.1223 +/-0.035
	b	0.5121 +/-0.083	-14.54	-32.33	-41.51	-71.6	-76.11
	c		NS	P<0.05	P<0.05	P<0.01	P<0.01
LIVER	a		1.2547 +/-0.047	1.0345 +/-0.096	0.7657 +/-0.103	0.5246 +/-0.055	0.5103 +/-0.07
	b	1.4956 +/-0.071	-16.1	-30.83	-48.8	-64.92	-65.87
	c		P<0.01	P<0.01	P<0.001	P<0.001	P<0.001

a: Mean enzyme activity (M=3) in u mol sodium pyruvate/mg pro./hr.
b: %Alteration from the mean value.
c: Level of significance.

TABLE 85
Etroplus maculatus: Effect of increasing concentration of GRAMOXONE invitro on the activity of GPT in Brain, Gill and Liver.

		CONTROL	C1 -8 10 ppm	C2 -7 10 ppm	C3 -6 10 ppm	C4 -5 10 ppm	C5 -4 10 ppm
BRAIN	a	1.3729 +/-0.078	1.3097 +/-0.012	1.1542 +/-0.103	0.9724 +/-0.029	0.6474 +/-0.046	0.4027 +/-0.025
	b		-4.6	-15.92	-29.17	-52.84	-70.66
	c		NS	P<0.05	P<0.01	P<0.001	P<0.001
GILL	a	0.7251 +/-0.041	0.6841 +/-0.022	0.5154 +/-0.079	0.4227 +/-0.019	0.2547 +/-0.011	0.1899 +/-0.03
	b		-5.65	-28.92	-41.7	-64.87	-73.81
	c		NS	P<0.05	P<0.001	P<0.001	P<0.001
LIVER	a	1.5121 +/-0.053	1.4563 +/-0.099	1.0067 +/-0.028	0.8643 +/-0.011	0.6893 +/-0.071	0.3349 +/-0.088
	b		-3.69	33.423	-42.84	-54.41	-77.85
	c		NS	P<0.001	P<0.001	P<0.001	P,0.001

a: Mean enzyme activity (M=3) in u mol sodium pyruvate/mg pro./hr.
b: %Alteration from the mean value.
c: Level of significance.

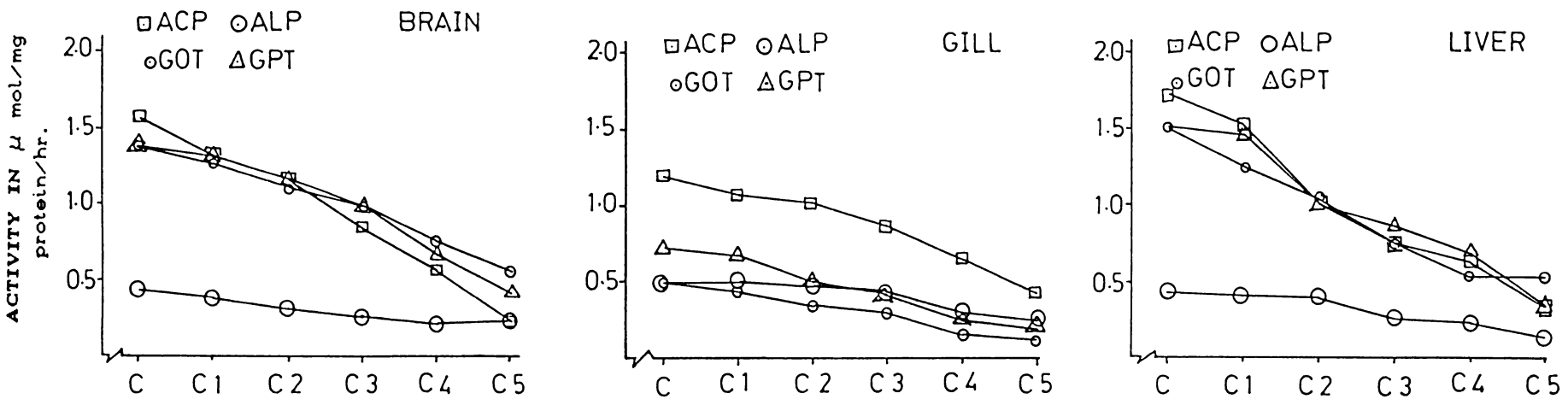

Fig.24. *Etroplus maculatus*: Effect of increasing concentration of Gramoxone *in vitro* on the activity of ALP, ACP, GOT, GPT in (a) Brain (b) Gill and (c) Liver.

of the five concentrations of DDT, Dimecron and Gramoxone. All the three pesticides caused inhibition of the four enzymes under study in brain, gills and liver. The enzymatic inhibition was pronounced and significant in higher concentrations indicating a definite dose dependent response. The inhibitory effect of the three pesticides was found to be more or less of the same pattern.

4.2.1.4.1 Alkaline phosphatase (ALP)

The maximum inhibition of ALP activity recorded in liver following in vitro exposure to gramoxone followed by the activity in brain due to DDT. In vitro Dimecron effect was comparatively low *(Table 74, 78 & 82)*.

4.2.1.4.2 Acid phosphatase (ACP)

Gramoxone caused maximum inhibition of ACP activity in all three tissues followed by DDT and Dimecron caused lesser inhibition *(Table 75, 79 & 83)*.

4.2.1.4.3 Glutamate oxaloacetate transaminase (GOT)

In vitro GOT activity showed maximum inhibition following gramoxone addition. However, in liver the GOT activity showed maximum

inhibition due to DDT. Of the three tissues, GOT showed maximum inhibition in liver following gramoxone addition (*Table 76, 80 & 84*).

4.2.*1*.4.4 Glutamate pyruvate transaminase (GPT)

Gramoxone caused the maximum inhibition of in vitro activity of GPT in all the three tissues and maximum inhibition was noted in liver. Compared to DDT, Dimecron caused higher inhibition of GPT activity in all the tissues *(Table 77, 81 & 85)*.

4.2.2 Sublethal Effects on Haematology: Under individual pesticide exposure.

A number of environmental variables have been proven to have reflections in the peripheral blood make-up. In some cases even the minutest environmental change is reflected by a measurable physiological change that may influence the results of the experiment (Klontz and Smith, *1968*). Changes in the physical and|or chemical environmental conditions do affect the physiology of fishes and reflect themselves as changes in the peripheral blood. Despite the fact that pesticide pollution of the aquatic environment is a present day major threat, only little attention have been given to the study of the haematological effects of pesticides poisoning in fishes.

4.2.2.*1* DDT

4.2.2.*1.1* Total erythrocyte count (TEC)

Short term:- TEC showed a general increasing trend with concentration in the three higher concentrations reaching statistically significant level in C_4, preceded by an initial decrease in C_1 during 24 hour exposure while it showed an increasing trend in lower three concentrations during 72 hours, but in the highest concentration it showed a lower value. During 120 hour exposure TEC found to decrease with increase in concentration, reaching statistically significant levels in higher three concentrations (C_2, C_3 and C_4). In general, TEC recorded an initial increase followed by decrease towards the final phase during short term exposure (*Table 86, 87 & 88, Fig. 25*).

Long term:- In general, TEC recorded statistically significant reduction in higher concentrations and was precisely evident during 30 day exposure. During 10 day exposure an initial lowering in TEC was found in lower concentrations followed by an increase during 20 day exposure. TEC response, in genera, found to be negatively affected due to DDT exposure(*Table 89, 90 & 91, Fig. 25*).

TABLE 86

Etropius maculatus: Haematological status of control fish and those pre-exposed to four SLC of DDT for 24 hrs.

		TEC 10^6 mm^3	Hb (g%)	Ht %	MCV μm^3	MCH pg	MCHC %	VI	CI	SI
CONTROL	a	2.984 +/-0.102	12.764 +/-0.307	29.175 +/-0.725	96.334 +/-2.377	42.117 +/-0.81	43.761 +/-0.992	1.00	1.00	1.00
	a	2.964 +/-0.092	12.834 +/-0.121	30.954 +/-0.314	104.486 +/-2.197	43.322 +/-0.069	41.462 +/-0.022	1.013 +/-0.022	1.068 +/-0.002	0.947
C1	b	-0.670	+0.548	+5.747	+7.802	+2.781	-5.544	---	---	---
0.00033	c	NS	NS	P<0.01	P<0.001	NS	P<0.001			
	a	3.09 +/-0.092	13.01 +/-0.104	31.007 +/-0.413	97.189 +/-3.647	40.769 +/-0.295	41.96 +/-0.027	0.985 +/-0.024	1.027 +/-0.006	0.959
C2	b	+3.552	+1.927	+5.908	+0.879	-3.258	-4.292			
0.00045	c	NS	NS	P<0.01	NS	NS	P<0.01			
	a	3.098 +/-0.099	13.074 +/-0.07	31.217 +/-0.401	100.817 +/-2.016	42.233 +/-1.196	41.884 +/-0.37	0.987 +/-0.027	1.031 +/-0.021	0.954 +/-0.011
C3	b	+3.820	+2.428	+6.541	+4.446	+0.274	-4.481			
0.0013	c	NS	NS	P<0.001	P<0.05	NS	P<0.01			
	a	3.256 +/-0.136	13.138 +/-0.069	31.269 +/-0.275	96.143 +/-3.281	40.4 +/-1.502	42.019 +/-0.478	0.94 +/-0.035	0.983 +/-0.034	0.960 +/-0.011
C4	b	+9.115	+2.930	+6.696	-0.198	-4.249	-4.145			
0.0026	c	P<0.01	P<0.05	P<0.001	NS	NS	P<0.01			

a: Mean value (N=5) with +/-SD.

b: %Alteration from the mean value.

c: Level of significance.

TABLE 37

Etroplus maculatus: Haematological status of control fish and those pre-exposed to four SLC of DDT for 72 hrs.

		TEC 10^6 mm^3	Hb g%	Ht %	MCV μm^3	MCH pg	MCHC %	VI	CI	SI
CONTROL	a	3.068 +/-0.128	12.799 +/-0.156	29.525 +/-0.703	96.108 +/-2.351	41.758 +/-0.540	43.446 1.00	1.00	1.00	1.00
	a	3.118 +/-0.086	12.88 +/-0.089	30.752 +/-0.499	98.667 +/-2.158	41.327 +/-0.89	41.888 +/-0.522	0.991 +/-0.021	1.027 +/-0.022	0.964 +/-0.012
C1	b	+1.629	+0.632	+3.989	+2.593	-1.042	-3.719			
0.00033	c	NS	NS	P<0.05	NS	NS	p<0.01			
	a	3.138 +/-0.177	13.2 +/-0.363	30.82 +/-0.408	98.487 +/-2.15	41.568 +/-3.329	42.824 +/-0.642	1.009 +/-0.032	1.024 +/-0.046	0.0986 +/-0.015
C2	b	+2.281	+3.133	+4.201	+2.415	-0.457	-1.452			
0.0045	c	NS	NS	P<0.01	NS	NS	NS			
	a	3.264 +/-0.118	14.044 +/-1.197	31.127 +/-0.026	95.464 +/-3.405	42.979 +/-2.524	45.117 +/-1.821	1.030 +/-0.060	0.993 +/-0.035	1.038 +/-0.088
C3	b	+6.388	+9.727	+5.146	-0.674	+2.840	+3.703			
0.0013	c	P<0.05	P<0.05	P<0.001	NS	NS	NS			
	a	2.972 +/-0.129	14.386 +/-1.384	31.244 +/-0.025	105.284 +/-3.44	48.355 +/-1.587	46.041 +/-1.402	1.159 +/-0.084	1.095 +/-0.046	1.059 +/-0.100
C4	b	-3.129	+12.399	+5.501	+8.715	+13.642	+5.636			
0.0026	c	NS	P<0.05	P<0.001	P<0.01	P<0.001	P<0.01			

a: Mean value (N=5) with +/-SD.

b: %Alteration from the mean value.

c: Level of significance.

TABLE 88

Etropius maculatus: Haematological status of control fish and those pre-exposed to four SLC of DDT for 120 hrs.

		TEC 10^6 mm^3	Hb g%	Ht %	MCV μm^3	MCH pg	MCHC %	VI	CI	SI
	a	3.01	12.684	28.975	96.434	42.217	43.757			
CONTROL		+/-0.116	+/-0.72	+/-0.455	+/-2.247	+/-1.81	+/-1.927	1.00	1.00	1.00
	a	2.922	12.945	31.097	106.781	44.44	41.628	1.055	1.108	0.951
		+/-0.188	+/-0.092	+/-0.076	+/-6.913	+/-2.679	+/-0.217	+/-0.064	+/-0.072	+/-0.005
C1	b	-2.923	+2.057	+6.823	+9.689	+5.002	-5.114			
0.00033	c	NS	NS	P<0.001	P<0.05	Ns	P<0.05			
	a	2.802	12.923	31.915	113.909	46.141	40.513	1.095	1.182	0.926
		+/-0.09	+/-0.216	+/-0.293	+/-2.821	+/-0.799	+/-0.408	+/-0.019	+/-0.029	+/-0.009
C2	b	-11.760	+0.638	+11.404	+21.881	+12.380	-12.111			
0.0013	c	P<0.01	NS	P<0.001	P<0.001	P<0.01	P<0.001			
	a	2.680	11.666	33.493	124.88	43.496	34.853	1.032	1.296	0.796
		+/-0.139	+/-0.184	+/-0.567	+/-4.45	+/-1.587	+/-0.825	+/-0.038	+/-0.046	+/-0.019
C4	b	-10.963	-8.025	-13.489	+22.778	+2.940	-25.547			
0.0026	c	P<0.01	P<0.05	P<0.001	NS	P<0.001				

a: Mean value (N=5) with +/-SD.

b: %Alteration from the mean value.

c: Level of significance.

TABLE 89

Etroplus maculatus: Haematological status of control fish and those pre-exposed to four SLC of DDT for 10 days.

		FEC 10^6 mm^3	Hb g%	Ht %	MCV μm^3	MCH pg	MCHC	CI	VI	SI
	a	3.36	12.505	28.626	85.266	37.251	43.685			
CONTROL		+/-0.14	+/-0.275	+/-1.648	+/-4.071	+/-1.047	+/-1.237	1.00	1.00	1.00
	a	3.258	12.005	29.135	89.466	36.869	42.904	0.991	1.049	0.982
		+/-0.105	+/-0.158	+/-0.437	+/-1.981	+/-0.842	+/-3.279	+/-0.023	+/-0.023	+/-0.075
C1	b	-3.035	-3.998	+1.147	+4.694	-1.036	-1.820			
0.00013	c	NS	P<0.01	NS	NS	NS	NS			
	a	3.104	11.777	30.816	99.365	38.002	38.219	1.021	1.165	0.875
		+/-0.155	+/-0.135	+/-0.951	+/-2.516	+/-1.527	+/-1.659	+/-0.041	+/-0.029	+/-0.038
C2	b	-17.619	-5.821	+7.106	+14.189	+1.976	-14.301			
0.00017	c	P<0.05	P<0.001	P<0.05	P<0.001	NS	P<0.001			
	a	3.15	11.672	35.254	111.9	37.089	33.118	0.997	1.313	0.758
		+/-0.116	+/-0.066	+/-0.603	+/-2.567	+/-1.191	+/-0.749	+/-0.032	+/-0.030	+/-0.017
C3	b	-6.249	-6.661	+18.80	+23.801	-0.436	-31.907			
0.00026	c	P<0.05	P<0.001	P<0.001	P<0.001	NS	P<0.001			
	a	3.406	10.883	36.265	106.63	32.019	29.971	0.860	1.251	0.686
		+/-0.172	+/-0.06	+/-0.58	+/-3.914	+/-1.504	+/-0.614	+/-0.040	+/-0.046	+/-0.014
C4	b	+1.369	-12.970	+21.06	+20.035	-16.340	-45.757			
0.00052	c	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001			

a: Mean value (N=5) with +/-SD.

b: %Alteration from the mean value.

c: Level of significance.

TABLE 90

Etroplus maculatus: Haematological status of control fish and those pre-exposed to four SLC of DDT for 20 days.

		TEC 10^6 /cu mm	Hb g%	Ht %	MCV cu mm	MCH pg	MCHC %	VI	CI	SI
CONTROL	a	3.38 +/-0.124	12.263 +/-0.219	29.159 +/-1.299	86.338 +/-3.37	36.302 +/-0.682	42.054 +/-3.438	1.00	1.00	1.00
C1	a	3.456 +/-0.143	11.303 +/-0.117	32.963 +/-0.284	96.3 +/-2.779	33.705 +/-0.867	34.76 +/-0.592	0.929 +/-0.024	1.142 +/-0.038	0.827 +/-0.014
	b	+2.248	-7.8284	+11.54	+12.168	-7.705	-20.9839			
0.00013	c	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.01			
C2	a	3.424 +/-0.077	10.902 +/-0.054	36.386 +/-1.13	106.255 +/-1.525	31.853 +/-0.581	28.657 +/-2.665	0.878 +/-0.016	1.232 +/-0.018	0.681 +/-0.063
	b	+1.301	-11.098	+19.85	+18.744	-13.967	-46.749			
0.00017	c	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001			
C3	a	3.172 +/-0.153	8.343 +/-0.912	38.101 +/-0.954	120.237 +/-3.328	26.252 +/-1.878	21.864 +/-1.909	0.724 +/-0.052	1.394 +/-0.039	0.519 +/-0.045
	b	-6.153	-31.966	+23.469	+28.193	-38.282	-92.343			
0.00026	c	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001			
C4	a	2.968 +/-0.144	7.227 +/-0.911	38.351 +/-0.841	129.35 +/-3.493	24.286 +/-2.135	18.814 +/-2.035	0.669 +/-0.059	1.499 +/-0.041	0.447 +/-0.048
	b	-12.189	-41.066	+23.968	+33.252	-49.477	-123.525			
0.00052	c	P<0.01	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001			

a: Mean value (N=5) with +/-SD.

b: %Alteration from the mean value.

c: Level of significance.

TABLE 91

Etroplus maculatus: Haematological status of control fish and those pre-exposed to four SLC of DDT for 30 days.

		TEC 10^6 mm^3	Hb g%	Ht %	MCV um^3	MCH pg	MCHC %	VI	CI	SI
CONTROL	a	3.364 +/-0.126	12.109 +/-0.294	30.916 +/-1.472	91.973 +/-5.414	36.025 +/-0.975	39.186 +/-4.501	1.00	1.00	1.00
C1 0.00013	a	2.74 +/-0.145	9.689 +/-0.291	34.085 +/-0.796	124.556 +/-3.748	35.413 +/-1.19	28.442 +/-0.974	0.984 +/-0.033	1.354 +/-0.041	0.726 +/-0.025
	b	-18.549	-19.985	+9.297	+26.159	-1.728	-37.775			
	c	P<0.001	P<0.001	P<0.01	P<0.001	NS	P<0.001			
C2 0.00017	a	2.568 +/-0.233	7.501 +/-0.195	36.504 +/-1.1	142.801 +/-9.046	29.348 +/-2.019	20.563 +/-0.714	0.816 +/-0.056	1.552 +/-0.098	0.525 +/-0.078
	b	-23.662	-38.054	+15.307	+35.593	-22.751	-90.565			
	c	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001			
C3 0.00026	a	2.426 +/-0.177	7.056 +/-0.272	40.267 +/-0.778	166.518 +/-9.2	29.145 +/-1.042	17.518 +/-0.372	0.809 +/-0.029	1.811 +/-0.1	0.447 +/-0.009
	b	-27.883	-41.729	+23.22	+44.766	-23.606	-123.689			
	c	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001			
C4 0.00052	a	2.322 +/-0.169	6.401 +/-0.223	41.939 +/-0.457	181.276 +/-11.438	27.631 +/-1.116	15.26 +/-0.4	0.768 +/-0.031	1.971 +/-0.124	0.389 +/-0.010
	b	-30.975	-47.138	+26.283	+49.263	-30.378	-156.789			
	c	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001			

a: Mean value (N=5) with +/-SD.

b: %Alteration from the mean value.

c: Level of significance.

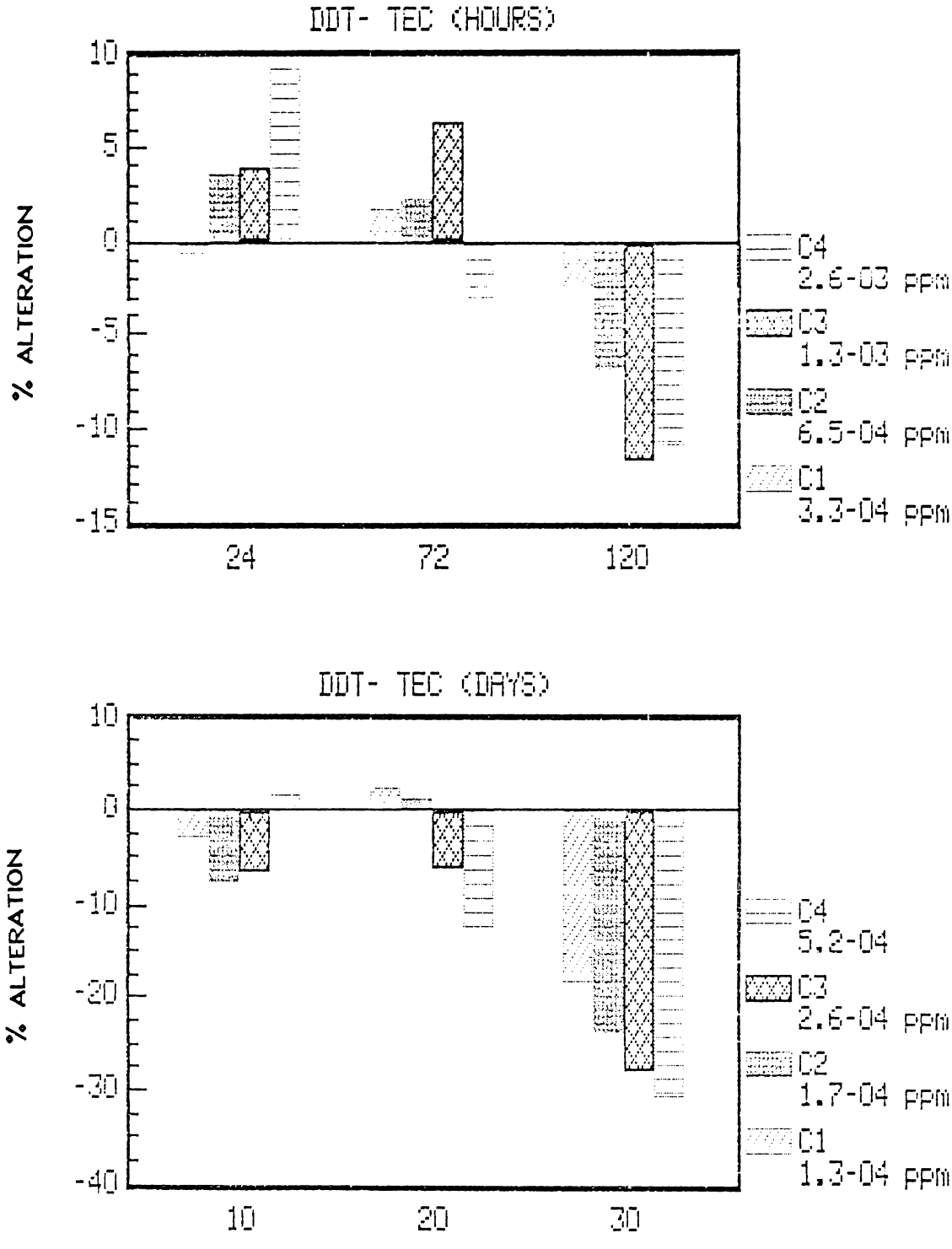

Fig. No. 25 *Etroplus maculatus* : Percentage alteration in TEC from mean control value following exposure to four SLC of DDT.

4.2.2.1.2 Haemoglobin Content (Hb)

Short term:- During short term exposure the Hb content showed an increasing trend at 24 and 72 hours exposure reaching statistically significant levels in higher concentrations. However, a comparative increase recorded in lower concentrations during 120 hour exposure narrowing down to reach a statistically significant reduction in Hb in the highest concentration (C_4). In general, the initial increase in Hb during early and middle phases of pesticide exposure was followed by a reduction in Hb towards the late phase of exposure *(Table 86, 87 & 88, Fig. 26)*.

Long term:- Hb content of the experimental groups showed a progressive, statistically significant, and both dose and duration dependent reduction during the long term exposure *(Table 89, 90 & 91, Fig. 26)*.

4.2.2.1.3 Haematocrit (Ht)

Short term:- A directly proportional, statistically significant increase in Ht was recorded in all pesticide concentrations. The increase in Ht with increase in exposure duration was also evident and found statistically significant in higher three concentrations *(Table 86, 87 & 88, Fig. 27)*.

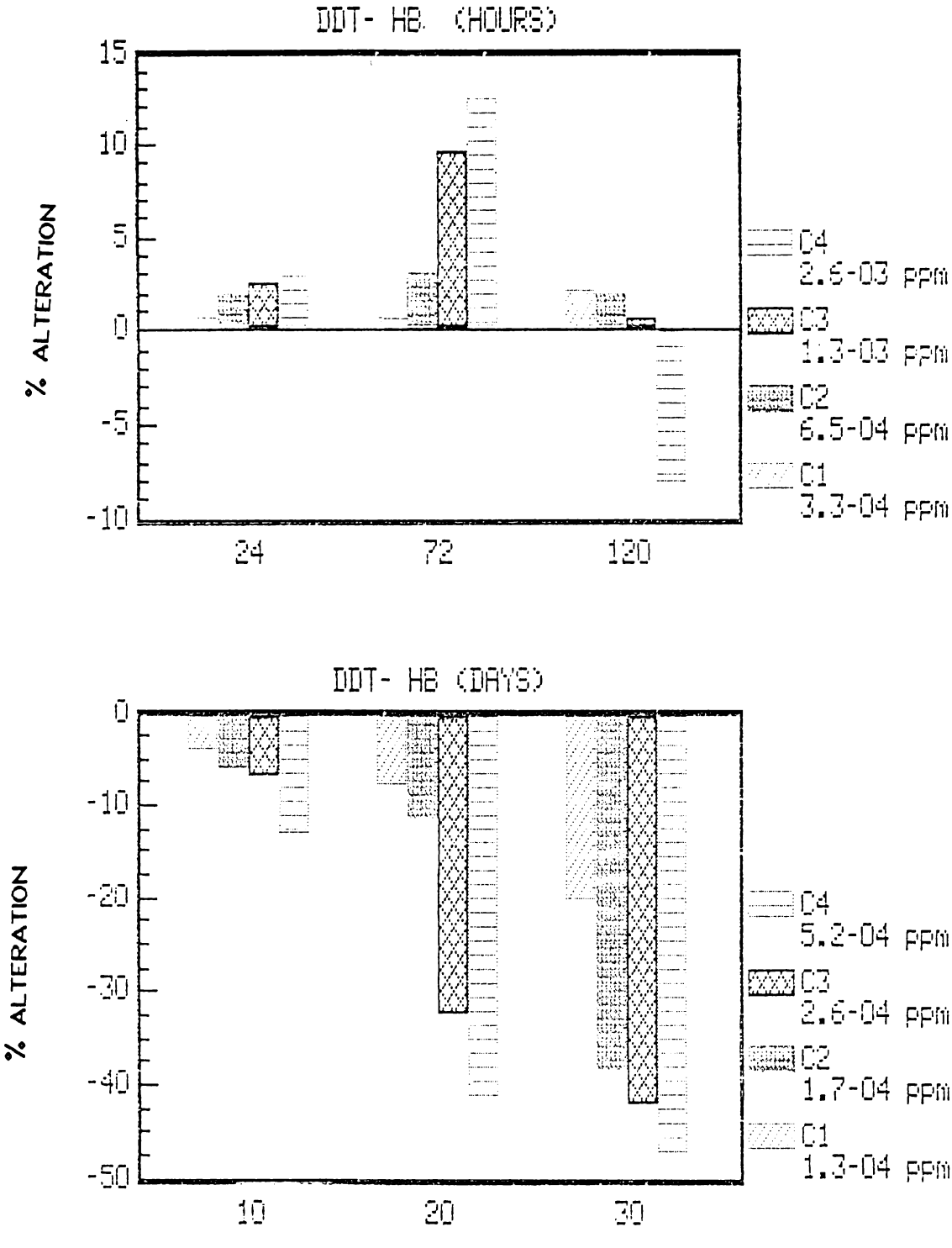

Fig. No. 26 *Etroplus maculatus* : Percentage alteration in HB from mean control value following exposure to four SLC of DDT.

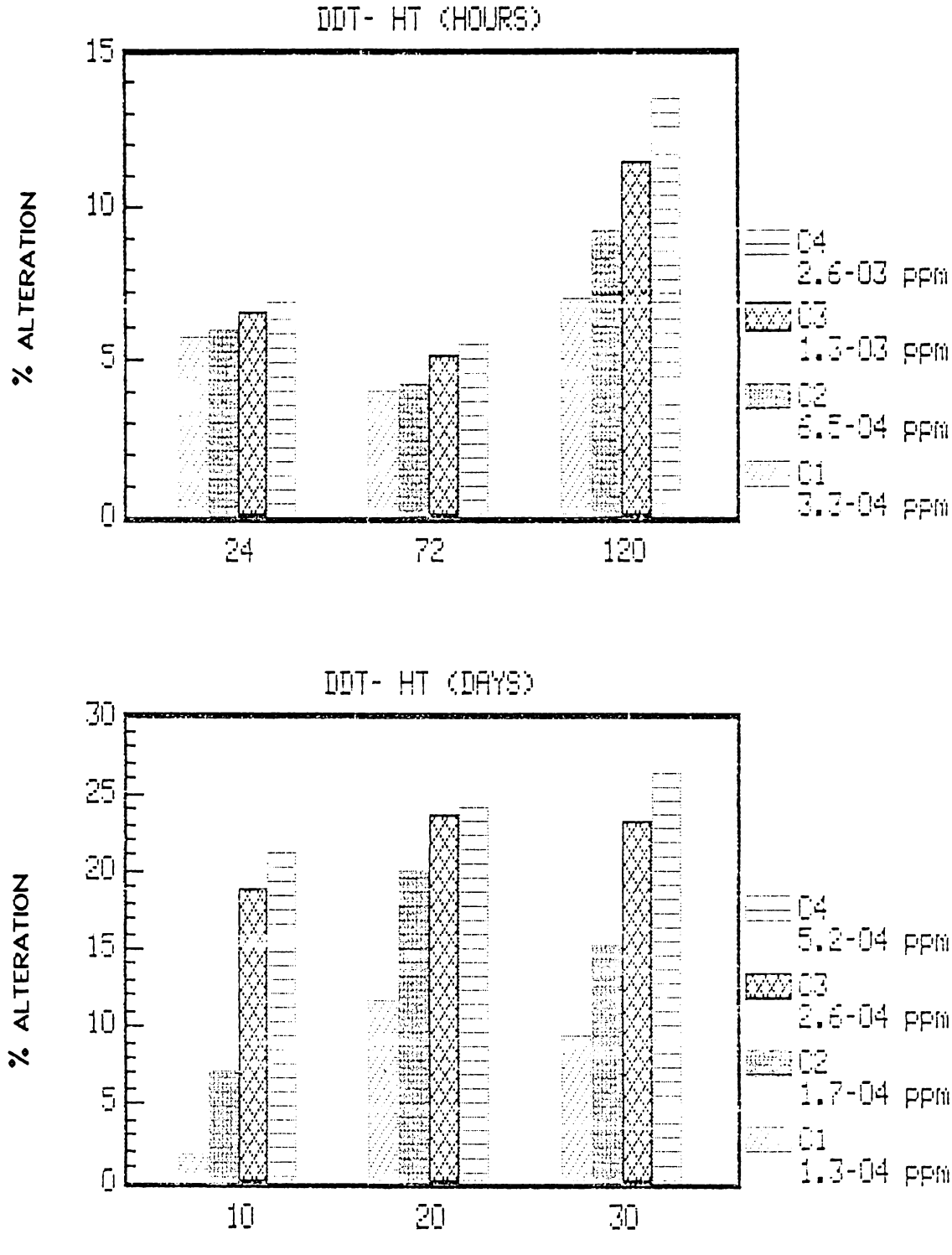

Fig. No. 27 *Etroplus maculatus* : Percentage alteration in HT from mean control value following exposure to four SLC of DDT.

Long term:- Similar to the results obtained during short term exposure, Ht showed a progressive and statistically significant (except in C_1 during 10 day exposure) increase during long term exposure. The increase in Ht with increase in exposure duration also found statistically significant *(Table 89, 90 & 91, Fig. 27)*.

4.2.2.*1*.4 Erythrocyte constants (MCV, MCH & MCHC)

Short term:- Mean corpuscular volume (MCV) showed an erratic response during initial and intermediate phases of exposure while towards late phase (120 hours) exposure, the MCV recorded statistically significant increase. Generally the MCV of experimental groups showed an increasing trend during 24 and 72 hours exposure with an exception in C_4 and C_3 during 24 and 72 hours exposure respectively where a slight reduction in MCV was recorded.

Mean corpuscular haemoglobin (MCH) recorded an erratic response during 24 and 72 hours exposure while during 120 hour exposure MCH found to be elevated and statistically significant in intermediate concentrations (C_2 and C_3). In general, the MCH recorded increase in experimental groups (since no significant change, except in C_4 during 72 hour, was noted during 24 and 72 hours exposure).

In general, mean corpuscular haemoglobin was found lowered in exposed groups and the reduction was statistically significant during 20 and 30 days exposure *(Table 86, 87 & 88, Fig. 28, 29 & 30)*.

Long term:- MCV recorded a dose proportional increase and the increase was found statistically significant. MCV showed progressive increase with increase in exposure duration.

A progressive and statistically significant (except in ' concentrations during 10 day exposure) reduction was recorded in MCH of experimental groups. This change in MCH was found comparatively higher during 20 day exposure.

Like-wise mean corpuscular haemoglobin concentration also recorded a progressive dose and duration dependent inverse change in exposed groups. The reduction in MCHC was statistically significant in higher concentrations *(Table 89, 90 & 91, Fig. 28, 29 & 30)*.

4.2.2.1.5 Erythrocyte indices (CI, VI & SI)

Short term:- In general, volume index recorded higher values than unity and high volume index was more obvious towards the late phase of exposure. The high volume index recorded, in confirmity with increased MCV, indicated the occurrence of macrocytosis of erythrocytes

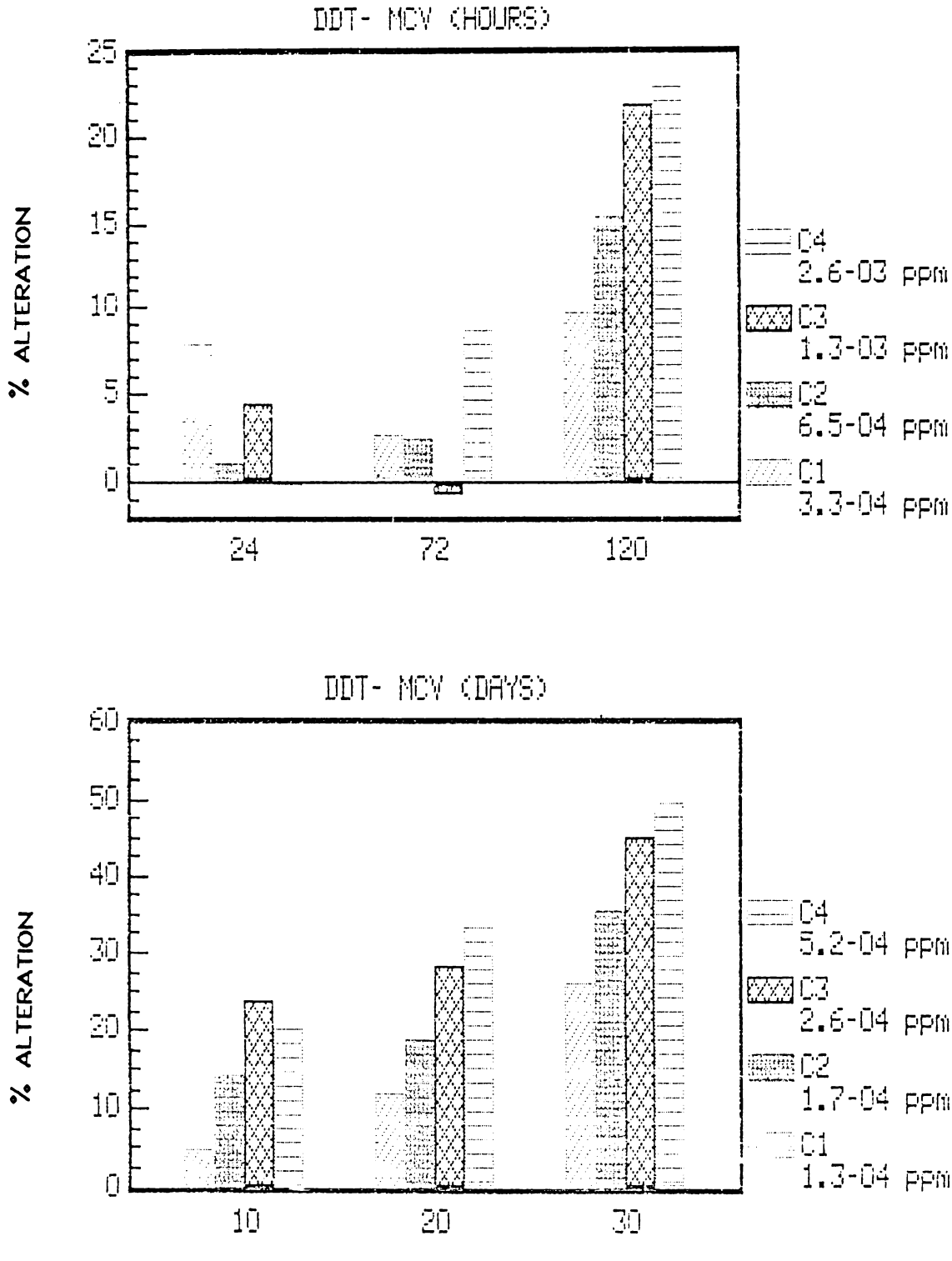

Fig. No. 28 *Etroplus maculatus* : Percentage alteration in MCV from mean control value following exposure to four SLC of DDT.

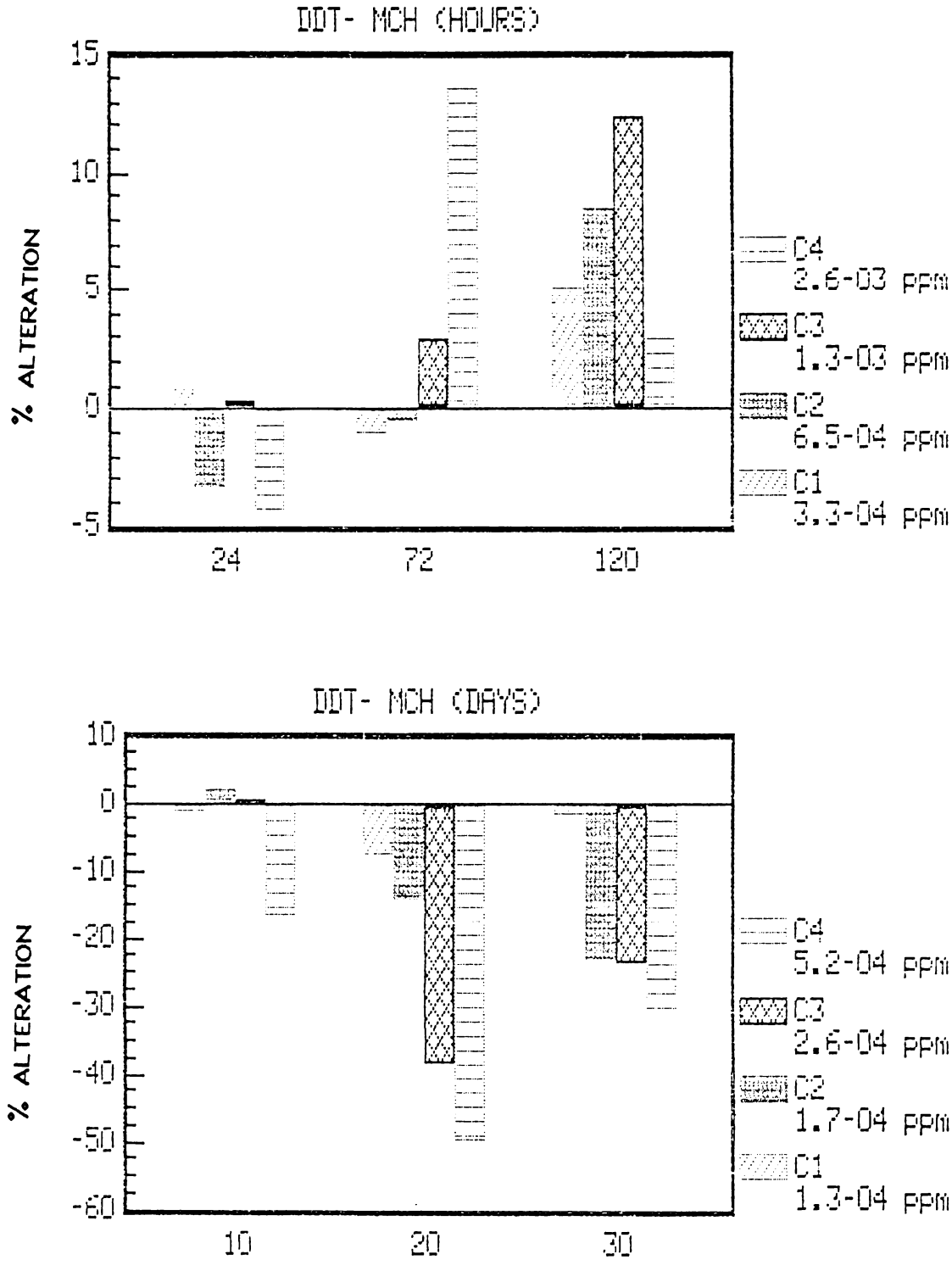

Fig. No. 29 *Etroplus maculatus* : Percentage alteration in MCH from mean control value following exposure to four SLC of DDT.

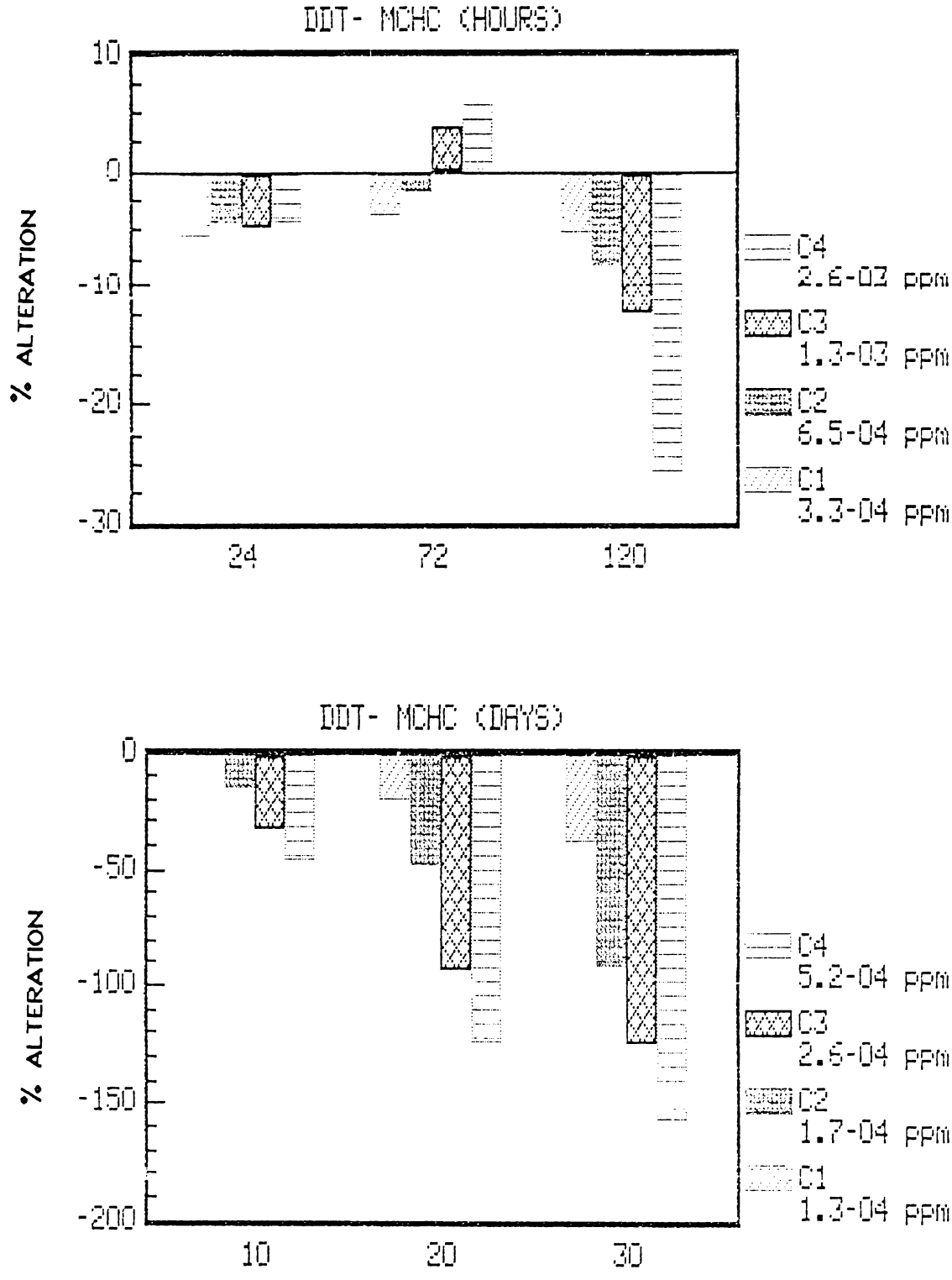

Fig. No. 30 *Etroplus maculatus* : Percentage alteration in MCHC from mean control value following exposure to four SLC of DDT.

towards the late phase of exposure. However, during early phase of exposure (24 hour) the erythrocytes appeared to be normocytic since the increase in volume index was comparatively less *(Table 86, 87 & 88)*.

Comparatively high colour index (CI) was recorded in higher concentrations and that too became more evident during 72 and 120 hours exposure. This indicated the hyperchromic state of RBC. However, during 24 hour exposure the colour index recorded lower value than unity indicating occurrence of hypochromia.

During short term exposure to DDT the erythrocytes were found less saturated as the saturation index recorded was lower than unity in all concentrations during three periods of exposure except in the highest concentration (C_4) during 72 hour exposure where saturation index was found higher than unity indicating supersaturation.

In general, the normocytic hypochromic state of RBC observed during initial phase of exposure to DDT turned to macrocytic hyperchromic condition towards the late phase during short term exposure.

Long term:- In confirmity with the higher MCV observed during long term exposure, volume index (VI) recorded higher values than unity indicating the swelling of erythrocytes (macrocytosis).

During long term exposure to DDT the erythrocytes were found to be hypochromic as the colour index recorded lower value than unity. This observation was in confirmity with the lower MCH recorded in all concentrations during the three periods of exposure under long term exposure to DDT.

The lower MCHC observed in all concentrations during long term exposure was further corroborated with the lower saturation index recorded. The lower SI than unity indicated a less saturation of erythrocytes with haemoglobin.

The higher volume index and lower colour and saturation indices recorded indicated the macrocytic hypochromic condition of erythrocytes and with the reduction in haemoglobin, macrocytic hypochromic anaemia occurred in experimental fishes during long term exposure to various concentrations of DDT *(Table 89, 90 & 91)*.

4.2.2.2 Dimecron

4.2.2.2.1 Total erythrocyte count (TEC)

Short term:- In general, TEC showed comparatively lower values in experimental group but no statistically significant variation could be noticed except in C_1 during 72 hours and in C_3 during

120 hours exposure. However, in the highest concentration (C_4 during 24 and 72 hours exposure), a slight increase was found *(Table 92, 93 & 94, Fig. 31)*.

Long term:- Generally a progressive reduction in TEC with increase in concentration (except in the lowest concentration, C_1 during 10 day exposure where a slight increase was found) was recorded and the reduction was statistically significant. The reduction in TEC was found more pronounced with the increase in exposure duration and this reduction in each concentration with exposure time was found statistically significant. During 30 days exposure a gradual decrease in TEC with increase of concentration was observed while no such dose-response relation could be found during 20 days exposure *(Table 95, 96 & 97, Fig. 31)*.

4.2.2.2.2 Haemoglobin content (Hb)

Short term:- Haemoglobin content showed a proportional increase with concentration and the increase was found statistically significant in all sublethal concentrations (except in C_1 during 72 hour exposure) of Dimecron during the three exposure periods under short term exposure. The increase in Hb content in each concentration was found more pronounced with increase in exposure duration*(Table 92, 93 & 94, Fig. 32)*.

TABLE 92

Etroplus maculatus: Haematological status of control fish and those pre-exposed to four SLC of DIMECRON for 24 hrs.

		TEC	Hb	Ht	MCV	MCH	MCHC	VI	CI	SI
		6 3 X10 mm	gX	X	3 um	pg	X			
	a	3.394	12.996	31.872	93.981	38.289	40.764			
CONTROL		+/-0.124	+/-0.516	+/-0.319	+/-2.542	+/-0.644	+/-1.231	1.00	1.00	1.00
C1	a	3.306	14.218	32.078	97.261	43.039	44.314	1.124	1.035	1.087
		+/-0.194	+/-0.629	+/-0.283	+/-4.915	+/-0.753	+/-1.609	+/-0.019	+/-0.052	+/-0.039
0.01	b	-2.596	+9.402	+0.646	+3.49	+12.405	+8.708			
	c	NS	P<0.01	NS	NS	P<0.001	P<0.01			
C2	a	3.336	14.724	32.134	95.326	44.147	46.417	1.153	1.014	1.139
		+/-0.191	+/-0.766	+/-0.264	+/-4.361	+/-0.385	+/-2.824	+/-0.01	+/-0.046	+/-0.069
0.022	b	-1.708	+13.296	+0.822	+1.431	+15.299	+13.867			
	c	NS	P<0.01	NS	NS	P<0.001	P<0.01			
C3	a	3.304	15.246	32.239	97.756	46.103	47.283	1.205	1.04	1.169
		+/-0.181	+/-0.859	+/-0.279	+/-4.537	+/-1.377	+/-2.263	+/-0.036	+/-0.048	+/-0.056
0.043	b	-2.651	+17.313	+1.151	+4.016	+20.407	+15.992			
	c	NS	P<0.01	NS	NS	P<0.001	P<0.001			
C4	a	3.458	15.569	32.393	93.746	45.58	48.648	1.19	0.998	1.193
		+/-0.156	+/-0.969	+/-0.836	+/-2.093	+/-0.855	+/-1.742	+/-0.022	+/-0.022	+/-0.043
0.086	b	+1.885	+19.798	+1.634	-0.29	+19.042	+19.34			
	c	NS	P<0.001	NS	NS	P<0.001	P<0.001			

a: Mean value (N=5) with +/-SD.

b: %Alteration from the mean value.

c: Level of significance.

TABLE 93

Etroplus maculatus: Haematological status of control fish and those pre-exposed to four SLC of DIMECRON for 72 hrs.

		TEC 10^6 mm^3	Hb g%	Ht %	MCV μm^3	MCH pg	MCHC %	VI	CI	SI
CONTROL	a	3.462 +/-0.188	13.086 +/-0.572	32.028 +/-0.261	92.67 +/-4.291	37.819 +/-0.749	40.848 +/-1.488	1.00	1.00	1.00
C1 0.01	a	3.204 +/-0.122	13.950 +/-0.939	31.268 +/-0.2	97.685 +/-3.109	43.511 +/-1.608	44.603 +/-2.728	1.151 +/-0.042	1.054 +/-0.034	1.092 +/-0.067
	b	-7.452	+6.602	-2.372	+5.411	+15.05	+9.192			
	c	P<0.05	NS	P<0.001	NS	P<0.001	P<0.05			
C2 0.022	a	3.318 +/-0.183	15.609 +/-0.883	31.286 +/-0.282	94.494 +/-4.61	47.043 +/-0.498	49.94 +/-2.474	1.253 +/-0.02	1.109 +/-0.049	1.223 +/-0.06
	b	-4.159	+19.28	-2.316	+1.968	+24.389	+22.258			
	c	NS	P<0.001	P<0.01	NS	P<0.001	P<0.001			
C3 0.043	a	3.38 +/-0.288	15.736 +/-1.109	31.674 +/-0.316	94.250 +/-7.449	46.618 +/-1.352	49.663 +/-3.111	1.233 +/-0.035	1.016 +/-0.081	1.216 +/-0.076
	b	-2.368	+20.25	-1.105	+1.704	+23.266	+21.58			
	c	NS	P<0.01	NS	NS	P<0.001	P<0.001			
C4 0.086	a	3.476 +/-0.184	15.961 +/-0.16	31.773 +/-0.543	91.549 +/-3.375	46.001 +/-1.963	50.243 +/-0.729	1.217 +/-0.051	0.988 +/-0.036	1.229 +/-0.018
	b	+0.404	+21.94	-0.796	-1.209	+21.634	+22.999			
	c	NS	P<0.001	NS	NS	P<0.001	P<0.001			

a: Mean value (N=5) with +/-SD.

b: %Alteration from the mean value.

c: Level of significance.

TABLE 94

Etroplus maculatus: Haematological status of control fish and those pre-exposed to four SLC of DIMECRON for 120 hrs.

		TEC 10^6 mm^3	Hb g%	Ht %	MCV μm^3	MCH pg	MCHC %	VI	CI	SI
CONTROL	a	3.456 +/-0.142	12.816 +/-0.767	31.918 +/-0.293	92.446 +/-2.996	37.064 +/-0.965	40.147 +/-2.186	1.00	1.00	1.00
C1	a	3.374 +/-0.132	14.949 +/-0.226	30.128 +/-0.141	89.391 +/-3.115	44.339 +/-1.089	49.617 +/-0.659	1.196 +/-0.029	0.967 +/-0.034	1.236 +/-0.016
0.01	b	-2.372	+16.643	-5.599	-3.304	+19.62	-23.588			
	c	NS	P<0.001	P<0.001	NS	P<0.001	P<0.001			
C2	a	3.372 +/-0.168	15.506 +/-0.395	29.224 +/-0.825	86.746 +/-2.167	46.031 +/-1.185	53.063 +/-0.256	1.241 +/-0.031	0.938 +/-0.023	1.322 +/-0.006
0.022	b	-2.43	+20.989	-8.431	-6.165	+24.193	+32.171			
	c	NS	P<0.001	P<0.001	P<0.01	P<0.001	P<0.001			
C3	a	3.176 +/-0.106	15.456 +/-0.432	29.177 +/-0.814	91.881 +/-0.523	48.672 +/-0.344	52.976 +/-0.654	1.313 +/-0.009	0.994 +/-0.006	1.319 +/-0.016
0.043	b	-8.101	+20.599	-8.579	-0.611	+31.318	+31.955			
	c	P<0.01	P<0.001	P<0.001	NS	P<0.001	P<0.001			
C4	a	3.416 +/-0.062	16.065 +/-0.919	28.626 +/-0.508	83.805 +/-0.942	47.01 +/-2.124	56.091 +/-2.360	1.286 +/-0.057	0.906 +/-0.01	1.397 +/-0.058
0.086	b	-1.157	+25.351	-10.305	-9.347	+26.834	+39.714			
	c	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001			

a: Mean value (N=5) with +/-SD.

b: %Alteration from the mean value.

c: Level of significance.

TABLE 9S

Etroplus maculatus: Haematological status of control fish and those pre-exposed to four SLC of DIMECRON for 10 days.

		TEC 10^6 mm^3	Hb g%	Ht %	MCV μm^3	MCH pg	MCHC %	VI	CI	SI
CONTROL	a	3.254 +/-0.102	13.089 +/-0.112	27.879 +/-1.338	85.724 +/-2.007	40.248 +/-1.912	46.958 +/-1.886	1.00	1.00	1.00
C1	a	3.376 +/-0.129	12.947 +/-0.286	29.117 +/-0.814	112.64 +/-1.037	37.784 +/-0.914	43.729 +/-1.872	0.9393 +/-0.022	1.009 +/-0.011	0.931 +/-0.039
0.0043	b	+3.749	-1.084	+4.44	+31.398	-6.122	-6.876			
	c	NS	NS	NS	P<0.001	P<0.05	P<0.05			
C2	a	3.188 +/-0.112	12.636 +/-0.232	31.819 +/-0.544	99.868 +/-2.202	40.268 +/-2.764	40.334 +/-0.657	1.001 +/-0.007	1.165 +/-0.626	0.859 +/-0.014
0.0058	b	-2.028	-3.460	+14.132	+16.499	+0.049	-14.106			
	c	NS	P<0.01	P<0.001	P<0.001	NS	P<0.001			
C3	a	3.084 +/-0.073	8.85 +/-0.85	32.232 +/-0.896	104.508 +/-1.148	28.657 +/-2.115	27.415 +/-1.932	0.712 +/-0.051	1.218 +/-0.011	0.584 +/-0.041
0.0087	b	-5.224	-32.385	+15.613	+21.912	-28.798	-41.618			
	c	P<0.05	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001			
C4	a	2.952 +/-0.084	7.056 +/-0.272	32.901 +/-1.109	111.468 +/-2.933	23.901 +/-5.418	21.455 +/-0.285	0.594 +/-0.013	1.3 +/-0.034	0.457 +/-0.006
0.017	b	-9.28	-46.092	+18.013	+30.031	-40.615	-54.31			
	c	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001				

a: Mean value (N=5) with +/-SD.

b: %Alteration from the mean value.

c: Level of significance.

TABLE 96
Etroplus maculatus: Haematological status of control fish and those pre-exposed to four SLC of DIMECRON for 20 days.

		TEC 10^6 mm^3	Hb g%	Ht %	MCV μm^3	MCH pg	MCHC %	VI	CI	SI
CONTROL	a	3.498 +/-0.22	13.045 +/-0.138	28.561 +/-0.587	81.827 +/-3.577	37.392 +/-2.824	45.684 +/-1.789	1.00	1.00	1.00
C1	a	2.728 +/-0.051	12.091 +/-1.337	30.724 +/-0.385	114.64 +/-1.237	43.518 +/-4.394	38.664 +/-15.366	1.167 +/-0.117	1.377 +/-0.013	0.846 +/-0.092
0.0043	b	-22.012	-7.313	+7.573	+40.1	+16.383	-15.366			
	c	P<0.001	NS	P<0.001	P<0.001	P<0.05	P<0.01			
C2	a	2.506 +/-0.163	9.462 +/-1.219	31.72 +/-0.541	126.902 +/-6.276	37.664 +/-3.065	29.796 +/-3.496	1.01 +/-0.082	1.551 +/-0.077	0.652 +/-0.077
0.0058	b	-28.359	-27.466	+11.06	+55.085	+0.727	-34.778			
	c	P<0.001	P<0.001	P<0.001	P<0.001	NS	P<0.001			
C3	a	2.56 +/-0.04	7.277 +/-1.011	33.325 +/-0.656	130.176 +/-5.362	28.376 +/-3.436	21.797 +/-2.628	0.761 +/-0.092	1.591 +/-0.007	0.477 +/-0.058
0.0087	b	-26.815	-44.216	-16.68	+59.086	-24.112	-52.287			
	c	P<0.001	P<0.001	P<0.001	P<0.001	P<0.01	P<0.001			
C4	a	2.656 +/-0.172	5.931 +/-0.292	34.246 +/-0.879	101.16 +/-1.272	17.512 +/-0.488	17.318 +/-0.695	0.599 +/-0.014	1.349 +/-0.154	0.379 +/-0.015
0.017	b	-24.07	-54.534	+19.904	+23.626	-53.166	-62.091			
	c	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001			

a: Mean value (N=5) with +/-SD.
b: %Alteration from the mean value.
c: Level of significance.

TABLE 97

Etroplus maculatus: Haematological status of control fish and those pre-exposed to four SLC of DIMECRON for 30 days.

		TEC $\acute{e}^3$ X10 mm	Hb g%	Ht %	MCV ^{3} um	MCH pg	MCHC %	VI	CI	SI
	a	3.386	13.2	29.092	85.956	38.997	45.373			
CONTROL		+/-0.107	+/-0.363	+/-0.462	+/-1.605	+/-0.893	+/-1.934	1.00	1.00	1.00
	a	2.684	8.584	32.393	118.53	31.906	26.584	0.818	1.404	0.586
		+/-0.075	+/-1.208	+/-0.836	+/-5.723	+/-3.651	+/-4.444	+/-0.093	+/-0.021	+/-0.097
C1	b	-20.732	-34.969	+11.346	+37.896	-18.183	-41.41			
0.0043	c	P<0.001	P<0.001	P<0.001	P<0.001	P<0.01	P<0.001			
	a	2.456	7.129	33.091	134.99	29.098	21.551	0.746	1.57	0.475
		+/-0.152	+/-0.103	+/-0.824	+/-5.287	+/-1.432	+/-0.409	+/-0.036	+/-0.062	+/-0.009
C2	b	-27.643	-45.992	+13.746	+57.045	-25.384	-52.502			
0.0056	c	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001			
	a	2.342	6.061	36.341	150.305	24.809	16.656	0.636	1.749	0.367
		+/-0.208	+/-0.802	+/-0.633	+/-3.016	+/-0.948	+/-1.946	+/-0.024	+/-0.182	+/-0.043
C3	b	-30.832	-54.083	+24.917	+74.862	-36.382	-63.29			
0.0087	c	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001			
	a	2.14	5.586	39.976	179.216	26.146	13.974	0.671	2.179	0.308
		+/-0.152	+/-0.224	+/-0.709	+/-4.014	+/-0.847	+/-0.589	+/-0.021	+/-0.118	+/-0.013
C4	b	-36.798	-57.681	+37.412	+108.497	-32.953	-69.201			
0.017	c	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001				

a: Mean value (N=5) with +/-SD.

b: %Alteration from the mean value.

c: Level of significance.

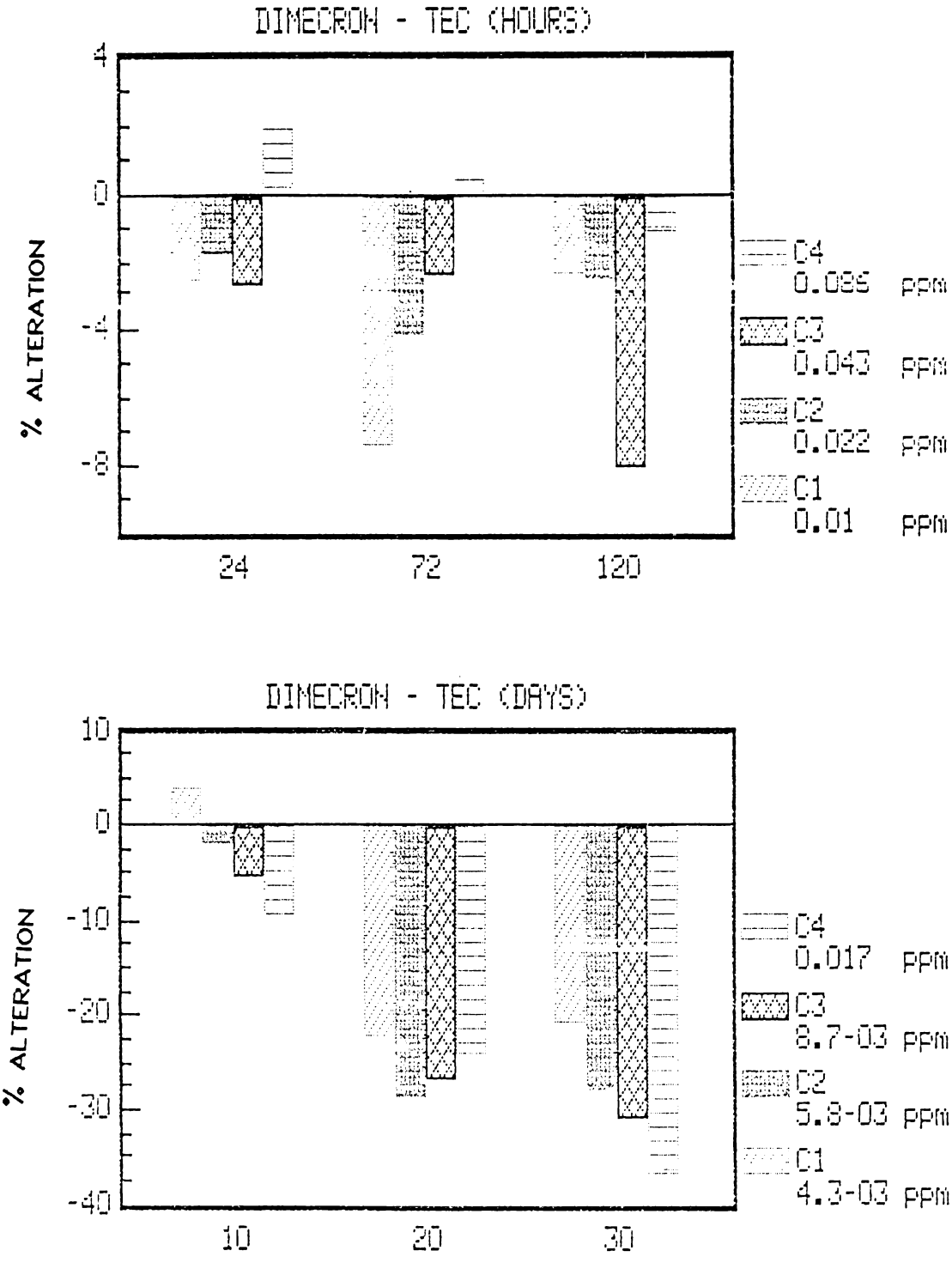

Fig. No. 31 *Etroplus maculatus* : Percentage alteration in TEC from mean control value following exposure to four SLC of Dimecron.

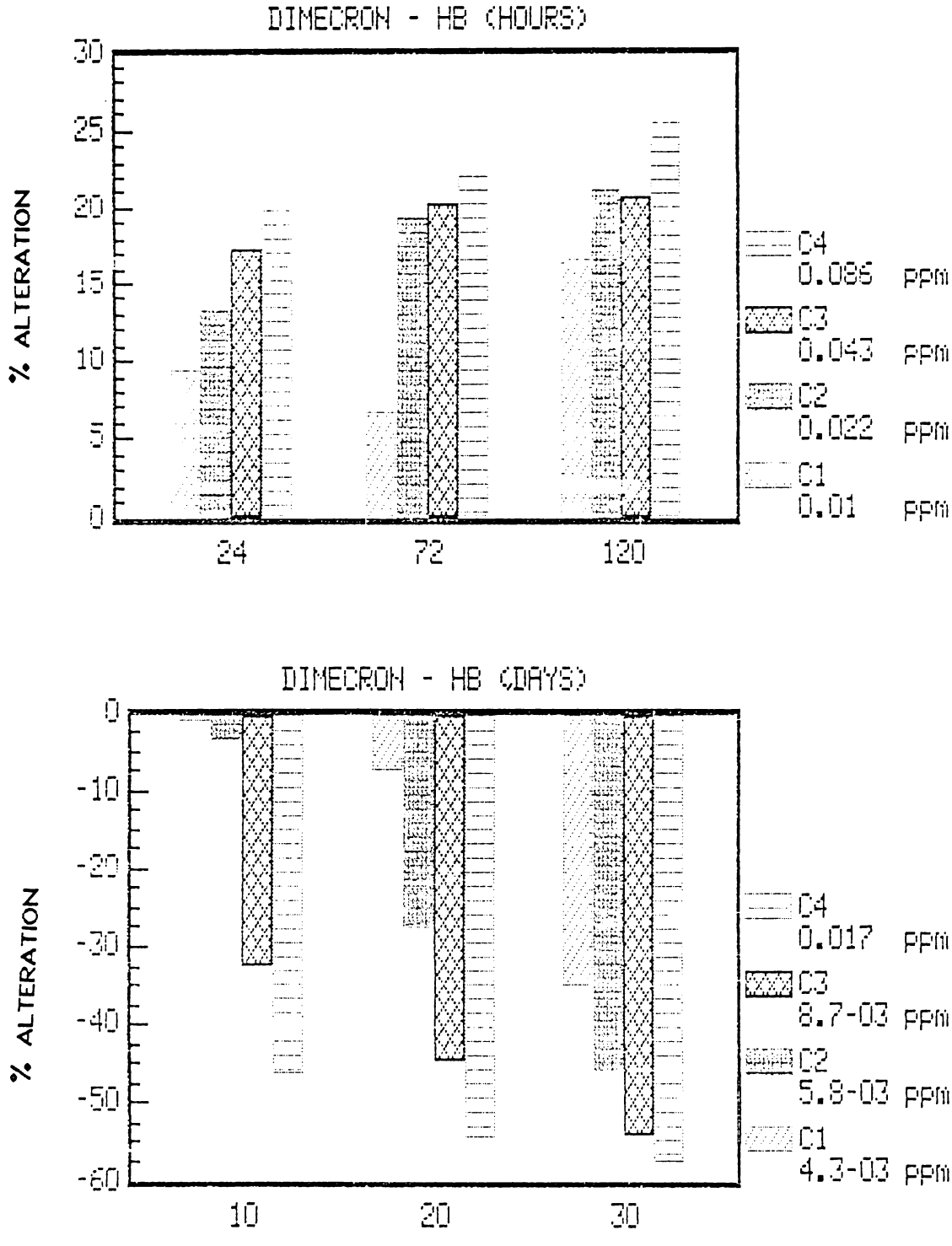

Fig. No. 32 *Etroplus maculatus* : Percentage alteration in HB from mean control value following exposure to four SLC of Dimecron.

Long term:- Progressive and inverse change in Hb with increase in concentration was recorded during the three periods of exposure under long term study. The reduction in Hb in each concentration was found statistically significant. Hb content in each concentration also recorded a gradual decrease with exposure duration *(Table 95, 96 & 97, Fig. 32)*.

4.2.2.2.3 Haematocrit (Ht)

Short term:- In short term exposure Ht showed a biphasic response. It showed a gradual increase, (not statistically significant) with concentration during initial phase (24 hour) while during intermediate and late phase Ht showed decrease and the reduction found statistically significant in lower concentrations during 72 hour and in all the concentrations during 120 hour exposure. A progressive reduction in Ht with exposure duration was evident and was statistically significant during 30 day exposure *(Table 92, 93 & 94, Fig. 33)*.

Long term:- In long term exposure, the Ht value of experimental fishes recorded a progressive and proportional increase with an increase in concentration and found statistically significant (except in C_1 during 10 day exposure) with the increase of exposure duration. Ht also showed a gradual increase reaching statistically significant levels during 30 day exposure *(Table 95, 96 & 97, Fig. 33)*.

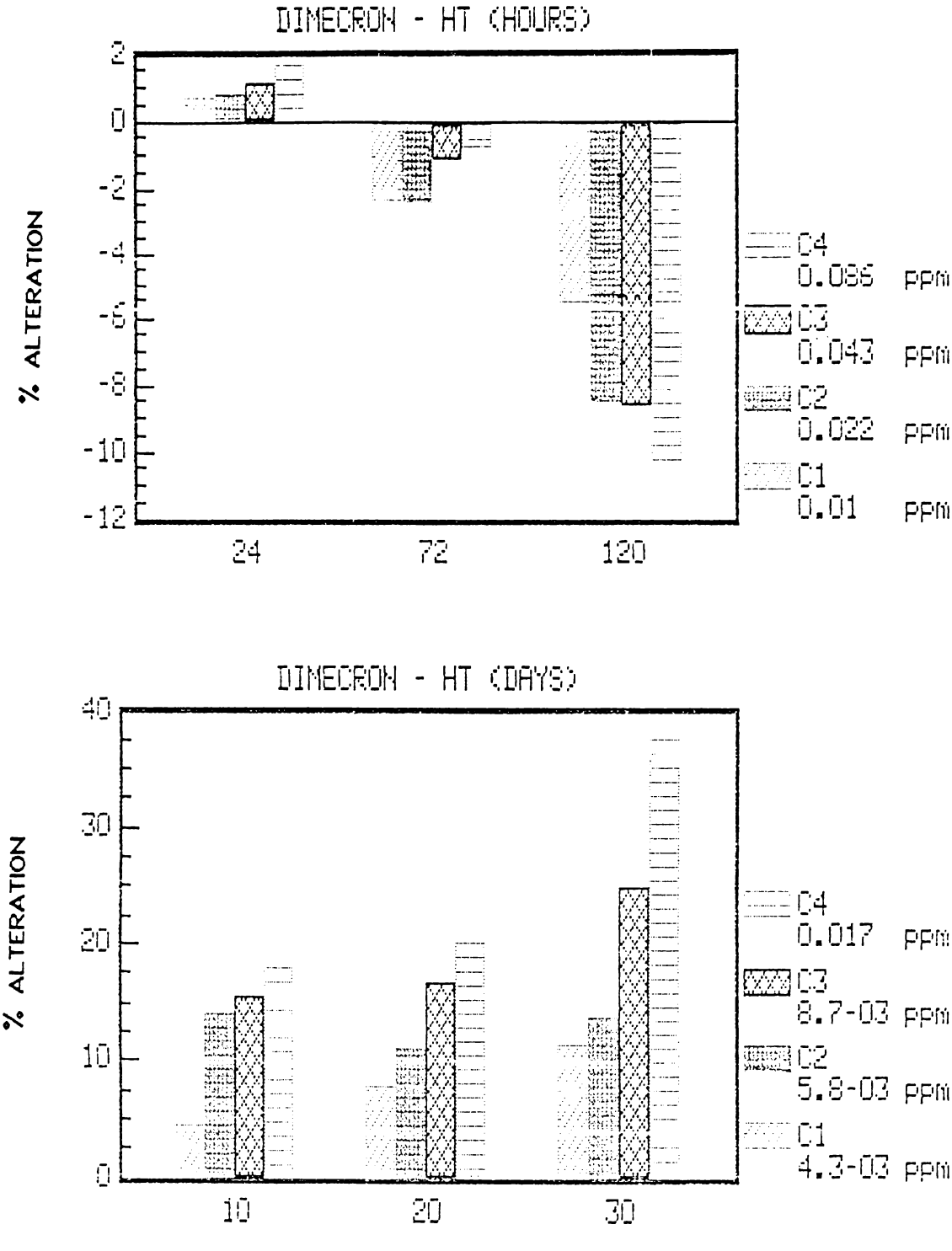

Fig. No. 33 *Etroplus maculatus* : Percentage alteration in HT from mean control value following exposure to four SLC of Dimecron.

4.2.2.2.4 Erythrocyte constants (MCV, MCH & MCHC)

Short term:- In short term exposure, MCV recorded a comparative increase in lower three concentrations during 24 and 72 hours exposure but not statistically significant. However, in the highest concentration (C_4) during the above period and in all the concentrations during 120 hour exposure, MCV recorded comparatively lower values reaching statistically significant level in C_4 during 30 day exposure.

A progressive and statistically significant increase in MCV was observed with increase in concentration. Comparatively the MCH recorded a gradual inrease with increase in exposure time.

Mean corpuscular haemoglobin concentration also found to increase with increase in concentration and was statistically significant in all the concentrations during the three exposure periods under long term study. The increase in MCHC was found more pronounced towards the final phase of the short term exposure *(Table 92, 93 & 94, Fig. 34, 35 & 36)*.

Long term:- Mean corpuscular volume (MCV) was found to increase statistically significant levels in experimental fishes in all concentrations during the three exposure periods under long term exposure. Increase in MCV was more pronounced during the late phases

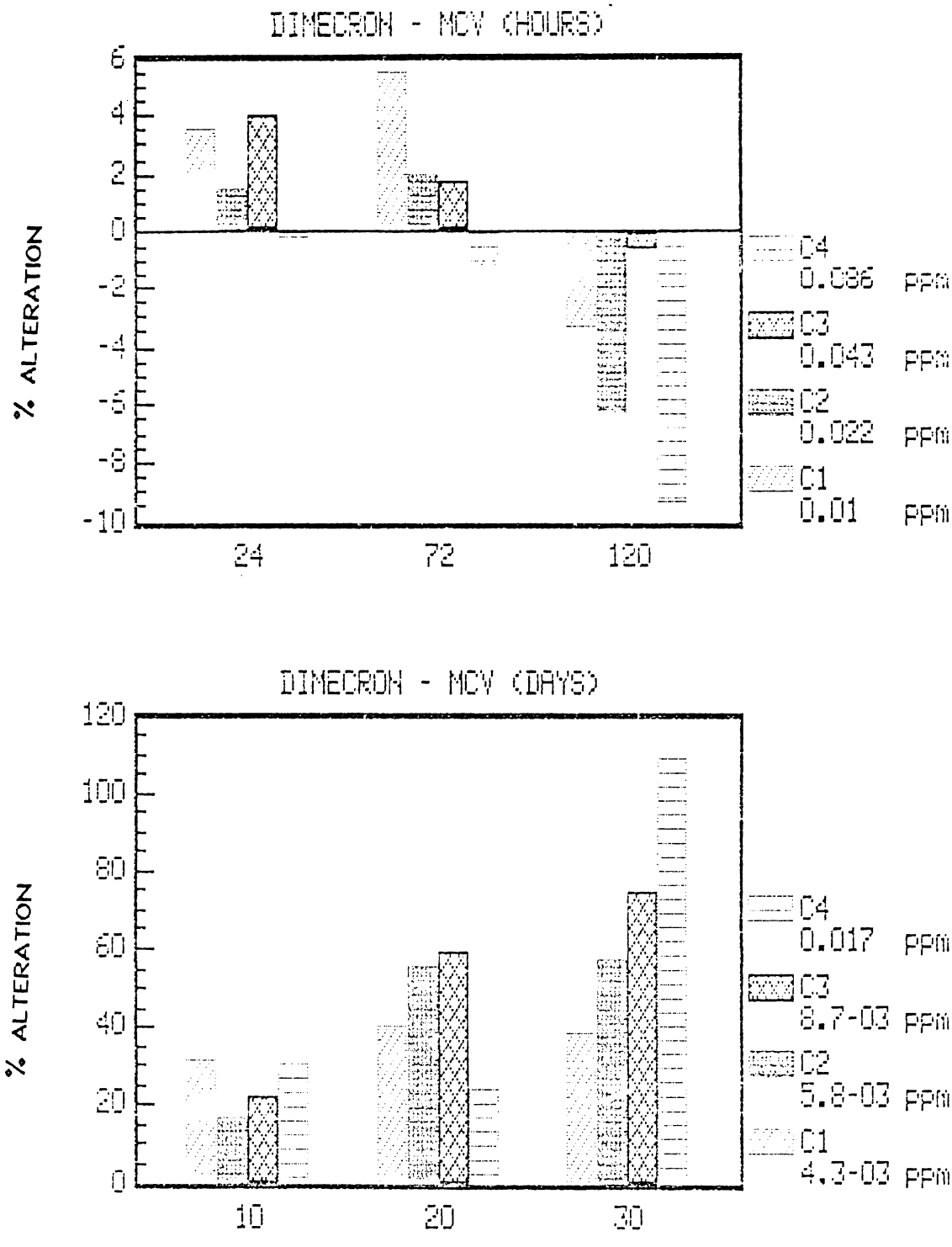

Fig. No. 34 *Etroplus maculatus* : Percentage alteration in from mean control value following exposure to SLC of Dimecron.

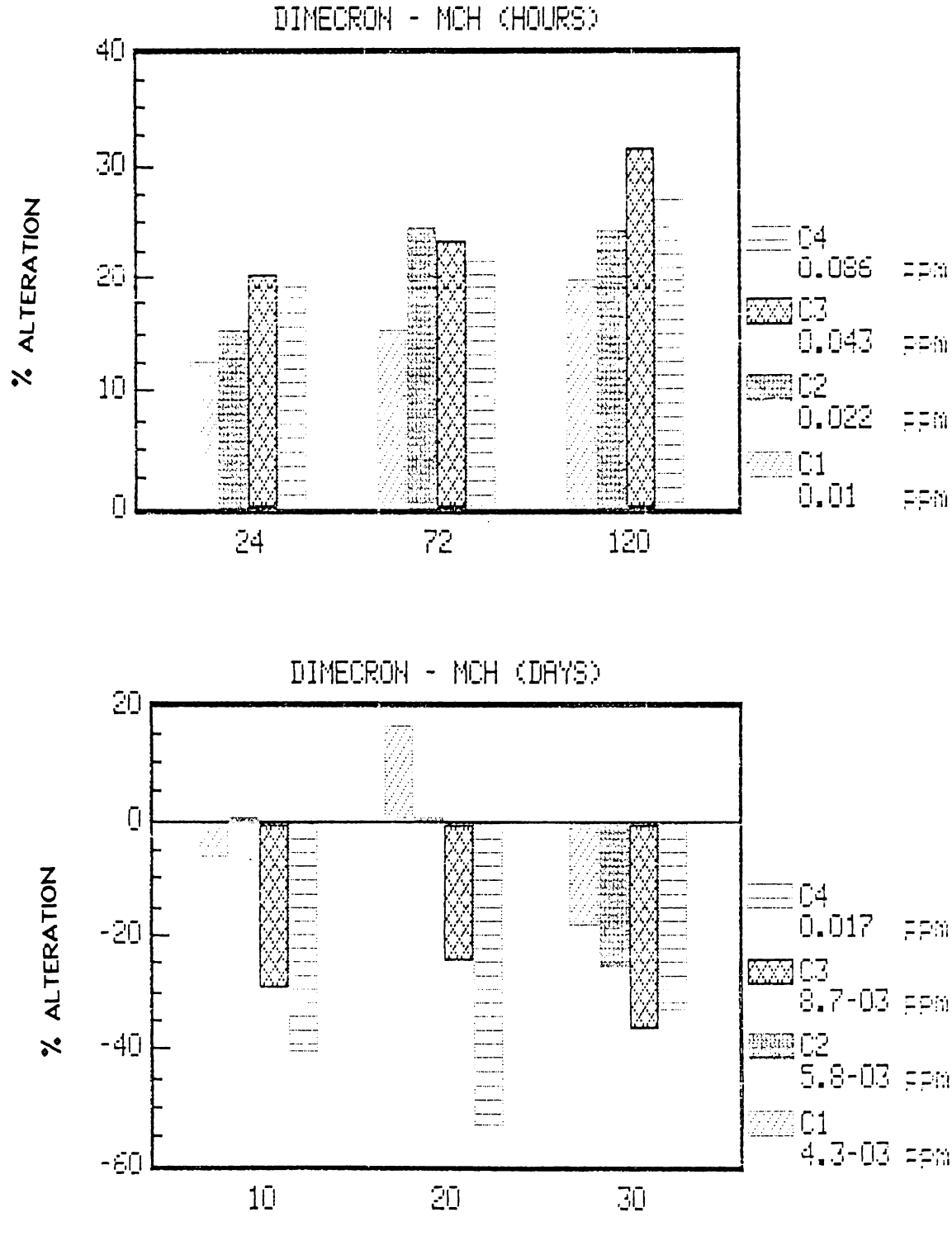

Fig. No. 35 *Etroplus maculatus* : Percentage alteration in MCH from mean control value following exposure to four SLC of Dimecron.

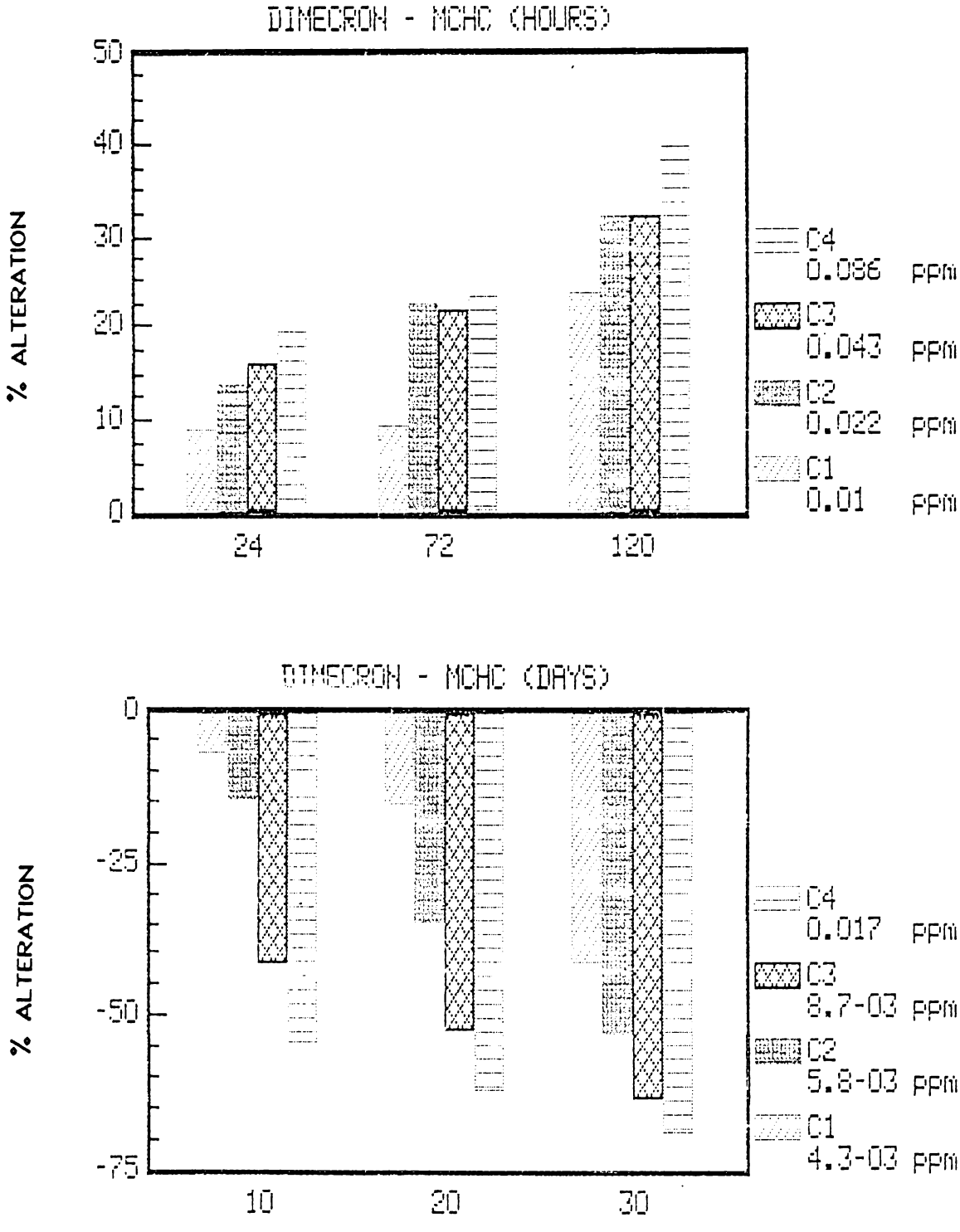

Fig. No. 36 *Etroplus maculatus* : Percentage alteration in MCHC from mean control value following exposure to four SLC of Dimecron.

of exposure reaching statistically significant levels during 30 day exposure in higher concentrations.

With the exception in C_2 during 10 day and in C_1 and C_2 during 20 day exposure, the MCH of experimental fishes recorded statistically significant inverse change with increase in concentration. The highest MCH recorded in C_4 during 20 day exposure.

Mean corpuscular haemoglobin concentration (MCHC) showed a progressive and statistically significant reduction with increase in concentration during three exposure periods. In each concentration MCHC recorded progressive and statistically significant reduction indicating both dose dependent and duration dependent and inverse change in MCHC during three exposure periods under long term exposure to Dimecron *(Table 95, 96 & 97, Fig. 34, 35 & 36)*.

4.2.2.2.5 Erythrocyte indices (CI, VI & SI)

Short term:- In lower three concentrations during 24 and 72 hours exposure, a slight swelling of erythrocytes were indicated by the higher volume index recorded. But in the highest concentration (C_4) during the above periods VI recorded a comparatively lower value than unity indicating normocytic condition. The much lower volume than unity recorded in the case of volume index during 120 hour

exposure in all the concentrations indicating the occurrence of microcytosis in the last phase of exposure to Dimecron. Microcytosis was more pronounced in higher concentrations as indicated by the comparatively lower VI recorded with increase in concentration.

In confirmity with higher MCH recorded in experimental fishes, colour index (C^{-}) recorded higher value than unity indicating the occurrence of hyperchromia, in experimental fishes in all concentrations.

Saturation index recorded higher value than unity in all the concentrations during three exposure periods indicating a supersaturation of haemoglobin in erythrocytes.

In general, the macrocytic hyperchromic condition observed in experimental fishes during initial phases of exposure had turned to microcytic hyperchromic condition towards last phase of exposure *(Table 92, 93 & 94)*.

Long term:- In confirmity with high MCV obtained the volume index showed higher value than unity in all exposed concentrations during long term exposure. This high VI indicated microcytosis of erythrocyte due to long term exposure to Dimecron.

The lower MCH recorded was responsible for the lower colour index recorded in all the concentrations, indicating hypochromia.

The hypochromic condition was further corroborated by the lower saturation index than unity recorded, in all the concentrations.

A comparative evaluation of the erythrocyte indices and the reduction of TEC and Hb recorded indicated the occurrence of macrocytic hypochromic anaemia in experimental fishes due to long term exposure to Dimecron *(Table 95, 96 & 97)*.

4.2.2.*3* Gramoxone

4.2.2.*3.1* Total erythrocyte count (TEC)

Short term:- The TEC showed a progressively increasing trend with increase in the concentration of the pesticide. The increase was statistically significant for higher two concentrations (C_3 and C_4) during 24 hour and higher three concentrations (C_2, C_3 and C_4) during 72 and 120 hours. This direct relation between TEC and pesticide concentration was evident at 24, 72 and 120 hours exposure. However, no significant change in TEC with exposure time for any concentration tested was noticed *(Table 98, 99 & 100, Fig. 37)*.

TABLE 98

Etroplus maculatus: Haematological status of control fish and those pre-exposed to four SLC of GRAMOXONE for 24 hours.

		TEC 10^6 X10 mm^3	Hb g%	Ht %	MCV μm^3	MCH pg	MCHC %	VI	CI	SI
CONTROL	a	3.138 +/-0.177	11.273 +/-0.738	30.713 +/-0.423	98.128 +/-4.374	35.922 +/-1.075	36.713 +/-2.565	1.00	1.00	1.00
C1 0.0034	a	3.232 +/-0.135	12.189 +/-0.958	30.7 +/-0.091	94.887 +/-3.681	37.68 +/-1.901	39.797 +/-3.158	1.049 +/-0.053	0.968 +/-0.022	1.084 +/-0.086
	b	+2.99	+8.13	-0.04	-3.3	+4.89	+8.4			
	c	NS	NS	NS	NS	NS	NS			
C2 0.0067	a	3.264 +/-0.097	13.111 +/-0.817	30.684 +/-0.308	94.057 +/-2.197	40.138 +/-1.524	42.733 +/-2.759	1.117 +/-0.042	0.959 +/-0.022	1.164 +/-0.075
	b	+4.02	+16.3	-9.09	-4.12	+11.74	+16.4			
	c	NS	P<0.01	NS	NS	P<0.001	P<0.01			
C3 0.013	a	3.382 +/-0.147	13.695 +/-1.101	30.68 +/-0.305	90.828 +/-3.281	40.45 +/-1.912	44.621 +/-3.304	1.126 +/-0.053	0.926 +/-0.033	1.215 +/-0.09
	b	+7.78	+21.48	-0.1	-7.44	+12.61	+21.54			
	c	P<0.05	P<0.01	NS	P<0.05	P<0.01	P<0.01			
C4 0.028	a	3.408 +/-0.18	12.853 +/-0.933	30.033 +/-0.187	88.305 +/-4.253	37.688 +/-1.038	42.786 +/-2.923	1.049 +/-0.028	0.9 +/-0.043	1.165 +/-0.079
	b	+8.6	+14.01	-2.21	-10.01	+4.92	+16.54			
	c	P<0.05	P<0.05	P<0.05	P<0.01	P<0.05	P<0.01			

a: Mean value (N=5) with +/-SD.

b: %Alteration from the mean value.

c: Level of significance.

TABLE 99

Etroplus maculatus: Haematological status of control fish and those pre-exposed to four SLC of GRAMOXONE for 72 hours.

		TEC 10^6 mm^3	Hb g%	Ht %	MCV fl	MCH pg	MCHC %	VI	CI	SI
CONTROL	a	3.072 +/-0.123	11.435 +/-0.958	30.556 +/-0.347	100.19 +/-2.299	37.172 +/-1.67	37.185 +/-3.127	1.00	1.00	1.00
C1 0.0034	a	3.184 +/-0.117	13.552 +/-1.119	31.038 +/-0.041	97.582 +/-3.402	42.513 +/-2.19	43.657 +/-3.552	1.142 +/-0.058	0.973 +/-0.033	1.174 +/-0.095
	b	+3.65	+18.51	+1.58	-2.6	+14.37	+17.4			
	c	NS	P<0.05	P<0.05	NS	P<0.01	P<0.05			
C2 0.0067	a	3.25 +/-0.111	14.417 +/-1.215	30.668 +/-0.349	93.861 +/-2.184	44.309 +/-2.629	47.282 +/-3.892	1.19 +/-0.07	0.955 +/-0.023	1.272 +/-0.104
	b	+5.79	+26.08	+0.4	-6.32	+19.2	+27.18			
	c	P<0.05	P<0.01	NS	P<0.001	P<0.001	P<0.01			
C3 0.013	a	3.388 +/-0.159	14.895 +/-1.159	30.515 +/-0.323	90.135 +/-2.535	43.913 +/-2.096	48.797 +/-3.56	1.179 +/-0.056	0.899 +/-0.029	1.312 +/-0.095
	b	+10.29	+30.26	-0.1	-10.04	+18.13	+31.23			
	c	P<0.01	P<0.001	NS	P<0.001	P<0.001	P<0.001			
C4 0.028	a	3.486 +/-0.222	14.83 +/-0.806	29.636 +/-0.566	85.214 +/-3.962	42.573 +/-1.289	50.014 +/-1.86	1.144 +/-0.034	0.851 +/-0.039	1.348 +/-0.05
	b	+13.48	+29.69	-3.01	-14.95	+14.53	+34.5			
	c	P<0.01	P<0.001	P<0.05	P<0.001	P<0.001	P<0.001			

a: Mean value (N=5) with +/-SD.

b: %Alteration from the mean value.

c: Level of significance.

TABLE 100

Etropius maculatus: Haematological status of control fish and those pre-exposed to four SLC of GRAMOXONE for 120 hrs.

		TEC 10^6 mm^3	Hb g%	Ht %	MCV μm^3	MCH pg	MCHC %	VI	CI	SI
CONTROL	a	2.972 +/-0.129	11.17 +/-0.784	30.711 +/-0.365	103.448 +/-3.282	37.59 +/-1.165	36.389 +/-2.851	1.00	1.00	1.00
C1	a	3.11 +/-0.105	13.773 +/-0.944	30.516 +/-0.327	98.189 +/-2.644	44.248 +/-1.651	45.122 +/-2.833	1.177 +/-0.043	0.949 +/-0.026	1.239 +/-0.077
	b	+4.64	+23.3	-0.6	-5.08	+17.84	+24.0			
0.0034	c	NS	P<0.01	NS	P<0.05	P<0.001	P<0.01			
C2	a	3.252 +/-0.133	15.015 +/-0.95	29.541 +/-0.56	90.907 +/-2.099	42.313 +/-1.208	50.796 +/-2.359	1.126 +/-0.032	0.879 +/-0.02	1.396 +/-0.064
	b	+9.42	+34.42	-3.81	-12.12	-12.68	+39.59			
0.0067	c	P<0.01	P<0.001	P<0.01	P<0.001	P<0.001	P<0.001			
C3	a	3.394 +/-0.147	15.615 +/-1.347	29.135 +/-0.437	85.934 +/-2.731	45.958 +/-2.581	53.575 +/-4.316	1.223 +/-0.068	0.831 +/-0.026	1.472 +/-0.118
	b	+14.2	+39.79	-5.13	-16.93	+22.39	+47.23			
0.013	c	P<0.01	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001			
C4	a	3.422 +/-0.104	15.573 +/-1.196	27.982 +/-0.421	81.803 +/-1.398	45.462 +/-2.292	55.612 +/-3.462	1.209 +/-0.06	0.791 +/-0.014	1.528 +/-0.095
	b	+15.14	+39.42	-8.89	-20.92	+21.07	+52.83			
0.028	c	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001			

a: Mean value (N=5) with +/-SD.

b: %Alteration from the mean value.

c: Level of significance.

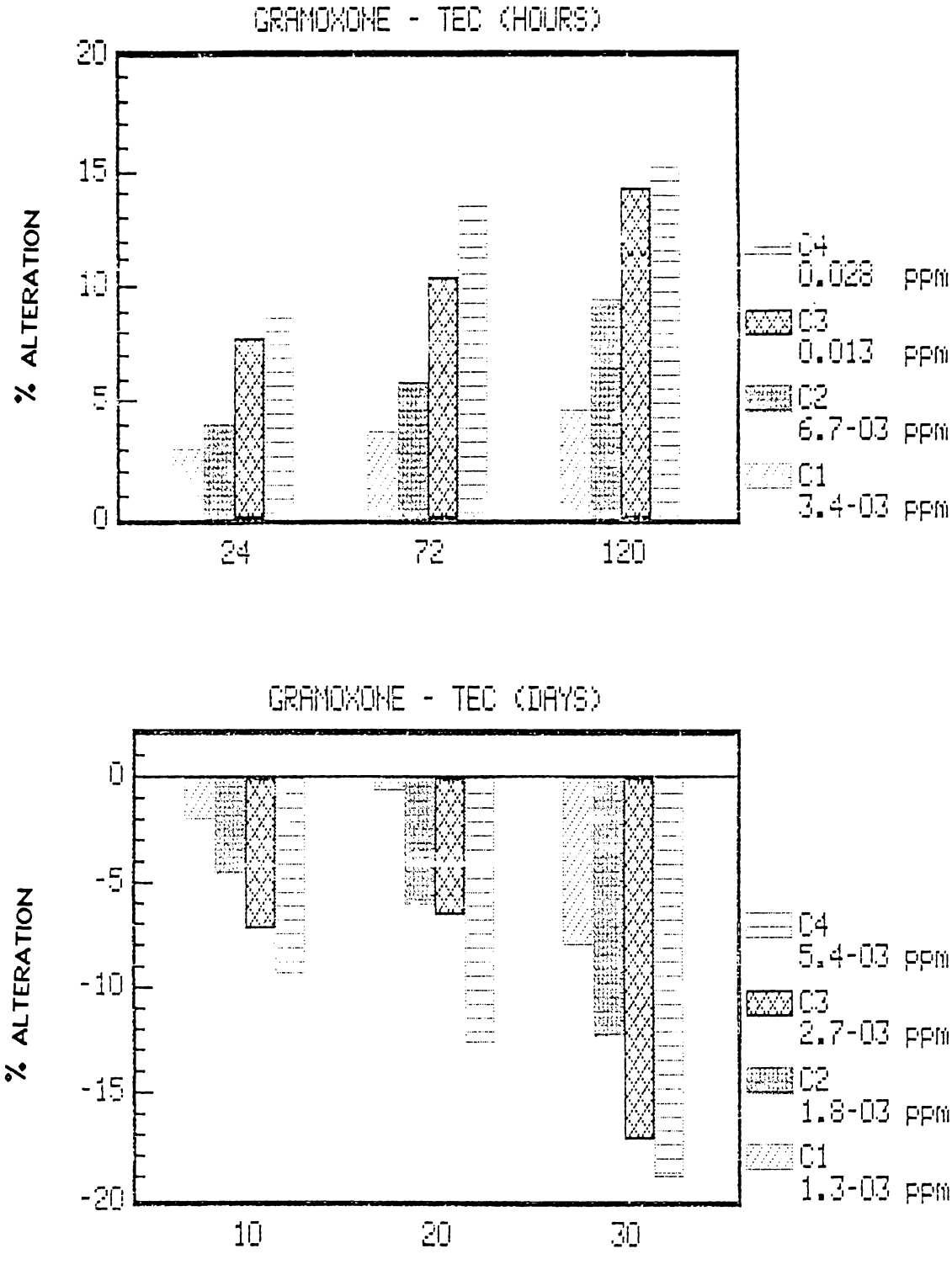

Fig. No. 37 *Etroplus maculatus* : Percentage alteration in TEC from mean control value following exposure to four SLC of Gramoxone.

Long term:- An inverse change in TEC was noticed with increase in pesticide concentration during long term exposure. Statistically significant reduction in TEC was found in the highest concentration (C_4) during 10 and 20 days and in three higher concentrations (C_2, C_3 and C_4) during 30 day exposure. No significant correlation in TEC with exposure time was evident in any of the pesticide concentrations *(Table 101, 102 & 103, Fig. 38)*.

4.2.2.3.2 Haemoglobin content (Hb)

Short term:- A progressive and statistically significant increase in Hb concentration was observed in three higher concentrations (C_2, C_3 and C_4) during 24 hour exposure and in all pesticide concentrations during 72 and 120 hours exposure. The maximum increase in Hb content was noted in C_3 during short term exposure. Besides the general trend of dose dependent increase in Hb content, a progressive increase in Hb concentration with exposure time was also evident in all pesticide concentrations *(Table 98, 99 & 100, Fig. 38)*.

Long term:- The Hb content showed an increasing trend with increase in pesticide concentration during 10 day exposure and the increase in the highest concentration was found statistically significant. However, during 20 and 30 days exposure, an increase, progressive with increase in pesticide concentration, change in Hb content

TABLE 101

Etroplus maculatus: Haematological status of control fish and those pre-exposed to four SLC of GRAMOXONE for 10 days

		TEC 10^6 mm^3	Hb g%	Ht %	MCV μm^3	MCH pg	MCHC %	VI	CI	SI
CONTROL	a	2.922 +/-0.188	11.828 +/-1.125	32.796 +/-0.252	112.583 +/-6.7	40.415 +/-1.645	36.047 +/-3.214	1.00	1.00	1.00
C1	a	2.862 +/-0.226	12.119 +/-1.275	32.921 +/-0.216	115.562 +/-8.462	42.287 +/-2.099	36.797 +/-3.738	1.045 +/-0.051	1.026 +/-0.073	1.021 +/-0.103
	b	-2.05	+2.46	+0.4	+2.65	+4.63	+2.08			
0.0013	c	NS	NS	NS	NS	NS	NS			
C2	a	2.79 +/-0.215	12.488 +/-1.342	32.976 +/-0.179	118.731 +/-8.7	44.675 +/-1.443	37.853 +/-3.871	1.224 +/-0.267	1.053 +/-0.077	1.08 +/-0.107
	b	-4.52	+5.58	+0.5	+5.46	+10.54	+5.01			
0.0018	c	NS	NS	NS	NS	NS	NS			
C3	a	2.71 +/-0.188	13.046 +/-1.546	34.222 +/-0.559	126.668 +/-6.997	47.325 +/-3.296	37.512 +/-3.958	1.169 +/-0.081	1.125 +/-0.062	1.104 +/-0.109
	b	-7.26	+10.3	+4.35	+12.51	+17.09	+4.06			
0.0027	c	NS	NS	P<0.001	P<0.05	P<0.05	NS			
C4	a	2.68 +/-0.182	15.27 +/-0.977	35.284 +/-0.603	133.414 +/-6.946	58.35 +/-1.265	43.88 +/-2.841	1.442 +/-0.03	1.185 +/-0.062	1.216 +/-0.078
	b	-9.31	+29.1	+7.49	+18.5	+44.38	+21.65			
0.0054	c	P<0.05	P<0.001	P<0.001	P<0.01	P<0.001	P<0.05			

a: Mean value (N=5) with +/-SD.

b: %Alteration from the mean value.

c: Level of significance.

TABLE 102
Etroplus maculatus: Haematological status of control fish and those pre-exposed to four SLC of GRAMOXONE for 20 days

		TEC 10^6 mm^3	Hb g%	Ht %	MCV μm^3	MCH pg	MCHC %	VI	CI	SI
CONTROL	a	2.982 +/-0.271	11.582 +/-0.959	33.109 +/-0.242	111.699 +/-9.2	38.918 +/-2.585	34.969 +/-2.727	1.00	1.00	1.00
C1	a	2.962 +/-0.231	9.337 +/-1.154	35.63 +/-0.72	121.545 +/-7.692	31.435 +/-1.576	26.161 +/-2.722	0.809 +/-0.04	1.081 +/-0.065	0.748 +/-0.077
	b	-0.6	-19.38	+7.61	+8.81	-19.23	-25.19			
0.0013	c	NS	P<0.05	P<0.001	NS	P<0.01	NS			
C2	a	2.802 +/-0.173	8.574 +/-1.029	37.178 +/-0.819	132.956 +/-5.729	30.516 +/-1.99	23.022 +/-2.284	0.789 +/-0.049	1.19 +/-0.051	0.658 +/-0.065
	b	-6.04	-25.97	+12.28	+19.03	-21.59	-34.16			
0.0018	c	NS	P<0.01	P<0.001	P<0.01	P<0.01	P<0.001			
C3	a	2.788 +/-0.225	8.101 +/-0.91	39.638 +/-0.747	142.626 +/-8.961	29.009 +/-1.532	20.415 +/-2.011	0.747 +/-0.039	1.278 +/-0.081	0.584 +/-0.057
	b	-6.51	-30.06	+19.71	+27.69	-25.46	-41.62			
0.0027	c	NS	P<0.001	P<0.001	P<0.01	P<0.001	P<0.001			
C4	a	2.606 +/-0.198	7.443 +/-0.718	41.168 +/-0.572	158.569 +/-10.044	28.297 +/-1.165	17.929 +/-1.711	0.729 +/-0.029	1.419 +/-0.089	0.513 +/-0.048
	b	-12.61	-35.74	+24.33	+41.96	-27.29	-48.73			
0.0054	c	P<0.01	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001			

a: Mean value (N=5) with +/-SD.
b: %Alteration from the mean value.
c: Level of significance.

TABLE 103

Etropius maculatus: Haematological status of control fish and those pre-exposed to four SLC ofGRAMOXONE for 30 days.

		TEC 10^6 mm^3	Hb g%	Ht %	MCV mm^3	MCH pg	MCHC %	VI	CI	SI
	a	3.172	11.529	32.194	102.037	36.437	55.807			
CONTROL		+/-0.263	+/-0.587	+/-0.128	+/-8.162	+/-2.569	+/-1.723	1.00	1.00	1.00
	a	2.92	7.598	34.249	117.847	25.907	22.139	0.713	1.155	0.618
		+/-0.257	+/-1.095	+/-0.834	+/-7.898	+/-1.533	+/-2.844	+/-0.042	+/-0.077	+/-0.079
C1	b	-7.94	-34.12	+6.38	+15.49	-28.9	-38.17			
0.0013	c	NS	P<0.001	P<0.001	P<0.05	P<0.001	P<0.001			
	a	2.784	7.186	36.862	133.044	25.731	19.463	0.708	1.304	0.544
		+/-0.248	+/-0.96	+/-0.753	+/-9.477	+/-1.313	+/-2.263	+/-0.036	+/-0.093	+/-0.063
C2	b	-12.23	-37.67	+14.5	+30.39	-29.38	-45.64			
0.0018	c	P<0.05	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001			
	a	2.628	6.872	39.487	150.978	26.033	17.362	0.716	1.479	0.495
		+/-0.236	+/-1.053	+/-0.946	+/-10.07	+/-1.729	+/-2.282	+/-0.047	+/-0.098	+/-0.063
C3	b	-17.15	-40.39	+22.63	+47.96	-28.55	-51.51			
0.0027	c	P<0.05	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001			
	a	2.572	6.095	42.296	164.973	23.614	14.453	0.649	1.617	0.404
		+/-0.185	+/-0.861	+/-0.7	+/-9.141	+/-1.798	+/-1.772	+/-0.049	+/-0.089	+/-0.049
C4	b	-18.92	-47.13	+31.38	+61.68	-35.19	-59.64			
0.0054	c	P<0.01	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001			

a: Mean value (N=5) with +/-SD.

b: %Alteration from the mean value.

c: Level of significance.

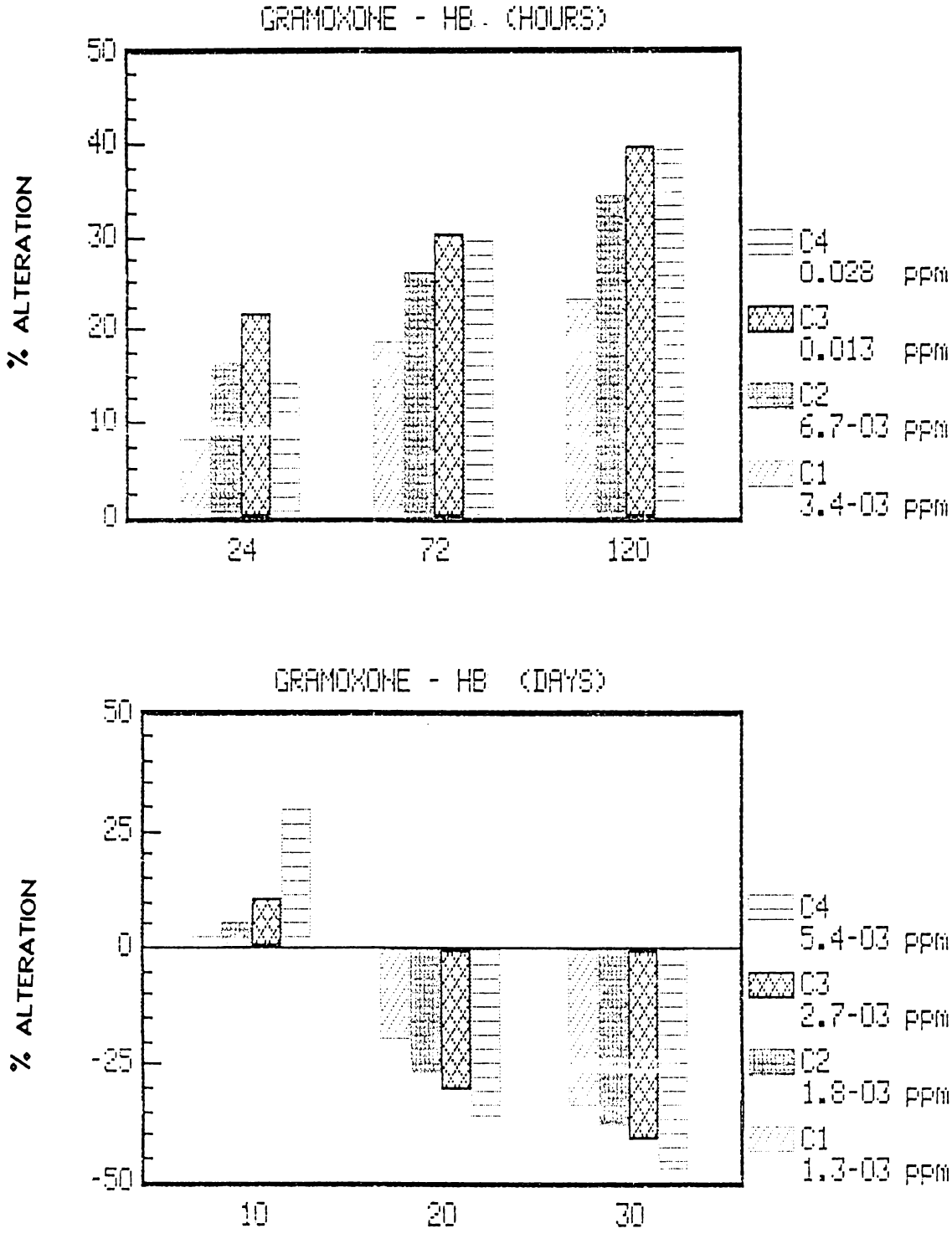

Fig. No. 38 *Etroplus maculatus* : Percentage alteration in HB from mean control value following exposure to four SLC of Gramoxone.

was observed and found statistically significant in all the concentrations. The reduction in Hb content was found more pronounced during 30 day exposure *(Table 98, 99 & 100, Fig. 38)*.

4.2.2.3.3 Haematocrit (Ht)

Short term:- The haematocrit response was found rather erratic in lower concentrations during 24 and 72 hours exposure, while in the highest concentration statistically significant reduction in Ht was found. During 120 hour exposure, the Ht showed progressive and inverse change with increase in pesticide concentration and the change in higher concentrations was statistically significant. In higher three concentrations statistically significant reduction in Ht was noted with increase in exposure time *(Table 98, 99 & 100, Fig. 39)*.

Long term:- A positively proportional change in Ht with increase in concentration was evident during long term exposure and the change in higher two concentrations during 10 day and in all pesticide concentrations, during 20 and 30 days exposure was found statistically significant. The maximum increase in Ht was noted during 30 day exposure and found increasing with increase in exposure time *(Table 101, 102 & 103, Fig. 39)*.

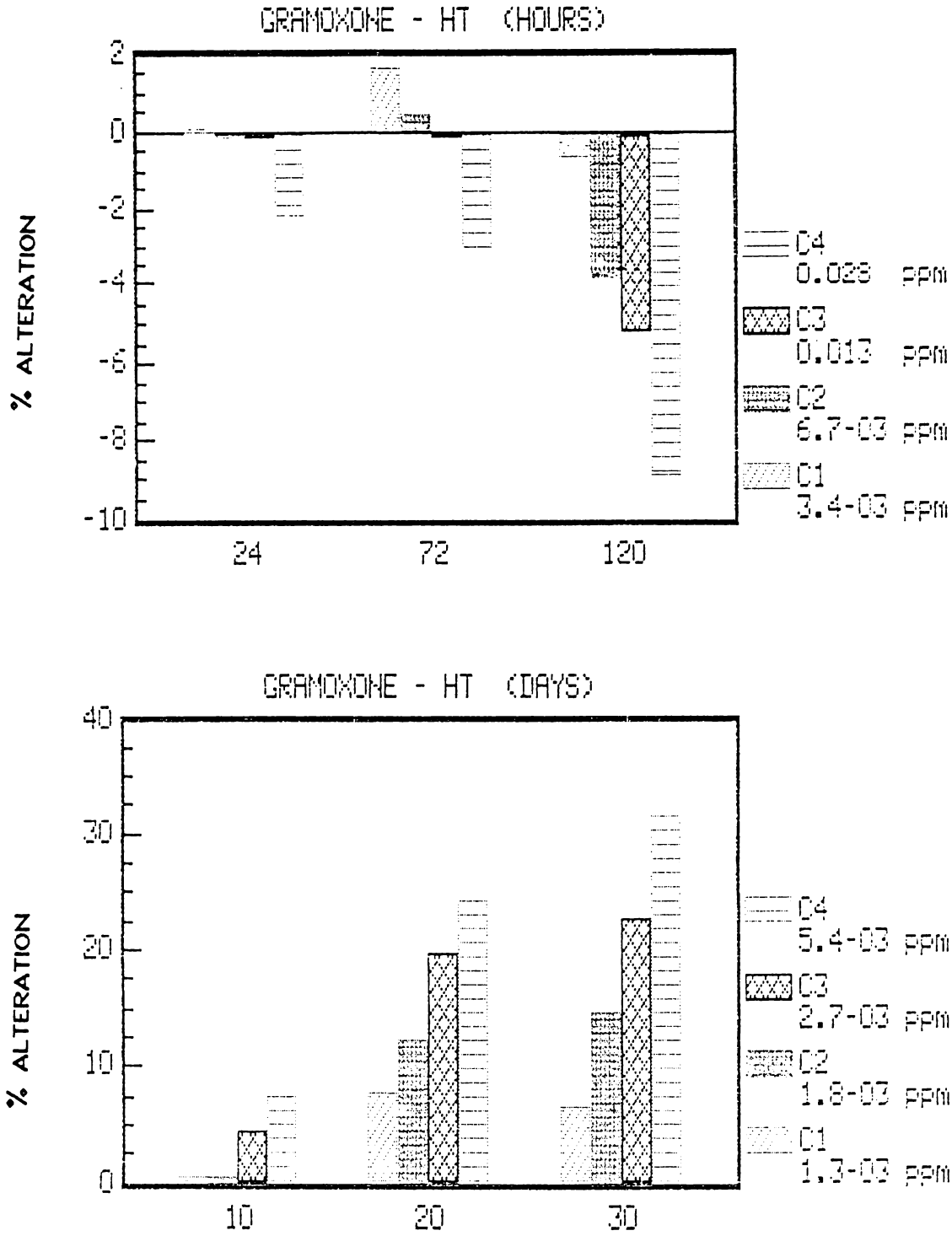

Fig. No. 39 *Etroplus maculatus* : Percentage alteration in HT from mean control value following exposure to four SLC of Gramoxone.

4.2.2.3.4 Erythrocyte constants (MCV, MCH & MCHC)

Short term:- Mean corpuscular volume (MCV) of experimental fishes showed a progressive, concentration dependent reduction in all the pesticide concentrations and found statistically significant in higher concentrations. This increase relation between MCV and pesticide concentration was evident in all the three short-term exposure periods. In higher three concentrations a definite reduction was noted in MCV with increase in exposure duration.

A progressive, and statistically significant (except in the lowest concentration during 24 hour exposure) increase in mean corpuscular haemoglobin (MCH) was recorded during short term exposure to gramoxone in all four sublethally exposed groups. Among exposed groups the MCH showed a progressive increase with concentration and exposure duration in the lowest (C_1) concentration and in higher two concentrations (C_3 and C_4) while MCH in C_2 recorded a comparatively lower value during 120 hour exposure. In higher two concentrations the increase in MCH with exposure duration was found statistically significant.

Mean corpuscular haemoglobin concentration (MCHC) recorded a progressive concentration dependent and duration dependent increase in experimental groups. The increase in MCHC was found statistically

significant in three higher concentrations (C_2, C_3 and C_4) during 24 and 72 hours and in all pesticide concentrations during 120 hour exposure (*Table 98, 99 & 100, Fig. 40, 41 & 42*).

Long term:- MCV in all experimental groups during long term exposure to gramoxone recorded a dose and duration dependent increase. The increase in MCV was found statistically significant in higher (1) two concentrations during 10 day exposure, (2) three concentrations during 20 day and (3) in all concentrations during 30 day exposure. However, the reduction in MCV noted in all concentration with increase in exposure time was not found statistically significant.

A biphasic pattern was noted (with respect to exposure duration) in MCH response during long term exposure. MCH recorded a dose dependent increase during 10 day exposure and the increase was found statistically significant in higher three concentrations. However, during 20 and 30 days exposure, statistically significant reduction in MCH was recorded in all the four exposed concentrations and the reduction found comparatively enhanced as the concentration and duration increased.

A biphasic response, similar to that of MCH, was recorded in MCHC of experimental groups. MCHC found elevated in all the concentrations during 10 day, and the high MCHC recorded in C_4 was

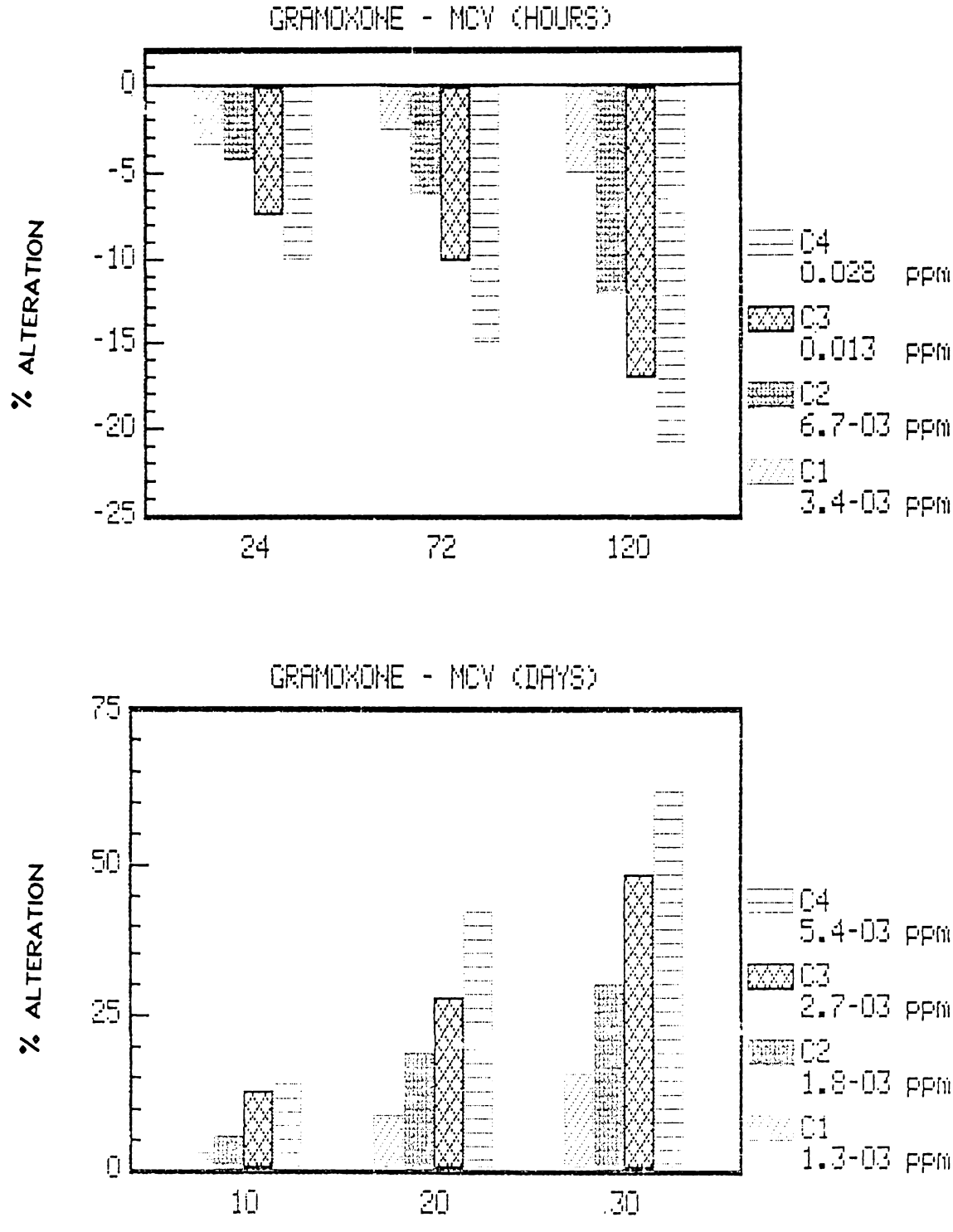

Fig. No. 40 *Etroplus maculatus* : Percentage alteration in MCV from mean control value following exposure to four SLC of Gramoxone.

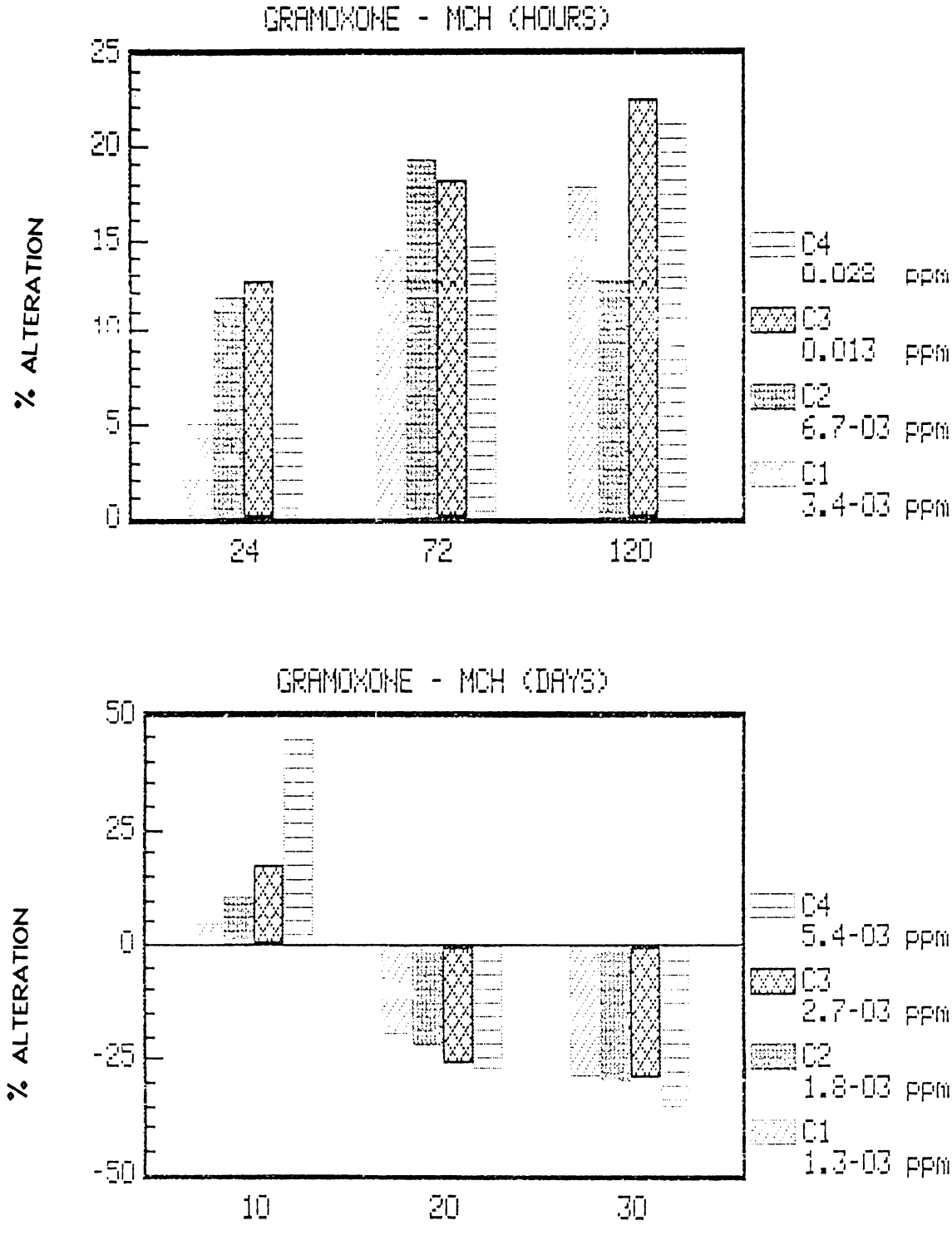

Fig. No. 41 *Etroplus maculatus* : Percentage alteration in MCH from mean control value following exposure to four SLC of Gramoxone.

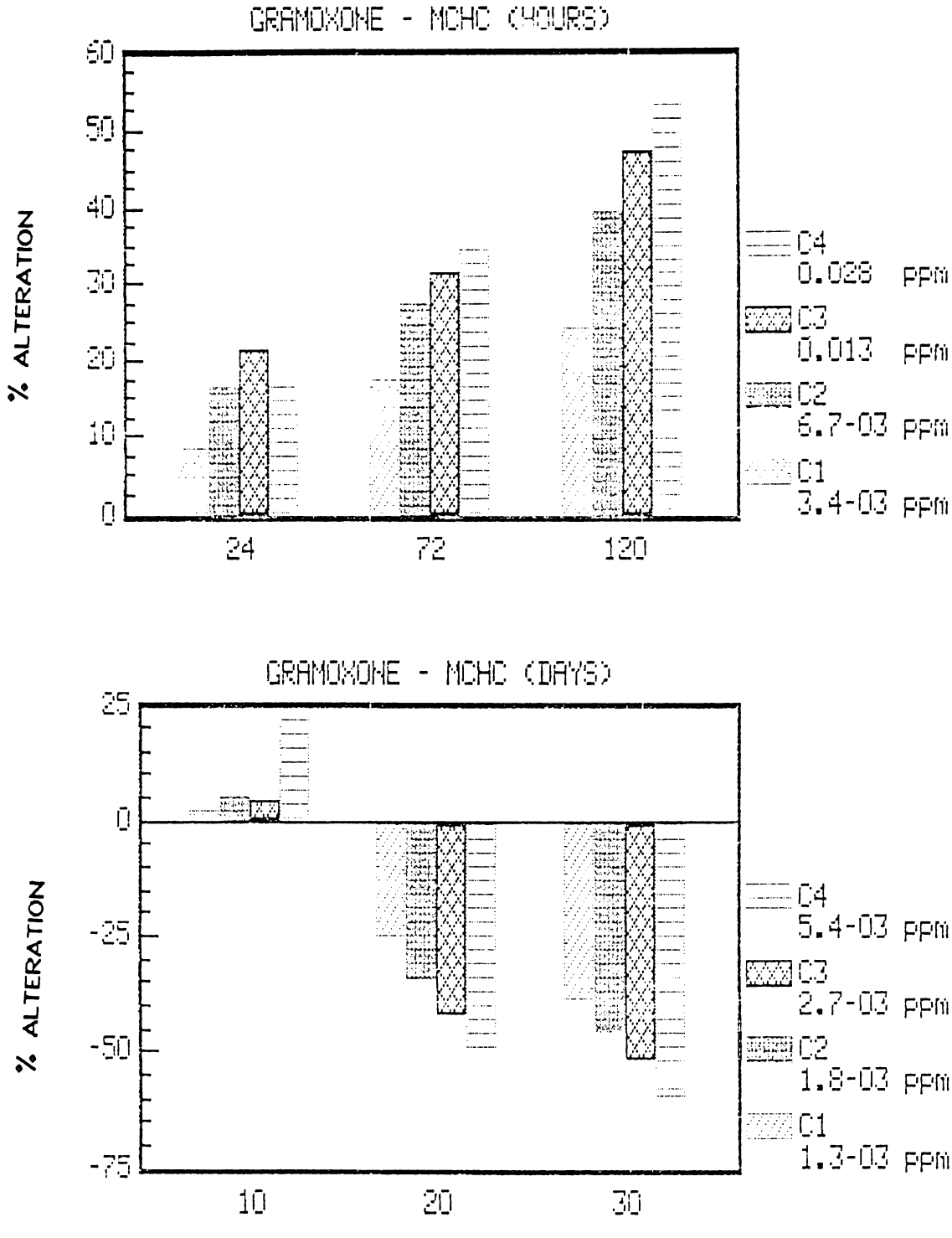

Fig. No. 42 *Etroplus maculatus* : Percentage alteration in MCHC from mean control value following exposure to four SLC of Gramoxone.

statistically significant. But an inverse change in MCHC noted during 20 and 30 days exposure and found pronounced with increase in concentration and exposure duration. The reduction in MCHC was statistically significant in all the concentrations except in the lowest concentration during 20 day exposure (*Table 101, 102 & 103, Fig. 40, 41 & 42*).

4.2.3.3.5 Erythrocyte indices (CI, VI & SI)

Short term:- In confirmity with the progressive reduction in MCV, the volume index (VI) recorded lower values than unity, indicating occurrence of microcytosis in all pesticide concentrations and in all the three exposure periods under short term exposure.

The elevated mean corpuscular haemoglobin (MCH) in all experimental groups was responsible for the higher colour index (CI) values recorded. The colour index greater than unity recorded in all the concentrations during short term exposure indicated the occurrence of hyperchromic state of RBC as a result of pesticide stress.

In all concentrations the saturation index (SI) recorded higher value than unity indicating supersaturation of RBC with haemoglobin. The high SI recorded during short term exposure as a result of high MCHC recorded in experimental fishes (*Table 98, 99 & 100*).

Long term:- Volume index was found higher than unity in all experimental concentrations during long term exposure indicating the swelling of erythrocytes (macrocytosis). The higher volume index recorded was in confirmity with the higher MCV of experimental fishes.

During the initial phase of exposure (10 day) colour index was found higher than unity indicating the hyperchromic condition of RBC. However, during 20 and 30 days exposure, as a result of lowering of MCH the colour index was found less than unity indicating hypochromic condition.

In confirmity with the increase in MCHC, saturation index recorded higher value than unity, indicating supersaturation RBC with haemoglobin, during 10 day exposure. While during 20 and 30 days exposure the SI recorded lower value than unity in all concentrations indicating a less saturated condition of erythrocytes *(Table 101, 102 & 103)*.

4.2.3 Histopathology

Many authors have made use of morphological and histological techniques to determine the toxic effects of poisons on fish tissue system. Histological effects of pesticides remain largely undefined and most of the recent work is inconclusive. Majority of the work

reported on fish and pesticides is directed at acute toxicity. Histopathological studies are useful in evaluating the pollution potential of pesticides since trace levels of pesticides which do not bring about animal mortality over a given period are capable of producing considerable organal damage (Kumar and Pant, *1984*). In the present investigation tissues like brain, gill and liver of Etroplus maculatus were examined histologically for the alterations caused in their general architecture following long term exposure of 30 days to the three pesticides individually. The fishes were exposed to the highest sublethal concentration selected for each pesticide during long term studies.

Brain

Control:- The teleost brain is similar in its basic components to the brain of higher animals, but with many differences in form and complexity. For the case of description it is usually divided into five divisions comprising, from the anterior : the telencephalon, the diencephalon, the mesencephalon, the metencephalon or cerebellum and the medulla oblongata (Ellis and Roberts, *1978*). There is only a very limited amount of information available on the detailed histology of fish brain. In the present study, transverse sections of the whole brain was prepared without assigning to a particular region (Plate I).

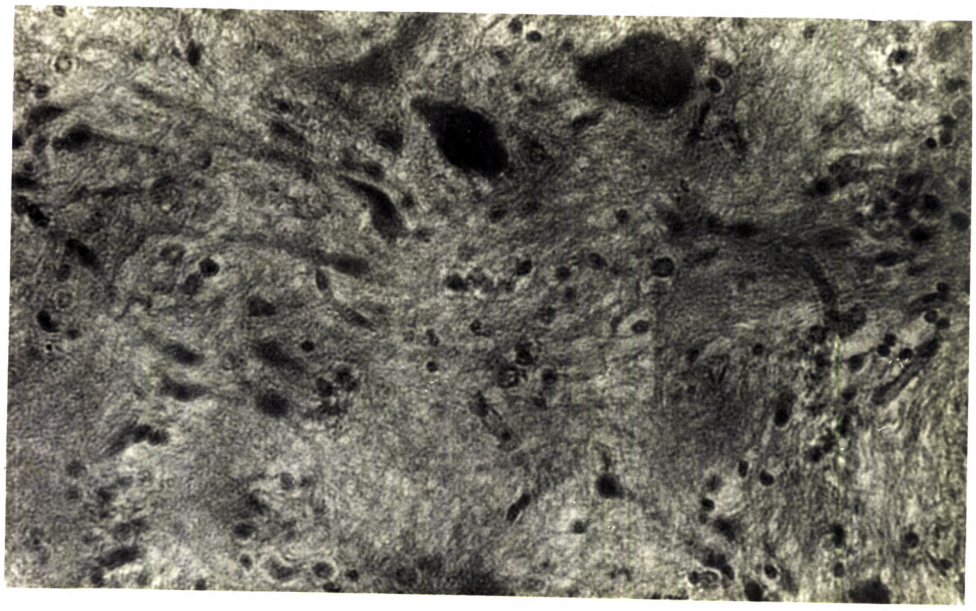

PLATE I

Etroplus maculatus : Brain - Normal (12.5 X 50x)

PLATE II

Etroplus maculatus : Brain - DDT exposed (12.5 X 50x)

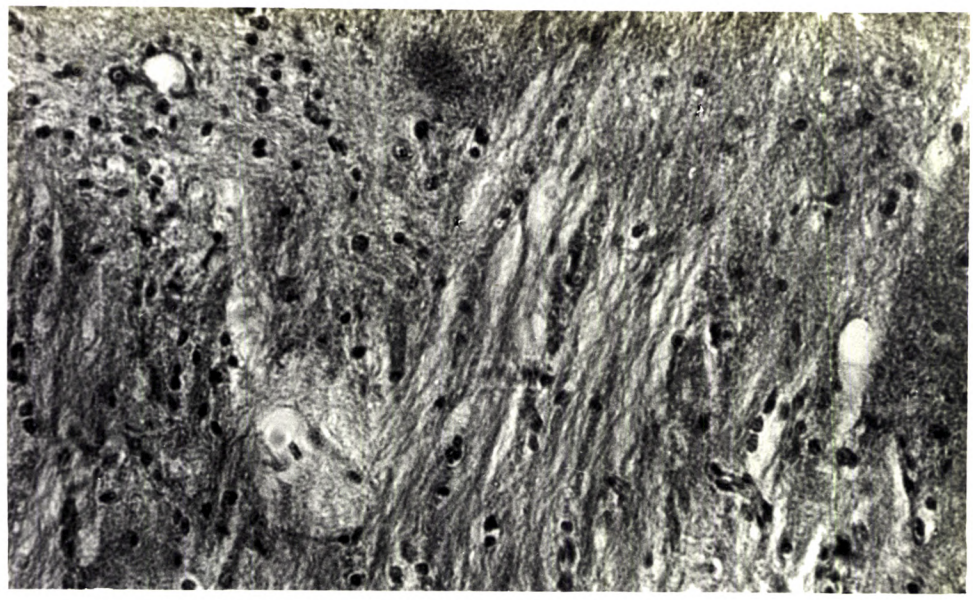

PLATE III

Etroplus maculatus : Brain - Dimecron exposed (12.5 X 50x)

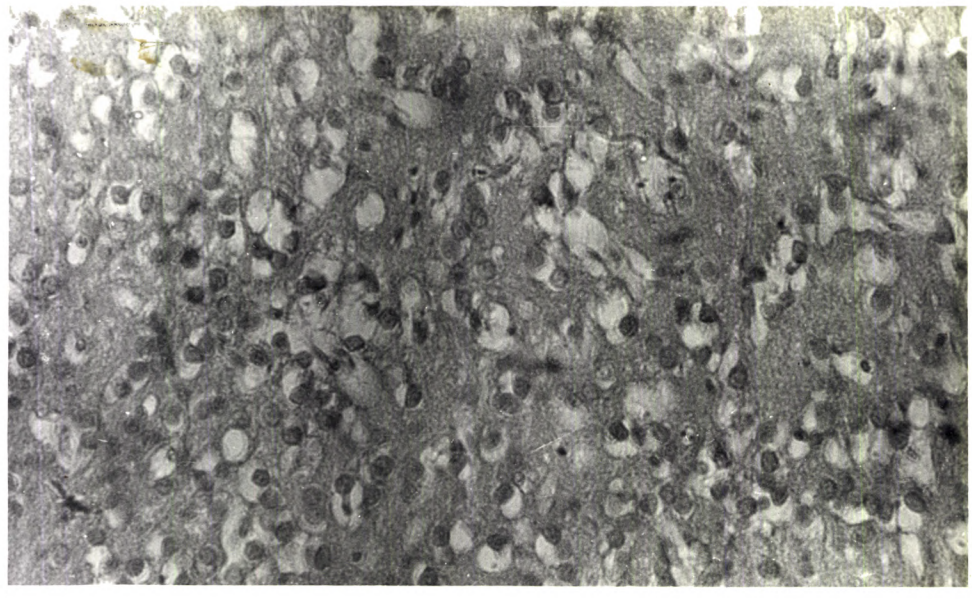

PLATE IV

Etroplus maculatus : Brain - Gramoxone exposed (12.5 X 50x)

DDT : Hyperemia of varying degree was evident in the brain of E. maculatus following exposure to DDT. Vacuolization of cells were also noted (Plate II).

Dimecron : Brain cells exposed to Dimecron showed vascular congestion and pyknosis. Slight vacuolization and the presence of eosinophilic globules were also noted (Plate III).

Gramoxone : No clear cut changes in the brain could be noticed following gramoxone exposure. However, nuclear pyknosis and vacuolization of cells were observed (Plate IV).

Gill

Control : The structure of the gill of E. maculatus, was in general, of the teleostan type described by Heghes and Morgan (*1973*). Control gills showed a row of long thin filaments, the primary lamellae, projecting from the gill arch. Secondary lamellae were found on its dorsal and ventral surfaces. The secondary lamallae consisted of a pillar cell system and epithelium. The pillar cells enclosed the lamellar blood space. The epithelial layer was one layer thick (Plate V).

DDT : Microscopic examination disclosed pathological gill lesions following DDT exposure. Oedema and epithelial hyperplasia of the secondary lamellae were evident. Hypertrophy of blood vessels and

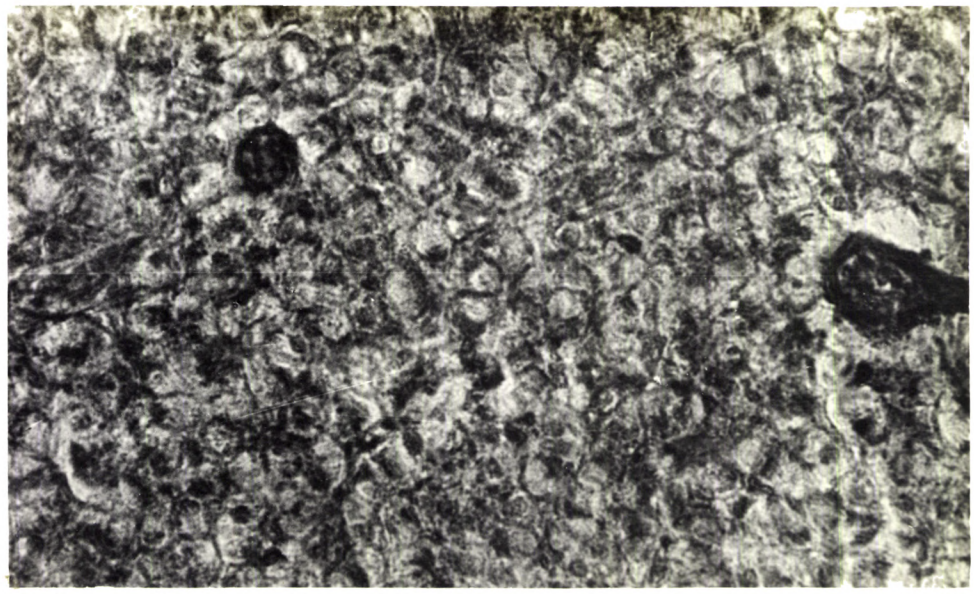

PLATE V

Etroplus maculatus : Liver - Normal (12.5 X 50x)

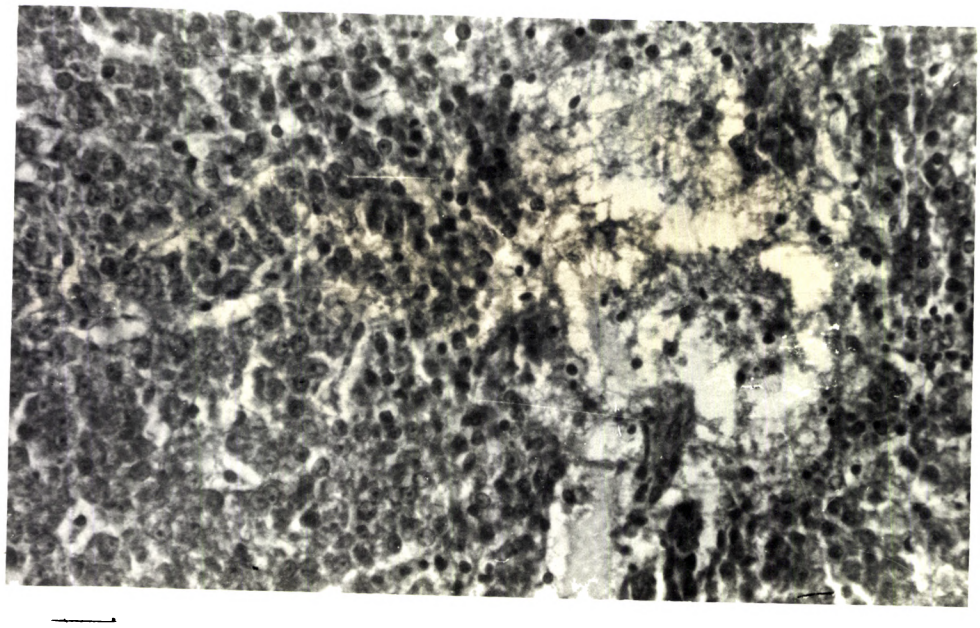

PLATE VI

Etroplus maculatus : Liver - DDT exposed (12.5 X 50x)

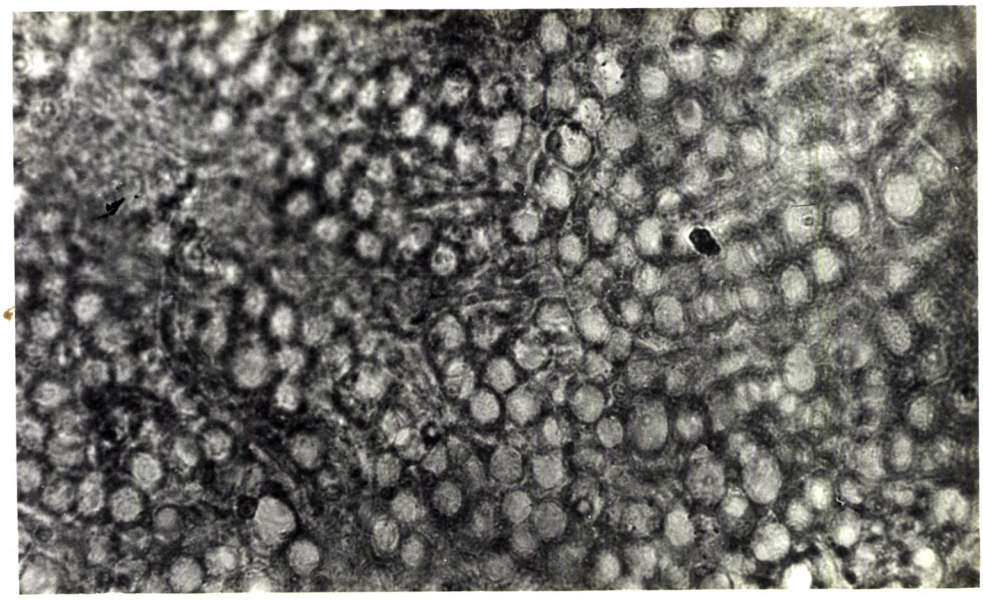

PLATE VII

Etroplus maculatus : Liver - Dimecron exposed (12.5 X 50x)

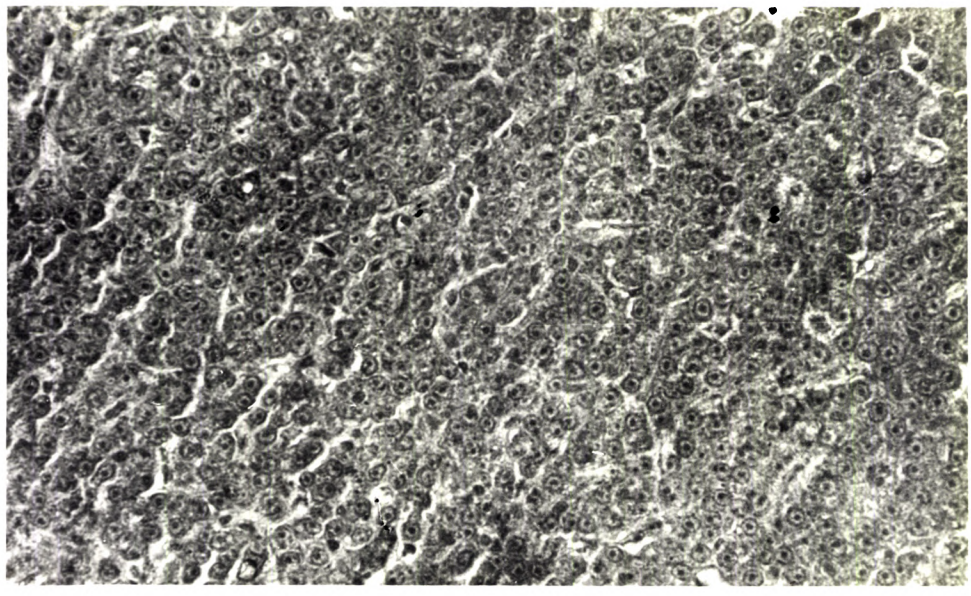

PLATE VIII

Etroplus maculatus : Liver - Gramoxone exposed (12.5 X 50x)

necrosis of inter-lamellar region and the tips of the filament were also noticed. The basement membrane of the gill lamellae were found ruptured at many places (Plate VI).

Dimecron : Dimecron caused branchial congestion in the gill filaments. Oedematous fluid lifted the respiratory epithelium in a few secondary lamellae. Secondary lamellae were found thickened. The cells between the secondary lamellae was thickened to such an extent that the inter-lamellar spaces occluded, which gave the filament a compact appearance (Plate VII).

Gramoxone : The gill filaments of fishes exposed to gramoxone showed swelling and elongation of the gill filaments as a result of branchial congestion. Destruction of the epithelial wall of the secondary filament and fusion of the basement membranes at certain places were clearly evident (Plate VIII).

Liver

Control : The liver composed of polyhydral hepatic cells which contained a granular cytoplasm. Nuclei of the liver cells were vesicular with large nucleoles. Blood capillaries and sinusoides were noticed. A typical tubulosinusoid pattern of arrangement of the parenchymal cells were clear. The lining of the sinusoids, formed by the reticuloendothelial cells was separated from the hepatic cells and engorged

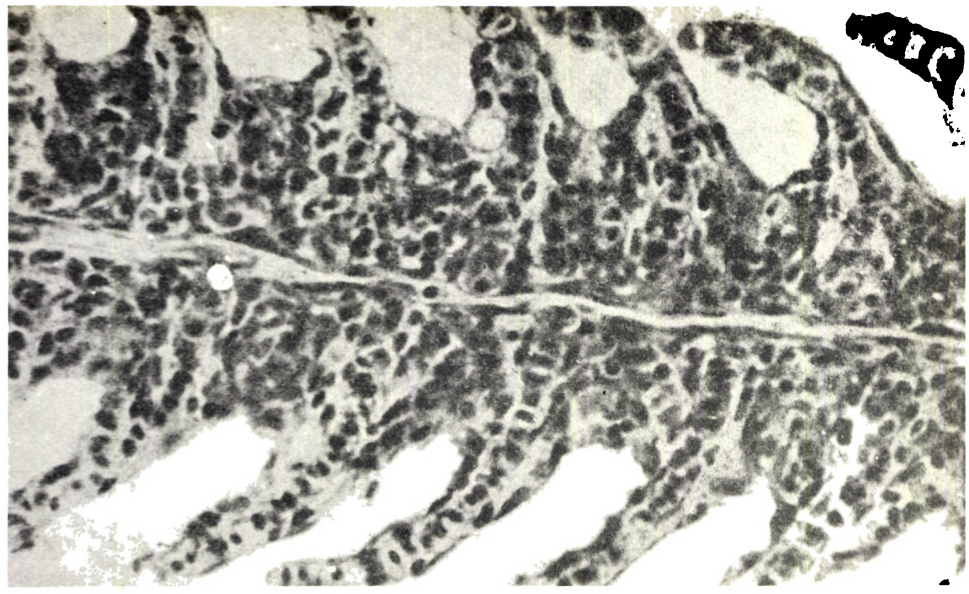

PLATE IX

Etroplus maculatus : Gill - Normal (12.5 X 40x)

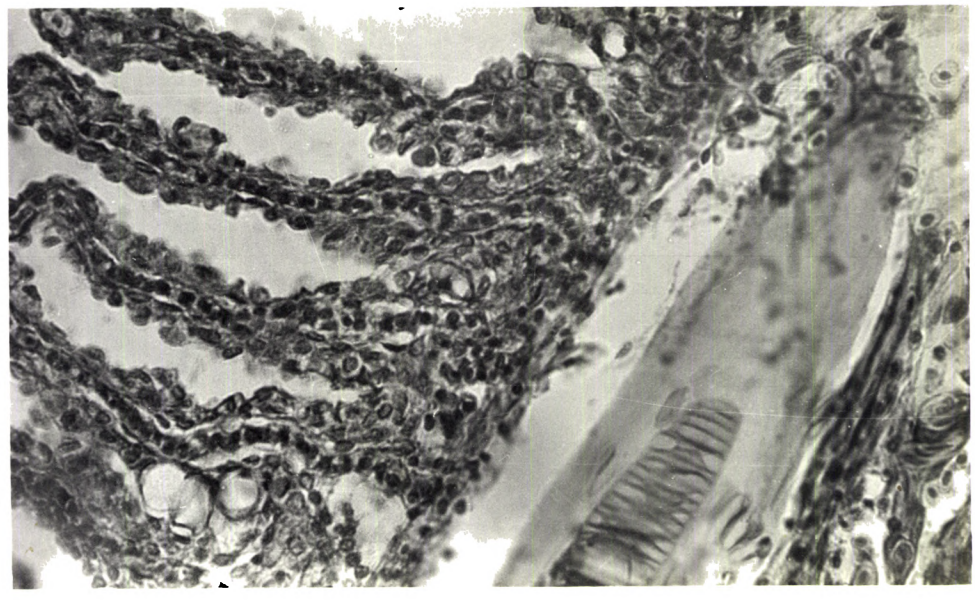

PLATE X

Etroplus maculatus : Gill - DDT exposed (12.5 X 50x)

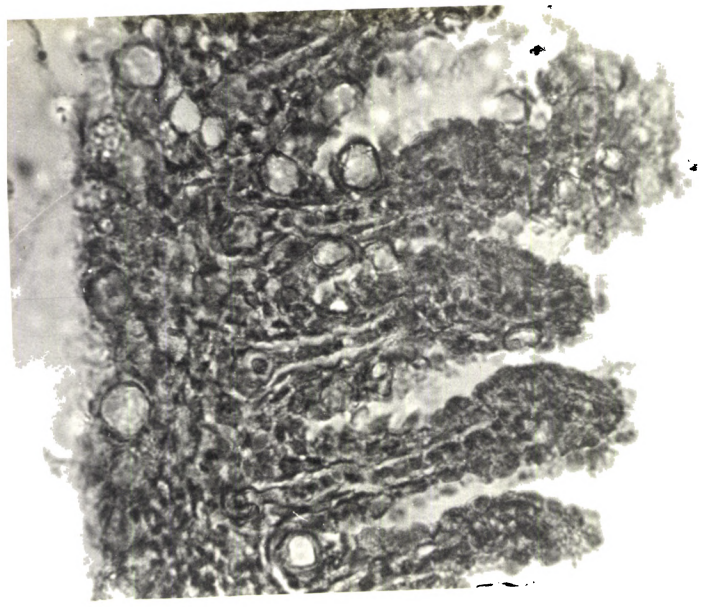

PLATE XI

Etroplus maculatus : Gill - Dimecron exposed (12.5 X 50x)

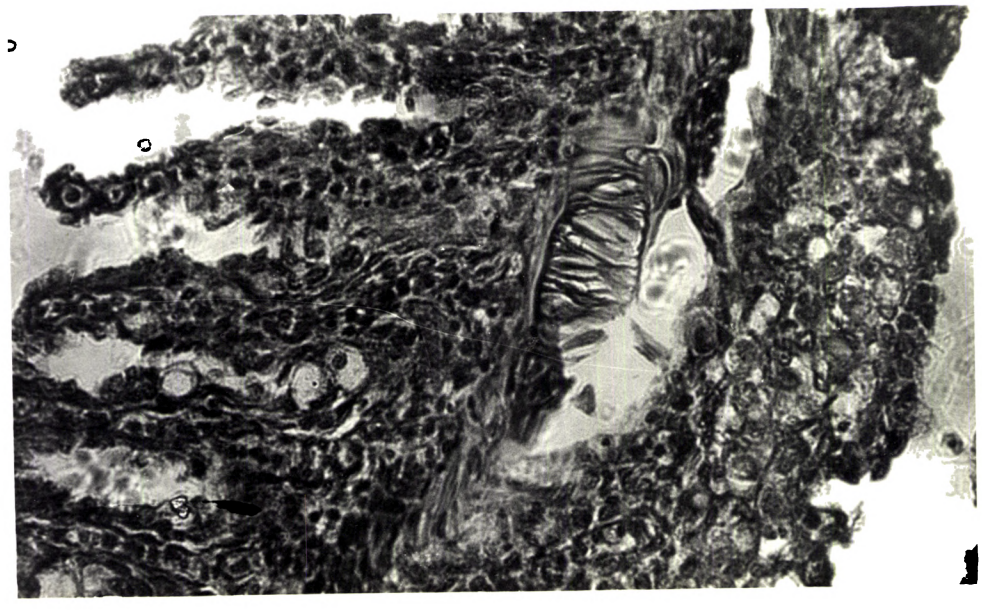

PLATE XII

Etroplus maculatus : Gill - Gramoxone exposed (12.5 X 50x)

with blood cells. Sinusoids, irregularly distributed between the hepatocytes were few in number (Plate IX).

DDT : Small cytoplasmic vacuoles were found throughout the parenchyma. DDT caused reduction in the fat vacuoles and hypertrophy were observed. Necrotic changes and pyknotic nuclei were noticed in the hepatic cells (Plate X).

Dimecron : Liver displayed cytoplasmic vacuolization, periportal atrophy and radial disorientation. The parenchymal nuclei were found to be enlarged and the tubulosinusoid arrangement was lost (Plate XI).

Gramoxone : Vacuolation of the parenchymal cells and pyknosis of the nuclei were observed following gramoxone exposure. Partial loss of radial orientation and blood vessel congestion could be seen in certain areas. Granulocytic infiltration was also noticed (Plate XII).

DISCUSSION

V - DISCUSSION

The main ecological concern about the damages that could be caused by pesticides arose with the finding that marine organism collected far off from the coasts contained traces of DDT. There was an over estimation regarding the capacity of aquatic organism to degrade and detoxify man-made chemicals, and further it is not possible to assess the effects of pesticides conclusively since, there are endless possibilities of these chemical compounds forming combinations. Probably, only in the case of highly toxic pesticides, a clear cut assessment on possible damages could be predicted. Kinne (*1984*) opined that the ecological consequences of these chemicals must be recorded and evaluated in long term extensive monitoring programmes.

A part of the enormous quantity of pesticides inducted into the biosphere through human activities is bound to reach the aquatic ecosystem in due course. Bio-concentration and bio-magnification will necessarily increase the load of these pesticides in aquatic biota and this can affect life and activity of aquatic organisms. New developments in aquatic toxicological research make it apparent that the xenobiotic compounds are not "benign", accumulating in fat depot or bound to proteins, affecting individual organism and populations. Contaminants may cause subtle genetic or biochemical effects in

aquatic biota that lead ultimately to activation of oncogenes, alterations in the developmental processes or depression of immune defense systems (O'connor and Huggeh, *1988*).

It is known that the capacity to accumulate toxicants due to both bioconcentration and biomagnification tends to outweigh elimination, and the resulting dynamic equilibrium level depends on the properties and ambient concentration of the pesticide concerned, the potential of the animals to counter-act, and environmental factors. It is possible that degradation may ultimately lead to the formation of inorganic end products. However, this aspect has to be viewed from the standpoint that the capacity of the natural ecosystem for degrading pesticides and technical organic chemicals is usually limited. Further, a number of degradation pathways have intermediate products which have very drastic deleterious effect on aquatic organisms (Jacob, *1988*).

The adverse effects of pesticide pollution on aquatic life fall into three general categories, (a) the direct toxicity of the chemical to aquatic organism, (b) the deterioration of the water quality and (c) imparting bad taste and odour to the edible flesh of fish and shell fish. The general theme of pesticides and aquatic organisms embraces many disciplines, each of which has been the subject of intense investigation in recent years. Fish are so frequently

the most obvious and economically the most important sufferers from exposure to pesticides and other toxic chemicals in the water, that there is a long history of investigation in many countries. Moreover, the vigorous development in the chemical control of undesirable fish populations has necessitated new and more critical standards of evaluation and has infused a wealth of new material and new concepts into this field. A widely accepted concept is that effects of pollution, clearly demonstrated on even a single individual or a local population, must be considered a cause for management action to protect the total population - just as is the case with humans. "All the mechanisms of public health protection come into force when even mediocre statistical correlations of pollution and human disease are made; and the same kinds of responses should be made by resource and environmental managers when correlations of pollution and fish abnormalities are found", as opined by Sindermann (*1979*). Therefore, any attempt to gather information on these lines has topical importance.

5.*1* LETHAL TOXICITY

Lethal toxicity study gives an opportunity for a quantitative appreciation of acute toxicity in relation to toxicants vs. time. In acute toxicity studies, time factor is usually compensated by employing unrealistic high concentrations, on the assumption that

external concentrations have direct bearing on the rate of intake, and thereby the resultant mode of activity of the animals. Acute toxicity tests have played a major role in aquatic toxicology because survival measured is considered the best index of pollutant stress (Moraitou Apostolopowlou et al., *1982*). Although mortality measured in acute toxicity tests appear to be a crude method of measurement of toxic response, its importance was highlighted by many workers (Duke, *1974*; Buikema Jr. et al., *1982*).

Three pesticides, in their commercial formulations, have been used to study lethal toxicity on Etroplus maculatus. Among the pesticides used, DDT an organochlorine was the most toxic followed by gramoxone, a bypyridilium herbicide. The organophosphate, Dimecron was least toxic to E. maculatus. Adamson (*1974*) generalized that protein denaturation and alteration in permeability and active transport due to toxic effects of chemicals are the most probable cause of death.

The *96* h LC 50 of DDT technical to river shiner, carp, gold fish, northern Dike brown trout, Atlantic salmon, rainbow trout, cutthroat trout and coho salmon were*5.8*, *9.7*, *14.7*, *2.7*, *1.8*, *1.8*, *11.4*, *7.9* and 5.5 ug|1 (ppt) respectively (Mayer and Eller Sieck, *1986*). Satyanarayanan (*1980*) has reported 0.0024 ppm for Cyprinus carpio,

0.049 ppm for Punctius ticto, and 0.003 ppm for Lebister reticulatus as the 96 h LC 50 of DDT. In the present study 0.005 ppm was recorded as the 96 h LC 50 of DDT to E. maculatus. The lethal concentration of the pesticide reduced as a function of time. The change in the lethal concentration as a function of time indicates that the quantity of the pesticide that enters the body could be dose dependent and that prolonged exposure brings about irreparable damage at high concentrations.

The toxic action of DDT in insects is more or less well established. The primary target of DDT is nervous system. Membrane surface recalcification is interfered by DDT which is essential to restore normal resting potential after a single depolarization of the axonic membrane (Welsh and Gordon, *1947*). Strong affinity of DDT made several authors to postulate that it acts on cholesterol in the lipoidmembrane, thereby reducing membrane permeability to Ca^{2+} (Langer et al., *1946*). Yamasaki and Ishii (*1952*) suggested that DDT alters the axonic membrane in such a way that the membrane constants, like conductance, resistance and capacity, themselves become different from those in normal membrane. Inhibition of membrane ATPase, especially Na^{+}-ATPase and Mg-ATPase, by DDT was reported by Koch et al. (*1969*). DDT binding to the nerve components involves a reaction of "Charge transfer complex formation" and this process induces the nervous

disturbance, i.e., symptom of 'Nerve Hyper excitation' (O'Brien, *1967*).

Though it is generally acknowledged that fish do not have efficient detoxification mechanism, Wedemeyer (*1968*) showed that liver of rainbow trout could convert DDT to DDE, indicating that atleast some of the activities come from the fish themselves apart from microbial action. Cherrington et al. (*1969*) found from their in vitro studies of intestinal contents of Atlantic salmon that degradation of DDT is possible in fishes.

It has been established that effective mechanisms that could detoxify DDT are absent in fish tissues (Lee et al., *1972*). However, maps summarizing the overall metabolic profile of DDT in fishes have been worked out and about fourteen metabolites of DDT have been identified in fishes by Cairns and Charles (*1980*).

Many authors worked out the toxic action of DDT to fishes. DDT was found more effective in inhibiting the oligomycian sensitive ATPase activity in fish kidney and liver (Khan and Cutcomp, *1982*).

Davis and Wedemeyer (*1971*), Janicki and Kinter (*1971*), Kinter et al. (*1972*) and Leadem et al. (*1974*) reported that DDT seriously interferes with Na^{+}K^{+}-ATPase activity in teleost which play a greater

role in osmoregulatory mechanism. Burdick et al. (*1972*) observed suppression of ovarian activity in salmonoids by DDT, by reducing the synthesis and release of gonadotropins from the pituitary gland. But Addinson et al. (*1977*) noted that even the hepatic MFOs which were stimulated by many organic pollutants were not influenced by exposure of fish to DDT. Radio-labelled uptake studies revealed that DDT has high partition coefficient and are rapidly taken up as a result of high affinity of cell membranes for such compounds (Aldays and Guthrie, *1982*). This high affinity leads to the tight association of DDT to the lipid constituents of cells reducing their further penetration. This, at length, leads to high bioconcentration and biomagnification noted in case of DDT in fishes.

The most conspicuous feature of all organophosphorous compounds is their structural complementary with the target enzyme molecule, cholinesterase, and one of the characteristics of OP inhibition of cholinesterase is that the rate of recovery (induced by the reactivating agents) becomes less and less, as the time of inhibitor-enzyme contact becomes longer. This phenomenon is often referred to as "aging" (Hobbiger, *1955*). Phosphamidon, the active ingredient in Dimecron, is essentially a diethylamide analogue of Bidirin. Studies in animals have showed that two factors make the metabolic pattern of this compound: (1) the extra chlorine atom is labile and therefore can

be subjected to hydrolytic dehalogenation which could be either chemical or biochemical or to reductive dechlorination which is enzymatic, and (2) an oxidative N-dealkylation process since this compound is a diethyl amide (Geiss-buhler et al., *1971*).

The *96* h LC 50 for phosphamidon technical reported by static bioassay for blue gill, channel catfish, fathead minnow and rainbow trout were 3.4 ppm, 70.0 ppm, 100.0 ppm and 7.8 ppm, respectively (Mayer and Ellersieck, *1986*). Vijayalakshmi et al. (*1986*) reported 1.25 and 0.54 ppm, respectively at 15 and 25‰ salinity as the 48 h LC 50 of phosphamidon to the prawn Metapenaeus monoceros. Hurlbert (*1975*) reported 8.8 ppb as *96* h LC 50 of phosphamidon to the amphipod Gammarus lacustris. The *96* h LC 50 of Dimecron to the brown mussel Perna indica and clam Villorita cyprinoides were found to be 117.58 ppm and 93.28 ppm, respectively (Jacob, *1988*).

Paraquat, the active ingredient in gramoxone, is a broad spectrum herbicide known to be highly lethal to animals. One of the proposed mechanisms whereby it produces toxic effects is through the generation of the super oxide anion (O^-_2). The biological consequences of O^-_2 generation include extensive damage to cell membranes with resultant loss of functional integrity (Geri et al., *1981*). The super oxide anion in liposomes perturbs the bilayer and increases the leakage of otherwise impermeable anions and this process is called

lipid peroxidation. Paraquat toxicity due to lipid peroxidation was observed in two species of carp Cyprinus carpio and C. carrasius in which lipid peroxidation was significantly elevated by 10 ppm Paraquat from 4 to *96* hours (Gabryelak and Klekot, *1985*). The damaging effects are excerted during its metabolism in the organism because of the extreme toxic effect of the formed free radicals (Stancliffe and Pirie, *1971*). The developed free radicals may lead to the degradation of the cell membrane, as has successfully been demonstrated in fish, with biochemical and electron microscopic methods (Rojith et al., *1983*). Paraquat also damages the nervous system of fish, through the inhibition of AChE (Nemscok et al., *1984*).

Comparative LC 50 study for gramoxone is rather limited. However, the *96* h LC 50 of 24% gramoxone by static bioassay was found to be 13 and 15 ppm, respectively to blue gill and rainbow trout while the *96* h LC 50 of paraquat technical to channel catfish was found to be more than 100 ppm (Mayer and Ellersieck, *1986*). The *96* h LC 50 of gramoxone to E. maculatus in the present study was found to be much lower than the above LC 50s. This wide difference in the toxicity of gramoxone may be due to a difference in formulation or may be due to the high sensitivity of tropical fishes to gramoxone than its temperate counterparts. About 0.1 ppm of paraquat was found toxic in carp fingerling (Singh and Yadav, *1978*).

The toxicity data presented here in the case of E. maculatus give an insight into chemical dependent variation in pesticide toxicity.

Results on the experiments of DDT on the life of E. maculatus are rather erratic, in the sense that the lethal dose is rather narrow with the concentration range between *0.006* and *0.008* ppm. The rate of mortality overlapping under a *0.006* and *0.007* ppm stress shows that the concentration that would have entered the body is more or less the same irrespective of the outside load. This is amply justified by the nearly asymptotic nature of the LC 50 curve. The asymptote denotes, based on the findings here, that the effects would be the same in concentrations above *0.008* ppm or below *0.006* ppm within the time frame. This further shows that the nature of availability of DDT in the media and the mode of entry are unconnected; for the results are indicative of concentration effects alone.

Dimecron is less toxic compared to DDT. The toxic chemicals may be entering the body and the sensitive tissues rather slowly. The entry is a function of both time and concentration, the latter ranging between 0.14 ppm to 0.20 ppm. An asymptote would have been obtained had the tested concentrations been reduced below 0.13 ppm. Dimecron is a proven neural toxicant to insects. However, the path of entry

is through trachea. In the case of these fishes, the only possibility of reaching the neural centre is through blood via the gills and the buccal cavity. It is not clear whether any sort of detoxification takes place at these loci. Notwithstanding this the elevated concentrations did have a clear cut increase in toxicity implying enhanced entry as a function of concentration and probably duration. The effective time ranged from two to three days indicating reduced toxicity of the chemical to the aquatic organism.

Relatively less toxic than DDT to these fishes, gramoxone, behaved more or less in conformity with these series of commercial insecticides. The toxic effect of this chemical seems to be short lived even at high concentrations as is evident from the effective time analysis.

5.2 SUBLETHAL TOXICITY

Sublethal effects of pollutants to fishes are carried out essentially to delineate responses of the organism that would be exemplified by (a) inhibition or enhancement of growth, (b) interference in predator-prey relations through the agency of selective toxicity, (c) modification in behaviour induced by the toxicant, including the avoidance of particular situations, and the motor functions related to swimming, (d) effects upon biochemical and physiological mechanisms

especially as they relate to respiration, osmoregulation, ionic regulations, the composition of body fluids, enzyme systems, bioaccumulation, and the development of tolerance and (e) predisposition to disease, parasites and histopathological changes (Perkins, *1979*). Alderdice (*1967*) defined sublethal effects of pollutants as debilitating and thus bringing about death only indirectly.

There is a lot of physiological processes which can be measured in aquatic organisms by their response to sublethal stress on exposure to a low concentration of pollutant. The stress of a pollutant measured on a laboratory test animal may be little more than the adaptation response exhibited by an organism in adjusting to normal environmental changes. Such acclimation capability may be essential for survival of the species through the various stressful conditions encountered in its life cycle. Adaptive physiological response can be distinguished from harmful physiological response in the ultimate expressions of biological performance, which contribute to survival, growth and reproduction of the species (Waldichuck, *1979*).

Waldichuk (*1979*) has remarked that a "response is not linear with pollutant concentration". Sublethal response can usually delineate linear and non-linear reactions. However, under laboratory conditions this will be decidely controlled by the concentration

ranges employed and the category of response tested. Usually, the concentrations used for the study, range between measurable sublethal response threshold and incipient lethal threshold. As mentioned earlier, the present study two sets of sublethal concentrations, half of the LC 50 and lower during short term exposure and 1|10 of LC 50 and lower during long term exposure, were employed.

5.2.*1* Effect on enzyme activity

It is apparent from the results obtained that the trend in activity of four enzymes studied can vary from linear to non-linear pattern. During the short term exposure to three pesticides, the activity of four enzymes in three tissues may be generalised as follows:

Regarding ALP activity in brain, a more or less inhibition was found following DDT exposure while in Dimecron exposure, an initial stimulation followed by inhibition was recorded, and this trend appeared both dose and time (of exposure) dependent. However, during gramoxone exposure the brain ALP activity showed an increasing trend. In gill, the ALP activity was found generally unaffected. DDT exposure caused a reduction in activity in higher concentration and during Dimecron exposure the ALP activity in gill was more or less unaffected. The ALP activity in gill of gramoxone treated fishes showed a reduction

during initial phase but seemed unaltered during later phases. The liver ALP activity during DDT exposure showed biphasic dose dependent response, inhibition in lower concentration and stimulation in higher concentration. During Dimecron exposure the ALP activity in liver showed an increasing trend. A biphasic response, stimulation in lower concentration and reduction in higher concentrations of liver ALP was recorded during short term intoxication following gramoxone exposure, and this response was quite reverse to the pattern of activity noted during DDT exposure.

During short term exposure to DDT, the brain ACP activity of experimental fishes followed an irregular pattern, nevertheless a general stimulatory response could be noticed. ACP activity in brain was found inhibited following Dimecron exposure while a biphasic response of ACP activity in brain, stimulation in lower concentration and reduction in higher concentration, was recorded due to gramoxone exposure. The gill ACP activity was found more or less unaffected following DDT exposure. But a reduction was observed in higher concentration during final phase of exposure. But stimulation of activity was recorded in gill of Dimecron-exposed fishes while gramoxone exposure generally failed to elicit any appreciable response in gill ACP activity. However, stimulation was noted during final phase. Biphasic response, reduction in lower concentration and stimulation

in higher concentration, of ACP activity in liver was noticed following DDT exposure. Dimecron exposure caused a stimulation which was pronounced towards the final phase of exposure of ACP activity in liver. A rather non-linear ACP activity in liver was recorded during initial phase of gramoxone exposure but the activity was found stimulated towards final phase of exposure.

The GOT activity showed an increasing trend towards the final phase of DDT exposure, while a biphasic response, inhibited in the lowest and highest concentrations and stimulated in intermediate ones, was noted in brain GOT activity following Dimecron exposure. In gramoxone exposed fishes, the brain GOT activity showed an increasing trend during final phase. DDT exposure caused a reduction of gill GOT activity in higher concentrations but an increase preceded the reduction of GOT activity in gill during final phase folowing Dimecron exposure. The GOT activity was found more or less unaffected in gill due to gramoxone exposure. A decreasing trend, more pronounced in higher concentrations, in liver GOT activity was caused by DDT exposure. Dimecron also caused reduction of liver GOT activity but more reduction was noticed in lower concentration while in higher concentrations a recovery trend could be observed. During gramoxone exposure the liver GOT exhibited a biphasic response showing higher activity in lower concentration and lower activity in higher concentration.

During short term exposure to DDT, dissimilar pattern in GPT activity was recorded in brain. However, a biphasic response was apparent during initial and final phases (lower activity in lowest and highest concentrations while in intermediate concentration higher activity was noted) of DDT exposure. Dimecron caused an increase in brain GPT activity during initial phase but was found irregular in final phase of exposure. But in gramoxone exposure, the brain GPT activity showed a reduction while in higher concentrations it was more or less unaffected. Like-wise DDT could not elicit any response in the activity of gill GPT while Dimecron caused an increasing trend in gill GPT activity and the activity was found generally unaffected following gramoxone exposure. However, in liver a bipahsic response, higher activity in lower concentration and lower activity in higher concentrations, of GPT was attributable to DDT exposure. The GPT activity was found decreased during initial Dimecron exposure in liver while during final phase higher activity was recorded in higher concentrations. A biphasic response of GPT activity, lower in higher concentrations and higher in lower concentrations, was evident in liver of gramoxone exposed fishes and the trend was found pronounced during final phase of exposure.

It is apparent, from a critical evaluation of the results, that no clear cut pattern of activity of these enzymes in the tissues

is forthcoming during short term exposure, and this non-linearity of activity response to pesticide exposure was more evident in the gills of pesticide exposed fishes. In most cases, the activity of many of the enzymes was found unaltered following exposure to pesticides. The author finds it difficult to explain this "no-response" pattern of activity of the many of the enzymes. One of the reasons attributable to this kind of result, may be due to the comparatively higher blood infusion of the gill tissue and the alterations of these enzymes, simultaneously occurring in blood following pesticide exposure.

During long term exposure to pesticides, the enzymes under study showed a definite response pattern. In DDT exposed fishes the brain ALP showed a dose dependent reduction during later phases of exposure while during initial phase the activity was found unaltered. The ALP activity in brain was found more or less unaffected following Dimecron and gramoxone exposure. However, in gill DDT exposure caused a dose dependent reduction in ALP activity. The reduction was more pronounced in higher concentrations of Dimecron during later phases. Though generally unaffected, a reduction trend in gill ALP activity could be observed, particularly in higher concentration, following gramoxone exposure. Liver ALP activity showed a reducing trend, obvious in the higher concentration, following DDT exposure. Dimecron also caused a reduction in liver ALP activity. However, gramoxone

exposure caused an elevation of liver ALP activity which was more obvious towards later phases of exposure. The activity of ALP in various tissues following exposure to the pesticides was found generally inhibited. But the stimulation of activity recorded following gramoxone exposure might be due to the chemical dependent variation of pesticide toxicity.

From the present study, it is apparent that evaluation of ALP activity did not offer itself a good indicator of pesticide toxicity. The dissimilar pattern, particularly the 'no-response' pattern of ALP activity in different tissues in many cases is quite inexplicable. During the short term exposure, only in gill the ALP showed a somewhat definite pattern following pesticide exposure.

The ACP activity showed a definite response pattern in all the three tissues following long term exposure to pesticides. DDT caused a clear cut inhibition of brain ACP activity and was found dose dependent. The inhibition of ACP activity in brain was more pronounced, and towards later phases of exposure, apart from dose dependent it showed time dependent response following Dimecron exposure. In gramoxone exposure, the brain ACP recorded reduction in activity but a trend of recovery could be noticed in higher concentrations. In gill, the activity of ACP was found inhibited following exposure to the three pesticides and comparatively pronounced inhibition was

recorded following Dimecron and gramoxone exposure. The gill ACP activity reduction showed a dose dependent pattern. Following DDT and Dimecron exposure, the liver ACP recorded a definite dose dependent reduction in activity while gramoxone caused clear cut inhibition during initial phase but towards later phases the activity of ACP showed a recovery phase.

Long term DDT exposure caused reduction of brain GOT activity and was more pronounced in higher concentrations and during later phases of exposure. Following Dimecron and gramoxone exposure, the brain GOT activity recorded definite dose dependent stimulation and this pattern of response was found more pronounced during gramoxone exposure. The gill GOT showed an increasing trend following exposure to the pesticides. Dimecron exposure caused a pronounced response in gill GOT activity during later phases while gramoxone caused comparatively higher response than caused by DDT. During long term exposure the activity of GOT in liver showed a definite dose dependent stimulation following pesticide exposure. Both Dimecron and gramoxone caused dose and time dependent elevation of GOT activity but in gramoxone exposure it was comparatively less.

The GPT activity in all the three tissues exhibited elevation following long term pesticide exposure. DDT caused dose and more or less time dependent elevation of GOT activity in brain, gill and

liver. The elevated enzyme activity showed a trend of inhibition in brain during later phase of exposure following Dimecron exposure while gramoxone brought about an inhibition trend in brain GPT activity in higher concentration towards the final phase. The liver GPT activity showed a biphasic response, reduction in lower concentration and stimulation in higher concentration, following gramoxone exposure.

The inhibition of ALP and ACP in brain, liver and muscle tissues of Sarotherodon mossambicus, following exposure to phenol was recorded and the pattern of decrease in activity showed a direct linear relationship. Maximum reduction recorded in muscle ACP activity at *60%o* salinity while ALP showed minimum reduction in brain at *60%* (Ravichandran and Ananthraj, *1984*). Shaffi (*1980*) reported on the effect of pesticide Thiodan on ALP, and ACP activities in various tissues including brain in a freshwater teleost. The brain ACP activity in Tilapia mossambica recorded an increase following Monocrotophos exposure (Joshi and Desai, *1983*). They opined that the rise in ACP activity during short term exposure to Monocrotophos could be due to increased lysosomal labilization and biochemical lateration as a result of AChE inhibition. During long term exposure to Monocrotophos, the ACP activity became normal while ALP activity showed stimulation and suggested that this pattern of activity is indicative of biochemical adaptation of sustained exposure. Lockhart et al. (*1975*) observed

an initial increase followed by decrease in serum ALP activity in Salmo gairdneri after chronic exposure to synthetic tri-aryl phosphate oil. Vegetable oil factory effluent caused reduction in ACP activity in liver and kidney of Channa punctatus. However the reduction of activity varied at different periods during long term exposure (Saxena et al., *1982*).

Verma et al. (*1981*) recorded decline in ALP and ACP activities in liver, gills and kidneys of Mystus vittatus following long term exposure to pesticides Thiotox, Dichlorvos and Carbofuran. They reported maximal inhibition of ALP activity in gill after Thiotox exposure. They offered no clear cut explanation for the alteration in enzyme activity. They suggested that the inhibition might be due to uncoupling of oxidative phosphorylation or structural alteration of lysosomes in response to toxic insult or both. Sastry and Sharma (*1980*) observed Diazinon, an organophosphate, induced reduction of brain ALP and ACP activity in Channa punctatus, but ALP showed increase after 30 day Diazinon exposure and opined that the reduction of activity might be due to enzyme inhibition (following structural alteration) or decreased synthesis of the enzymes. Phenol and penta-Chlorophenol inhibited ALP and ACP activity in liver of Notopterus notopterus. Maximum reduction was noted in ACP activity followed by ALP (Dalela et al., *1980*). Marked inhibitory changes in ALP and ACP

activity in liver of channel catfish following methyl mercuric chloride exposure has been recorded (Hinton et al., *1973*).

ACP and ALP are the enzymes concerned with biosynthesis of fibrous protein (Johnson and Mc Minn, *1958*), mucopolysaccharides (Kroon, *1952*) or they may serve as a regulator of intracellular phosphate concentration (Gutman, *1959*). They are also hydrolytic enzymes which play an active part in the dissolution of the body's cells. ALP and ACP are also believed to be involved in permeability processes and associated with nucleic acid synthesis (Cox and Griffin, *1965*). Stimulation or inhibition of these enzymes will thus result in disturbances in metabolism. The elevation of activity of ALP and ACP may be due to increased discharge from lysosomes (Vijeyendra Babu and Vasudev, *1984*; Viswanathan et al., *1973*). Several mechanisms have been suggested for the release of hydrolytic enzymes from lysosomes. The elevation of ALP and ACP following pesticide exposure could be due to (i) alteration in osteoblasts resulting in more production and liberation (Cantarow and Schepartz, *1967*), (ii) proliferation of smooth endoplasmic reticulum in parenchymatous cells, that leads to more production and release of microsomal enzymes resulting in an increased enzyme activity (Hart and Fouts, *1965*), (iii) peroxidation of lysosomal membrane leading to membrane break down or increase in permeability of lysosomal membrane or both (Novikoff, *1961*), (iv) degeneration and necrosis induced in tissues (Reddy et al., *1986*) and

(v) changes in energy supply metabolism as it is associated with carbohydrate metabolism (Rosenthal et al., *1960*) and may be due to increased uptake of certain metabolites and ions since these enzymes are reported to be involved in this process (Simkiss, *1964*).

Uncoupling of oxidative phosphorylation is thought to be mainly responsible for the inhibition of hydrolytic enzymes (Mitchell, *1961*; Weinbach and Garbus, *1969*; Yap et al., *1975*). Toxicants prevent orderly formation of energy rich compounds prior to synthesis of ATP, thus acting as uncoupling agents that hinder phosphorylation that normally accompany oxidation (Kelly and Syrett, *1964*). Action of uncouplers of oxidative phosphorylation has been pointed out on the basis of chemical and chemi-osmotic interactions (Mitchell, *1961*; Pressman, *1963*). Simon (*1953*) opined that concentrations higher than those needed to prevent oxidative phosphorylation may injure the mitochondrial systems so markedly as to block the action of enzymes. He further noted that uncoupling of oxidative phosphorylation was not only the mechanism but various other processes, such as oxidation accompanied by phosphorylation, which inhibited enzyme activity. Another possibility for the diminution of enzyme activity is probably due to the inhibition of the enzyme by conjugation with toxicant, (binding affinity of protein to heavy metal cation is established *[*Hilmy et al., *1981]*), or replacement of cofactors. Ikehara et al.

(*1978*) showed that the EDTA inhibition on ALP activity was due to the removal of Zn^{2+} from the enzyme protein and this inactivation is overcome by the addition of excess Mg^{2+}.

As generally known, there exists a dependence between the increase in transaminase activity and the influence of various toxicants in fish. This connection has been proved for carbon tetrachloride (Racicot et al., *1975*), copper (Mclcim et al., *1970*), dieldrin (Lane and Scura, *1970*), bromobenzene (Bell, *1968*) and phenol (Tiedge et al., *1986*; Gupta and Dalela, *1985*). The transaminases which represent link between carbohydrate and amino acid metabolism recorded decrease in liver, kidney, brain and gills of Tilapia mossambica following ammonia toxicity (Chetty et al., *1980*). Cheng (*1965*) showed that stimulation in activity of GOT and GPT occured in fish at 0.1 ppm potassium pentachlorophenol while exposure to 0.2 ppm potassium pentachlorophenol caused inhibition. Significant increase was recorded in GOT and GPT activity in the blood serum of Notopterus notopterus following exposure to very low concentrations of phenol, dinitrophenol and pentachlorophenol (Verma et al., *1981*). GOT and GPT at different levels increased in liver, muscle, gills and brain of Oreochromis mossambicus following exposure to Lindane, indicating that the fish is under toxic stress and energy crisis caused by Lindane thus promoting the utilization of amino acids for energy synthesis (Murthy et al., *1985*).

Aminotransferase are widely acknowledged for their significance in protein metabolism, by virtue of their ability to regulate both the synthesis and degradation of amino acids. Changes in their activities, whether induced by endogenous or exogenous factors, are often associated with changes in many other metabolic functions and may thus represent widespread alterations in the organism's physiological state. Environmental pollution appears to be one of the factors that affects aminotransferase activities in animal tissues (Lane and Scura, *1981*). The stress of 10 weeks exposure to chronically sublethal concentrations of hydrocarbons such as naphthalene, toluene and phenol stimulated GOT and GPT activity in liver and muscle tissues of Oreochromis mossambicus (Dange, *1986*). In the liver of Tautogolabrus adspersus exposed to 0.1 ppm cadmium chloride for 30 days caused significant reduction of GOT activity and it seems probable that the cadmium inhibitory effects on GOT activity is at the point of pyridine phosphate synthesis (Mc Innes et al., *1976*).

Anoxia or hypoxia is known to increase carbohydrate consumption and is evidenced by reductions in stored glycogen content in tissues (Dezwann and Zandee, *1972*). The exposure of Sarotherodon mossambicus to Thiodon elicited a severe hypoxia resulting in the utilization of stored glycogen by way of anaerobic glycolysis to meet the energy demand during pesticide stress and the failure of aerobic

metabolic pathway hints upon the possibility of a shift from aerobic to anaerobic mode of energy metabolism in tissues (Vasanthi and Rama-Swamy, *1987*). Rao et al. (*1987*) recorded decrease in sucrose-soluble and insoluble protein in tissue of Sarotherodon mossambicus following Benthiocarb exposure. Similar decease in protein content was reported in animals exposed to pesticides (Kabeer Almeed et al., *1978*). The increased proteolytic activity could lead to enhanced free amino acid levels resulting a shift in nitrogen metabolism (Rao et al., *1987*). Higher free amino acid content may also be attributed to decreased utilization of amino acids in gluconeogenesis as a consequence of pesticide exposure (Murthy, *1983*). The increase in GOT and GPT suggests the existence of heavy drain the metabolites during pesticide exposure. Since stress is known to induce elevation of amino-transferases, toxic impact caused by pesticide exposure should be the reason for their elevation (Knox and Greengaurd,*1965*).

Alanine and aspartate serve as two major glucogenic amino acids which through the activities of the enzymes GOT and GPT give rise to glucose precursors (Lehinger, *1979*). The high rate of GOT activity could result only from an enhanced rate of aspartate formation (Malhotra et al., *1986*). The decreased GOT activity may be due to the damage caused to mitochondrial membranes, loss of matrix and swelling of mitochondrion (Chow and Pond, *1972*). The decrease of GOT may also be attributable to the decreased oxaloacetate availability.

The increase in GPT activity might partly be to compensate the loss of GOT activity or to the increased pyruvate availability (Chetty et al., *1980*). Cortisol is known to stimulate the amino transferase activities in fish tissues (Freeman and Idler, *1973*).

The development of an understanding of the role of stress responses in fish is the key to a better understanding of the problems associated with the intensive culture of fish during all or as part of their life cycle. The primary stress responses or effects induced by exogenous or endogenous factors, have been divided into two categories, increased production of corticosteroids and increased production of catecholamines (Mazeaud et al., *1977*). These two primary neuroendocrine responses bring about a number of biochemical, physiological and immunological changes which have been described as the secondary effects(Mazeaud et al., *1977*). Fishes have shown to have a functional hypothalamic-pituitary-inter-renal (HPI) axis which is sensitive and responsive to stresses (Donaldson, *1981*).

Transicent elevation in plasma cortisol (Hill and Fromm, *1968*) and increase in stress hormone metabolites in urine (Mc Kinn et al., *1966*), have been reported in fish after acute exposure to sublethal levels of pollutants. A variety of stressors stimulate the adrenal gland tissue of teleost fishes, resulting in increased levels of circulating gluco-corticoids (Mc Kim, *1966*; Wedemeyer, *1969*) and

catecholamines (Nakno and Tomlinson, *1967*). The stress mediated secretion of corticosteriods, it is thought, play a role in the biochemical changes recorded in the present study following exposure to the pesticides.

The result of the present study on in vitro effect of three pesticides on the activity pattern of the four enzymes suggested a definite inhibitory action of pesticides. The difference in enzyme responses associated with in vivo and in vitro addition of pesticides suggests that different molecular processes are involved in the two types of exposure. Lack of correlation between in vivo and in vitro inhibition could be explained by cellular or tissue barriers that could exclude specific cations from active sites. In vivo activation could be indirect, through endocrine mechanisms such as those demonstrated in mammals (Knox et al., *1956*). Other mechanism could be postulated such as interactions with regulators or cofactors (Hilmy et al., *1985*). The biphasic response of enzymes to pesticide toxicity could be envisioned as an initial induction or stimulation followed by direct inhibition as the toxic radicals increased.

5.3 EFFECT ON HAEMATOLOGY

Peripheral haematological make up is so seriously affected by toxicants that under sufficient toxicant stress one or more of the

haematological parameters will be perceivably altered (Van Vuran and Hatting, *1978*). Since the fish used in the present study were collected from the same location, and were almost of comparable size and condition, and since the conditions under which the experiments were run were the same for the experimentals and the controls, the differences in the haematological parameters observed between the control and exposed fish are obviously caused by the effects of pesticide or may be the indirect effect of stress attributable to the pesticide.

The observations of the present study on the haematological effects of sublethal doses of three pesticides, DDT, Dimecron and Gramoxone, in Etroplus maculatus may be summarised as follows. During short term exposure, DDT and Dimecron caused reduction in TEC while TEC increased following Gramoxone exposure. The long term exposure to three pesticides caused reduction in TEC and the reduction was more prominent in higher concentrations, and towards late phases of exposure indicating a dose as well as time dependent response. The haemoglobin content showed increase during short term exposure to pesticides while long term exposure to three pesticides caused marked reduction in the Hb content. However, a slight increase was recorded in Hb content during the initial phase of long term exposure to Gramoxone. Following short term exposure to Dimecron and Gramoxone, the haematocrit recorded reduction, while DDT caused increase in

haematocrit and was pronounced in higher concentrations and towards the late phases of exposure indicating a dose and time dependent response. The haematocrit recorded marked increase during long term exposure to the three pesticides.

The short term DDT exposure caused an increase in MCV and was pronounced during the final phase of exposure, and showed a dose dependent pattern. Though initially increased, MCV showed reduction during final phase of short term exposure to Dimecron while during gramoxone exposure the MCV showed dose as well as time dependent reduction. During long term exposure to the three pesticides, the MCV recorded a marked increase of definite dose and time dependent pattern. Of the three pesticides, Dimecron caused the maximum increase in MCV during long term exposure followed by gramoxone and DDT. Following Dimecron and gramoxone exposure the mean corpuscular haemoglobin (MCH) recorded an increase (comparatively high in Dimecron exposed groups) during short term exposure. DDT caused an increase in MCH during the final phase of short term exposure, however, during initial phases the MCH recorded dissimilar pattern. During long term exposure to three pesticides the RBC was hyperchromic as revealed by erythrocyte indices. Though hyperchromic, the RBC were less saturated with haemoglobin as revealed by low saturation index recorded. The Dimecron and gramoxone, during short term, caused microcytic hyperchromic condition

in RBC. With low colour and saturation indices and high volume index, macrocytic hypochromic anaemia developed following long term exposure to DDT, Dimecron and gramoxone. However, during initial phase of long term exposure, gramoxone caused macrocytic hyperchromic condition of RBC with super saturation of haemoglobin. Respiratory impairment caused by the pesticides might have induced a reaction to increase the surface area of RBC (macrocytosis) that it would help to absorb more oxygen or more likely, enhancement of haematopoiesis, induced by the pesticides, might have released more immature RBC into circulation that are macrocytic and hypochromic. The impairment of haematopoiesis, haemoglobin synthesis or its incorporation or impairment of iron metabolism may be held responsible for the hypochromic condition developed following pesticide exposure. The pesticide might have induced enhanced haematopoiesis and haemoglobin synthesis so that the RBC entering circulation were microcytes overloaded with haemoglobin (Microcytic hyperchromic condition).

The macrocytic hypochromic condition indicated that one of the adverse effects of pesticide exposure was the development definite anaemic condition, probably due to inhibition of erythropoiesis or haemoglobin synthesis and its incorporation. The reduction of cell volume with more amount of haemoglobin (microcytic hyperchromia) may be an effective compensatory response (so that the unit volume of

blood will contain more number of RBC with high haemoglobin content) to combat the oxygen tension caused by the pesticide exposure.

The most common responses were the development of moderate to severe anaemia as evidenced by significant reduction of RBC count, Hb content and haematocrit and is secondary to possible accelerated haemolysis, haemorrhage and or reduced erythropoiesis inflicted by the pesticides. Organic pesticide belonging to different classes induced more or less similar haematologic disorders. Fish blood is increasingly studied for toxicological research and for environmental monitoring and is considered as a possible indicator of physiological or pathological change in fishery management. The fish haematology under chemical stress has been studied by many workers.

In fishes, an alteration of the blood cell distribution has been correlated with changes in environment conditions (De wilde and Houston, *1967*). Blood alteration or damage to haemopoietic organs in fishes may also be associated with pathological conditions related to water borne pollutants (Gardner and Yevich, *1969*). The subacute concentrations of sodium lauryl sulphate caused alterations in several haematological parameters during long term exposure (Dalela et al., *1981*). Haematological parameters such as RBC and Hb increased following 4 day treatment of Malathion in freshwater catfish Heteropneustus

fossilis and their levels returned to normal after *8* and *16* days' exposure (Lal et al., *1986*) and the recovery of RBC and Hb might be attributed to a certain degree of tolerance during pesticide exposure. Erythrocyte counts, haematocrit values, haemoglobin content and number of erythrocyte|1000 cells were found significantly reduced following 90 hour lead exposure in Colisa fasciatus (Srivastava and Mishra, *1979*), and it was suggested that haemolytic anaemia and the numerical increase in circulating immature erythrocytes could be used as indicators of toxicity. Puntius conchonius under chronic exposure to mercury registered an initial fall, albeit slight in the RBC count and Hb content, but after 2-3 weeks an increment occured in both indices (Gill and Pant, *1981*). They attributed the incipient decrease could be due to haemolysis caused by mercury whilst the subsequent recovery and then a rise in RBC number could be ascribed to enhanced erythropoiesis which was triggered as a typical stress response.

An increase in haematocrit has been reported as a result of oxygen deficiency in channel catfish Ictalurus punctatus (Scott and Rogers, *1981*). According to them the redistribution of water from the general circulation to tissues, erythrocyte swelling and spleenic contraction might be responsible for the elevated haematocrit. Spleenic contraction would increase haematocrit by introducing additional erythrocytes to the circulatory system (Black, *1955*). Elevated

haematocrit associated with short term hypoxia appears to be an initial response to reduced oxygen levels (Scott and Rogers, *1981*).

Verma et al. (*1982*) recorded an increase in prothrombin time (PT), WBC, haematocrit and MCV, while RBC, clotting time (CT), Hb, ESR and MCH decreased in Mystus vittatus at most concentrations of pesticides, Thiotox, Dichlorvos and Carbofuran, and combinations with the exception in Dichlorvos and Thiotox-Dichlorvos combination where RBC recorded increase and MCV a decrease. They suggested that the lowering in RBC count might be due to the destructive action of pesticides on peripheral red cells as a result of which the viability of the cells was affected. However, the damaging effects on erythrocytes may be secondary, resulting from a primary action of the toxicant on the erythropoietic tissue. The increase in haematocrit might be due to the catalysing action of pesticides. Channel catfish, Ictalurus punctatus, anaesthetized with 2 ppm Etomidate per 30 min had haemoconcentration indicated by increased Hb, RBC and haematocrit and the reduced MCH and MCHC might be caused by a lower haemoglobin concentration in the new immature erythrocytes released into the peripheral circulation (Limsuvan et al., *1983*). Sharma and Joshi (*1985*) observed increase in RBC, Hb content and haematocrit following asphyxiation in Noemacheilus rupicola, but MCHC was found lowered.

Thakur and Sahai (*1987*) recorded development of anaemia due to loss of haemoglobin and low RBC following long term exposure to BHC in Channa punctatus. The chronic Aldicarb exposure caused moderate polycythemia, increase in Hb content and haematocrit after 15 and 30 days exposure in Barbus conchonius (Pant et al., *1987*). Short term Mahua oil cake exposure caused reduction in TEC, Hb content and haematocrit in Heteropneustus fossilis and Cyprinus carpio.

It has become apparent that a close scrutiny of the haematological results following short and long term exposure to individual pesticides revealed responses more or less of similar nature. However, deviations from the general pattern were also recorded. These haematological responses exhibited were initially of a compensatory nature, gradually transcended to adaptive, and at length sustained toxic action of pesticide induced certain pertinent changes of irrecoverable nature. The results, in general, corroborate the views of Selye (*1950*) on G.A.S. - general adaptive syndrome - of animals to stressors.

The physiological response of higher vertebrates to stressors has been described by Selye (*1950*, *1973*) and incorporated into a response classification called the general adaptative syndrome (G.A.S). The physiological response to each of the stages of G.A.S is characteristic, and varies little from species to species. The physiological responses are also remarkably independent of the nature

of stressor. There are many similarities between metabolic changes seen in stressed fish and those recognized in higher vertebrates (Hoar, *1957*). The biochemical and physiological adjustments initiated through the G.A.S, such as hormonal and neurological changes and the morphological alterations resulting from these adjustments, may serve as valuable indicators of polutant stress in fishes (Scott and Rogeres, *1980*).

More intensive analysis of haematological responses under precisely controlled experimental conditions and right from shortly after exposure to the stressor through longer periods of substained stress, would be rewarding in getting an insight into the pattern and sequence of G.A.S of blood in the toxic-test-combination of Etroplus maculatus and the three pesticides, viz. DDT, Dimecron and Gramoxone.

5.4 HISTOPATHOLOGY

Tissue lesions in fishes induced by controlled exposures to pesticides have been described, however, these reports are few in number. Most lesions have been extremely non-specific and merely indicative of toxic insult by pesticides and generally reflects a wide spread degenerative and necrotic inflammatory condition. By virtue of their non-specificity, the pathological changes caused by pesticides make diagnosis of a single causative compound or group of

compounds difficult, if not impossible (Meyers and Hendricks, *1982*).

In the present study only brain, gill and liver were considered. Hyperaemia, vascular congestion and pyknosis produced in the brain cells of E. maculatus exposed to pesticides agree with the findings of Walsh and Ribelin (*1975*); Kennedy et al. (*1975*) and Cope et al. (*1970*). The formation of vacuoles might be another effect of pesticide insult in brain cells. King (*1962*), Mathur (*1962*), Halver et al. (*1962*) and Walsh and Ribelin (*1975*) have reported various histopathological changes produced by pesticides in the liver of fishes. The reduction of fat content, hypertrophy, formation of pyknotic nuclei and necrosis observed in the present study are in conformity with previous reports. Liver lesions were observed in fish exposed to Heptachlor (Cope, *1963*), Methoxychlor (Kennedy et al.,*1970*) and Endrin (Eller, *1971*).

The respiratory epithelial hyperplasia of secondary lamellae, as observed in the present study, could reduce the efficiency of oxygen uptake by gills. Cope (*1963*) and Eller (*1971*) are of the opinion that the hyperplasia on the gill filaments are associated with prolonged exposure to chronic levels of pesticide. The branchial congestion, haemorrhage, reduction of mucus cells, swelling and elongation of gill filaments, destruction of epithelial cells and fusion

of the filaments at the base are also considered to be due to pesticide insult.

Pesticides by design are meant to be toxic. Although a major goal of the discipline of modern pesticide chemistry is to develop pesticides and consequent use patterns that confine pesticide toxicity to pest organisms, such a goal is seldom attained easily. All living organisms have much in common biochemically, and successful exploitation, often relatively minor biochemical difference between pest and non-pest species, is almost always difficult and is, in fact, sometimes impossible. The interactions of these chemicals or their transformation products with non-target species may result in some unforeseen toxic consequences.

The advantage of utilizing physiological response as an index of stress lies in the fact that early detection of potential biological harm in an impacted area may be possible. This level of examination allows one to investigate the initial interaction between an organism and a potential stress. There are a number of parameters which appear to be appropriate measures of stress. The parameters selected in the present study to evaluate the toxic impact of pesticides on Etroplus maculatus is thought to be meaningful in view of the foregoing account. It is obvious that simultaneous observations of

a number of physiological parameters will be necessary to distinguish between responses due to temporary environmental or natural stresses and those which are an early reflection of potentially lethal condition. The major obstacle remaining in physiological monitoring is the difficulty in discriminating the responses due to man-induced stress against a background of natural stress.

SUMMARY

VI - SUMMARY

The work presented here centers around the toxic action of three pesticides, comprising organochlorine, organophosphate and by bypyridilium compounds, on the euryhaline fish Etroplus maculatus (Bloch) (family : cichlidae). Aspects like individual toxicity, modulations in the activities of some selected enzymes, consequent to exposure to sublethal levels of pesticides, sublethal effects on peripheral haematology and alterations caused on the tissue architecture of brain, gills and liver, have been documented.

The chapter on Introduction presents material and notes on various aspects of the toxicants and the relevance of the study.

In general, information on the toxic effects of pesticides on fishes are detailed out in the Review of Literature. In this chapter, available papers on lethal and sublethal toxicity of pesticides are critically reviewed.

The chapter on Material and Methods details out, the animal used for the present day, methods of collection, instrumentation employed, chemical methods followed and the experimental designs to evaluate lethal toxicity and sublethal toxicity on enzyme activity, peripheral haematology and histopathology. The enzymes selected for

the study are Alkaline phosphatase (ALP E.C. *3.1.3.1*), Acid phosphatase (ACP E.C. *3.1.3.2*), Glutamate oxaloacetate transaminase (GOT E.C. *2.6.1.1*) and Glutamate pyruvate transaminase (GPT E.C. *2.6.1.2*) from the tissues like brain, gill and liver. The haematological parameters under study are Total erythrocyte counts (TEC), Haemoglobin content (Hb), Haematocrit (Ht), Erythrocyte constants like Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) and Erythrocyte indices like Colour index (CI), Volume index (VI) and Saturation index (SI). Histopathological studies are restricted to the brain, gill and liver tissues. The statistical techniques used for analysis and computation of data are also outlined in this chapter.

The Experimental Results are presented under different sub heads. Two sets of concentrations, based on corresponding *96* h LC 50, are selected to evaluate the sublethal effects of individual pesticides, viz. DDT, Dimecron and Gramoxone. In short term exposure comparatively higher concentrations are used to delineate the effects caused due to high concentration over a short term period while long term experiments are designed to get an insight into the effects of low concentrations of pesticides over a long period of exposure.

Among the pesticides used DDT, an organochlorine was the most toxic for Etroplus maculatus giving 96 h LC 50 value of 0.005 ppm. The organophosphate, Dimecron, was found least toxic (96 h LC 50 0.17 ppm) and the Gramoxone, bipyridilium compound (Paraquat Dichloride) recorded an intermediate 96 h LC 50 value of 0.05 ppm. It was noticed that lethal concentrations of pesticides reduced as a function of time.

In general, during short term exposure studies the activity of two phosphomonoesterases, viz. alkaline and acid phosphatases, were found elevated following pesticide exposure in brain, gills and liver of E. maculatus. However, the alkaline phosphatase activity in gill showed a more or less "no response" in many cases. But in case of transaminases, viz. Glutamate oxaloacetate transaminase (GOT) and Glutamate pyruvate transaminase (GPT) activity elevated was recorded following short term exposure to pesticides.

In most cases, during long term exposure, pesticides caused more or less dose dependent modulation in enzymatic activity. Both phosphatases, ALP and ACP, showed inhibition while transaminases, GOT and GPT were found stimulated. However, non-linear pattern of enzymatic activity were also recorded, especially in gill Of the three tissues, liver enzymes showed maximum activity response towards

pesticide insult. Generally no chemical-specific changes in activity could be observed.

Since lysosomes, cell membranes and endoplasmic reticulum are the major sub cellular units to encounter xenobiotics, a variation in the activity of both phosphatases bound to these cellular components is inevitable, while the animal is under stress. In animals, the two transaminases, GOT and GPT, are two important enzymes that catalyse the process of biological transamination. The variation in their activity can be considered as an indicator of pathological influence of pesticides.

During short term exposure to three pesticides, compensatory responses were noted in haematological parameters which in many cases were found non-linear. These compensatory haematological adjustments exhibited by E. maculatus were indicative of the immediate response to the respiratory stress following pesticide exposure. However, in long term exposure, pesticide caused negative effects on haematological parameters like reduction in TEC, Hb, Ht and corresponding reduction in erythrocyte constants and indices indicating development definite anaemic condition induced by pesticide. Further the alterations in the haematological make up of E. maculatus are indicative of, apart from respiratory stress and consequent changes in metabolism,

physiological impairment such as reduced erythropoiesis, haemoglobin synthesis and its incorporation in red cells.

The histopathological examination of tissues like brain, gills and liver revealed pertinent changes in their tissue architecture, however, these change were of non-specific nature. Nuclear pyknosis, vascular congestion and hyperaemia were the changes noted in brain following pesticide exposure. Gill lesions included oedema and hyperplasia of secondary filaments, hypertrophy and necrosis of inter-lamellar region, branchial congestion and swelling and elongation of filaments. Pesticide exposed liver of E. maculatus showed cytoplasmic vacuolization, periportal atrophy, radial disorientation, granulocytic infiltration, hypertrophy and nuclear pyknosis.

The chapter on Discussion enlightens the result obtained in the light of the available literature. It becomes clear that more investigations are warranted on the sublethal exposure of fishes to low levels of pesticides. List of scientific papers consulted with for the enrichment of the present work are provided under References.

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VII - REFERENCES

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