COMBINED TOXIC EFFECTS OF OIL AND PESTICIDES ON SELECTED MARINE INVERTEBRATES

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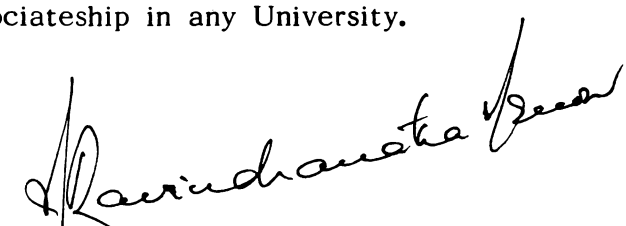
" Lives of great men all remind us
That we can make our lives sublime
And departing, leave behind us
Foot prints on the sands of time "

Longfellow, H.W.

This thesis is dedicated to
MY BELOVED PARENTS
Whose love, constant encouragement
and reproof has made me what I am.

CERTIFICATE

This is to certify that this thesis is an authentic record of research work carried out by Shri. Jose P. Jacob, under my scientific supervision and guidance in the Division of Marine Biology, Microbiology and Biochemistry; 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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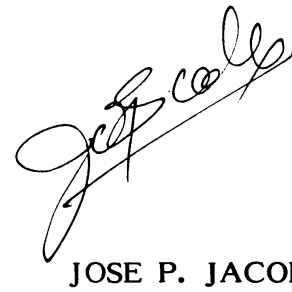
DECLARATION

I, Shri. Jose P. Jacob, do hereby declare that this thesis, entitled "COMBINED TOXIC EFFECTS OF OIL AND PESTICIDES ON SELECTED MARINE INVERTEBRATES" is a genuine record of the research work done by me under the scientific supervision of Prof. Dr. N. Ravindranatha Menon, Head, Division of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology, and has not been previously formed the basis of the award of any degree, diploma or associate-ship in any University.



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JOSE P. JACOB

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With this acknowledgement, I leave this thesis for the scientific community at large and would be gratified if this thesis offers even a little help in making our environment less polluted and more liveable to a fuller life for all mankind.

C O N T E N T S

PREFACE

Pollution of the sea by organic chemicals was recognized 20-25 years ago when highly accumulating materials such as DDT were found in marine organisms, far away from locations of intended application. Man has now become aware of the fact that, organic chemicals can be transported over long distances by water movements, wind and precipitation, accumulating in oceans and coastal waters as the ultimate sink. It is now known that the discharged materials do not undergo degradation or even mineralization as rapidly as expected previously. Organic compounds are subjected to specific usage patterns resulting in their continuous or increased production or in their abolishment and replacement. Many of the 60,000 organic chemicals now used (Maugh, 1978) may finally reach estuaries and oceans. It is quite unlikely that a detailed critical assessment of the specific risk can ever be accomplished for such a large number of compounds.

Realising these, there is an intense search for viable alternatives, for example, Cycloprothrin is the latest find. It is said to act several times better than DDT, offering only less toxicity to mammals, fish and invertebrates. It does not chemically interfere with the body metabolism. Development of a range of new insecticides, fungicides and herbicides known as 'designer chemicals' because these are designed at the molecular level and not obtained by screening large number of chemical substances, is the latest trend in pesticide research.

Marine scientists continue to make extensive investigations on the effects of hazardous chemicals on estuarine and marine organisms. Usually the studies are confined to delineate the cause and effect of pollution due

to most common pesticides. It is understood that documentation of marine pollution in terms of concentration of the contaminant in water alone is no longer sufficient The use of bioassays as part of a comprehensive approach to pollution assessment is widely accepted. Information so gathered can be of use in management of pollution for different purposes such as prediction of environmental damage of a waste, comparison of various toxicants, animals or test conditions, regulation of waste discharge

Lethal and sublethal toxicity studies open up a very interesting vista of information on the probable consequences of presence of pollutants and its influence on the life and activity of marine animals It has been recog nized that, chemicals seldom occur alone, which opens up another important facet of pollution effects brought about by combined toxicity Therefore it is essential that any study directed to analyse the effects of the common contaminants on aquatic organisms, should take into consideration the above aspects.

The material presented here explains the usefulness of such studies especially with reference to pesticides and oil. It is realised that any study of the above sort requires further continuation to explain scientific results in a more detailed manner. However, dearth of information on combined toxicity necessitates studies to understand the basics of these aspects. The lipophilic nature of the pesticides, associated with the possibility of pesticides and WAFs of oils occuring in concert in coastal and estuarine waters demand proper documentation of the combined effect of these components on the life and activity of marine and estuarine organisms The present study is carried out considering these factors. It is earnestly hoped that the information provided here in offers an excellent background data to follow up the investigations to organic, cellular and sub-cellular levels.

INTRODUCTION

I INTRODUCTION

In recent years, pollution in general and sea water pollution in particular, has become an important topic for national and international considerations. Because of its impact on society, marine pollution has attracted great attention from politicians, administrators, natural scientists and technologists all over the world.

To save our environment from further deterioration, it is essential to have an assessment of this problem. Pollution is the result of industrialization and technological achievements, but its increase is also correlated with the rising population. The need for pollution monitoring and control in countries undergoing rapid industrialization is well established because of the tendency for environmental concerns to be ignored in favour of rapid development and due to the lack of antipollutional legislation or laws and inability to enforce such laws.

Coastal zones are more prone to vulnerability of pollution, as this zone receives pollutants both from land and waters and major developments in industries, transport and other maritime activities causing pollution tend to take place in the vicinity of coastal zones. Besides, coastal areas are densely populated and 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, including shell fisheries and are nursery areas for off-shore fish stocks. Coastal area dumping grounds have a 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). Hence pollution levels of coastal sea water need to be constantly monitored to establish an early warning system and to yield standardised data for environmental management.

Among the various animal groups, the use of mussels or clams as sentinels of pollution is currently gaining importance. It appears to be generally accepted that enough is known to pursue a productive programme of coastal pollution-monitoring using the 'mussel watch' strategy. In the development and application of the sentinel organism concept for pollution-monitoring, an integrated and interdisciplinary approach to the management of these complex pollution problem is imperative. In this context, it is a pre-requisite to take stock of the information available and the state-of-the art prevailing in the country at present. For assessing the total biological effects and long-term consequences of pollution on aquatic organisms it is essential to collect available and relevant information on possible sentinel species. The class bivalvia is of interest to toxicologists, as it comprises sedentary, filter-feeding invertebrates which are likely to accumulate pollutants from the environment and demonstrate clearly, the deleterious effects of its changing surrounding.

It is no longer sufficient to document marine pollution in terms of the chemical concentration of the contaminant. The use of bioassays as part of a comprehensive approach to marine 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. Bioassay (=toxicity) tests are defined here as estimation of the amount of biologically active substances by the level of their effect on test organisms (Chapman and Long, 1983). The majority of present bioassays are concerned with determination of survival related to effluent or single aqueous toxicant concentration. Information generated from various toxicity tests can be of use in the management of pollution for different purposes like, prediction of environmental damage of a waste; comparison of various toxicants, animals or test conditions and regulation of waste discharge.

In general, sublethal effect covers, 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, reproduction or which impair the defence mechanism of an organism are referred to as sublethal effects. In the present investigation sublethal effects of pesticides and water accommodated fractions of Light Diesel Oil and Persian Gulf Crude oil on two selected bivalves were looked into in detail. Physiological/behavioural responses like rate of filtration, oxygen consumption and byssogenesis are the parameters used for the assessment of the sublethal effects.

In the process of the seas and estuaries around us becoming the ultimate sink and as the problem of pollution being multifarious, it is unlikely that a toxicant occurs singly in the surrounding. Sprague (1970) remarked, "probably the most exciting and potentially useful recent development in pollution biology has been a method of predicting toxicity of mixtures of

toxicants". Recently the concept of prediction of toxicity of mixtures of pollutants has received wide approval in aquatic toxicological studies as it provides scope to study simultaneous effects of several pollutants in a single set of experiment, the result of which can be expressed as a single number.

The present study involved investigation of the lethal and sublethal effects of four pesticides and two petroleum oil, individually and in combinations on two commercially important bivalves. Among the four pesticides used two are organophosphates and the other two are organochlorines. Synthetic pesticides, especially organophosphates and organochlorines have become increasingly important additions to chemical wastes polluting natural aquatic communities. Many of these are considered hazardous because of their ability to kill or immobilize organisms even at very low concentrations.

Most pesticides are synthetic chemicals and could be classified by chemical types. Organophosphorous pesticides (o.p) or simply organophosphates are esters of phosphoric, phosphinic or phosphonic acid. Ekalux^{R} and Dimecron^{R} are the two organophosphates used in the present study.

Organochlorine pesticides also known as organochlorines or simply as o.c.s. consist of two different major groups based on their molecular structure; namely the cyclodiene or diene group and the DDT group. Cyclodiene group are cyclic groups with characteristic 'endomethylene bridged' structure. With the exception of Toxaphene, all the cyclodiene insecticides are the Diels-Alder reaction products of hexachlorocyclopentadiene and a suitable unstable compound. DDT and its analogues, that contain two aromatic rings represent the second group in organochlorines (Lee et al., 1977).

Among the two organochlorines used in this study Aldrex^{R} belongs to diene group and DDT^{R} belongs to DDT group.

Most of the pesticides are neurotoxins and their effect on animals is manifested through the nerve tissue, inhibiting or poisoning cholinesterase, an enzyme which is essential to the orderly operation of the nervous system.

There are several sources for the hydrocarbons found in marine samples (Farrington et al., 1976). Petroleum is an extremely complex mixture of thousands of different hydrocarbons and related compounds. When exposed to oil-contaminated sea water, marine animals rapidly accumulate in their tissues a wide spectrum of petroleum hydrocarbons (Neff and Anderson, 1975., Gilfillan et al., 1977; Neff and Anderson, 1981) and concentrate them to a marked degree over sea water levels. The lethal as well as sublethal effects of the water accommodated fractions of two petroleum products, Light Diesel Oil (LDO) and Persian Gulf Crude (P.G. Crude) are assessed in the present investigation. Many of the components of the petroleum fractions are carcinogenic agents, which combine with various cellular constituents, so that the heritable cellular physiology is altered. Moreover, it is likely that pesticides being lipophilic, combine with the oil fraction to produce a combined effect which might be rapdily toxic and more lethal to the biota. Therefore, special attention is given in the present investigation to delineate the combined toxic effect of oil and pesticides.

The results are presented under different sections to make the presentation meaningful. 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 and oil, individually and in combination on the intertidal and subtidal biota of the coastal ecosystem.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

Among the pollutants which have received increased attention of pollution biologists and ecologists, 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. Aquatic ecosystem in general and marine ecosystem in particular have no or only limited capabilities for metabolising and degrading such compounds and their derivatives. Therefore, pesticides and technical organic chemicals released into marine and freshwaters 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 marine ecosystems, quite a few reach the seas in appreciably copious quantities (Eknath and Menon, 1979). It is known that these compounds tend to accumulate in water, sediments and biota causing as yet insufficiently known and proved modifications. Many of these chemicals are toxic, cannot be recycled and hence cause additional problems. As an example, the fungicide pentachlorophenol contains as impurities; tetrachlorophenol, other lower chlorinated phenols, chlorinated diphenyl ethers, dibenzofurans, dihydroxybiphenyls and phenoxyphenols; an array of chemicals which are precursors of highly toxic dioxins. Separation, identification and quantification of these chemicals in sea water when present in very low levels, create problems of procedural contamination during sampling and analysis and therefore require sophisticated methods of analysis.

Bioconcentration, biomagnification and degradation of pesticides are not sufficiently understood. The bioconcentration and biomagnification have got differences, the former being a function of increase in the tissue load subsequent to exposure of the animals to polluted water and bioaccumulation is stepwise increase in pollutant accumulation to supranormal levels via the food chain. A factor which has created concern among the pollution biologists is that, during the course of degradation intermediate products that are formed may inflict more negative effects.

Our concern on oil pollution mainly arises out of accidental spillage of oils which accounts for 38% of the oil that causes aquatic pollution (Johnston, 1984). Natural seepage is also known to contribute in considerable quantity to result in pollution of water. River run off brings about 29% of the total oil that pollutes the sea. A holistic ecological consideration of oil pollution must take into account many facets in addition to the evaluation of the impacts on marine species and on the food web. However, usually laboratory studies are employed to find out the effects of petroleum hydrocarbon derivatives on aquatic organisms. Spies and co-authors (1980) have evaluated the effect of C_2 - C_5 alkanes on the benthos employing Shannon - Wiener diversity index, evenness value, dominance-diversity curves and Spearman's ranking correlation, based on observation from areas receiving natural seepages of oil. The oil that reaches the sea can never be totally recovered. Remedial measures for oil spills are limited, inefficient and restricted in application. They are also expensive and could be damaging to the environment.

Toxicological studies involving short-term mortality tests, extended laboratory tests and model ecosystem experiments have been conducted to study

the different aspects of the toxicology of oil. Because of the diverse forms and distribution of petroleum oils in water it is difficult to determine and indicate toxic and non-toxic concentrations of these materials. The toxicity of breakdown products of petroleum hydrocarbons is largely directly related to its dispersion throughout the watermass. Water accommodated fraction (WAF) of oil is used as the most common toxic component of crude oil to study the effect of oil on aquatic organisms. The water accommodated fractions based on the gas chromatographic analysis have shown that the main hydrocarbons present are, n-paraffins from C_{14} to C_{24}, aromatics like naphthalene, methyl naphthalenes, di and trimethylnaphthalenes, biphenyls, fluorenes, phenanthrenes and dibenzothiophenes. Among the hydrocarbons analysed, the normal paraffins present at highest concentrations in oil and their WAFs are those with chain lengths of 14 to 17 carbons. Due to greater water solubility, naphthalenes represent the most prominent diaromatic hydrocarbon in the WAFs (Anderson et al., 1974). The refined oils are known to be more toxic than the crude. When the marine or estuarine animals are exposed to oil-water mixtures they ordinarily come into contact only with those petroleum hydrocarbons actually present in the aqueous phase either in solution or in dispersed form. Therefore research dealing with the effects of oil on marine and estuarine organisms must include a careful consideration of the concentration and composition of petroleum hydrocarbons present in the aqueous phase during exposure.

Pesticides were conceived and developed to kill pests and therefore must be detrimental to other forms of life. It is proved that aquatic life is specifically sensitive to most pesticides and to various other organic chemicals. Acute toxicity is probably the most useful method of testing the effects of

pesticides on aquatic organisms. Eisler (1970a) has documented on the methodologies to be followed to investigate acute toxicity with reference to fishes. Holden (1973) has worked out the effect of temperature on the toxicity of numerous chemicals to fishes. It was proved that increased temperature enhances the toxicity of pesticides especially in the case of rainbow trout (Macek et al., 1969). Eisler (loc cit.) in an illuminating paper on the comparative toxicity of organochlorines and organophosphates on estuarine fishes reported a sharp increase in toxicity with rising temperature, when the fishes were exposed to organophosphates. On the other hand, organochlorines like DDT, Heptachlor and Endrin were most effective between 20^{o} and 25^{o}C. Powell and Fielder (1982) looking into the effect of temperature and toxicity of DDT, to Mugil cephalus on the other hand stated that an increase in temperature produced a decrease in toxicity. Discussing on the possible reasons for this they suggested that DDT appears to interfere with the thermal acclimation mechanism of M. cephalus, so as to alter the temperature responses. Further, they felt that if the mode of action of DDT involvled 'complex formation', then 25^{o}C must represent a point of chemical breakdown.

Much of the quantitative information available on the effects of pesticides on marine organisms relates acute toxicities of individual organisms. The LC50 values recorded in the literature are far above pollutant concentrations likely to occur in the marine environment except in cases of accidental spills or dumping in restricted sites. The 96 h LC50 on Crassostrea virginica was recorded to be 0.0102 mg l^{-1} of Aroclor 1016 (Hansen et al., 1974). On the other hand the grass shrimp, Palaemonetes pugio died when the concentration reached 0.0078 mg l^{-1} of Aroclor 1254 (Roesijadi et al., 1976). The toxicity

of DDT to marine fishes was found to vary from species to species (Eisler, 1970b). Similarly, the lethal dose to kill 50% of the test population of fishes when exposed to Heptachlor also was found to show fluctuations from 0.194 to 0.0008 mg l^{-1}. Similar results were obtained when shrimps or oysters were exposed to Dieldrin (Portman and Wilson, 1971; Eisler, 1970b; Parrish et al., 1974). Cardium edule was found to succumb to Parathion when the concentration ranged between 3.3 and 10 mg l^{-1} (Portman and Wilson, 1971). The varying concentrations that resulted in the death of freshwater and estuarine fishes has led to the calculations of maximum acceptable toxicant concentration (MATC) of organic chemicals, by Ernst (1984) and the values represented are at ug l^{-1} level.

D'Silva (1980) worked out the toxic effect of Malathion on a few marine invertebrates. Malathion was found to be highly toxic to Perna viridis. The 96 h LC50 was 14.0 µl l^{-1} for P. viridis while it was 12.5 µl l^{-1} for Modiolus carvalhoi. Meretrix casta was comparatively more tolerant, the 96 h LC50 being 18.0 µl l^{-1}. The marine intertidal burrowing bivalve Donax spiculum, a very sensitive organism recorded 7.0 µl l^{-1} as the 36 h LC50 value. The 96 h LC50 recorded for the marine polychaete, Sabellaria clandestinus was 12.75 µl l^{-1} (D'Silva, 1980).

Eisler (1969) reviewed the acute toxicities of insecticides to marine decapod crustaceans. He found that with minor exceptions the LC25 96 h value for each pesticide ranged between 20 and 80% of the 96 h LC50 values. The LC75 96 h values, with some exceptions ranged between 110 and 320% of LC50 96 h levels. As in the case of results obtained and presented in the literature, a meaningful statistical treatment of these observations was not

possible owing to the demonstrably large variations in resistance (ie, 96 h LC50 values) to the twelve pesticides tested both within and between species. Further, Eisler (1969) found that the reaction of shrimps subjected to pesticides was controlled by salinity and those exposed to DDT, Endrin or Heptachlor are most susceptible at the lowest salinity tested (12‰) and appears slightly more resistant at higher salinity (36‰). Discussing on the relative toxicity of organochlorines and organophosphates on crustaceans, Eisler (loc cit.) concluded that organochlorines were more toxic to marine fauna than other organic chemicals including organophosphates. In an attempt to investigate the effect of factor combinations Jacob and Menon (1987) looked into the combined influence of salinity and pesticides on responses of the black clam, Villorita cyprinoides var. cochinensis. These animals showed maximum tolerance to the pesticide in salinities ranging from 5‰ to 20‰ , their normal habitat salinity. The results proved that the response surface of these animals, with reference to two parameter alterations, tend to rotate in a limited fashion, when encountered with one totally unencountered (pesticide) and another normal environmental variable. Comparing the relative responses of fishes and crustaceans to organophosphates, Butler (1966a) stated that crustaceans are more sensitive than marine fishes by several magnitude. He suggested that it was possible that inter and intraspecies variations in sensitivity to organophosphate pesticides was related to the number and types of esterases present. Negherbon (1959) and O'Brien (1966) demonstrated that organophosphate pesticides inhibit competitively and irreversibly the action of several enzymes, especially choline esterases, the chemical mediators of transmission between nerve and effector, resulting in parasympathetic, somatic motor nerve and central nervous system stimulation effects.

Butler et al.(1968) investigated the effect of insecticide Sevin on the survival and growth of cockle clam, Clinocardium nuttalli. They worked out the effect of the insecticide Sevin and its hydrolytic product, 1-naphthol on the survival, growth and food consumption of the larval and juvenile clams. Larvae exposed to Sevin concentration of 0.8 mg l^{-1} were dead by 7th day and the growth of those exposed to 0.4 mg l^{-1} was reduced by 15%. Sevin was less toxic than 1-naphthol to juvenile clams. The 96 h median tolerance limits (TLm), ranged between 2.70 and 3.75 mg l^{-1}. The growth of juvenile clams was reduced more by 1-naphthol than by Sevin. Adult clams concentrated the toxicants in the tissue and maximum concentration was reached after 12 h of exposure. Curiously enough, clams exposed at 11°C accumulated more toxicants than those exposed at 20°C. Quick cleaning up of tissues was noticed by the authors, (Butler et al., loc cit.) when the pre-exposed clams were returned to clean sea-water Stewart et al., (1967) found that Sevin was less toxic to adult cockle clam, than 1-naphthol. Contrary to the findings of Butler et al., (1968) and Stewart et al., (1967) Armstrong and Millemann (1974a) reported that there was no difference between Sevin and its first hydrolytic product, in toxicity to the early stages of Mytilus edulis. They suggested that embryos were probably most sensitive to 1-naphthol after the first polar body stage. One of the most pronounced effect of Sevin on M. edulis embryo was retardation of development or complete cessation of cleavage. Information is available in the literature on the causative factors of toxicity of Sevin to developmental stages of embryos and larvae. Casida (1963) found that Sevin is cholinesterase inhibitor. However, Grosch and Hoffman (1973) doubted the effect of cholinesterase inhibition on other enzymatic systems. Grosch and Hoffman (loc cit.)

stated that the breakdown product of Sevin have severe effects on the reproductive potential of the brown shrimp, Artemia salina. They also found that naphthalene compounds related to Sevin and its derivatives are spindle poisons affecting the mitotic apparatus and therefore cleavage. Armstrong and Millemann (loc cit.) felt that reduction in cleavages or failure of cleavages in the embryos of M. edulis exposed to Sevin and 1-naphthol could be due to destruction of enzyme system responsible for spindle formation.

Armstrong and Millemann (1974b) tested the 96 h toxicity of Sevin on Macoma nasuta. They found the 96 h TL50 to be 17.0 mg l^{-1}. When these 'dead' clams were returned to clean sea-water none of them recovered. The histopathology primarily consisted of necrosis of epithelial tissue of the gill, mantle, siphon and supra branchial glands and the degree of damage was directly proportional to the test concentrations. The gills were found to be the most affected organs. Epithelial cells of the gill filaments bearing the frontal, laterofrontal and lateral cilia were sloughed off as early as 24 h during exposure.

Schimmel et al., (1976a) employed the technical grade Heptachlor to study the toxicity on estuarine animals. They found that the American oyster recorded LC50 96 h value of 1.5 μg l^{-1}. Their study also revealed that the analytical grade Heptachlor was much more toxic than the commercial grade. Fish was found to accumulate Heptachlor in greater quantity than crustaceans. Gills which are the major organ of pesticide uptake have greater permeability in the case of fish, and it was the reason for this increased uptake. It was also noticed that relatively high amounts of fat in the fish tissues might be a reason for the enhanced toxicity of this organochlorine pesticide to fish.

Hansen et al., (1974) studied the acute and chronic effects of Aroclor 1016 to various estuarine animals. They found that the shell growth of Crassostrea virginica was inhibited greatly when they were exposed to 100 $\mu g\ l^{-1}$ for 96 h. Parrish et al., (1974) found that the 96 h LC50 of oysters was 12.5 $\mu g\ l^{-1}$ when they were exposed to Dieldrin.

Intertidal crabs, being an important group of animals which come into contact with pesticide residues have received increased attention with reference to acute toxicity and subacute effects. Armstrong et al., (1976) looked into the toxicity of Methoxychlor on the crab, Cancer magister, using larval, juvenile and adult stages. They found that the toxicity was inversely related to the age of the crab after hatching and increased with length of exposure. The variation in the 96 h LC50 values were enormous and the zoea recorded 0.42 $\mu g\ l^{-1}$ whereas, the adult 130 $\mu g\ l^{-1}$. When these stages were exposed for a longer duration beyond 50 d the values reduced to 0.05 and 4.0 $\mu g\ l^{-1}$. Prolonged exposure conspicuously affected the tolerance levels of both the larvae and the adults, the adults becoming more sensitive than the larvae. The rate at which the insecticide was removed from the body was quicker in the case of adult crabs. The concentration of Methoxychlor in individual tissues were found to be higher in the exoskeleton, gill and hepatopancreas in declining order. About 81% of the Methoxychlor measured in wholebody samples was associated with exoskeleton and the author did not know whether or not the pesticide was transported through the cuticle. Caldwell et al., (1978) studied the effect of the fungicide Captan (N-trichloromethylothio - 4- cyclohexane-1, 2 - dicarboximide) on the survival of the larvae of Cancer magister. Apart from causing death at high concentrations, concentrations as low as 30 $\mu g\ l^{-1}$

resulted in delayed moulting of the larvae. Survival of juvenile crabs was not reduced by exposure to Captan for 36 d at 510 $\mu g\ l^{-1}$ or for 80 d at 290 $\mu g\ l^{-1}$. The adults did not die when they were exposed to 340 $\mu g\ l^{-1}$ for 70 d. Because of the relatively low toxicity of captan to crab stages and due to its high rate of degradation in sea-water the authors felt that application of this fungicide was not likely to affect natural crab populations.

Information on the rate of accumulation of pesticides by mussels and clams is available in the literature. Duke and Dumas (1974) opined that it is necessary to analyse the capacity of an organism to concentrate a pesticide and that this factor must be considered when evaluating the impact of these chemicals on a coastal system. Butler (1966b) worked on the concentration factor with reference to accumulation of organochlorines by the hard shell clam, Mercenaria mercenaria and the Pacific oyster, Crassostrea gigas. In the case of M. mercenaria exposure to 1.0 ppb DDT for 1 week resulted in a concentration factor of 6,000 whereas, the soft shell clam, Mya arenaria recorded a concentration factor of 8,800 when they were exposed to 0.1 ppb of the same pesticide for 5 days. On the other hand, the Pacific oyster showed a concentration factor of 20,000 when they were exposed to 1.0 ppb of DDT for 7 days. The fact that depending on the composition of the pesticide the concentration factor could vary in the same species was amply justified in another paper by Butler (1971). Here, he exposed M. mercenaria to Dieldrin, Endrin and Methoxychlor at concentration varying from 0.5 ppb to 1.0 ppb for five days. The concentration factor ranged between 470 and 760, Methoxychlor though present at 1.0 ppb level resulted in reduced concentration factor. Parrish (1974) studied the accumulation and loss of Aroclor 1254, DDT, DDD

and Dieldrin by American oyster exposed at very low concentrations for 56 weeks. He found that maximum concentration of these toxicants based on total body weight occurred after 8 week of exposure and maximum concentration based on absolute amount of toxicants accumulated (in μg) occurred after 56 weeks of exposure. Differential uptake of Endosulfan by the tissues of Mytilus edulis was the topic of scientific enquiry by Roberts (1976a). The patterns of uptake and elution of pesticides during and after exposure to a range of concentrations of Endosulfan indicated slower equilibration with the pesticides and more rapid elution of pesticides at higher concentrations. He further demonstrated that the rate of accumulation and elution varied between tissues. Variations in PCB concentration in the tissues of different species of molluscs occupying the same polluted area was demonstrated by Riley and Wahby (1977). Molluscs were found to be the most sensitive taxonomic group in an experimental estuarine taxonomic communities exposed to Dowicide G-ST a pentachlorophenol (PCP) by Tagatz et al., (1978). Contardi et al., (1979) determined the concentrations of BHCs, DDT and PCBs on Mytilus galloprovincialis along with a set of other species belonging to other taxa. He found that Mytilus showed a notable decrease in the concentration of chlorinated residues. The Mytilus of Mediterranean coasts, however, were found to contain more of chlorinated residues (Marchand et al., 1976; Duursma et al., 1974). Variations in the quantity of organochlorine residues in five species of estuarine animals including a mollusc, polychaete, crustaceans and a fish were estimated by Goerke et al., (1979). They found that the patterns of residue concentrations in the five aquatic species were remarkably different indicating species and compound specific bioaccumulation. Accumulation of

PCBs by Mercenaria mercenaria from contaminated harbour sediment was analysed by Rubinstein et al., (1983). They found that the uptake was affected by the organic content of the sediment. The authors supported the contention that sediment concentration alone does not reflect bioavailability and that toxicity tests remain the most direct method for estimating bioaccumulation potential of sediment bound contamination. Risebrough et al., (1983) analysed the distribution of hydrocarbon in mussels as a part of 'Mussel watch' programme. They found that there could be variations in the quantity of the pesticides accumulated in the tissues by mussels distributed in different areas in the coastal zone. Satsmadjis and Taliadouri (1983) suggested Mytilus galloprovincialis as a useful indicator to organochlorine pollution. Their results suggested that the mussels accumulate organochlorines to a greater extent than shrimps which co-habit in the locality. Perna viridis according to Phillips (1985) could be a useful bivalve which could be utilized as a bioindicator of PCBs and organochlorines. He noticed that variations in the background concentration of organochlorines in the Hong Kong waters clearly reflected in the tissues of the mussels collected from such localities. The bioaccumulation factor for total DDT was approximately 100,000 in the case of mussels which were transplanted to coastal waters polluted by chlorinated hydrocarbons. Mussels, therefore could be used as a suitable animal to measure the bioavailability of chlorinated hydrocarbons (Green et al., 1986). As part of the national pesticide monitoring programme (1965-'72), 8095 samples were analysed for fifteen persistent organochlorine compounds by Butler (1973). The results showed that DDT residues were ubiquitous and the maximum DDT residue detected was 5.39 ppm. Dieldrin was the second most commonly detected compound. Endrin,

Mirex, Toxaphene and PCBs were found only occasionally. Three species of oysters, (Crassostrea gigas, C. virginica and Ostrea lurida), four species of mussels (Modiolus demissus, M. modiolus, Mytilus californianus and M. edulis) and two species of clams (Mya areneria and Mercenaria mercenaria) were found to be reliable indicators of the magnitude of organochlorine pollution. Radhakrishnan et al., (1986) analysed mussel, prawns and fish of same age group for eleven chlorinated pesticides and reached in the conclusion that compared to fish and prawn, mussels have a remarkably high capacity to accumulate pollutants and hence mussels can be used as an indicator of pollution.

Reinert et al., (1974) working on the effects of temperature on the uptake of p,p'-DDT by rainbow trout found that fishes exposed to DDT at concentration of 133-176 parts per trillion, accumulated 3.76, 5.93 and 6.82 ppm of this pesticide when the temperature regimes were 5,10 and 15^{o}C respectively. They suggested that the extremely high lipid/water partition coefficient of p,p'-DDT is probably the single most important factor in explaining the high concentration factor for this compound. Concomitant increase in the concentrations, accompanying elevation in temperature was suggested to be related to increase in metabolic rate. The concentration of commercial Heptachlor in the tissues of Leiostomus xanthurus varied, depending on the duration of exposure. The maximum concentration of Heptachlor was observed on the third day and the maximum concentrations three other compounds namely trans-chlordane, cis-chlordane and nonachlor were observed on the day 17. Nearly all of the Heptachlor was eliminated or metabolized to its epoxide (Schimmel et al., 1976b). Epifanio, (1973) worked out the rate of uptake of ^{14}C -Dieldrin by crab larvae, (Leptodius floridanus) from 0.5 ppb in sea water and from

213 ppb (dry wt.) in their food. It was found that, if equal concentrations of Dieldrin were available to the larvae in their food and in sea water, the animals would accumulate the pesticide about 8,000 times as fast from the water as from the food.

The biological problems that affect the interpretation of monitoring data on pesticides were discussed by Butler (1974). In this paper the factors which should be looked into were discussed in detail. They were the type of species to be sampled, the age of the individuals, seasonal variation and selection of tissues to be analysed. As in the case of heavy metals, in the case of pesticides also, molluscs have been recognized as suitable indicators of the presence of organochlorine pesticides. A model developed by Couch (1974) in general, gives the impact of a stressor on a biological system. Three distinct phases have been recognized such as, a normal steadystate, overlapping to a compensation state, followed by decompensation and death. Therefore, a pesticide could be considered to have adverse effect, if it temporarily or permanently altered the normal steady state of a particular biological system to such an extent as to render the compensating mechanism incapable of maintaining an acceptable altered steady-state. Among these three phases, the normal steady-state and compensation can be seen in the performance of any animal subjected to an environmental alteration. In experimentation using laboratory techniques, it is assumed that the conditions prevailing in the laboratory offer an environment for the animal to maintain a steady-state. To explain a situation of compensation at work Couch (1974) found that the fish Leiostomus xanthurus, when exposed to Aroclor 1254 under laboratory conditions did not show any outward signs of stress, on the other hand the livers of the fish

accumulated excess fat during the test. The liver was able to contend with excessive fat accumulation. But eventually chronic damage leading to necrosis occured forcing the fish to enter to another biological state.

Information available on the behaviour of organisms subjected to exposure of pesticides is limited especially for marine molluscs. The behavioural responses of marine biota used as sublethal indicators of stress of pesticides are mainly confined to fishes and crustaceans. The responses worked out include chemotaxis, temperature preferences, tactile inhibitions, lateral line sensitivity in fishes, avoidance reactions, equilibrium, swimming performance, burrowing, feeding, respiration, filtration, etc. (Eisler, 1979). DDT was found to affect the chemoreception of larvae of Balanus amphitrite, (Meith-Avcin, 1974). The tactile responses of Mercenaria mercenaria was found to get inhibited in the presence of pesticides (Eisler, 1970c). Maintenance of equilibrium, while swimming, was affected by the presence of pesticides in the case of Cyprinodon variegatus, Fundulus similis and Fundulus majalis (Eisler, 1969; Hansen et al., 1977; Dixit and Anderson, 1977).

Hansen (1969) showed that the estuarine fish Cyprinodon variegatus, avoided water containing DDT, Endrin etc. in controlled laboratory experiments although, they did not avoid test concentrations of Malathion or Sevin. Similarly, Palaemonetes pugio, an important forage food of estuarine organisms in the temperate area avoided 1.0 and 10.0 ppm of Dursban by seeking, water free of this herbicide although they did not avoid the other five insecticides tested (Hansen et al., 1973). Although, avoidance is a temporary behaviour which will help the animals to escape contaminated water this movement in

large numbers might lead to disastrous ecological effects on the particular population. Coppage and Duke (1972) worked on the capacity of a fish population to compensate the effect of Malathion. Immediate application of this pesticide in a natural environment resulted in conspicuous inhibition in the acetylcholinesterase (AChE) activity of the brain and the levels approached to those recorded under acute toxicity condition in the laboratory. However, by migrating away from the contaminated area the fishes could regain normalcy and the duration involved for this process was around forty days.

Egg hatchability, mean time to hatch and survival and growth of larvae of coho salmon, Onchorhynchus kisutch exposed to 4.4 $\mu g\ l^{-1}$ of Aroclor 1254 for four weeks and to 15 ug l^{-1} for two days were worked out by Halter and Johnson (1974). Premature hatching occurred in all egg groups exposed to the PCB. The median survival times (MST) of fry exposed to Aroclor 1254 and DDT combination for 2 wks. were similar to those after exposure to the various concentrations of DDT alone. The more rapid reaction time to DDT was suggested as the basis for additive toxicity.

The effect of PCB preparations Aroclor 1242 and 1254 on colour changes, moulting and limb regeneration of Uca pugilator are available (Fingerman and Fingerman; 1977, 1978, 1979a & b). Fingerman and Fingerman (1977) found that low concentrations of PCBs greatly inhibit moulting and the regeneration of limbs by U. pugilator. Fingerman and Fingerman (1980) studied the inhibition on limb regeneration of the fiddler crab by Aroclor 1242 under different salinity regimes. They found that prolonged exposure of amputed crabs to higher and lower salinity ranges along with PCBs, affected the rate of regener-

ation of the limbs. The effect of Malathion on the development of mud crab, Rhithropanopeus harrissii and Callinectes sapidus from the time of hatching to the first crab stage was studied by Bookhout and Monroe (1977). They found that there was a reduction in the survival of the larvae when the background concentration of Malathion was raised, from 0.011 ppm to 0.02 ppm. Buchanan et al., (1970) studied the effect of Sevin on various stages of Cancer magister. They found that a concentration of 1.0 mg l^{-1} did not affect egg hatching but prevented moulting of protozoea to zoea. At 0.01 mg l^{-1} more than 50% of the test population of the first zoeal stages were killed. Young juvenile crabs were more sensitive to Sevin than old juveniles and adults. Fingerman et al., (1979) observed that DDT produced an increased level of spontaneous locomotor activity in the fiddler crabs. Bookhout et al., (1976) conducted laboratory experiments to determine the effects of Methoxychlor on the larval development of the mud crab, Rhithropanopeus harrissii and the blue crab Callinectus sapidus. They found that the larvae of mud crabs were much more resistant than that of blue crabs. The difference in sensitivity to Methoxychlor between R. harrissi and C. sapidus was found to be marked, although the same species showed similar degrees of sensitivity when they were reared in a range of concentrations of Mirex from 0.01 ppm to 10.0 ppb (Bookhout et al., 1972; Bookhout and Costlow, 1975).

Patho-histological changes, although not specific to pesticide toxicity, have been noticed in fishes by various authors, when the fishes were exposed to sublethal concentrations of pesticide. Lowe (1964) found that gill lamellae thickened when exposed to sublethal concentrations of Toxaphene. Lowe et al., (1971) described epithelial necrosis and the damages in oysters chronically

poisoned with mixtures of DDT, Toxaphene and Parathion. Atrophy of the diverticular epithelium was found as the major histopathology in oysters treated for 24 weeks to Aroclor (Lowe et al., 1972). Pauley and Sparks (1965, 1966) studied inflammatory reactions and histological changes in oysters injected with turpentine and reported necrosis and sloughing off of intestine and kidney epithelium.

Armstrong and Millemann (1974c) found out that the insecticide Carbaryl (Sevin) significantly affected the population concentration of juvenile clam, Macoma nasuta. These were the results of experiments conducted using Carbaryl as a pest control chemical in oyster beds.

Calabrese (1972) reported the effects of pesticides on the development of embryos, on the survival and growth of the larvae of American oyster and hard shell clam. He found that most of the pesticides tested affected embryonic development more than survival or growth of larvae. Some, however, drastically reduced growth of larvae at concentrations that had relatively little effect on embryonic development. Davis and Hidu (1969) looked into the effects of pesticides on the embryonic development, survival and growth of larvae of clams and oysters. Most of the compounds tested affected embryonic development more than survival or growth of larvae of Mercenaria mercenaria and Crassostrea virginica. Some other pesticides however, drastically reduced growth of larvae at concentrations that had little effect on embryonic development. Sheridan (1981) noticed reduced survival and growth of newly settled spats of American oyster when exposed to chronic chlorination. Spat growth was retarded at very low concentration of chlorine (0.125 mg l^{-1}). The

burrowing bivalve, Macoma balthica was found to get affected when exposed to pesticide contaminated sediments (Mohlenberg and Kiorbe, 1983). The authors found that there was reduction in the burrowing rates when the sediments contained residues of pesticide.

Generally, under sublethal responses studied, effects on respiration and filtration have received very little attention with reference to molluscs.

Menon et al., (1983) proved that Heptachlor is highly poisonous to Perna viridis even at very low concentrations. Considerable quantities of mucus secreted on the gill as a result of exposure to Heptachlor was found to result in decreased gill irrigation. Dasaratharamaiah (1984) studied the metabolic profile of two species of prawns exposed at sublethal concentrations of Dimecron. Employing carbohydrate breakdown as an index of subacute stress, Reddy et al., (1985) found that the levels of carbohydrates, glycogen-lactate dehydrogenase and succinate dehydrogenate decreased in Phosphomidon exposed prawns. Vinod (1986) worked out the rate of oxygen consumption by juveniles of Penaeus indicus belonging to different size groups, exposed to sublethal concentration of Malathion. Results indicated that although oxygen consumption in larger forms (70 $\pm$ 5mm) increased to that of control animals, in smaller juveniles (20 to 30 and 30 to 40mm length) oxygen consumption was reduced drastically when exposed to sublethal Malathion concentrations. Considerable reduction in oxygen consumption occurred in the case of Perna viridis, when subjected to sublethal concentration of 7.5 $\mu l\ l^{-1}$ of Malathion. Presence of Malathion was found to act as a respiratory depressor in the case of Meretrix casta. The green mussel, P. viridis passed through phases of in-

activity and hyperactivity with reference to filtration under Malathion stress. On the other hand the rate of filtration in M. casta was found to be inversely proportional to Malathion concentration, (D'Silva, 1980). While studying the effect of organophosphates on the life and activity of the black clam, Villorita cyprinoides var. cochinensis Jacob and Menon (1987a) found that the filtering mechanism of this animal was severely impaired with when they were exposed to concentrations above 500 ppb in the case of Ekalux and 2.5 ppm for Dimecron. Changes in the rate of ciliary activity of the black clam, Villorita cyprinoides var. cochinensis under stress imposed by variations in temperature, hydrogen-ion concentration, salinity and under different concentrations of heavy metals and pesticides like Dimecron, Nuvan and Ekalux were studied by Rita et al., (1987). They observed that the biocides have depressive effect upon the ciliary activity of the clam.

An investigation of byssus formation by mussels seems to offer a simple technique for the assessment of pesticide toxicity. Byssogenesis by Perna viridis was found to be interfered in the presence of Malathion. Presence of 1.0 $\mu l\ l^{-1}$ of Malathion was found to disrupt byssogenesis. Also a concentration of 12.5 $\mu l\ l^{-1}$ of Malathion was found to result in retardation of byssogenesis in Modiolus carvalhoi (D'Silva, 1980). The production of byssus threads by P.viridis was nearly stopped at 2.5 $\mu l\ l^{-1}$ of Ekalux. It was opined that since there was not much difference between the highest range where cessation of byssus threads production occurred and the lowest concentration at which P. viridis died, involvement of deleterious effect on important basic functions might be the reason for this difference (Eknath, 1978). Roberts (1975) suggested that byssogenesis tests offer a rapid and convenient technique for the

routine secreening of potential marine pollutants. Seed mussels (Mytilus edulis) were exposed to a range of pesticides and PCBs and it was found that the byssal attachment was impaired at higher concentrations. In queen scallops (Chlamys opercularis) byssus formation was similarly affected although this species was more sensitive than M. edulis. The sensitivity of mussels was greater at higher temperatures and decreased with increase in size.

Petroleum is an extremely complex mixture of thousands of different hydrocarbons and so it is difficult to define the acute toxicity of a particular component. Notwithstanding this, literature is available on the relative toxicity of different petroleum products and sensitivity of different marine species to oil. It is rather difficult in many cases to ascertain the actual concentration of petroleum hydrocarbons in the aqueous phase of the exposure medium. Boyland and Tripp (1971); Anderson et al., (1974a) and Lee et al., (1974) have shown that, different crude and refined oil vary tremendously in their relative concentrations of different components and as a result show substantial variability in solubility. Crude petroleum is generally less toxic to marine animals than refined products. In seeking useful generalization, Ottway (1971) found that the toxicity of twenty crude oils rank fairly consistently with the proportion of low boiling point fractions especially, the aromatics. Recent evidences strongly indicate that in majority of cases acute toxicity of petroleum products are directly correlated to its content of soluble aromatic derivatives (Moore and Dwyer 1974; Anderson et al., 1974a). These compounds include benzene, naphthalene, phenanthrene and their alkyl homologues.

The review of Hyland and Schneider (1976) indicated that the lethal effects of water accommodated fraction of petroleum and petrochemicals occur

in the 1-100 ppm range. Larval and juvenile life stages are usually more sensitive to oil pollutants and the lethal concentration range from 0.1 - 1.0 ppm. There is a feeling that the coastal and estuarine species are less sensitive to oil than are their open sea counterparts. This may be because of the fact that the coastal animals are more tolerant to environmental stress and hence, more resistant to pollutional stress. Evidences exist which showed that the post-larvae of the brown shrimp Penaeus aztecus were significantly more tolerant to the Water Soluble Fraction (WSF) of No.2 fuel oil than were either the early or late juvenile stages. On the other hand post-larvae and juveniles of the white shrimp, P. setiferus did not show conspicuous variations in tolerance limits when the larvae, postlarvae and juveniles were exposed to WSF of No.2 fuel oil. (Neff and Anderson, 1981). Mc Auliffe (1966) showed that among the hydrocarbons analysed, the n-paraffin present at the highest concentration in the oil and that their WAFs are those with chain lengths of 14 to 17 carbons. The n-alkanes as a group are present at very low quantity. The dimethyl naphthalenes are the hydrocarbons present at highest concentration in the oils. However, due to greater water solubility, naphthalenes represent the most prominent diaromatic hydrocarbons in the WAFs (Anderson et al., 1974). Reddy and Meon (1980) found that at comparatively higher concentrations of WAF, the maximum mortality occurred between 24 and 48 h of exposure. The LC50 values of 72 and 96 h usually show only minimal variation from 48 h LC50 indicating that the concentration of WAF reduces as a function of time. Comparable results are documented by Blumer et al., (1970); Lee et al., (1972a,b); Corner et al., (1973); Anderson (1973); Tatem and Anderson (1973); Neff and Anderson (1974) etc. The documented evidence on oil

toxicity can broadly be brought under the following categories. a) Toxicity of different oil and specific petroleum hydrocarbons b) Effect of exposure to sublethal concentrations of oil on various physiological processes of the target organisms and c) Uptake and release of petroleum hydrocarbons (Fry, 1971; Newell, 1973; Dunning and Major, 1974; Stegeman, 1974).

Looking into the fate and effects of petroleum hydrocarbons in marine ecosystem, various authors have discussed biological effects, bioaccumulation and metabolism, and distribution and movement of petroleum hydrocarbons (Wolfe, 1977). Investigating the comparative oil toxicity and comparative animal sensitivity, Rice et al., (1977) concluded that it would be better to employ the same species to assess toxicities of different oils. Refined oils are generally considered more toxic than crude oils on a volume added basis and the toxicity of an oil is controlled by the concentration of toxic compounds in the oil. It was also noticed that the toxicity of aromatic hydrocarbons increases with the number of rings and with the degree of alkyl substitution. Since the toxic aromatic hydrocarbons persist for a longer period under lower temperature conditions, oil derivatives are more toxic in temperate species. Anderson (1977) found that sensitivity factors, usually used to estimate a safe environmental level based on 96 h toxicity data alone may not be reliable to assess the toxicity of a toxicant.

Emphasizing the significance of measurement of responses of aquatic animals on pollutional stress, Bayne et al., (1979) remarked, "although the vitality of populations may be of ultimate concern when assessing the effect of pollution, it is on the survival, growth and reproduction of individuals in a population that this vitality depends". Physiological responses should provide

both an integration of the biochemical and cytological effects and an indication of the likely consequences of environmental change to a population. Dicks (1973) proved that natural rhythm in activity affect the susceptibility of the limpet population to crude oil, greatest toxic effects occuring during times of greatest activity. Anderson et al., (1974) found that exposure of fish to WSF of number 2 fuel oil cause stimulation of the respiratory rate. Anderson and Anderson (1975) did notice changes in the osmotic pressure of the pericardial fluid of oysters when exposed to a combination of oil and varying salinities. These authors found that, short-term oil exposure did affect chloride regulation and the effects were found to be reversible. Chronic exposure to oil fractions resulted, reduction in respiration and growth rate in Crangon crangon (Edwards, 1978). Involvement of metabolic process in breaking down and synthesis of oil ingested by mussels, Mytilus galloprovincialis, has been proved by Mironov and Shchekaturina (1979). Reddy and Menon (1980) found that different percentages of soluble hydrocarbons present in the WAFs of light diesel oil and fuel oil would impair not only the basic physiology of the animal but also the structure of organs or organelles under the pollutional stress of these oils. This finding was based on the structure and morphology of byssus threads secreted by Perna viridis subjected to the pollutional stress of the oils. The ability to maintain hyper-osmolarity was reduced after exposure to oil, although the effect was reversible in Palaemon adspersus (Baden, 1982). Stress indices, such as scope for growth and lysosomal latency were negatively correlated with tissue aromatic hydrocarbon in the case of Mytilus edulis, on exposure to the WAF of North Sea oils (Widdows et al., 1982). Axiak and George (1987) opined that the ciliary action of the gill of the clam,

Venus verrucosa was considerably modified accompanied by enhanced mucus production, when this marine bivalve was exposed to petroleum hydrocarbon, derived from Kuwait crude oil. They have suggested that this would have a significant effect on the energy budget of the clam.

The patterns of accumulation and release of petroleum hydrocarbons by marine animals were reviewed by Varanasi and Malins (1977) and Neff and Anderson (1981). High quantities of petroleum hydrocarbons were detected in the tissues of Crassostrea virginica (Ehrhardt, 1972). Neff and Anderson (loc cit.) have presented certain details on the uptake and release of petroleum hydrocarbons in oysters and clams. They found time dependent variations in the PHC concentrations in the tissues. Clams were found to accumulate less than oysters. Exposure to oil free water was found to result in release of accumulated hydrocarbons by both oysters and clams. The uptake of aromatic hydrocarbons into the tissues of snails and mussels was found to be directly related to the amount of hydrocarbons in WAF. Looking into the effect of an experimental oil spill on the distribution of aromatic hydrocarbons Cox et al., (1975) found that the accumulated aromatic hydrocarbons were released quickly and the tissue levels reached background level in the case of both shrimp, Penaeus setiferous and clam, Rangia cuneata. Neff and Anderson (1975) found that, when exposed to oil contaminated sea water, marine animals rapidly accumulated in their tissues, a wide spectrum of aromatic hydrocarbons. They also found that, when such animals were returned to clean seawater they rapidly released the accumulated hydrocarbons. Faecal material was found to be the major pathway of release of hydrocarbons in the case of Callinectus sapidus, the blue crab (Lee et al., 1976). High concentrations

of petroleum derived hydrocarbons were detected in the tissues of the oyster Pinctada margaratifera distributed in the coastal waters adjacent to oil loading facilities (Anderlini et al., 1981). Using mussels as indicators to determine the nature and extent of petroleum hydrocarbon (PHC) pollution along the coastal waters Risebrough et al., (1983), studied the distribution of hydrocarbons in the tissue of Mytilus galloprovincialis, Ostrea edulis and Venus gallinae. Mussels were found to accumulate high levels of PHC. A connection between the type of sediments and the PHC levels in the tissues of Mytilus edulis was demonstrated by Law and Andrulewicz (1983). Assigning influence of seasons on the distribution of polycyclic aromatic hydrocarbon (PAH) in soft shell clam, Mya arenaria, Mix and Schaffer (1983) found that PAH concentrations were lowest in the fall winter and highest during spring-summer period.

Information on the combined effects of pesticides and oil is generally lacking. However, unpublished work on this aspect is available (D'Silva, 1980). In the case of Malathion and LDO-WAF-it was noticed that pesticides become less toxic in the case of Perna viridis. On the other hand, in the case of Meretrix casta, the toxicity of Malathion increased when supplied along with WAF of LDO. The toxicity of Ekalux to Perna viridis and Meretrix casta was increased, when supplied along with WAF of HSD or LDO. Marginal variations occurred when a marine polychaete, Sabellaria clandestinus was exposed to Ekalux and HSD - WAF. Sinderman (1979) has suggested that there is still uncertainty about multiple pollutant effects on marine organisms and the critical problem of resistance to contaminant toxicity is only now beginning to be elucidated. A clear understanding,

of this can be had only if there is a shift of emphasis towards elucidating sublethal effects and paying more attention to analyse the metabolic pathways and resistant mechanisms.

In the present review, all the relevant papers available have been looked into and it is clear that information on combined toxicity is generally lacking with reference to pesticides and water accommodated fraction (WAF) of crude and refined oils.

MATERIAL AND METHODS

III. MATERIAL AND METHODS

3.1 INTRODUCTION

Quantitative assessment of the effect of pollutants has got cardinal importance in any pollution research, both from the biological and ecological point of view. Efforts were made to evaluate the lethal and sublethal effects of pesticides and petroleum hydrocarbons individually and in combination on two selected non-target invertebrates. Both the animals used for the study were bivalves, one of them the brown mussel Perna indica is a representative of the marine environment and the other, the black clam, Villorita cyprinoides var. cochinensis has an estuarine distribution.

3.2 TEST ANIMALS

3.2.1 *PERNA INDICA*

Perna indica (Kuriakose and Nair, 1976) the common brown mussel, extensively distributed along the intertidal and subtidal rocky beaches of the South West coast of India was used for the present study. These marine bivalves attain a maximum length of 120 mm (from umbo to the posterior tip) and animals with a size range of 25 - 30 mm are abundant during October to February. Extensive beds of these animals are available on the rocks which are laid to construct a wave breaker at the mouth of the Ashtamudi Lake at Sakthikulangara, 8 km north of Quilon ($8^{o}56'$ N- $76^{o}35'$E). Using sharp chisel these animals were detached, cleaned and transported to the laboratory in large polyethylene drums of 50 l capacity with sea water from the site of collection. (See Map.)

3.2.2 *VILLORITA CYPRINOIDES VAR. COCHINENSIS*

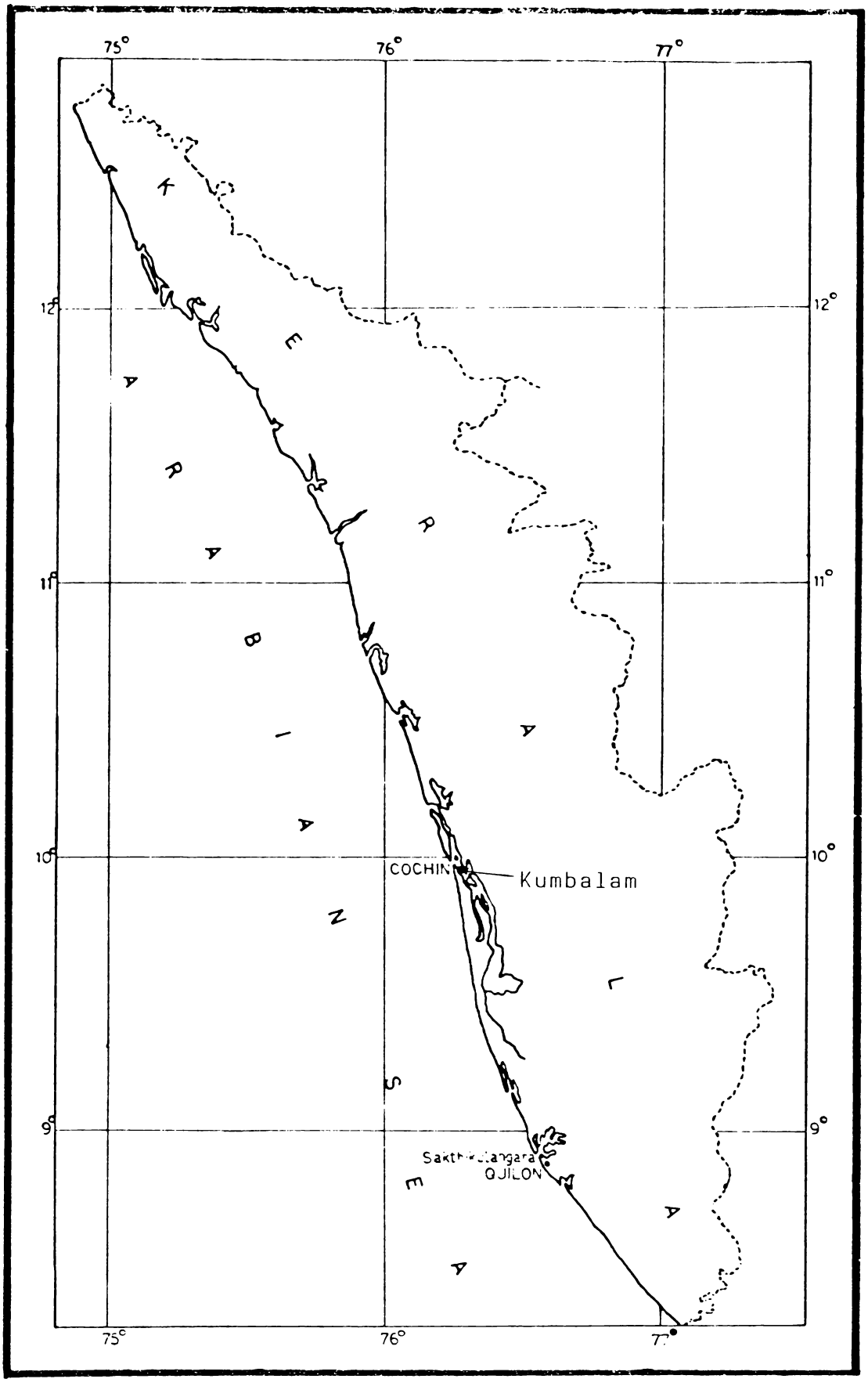
Villorita cyprinoides var. cochinensis (Hanley), popularly known as black clam, is a hardy bivalve abundantly distributed along the mesohaline and oligohaline stretches of the Cochin backwater. Though these animals are capable of tolerating wide fluctuations in salinity (Sivankutty Nair and Shynamma, 1975), the density of population had been found to decrease when the salinity exceeds ca. 20‰.

Animals were collected from the Cochin backwaters (See Map) near Kumbalam ($9^{o}53'$ W-$76^{o}17'$E) where the salinity ranges from fresh water during monsoon periods (June-August) to more or less 20‰ during high tides of peak summer (March-May). Collections for the studies were mainly made during post monsoon to pre monsoon months (September-May) when the salinity of water at the collection site ranged from 10‰ to 20‰. Individuals of V. cyprinoides var. cochinensis of the size range of 20-25 mm were collected in plastic buckets with water from the collection site and brought to the laboratory.

3.3 LABORATORY PROCEDURES

3.3.1 LABORATORY CONDITIONING OF TEST ANIMALS

The animals brought to the laboratory were maintained in polyethylene tubs of 50 l capacity with well aerated sea-water of salinity ca. 32‰ and 15‰ respectively for P. indica and V. cyprinoides var. cochinensis at room temperature ($28^{o}C \pm 1.5^{o}C$). In the case of V. cyprinoides var. cochinensis, care was taken to bring about a gradual change in salinity of water in the conditioning tank when the salinity of water in the locality of collection



Map showing the locations of collection of Perna indica (Sakthikulangara) and Villorita cyprinoides var. cochinensis (Kumbalam)

ranged widely from 15‰. The period of conditioning lasted from 36 to 48 h, during which time they were fed with the algae Synechocystis salina. The water in the holding tank was changed periodically. All organisms used for any one set of experiment belonged to one population. Only healthy and active animals of the same size were used for the experiments.

The seawater used for the experiments was collected from Arabian Sea, off Cochin. Black polyethylene carboys of 50 l capacity were used to bring the collected water. Before being employed for the experiment, the seawater was filtered through a fibre glass filter (length 32 cm, breadth 16 cm), containing glass wool and activated charcoal, using a 0.15 H.P. pump and stored in the dark in fibre glass tanks of 200 l capacity, upto 15 days. Salinity adjustments were done by diluting with de-ionised water, whenever necessary. The pH of the experimental water was 8.2 $\pm$ 0.1. All the experiments were carried out at laboratory temperature ($28.0^{o}C \pm 1.5^{o}C$)

3.3.2 TOXICANTS

The toxicants used for the study were four pesticides and petroleum hydrocarbon (PHC) derived from the Water Accommodated Fractions (WAF) of oils. The four pesticides used were Ekalux, Dimecron, Aldrex and DDT, the former two belonging to organophosphates and the latter two representing organochlorines. Both these groups are being used extensively for the control of pests in the agricultural fields. The PHCs used were derived from the Water Accommodated Fractions of Light Diesel Oil - LDO (WAF) - and Persian Gulf Crude Oil-PG Crude (WAF).

The toxicant solutions were prepared separately and were added to the test media to get respective pesticide, PHC or Pesticide-PHC concentrations.

The pesticide concentrations were prepared by mixing the commercial grade pesticide with acetone in 1:1 ratio. For getting the sublethal concentrations the initial stock solution with acetone was further diluted with distilled water.

3.3.2.1 Ekalux^{R} EC 25

Ekalux^{R} EC 25 was supplied by Sandoz India Ltd. It is a wide spectrum emulsifiable concentrate containing 25% (W/W) of the organophosphate (o.p) active ingredient 'Quinalphos' (o,o diethyl - o - quinosxalinyl (2) - thionophosphate) and 75% (W/W) of stabilizers, emulsifers and adjuvants. A suspension of this pesticide was prepared with acetone in 1:1 ratio and added to the test solution in the required concentration. Ekalux was used individually and in combination with LDO (WAF). The stock solution was prepared afresh for each experiment.

3.3.2.2 Dimecron^{R}

Dimecron^{R} is the commercial product of Hindustan Ciba Geigy Ltd. It is a systemic water soluble pesticide based on 'phosphomidon'

(C_{10} H_{19} Cl N O_5)

The procedure followed for the preparation of the test solution was similar to that followed for Ekalux. The effect of this pesticide also was studied individually and in combination with LDO (WAF).

3.3.2.3 Aldrex^{R} 30

Aldrex^{R} 30 is an organochlorine pesticide (o.c.s.) and was manufactured by National Organic Chemical Industries Ltd. It is based on Aldrin and

contain 300gms, Hexachloro Hexahydro Dimethano Naphthalene (HHDN) per Kilogram. Though it is a soil insecticide, it can also be used for foliar application in agricultural fields. Lethal as well as sublethal effects of this pesticide was studied individually and in combination with LDO (WAF).

3.3.2.4 DDT^R 25 EC

DDT^R 25 EC (Dichloro Diphenyl Trichloroethane) another representative of o.c.s. studied is an emulsion concentrate containing 25% (W/W) DDT technical. It is marketed by Premier Pesticides (P) Ltd. and is widely used in municipal drainages for the control of mosquitoes. Experiments were carried out to evaluate its individual and combined effect with LDO (WAF).

3.3.2.5 Water Accommodated Fractions of Oils

LDO and P.G. Crude supplied by the Cochin Refineries Ltd. (CRL) of Indian Oil Corporation (IOC) were brought to the laboratory in carboys of 20 l capacity and kept in the dark. The oil obtained once were used for only a month and after that fresh supplies were obtained.

The water accommodated fractions (WAFs) were prepared daily by continuously stirring a mixture of the oil and sea water of the required salinity at a ratio of 1:20 for periods upto 14 hours, using a vortex mixer. A round perspex container of 20 l capacity, fitted with an outlet at the bottom was used for this purpose. After allowing to settle the mixture for ca. 10 minutes, the aqueous fraction was drained out leaving the supernatant scum in the container. The resultant emulsion of the aqueous fraction was transferred into a thoroughly cleaned separatory funnel of 2 l capacity, for further separation. After ca. 2 h the aqueous fraction from this was collected into clean beakers of 5 l capacity and this was considered 100% WAF

of the respective oil. The dosing of the WAFs were in ppm (PHC) basis. The PHC concentration of the WAF was estimated as follows:

125 µl of the respective WAF was dissolved in 100 ml n-hexane (Spectroscopic grade) to obtain a 1000 ppm stock solution and this was filtered using a Whatman No.42 filter paper to remove the insoluble materials (Levy, 1972) especially in the case of crude oil. From this stock solution 1 ppm, 5 ppm, 10 ppm, 20 ppm, and 30 ppm of the respective oils were made by appropriately diluting with 100 ml of n-hexane. These standards were scanned over the range of 200 - 300 nm, with a spectrophotometer (Hitachi 200-20-UV-Vis)and the absorption spectra of the standards relative to n-hexane were obtained. The absorbance of the above standard solution at 225 nm (λmax), were noticed and from these readings, standard plots indicating the PHC concentrations of both the WAFs were found out.

To find out the concentration of PHC in the WAF, 50 ml of the 100% WAF prepared was taken in a hexane cleaned, dry beaker, and its pH brought down below 2 by acidifying with 1 ml of concentrated HCl. After this it is taken in a hexane cleaned separatory funnel of 100 ml capacity and extracted with about 15 ml of spectroscopic grade n-hexane, by thoroughly shaking for 2 minutes. Then the separatory funnel was allowed to remain undisturbed till the hexane fraction was completely separated. The supernatant was separated and this process of extraction was repeated twice. The combined hexane extract was passed through a column of anhydrous sodium sulphate (Gupta et al., 1980) to free it from residual water and later made up into 50 ml in a hexane cleaned, dry standard flask. The absorbance of the hexane extract was measured at 225 nm and the concentration of the PHC in the respective WAFs were computed from the standard curve.

Once the concentration of the 100% WAF's of the respective PHCs were known, required volumes to be added to the test media to obtain the desired concentrations were calculated out. The addition of WAF to the test media did not produce any significant variation in the quality of the media. The WAF to be added, was prepared daily. Variations in the PHC concentrations were observed in the 100% WAF of the P.G. Crude. PHC concentration in the 100% WAF of LDO did not vary much. However, the PHC concentration in both the WAFs were estimated every day before its application.

3.3.3 TOXICITY STUDIES

3.3.3.1 Lethal Toxicity Studies

Lethal toxicity studies give 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 test organisms. Though this 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). With all its imperfections, lethal toxicity studies were considered ecologically significant, most scientifically and legally defensible, modest in predictive capacity, simple and less expensive.

3.3.3.1.1 Lethal toxicity of individual toxicants

Experiments were carried out to assess the individual lethal toxic responses to four pesticides, Ekalux, Dimecron, Aldrex and DDT and PHCs derived from LDO (WAF) and P.G. Crude (WAF) by both the bivalves, the brown

mussel, P. indica and black clam, V. cyprinoides var. cochinensis. Laboratory conditioned mussels and clams of uniform size (mussels 28-30 mm; clams 20-24 mm) were exposed to 5 l of test solution that contain graded, logarithmic series of concentrations of the toxicants. Exposure to pesticides were carried out in glass troughs (15 x 30 cm) while fibre glass troughs of the same size were used for exposing to WAFs. Ten animals were used for each test concentration of the toxicant. The experimental vessels were kept closed with a perpex sheet. The experiments were carried out at laboratory temperature ($28^{o}C \pm 1.5^{o}C$) and the animals were not fed during the duration of experiment. Duplicates and controls were run for all the experiments. The test media were replenished totally every 24 h. The animals were inspected every 12 h and were considered dead, if the valves gaped beyond 5 mm and showed no movement even under mechanical stimulation. The dead animals were removed and the cumulative percentage mortality at every 12 h recorded. The LC 50 values and their 95% confidence limits were calculated using probit analysis, (Finney, 1971).

3.3.3.1.2 Combined toxicity of toxicant mixtures

After assessing the toxic responses to the individual toxicants, efforts were made to study the combined toxic effects of a mixture of toxicants. The concentrations employed for the combination studies were derived from the respective 96 h LC50 of the individual toxicants. For studying the combined effect, the concentration of one toxicant employed (Toxicant 'A') was kept constant while that of the other (Toxicant 'B') varied. Usually 25 (5x5) combinations were tested to complete one set of experiments on combined toxicity of toxicants. All procedures observed for the study of individual toxicity were followed here.

The toxic effects of Ekalux, Dimecron, Aldrex and DDT in combination with LDO (WAF) on P. indica and in the case of V. cyprinoides var. cochinensis, toxic effects of Ekalux and Aldrex with LDO (WAF) were studied. The LC50 levels and their 95% confidence limits were found out and the additive indices were assessed following the methods of Marking and Dawson (1975).

3.3.3.2 Short-Term Sublethal Toxicity Studies

The objective of these toxicity tests were to find out the concentrations of the toxicants capable of inducing abnormal responses on the activities of the organisms. The activities studied were rate of oxygen consumption and filtration. In the case of P. indica byssogenesis was also used as an index of sublethal response.

The concentrations of different toxicants employed for the studies were derived based on the recorded 96 h LC50 values for individual toxicants. Normally 1/10 of the individual 96 h LC50 values were taken as the highest concentration along with four other concentrations fixed in the descending order. For studying the combined sublethal effect, two unvarying concentrations of one toxicant were combined with five varying concentrations of the other toxicant and concentrations that did not produce any lethality during 96 h study were selected for this study. The duration of these experiments never exceeded a maximum of 10 h. Animals used for this studies were pre-exposed to different sublethal concentrations for 2 h and 48 h to pesticides and petroleum hydrocarbons respectively. The test solutions were not aerated.

3.3.3.2.1 Rate of oxygen consumption

Mussels and clams pre-exposed to the toxicants for a period of 2 h in the case of pesticides and 48 h in the case of oil were used for this study . Experiments were conducted in conical flasks (2 l) with 2 l of test solution, each with five animals. Test solution was sealed with inert liquid paraffin (Burgoyne^{R}) to prevent gaseous exchange with the atmosphere. Duration of the experiment was 8 h and the reduction in the oxygen content was determined every 2 h by siphoning out about 30 ml of the test solution with a flexible polyethylene tube. Quantity of oxygen in the test solution was determined by Winkler's method. After the experiment, soft tissues of the animals were cleaned with distilled water, removed to a previously weighed aluminium foil, dried at 70^{o}C for 48 h and dried tissue weight taken to constancy. The results are expressed as μg oxygen h^{-1} mg^{-1} dry wt of the animal.

3.3.3.2.2 Rate of filtration

Dye clearance technique was employed to assess the rate of filtration (Abel, 1976). Animals were pre-exposed for 2 h to 2 ppm neutral red dye (A.R. Koch-Light Lab, England) along with the respective toxicants. This was done to minimize a possible initial shoot up of filtration that could be induced by the dye particle. Two animals each were introduced in glass beakers (1 litre) with 500 ml of test solution, containing 2 ppm of neutral red. Five sublethal concentrations and a control (in quadruplets) were employed. Reduction in dye concentration was recorded at 1 h interval by withdrawing 10 ml of the test solution. The experiments were terminated after 2 h. Filtration was estimated using Abel's equation (Abel, 1976). After the experiments, the soft tissues of the animals were cleaned with distilled water, removed

to a previously weighed aluminium foil and dried at $70^{o}C$ for 48 h and weighed to constancy. The results are expressed as volume of sea water (in ml) filtered h^{-1} mg^{-1} dry tissue weight of the animal.

3.3.3.2.3 Rate of byssogenesis

Five mussels each, were pre-exposed to five sublethal concentrations of pesticides and oil for 2 h and 48 h respectively. Such pre-exposed animals were allowed to produce byssus thread under the stress of pesticides and oil for a period of 10 h. Five sublethal concentration of pesticides and oil were used to assess the capacity of byssus production. After the experiment, the test solutions in the beaker was siphoned out carefully and the number of byssus threads produced by each individual were counted using a biconvex lens. Byssus threads with adhesive discs at the tip were only considered. The rate of byssus production is expressed as number of threads produced $individual^{-1}$ 10 h^{-1}.

3.3.3.3 Long-Term Sublethal Toxicity Studies

A study on the toxicity after prolonged exposure of the test organism to toxicant is a recent development in pollution experimentation.

30 mussels of uniform size were exposed to three very low concentrations (1/100 of the 96 h LC50 values were the highest) of the pesticides together with a control group. Polythene tubs of 35 l capacity containing 10 l of the toxicant solution were used for the exposure. The test media were changed daily with fresh media and the animals were fed with the algae, Synechocystis salina for 30 minutes before changing the media. The period of exposure to toxicant solution lasted for 14 days and subsequently they were

transferred to toxicant free, raw sea water for a period of 7 days. After 7 and 14 days during the period of toxicant exposure and after 7 days of exposure to toxicant free sea water 9 animals were withdrawn from each tub and used for the study of oxygen consumption and filtration.

Long-term studies involving PHCs were also conducted with mussels. For this, 100 mussels were exposed to 3 different sublethal concentrations of the respective PHCs (1/10 of the 96 h LC50 values were the highest) and a control. All the experiments were done in triplicates. Polythene tubs with 30 l of the test solution were used for this study. Test solution was replenished daily and the animals were fed with the algae Synechocystis salina for 30 minutes before replenishing the test media. After 21 days of continuous exposure the animals were transferred to toxicant free raw sea water for a period of 7 days. 20 animals were withdrawn from each tub after 7, 14 and 21 days during the period of exposure to the toxicant and at the end of the 7th day after transferring to raw sea water and these animals were pooled and used for the study of oxygen consumption, filtration and accumulation/depuration.

3.3.3.3.1 Rate of oxygen consumption during different duration of long-term exposure

The mussels removed from the toxic medium at different intervals of exposure were used for the study. Experiments were conducted in conical flasks (2 l) with 2 l of test solution each with 5 animals. 3 sublethal concentrations and a control were used in duplicate. All other procedures were the same as stated in section 3.3.3.2.1.

3.3.3.3.2 Rate of filtration during different duration of long-term exposure

Individuals of mussels removed from the toxic medium at different intervals of exposure and those transferred to raw sea water were used to asses the rate of filtration. Experiments were conducted with two animals in glass beaker (1 litre) containing 500 ml of test solution. 3 sublethal concentrations and a control were employed, in duplicate. The rest of the procedures were same as described in section 3.3.3.2.2.

3.3.4 ACCUMULATION AND DEPURATION STUDIES

Mussels have the remarkable ability to accumulate petroleum hydrocarbons (PHC) in their tissue. Bioaccumulation by an organism is one of the major factors that plays a crucial role in the biomagnification of a pollutant in the trophic system. Hence efforts were made to assess the rate of accumulation and depuration of the PHCs by the mussels.

40 mussels pre-exposed to different PHC concentrations were withdrawn at different intervals of accumulation/depuration as the case may be (0,7,14 and 21st day of accumulation and 7 days after transferring to raw sea water) and pooled into 4 sets each with 10 animals. The soft tissues of these animals were shucked out, cleaned in distilled water, drained, blotted, weighed in a preweighed aluminium foil, wrapped and preserved at $-20^{o}C$ for the analysis of tissue load.

3.3.4.1 Analysis of Petroleum Hydrocarbons

Petroleum hydrocarbons in the whole tissue of mussels were analysed by steam distillation using U.V. spectrophotometry (Donkin and Evans, 1984; Neff and Anderson, 1975).

3.3.4.1.1 Reagents

1. n-hexane (Spectroscopic grade)
2. Concentrated hydrochloric acid
3. Sodium hydroxide (G.R)
4. Silica gel (60-120 mesh, chromatographic grade)
5. Alumina
6. Sodium sulphate (Anhydrous)

3.3.4.1.2 Sample digestion and preparation

About 5 g of the soft tissues of the mussels preserved at-20^{o}C were homogenized well, with a mortar and pestle. Before use, the mortar and pestle were kept in a freezer to maintain a very low temperature preferably 0^{o}C, during homogenization.

The homogenized samples were transferred to a round bottomed distillation flask of 500 ml capacity, containing 15 ml of n-hexane and 50 ml of distilled water. The apparatus used for distillation was similar to that of Dean-Stark water estimator. About 5 ml of 4 M sodium hydroxide was poured into the flask and more distilled water was added to make the total volume ca. 250 ml. Maintaining the temperature of the homogenate sample at ca. 80^{o}C it was distilled for 2 h. Then the apparatus was allowed to cool and 20 ml of 1 M hydrochloric acid was added to the homogenate. The sides of the condenser was rinsed by pouring down 10 ml of distilled water and the process of distillation continued for 2 h at the end of which time, the distillate was collected in the condenser.

The product of steam distillation containing the accumulated oil was passed through a double layered column of anhydrous sodium sulphate and

activated alumina to free it from residual water and biogenic hydrocarbon, that might otherwise interfere with UV absorption. Finally the column was eluted with sufficient n-hexane and the extract was made upto 25 ml in a volumetric flask.

3.3.4.1.3 Sample estimation

Estimation of the hexane extracted sample was done with a spectrophotometer (Hitachi 200-20-UV-Vis.) equipped with a recorder. Using n-hexane as the blank the absorbance of the sample was measured at 225 nm and the concentration of respective PHCs was computed from the standard curve. The tissue concentration has been expressed as $\mu g\ g^{-1}$ dry wt. (for details of conversion of wet tissue wt. to dry tissue wt. see section 3.4)

3.4 COMPUTATION AND PRESENTATION OF DATA

Median lethal concentration (LC50) levels and their 95% confidence limits were calculated by probit analysis (Finney, 1971) and explained with graphs and tables. The ET50 values and toxicity curves also have been represented graphically to demonstrate the lethal effects of pollutants, following approved methods (Sprague, 1973)

Combined toxicity of toxicant mixtures on the lethal as well as sublethal responses of the animals were calculated following the methods of Marking and Dawson (1975). The data are presented with graphs and tables. The effect of mixtures of two or more chemicals was commonly referred to as additive, synergistic or antagonistic, based on the relation of the toxicity of the mixtures of that of individual toxicants. Marking and Dawson (1975) have coined a better terminology which was more clear and quantitative and there-

fore, that have been used in the present description.

The additive indices of mixtures of toxicants were derived as follows:

$$\frac{Am}{Ai} + \frac{Bm}{Bi} = S$$

Where, A and B are the toxicants; i and m are the toxicities (LC50's or EC50's) of individual and mixtures respectively for A and B and S is the sum of biological activity.

If the sum of 'S' = 1.0, then the combined action is referred to as simple additivity. If the value of 'S' is greater than or less than 1.0 it indicates that the combined toxicity is either less than additive or more than additive, respectively. Thus the value of 'S' alone could give a quantitative indication of the additive toxicity, except that values greater than 1.0 are not linear with values less than 1.0. This non-linearity can be corrected as follows and a system in which the index represents simple additivity by values '0', greater than additivity by value '+'ve and less than additivity by values '-'ve were developed (Marking and Dawson, 1975).

The linearity between values greater than and less than 1.0 are established by using the reciprocal of the values of 'S' which are less than 1.0 and by substracting 1.0 from the reciprocal ie; $\frac{(1)}{S} - 1$, to obtain a zero reference point. Index values representing less than additive toxicity were obtained by multiplying the values of 'S' which are greater than 1.0 by -1, to make them negative and a zero reference point is achieved by adding 1.0 to this negative value ie; S (-1)+1. Thus, greater than additive toxicity is represented by index values greater than zero, less than additive by negative index values and

simple additivity by values '0'. The significance of the additive indices close to zero can be assessed by substituting the lower confidence limits of the LC50 of the individual toxicants (Ai and Bi) and the upper limits of the mixtures (Am and Bm) to find out the lower limit of the index. Correspondingly, the upper limits of the individual toxicants (Ai and Bi) and the lower limits of the mixtures (Am and Bm) were substituted into the formula to determine the upper limit of the index.

Filtration rate was expressed as the quantity of sea water (in ml) filtered h^{-1} mg^{-1} dry tissue weight of the animal. This was estimated using Abel's equation (Abel, 1976) which states,

$$m = \frac{M}{nt} \quad Log_e \quad \frac{Co}{Ct}$$

Where, m = the rate of filtration in ml individual^{-1} h^{-1}

M = Volume of test solution in ml

n = number of animals in the test vessel

Co = dye concentration in the initial sample

Ct = dye concentration in the final sample

t = time between dye sampling, in hour

The value obtained in the above equation was divided by the mean dry tissue weight of the animals, to get the filtration rate in ml h^{-1} mg^{-1} (dry wt.).

Graphical representation together with tables have been used to explain the results on filtration rate, oxygen consumption and byssogenesis. These data have been analysed statistically using the student's t test to manifest the variation in response from the control at 5% level of significance.

The median effective concentration (EC50) ie; the concentration causing 50% response in the performance of the test animals was calculated, wherever possible, using regression analysis.

The tissue load of PHCs was expressed as $\mu g\ g^{-1}$ (dry wt.). For the conversion of wet weight of the mussel tissue to dry weight a ratio has been developed (Baby, 1987). Mussels used for the experiments were pooled into 10 groups of 4 animals each. The soft tissue was shucked out, cleaned in distilled water, blotted and weighed to find out the wet weight. Later these tissues were dried, and weighed to constancy. Using these data the water content of the tissues, the relationship in weight between wet and dry tissue etc. and their mean were computed, and used to express the dry weight of the tissue.

All the computations involved in the work are carried out with a personal computer (Casio, model PB700).

EXPERIMENTAL RESULTS

IV. EXPERIMENTAL RESULTS

In this section the individual toxicity and combined toxicity of four pesticides and water accommodated fractions (WAFs) of Light Diesel Oil (LDO) and Persian Gulf Crude (P.G. Crude), with reference to lethality on Perna indica, the common brown mussel of the south west coast of India and Villorita cyprinoides var. cochinensis, the black clam inhabiting the mesohaline stretches of Cochin backwaters are presented. The sublethal effects assessed with reference to oxygen consumption and filtration rate in P. indica and V. cyprinoides var. cochinensis are documented. In the case of P. indica, the rate of byssus production under toxicant stress was also worked out.

The long-term effects delineated employing filtration and oxygen consumption as indices of sublethal toxicity were also looked into in the case of P. indica. The results on this aspect were obtained utilizing test individuals subjected to accumulation and depuration. The load of petroleum hydrocarbons (PHC) in P. indica, on exposure to sublethal concentrations of LDO (WAF) and P.G. Crude (WAF) for varying periods and subsequent depuration were also looked into and presented.

For effective representation the results are categorized under lethal toxicity and sublethal toxicity (short-term and long-term) and dealt with, with reference to individual species and toxicants.

4.1 LETHAL TOXICITY

Lethal toxicity of both the animals employed for the studies, P. indica and V. cyprinoides var cochinensis was assessed from two standpoints viz.

toxicity relating to exposure to individual chemicals and to the mixtures of chemicals.

4.1.1 LETHAL TOXICITY TO INDIVIDUAL TOXICANTS

Lethal toxicity of toxicants belonging to organochlorines, organophosphates and refined and crude oils were assessed and the results presented under this heading. The organophosphates were Ekalux and Dimecron and the organochlorines were DDT and Aldrex. Two species of molluscs namely, P. indica, a typical intertidal marine bivalve and Villorita cyprinoides var. cochinensis, the common clam of the Cochin backwaters were the animals used as test organisms.

4.1.1.1 Individual Lethal Toxicity on Perna indica

Perna indica, commonly known as brown mussel has only a restricted distribution along the south west coast of India. This species is found along rocky coasts from intertidal region to a depth of about 10 fathoms. Fresh settlement of this animal occurs around September. This animal is an ecologically and economically relevant species. Earlier studies have proved that this animal is an excellent species for toxicological studies.

4.1.1.1.1 Ekalux

Representatives of the species P. indica were exposed to Ekalux for a period of 96 h. The results obtained are presented in Table 1 and Fig. 1 a-d Animals exposed to 5.0, 7.5 and 10.0 ppm died quickly resulting in an LC50 of 3.22 ppm. The slope functions registered conspicuous variations between 48 h and 96 h, indicating immediate effect on exposure to higher

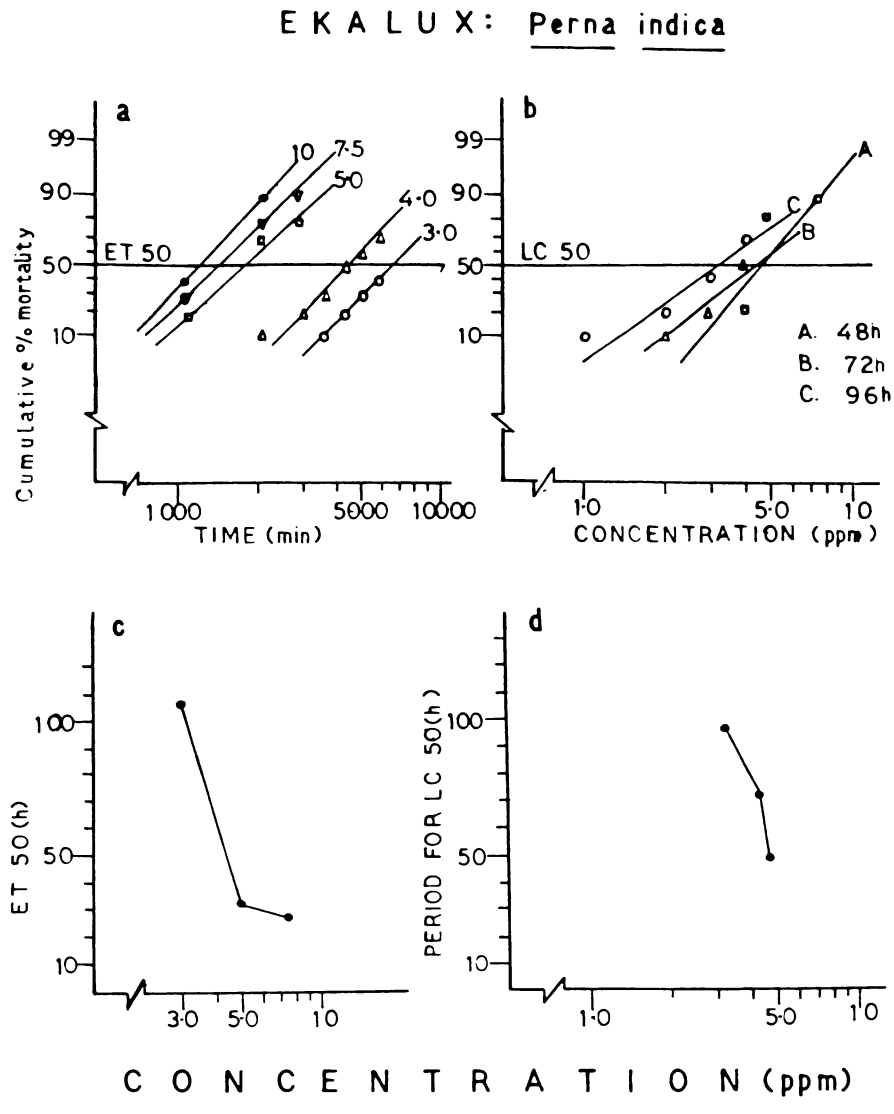


Fig. 1. Perna indica. Lethal effects of Ekalux
a. Progress of mortality against time
b. Progress of mortality against concentration
c & d. Toxicity curves

concentrations. The animals exposed to concentrations above 1.0 ppm were not byssally attached. Excessive quantities of mucus of brownish hue was secreted by the test organisms throughout the course of the experiments.

4.1.1.1.2 Dimecron

This pesticide was found to be comparatively less toxic to P. indica. The 96 h LC50 recorded was 117.58 ppm (Table 1, Fig. 2a-d). P. indica did not record conspicuous rate of mortality even when exposed to 100 ppm of Dimecron. Only 20% of the test animals died after 96 h. In higher concentrations, death when occurred was found to progress quickly as a function of time. In majority of cases maximum mortality occurred between 60 and 90 h. In the case of those animals which could move the foot and keep it outside the shell, it was noticed that the foot has become flabby and listless and the animals had lost the ability to withdraw the foot quickly, on mechanical stimulation.

4.1.1.1.3 DDT

P. indica was exposed to DDT concentrations ranging from 5.0 to 50 ppm. Mortality was recorded in all concentrations from 5.0 ppm onwards. The 96 h LC50 was 10.66 ppm (Table 1, Fig. 3 a-d). Death was gradual as a function of concentration although the time required to kill 100% of the animals was only 48 h when the external concentration was 50 ppm. Reduction in slope function clearly indicates influence of higher concentrations on mortality.

4.1.1.1.4 Aldrex

The concentrations employed to assess the lethal toxicity of Aldrex on P. indica were 5.0 to 17.5 ppm. No animal died when exposed to

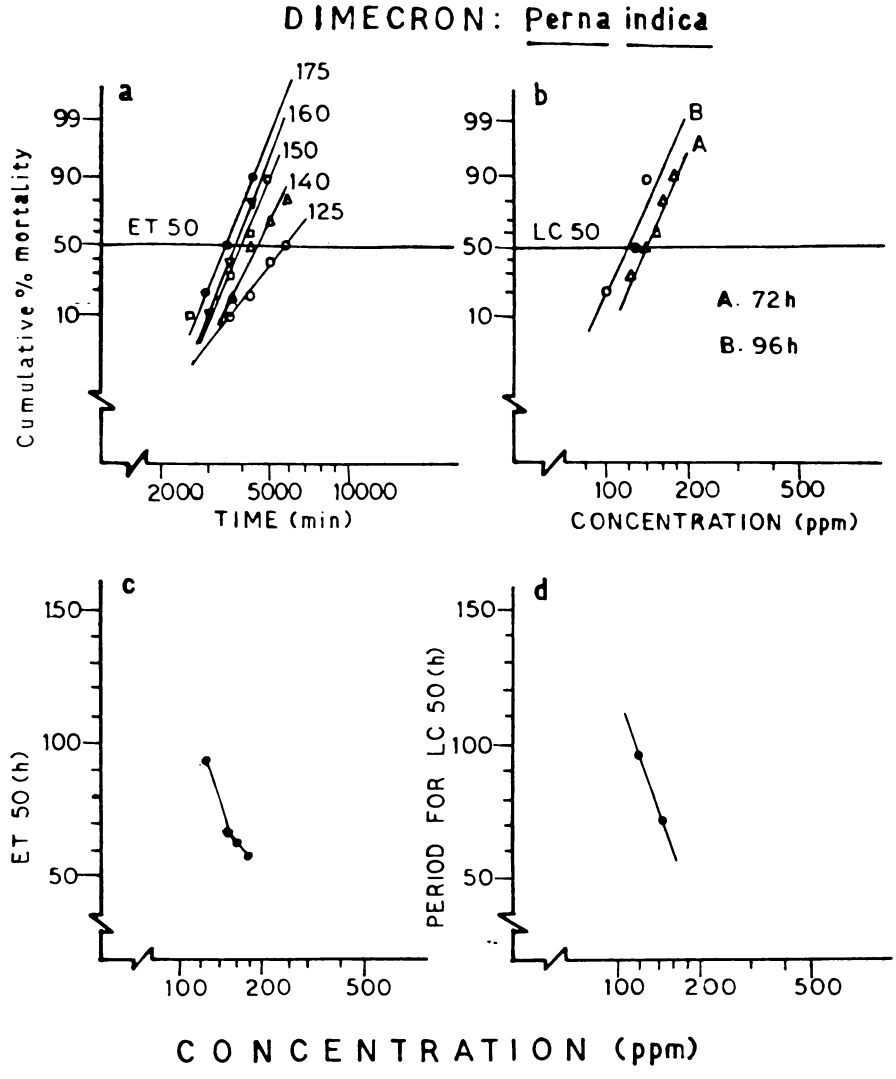


Fig. 2. Perna indica. Lethal of Dimecron

a. Progress of mortality against time

b. Progress of mortality against concentration

c & d. Toxicity curves

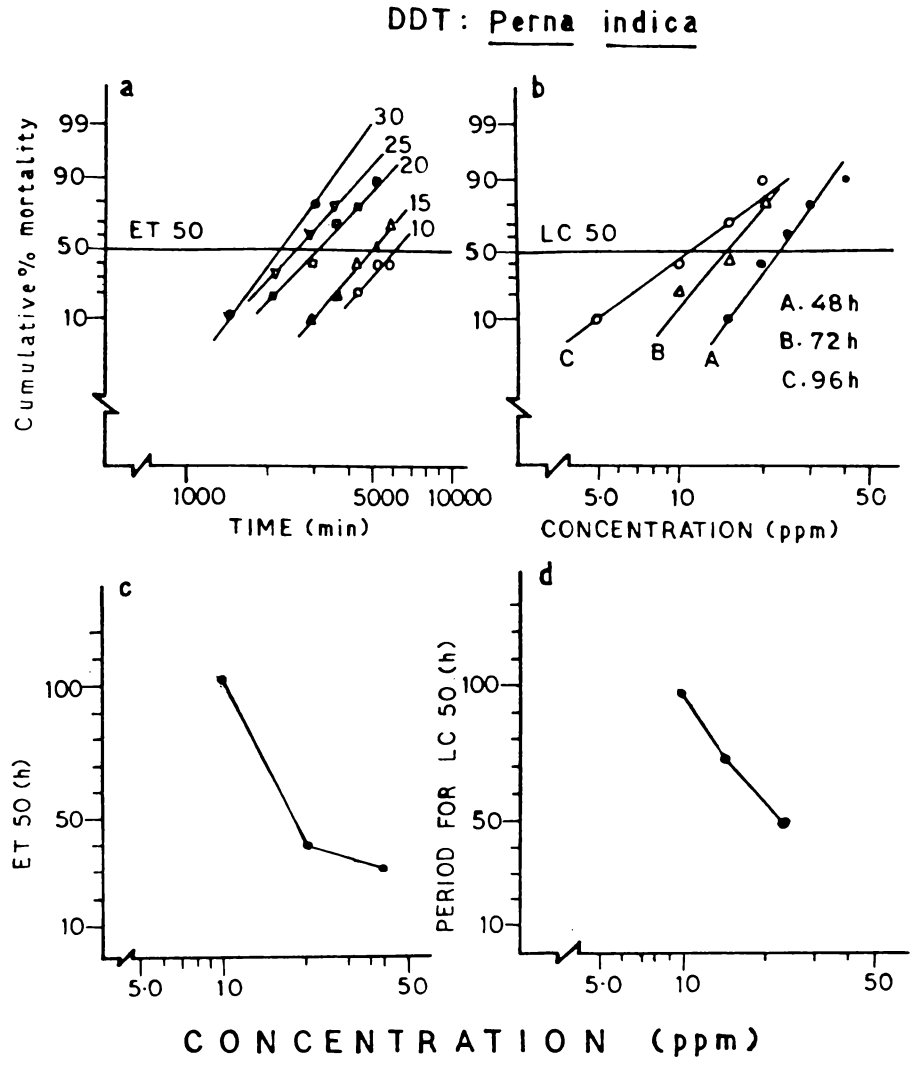


Fig. 3. Perna indica. Lethal effects of DDT

a. Progress of mortality against time

b. Progress of mortality against concentration

c & d. Toxicity curves

5.0 ppm of Aldrex even after 96 h. 100% mortality occurred only when the concentration was maintained at 17.5 ppm. The 96 h LC50 was 8.76 ppm. 50% mortality occurred much before 96 h in 12.5, 15.0 and 17.5 ppm (Table 1, Fig. 4 a-d).

4.1.1.1.5 Light Diesel Oil-Water Accommodated Fraction-LDO (WAF)

The concentration of PHC in the WAF employed varied between 2.0 to 10 ppm and the 96 h LC50 was 4.6 ppm. Only negligible numbers of animals died at experimental concentrations ranging between 2.0 to 4.0 ppm. In higher concentrations death was gradual (Table 1, Fig. 5 a-d).

4.1.1.1.6 Persian Gulf Crude Oil-P.G. Crude (WAF)

P. indica exposed at 6.0 to 13.2 ppm (100% WAF) of PHC derived from the WAF of P.G. Crude did not result in conspicuous mortality. It was noticed that only 30% of the animals died after 96 h when they were exposed to 13.2 ppm of PHC contained in 100% WAF of P.G. Crude.

4.1.1.2 Individual Lethal Toxicity on Villorita cyprinoides var. cochinensis

Villorita cyprinoides var. cochinensis is a typical mesohaline clam and is distributed along the salinity regime ranging between around 15‰ to 4‰. However, this species is capable of tolerating considerable variations in salinity and juveniles are more tolerant than adults, especially at lower salinities. Efforts were made to analyse the individual lethal toxic responses of this animal to Ekalux, Dimecron, DDT, Aldrex and WAFs of LDO and P.G. Crude. The results are presented in Table 2 and Fig. 6-10.

4.1.1.2.1 Ekalux

V. cyprinoides var. cochinensis was exposed to Ekalux at varying concentrations ranging between 1.5 to 50 ppm. The rate of mortality was in-

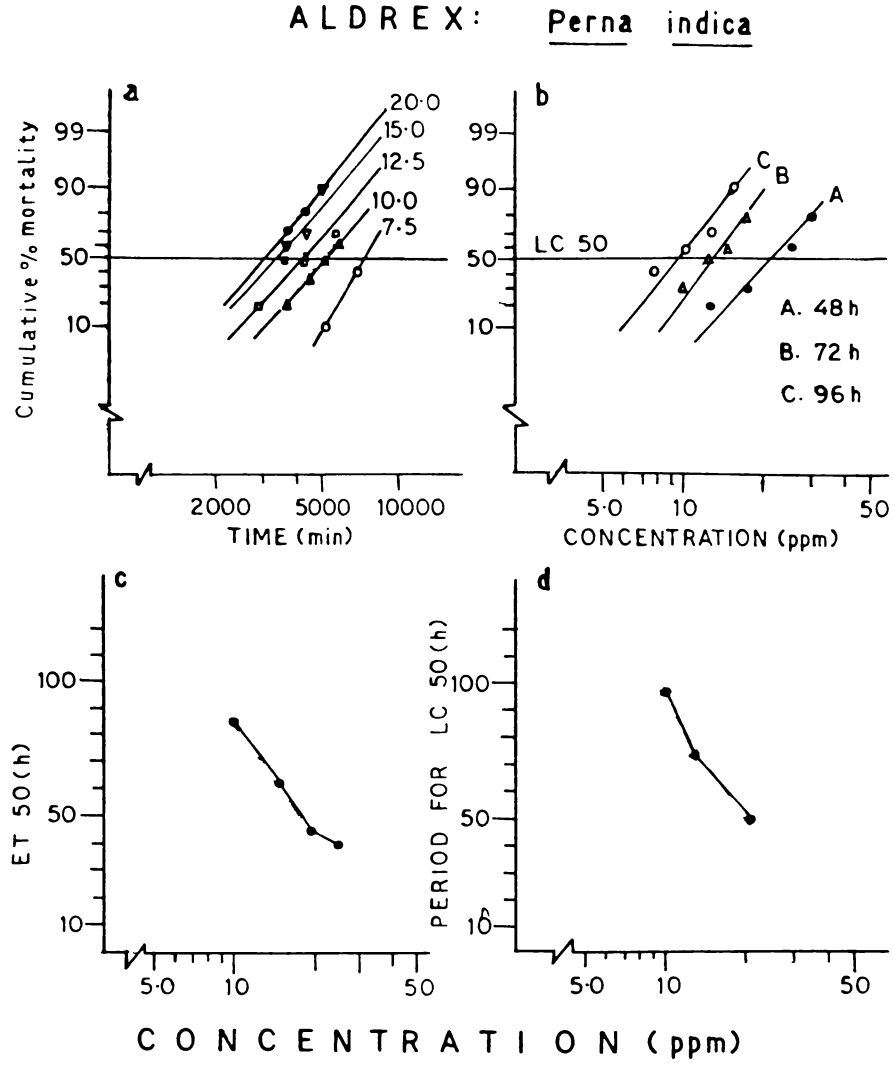


Fig. 4. Perna indica. Lethal effects of Aldrex

a. Progress of mortality against time

b. Progress of mortality against concentration

c & d. Toxicity curves

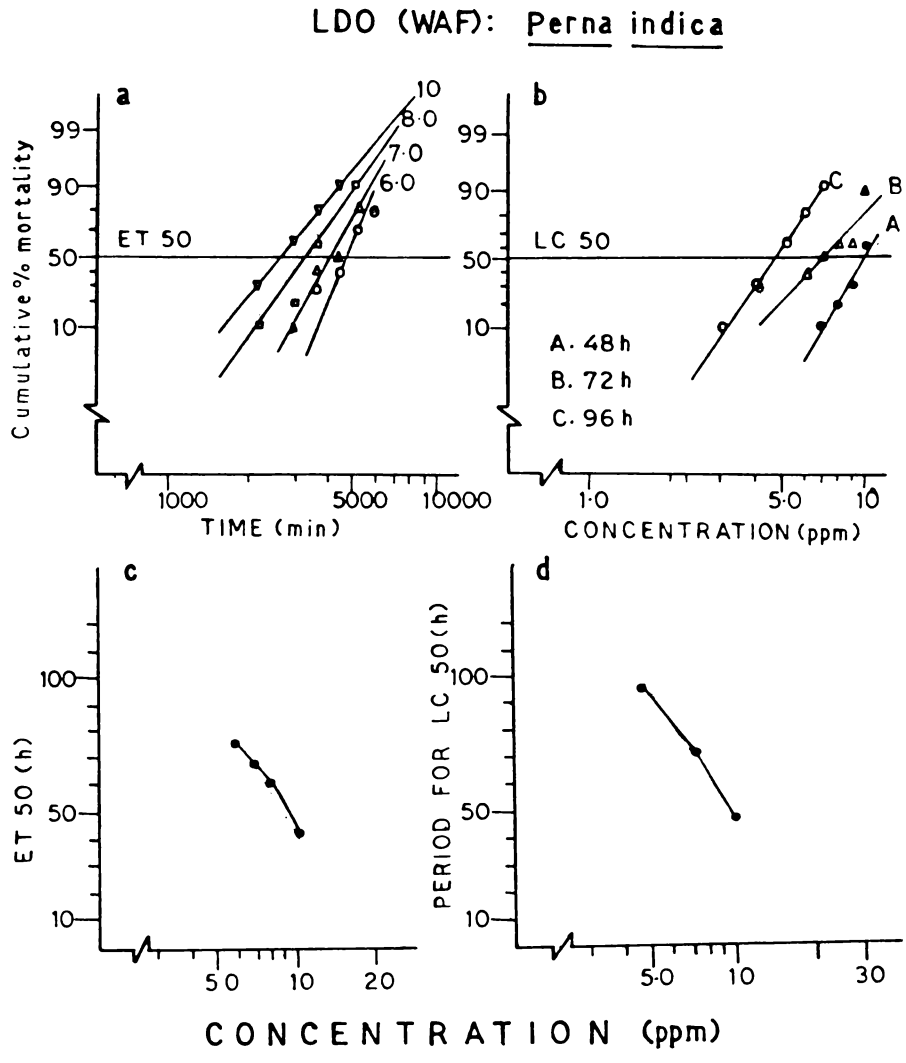


Fig. 5. Perna indica. Lethal effects of PHC in LDO (WAF)

a. Progress of mortality against time

b. Progress of mortality against concentration

c & d. Toxicity curves

Table 1. Perna indica. LC50 (ppm), when exposed to Ekalux, Dimecron, DDT, Aldrex, Light Diesel Oil (LDO-WAF) and Persian Gulf Crude (P.G. Crude-WAF) over periods upto 96h, along with respective 95% confidence limits* and slope functions.

Toxicant	48h		72h		96h	
	LC50 (ppm) (95% confidence limits)	slope 'b'	LC50 (ppm) (95% confidence limits)	slope 'b'	LC50 (ppm) (95% confidence limits)	slope'b'
Ekalux	4.63 (3.39 - 6.31)*	7.13	4.28 (3.26 - 5.60)*	4.14	3.22 (2.39 - 4.34)*	2.84
Dimecron	-	-	139.27 (136.41 - 142.18)*	12.59	117.58 (107.70 - 128.35)*	13.71
D D T	23.07 (21.76 - 24.46)*	6.05	14.95 (12.79 - 17.46)*	5.44	10.66 (9.73 - 11.67)	4.17
Aldrex	20.68 (19.09 - 22.40)*	4.39	12.66 (11.99 - 13.35)*	5.34	8.76 (7.75 - 9.89)	4.78
LDO (WAF)	9.75 (9.13 - 10.41)*	9.44	6.98 (6.09 - 7.98)*	5.84	4.62 (4.54 - 4.69)	7.10
P.G. Crude (WAF)	No mortality even after exposure to 96h at concentrations upto 100% WAF (PHC - 13.2 ppm)					

significant even after 96 h upto a concentration of 3.12 ppm. Concentrations above 6.25 ppm were proved to be highly lethal. All the test individuals exposed to 50 ppm collapsed within 72 h. 5.07 ppm was the 96 h LC50 whereas, this was 29.35 ppm at 48 h. (Table 2, Fig. 6 a-d). The data on ET50 show that concentrations above 25 ppm are highly lethal to this animal. The rate of increase in mortality was directly proportional to concentration and time.

4.1.1.2.2 Dimecron

Higher concentrations of this pesticide were used to find out the cumulative percentage mortality of V. cyprinoides var. cochinensis, exposed to this. Preliminary experiments have shown that this animal is more tolerant to Dimecron. Therefore, the concentrations selected for assessing mortality were 50 to 500 ppm. In 50 ppm mortality was negligible even after 96 h. 50% of the animals were killed in 100 ppm at 96 h and no animals survived beyond 84 h when the concentration was enhanced to 300 ppm and above. A clear cut morphological peculiarity noticed was considerable enlargement of the foot, which protruded out when the animals became moribund. This was especially so in the case of those animals exposed to 300, 400 and 500 ppm. The 96 h LC50 was 93.28 ppm. This concentration, curiously enough was less than that recorded in the case of P. indica, which has been recognized as a very sensitive intertidal mollusc (Table 2, Fig. 7 a-d).

4.1.1.2.3 DDT

DDT was found less toxic to V. cyprinoides var. cochinensis. The concentrations to which these animals were exposed ranged from 25 ppm to

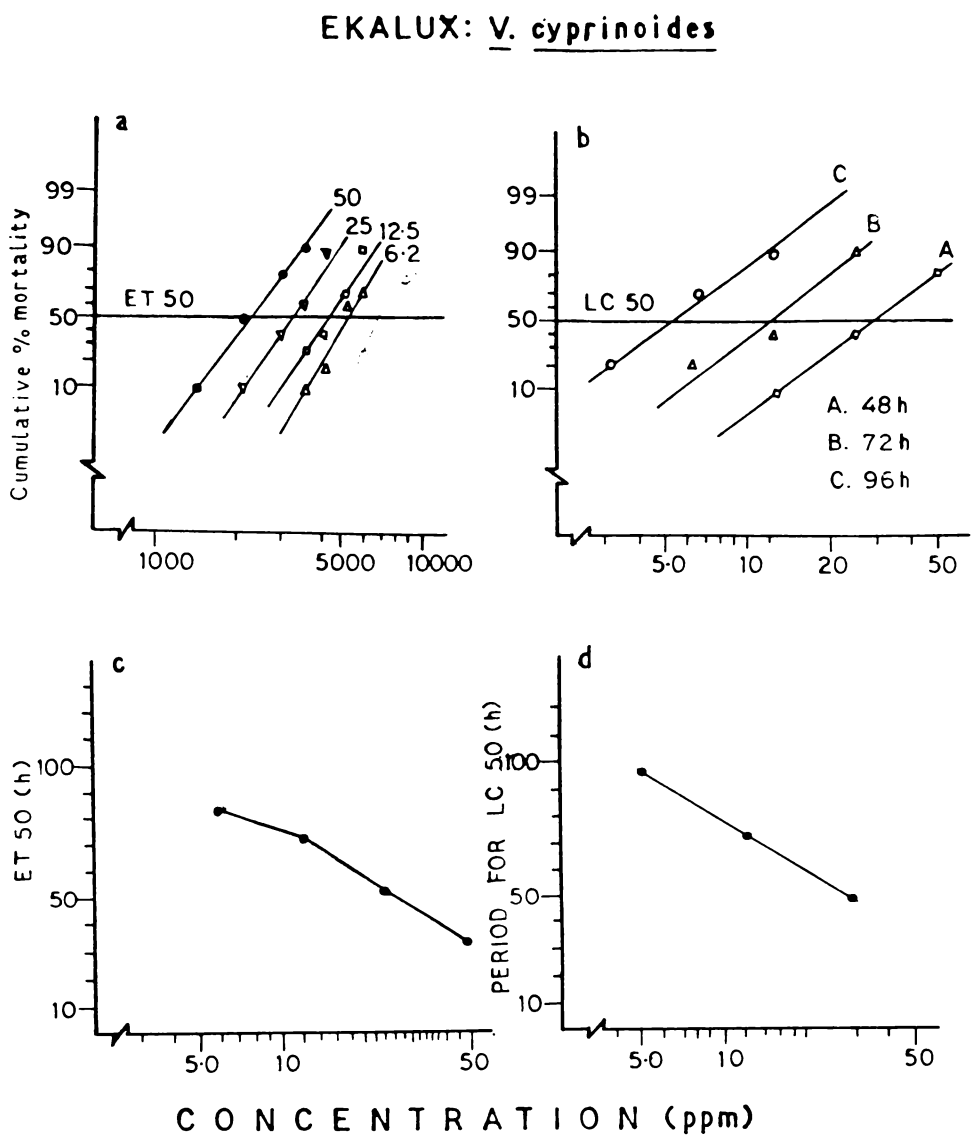


Fig. 6. Villorita cyprinoides var. cochinensis.
Lethal effects of Ekalux
a. Progress of mortality against time
b. Progress of mortality against concentration
c & d. Toxicity curves

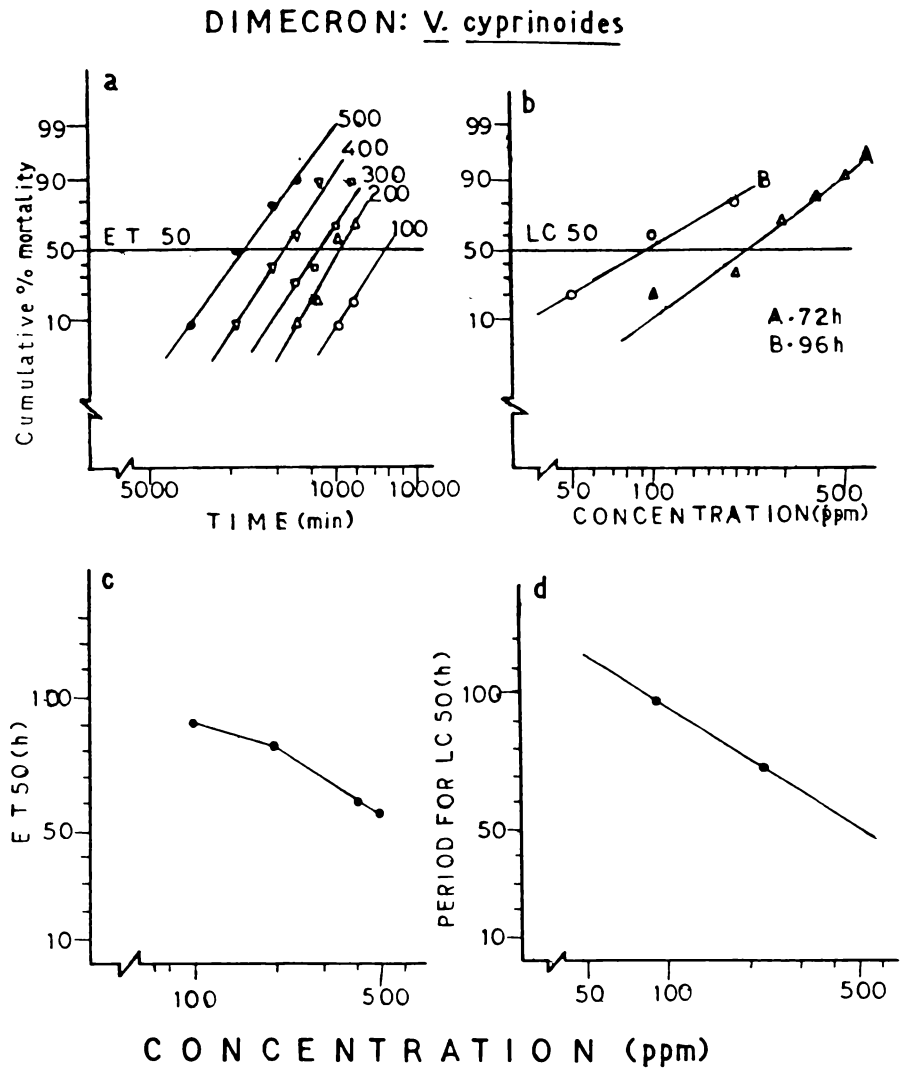


Fig. 7. Villorita cyprinoides var. cochinensis. Lethal effect of Dimecron

a. Progress of mortality against time

b. Progress of mortality against concentration

c & d. Toxicity curves

1000 ppm. However, 250 ppm and above were found to be highly lethal even during the early phase of the experiment. The 96 h LC50 worked out was 74.77 ppm. Mortality occurred in all the concentrations employed, although the rate of mortality was not very significant in 25 ppm and 50 ppm. It is not sure whether the higher concentrations employed were realistic, since a certain amount of precipitation was observed when the concentrations employed exceeded above 100 ppm. (Table 2, Fig. 8 a-d).

4.1.1.2.4 Aldrex

The test concentrations of Aldrex used for the experiment ranged from 10 to 75 ppm. The animals were found to survive beyond 96 h when the external concentration was 10 ppm. It was seen from the experiments that upto 25 ppm of Aldrex, rate of mortality was negligible. 50 ppm and 75 ppm resulted in pronounced lethality and no animals survived beyond 60 h in 75 ppm. The 96 h LC50 was 48.43 ppm (Table 2, Fig. 9 a-d).

4.1.1.2.5 Light Diesel Oil-Water Accommodated Fraction - LDO (WAF)

The WAF of LDO was employed to find out the lethal toxicity of this component on V. cyprinoides var. cochinensis. The concentrations of PHC contained in the WAF varied from 12 to 24.8 ppm (100% WAF). During the early 48 h practically no animals died even when the external concentration reached 22 ppm. However, with the passage of time the oil fraction was found to exert a certain degree of lethal effect on this animal. The 96 h LC50 worked out was 17.28 ppm. When 100% LDO (WAF) was used none of the animals survived after 96 h (Table 2, Fig. 10 a-d).

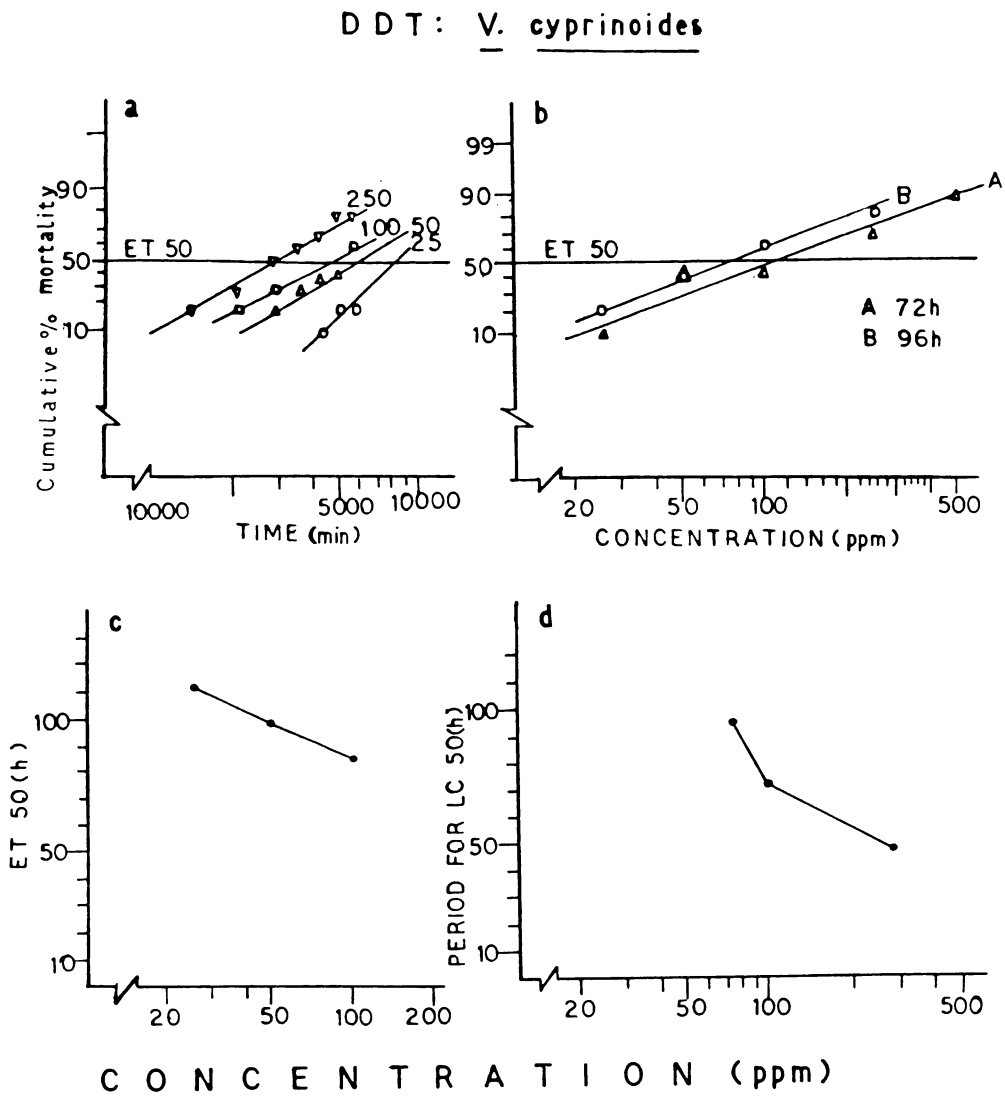


Fig. 8. Villorita cyprinoides var. cochinensis.
Lethal effects of DDT
a. Progress of mortality against time
b. Progress of mortality against concentration
c & d. Toxicity curves

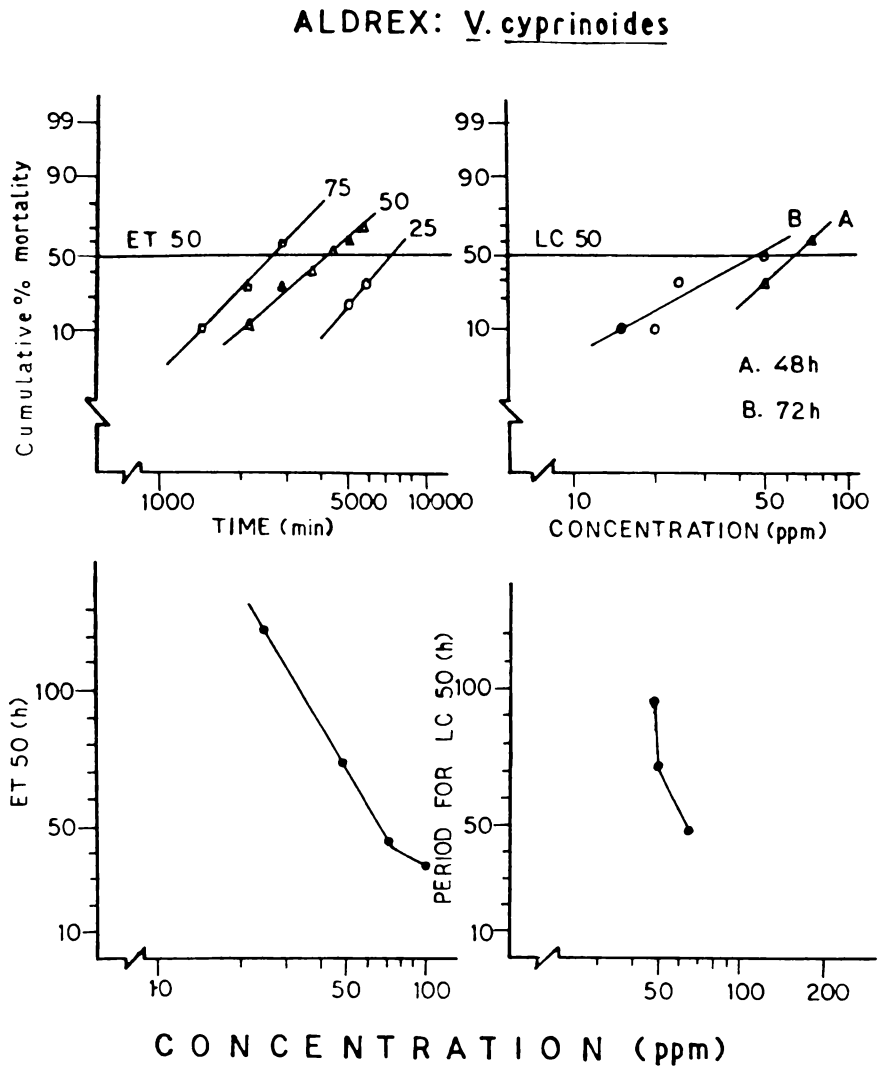


Fig. 9. Villorita cyprinoides var. cochinensis
Lethal effects of Aldrex
a. Progress of mortality against time
b. Progress of mortality against concentration
c & d. Toxicity curves

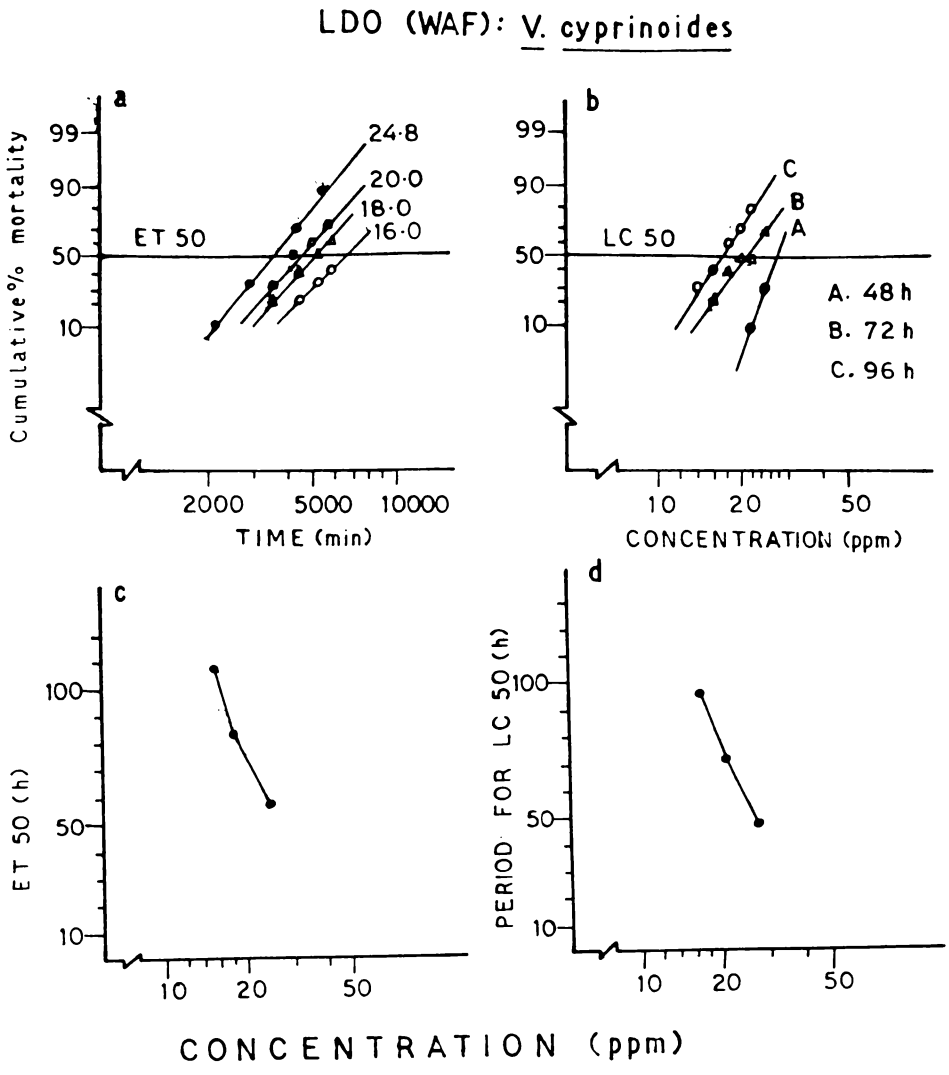


Fig. 10. Villorita cyprinoides var. cochinensis
Lethal effects of PHC in LDO (WAF)
a. Progress of mortality against time
b. Progress of mortality against concentration
c & d. Toxicity curves

Table 2. Villorita cyprinoides var. cochinensis. LC50 (ppm), when exposed to Ekalux, Dimecron, DDT, Aldrex, Light Diesel Oil (LDO-WAF) and Persian Gulf Crude (P.G. Crude-WAF) over periods upto 96h, along with respective 95% confidence limits* and slope functions.

Toxicant	48h		72h		96h	
	LC50 (ppm) (95% C.L.)	Slope 'b'	LC50 (ppm) (95% C.L.)	Slope 'b'	LC50 (ppm) (95% C.L.)	slope 'b'
Ekalux	29.35 (-)	3.63	12.00 (9.01 - 15.98)*	3.53	5.07 (4.17 - 6.16)*	3.53
Dimecron	766.17 (-)	4.54	224.25 (178.51 - 281.72)*	2.96	93.28 (76.82 - 113.27)*	2.80
D D T	280.84 (195.36 - 403.73)*	1.17	100.77 (76.76 - 132.28)*	1.89	74.77 (69.20 - 80.79)*	1.68
Aldrex	65.72 (-)	4.42	50.00 (-)	-	48.43 (32.6 - 71.94)*	2.65
LDO (WAF)	26.95 (-)	14.56	20.76 (19.74 - 21.83)*	6.45	17.28 (16.96 - 17.61)*	8.52
P.G. Crude (WAF)	No mortality even after exposure to 96h at concentrations upto 100% WAF (PHC 13.2 ppm)					

4.1.1.2.6 Persian Gulf Crude - P.G. Crude (WAF)

No mortality of V. cyprinoides var. cochinensis occurred even when the animals were exposed to 100% WAF in which the PHC concentration was 13.2 ppm.

4.1.2 COMBINED LETHAL TOXICITY

The toxicity of combinations of pesticides and WAFs of refined oil was studied to delineate whether the reactions were less than additive, more than additive or simple additive. It is known that the additivity can be a function of concentrations, duration of exposure, rate of uptake and detoxification. However, in lethal toxicity experiments the animals are exposed to unrealistic concentrations of pollutants which bring about sudden shock reaction and death of the animals. Combined toxicity studies would help to evaluate the above points. Therefore, in the following series of experiments presented here, both P. indica and V. cyprinoides var. cochinensis were subjected to exposure to series of combinations of organophosphates, organochlorines vs. petroleum hydrocarbon (PHC) contained in the WAF of LDO.

4.1.2.1 Combined Lethal Toxicity on Perna indica

The lethal toxicity of combinations of pesticides and PHC in WAF of LDO was worked out to analyse the impact of these combinations on the survival rates of Perna indica. These observations, are of topical importance since, P. indica is likely to encounter combinations of pesticides and oil fractions in their natural habitat, which is usually the intertidal and subtidal reaches of the rocky shores along the south west coast of India in the vicinity of which are located some important ports and fishing harbours.

4.1.2.1.1 Ekalux unvarying and PHC in LDO (WAF) varying

The cumulative percentage mortality of P. indica exposed to Ekalux (unvarying) with varying concentrations of PHC in LDO (WAF) was assessed. The concentrations of PHC in LDO (WAF) ranged between 2.0 to 3.0 ppm and no mortality occurred in 96 h time in combination with 1.5 ppm Ekalux. The concentration of Ekalux was increased to 1.75 ppm, 2.0 ppm and 2.5 ppm, to find out whether enhancement in the Ekalux concentration would influence mortality. However, only negligent mortality was recorded by increasing the Ekalux component in the mixture. It may be pointed out here, that the 96 h LC50 of Ekalux alone was 3.22 ppm and that of LDO (WAF) was 4.62 ppm (Table 3).

4.1.2.1.2 PHC in LDO (WAF) unvarying and Ekalux varying

In another series of experiment the concentrations of Ekalux were varied retaining the PHC concentrations in LDO (WAF) unvarying. The concentrations employed in the combinations were 1.50 ppm to 2.50 ppm of Ekalux and PHC in LDO (WAF) as individual component ranged from 2.0 ppm to 3.0 ppm. The results obtained from 25 such observations substantiated that in these combinations, P. indica did not show any lethal stress evidenced by mortality (Table 3).

4.1.2.1.3 Dimecron unvarying and PHC in LDO (WAF) varying

After finding out the individual toxicity of Dimecron and LDO (WAF) a series of experiments were conducted to find out the combined toxicity of these two components. The criterion employed to select the concentrations and their combinations was preparing mixtures containing around 8%

Table 3. Perna indica. Lethal effect on exposure to combinations of Ekalux and LDO (WAF) concentrations (ppm), over periods upto 96h.

Concentrations (ppm) in combinations		Toxic response after 96h	96h LC50 (ppm)	
Ekalux	PHC in LDO (WAF)		Ekalux	LDO (WAF)
1.50				
1.75				
2.00	Varying from 2.0 to 3.0	No Mortality		
2.25				
2.50				
			3.22	4.62
	2.00			
	2.25			
Varying from 1.5 to 2.5	2.50	No Mortality		
	2.75			
	3.00			

to 50% of 96 h LC50 value in the case of LDO (WAF) and about 25 to around 60% in the case of Dimecron. A series of 25 experiments were conducted and the concentration of the individual component varied between 15 ppm to 60 ppm of Dimecron and 1.0 ppm to 2.5 ppm of PHC in LDO (WAF). No mortality was recorded in any of these combinations employed (Table 4).

4.1.2.1.4 PHC in LDO (WAF) unvarying and Dimecron varying

In this set of experiments the concentrations of Dimecron were varied, keeping the PHC concentration in LDO (WAF) unchanged. The concentrations used in the combinations were 15 ppm to 60 ppm of Dimecron along with a PHC in LDO (WAF) concentration. 25 such experiments were conducted in which PHC concentration in LDO (WAF) as individual component ranged from 1.0 ppm to 2.50 ppm. The results clearly indicated that P. indica could survive in these concentrations without showing any lethal stress as evidenced by mortality (Table 4).

4.1.2.1.5 DDT unvarying and PHC in LDO (WAF) varying

DDT in combination with PHC in LDO (WAF) was employed to find out the combined toxicity of these two components on the rate of survival of P. indica. 1.0 ppm of DDT along with 1.0 to 2.5 ppm PHC in LDO (WAF) did not bring in mortality to the test individuals upto 60 h. However, 50% of the test organisms died, when the PHC concentration in LDO (WAF) was 2.39 ppm (computed value). 2.0 and 3.0 ppm of DDT were also found to be non-lethal at least upto 48 h in combination with 1.0 to 2.5 ppm of PHC in LDO (WAF). Death occurred in these combinations after 60 h and

Table 4. Perna indica. Lethal effects on exposure to combinations of Dimecron and LDO (WAF) concentrations (ppm), over periods upto 96h.

Concentrations (ppm) in combinations		Toxic response after 96h	96h LC50 (ppm)	
Dimecron	PHC in LDO (WAF)		Dimecron	LDO (WAF)
15.0 25.0 40.0 50.0 60.0	Varying from 1.0 to 2.5	No Mortality	117.58	4.62
Varying from 15.0 to 60.0	1.00 1.25 1.75 2.00 2.50	No Mortality		

at the end of 96 h 2.0 ppm of DDT with 1.96 ppm PHC in oil and 3.0 ppm of DDT with 1.27 ppm PHC in oil recorded 50% mortality. An increase in the concentration of DDT to 4.0 and 5.0 ppm resulted in drastic increase in mortality rates. Animals were found to die within 24 h in both these concentrations and none of the individuals maintained in 5.0 ppm of DDT with 2.0 and 2.5 ppm PHC in oil remained alive after 84 h. The different LC50 values have been worked out and are presented in Table 5 and Fig. 11 a-c to 15 a-d. The 96 h LC50 values for the higher concentrations were 0.74 ppm PHC in oil with 4.0 ppm of DDT and 0.65 ppm PHC in oil with 5.0 ppm of DDT. The combined toxicity of these two components was found to be more than additive even in the lowest concentrations viz. 1.0 ppm of DDT with 1.0 to 2.5 ppm PHC in LDO (WAF).

4.1.2.1.6 PHC in LDO (WAF) unvarying and DDT varying

In a reciprocal series of experiments the PHC concentration in LDO (WAF) was retained unvarying and DDT varying. In this series of experiments 1.0 ppm PHC in LDO along with 1.0 to 5.0 ppm of DDT recorded death after 60 h in concentrations, where the DDT was 5.0 ppm. The computed LC50 value for this combination was 5.47 ppm after 72 h. Increase in the PHC concentration in LDO (WAF) to 2.5 ppm was found to drastically enhance the toxicity of DDT, thus 2.5 ppm PHC in LDO (WAF) along with 2.32 ppm of DDT resulted in 50% mortality of the test organisms after 72 h. The DDT value was reduced to 1.0 ppm after 96 h. The 96 h LC50 value ranged between 1.0 ppm PHC in LDO (WAF) with 3.47 ppm of DDT and 2.5 ppm PHC in LDO (WAF) with 1.0 ppm of DDT. The death was found to be brought about by a more than additive reaction as evidenced by the computed values on additive indices. (Table 6; Fig. 16 a-d to 20 a-d).

Table 5. Perna indica. LC50 (ppm), when exposed to unvarying concentration of DDT, with varying concentrations of PHC in LDO (WAF), over periods upto 96h, along with respective 95% confidence limits*, slope functions and additive indices (AI)

D D T Concentration (ppm)	48h			72h			96h		
	PHC in LDO (WAF) LC50 (ppm) (95% C.L)*	slope 'b'	AI	PHC in LDO (WAF) LC50 (ppm) (95% C.L)*	slope 'b'	AI	PHC in LDO (WAF) LC50 (ppm) (95% C.L)*	slope 'b'	AI
1.0	-	-	-	-	-	-	2.39 (2.21 - 2.59)*	4.35	0.64 (M.A)
2.0	-	-	-	3.16 (-)	4.76	0.71 (M.A.)	1.96 (1.91 - 2.00)*	4.43	0.61 (M.A)
3.0	-	-	-	2.68 (2.28 - 3.16)*	2.88	0.71 (M.A.)	1.27 (1.19 - 1.37)*	2.66	0.79 (M.A)
4.0	3.89 (0.96 - 15.74)*	2.89	0.75 (S.A.)	1.66 (1.33 - 2.08)*	2.84	0.98 (M.A.)	0.74 (0.46 - 1.20)*	2.77	0.87 (M.A)
5.0	3.09 (1.26 - 7.60)*	2.87	0.87 (S.A.)	1.18 (0.98 - 1.43)*	3.50	0.50 (M.A.)	0.65 (-)	4.54	0.64 (M.A)

M.A More than additive S.A. Simple additive

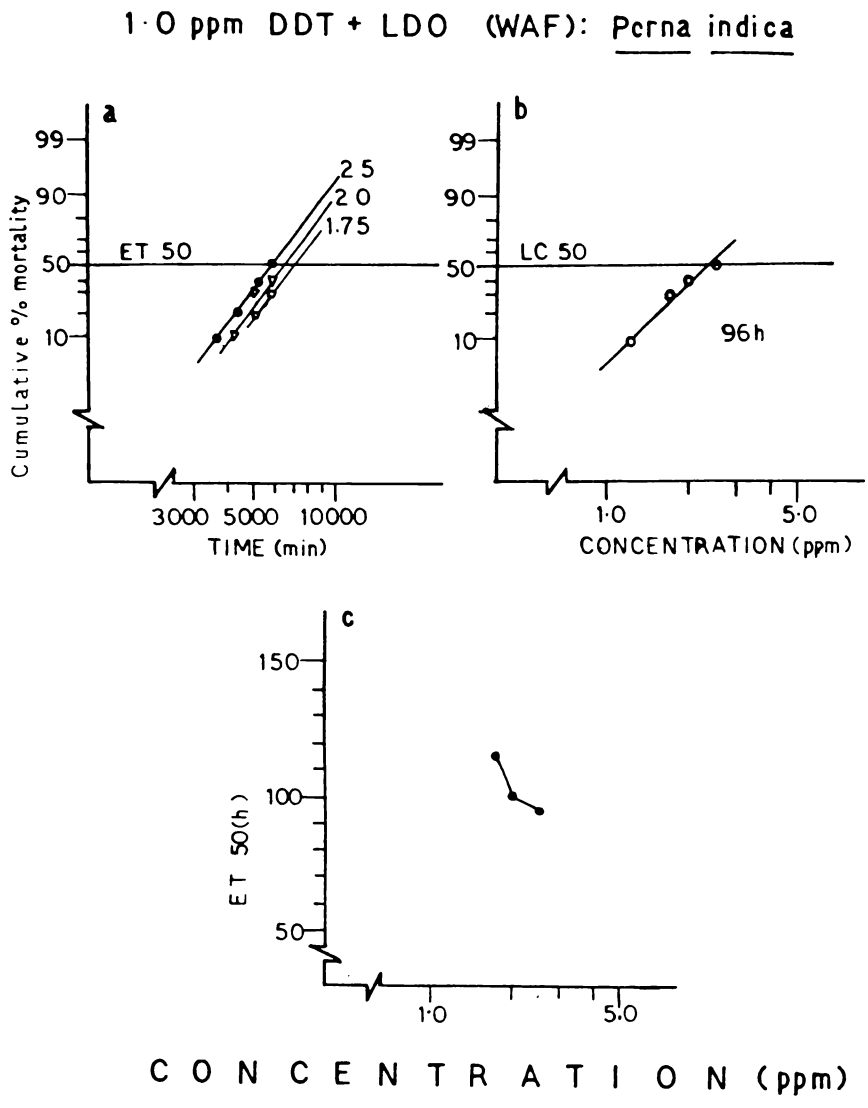


Fig. 11. Perna indica. Combined lethal effects of 1.0 ppm DDT (unvarying) and PHC in LDO-WAF-(Varying)

a. Progress of mortality against time

b. Progress of mortality against concentration

c & d. Toxicity curves

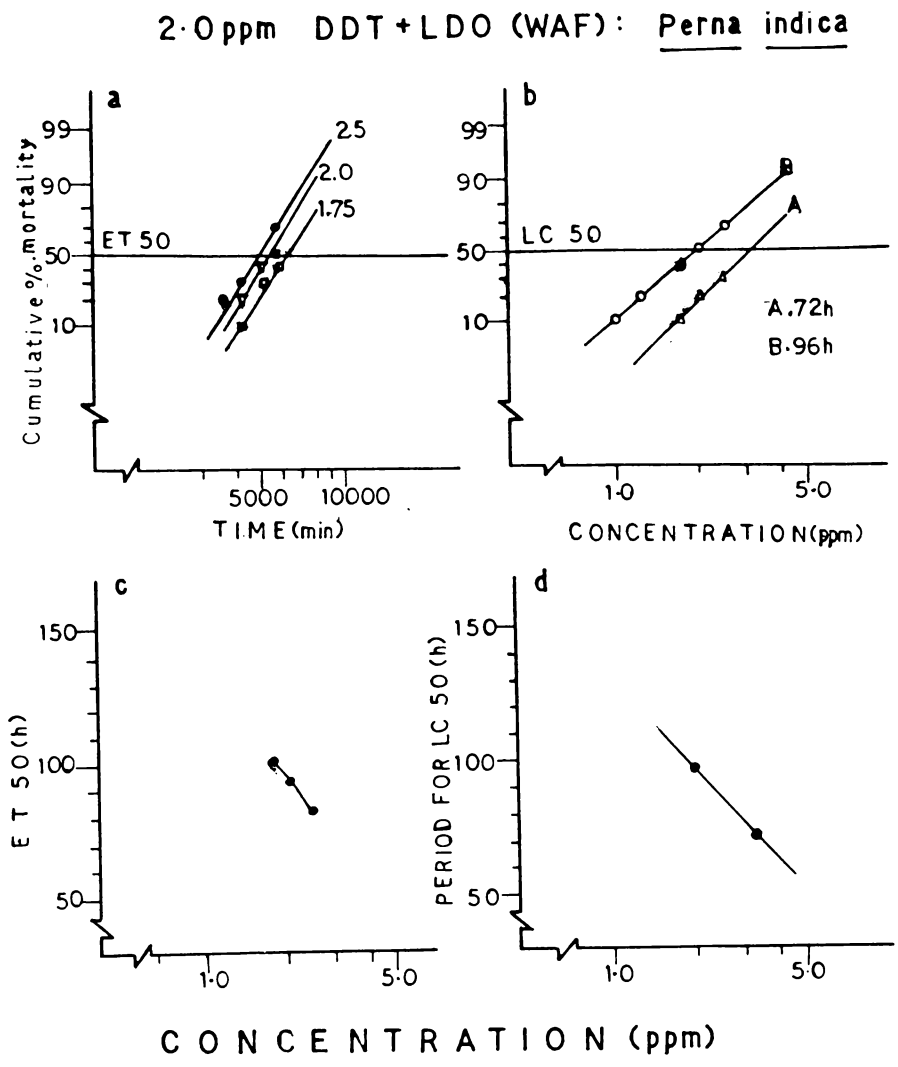


Fig. 12. Perna indica. Combined lethal effects of 2.0 ppm DDT (unvarying) and PHC in LDO-WAF-(varying)

a. Progress of mortality against time

b. Progress of mortality against concentration

c & d. Toxicity curves

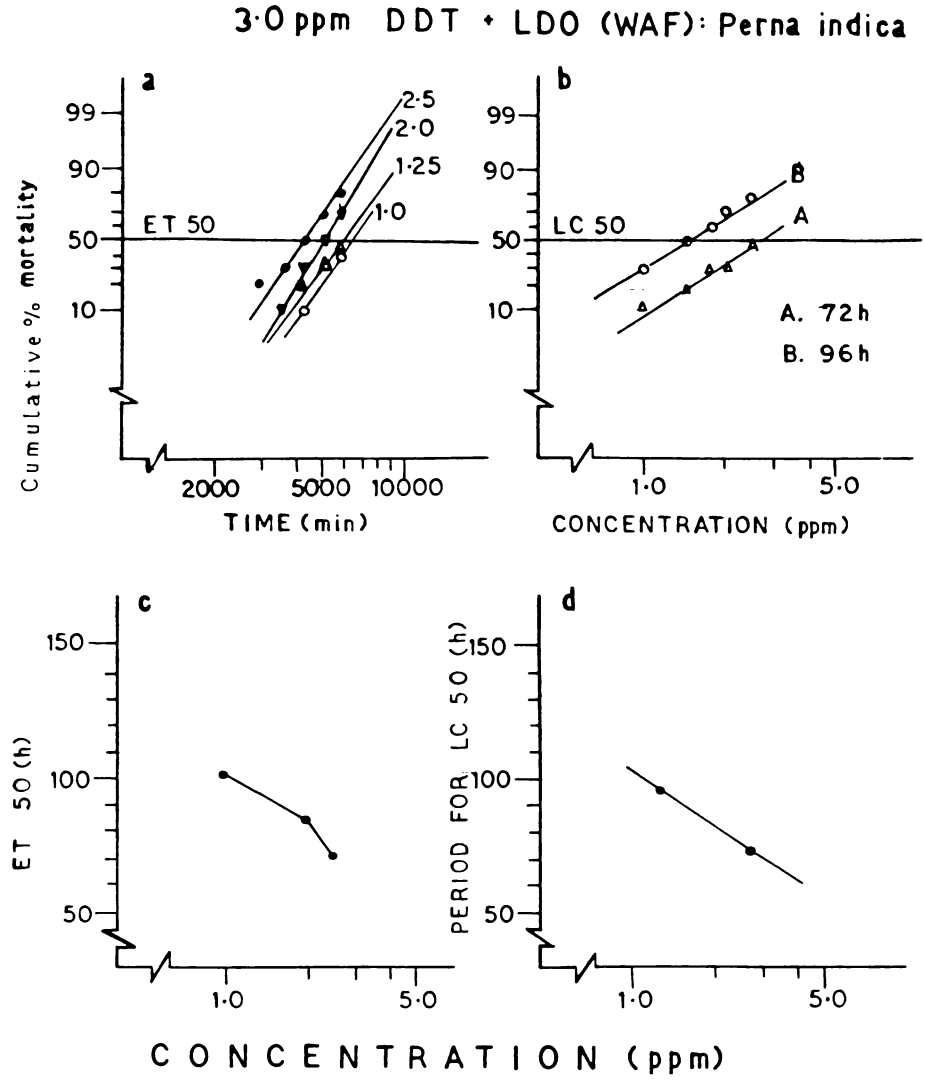


Fig. 13. Perna indica. Combined lethal effects of 3.0 ppm DDT (unvarying) and PHC in LDO-WAF-(varying)

a. Progress of mortality against time

b. Progress of mortality against concentration

c & d. Toxicity curves

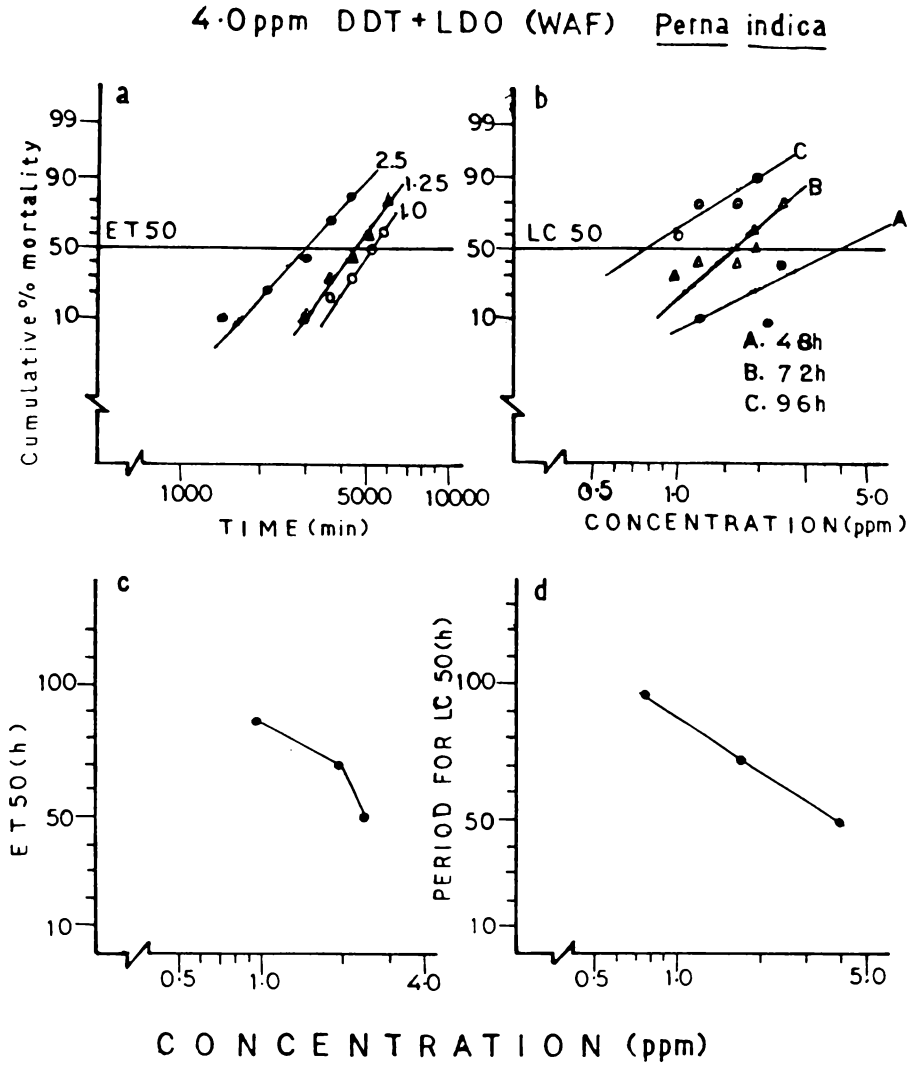


Fig. 14. Perna indica. Combined lethal effects of 4.0 ppm DDT (unvarying) and PHC in LDO-WAF-(varying)

a. Progress of mortality against time

b. Progress of mortality against concentration

c & d. Toxicity curves

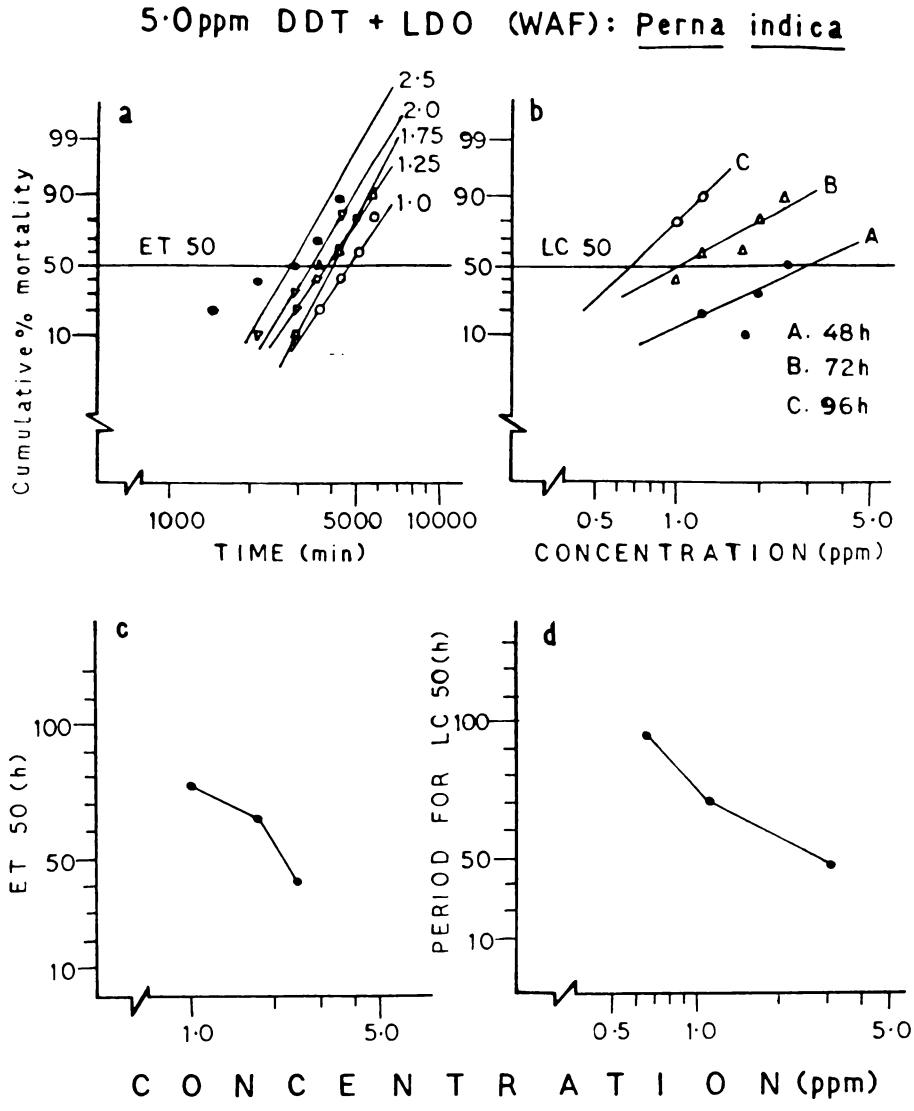


Fig. 15. Perna indica. Combined lethal effects of 5.0 ppm DDT (unvarying) and PHC in LDO-WAF-(varying)

a. Progress of mortality against time

b. Progress of mortality against concentration

c & d. Toxicity curves

Table 6. Perna indica. LC50 (ppm), when exposed to unvarying concentration of PHC in LDO (WAF), with varying concentrations of DDT, over periods upto 96h, along with respective 95% confidence limits*, slope functions and additive indices (AI)

PHC in LDO (WAF) Concentration (ppm)	48h			72h			96h		
	D.D.T., LC50 (ppm) (95% C.L)*	slope 'b'	AI	D.D.T., LC50 (ppm) (95% C.L)*	slope 'b'	AI	DDT., LC50 (ppm) (95% C.L)*	slope 'b'	AI
1.00	-	-	-	5.47	4.72	0.96 (M.A.)	3.47 (3.36 - 3.59)*	5.23	0.84 (M.A.)
1.25	-	-	-	4.46 (4.40 - 4.53)*	4.92	1.09 (M.A.)	2.61 (2.16 - 3.14)*	3.77	0.94 (M.A.)
1.75	-	-	-	4.38 (4.04 - 4.75)*	3.69	0.84 (M.A.)	2.03 (1.55 - 2.67)*	2.16	1.76 (M.A.)
2.00	5.81 (-)	7.93	1.18 (M.A.)	3.53 (2.61 - 4.77)*	2.74	0.91 (M.A.)	1.52 (1.03 - 2.25)*	2.38	0.74 (M.A.)
2.50	4.87 (4.45 - 5.34)*	3.84	1.14 (M.A.)	2.32 (1.83 - 2.96)*	3.05	0.95 (M.A.)	1.00 (0.99 - 1.01)*	1.76	0.57 (M.A.)

M.A: More than additive

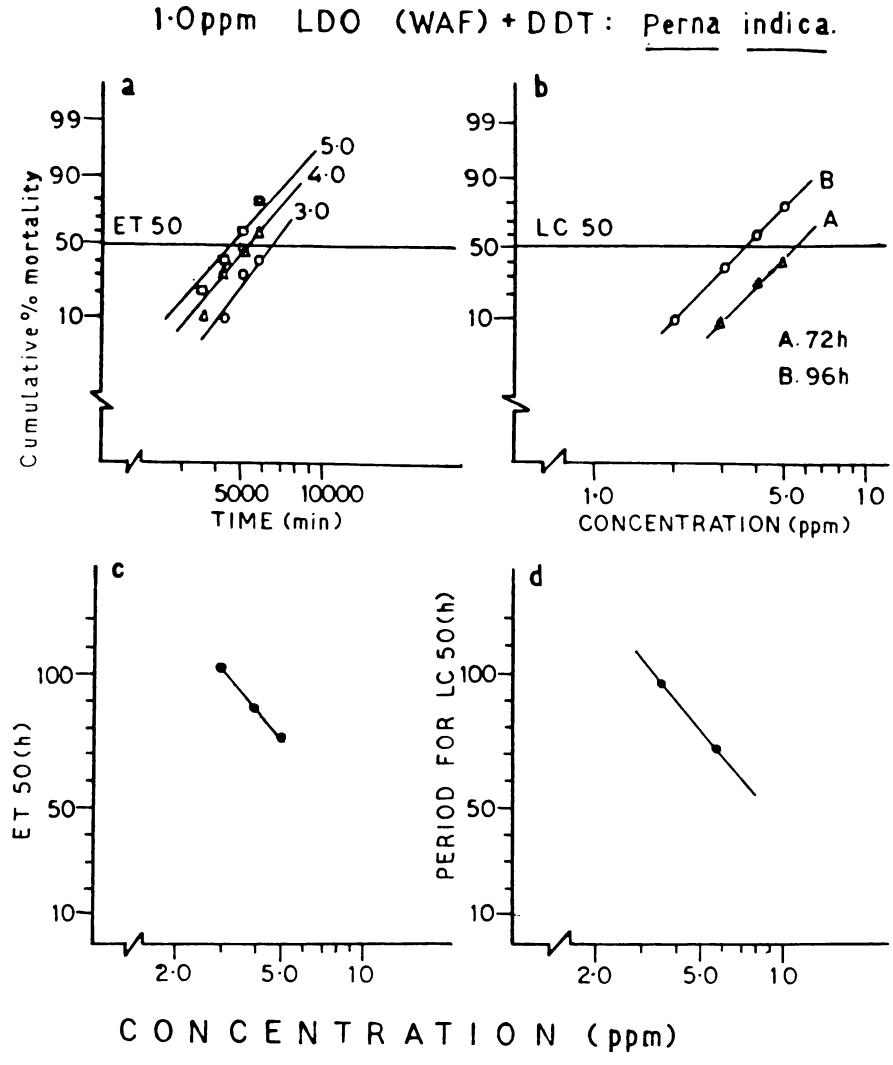


Fig. 16. Perna indica. Combined lethal effects of 1.0 ppm PHC in LDO-WAF-(unvarying) and DDT (varying)

a. Progress of mortality against time

b. Progress of mortality against concentration

c & d. Toxicity curves

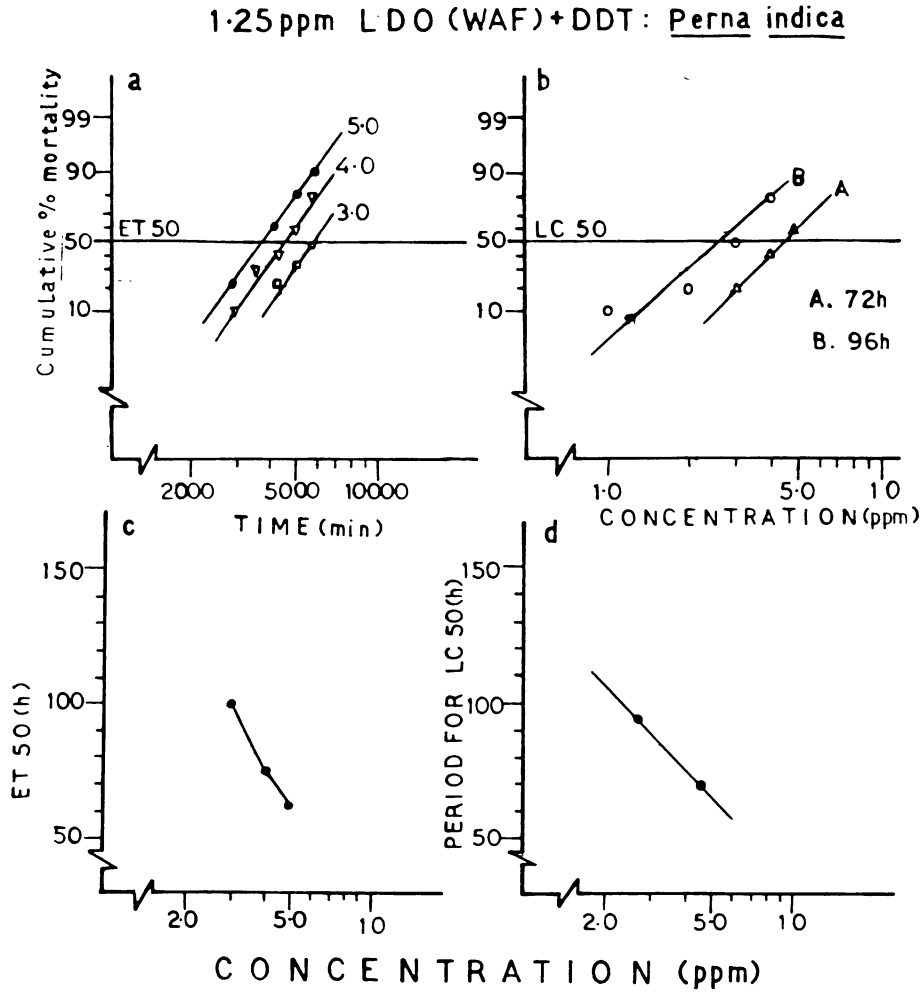


Fig. 17. Perna indica. Combined lethal effects of 1.25 ppm PHC in LDO-WAF-(unvarying) and DDT (varying)

a. Progress of mortality against time

b. Progress of mortality against concentration

c & d. Toxicity curves

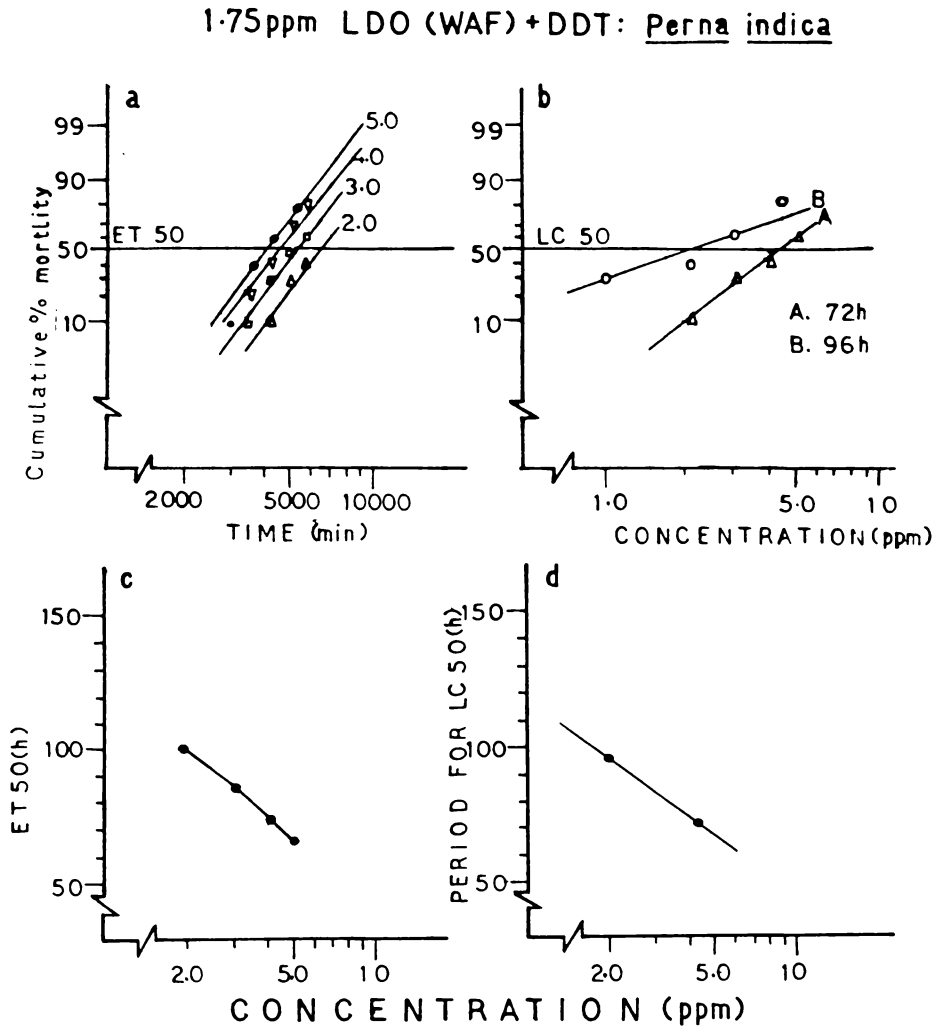


Fig. 18. Perna indica. Combined lethal effects of 1.75 ppm PHC in LDO-WAF-(unvarying) and DDT (varying)

a. Progress of mortality against time

b. Progress of mortality against concentration

c & d. Toxicity curves

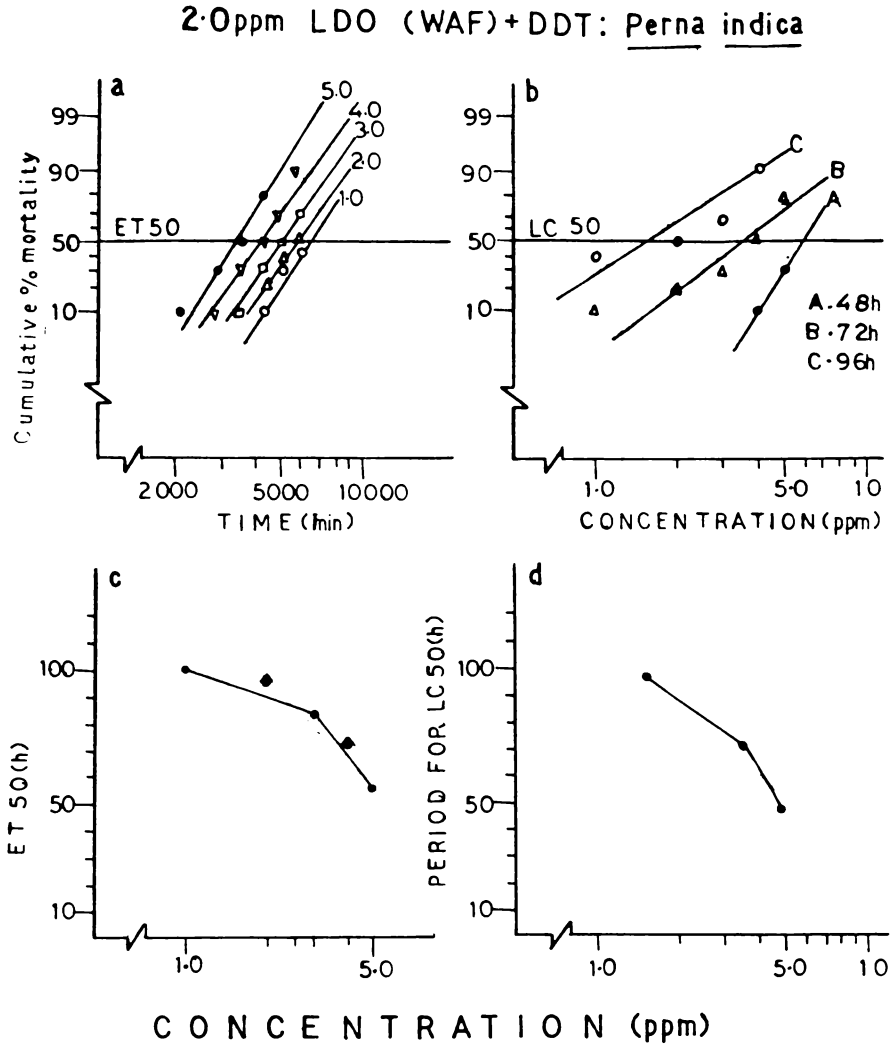


Fig. 19. Perna indica. Combined lethal effects of 2.0 ppm PHC in LDO-WAF- (unvarying) and DDT (varying)

a. Progress of mortality against time

b. Progress of mortality against concentration

c & d. Toxicity curves

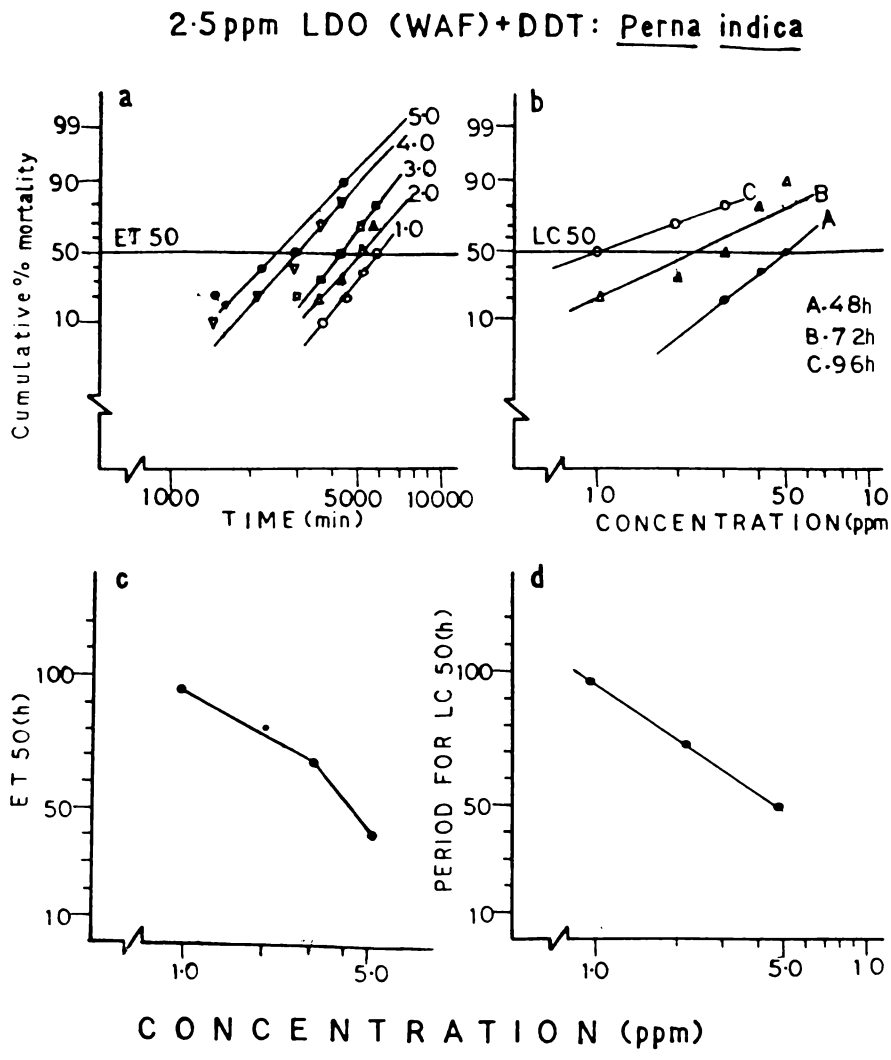


Fig. 20. Perna indica. Combined lethal effects of 2.5 ppm PHC in LDO-WAF-(unvarying) and DDT (varying)

a. Progress of mortality against time

b. Progress of mortality against concentration

c & d. Toxicity curves

4.1.2.1.7 Aldrex unvarying and PHC in LDO (WAF) varying

In the case of Aldrex it was noticed that its individual 96 h LC50 was 8.76 ppm. A combination of Aldrex and PHC in LDO (WAF) containing various concentrations of these two components ranging from 10 to around 40% of 96 h LC50 values did not register any mortality. Even after raising the LDO (WAF) concentration to 3.0 ppm the animals did not die. Therefore another set of experiment was conducted in which the Aldrex concentration was increased upto 8.0 ppm in combination with 2.0 to 3.0 ppm PHC in LDO (WAF). The results are given in Table 7. Practically no death occurred within 48 h in combinations which contained 4.0 or 5.0 ppm of Aldrex and 2.0 to 2.75 ppm PHC in LDO (WAF). Whereas, in those combinations which contained 6.0 to 8.0 ppm of Aldrex and 2.0 to 3.0 ppm PHC in LDO (WAF) very high mortality occurred within 48 h. The mortality rate drastically increased in all combinations by 72 h. The 96 h LC50 recorded was 4.0 ppm Aldrex and 2.53 ppm PHC in LDO (WAF), 5.0 ppm Aldrex and 2.33 ppm PHC in LDO (WAF) and 7.0 ppm of Aldrex with 1.99 ppm PHC in LDO (WAF). None of the animals kept in 8.0 ppm of Aldrex and 2.5 to 3.0 ppm of PHC in LDO (WAF) survived beyond 72 h. Mortality was brought about by a more than additive reaction of these two toxicants. Curiously enough in all the concentrations, the results recorded after 96 h was found to be simple additive in nature (Table 7; Fig 21 a-d to 25 a-d).

4.1.2.1.8 PHC in LDO (WAF) unvarying and Aldrex varying

Table 8 represents the results obtained in lethality of P. indica exposed to unvarying concentrations of PHC in LDO (WAF) with varying concentrations of Aldrex over periods upto 96 h along with 95% confidence limits etc.

Table 7. Perna indica. LC50 (ppm), when exposed to unvarying concentration of Aldrex, with varying concentrations of PHC in LDO (WAF), over periods upto 96h, along with respective 95% confidence limits*, slope functions and additive indices (AI)

Aldrex concentration (ppm)	48h			72h			96h		
	PHC in LDO (WAF) LC50 (ppm) (95% C.L)*	slope 'b'	AI	PHC in LDO (WAF) LC50 (ppm) (95% C.L)*	slope 'b'	AI	PHC in LDO (WAF) LC50 (ppm) (95% C.L)*	slope 'b'	AI
4.0	-	-	-	2.83 (2.57 - 3.12)*	6.29	0.39 (M.A.)	2.53 (2.47 - 2.59)*	10.42	-0.004 (S.A.)
5.0	-	-	-	2.57 (2.42 - 2.72)*	15.18	0.31 (M.A.)	2.33 (2.28 - 2.38)*	12.68	-0.08 (S.A.)
6.0	2.78 (2.74 - 2.83)*	17.30	0.74 (M.A.)	2.37 (2.33 - 2.4)*	12.54	0.23 (M.A.)	2.14 (1.99 - 2.30)*	12.59	-0.15 (L.A.)
7.0	2.74 (2.73 - 2.75)*	6.39	0.62 (M.A.)	2.20 (2.15 - 2.27)*	21.8	0.15 (M.A.)	1.99 (-)	25.05	-0.23 (L.A.)
8.0	2.40 (2.32 - 2.49)*	18.89	0.63 (M.A.)	1.99	16.45	0.09 (M.A.)	-	-	-

M.A: More than additive, L.A: Less than additive; S.A: Simple additive.

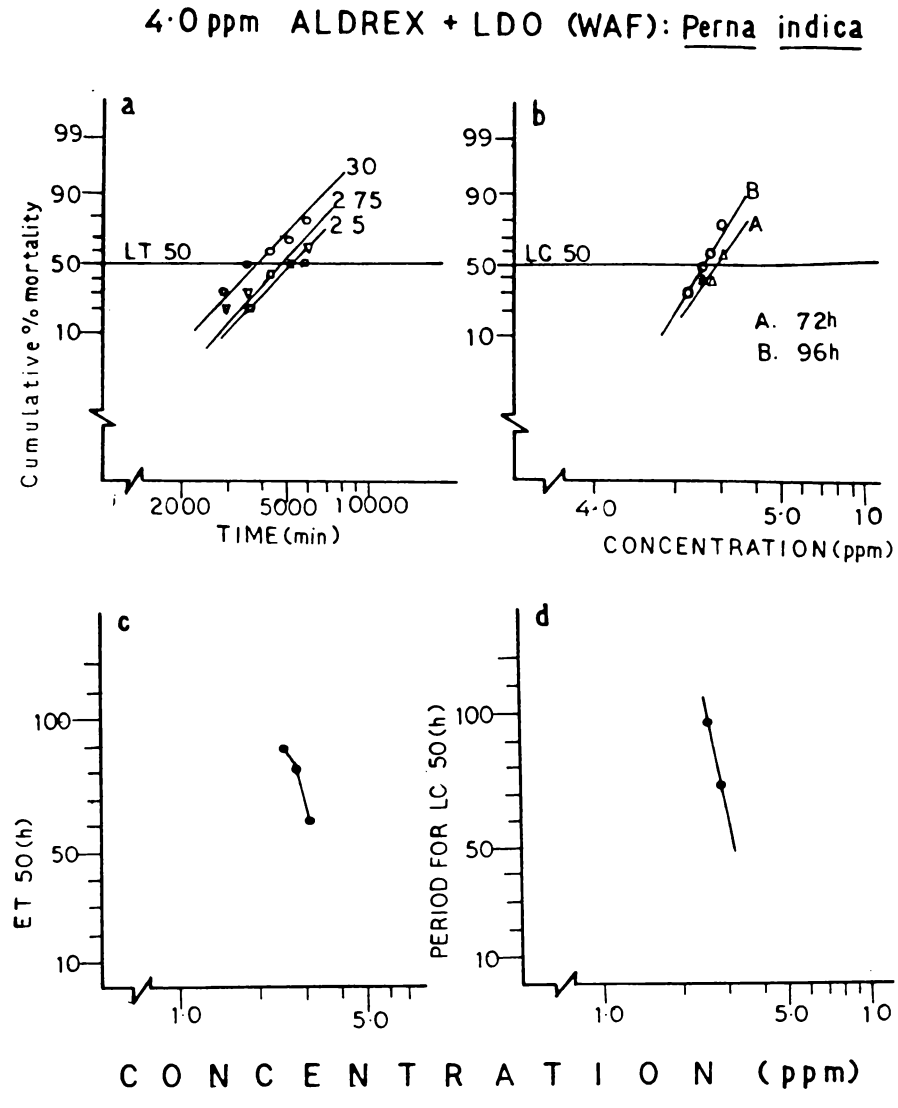


Fig. 21. Perna indica. Combined lethal effects of 4.0 ppm Aldrex (unvarying) and PHC in LDO-WAF- (varying)

a. Progress of mortality against time

b. Progress of mortality against concentration

c & d. Toxicity curves

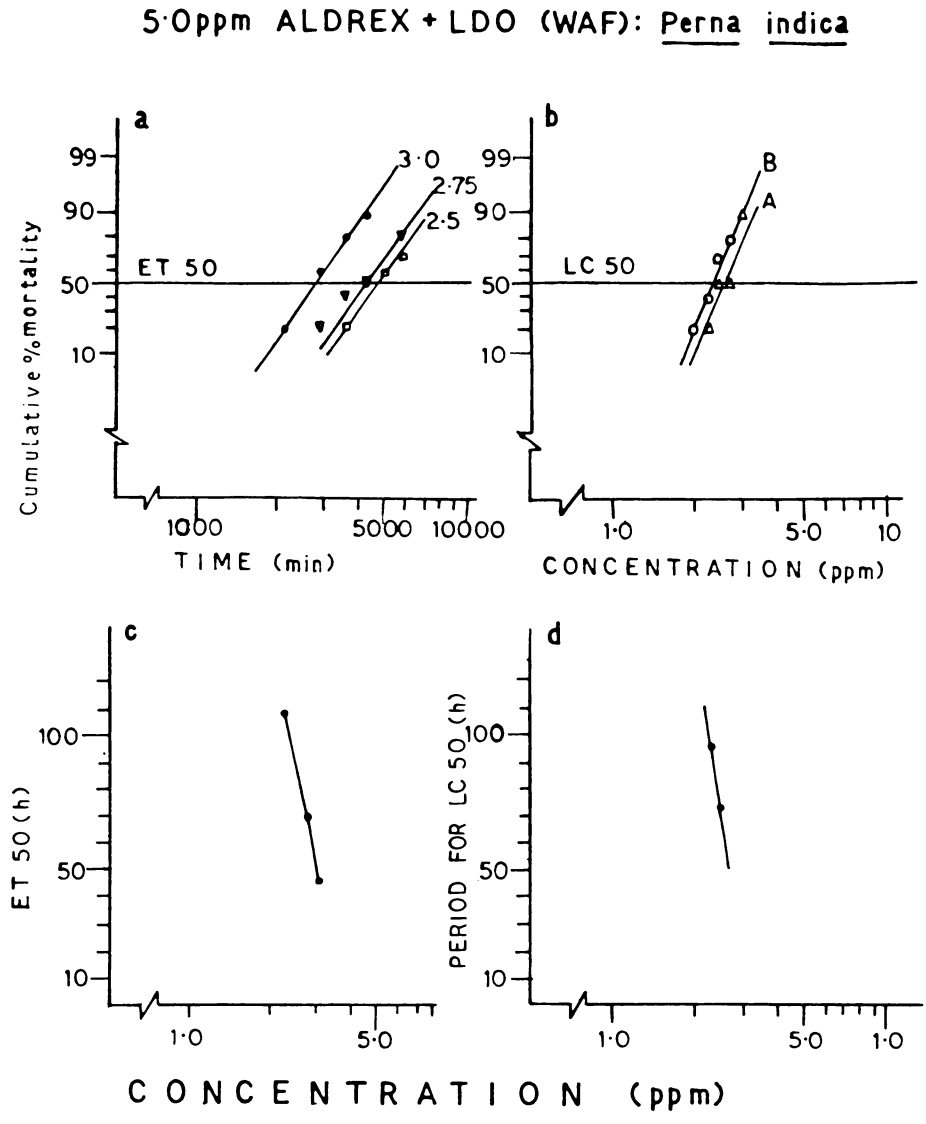


Fig. 22. Perna indica. Combined lethal effects of 5.0 ppm Aldrex (unvarying) and PHC in LDO-WAF- (varying)

a. Progress of mortality against time

b. Progress of mortality against concentration

c & d. Toxicity curves

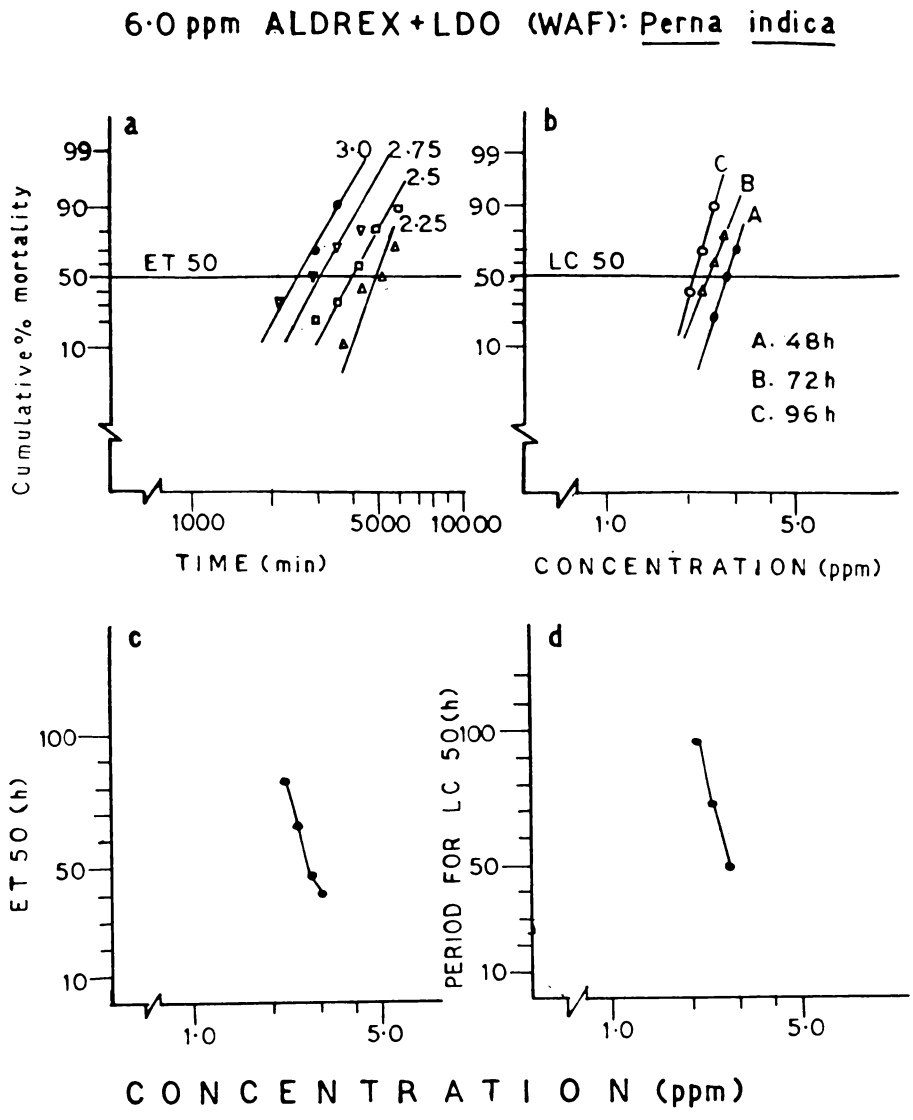


Fig. 23. Perna indica. Combined lethal effects of 6.0 ppm Aldrex (unvarying) and PHC in LDO-WAF- (varying)

a. Progress of mortality against time

b. Progress of mortality against concentration

c & d. Toxicity curves

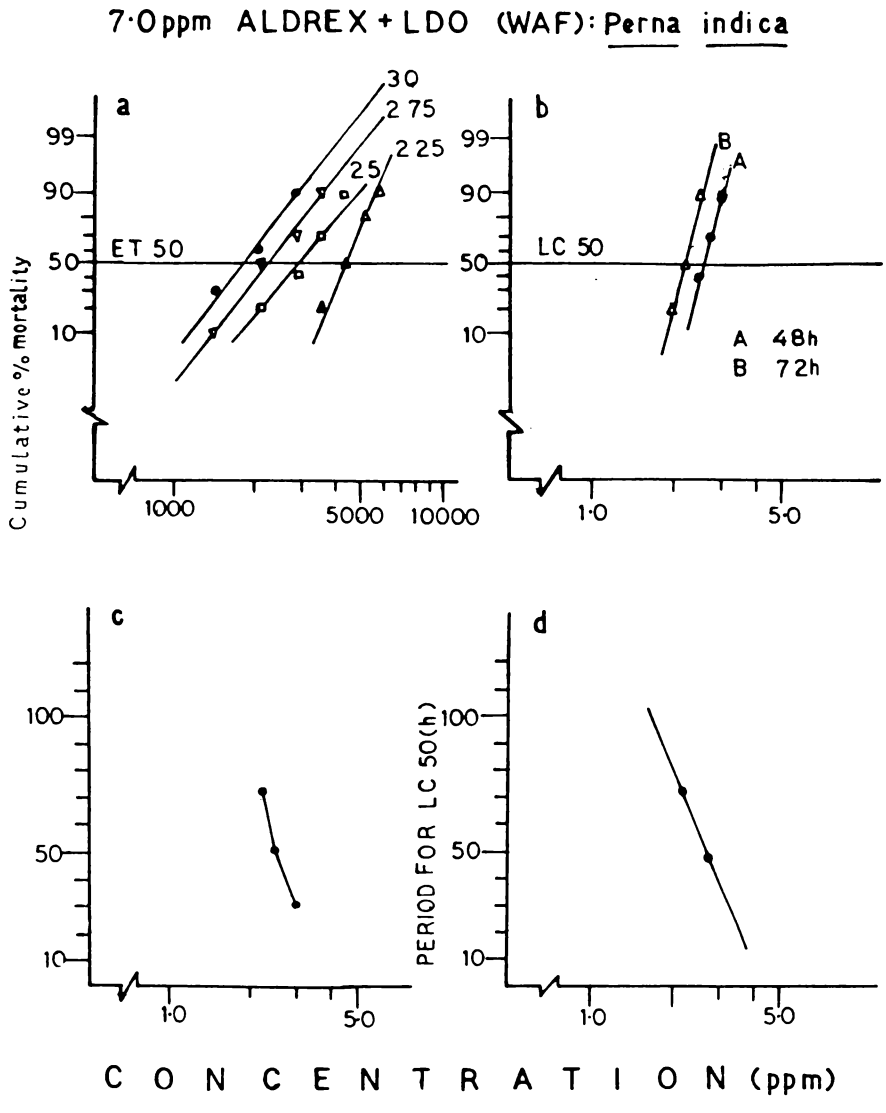


Fig. 24. Perna indica. Combined lethal effects of 7.0 ppm Aldrex (unvarying) and PHC in LDO-WAF- (varying)

a. Progress of mortality against time

b. Progress of mortality against concentration

c & d. Toxicity curves

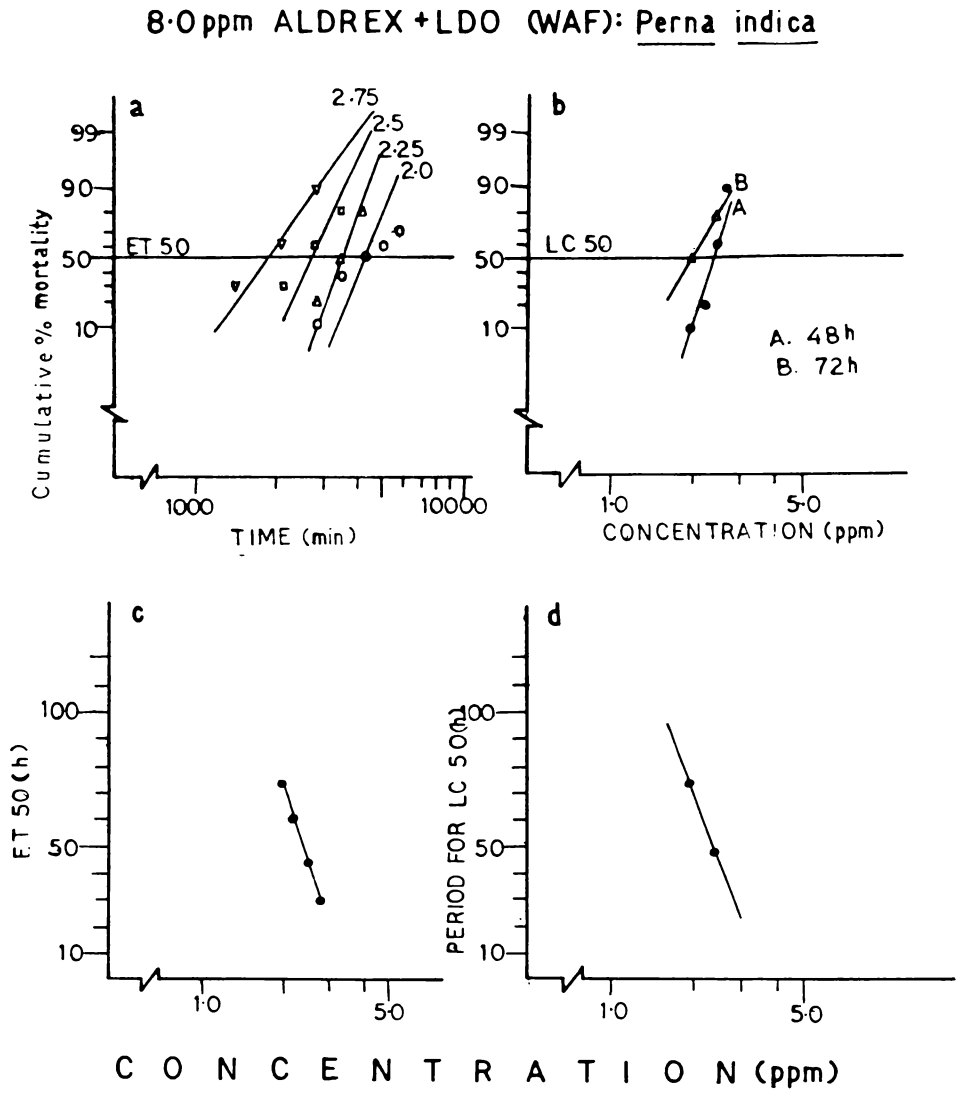


Fig. 25. Perna indica. Combined lethal effects of 8.0 ppm Aldrex (unvarying) and PHC in LDO-WAF- (varying)

a. Progress of mortality against time

b. Progress of mortality against concentration

c & d. Toxicity curves

2.0 ppm PHC in LDO (WAF) and 4.0 to 8.0 ppm of Aldrex did not bring about considerable death of the test organisms in 72 h. Whereas, increase in the concentration of PHC in LDO (WAF) to 2.5 ppm resulted in the sporadic death of the animals from 48 h onwards. Here also, none of the animals retained in test media which contained 3.0 ppm of PHC in LDO (WAF) with 7.0 or 8.0 ppm of Aldrex survived beyond 60 h. A conspicuous toxic effect of Aldrex was noticed after 72 h in those combinations which contained 2.5 and 3.0 ppm of PHC in LDO (WAF). The results are given in Table 8 and Fig. 26 a-d to 30 a-d.

4.1.2.2 Combined Lethal Toxicity on Villorita cyprinoides var. cochinensis

V. cyprinoides var. cochinensis is a common brackish water clam encountered in Cochin backwaters. Benthic in habitat, this animal occupies even very shallow areas of the backwaters. Therefore it is likely that under chronic pollutional conditions these animals would encounter a variety of pollutants. This was one of the reasons why this animal was utilized to assess the combined toxicity of pesticides and oil, two common pollutants encountered in natural waters. In the case of this animal only Ekalux an organophosphate and Aldrex an organochlorine were employed in combination with the PHC derived from LDO (WAF). This was mainly because of the fact that while analysing the combined toxicity of the other organophosphate, Dimecron and the other organochlorine, DDT it was noticed that no mortality occurred at conceivable concentrations. Therefore, it was felt that combined toxicity need not be assessed as it was likely to give results which may not be reliable.

Table 8. Perna indica. LC50 (ppm), when exposed to unvarying concentration of PHC in LDO (WAF) with varying concentrations of Aldrex, over periods upto 96h, along with respective 95% confidence limits*, slope functions and additive indices (AI)

PHC in LDO (WAF) Concentration (ppm)	48h			72h			96h		
	Aldrex, LC50 (ppm) (95% C.L)*	slope 'b'	AI	Aldrex, LC50 (ppm) (95% C.L)*	slope 'b'	AI	Aldrex, LC50 (ppm) (95% C.L)*	slope 'b'	AI
2.0	-	-	-	-	-	-	6.74 (6.51 - 6.98)*	6.39	-0.20 (L.A.)
2.25	-	-	-	6.52 (6.14 - 6.93)*	7.72	0.19 (M.A.)	4.97 (4.56 - 5.42)*	7.49	-0.06 (L.A.)
2.50	7.48 (-)	8.78	0.62 (M.A.)	4.74 (4.03 - 5.58)*	5.80	0.37 (M.A.)	4.07 (3.75 - 4.42)*	7.21	-0.006 (S.A.)
2.75	5.78 (5.27 - 6.34)*	7.28	0.78 (M.A.)	4.58 (4.01 - 5.23)*	6.09	0.32 (M.A.)	3.25 (-)	2.8	0.035 (M.A.)
3.0	4.75 (4.46 - 5.05)*	7.00	0.86 (M.A.)	3.80 (-)	10.80	0.37 (M.A.)	-	-	-

M.A: More than additive; L.A: Less than additive; S.A: Simple additive

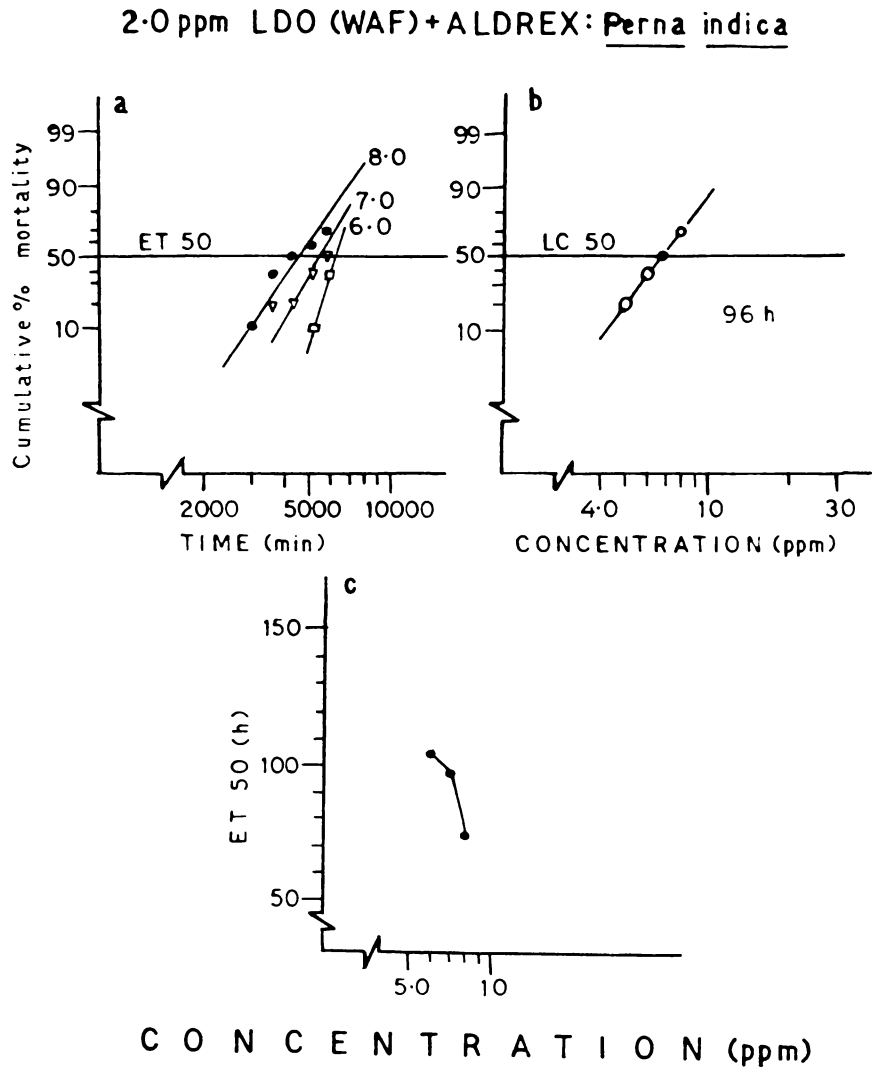


Fig. 26. Perna indica. Combined lethal effects of 2.0 ppm PHC in LDO - WAF - (unvarying) and Aldrex (varying)

a. Progress of mortality against time

b. Progress of mortality against concentration

c & d. Toxicity curves

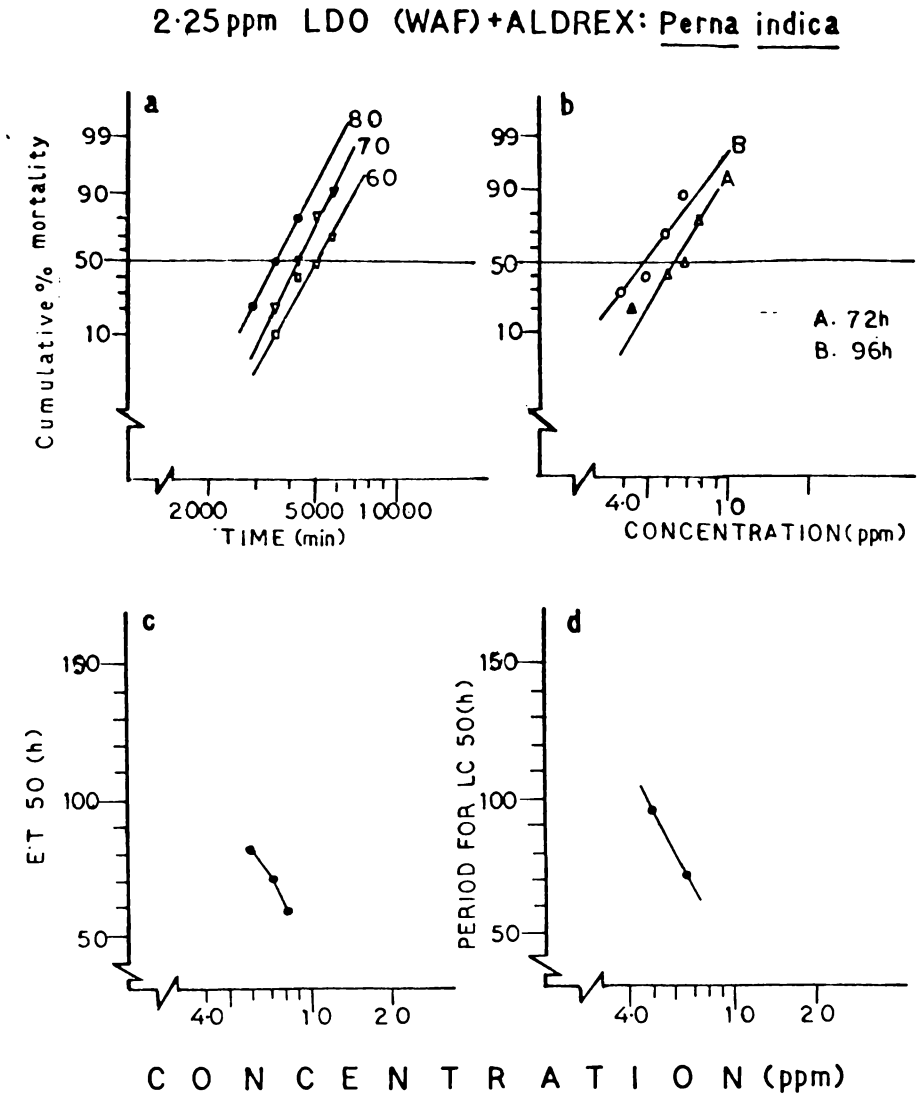


Fig. 27. Perna indica. Combined lethal effects of 2.25 ppm PHC in LDO-LAF- (unvarying) and Aldrex (varying)

a. Progress of mortality against time

b. Progress of mortality against concentration

c & d. Toxicity curves.

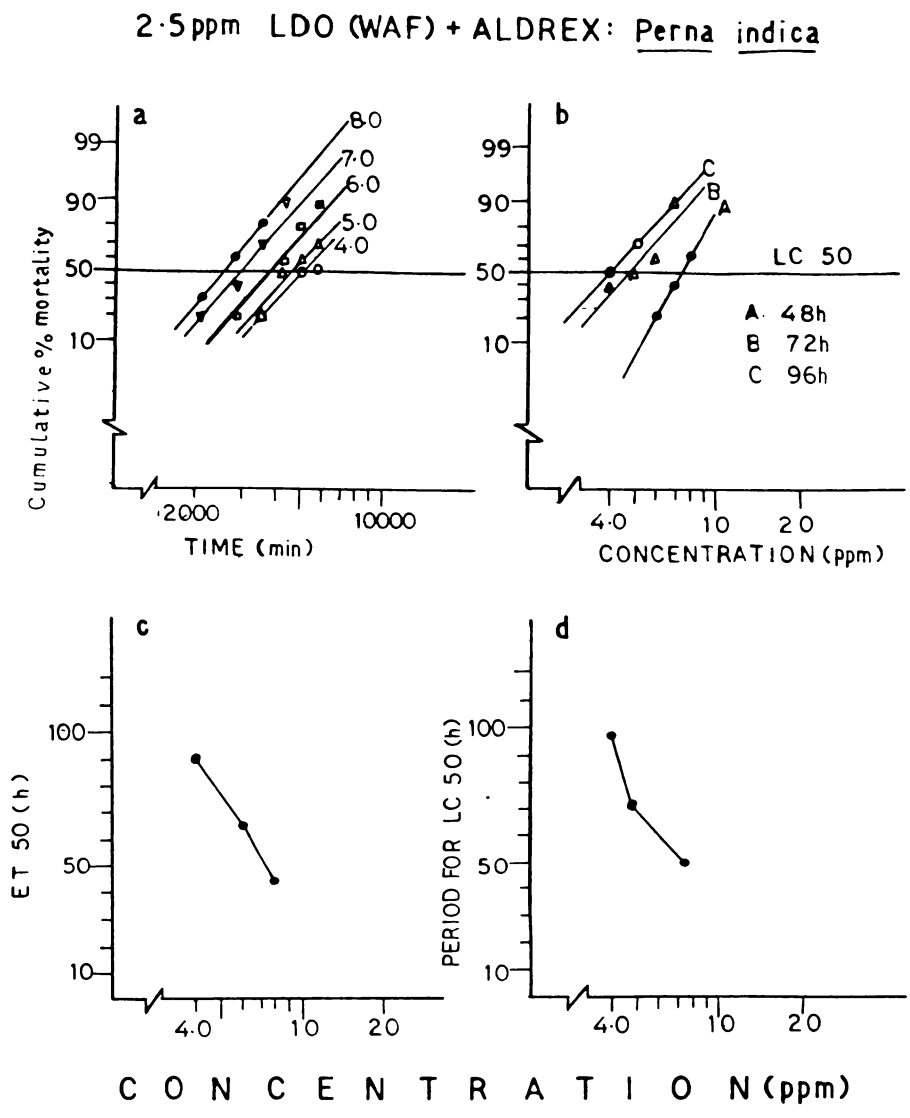


Fig. 28. Perna indica. Combined lethal effects of 2.50 ppm PHC in LDO-WAF- (unvarying) and Aldrex (varying)

a. Progress of mortality against time

b. Progress of mortality against concentration.

c & d. Toxicity curves.

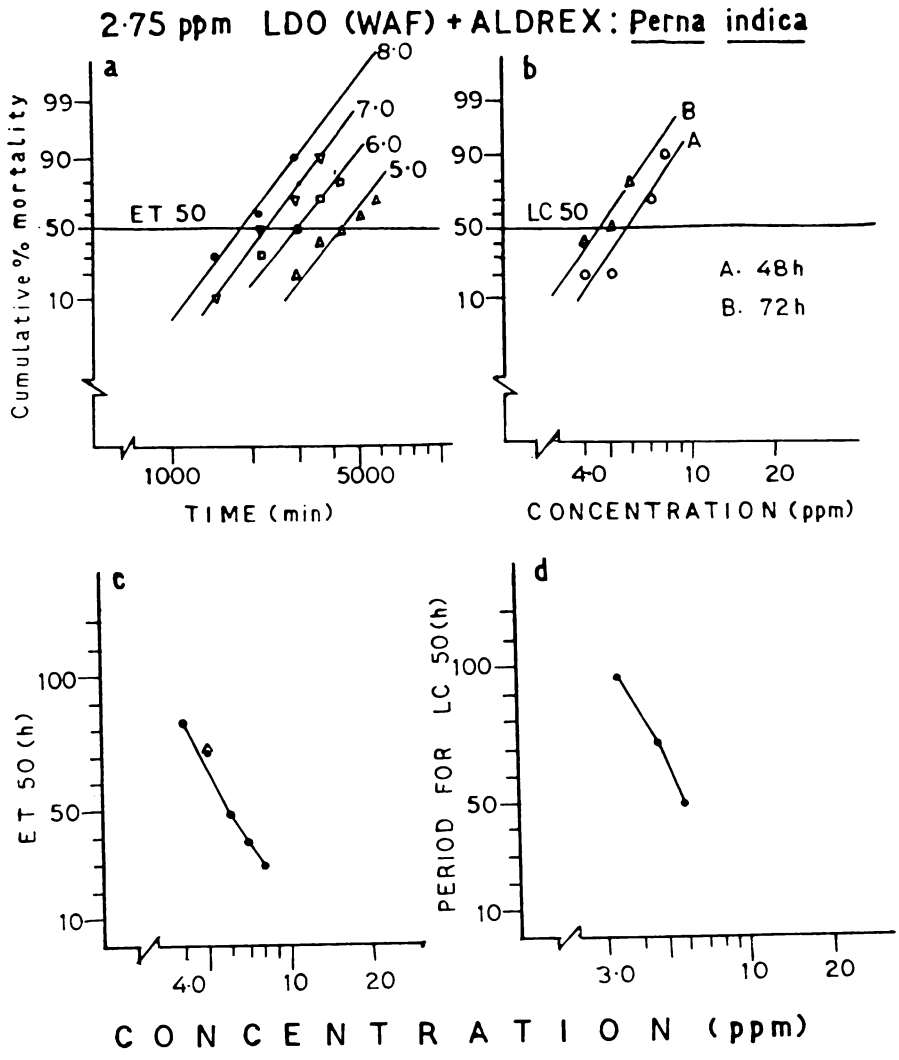


Fig. 29. Perna indica. Combined lethal effects of 2.75 ppm PHC in LDO-WAF- (unvarying) and Aldrex (varying)

a. Progress of mortality against time

b. Progress of mortality against concentration

c & d. Toxicity curves

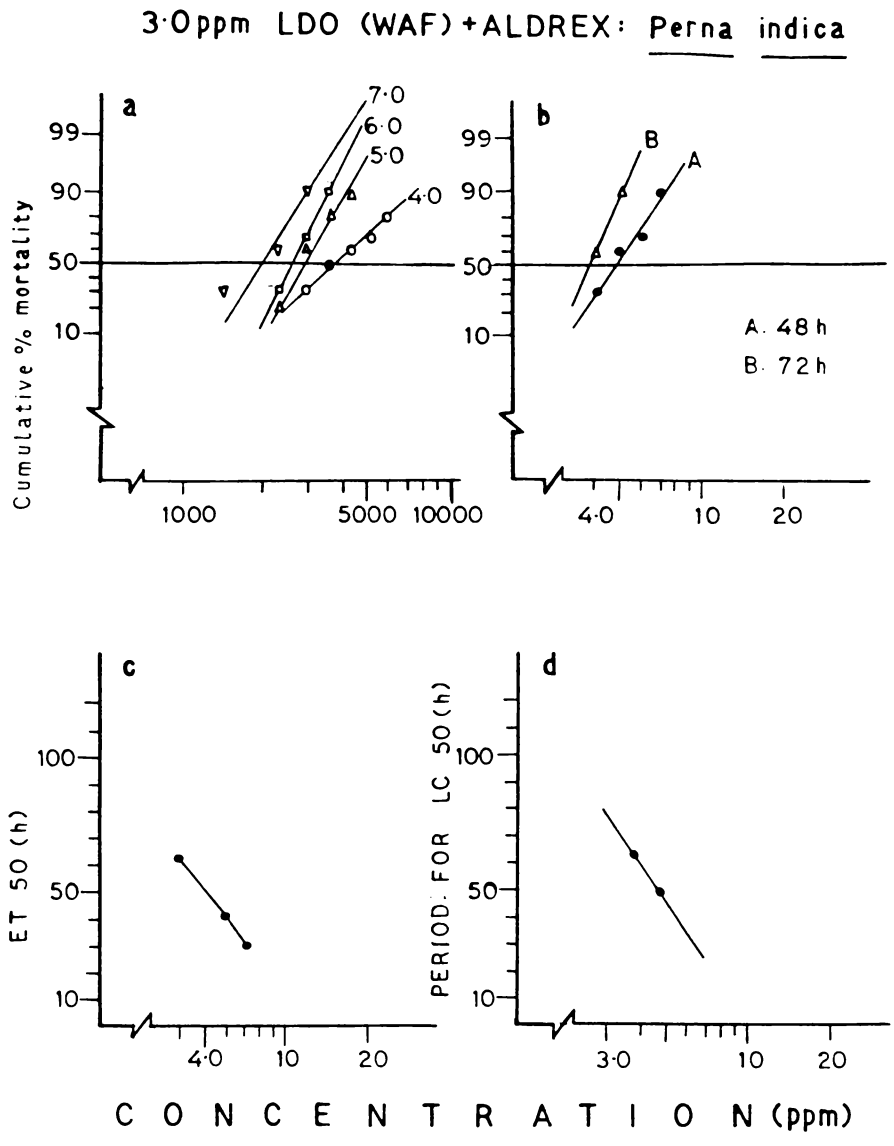


Fig. 30. Perna indica. Combined lethal effects of 3.0 ppm PHC in LDO-WAF- (unvarying) and Aldrex (varying)

a. Progress of mortality against time

b. Progress of mortality against concentration

c & d. Toxicity curves

4.1.2.2.1 Ekalux unvarying and PHC in LDO (WAF) varying

The concentrations of Ekalux used ranged between 0.75 to 2.5 ppm in combination with 2.0 to 9.0 ppm of PHC in LDO (WAF). The rate of mortality in the combinations which contained 0.75 ppm of Ekalux with 2.0 to 9.0 ppm of PHC in oil was negligible. Only when the concentration of Ekalux was raised above 1.5 ppm considerable mortality occurred, although the rate was not conspicuous during the early 48 h. 50% or above mortality was recorded only in those combinations which contained 7.0 or 9.0 ppm of PHC in LDO (WAF). The LC50 96 h recorded was as high as 7.01 ppm of PHC in LDO (WAF) with 1.0 ppm of Ekalux giving a less than additive reaction. Recording death in an ascending pattern, combinations which contained 1.5, 2.0 or 2.5 ppm of Ekalux and 3.0 to 9.0 ppm of PHC in LDO (WAF) registered noticeable rate of mortality. Drastic variation in the LC50 values could be noticed between 72 and 96 h. To cite one example, when the test medium contained 2.5 ppm of Ekalux and 2.0 to 9.0 ppm of PHC in oil the 72 h LC50 recorded was 7.6 ppm of PHC. This value was reduced to 4.84 ppm of PHC in LDO (WAF) at 96 h with the same Ekalux concentration of 2.5 ppm (Table 9; Fig. 34 a-d). It is evident from Table 9 and Fig. 31 a-d to 34 a-d, that lower concentrations were ineffective and even higher concentrations showed toxic effect of a noticeable nature only after the lapse of 36 to 48 h of exposure.

4.1.2.2.2 PHC in LDO (WAF) unvarying and Ekalux varying

Reciprocal to the above set of experiments, in another series of experiment the concentration of PHC in LDO (WAF) was retained constant and Ekalux varied. It has become clear that when the test medium contained

Table 9. Villorita cyprinoides var. cochinensis. LC50 (ppm), when exposed to unvarying concentration of Ekalux, with varying concentrations of PHC in LDO (WAF) over periods upto 96h, along with respective 95% confidence limits*, slope functions and additive indices (AI)

Ekalux Concentration (ppm)	48h			72h			96h		
	PHC in LDO(WAF) LC50 (ppm) (95% C.L)*	slope 'b'	AI	PHC in LDO (WAF) LC50 (ppm) (95% C.L)*	slope 'b'	AI	PHC in LDO (WAF) LC50 (ppm) (95% C.L)*	slope 'b'	AI
0.75	-	-	-	-	-	-	-	-	-
1.00	-	-	-	-	-	-	7.01 (-)	2.36	-1.66 (L.A.)
1.50	-	-	-	8.98 (-)	2.36	-9.31 (L.A.)	6.27 (6.25 - 6.29)*	5.35	0.52 (M.A.)
2.00	-	-	-	7.60 (-)	7.12	0.81 (M.A.)	5.18 (4.70 - 5.71)*	5.68	0.34 (M.A.)
2.50	-	-	-	7.60 (4.82 - 11.98)*	4.09	0.68 (M.A.)	4.84 (3.94 - 5.94)*	3.69	0.22 (M.A.

M.A: More than additive, L.A: Less than additive

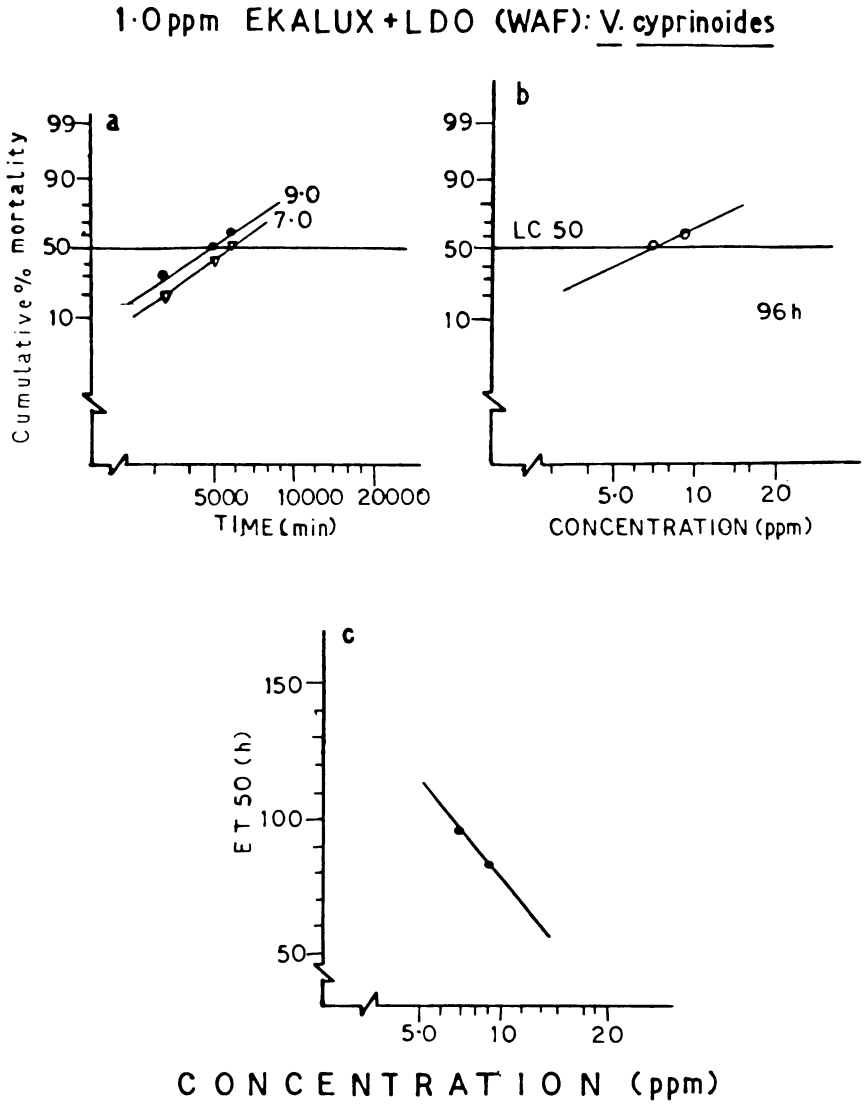


Fig. 31. Villorita cyprinoids var. cochinensis. Combined lethal effects of 1.0 ppm Ekalux (unvarying) and PHC in LDO-WAF-(varying)

a. Progress of mortality against time
b. Progress of mortality against concentration
c. Toxicity curve

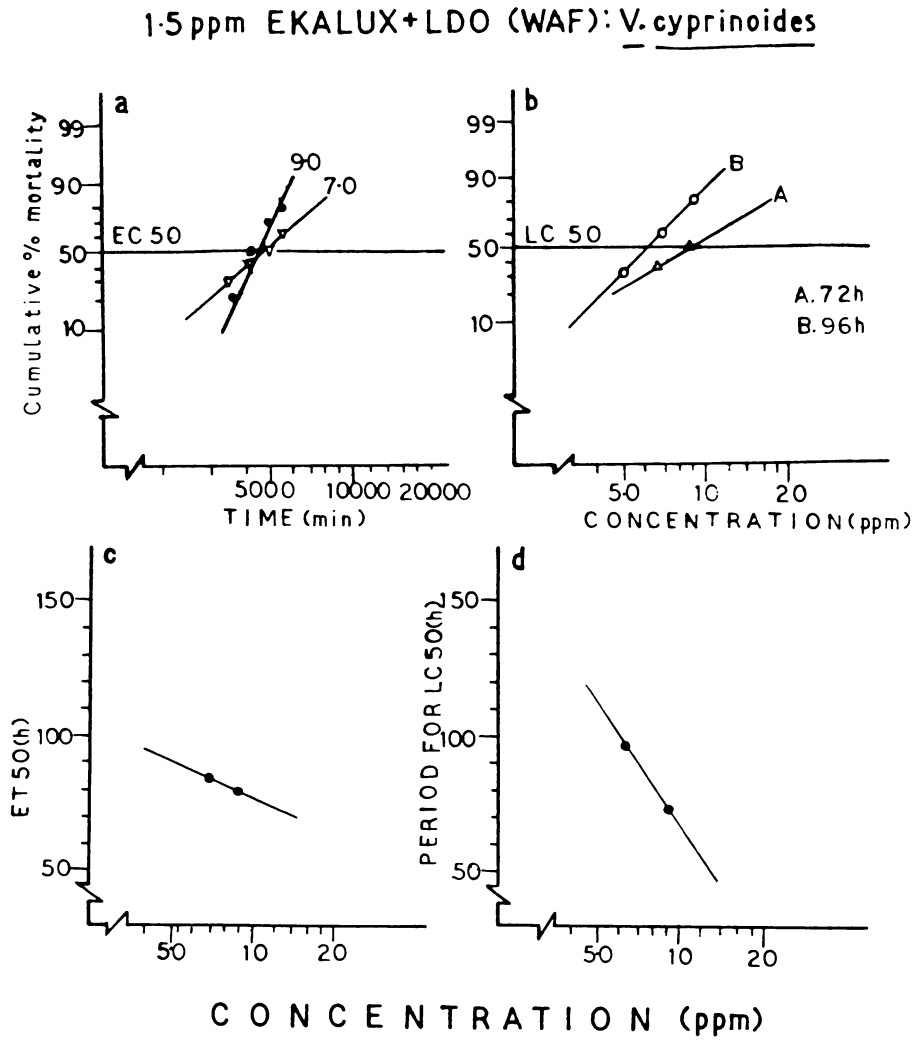


Fig. 32. Villorita cyprinoids var. cochinensis. Combined lethal effects of 1.50 ppm Ekalux (unvarying) and PHC in LDO-WAF- (varying)

a. Progress of mortality against time

b. Progress of mortality against concentration

c & d. Toxicity curves

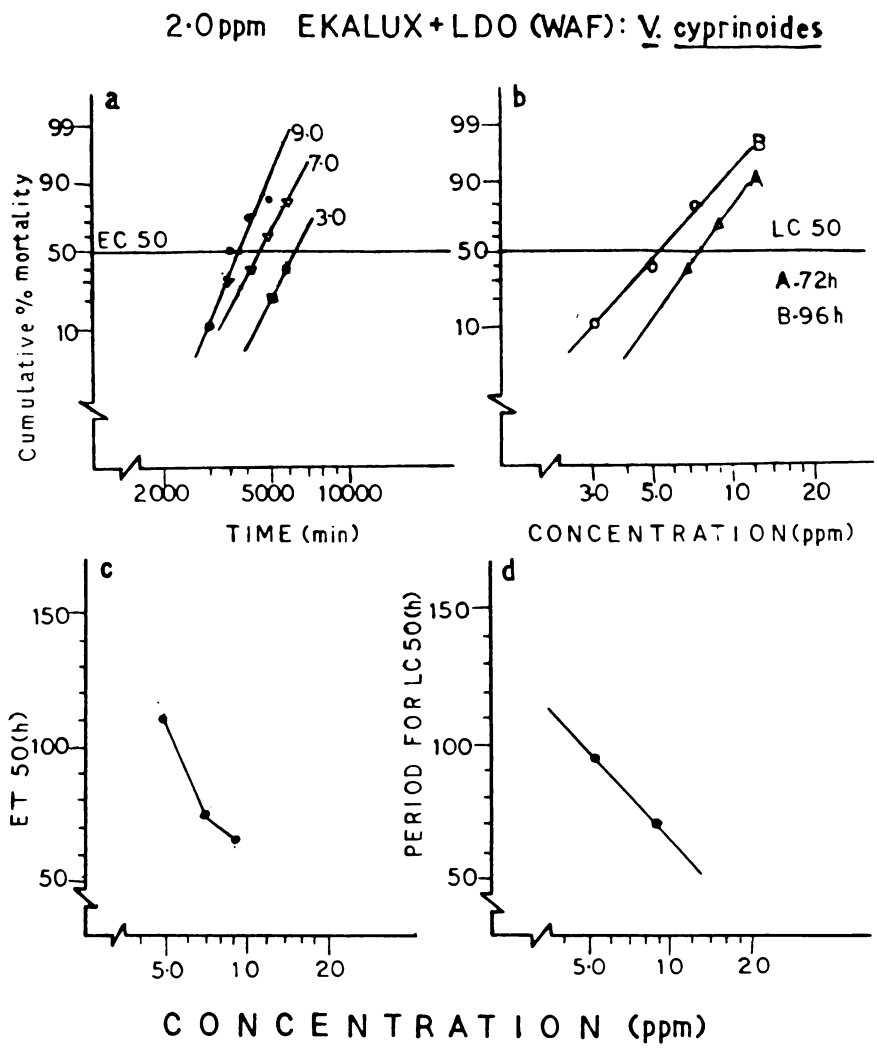


Fig. 33. Villorita cyprinoides var. cochinensis. Combined lethal effects of 2.0 ppm Ekalux (unvarying) and PHC in LDO-WAF-(varying)

a. Progress of mortality against time

b. Progress of mortality against concentration

c & d. Toxicity curves

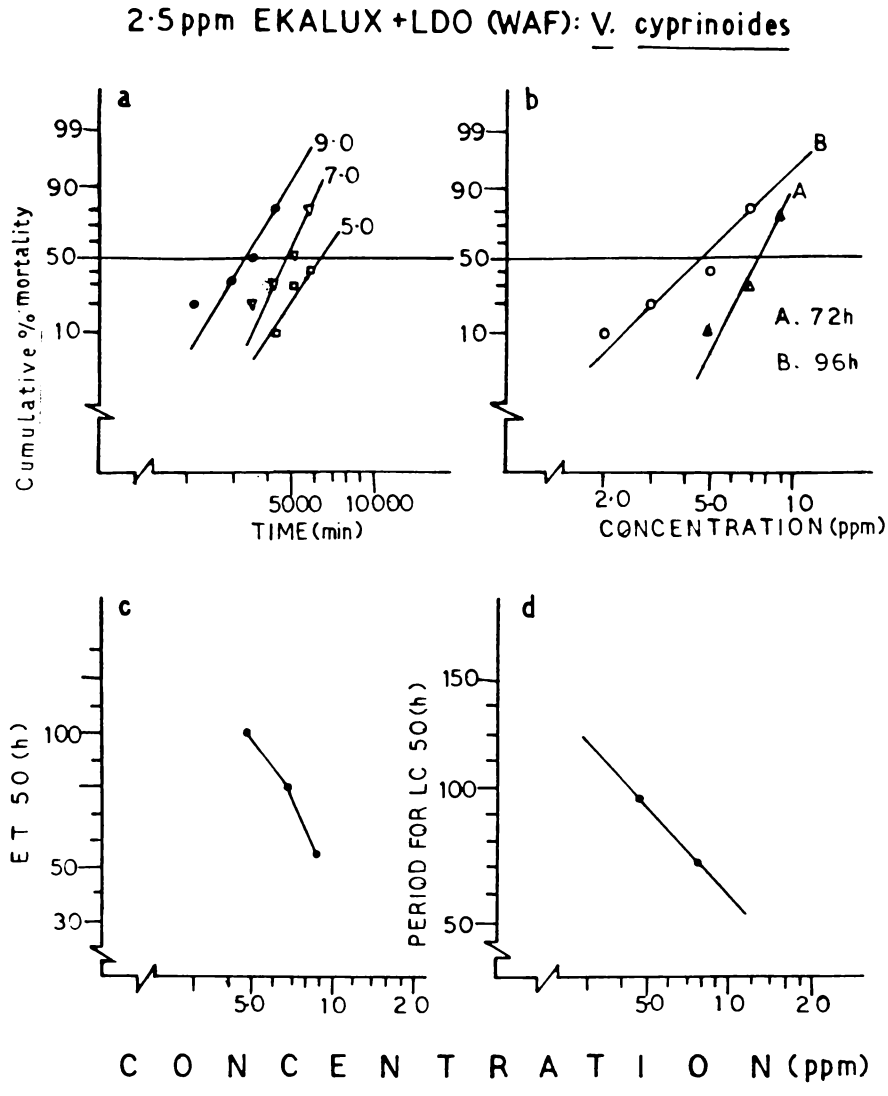


Fig. 34. Villorita cyprinoides var. cochinensis. Combined lethal effects of 2.50 ppm Ekalux (unvarying) and PHC in LDO-WAF- (varying)

a. Progress of mortality against time

b. Progress of mortality against concentration

c & d. Toxicity curves

low concentrations of PHC ie. 2.0, 3.0, or 5.0 ppm the rate of mortality was very negligible. Increase in the concentration to 7.0 ppm and above resulted in clear cut mortality. An examination of LC50 values show that when the test medium contained 5.0 ppm of PHC in LDO (WAF) even with 2.5 ppm of Ekalux, 50% of the animals did not die in 96 h. However, the computed LC50 value was 3.8 ppm of Ekalux with 5.0 ppm of PHC in oil. The death was brought about by a simple additive reaction. Increase in the PHC concentration to 7.0 and 9.0 ppm enhanced the toxicity of Ekalux. The combination which contained 9.0 ppm PHC in LDO (WAF) with 1.47 ppm of Ekalux, registered 50% mortality in 72 h. After 96 h this was reduced to 0.87 ppm of Ekalux, indicating a clear cut more than additive reaction (Table 10; Fig. 35 a-d to 37 a-d).

4.1.2.2.3 Aldrex unvarying and PHC in LDO (WAF) varying

Organochlorines were found to be less toxic to V. cyprinoides var. cochinensis. The concentration of Aldrex used varied from 6.0 to 18 ppm. When employed individually the LC50 96 h of Aldrex was 48.43 ppm and of LDO (WAF) was 17.28 ppm. The experiments performed with the combination of these two pollutants, however, proved that when present in combination the toxicity of both these compounds increased and in general the reaction bringing about mortality was more than additive in nature. When the media contained 6.0, 9.0 or 12.0 ppm of Aldrex and 3.0 or 4.5 ppm of PHC in LDO (WAF) no mortality occurred upto 48 h. With the passage of time mortality increased drastically. From the table 11 and Fig. 38 a-d to 42 a-d it become apparent that when Aldrex was present in higher concentrations death occurred even when the PHC concentration was very low (3.0 ppm).

Table 10. Villorita cyprinoides var.cochinensis. LC50 (ppm), when exposed to unvarying concentration of PHC in LDO (WAF), with varying concentrations of Ekalux, over periods upto 96h, along with respective 95% confidence limits*, slope functions and additive indices (AI)

PHC in LDO (WAF) Concentration (ppm)	48h			72h			96h		
	Ekalux LC50 (ppm) (95% C.L)*	slope 'b'	AI	Ekalux LC50 (ppm) (95% C.L)*	slope 'b'	AI	Ekalux LC50 (ppm) (95% C.L)*	slope 'b'	AI
2.0	-	-	-	-	-	-	-	-	-
3.0	-	-	-	-	-	-	-	-	-
5.0	-	-	-	-	-	-	3.8 (3.0 - 4.15)*	-	0.03 (S.A.)
7.0	-	-	-	-	-	-	1.24 (1.04 - 1.46)*	2.85	0.54 (M.A.)
9.0	-	-	-	1.47 (1.44 - 1.51)*	2.98	0.80 (M.A.)	0.87 (0.84 - 0.90)*	3.62	0.44 (M.A.)

M.A: More than additive, S.A: Simple additive

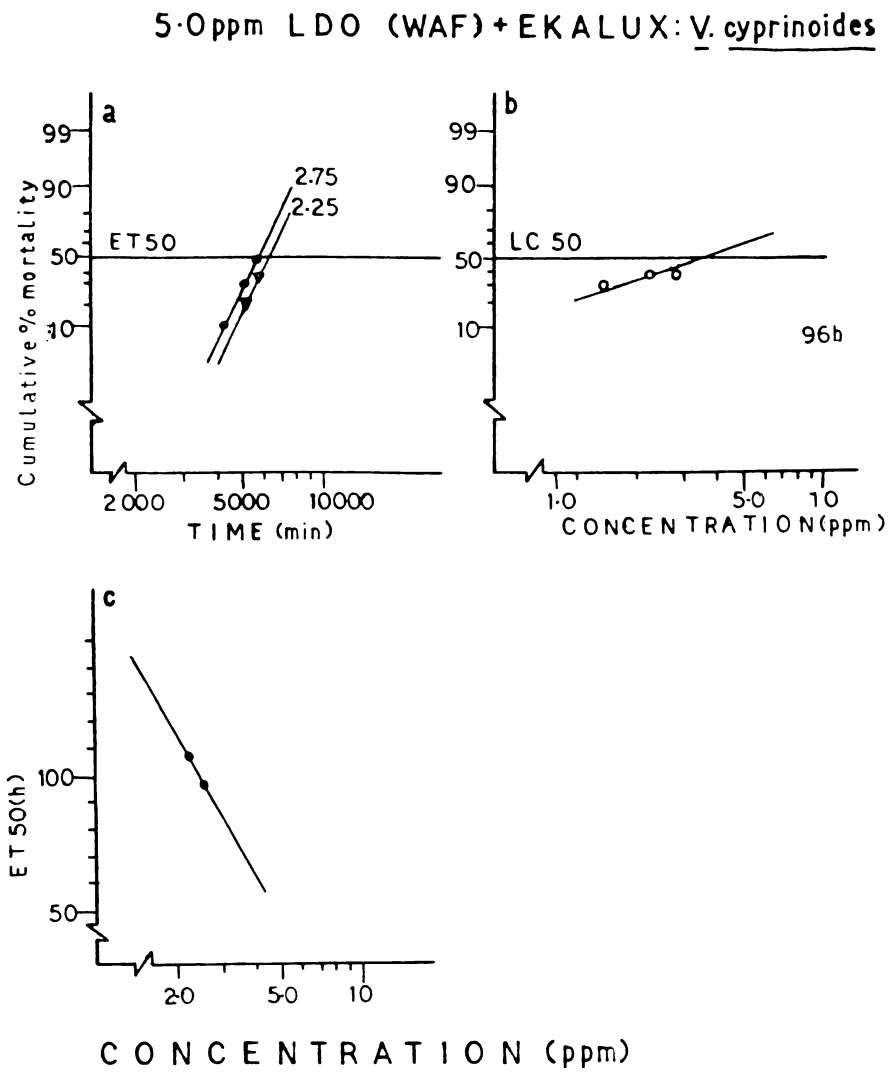


Fig. 35. Villorita cyprinoides var. cochinensis. Combined lethal effects of 5.0 ppm PHC in LDO-WAF- (unvarying) and Ekalux (varying)

a. Progress of mortality against time

b. Progress of mortality against concentration

c & d. Toxicity curves

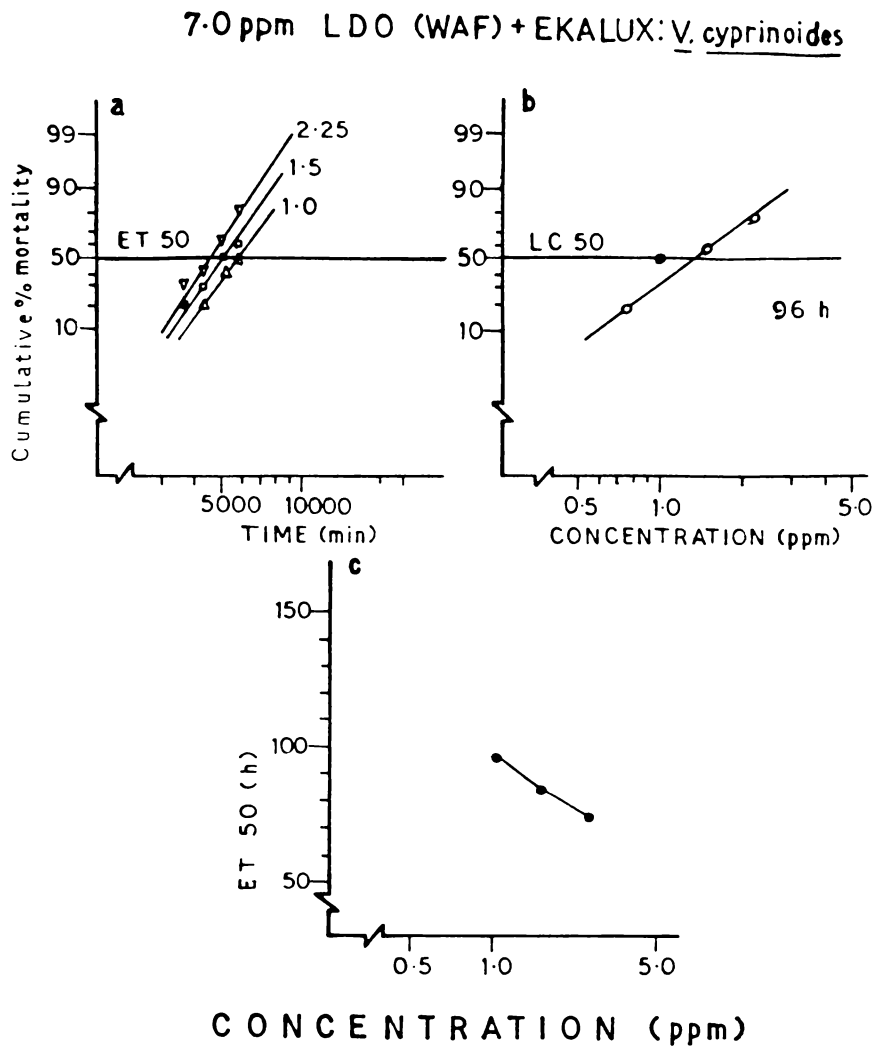


Fig. 36. Villorita cyprinoides var. cochinensis. Combined lethal effects of 7.0 ppm PHC in LDO-WAF- (unvarying) and Ekalux (varying)

a. Progress of mortality against time

b. Progress of mortality against concentration

c & d. Toxicity curves

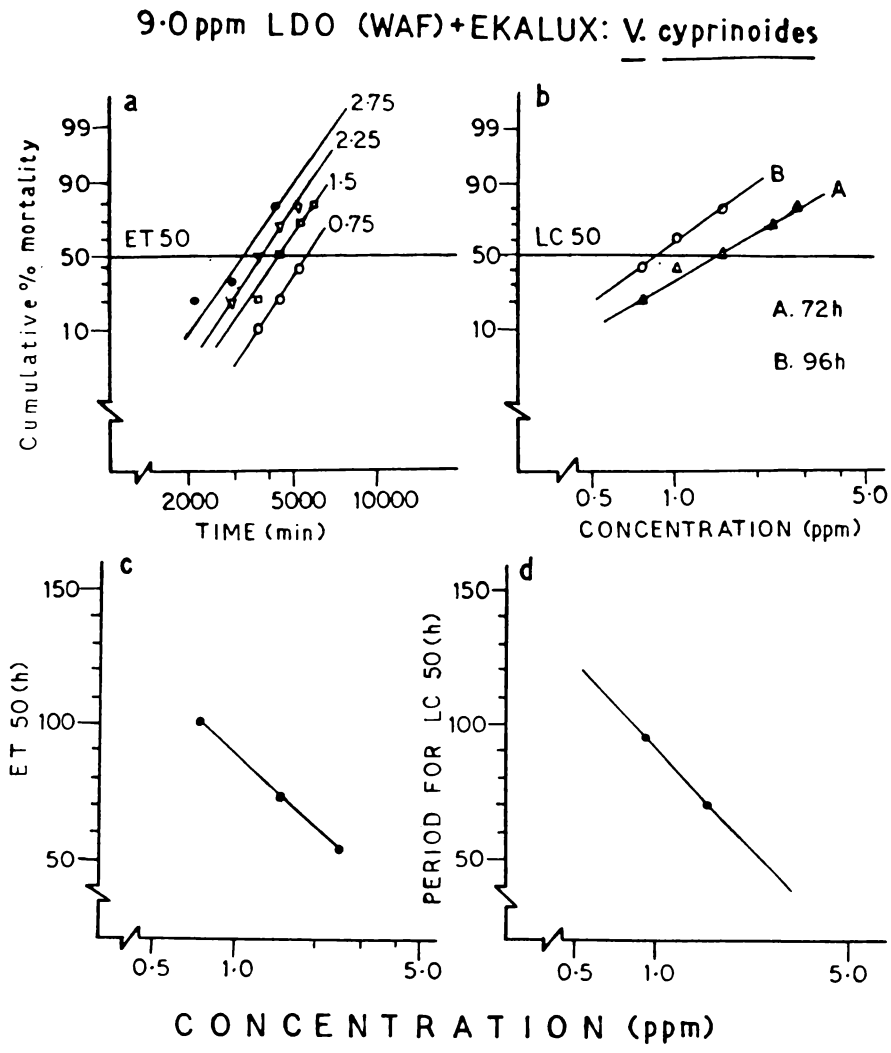


Fig. 37. Villorita cyprinoides var. cochinensis. Combined lethal effects of 9.0 ppm PHC in LDO-WAF- (unvarying) and Ekalux (varying)

a. Progress of mortality against time

b. Progress of mortality against concentration

c & d. Toxicity curves

Table 11. Villorita cyprinoides var.cochinensis. LC50 (ppm), when exposed to unvarying concentration of Aldrex, with varying concentrations of PHC in LDO (WAF) over periods upto 96h, along with respective 95% confidence limits*, slope functions and additive indices (AI)

Aldrex Concentration (ppm)	48h			72h			96h		
	PHC in LDO (WAF) LC50 (ppm) (95% C.L)*	slope 'b'	AI	PHC in LDO (WAF) LC50 (ppm) (95% C.L)*	slope 'b'	AI	PHC in LDO (WAF) LC50 (ppm) (95% C.L)*	slope 'b'	AI
6.0	-	-	-	9.24 (7.62 - 11.20)*	3.87	0.77 (M.A.)	6.04 (5.74 - 6.36)*	4.24	1.11 (M.A.)
9.0	-	-	-	8.09 (6.1 - 10.74)*	3.36	0.76 (M.A.)	4.43 (3.98 - 4.94)*	3.68	1.26 (M.A.)
12.0	9.00 (-)	10.63	0.94 (M.A.)	5.80 (4.36 - 7.73)*	2.34	0.92 (M.A.)	3.71 (3.11 - 4.44)*	3.58	1.15 (M.A.)
15.0	8.48 (6.34 - 11.35)*	3.48	0.54 (M.A.)	4.38 (3.36 - 5.70)*	2.69	0.96 (M.A.)	2.81 (1.37 - 5.80)*	3.19	1.11 (M.A.)
18.0	6.14 (5.05 - 7.47)*	3.21	0.99 (M.A.)	2.79 (0.92 - 8.46)*	1.34	1.02 (M.A.)	1.90 (1.36 - 2.64)*	2.47	1.08 (M.A.)

M.A: More than additive.

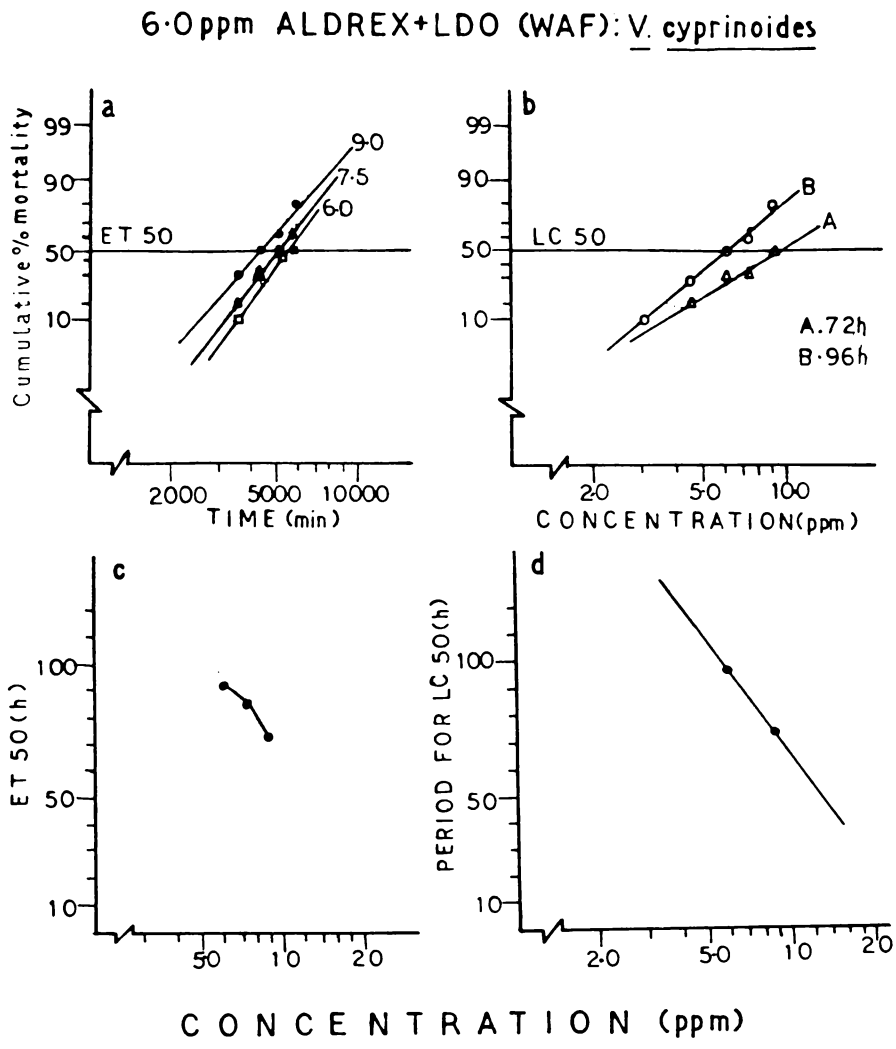


Fig. 38. Villorita cyprinoides var. cochinensis. Combined lethal effects of 6.0 ppm Aldrex (unvarying) and PHC in LDO-WAF- (varying)

a. Progress of mortality against time

b. Progress of mortality against concentration

c & d. Toxicity curves

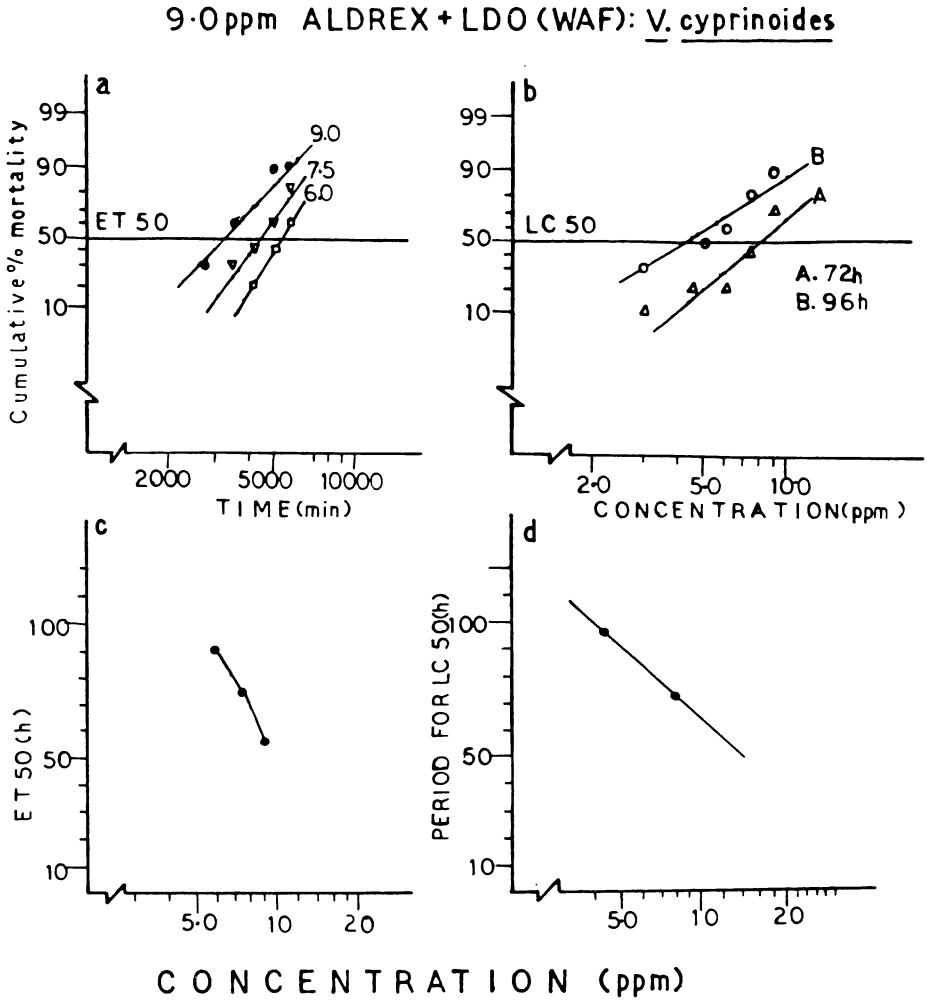


Fig. 39. Villorita cyprinoides var. cochinensis. Combined lethal effects of 9.0 ppm Aldrex (unvarying) and PHC in LDO-WAF- (varying)

a. Progress of mortality against time

b. Progress of mortality against concentration

c & d. Toxicity curves

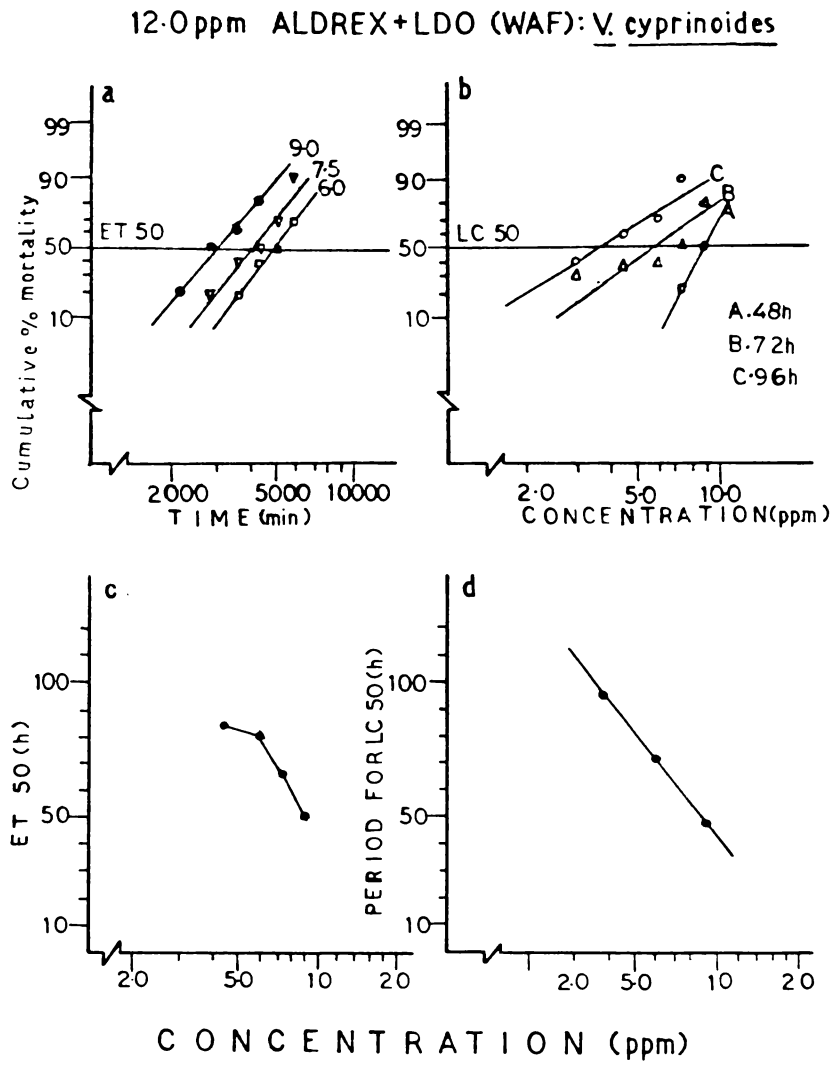


Fig. 40. Villorita cyprinoides var. cochinensis. Combined lethal effects of 12.0 ppm Aldrex (unvarying) and PHC in LDO-WAF- (varying)

a. Progress of mortality against time

b. Progress of mortality against concentration

c & d. Toxicity curves

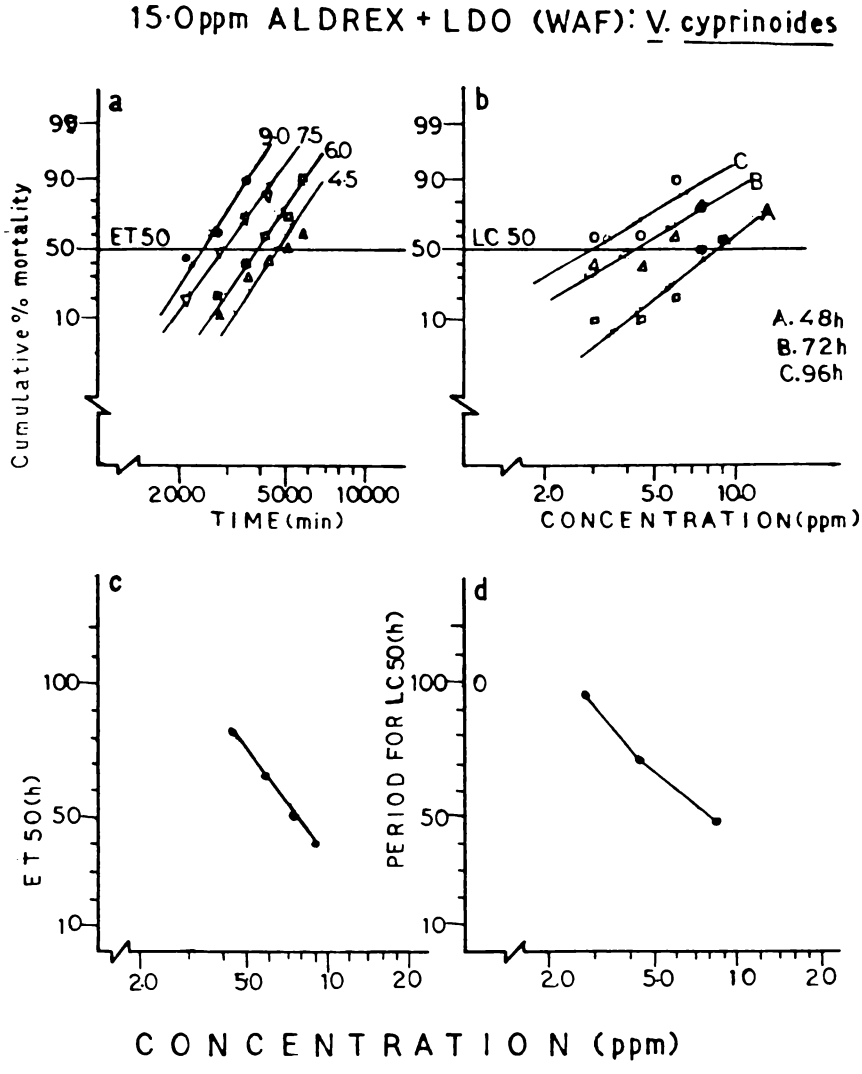


Fig. 41. Villorita cyprinoides var. cochinensis. Combined lethal effects of 15.0 ppm Aldrex (unvarying) and PHC in LDO-WAF- (varying)

a. Progress of mortality against time

b. Progress of morality against concentration

c & d. Toxicity curves

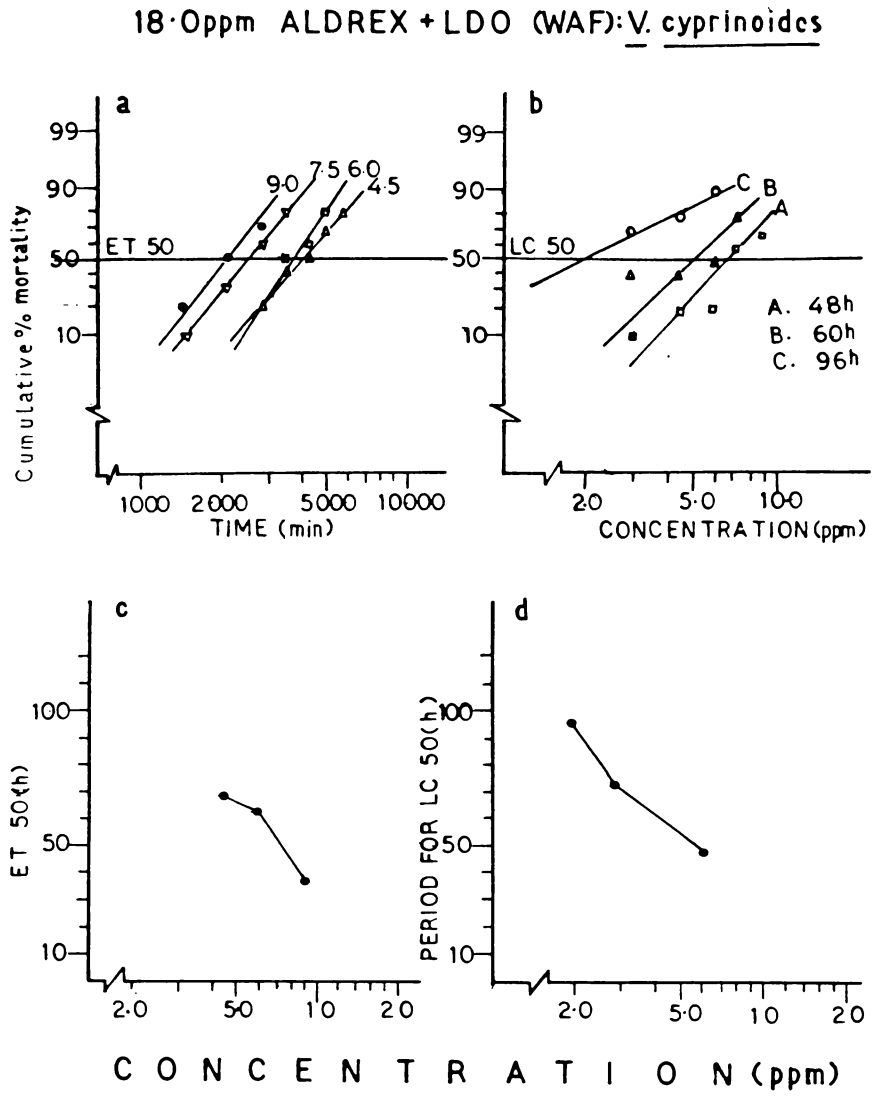


Fig. 42. Villorita cyprinoides var. cochinensis. Combined lethal effects of 18.0 ppm Aldrex (unvarying) and PHC in LDO-WAF-(varying)

a. Progress of morality against time

b. Progress of morality against concentration

c & d. Toxicity curves

PHC assumes a very high toxic nature with the passage of time in the presence of Aldrex. For example, the 48 h LC50 value of 8.48 ppm of PHC in LDO (WAF) was reduced to 2.81 ppm of PHC in LDO (WAF) after 96 h with 15 ppm of Aldrex. Similarly with 18 ppm of Aldrex, the 48 h LC50 value of 6.14 ppm PHC in LDO (WAF) was lowered to 1.9 ppm of PHC in LDO (WAF). The toxic reactions were uniformly more than additive in nature (Table 11; Fig. 38 a-d to 42 a-d).

4.1.2.2.4 PHC in LDO (WAF) unvarying and Aldrex varying

In this series of experiments the PHC concentrations in LDO (WAF) ranged from 6.0 to 18 ppm. Death of a noticeable nature did not occur during the early 48 h of exposure, when the test medium contained a combination of 3.0, 4.5 or 6.0 ppm of PHC in LDO (WAF) with 6.0 to 18 ppm of Aldrex (Table 12). When the PHC concentration was raised to 7.5 or 9.0 ppm in the medium, sporadic death occurred. Increase in mortality was drastic and with 9.0 ppm PHC in LDO (WAF) 2.79 ppm of Aldrex resulted in 50% mortality at 96 h. This should be considered as a highly toxic reaction of Aldrex in the presence of LDO (WAF) taking into account its comparative less lethal effect when present alone in the test media ie; 96 h LC50 of Aldrex was 48.43 ppm. The results were uniformly of a more than additive nature (Table 12; Fig. 43 a-d to 47 a-d).

4.2 SUBLETHAL TOXIC RESPONSE

Investigations on the sublethal toxicity of pesticides and WAFs of oil, individually and in combinations have been carried out. Both the species of molluscs, namely Perna indica and Villorita cyprinoides var. cochinensis were used to assess the responses. The responses delineated were, rate of oxygen

Table 12. Villorita cyprinoides var.cochinensis. LC50 (ppm), when exposed to unvarying concentration of PHC in LDO (WAF), with varying concentrations of Aldrex, over periods upto 96h, along with respective 95% confidence limits*, slope functions and additive indices (AI)

PHC in LDO (WAF) Concentration (ppm)	48h			72h			96h		
	Aldrex, LC50 (ppm) (95% C.L)*	slope 'b'	AI	Aldrex, LC50 (ppm) (95% C.L)*	slope 'b'	AI	Aldrex, LC50 (ppm) (95% C.L)*	slope 'b'	AI
3.0	-	-	-	16.16 (15.17 - 17.23)*	4.89	1.14 (M.A.)	13.10 (12.48 - 13.76)*	3.73	1.25 (M.A.)
4.5	-	-	-	17.46 (15.17 - 20.1)*	2.72	0.77 (M.A.)	9.61 (8.32 - 11.11)*	2.51	1.18 (M.A.)
6.0	-	-	-	14.27 (9.96 - 20.46)*	2.10	0.74 (M.A.)	6.65 (5.17 - 8.56)*	2.96	1.06 (M.A.)
7.5	15.90 (14.56 - 17.35)*	6.31	0.92 (M.A.)	9.81 (7.88 - 12.22)*	3.12	0.79 (M.A.)	5.07 (4.98 - 5.16)*	3.41	0.86 (M.A.)
9.0	11.80 (10.08 - 13.81)*	2.34	0.95 (M.A.)	4.54 (3.72 - 5.54)*	1.93	0.91 (M.A.)	2.79 (-)	2.79	1.27 (M.A.)

M.A: More than additive

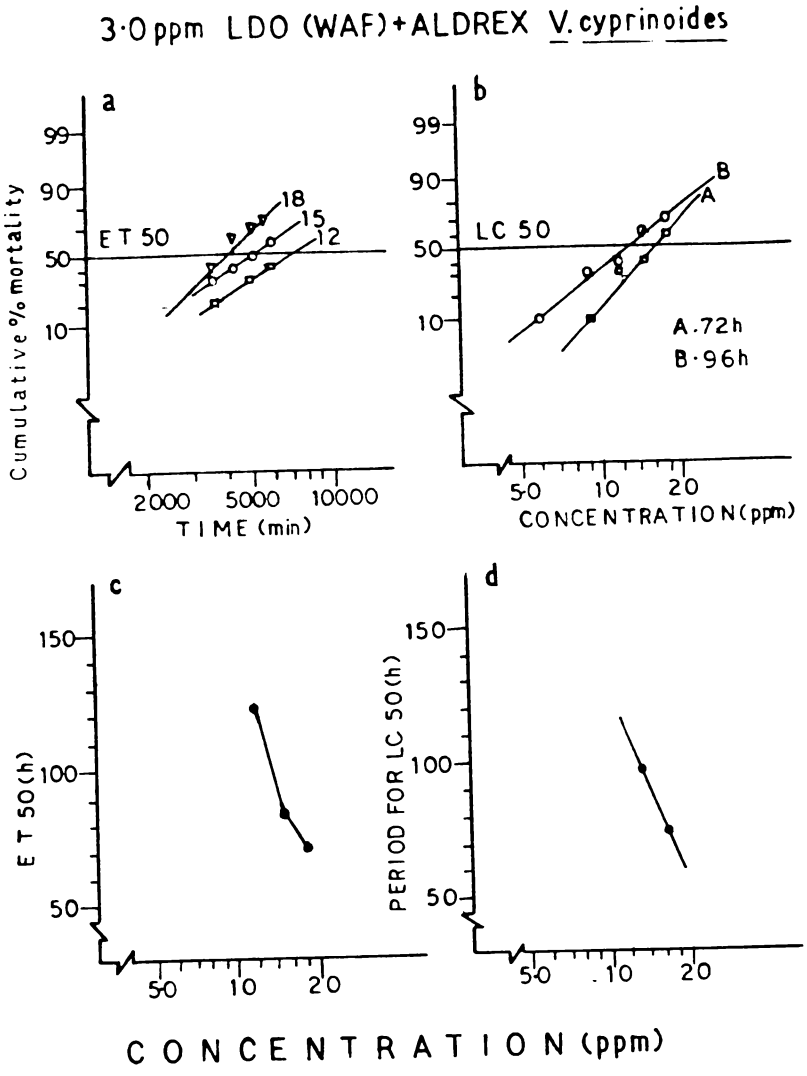


Fig. 43. Villorita cyprinoides var. cochinensis. Combined lethal effects of 3.0 ppm PHC in LDO-WAF- (unvarying) and Aldrex (varying)

a. Progress of morality against time

b. Progress of morality against concentration

c & d. Toxicity curves

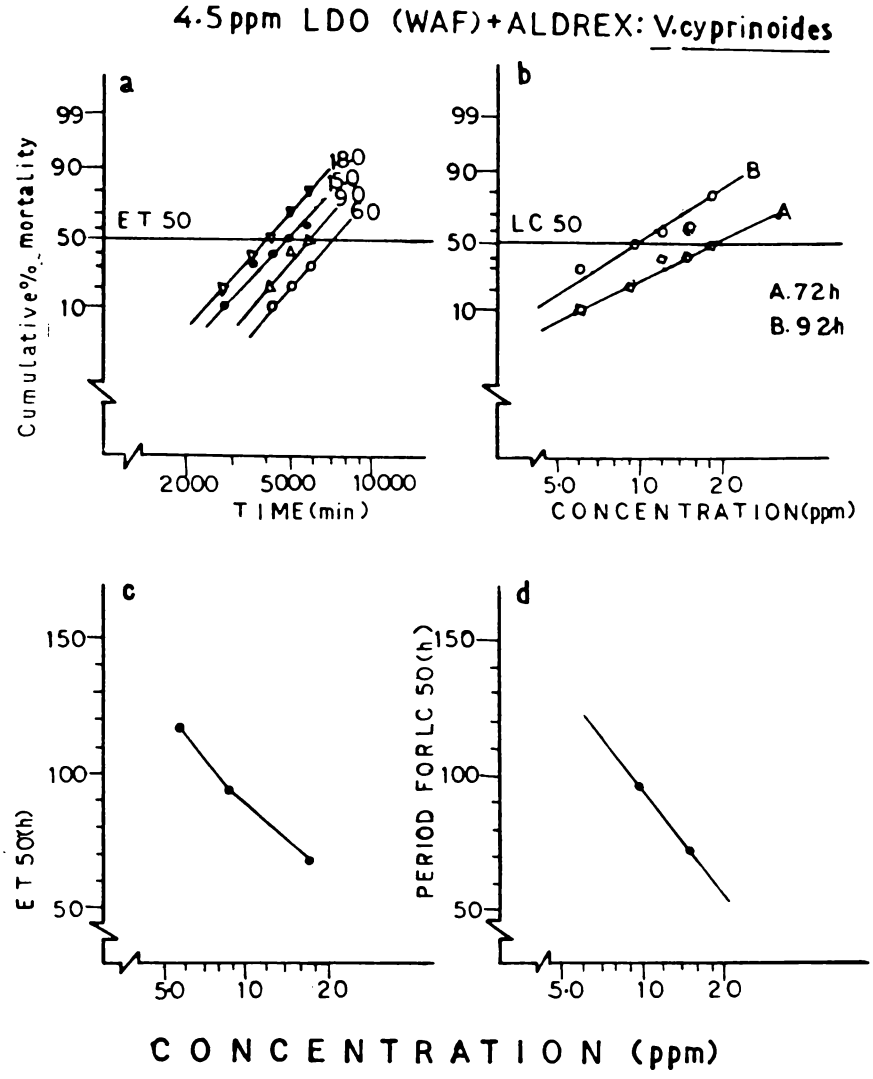


Fig. 44. Villorita cyprinoides var. cochinensis. Combined lethal effects of 4.5 ppm PHC in LDO-WAF- (unvarying) and Aldrex (varying)

a. Progress of morality against time

b. Progress of morality against concentration

c & d. Toxicity curves.

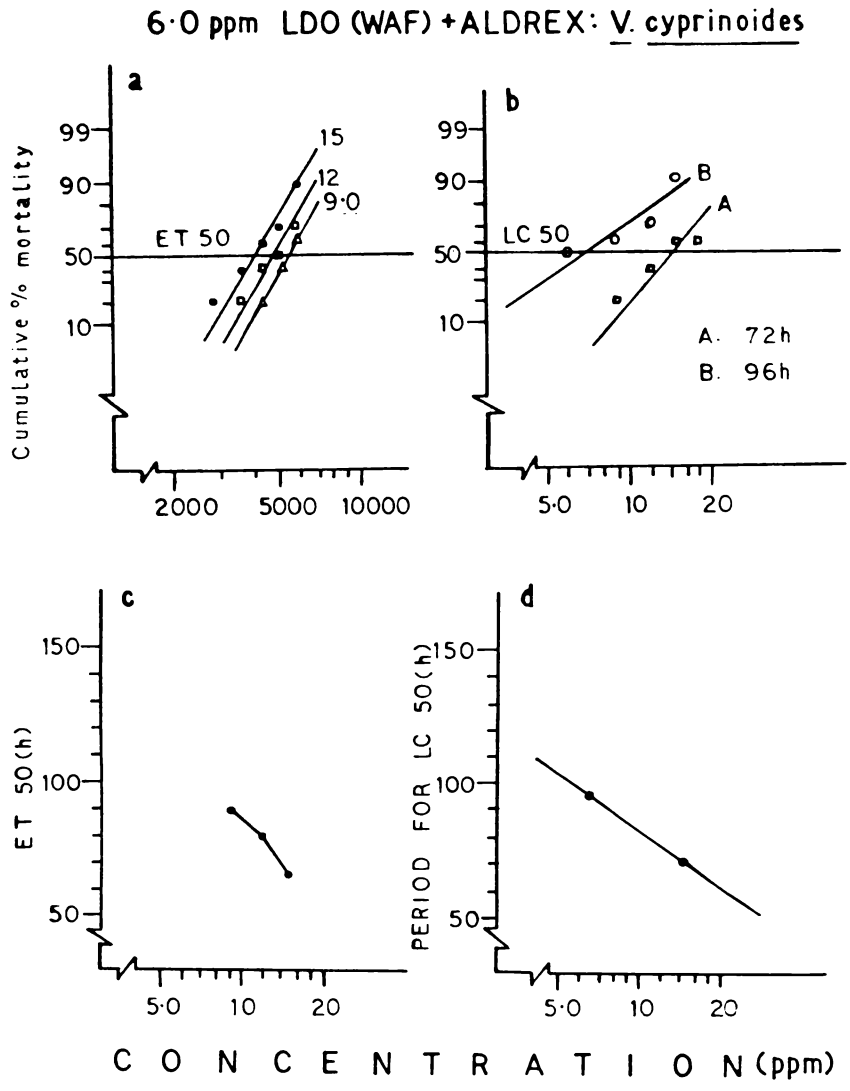


Fig. 45. Villorita cyprinoides var. cochinensis. Combined lethal effects of 6.0 ppm PHC in LDO-WAF- (unvarying) and Aldrex (varying)

a. Progress of morality against time

b. Progress of morality against concentration

c & d. Toxicity curves.

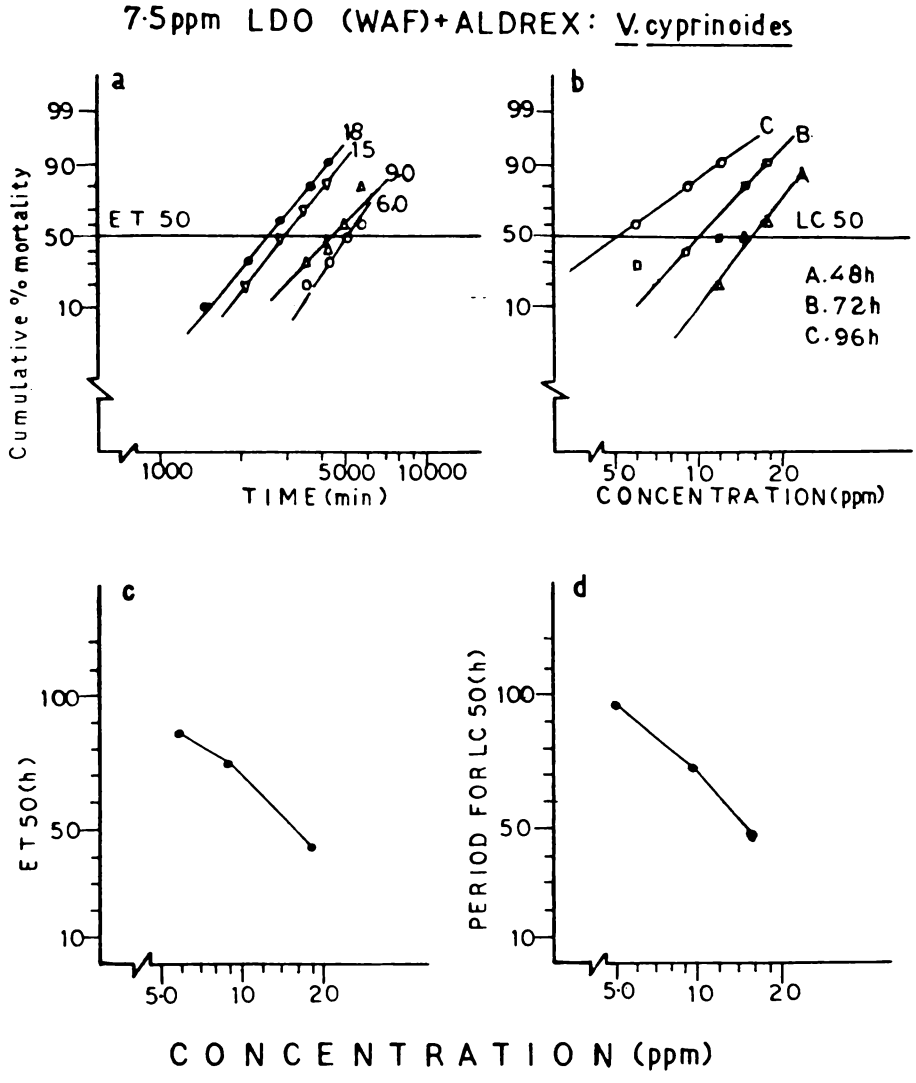


Fig. 46. Villorita cyprinoides var. ccchinensis. Combined lethal effects of 7.5 ppm PHC in LDO-WAF- (unvarying) and Aldrex (varying)

a. Progress of morality against time

b. Progress of morality against concentration

c & d. Toxicity curves.

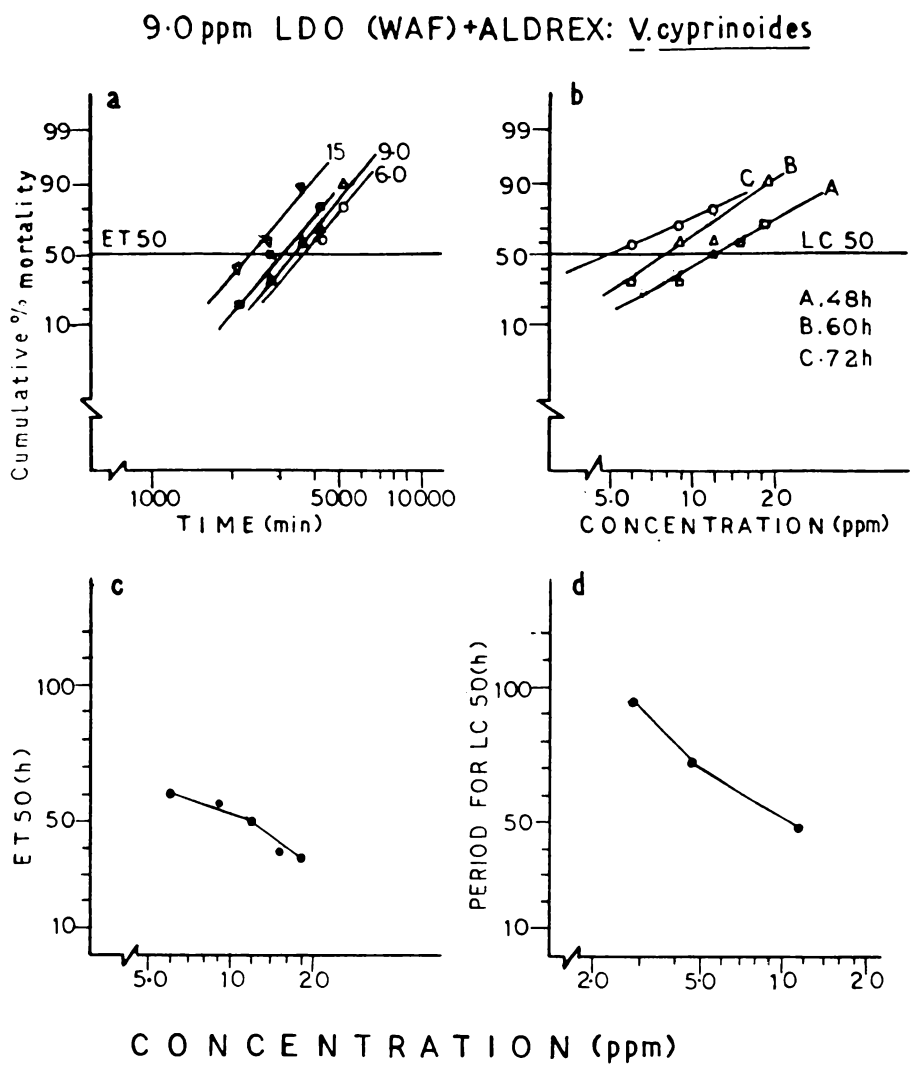


Fig. 47. Villorita cyprinoides var. cochinensis. Combined lethal effects of 9.0 ppm PHC in LDO-WAF- (unvarying) and Aldrex (varying)

a. Progress of morality against time

b. Progress of morality against concentration

c & d. Toxicity curves.

consumption, rate of filtration and rate of byssogenesis. The results are presented under short-term and long-term sublethal toxic response to enable effective presentation. A total of around 85 experiments were conducted to analyse the short-term effects and around 120 experiments to delineate the long-term effects.

4.2.1 SHORT-TERM SUBLETHAL TOXIC RESPONSE

The main objective of this series of experiments was to delineate concentrations which would induce alterations in basic responses of the animal, as well as to segregate toxicity–bound hyper or lower activity exemplified by three important physiological/behavioural functions namely, oxygen consumption, filtration and byssogenesis.

4.2.1.1 Rate of Oxygen Consumption: Under Individual Toxicant Stress

The quantity of dissolved oxygen consumed by experimental animals from the culture medium is a very useful index to assess the general well-being of the animal. Usually when bivalves are used as experimental animals, to analyse oxygen consumption, certain amount of variability could be expected, which is controlled by behaviour pattern. The bivalves tend to 'clam up' and reduce the rate of consumption. This is a reaction, which is normally elicited by these animals irrespective of the presence or absence of a stress factor under laboratory conditions. With a view to overcome the possible influence of this factor on the results, controls are run along with all the experiments. However, experiences have shown that if the animals are brought to the laboratory from the field and retained under laboratory conditions for periods ranging from 24 to 48 h they get adjusted and the time

course for adjustments in the case of bivalves vary from 24 to 72 h. The rate of oxygen consumption of animals exposed to different fashions of toxicant concentrations is worked out and presented in this section.

4.2.1.1.1 *Perna indica*

Perna indica was exposed to sublethal concentrations of Ekalux. Dimecron, Aldrex, DDT and WAFs of Light Diesel oil and Persian Gulf crude to assess the alterations induced by these toxicants on the rate of oxygen consumption.

4.2.1.1.1.1 Ekalux

The concentrations of Ekalux employed for the study ranged from 0.05 ppm to 0.30 ppm. General trend in oxygen consumption was, marginal increase in consumption at the lowest concentration and subsequent decrease in higher concentrations (Table 13; Fig. 48 a). It is seen from the results that drastic reduction in oxygen consumption did not occur even when the Ekalux concentration reached 0.30 ppm. The effective concentration that would have caused 50% reduction was calculated and this was 1.53 ppm.

4.2.1.1.1.2 Dimecron

Table 14 and Fig. 48b give the data on the oxygen consumption of P. indica exposed to various concentrations of Dimecron. The concentrations of Dimecron in the experimental medium ranged from 2.50 to 12.0 ppm. A conspicuous feature of the result was that animals maintained in all this concentrations respired more or less in a unifrom manner. The variation in percentage performance was erratic. At one instance it was noticed that

Table 13. Perna indica. Average oxygen consumption ($\mu gO_2\ h^{-1}\ mg^{-1}$ dry wt) under sublethal concentrations of Ekalux, along with respective standard error, percentage performance and EC50 level.

Ekalux Concentration (ppm)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.05	1.84	0.69	102.81	
0.075	1.59	0.47	88.44	
0.10	1.31	0.67	73.07	1.5
0.20	1.44	0.38	80.18	
0.30	1.31	0.35	73.15	
Control	1.80	0.33	-	

Table 14. Perna indica. Average oxygen consumption ($\mu gO_2\ h^{-1}\ mg^{-1}$ dry wt) under sub lethal concentrations of Dimecron, along with respective standard error and percentage performance.

Dimecron Concentration (ppm)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt)		Performance (% of control)
	Mean	SE	
2.5	1.11	0.24	62.03
5.0	1.09	0.43	60.86
7.5	1.06	0.47	59.13
10.0	1.07	0.14	59.52
12.0	1.27	0.39	70.57
Control	1.80	0.33	-

P. indica was respiring more, when the external concentration of Dimecron was 12.0 ppm. Although, this variation was marginal, it upsets the general trend of reduction in consumption, with increase in concentration. The difference in consumption between 2.50 ppm maintained animals and 10.0 ppm maintained animals was only around 2.5% of that of control.

4.2.1.1.1.3 Aldrex

P. indica when exposed to various concentrations of Aldrex recorded elevation in oxygen consumption. As it is seen from Table 15 and Fig. 48c, variation in oxygen consumption between 0.10 ppm and 1.0 ppm maintained animals was negligible. It is seen that all the test animals recorded hyperactivity when exposed to sublethal concentrations of Aldrex.

4.2.1.1.1.4 DDT

Recording marginal decrease in oxygen consumption the reaction of P. indica to DDT was also not very drastic. The animals maintained in 0.25 ppm to 2.50 ppm of DDT recorded erratic variation in oxygen consumption. The EC50 was calculated and was found to be 2.0 ppm (Table 16; Fig. 48d).

4.2.1.1.1.5 Light Diesel Oil (WAF)

The presence of 0.075 ppm to 0.50 ppm PHC in LDO (WAF) did not bring about any conspicuous reduction in oxygen consumption. But when the concentration was increased to 0.75 ppm, the animals did show reduction in oxygen consumption. The effective concentration that would cause 50% reduction in oxygen consumption was 1.2 ppm of PHC (Table 17; Fig. 49a).

Table 15. Perna indica. Average oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt) under sublethal concentrations of Aldrex, along with respective standard error and percentage performance.

Aldrex Concentration (ppm)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt)		Performance (% of control)
	Mean	SE	
0.10	2.14	0.41	117.70
0.25	2.21	0.42	121.67
0.50	1.83	0.43	100.95
0.75	2.29	0.50	126.15
1.00	2.23	0.39	122.73
Control	1.81	0.30	-

Table 16. Perna indica. Average oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt) under sublethal concentrations of DDT, along with respective standard error, percentage performance and EC50 level.

DDT Concentration (ppm)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.25	1.54	0.35	85.01	2.0
0.50	1.77	0.33	97.32	
0.75	1.68	0.47	92.45	
1.00	1.49	0.43	82.25	
2.50	1.67	0.31	91.83	
Control	1.81	0.29	-	

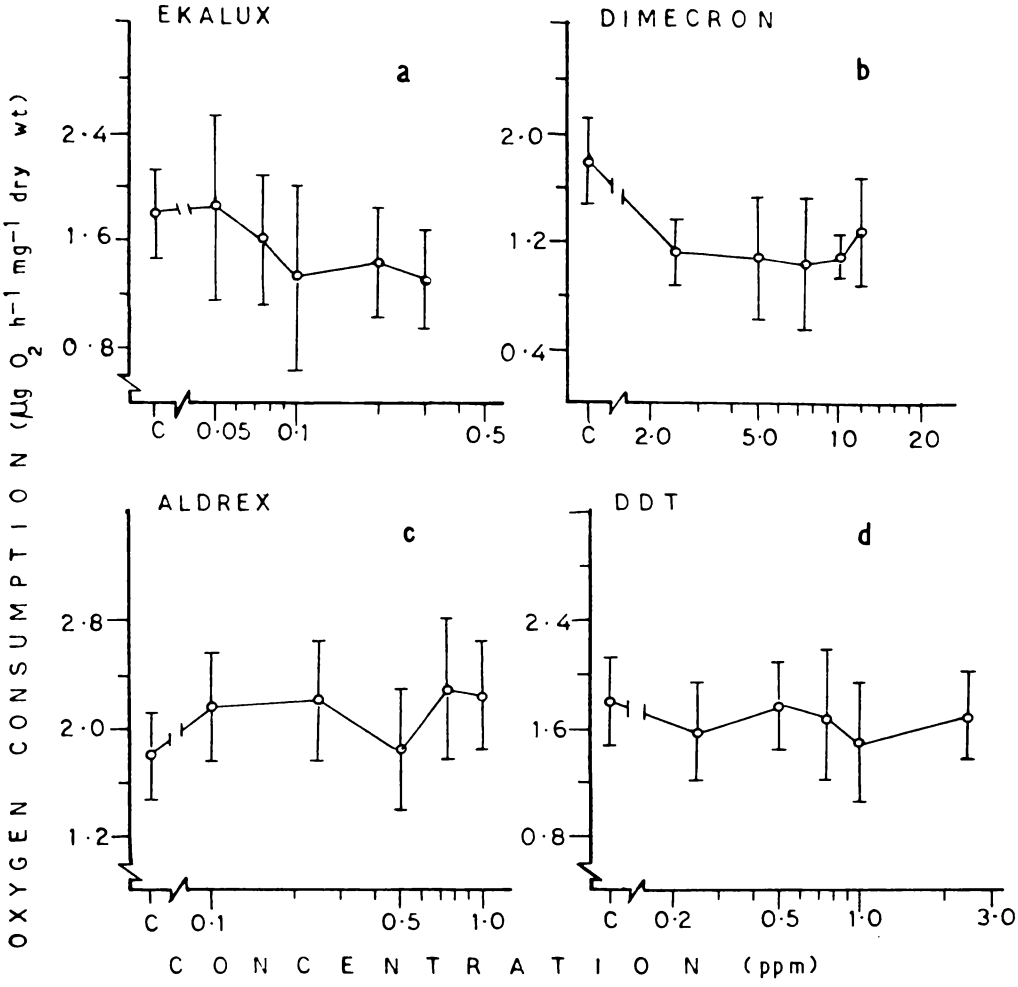


Fig. 48. Perna indica. Average oxygen consumption under different sublethal concentrations of pesticides, along with respective standard errors (Vertical bars) a) Ekalux b) Dimecron c) Aldrex d) DDT

4.2.1.1.1.6 Persian Gulf Crude (WAF)

Water accommodated fraction of P.G. Crude also did not seem to conspicuously affect the oxygen consumption of P. indica. It is clear from Table 18 and Fig. 49b, that at times the animals recorded only marginal reduction irrespective of the variations in concentration. The EC50 was 3.6 ppm of PHC.

4.2.1.1.2 *Villorita cyprinoides var. cochinensis*

This species also was exposed to various sublethal concentrations of Ekalux, Dimecron, Aldrex, DDT and WAF of Light Diesel Oil and the variations in the rate of oxygen consumption was assessed.

4.2.1.1.2.1 Ekalux

A noticeable feature of the results obtained on the oxygen consumption of this species on exposure to various sublethal concentrations of Ekalux was that in the presence of 0.60 ppm of Ekalux drastic increase in oxygen consumption resulted. Among the five concentrations namely, 0.10, 0.25, 0.40, 0.50 and 0.60 ppm employed, only those animals exposed to 0.10 ppm showed a clear cut reduction in oxygen consumption. Considerable variations in the rate of oxygen consumption was observed resulting in relatively higher standard error, indicating that at times the animals remain clammed up maintaining consumption at a very minimum level (Table 19; Fig. 50a).

4.2.1.1.2.2 Dimecron

The concentrations of Dimecron used to study oxygen consumption, ranged from 1.0 ppm to 10.0 ppm. Even those animals facing a stress of 1.0 ppm of Dimecron, consumed oxygen at a level comparable to those ani-

Table 17. Perna indica. Average oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.) under sublethal concentrations of PHC in LDO (WAF), along with respective standard error, percentage performance and EC50 level.

Concentration of PHC in LDO-WAF (ppm)	Oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.075	1.04	0.27	87.90	1.2
0.150	1.17	0.17	98.77	
0.300	1.12	0.22	94.26	
0.500	1.13	0.27	95.43	
0.750	0.83	0.13	70.27	
Control	1.19	0.21		

Table 18. Perna indica. Average oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.) under sublethal concentrations of PHC in P.G. Crude (WAF), along with respective standard error, percentage performance and EC50 level.

Concentration of PHC in P.G.Crude WAF (ppm)	Oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.75	1.16	0.06	95.92	3.6
1.00	0.80	0.33	66.70	
1.25	1.13	0.08	94.50	
1.50	0.78	0.22	64.96	
1.75	0.90	0.28	74.81	
Control	1.20	0.07	-	

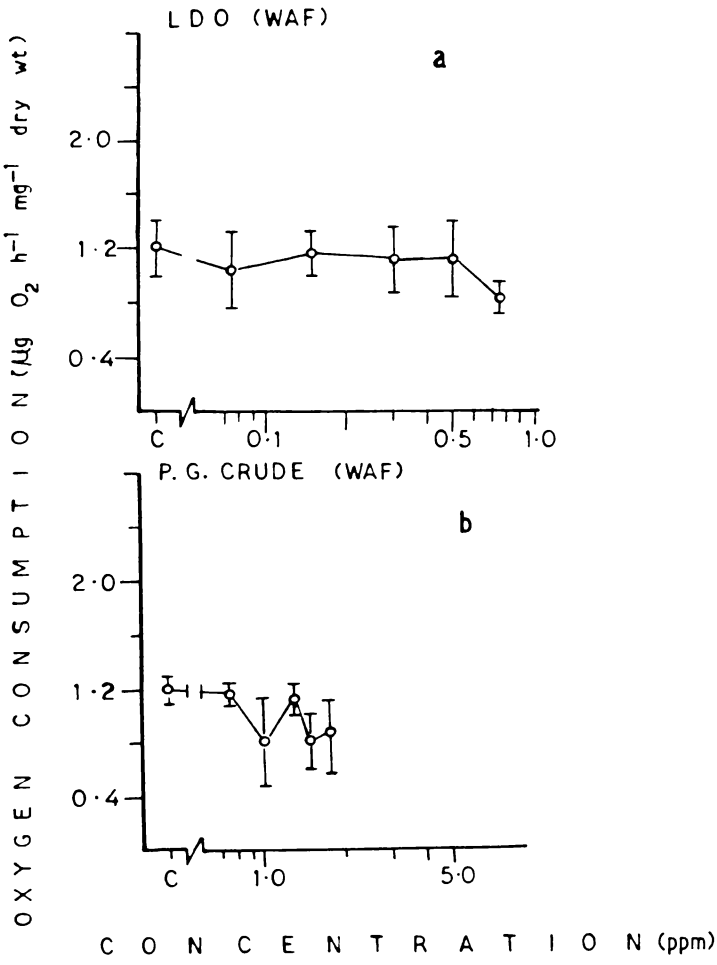


Fig. 49. Perna indica. Average oxygen consumption under different sublethal concentrations of petroleum hydrocarbons (PHC , along with respective standard errors (vertical bars) a) PHC in LDO (WAF) b) PHC in P.G.Crude (WAF)

Table 19. Villorita cyprinoides var. cochinensis. Average oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.) under sublethal concentrations of Ekalux, along with respective standard error and percentage performance.

Ekalux Concentration (ppm)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.)		Performance (% of control)
	Mean	SE	
0.10	1.02	0.37	92.08
0.25	1.06	0.55	96.20
0.40	1.09	0.64	99.09
0.50	1.14	0.52	102.73
0.60	1.47	0.69	133.22
Control	1.11	0.40	-

Table 20. Villorita cyprinoides var. cochinensis. Average oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.) under sublethal concentrations of Dimecron, along with respective standard error and percentage performance.

Dimecron Concentration (ppm)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.)		Performance (% of control)
	Mean	SE	
1.0	1.20	0.30	100.00
2.5	1.37	0.57	114.17
5.0	1.35	0.50	113.01
7.5	1.41	0.24	117.54
10.0	1.64	0.71	137.47
Control	1.20	0.75	-

Table 21. Villorita cyprinoides var. cochinensis. Average oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt) under sublethal concentrations of Aldrex, along with respective standard error and percentage performance.

Aldrex Concentration (ppm)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.)		Performance (% of control)
	Mean	SE	
0.50	0.88	0.06	114.36
0.75	0.72	0.10	93.28
1.00	0.77	0.20	99.74
2.50	1.07	0.20	138.47
5.00	0.74	0.10	95.68
Control	0.77	0.04	-

Table 22. Villorita cyprinoides var. cochinensis. Average oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt) under sublethal concentrations of DDT, along with respective standard error and percentage performance.

DDT Concentration (ppm)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt)		Performance (% of control)
	Mean	SE	
1.00	0.77	0.12	100.00
2.50	0.82	0.06	106.97
5.00	0.77	0.05	99.99
7.50	0.90	0.22	116.91
10.00	0.86	0.26	111.35
Control	0.77	0.04	-

Table 23. Villorita cyprinoides var. cochinensis. Average oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.) under sublethal concentrations of LDO (WAF), along with respective standard error and percentage performance.

PHC in LDO (WAF) Concentration (ppm)	Oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
0.75	1.11	0.15	103.04
1.00	1.07	0.10	98.66
1.25	1.12	0.10	103.54
1.75	1.25	0.18	116.08
2.00	1.22	0.16	112.81
Control	1.08	0.19	-

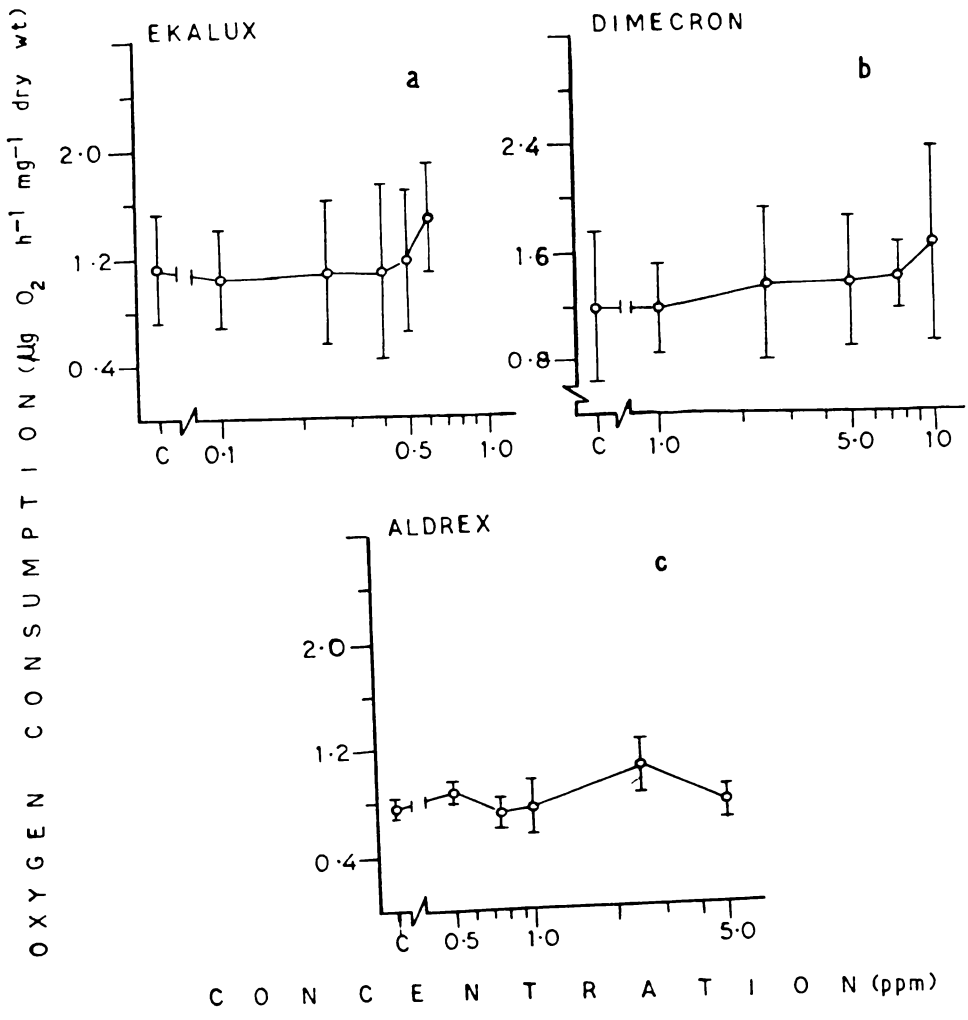


Fig. 50. Villorita cyprinoides var. cochinensis. Average oxygen consumption under different sublethal concentrations of pesticides, along with respective standard errors (vertical bars)
a) Ekalux b) Dimecron c) Aldrex

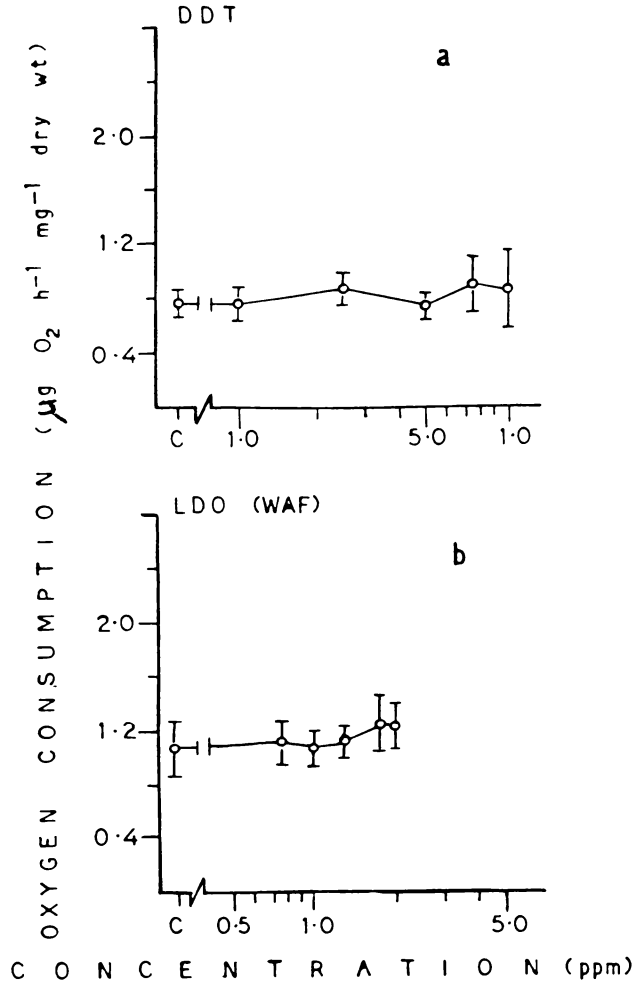


Fig. 51. Villorita cyprinoides var. cochinensis. Average oxygen consumption under different sublethal concentrations of a) DDT and b) Petroleum hydrocarbon (PHC) in LDO-WAF-,along with respective standard errors (vertical bars)

these studies were usually derived from such combined concentrations which did not produce lethal effect on the test animals. This criterion has resulted in using unusually high concentrations of some insecticides, especially Dimecron which was found to be a relatively less sensitive insecticide.

4.2.1.2.1 *Perna indica*

Both the organochlorine and organophosphate pesticides in combination with petroleum hydrocarbon derived from the water accommodated fraction of Light Diesel Oil were used to assess their combined effect on the rate of oxygen consumption by this animal.

4.2.1.2.1.1 PHC in LDO (WAF) unvarying and Ekalux varying

P. indica was exposed to a combination of LDO (WAF) and Ekalux in a fashion, where the PHC concentration in oil was maintained constant at 0.75 ppm and Ekalux varied from 0.50 to 1.50 ppm. The results are presented in Table 24; Fig. 52a. Decrease in the oxygen consumption was considerable, when the Ekalux concentration reached 0.75 ppm. This trend was maintained in higher concentrations of Ekalux too. The effective concentration which resulted in 50% reduction of oxygen consumption was 1.9 ppm of Ekalux.

Increase in the PHC concentration in LDO (WAF) to 2.0 ppm resulted in sudden decrease in oxygen consumption, even when the medium contained only 0.50 ppm of Ekalux. The effective concentration was 1.2 ppm, which shows that after a sudden decrease in oxygen consumption further decrease was minimal by the animals maintained in higher concentration combinations (Table 25; Fig. 52b).

Table 24. Perna indica. Average oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 0.75 ppm PHC in LDO-WAF (unvarying) and Ekalux (varying), along with respective standard errors, percentage performance and EC50 level.

Ekalux Concentration (ppm)	Oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.50	1.54	0.28	98.41	
0.75	1.14	0.19	72.91	
1.00	0.96	0.20	61.45	1.9
1.25	0.87	0.18	55.90	
1.50	0.92	0.20	58.57	
Control	1.56	0.23		

Table 25. Perna indica. Average oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 2.0 ppm PHC in LDO-WAF (unvarying) and Ekalux (varying), along with respective standard errors, percentage performance and EC50 level.

Ekalux Concentration (ppm)	Oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.50	1.07	0.29	68.64	
0.75	1.03	0.20	65.55	
1.00	0.82	0.24	52.63	
1.25	0.79*	0.15	50.83	1.2
1.50	0.68*	0.25	43.23	
Control	1.56	0.23	-	

* $P < 0.05$

4.2.1.2.1.2 Ekalux unvarying and PHC in LDO (WAF) varying

The concentrations employed varied from 0.75 to 2.0 ppm of petroleum hydrocarbons and 0.50 ppm of Ekalux. A clear cut reduction in oxygen consumption occurred was concentration dependent. The percentage performance of those animals maintained in a medium which contained 2.0 ppm of PHC and 0.50 ppm of Ekalux was 68.64. It was found that when the PHC concentration in LDO (WAF) was 2.7 ppm along with 0.50 ppm of Ekalux, the animal's performance rate would be 50% when compared to that of control (Table 26; Fig. 52c).

In another set of experiments the same concentrations of PHC in LDO (WAF) was maintained and Ekalux was enhanced to 1.50 ppm. Then, even in the lowest concentration viz. 0.75 ppm of PHC in LDO (WAF) and 1.50 ppm of Ekalux, the animals tend to respire less. The reduction in performance recorded was found to be statistically significant. The recorded EC50 value was 1.3 ppm of PHC (Table 27; Fig. 52d).

4.2.1.2.1.3 PHC in LDO (WAF) unvarying and Dimecron varying

To find out how far, varying concentration of Dimecron with an unvarying PHC concentration in LDO (WAF) affect the rate of oxygen consumption in P. indica, two sets experiments were performed. In one, the PHC concentration in LDO (WAF) was 0.75 ppm and in another it was 2.0 ppm. In both sets of these experiments the animals were found to consume more of oxygen. Some variations were noticed which cannot be explained properly (Table 28, 29 and Fig. 53 a&b).

Table 26. Perna indica. Average oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.) under the sublethal concentrations of 0.5 ppm Ekalux (unvarying) and PHC in LDO-WAF (varying), along with respective standard errors, percentage performance and EC50 level.

Concentration of PHC in LDO-WAF (ppm)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.75	1.54	0.28	98.41	
1.00	1.50	0.40	95.86	
1.25	1.09	0.21	70.19	2.7
1.75	1.16	0.12	74.03	
2.00	1.07	0.29	68.64	
Control	1.56	0.23		

Table 27. Perna indica. Average oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.) under the sublethal concentrations of 1.5 ppm Ekalux (unvarying) and PHC in LDO-WAF (varying), along with respective standard errors percentage performance and EC50 level.

Concentration of PHC in LDO-WAF (ppm)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.75	0.92	0.20	58.57	
1.00	1.04	0.20	66.46	
1.25	0.81*	0.16	51.99	1.3
1.75	0.69*	0.18	44.31	
2.00	0.68*	0.25	43.23	
Control	1.56	0.23	-	

* $P < 0.05$

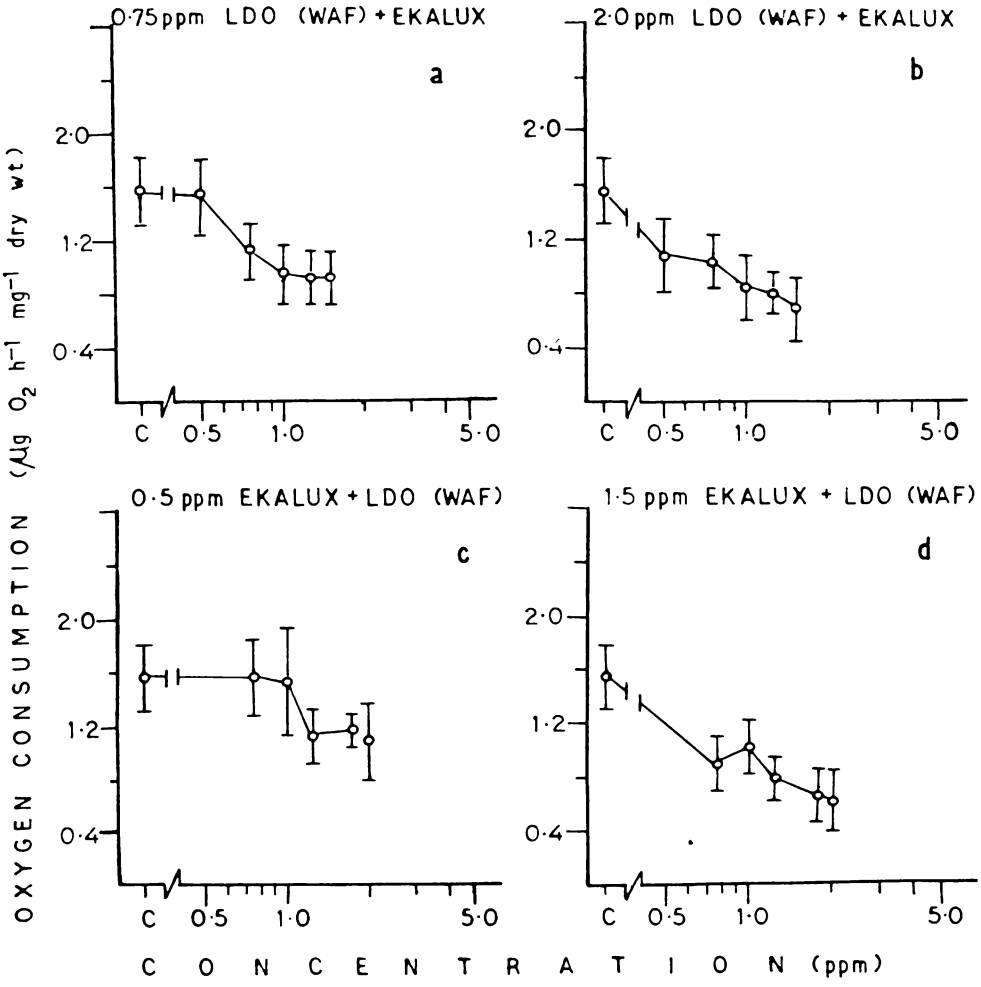


Fig. 52. Perna indica. Average oxygen consumption under different sublethal concentrations of pesticide and petroleum hydrocarbon (PHC) in combinations, along with respective standard errors (vertical bars) a & b. PHC in LDO-WAF- (unvarying) and Ekalux (varying).c & d. Ekalux (unvarying) and PHC in LDO-WAF- (varying)

Table 28. Perna indica. Average oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 0.75 ppm PHC in LDO-WAF (unvarying) and Dimecron (varying), along with respective standard errors, and percentage performance.

Dimecron Concentration (ppm)	Oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
10.0	1.47	0.34	96.58
15.0	1.62	0.24	106.50
25.0	1.46	0.71	95.87
40.0	1.93	0.31	125.63
50.0	1.75	0.66	115.06
Control	1.52	0.10	-

Table 29. Perna indica. Average oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 2.0 ppm PHC in LDO-WAF (unvarying) and Dimecron (varying), along with respective standard errors, and percentage performance.

Dimecron Concentration (ppm)	Oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
10.0	1.58	0.50	103.59
15.0	1.48	0.57	97.18
25.0	1.71	0.15	112.38
40.0	1.76	0.34	115.91
50.0	1.85	0.11	121.84
Control	1.52	0.10	-

4.2.1.2.1.4 Dimecron unvarying and PHC in LDO (WAF) varying

P. indica, when exposed to concentrations of Dimecron maintained at a constant level and PHC in LDO (WAF) varying, gave totally different results. The concentrations of PHC in LDO (WAF) was 0.75 to 2.0 ppm with 10.0 ppm of Dimecron. The animals respired at a higher rate than their counterparts in control. However, increase in consumption did not show any pattern (Table 30; Fig. 53c).

Concentration of Dimecron was raised to 50.0 ppm in another set of experiment, where the PHC concentration in LDO (WAF) ranged from 0.75 to 2.0 ppm. Here also, the rate of oxygen consumption was found to increase. The animals maintained in those combinations which contained 0.75 ppm of PHC and 2.0 ppm of PHC with 50.0 ppm of Dimecron consumed more oxygen than the rest (Table 31; Fig. 53d). Very great variations were found to occur in the same concentrations which has resulted in considerable range of standard error.

4.2.1.2.1.5 PHC in LDO (WAF) unvarying and Aldrex varying

Combination of LDO (WAF) and Aldrex were employed to find out the effect of these combinations on the oxygen consumption performance of P. indica. The results obtained are presented in Tables 32, 33 and Fig. 54 a&b. The unvarying concentrations of PHC in LDO (WAF) was either 0.50 ppm or 2.0 ppm along with the Aldrex concentrations varying from 1.0 ppm to 4.0 ppm. In both these experiments it was noticed that, there was reduction in oxygen consumption in all the concentration combinations. However, reduction in the percentage performance was not very conspicuous.

Table 30. Perna indica. Average oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 10.0 ppm Dimecron (unvarying) and PHC in LDO-WAF (varying), along with respective standard errors and percentage performance.

Concentration of PHC in LDO-WAF (ppm)	Oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
0.75	1.47	0.34	96.58
1.00	1.69	0.11	111.13
1.25	1 83	0.13	120.13
1.75	1.88	0.37	123.17
2.00	1.58	0.50	103.59
Control	1.52	0.10	

Table 31. Perna indica. Average oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 50.0 ppm Dimecron (unvarying) and PHC in LDO-WAF (varying), along with respective standard errors and percentage performance.

Concentration of PHC in LDO-WAF (ppm)	Oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
0.75	1.75	0.66	115.06
1.00	1.56	0.23	102.34
1.25	1.62	0.39	106.52
1.75	1.65	0.11	108.06
2.00	1.85	0.11	121.84
Control	1.52	0.10	-

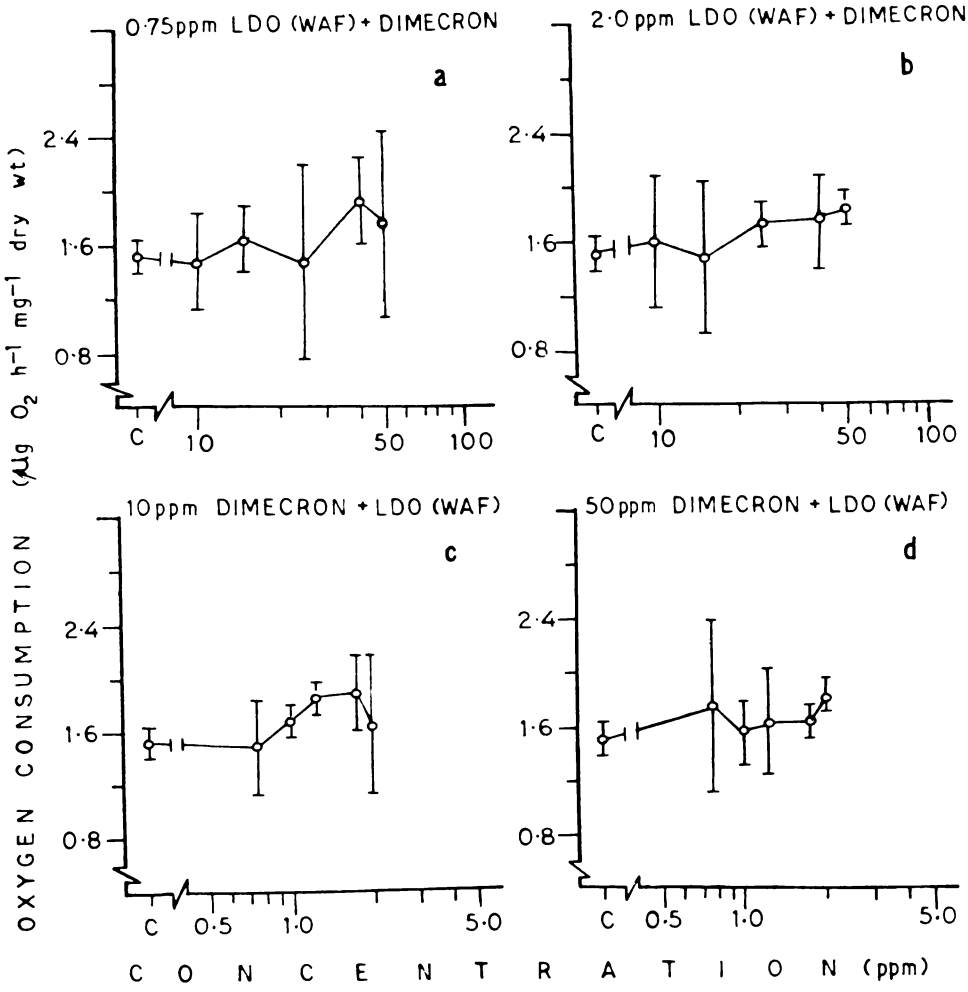


Fig. 53. Perna indica. Average oxygen consumption under different sublethal concentrations of pesticide and petroleum hydrocarbon (PHC) in combinations, along with respective standard errors (vertical bars) a&b. PHC in LDO-WAF- (unvarying) and Dimecron (varying) c&d. Dimecron (unvarying) and PHC in LDO-WAF- (varying).

Table 32. Perna indica. Average oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.) under the sublethal concentrations of 0.5 ppm LDO-WAF (unvarying) and Aldrex (varying), along with respective standard errors, percentage performance and EC50 level.

Concentration (ppm) Aldrex	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
1.0	1.35	0.19	81.56	
1.5	1.55	0.48	94.09	
2.0	1.31	0.34	79.50	7.8
3.0	1.19	0.40	72.53	
4.0	0.93 *	0.17	56.03	
Control	1.65	0.12	-	

Table 33. Perna indica. Average oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.) under the sublethal concentrations of 2.0 ppm PHC in LDO-WAF (unvarying) and Aldrex (varying), along with respective standard errors, and percentage performance.

Concentration (ppm) Aldrex	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.)		Performance (% of control)
	Mean	SE	
1.0	1.15	0.26	69.60
1.5	1.36	0.37	82.27
2.0	1.56	0.32	94.52
3.0	1.27	0.22	77.12
4.0	1.06	0.37	64.18
Control	1.65	0.12	-

* $P < 0.05$

4.2.1.2.1.6 Aldrex unvarying and PHC in LDO (WAF) varying

Maintenance of unvarying concentration of Aldrex and varying concentrations of PHC in LDO (WAF) also gave similar results. The performance levels of the animals were more or less comparable irrespective of the concentration (Table 34, 35 and Fig. 54 c&d).

4.2.1.2.1.7 PHC in LDO (WAF) unvarying and DDT varying

Perna indica was exposed to a combination of either 0.15 ppm or 0.75 ppm PHC in LDO (WAF) with 0.25 to 1.0 ppm DDT. In both the cases there was reduction in oxygen consumption. The effective concentrations were 0.8 and 0.5 ppm of DDT, respectively (Table 36, 37 and Fig. 55 a&b).

4.2.1.2.1.8 DDT unvarying and PHC in LDO (WAF) varying

In a reciprocal series of experiments, P. indica was exposed to a combination of 0.25 ppm of DDT and 0.15 to 0.75 ppm of PHC in LDO (WAF). The results indicated a reduction in oxygen consumption. Those animals maintained in the combination of 0.75 ppm of PHC and 0.25 ppm of DDT, consumed only 60% of oxygen to that of controlled animals. The effective concentration that caused 50% reduction in consumption was 1.1 ppm (Table 38; Fig. 55c).

When the DDT concentration was increased to 1.0 ppm the reduction in consumption was not very different from that observed in the previous case. The EC50 was 1.0 ppm of PHC with 1.0 ppm of DDT (Table 39; Fig. 55d).

4.2.1.2.2 *Villorita cyprinoides var. cochinensis*

This backwater clam was exposed to varying and unvarying concentrations of PHC in LDO (WAF), Ekalux and Aldrex. Since the rate of mortality in Dimecron and DDT was rather low when exposed individually, these pesticides

Table 34. Perna indica. Average oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 1.0 ppm Aldrex (unvarying) and PHC in LDO-WAF (varying), along with respective standard errors, percentage performance and EC50 level.

Concentration (ppm) PHC in LDO(WAF)	Oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.50	1.35	0.19	81.56	2.1
0.75	1.05	0.28	63.43	
1.00	1.02	0.30	61.61	
1.50	1.03**	0.11	62.21	
2.00	1.15	0.26	69.60	
Control	1.65	0.12	-	

Table 35. Perna indica. Average oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 4.0 ppm Aldrex (unvarying) and PHC in LDO-WAF (varying), along with respective standard errors, and percentage performance.

Concentration (ppm) PHC in LDO (WAF)	Oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
0.50	0.93*	0.17	56.03
0.75	1.08	0.31	65.68
1.00	1.17	0.44	71.10
1.50	1.16**	0.033	70.56
2.00	1.06	0.37	64.18
Control	1.65	0.12	-

* $P < 0.05$

** $P < 0.01$

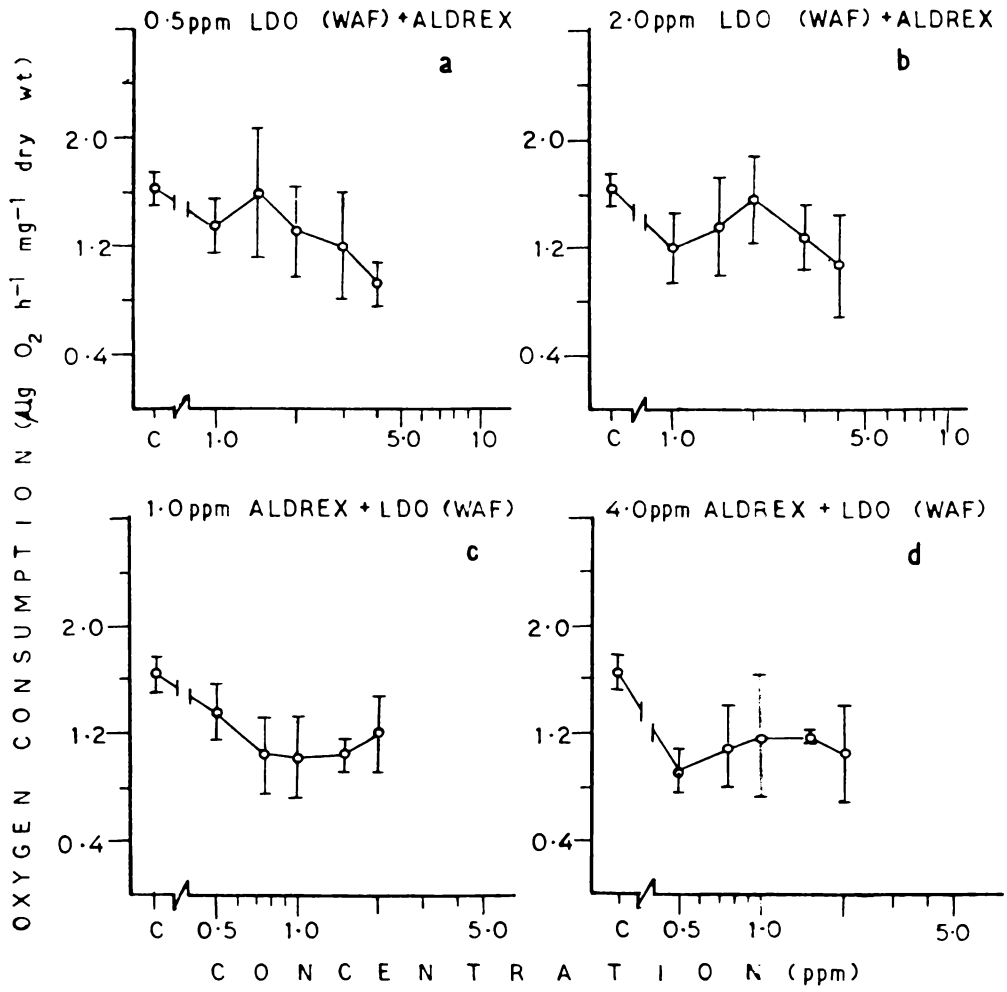


Fig. 54. Perna india. Average oxygen consumption under different sublethal concentrations of pesticide and petroleum hydrocarbon (PHC) in combinations, along with respective standard errors (vertical bars) a&b. PHC in LDO-WAF- (unvarying) and Aldrex (unvarying) and PHC in LDO-WAF- (varying).

Table 36. Perna indica. Average oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 0.15 ppm PHC in LDO-WAF (unvarying) and DDT (varying), along with respective standard errors, percentage performance and EC50 level.

DDT Concentration (ppm)	Oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.25	1.46	0.50	87.85	
0.40	1.26	0.39	75.42	
0.50	1.51	0.71	90.71	0.8
0.75	0.86	0.25	51.44	
1.00	1.11	0.47	66.84	
Control	1.67	0.31	-	

Table 37. Perna indica. Average oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 0.75 ppm PHC in LDO-WAF (unvarying) and DDT (varying), along with respective standard errors, percentage performance and EC50 level.

DDT Concentration (ppm)	Oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.25	1.01	0.47	60.60	
0.40	1.15	0.33	68.81	
0.50	0.74*	0.22	44.15	0.5
0.75	0.62*	0.22	37.26	
1.00	0.92	0.18	55.12	
Control	1.67	0.31	-	

* $P < 0.05$

Table 38. Perna indica. Average oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 0.25 ppm DDT (unvarying) and PHC in LDO-WAF (varying), along with respective standard errors, percentage performance and EC50 level.

Concentration of PHC in LDO-WAF (ppm)	Oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.15	1.46	0.50	87.85	
0.25	1.09	0.41	65.40	
0.40	1.15	0.35	69.21	
0.50	1.04	0.32	62.57	1.1
0.75	1.01	0.47	60.60	
Control	1.67	0.31	-	

Table 39. Perna indica. Average oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 1.0 ppm DDT (unvarying) and LDO-WAF (varying), along with respective standard errors, percentage performance and EC50 level.

Concentration of PHC in LDO-WAF (ppm)	Oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.15	1.11	0.47	66.84	
0.25	0.94	0.26	59.99	
0.40	1.19	0.31	71.50	1.0
0.50	1.24	0.50	74.45	
0.75	0.92	0.18	55.12	
Control	1.67	0.31	-	

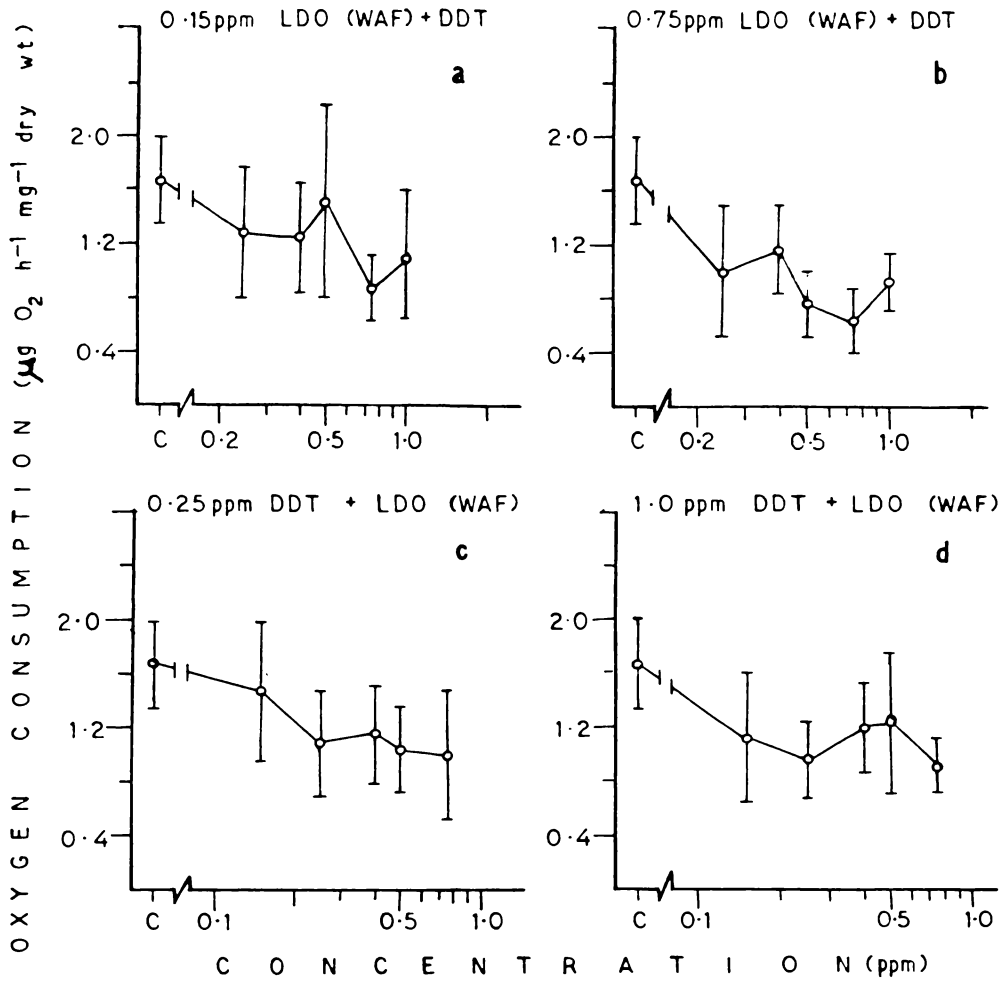


Fig. 55. Perna india. Average oxygen consumption under different sublethal concentrations of pesticide and petroleum hydrocarbon (PHC) in combinations, along with respective standard errors (vertical bars) a&b. PHC in LDO-WAF- (unvarying) and DDT (varying) c&d. DDT (unvarying) and PHC in LDO-WAF- (varying).

were not used to analyse their sublethal effect in combination with LDO (WAF).

4.2.1.2.2.1 PHC in LDO (WAF) unvarying and Ekalux varying

Two concentrations of PHC in LDO (WAF) namely 0.50 ppm and 2.0 ppm, in combination with varying concentrations of Ekalux, ranging from 0.25 to 1.50 ppm were employed to analyse the pattern of oxygen consumption by this animal. Clear cut concentration dependent reduction in oxygen consumption was delineated. The presence of 0.50 ppm PHC in LDO (WAF) and 0.25 ppm of Ekalux did not produce conspicuous reduction in oxygen consumption, but when the concentration of Ekalux was increased, the animals continued to reduce the consumption of oxygen. The worked out EC50 value was 1.8 ppm of Ekalux with 0.50 ppm PHC in LDO (WAF) (Table 40; Fig. 56a).

Even when the concentration of PHC in LDO (WAF) was increased to 2.0 ppm the resultant decrease in oxygen consumption was not drastic. This is clearly evident from the EC50 value of 1.4 ppm of Ekalux with 2.0 ppm of PHC in LDO (WAF). However, even in the lowest concentration of Ekalux with PHC, the stress was felt. It seems from the results that in the presence of LDO (WAF), varying concentrations of Ekalux resulted in a rather uniform reduction in oxygen consumption (Table 41; Fig. 56b).

4.2.1.2.2.2 Ekalux unvarying and PHC in LDO (WAF) varying

Reciprocal to the above series of experiment, the concentration of Ekalux was maintained constant and that of PHC in LDO (WAF) fluctuating from 0.50 to 2.0 ppm. In the presence of 0.25 ppm of Ekalux only a concentration of 2.0 ppm PHC in LDO (WAF) produced clear cut reduction in consumption, reaching around 50% to that of control. The EC50 was 3.2 ppm which means that

Table 40. Villorita cyprinoïdes var. cochinensis. Average oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.) under the sublethal concentrations of 0.5 ppm PHC in LDO-WAF (unvarying) and Ekalux (varying), along with respective standard errors, percentage performance and EC50 level.

Ekalux Concentration (ppm)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.25	1.13	0.09	88.47	
0.50	0.98*	0.01	76.77	
0.75	0.79*	0.15	62.65	1.8
1.00	0.82*	0.07	64.56	
1.50	0.72**	0.07	56.24	
Control	1.27	0.11	-	

Table 41. Villorita cyprinoides var. cochinensis. Average oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.) under the sublethal concentrations of 2.0 ppm LDO-WAF (unvarying) and Ekalux (varying), along with respective standard errors, percentage performance and EC50 level.

Ekalux Concentration (ppm)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.25	0.72**	0.06	56.82	
0.50	0.81*	0.12	63.91	
0.75	0.75**	0.06	58.82	1.4
1.00	0.71*	0.13	55.85	
1.50	0.61**	0.05	48.19	
Control	1.27	0.11	-	

* $P < 0.05$

** $P < 0.01$

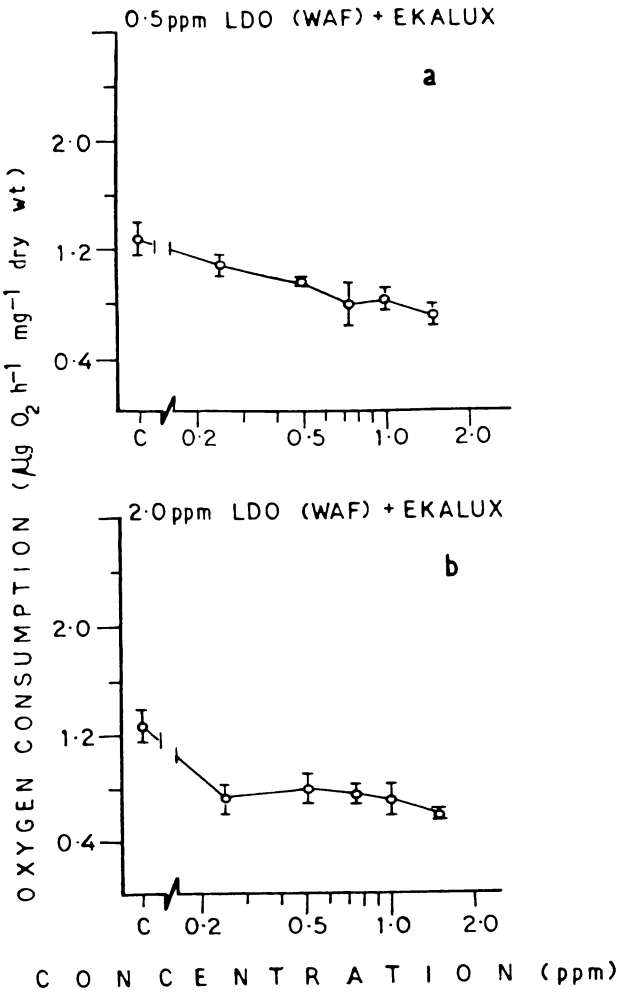


Fig. 56. Villorita cyprinoides var. cochinensis. Average oxygen consumption under different sublethal concentrations of pesticide and petroleum hydrocarbon (PHC) in combinations, along with respective standard errors (vertical bars) a&b. PHC in LDO-WAF- (unvarying) and Ekalux (varying).

3.2 ppm PHC in LDO (WAF) is required in the media that contained 0.25 ppm of Ekalux to produce 50% reduction in oxygen consumption (Table 42; Fig. 57a).

In another series of experiment the concentration of Ekalux was raised to 1.50 ppm keeping the PHC concentration in LDO (WAF) ranging from 0.50 to 2.0 ppm as in the previous case. The increased concentration of Ekalux resulted reduction in oxygen consumption and the profile in consumption centred around 50% of control. However, the calculated EC50 value was 2.2 ppm of PHC with 1.50 ppm of Ekalux. (Table 43; Fig. 57b).

4.2.1.2.2.3 PHC in LDO (WAF) unvarying and Aldrex varying

Another insecticide which was employed to analyse the pattern of oxygen consumption in V. cyprinoides var. cochinensis, in combination with unvarying and varying concentration of PHC in LDO (WAF) was Aldrex. This is a relatively less toxic insecticide with reference to V. cyprinoides var. cochinensis. The concentrations of Aldrex varied between 1.0 to 4.0 ppm either with 0.50 or 2.0 ppm of PHC. Table 44 and 45 give the results obtained in oxygen consumption by V. cyprinoides var. cochinensis when exposed to a concentration of 0.50 or 2.0 ppm of PHC in LDO (WAF) in the presence of varying concentrations of Aldrex. 0.50 ppm of PHC in LDO (WAF) in combination with 1.0 to 4.0 ppm of Aldrex produced toxic effect of a marginal nature, especially at lower concentrations of Aldrex. It was found that 7.0 ppm of Aldrex, with 0.50 ppm PHC in LDO (WAF) is needed to produce 50% reduction in oxygen consumption performance. Minimal variations in standard error in both the concentration series indicate that the performance of animals, whether reduction or increase was uniform (Fig. 58 a&b).

Table 42. Villorita cyprinoides var. cochinensis. Average oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.) under the sublethal concentrations of 0.25 ppm Ekalux (unvarying) and PHC in LDO-WAF (varying), along with respective standard errors, percentage performance and EC50 level.

PHC in LDO (WAF) Concentration (ppm)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.50	1.13	0.09	88.47	
0.75	0.98	0.12	76.99	
1.00	0.96	0.17	78.54	3.2
1.50	0.93*	0.03	72.87	
2.00	0.72**	0.06	56.82	
Control	1.27	0.11	-	

Table 43. Villorita cyprinoides var. cochinensis. Average oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.) under the sublethal concentrations of 1.50 ppm Ekalux (unvarying) and PHC in LDO-WAF (varying), along with respective standard errors, percentage performance and EC50 level.

PHC in LDO (WAF) Concentration (ppm)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.50	0.72**	0.07	56.24	
0.75	0.71*	0.12	55.82	
1.00	0.78**	0.06	61.01	2.2
1.50	0.69**	0.06	54.54	
2.00	0.61**	0.05	48.19	
Control	1.27	0.11	-	

* $P < 0.05$

** $P < 0.01$

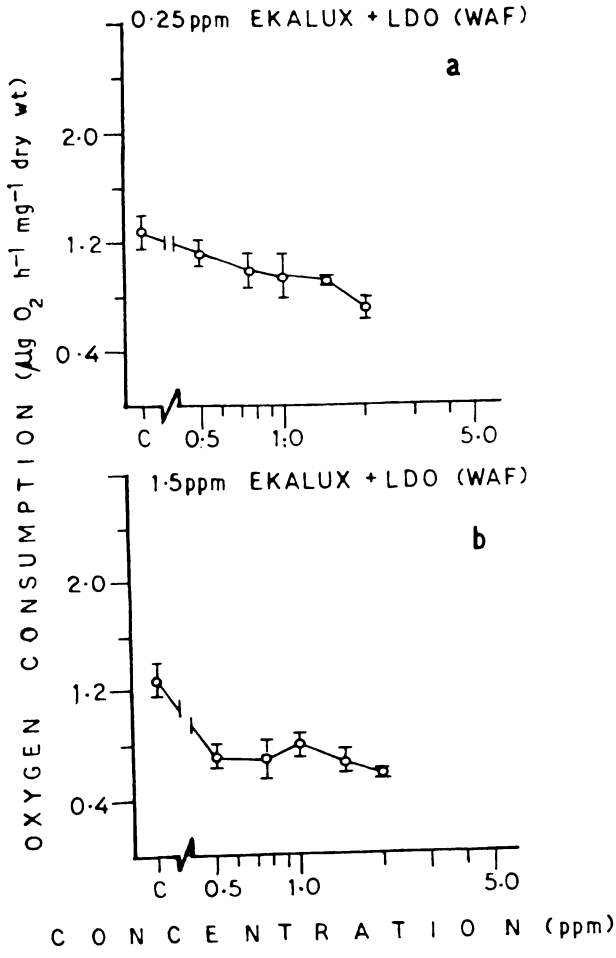


Fig. 57. Villorita cyprinoides var. cochinensis. Average oxygen consumption under different sublethal concentrations of pesticide and petroleum hydrocarbon (PHC) in combinations, along with respective standard errors (vertical bars) a&b. Ekalux (unvarying) and PHC in LDO-WAF- (varying).

Table 44. Villorita cyprinoides var. cochinensis. Average oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.) under the sublethal concentrations of 0.5 ppm LDO-WAF (unvarying) and Aldrex (varying), along with respective standard errors, percentage performance and EC50 level.

Aldrex Concentration (ppm)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
1.00	1.09	0.07	87.01	
1.50	0.96	0.11	76.80	
2.00	0.87*	0.10	69.42	7.0
3.00	0.84*	0.11	67.11	
4.00	0.82**	0.05	65.46	
Control	1.25	0.07	-	

Table 45. Villorita cyprinoides var. cochinensis. Average oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.) under the sublethal concentrations of 2.0 ppm LDO-WAF (unvarying) and Aldrex (varying), along with respective standard errors, percentage performance and EC50 level.

Aldrex Concentration (ppm)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
1.00	0.70**	0.07	56.05	
1.50	0.79**	0.09	63.20	
2.00	0.75**	0.07	60.22	3.4
3.00	0.66**	0.10	52.34	
4.00	0.58**	0.08	46.60	
Control	1.25	0.07	-	

* $P < 0.05$

** $P < 0.01$

When the PHC in LDO (WAF) concentration was enhanced to 2.0 ppm the toxicity of Aldrex was found to increase. The EC50 obtained was 3.4 ppm of Aldrex. Here, irrespective of the variation in the Aldrex concentration, animals continued to consume only less quantities of oxygen (Table 45; Fig. 58b).

4.2.1.2.2.4 Aldrex unvarying and PHC in LDO (WAF) varying

Tables 46 and 47, detail out the results obtained on the oxygen consumption pattern of V. cyprinoides var. cochinensis exposed to unvarying concentrations of Aldrex (either 1.0 or 4.0 ppm) with varying PHC concentrations in LDO (WAF) ranging from 0.50 to 2.0 ppm. The most interesting feature of the results was that irrespective of the variations in Aldrex concentrations, more or less the same concentration of PHC in LDO (WAF) produced 50% reduction in oxygen consumption. The only difference was that in the presence of 1.0 ppm of Aldrex, animals maintained in 0.50 and 0.75 ppm of PHC consumed relatively higher quantities of oxygen. Reduction occurred only when the PHC concentration was elevated to 2.0 ppm. On the other hand animals maintained in all the concentrations of PHC in combination with 4.0 ppm of Aldrex consumed less oxygen (Fig. 58 c&d).

4.2.1.3. Rate of Filtration: Under Individual Toxicant Stress

Rate of filtration is a more reliable parameter for assessing sublethal toxic response. Results obtained and presented in the literature available, on the rate of filtration show that the quantity of water filtered is a dependable measure of toxicity stress and well-being of bivalves used for the experiments. It was found that P. indica, which has got a byssal mass is not in a position to close the valves completely so as to prevent entry of water into the mantle cavity. The interference by the byssal mass is an important factor which

Table 46. Villorita cyprinoides var. cochinensis. Average oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.) under the sublethal concentrations of 1.0 ppm Aldrex (unvarying) and PHC in LDO-WAF (varying), along with respective standard errors, percentage performance and EC50 level.

Concentration of PHC in LDO-WAF (ppm)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.50	1.09	0.08	87.01	
0.75	1.02	0.07	81.78	
1.00	0.76**	0.01	60.39	2.2
1.50	0.79**	0.04	63.35	
2.00	0.70**	0.07	56.05	
Control	1.25	0.14	-	

Table 47. Villorita cyprinoides var. cochinensis. Average oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.) under the sublethal concentrations of 4.0 ppm Aldrex (unvarying) and PHC in LDO-WAF (varying), along with respective standard errors, percentage performance and EC50 level.

Concentration of PHC in LDO-WAF (ppm)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.50	0.82**	0.10	65.46	
0.75	0.78**	0.06	61.89	
1.00	0.77**	0.17	61.36	2.3
1.50	0.74**	0.12	58.73	
2.00	0.58**	0.17	46.60	
Control	1.25	0.14	-	

** $P < 0.01$

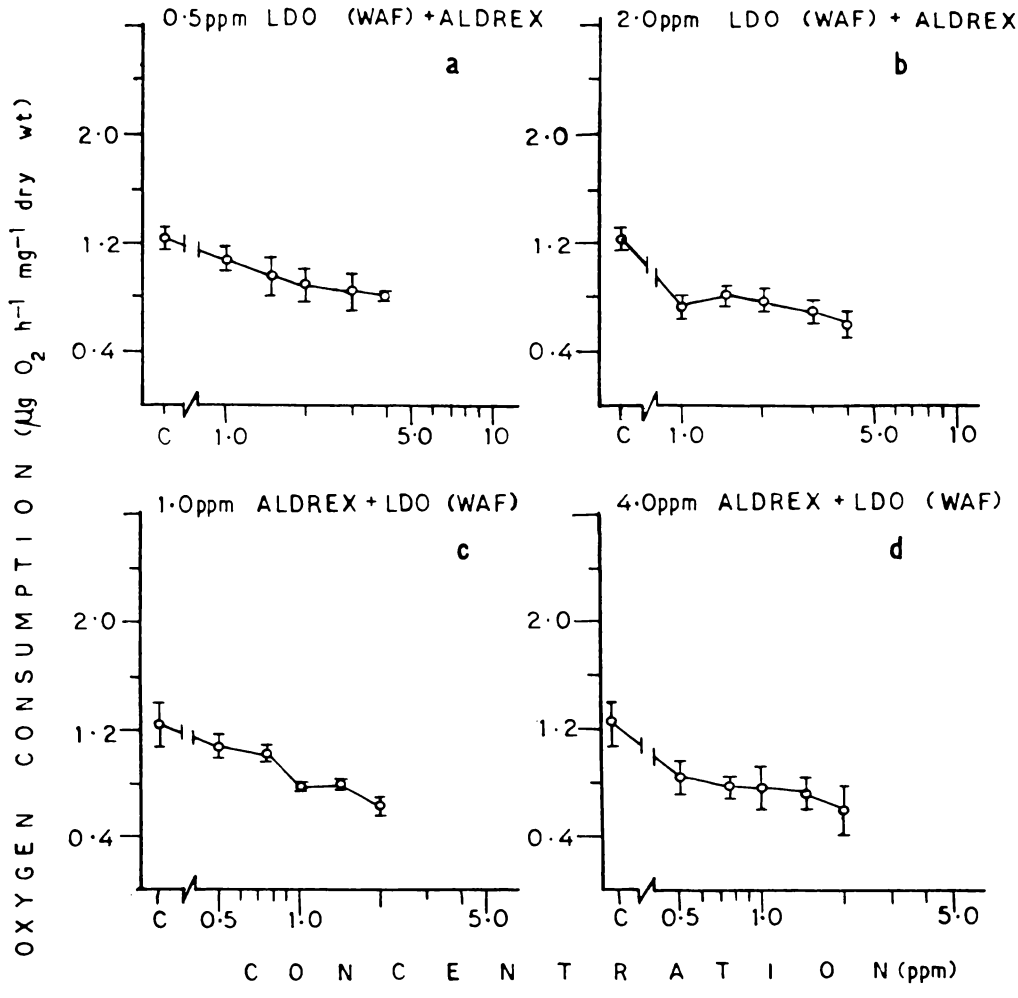


Fig. 58. Villorita cyprinoides var. cochinensis. Average oxygen consumption under different sublethal concentrations of pesticide and petroleum hydrocarbon (PHC) in combinations, along with respective standard errors (vertical bars). a&b. PHC in LDO-WAF- (unvarying) and Aldrex (varying) c&d Aldrex (unvarying) and PHC in LDO-WAF- (varying).

influences the filtration rate of mussels, even when exposed to highly toxic chemicals. On the other hand clams in general can 'clam up' resulting in almost total cessation of entry of water into the mantle cavity. Wedging the shells or drilling holes on the shell have been suggested as methods to prevent a complete closure of the shells of clams while employing for experiments. However, laboratory trials have shown that the inert material wedged in between the shells could be easily rejected by the animal thereby bringing in considerable fluctuations in the quantity of water entering the mantle cavity. Therefore, such methods were not used during the course of present experiment. The rate of filtration by animals exposed to sublethal concentrations of individual toxic components have been delineated and presented in this section.

4.2.1.3.1 *Perna indica*

Representatives of this animal were exposed to sublethal concentrations of Ekalux, Dimecron, Aldrex, DDT and water accommodated fractions of both Light Diesel Oil and Persian Gulf Crude. The quantity of water filtered under the sublethal stress of individual toxic components of these toxicants were worked out and presented.

4.2.1.3.1.1 Ekalux

Sublethal concentrations selected to investigate the effects of Ekalux, on the rate of filtration of P. indica ranged between 0.05 to 0.30 ppm. The data obtained are given in Table 48 and Fig. 59a. Concentration dependant decrease in filtration rate was evinced. However, those levels which ranged from 0.05 to 0.20 ppm resulted reduction in performance levels from 98 to 73% of control only. But when the concentration was increased to 0.30 ppm there

was a drastic reduction in filtration rate. This is the reason why the effective concentration to result in 50% reduction of filtration was found to be 0.27 ppm. An increase in the Ekalux level by a factor of 0.07 ppm from 0.20 ppm resulted a drop in filtration rate from 75% to 50%.

4..2.1.3.1.2 Dimecron

Dimecron is relatively a less toxic pesticide to P. indica. The concentrations employed to study filtration rate varied between 2.50 and 12.0 ppm. The reduction in filtration rate was not conspicuous even when P. indica was exposed to 12.0 ppm of Dimecron. The calculated EC50 value was 35.4 ppm (Table 49; Fig. 59b).

4.2.1.3.1.3 Aldrex

The results obtained on the rate of filtration of P. indica exposed to sublethal concentrations of Aldrex are presented in Table 50 and Fig. 59c. A clear cut decrease in filtration was found to occur with every 0.25 ppm increase in the Aldrex concentrations in the test medium. When the concentration of Aldrex was 1.0 ppm the performance level of the animal was 52% to that of control. The EC50 was 1.4 ppm. All the results obtained were found to be significant either at 0.01 or 0.05 levels.

4.2.1.3.1.4 DDT

DDT was also found to be highly toxic to P. indica. A concentration of 2.50 ppm brought down the rate of filtration around 40%. Even the presence of 0.25 ppm of DDT also brought about clear cut reduction in filtration rate. Further increase resulted a drop in the rate of filtration. A more or less uni-

Table 48. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under sublethal concentrations of Ekalux, along with respective standard error, percentage performance and EC50 level.

Ekalux Concentration (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.05	0.596	0.03	97.55	
0.075	0.512*	0.02	83.80	
0.10	0.479*	0.05	78.40	
0.20	0.446**	0.04	73.00	0.27
0.30	0.270**	0.02	44.19	
Control	0.611	0.03	-	

Table 49. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under sublethal concentrations of Dimecron, along with respective standard error, percentage performance and EC50 level.

Ekalux Concentration (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
2.5	0.573	0.04	93.78	
5.0	0.517	0.04	84.62	
7.5	0.487*	0.03	79.71	35.4
10.0	0.552	0.04	90.34	
12.0	0.461**	0.02	75.45	
Control	0.611	0.03	-	

* $P < 0.05$

** $P < 0.01$

Table 50. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under sublethal concentrations of Aldrex, along with respective standard error, percentage performance and EC50 level.

Aldrex Concentration (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.1	0.521*	0.03	82.44	
0.25	0.498**	0.03	78.80	
0.50	0.419**	0.02	66.30	1.4
0.75	0.375**	0.03	59.30	
1.0	0.328**	0.01	51.89	
Control	0.632	0.02	-	

Table 51. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under sublethal concentrations of DDT, along with respective standard error, percentage performance and EC50 level.

DDT Concentration (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.25	0.501*	0.04	79.27	
0.50	0.355**	0.03	56.17	
0.75	0.358**	0.02	56.65	1.1
1.00	0.323**	0.01	51.11	
2.50	0.250**	0.01	39.56	
Control	0.632	0.02	-	

* $P < 0.05$

** $P < 0.01$

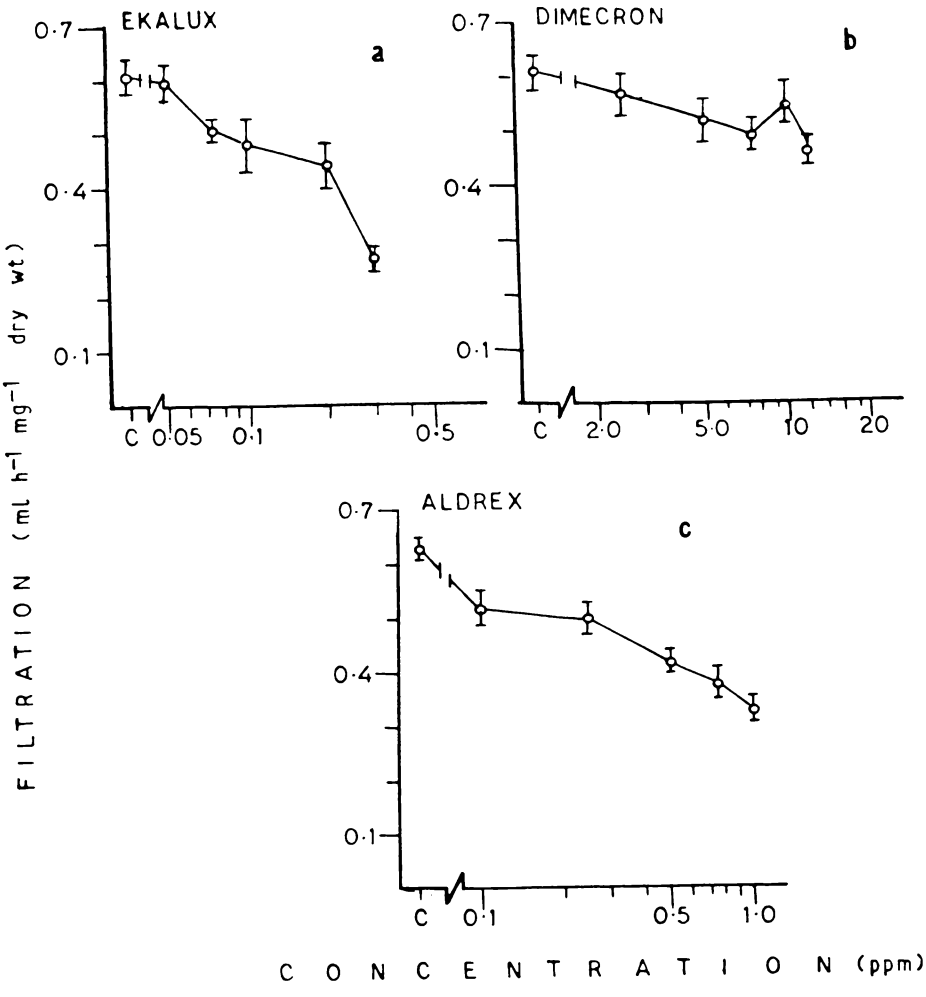


Fig. 59. Perna indica. Average filtration under different sublethal concentrations of pesticides, along with respective standard errors (vertical bars) a) Ekalux b) Dimecron c) Aldrex.

formity in the rate was noticed until when the external concentration of DDT was 1.0 ppm. The results obtained were found to be statistically significant (Table 51; Fig. 60a).

4.2.1.3.1.5 Light Diesel Oil (WAF)

The water accommodated fraction of LDO did not produce any clear cut reduction in the rate of filtration even when the petroleum hydrocarbon content in the WAF was 0.50 ppm. The amount of water filtered remained more or less uniform at all the various concentrations used (Table 52; Fig. 60b).

4.2.1.3.1.6 Persian Gulf Crude (WAF)

The PHC concentrations used to study the effect of P.G. Crude (WAF) in P. indica ranged from 0.75 to 1.75 ppm. None of the concentrations could bring about drastic reduction in filtration rate (Table 53; Fig. 60c). The calculated EC50 value was 4.8 ppm. It may be recalled that P.G. Crude (WAF) was a relatively less toxic material to P. indica.

4.2.1.3.2. *Villorita cyprinoides var. cochinensis*

As in the case of P. indica, representative of V. cyprinoides var. cochinensis was also used to assess the sublethal effect of individual components of the pesticides and WAFs on the rate of filtration.

4.2.1.3.2.1 Ekalux

Concentrations ranging from 0.10 to 0.60 ppm of Ekalux were used in the test medium to study the resultant effect on the rate of filtration of V. cyprinoides var. cochinensis. Presence of 0.50 and 0.60 ppm of Ekalux could produce clear cut deleterious effects. The animals practically stopped filtration

Table 52. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under sublethal concentrations of LDO (WAF), along with respective standard error and percentage performance.

Concentration of PHC in LDO (WAF) (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
0.075	0.477*	0.02	84.13
0.150	0.465	0.04	82.01
0.300	0.467	0.04	82.36
0.500	0.485	0.05	85.54
0.750	0.451	0.05	79.54
Control	0.567	0.03	-

Table 53. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under sublethal concentrations of P.G. Crude (WAF), along with respective standard error, percentage performance and EC50 level.

Concentration of PHC in PG Crude (WAF)(ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0 75	0.516	0.02	91.01	
1.00	0.409*	0.04	72.13	
1.25	0.493	0.05	86.95	4.8
1.50	0.484	0.04	85.36	
1.75	0.406**	0.04	71.60	
Control	0.567	0.03	-	

* $P < 0.05$

** $P < 0.01$

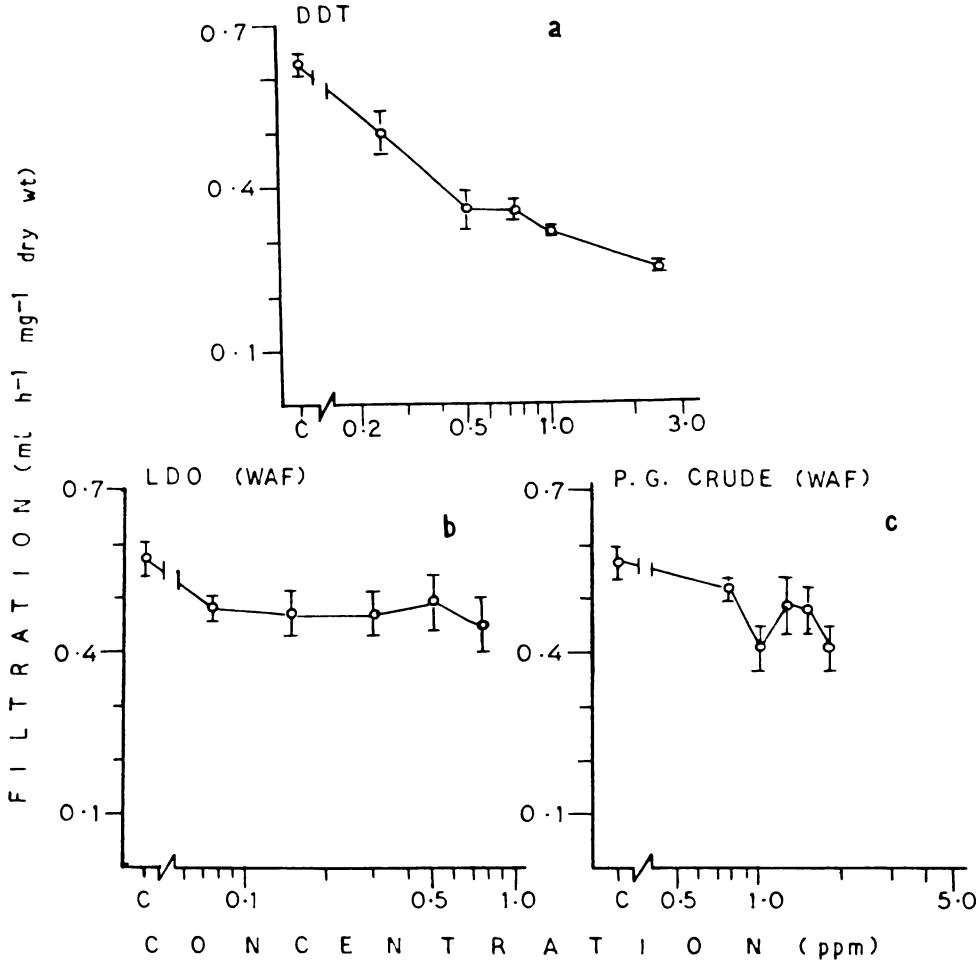


Fig. 60. Perna indica. Average filtration under different sublethal concentrations of pesticide and petroleum hydrocarbons (PHC), along with respective standard errors (vertical bars) a) DDT b) PHC in LDO-WAF- c) PHC in P.G.Crude WAF.

in these concentrations or maintained only minimal quantities of water in circulation. The EC50 value was 0.4 ppm (Table 54; Fig. 61a).

4.2.1.3.2.2 Dimecron

1.0 to 10.0 ppm of Dimecron were added to the test media to find out the effect of this on the rate of filtration. All these concentrations were found to be toxic to the animals. More than 50% of reduction occurred when the concentration was enhanced from 1.0 to 2.50 ppm. The results obtained were highly significant. It may be stated that 7.50 and 10.0 ppm of Dimecron nearly stopped filtration activity in V. cyprinoides var. cochinensis. The EC50 was 1.7 ppm (Table 55; Fig. 61b).

4.2.1.3.2.3 Aldrex

Aldrex was also proved to be a very toxic chemical with reference to filtration in V. cyprinoides var. cochinensis. The presence of even 0.50 ppm of Aldrex reduced filtration rate by more than 50%. It was noticed that when the experimental medium contained 5.0 ppm of Aldrex, animals nearly stopped filtering. The worked out EC50 was 0.3 ppm, a concentration which was not used for the test. All the results were found to be statistically highly significant (Table 56; Fig. 61c).

4.2.1.3.2.4 DDT

1.0 to 10.0 ppm of DDT were supplied in the test medium to assess the rate of filtration. The results show that 2.50 ppm and above are concentrations which would result in clear cut reduction in filtration. 7.50 and 10.0 ppm of DDT were highly toxic from a sublethal stand point. The EC50 was 2.4 ppm. The data obtained were highly significant (Table 57; Fig. 61d).

Table 54. Villorita cyprinoides var. cochinensis. Average filtration (ml h^{-1} mg^{-1} dry wt.) under sublethal concentrations of Ekalux, along with respective standard error, percentage performance and EC50 level.

Ekalux Concentration (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.10	0.237	0.03	89.77	
0.25	0.179*	0.03	67.80	
0.40	0.183	0.04	69.32	
0.50	0.067**	0.01	25.38	0.4
0.60	0.050**	0.01	18.93	
Control	0.264	0.02	-	

Table 55. Villorita cyprinoides var. cochinensis. Average filtration (ml h^{-1} mg^{-1} dry wt.) under sublethal concentrations of Dimecron, along with respective standard error, percentage performance and EC50 level.

Dimecron Concentration (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
1.0	0.169**	0.02	64.02	
2.5	0.106**	0.02	40.15	
5.0	0.067**	0.01	25.38	1.7
7.5	0.050**	0.01	18.94	
10.0	0.032**	0.01	12.12	
Control	0.264	0.02	-	

* $P < 0.05$

** $P < 0.01$

Table 56. Villorita cyprinoides var. cochinensis. Average filtration (ml h^{-1} mg^{-1} dry wt.) under sublethal concentrations of Aldrex, along with respective standard error, percentage performance and EC50 level.

Aldrex Concentration (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.50	0.117**	0.01	44.49	
0.75	0.117**	0.01	44.48	
1.00	0.064**	0.01	24.33	0.3
2.50	0.065**	0.01	24.72	
5.00	0.036**	0.003	13.69	
Control	0.263	0.01	-	

Table 57. Villorita cyprinoides var. cochinensis. Average filtration (ml h^{-1} mg^{-1} dry wt.) under sublethal concentrations of DDT, along with respective standard error, percentage performance and EC50 level.

DDT Concentration (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
1.00	0.206*	0.02	78.33	
2.50	0.107**	0.01	40.68	
5.00	0.076**	0.01	28.90	2.4
7.50	0.049**	0.01	18.63	
10.00	0.048**	0.01	18.25	
Control	0.263	0.01	-	

* $P < 0.05$

** $P < 0.01$

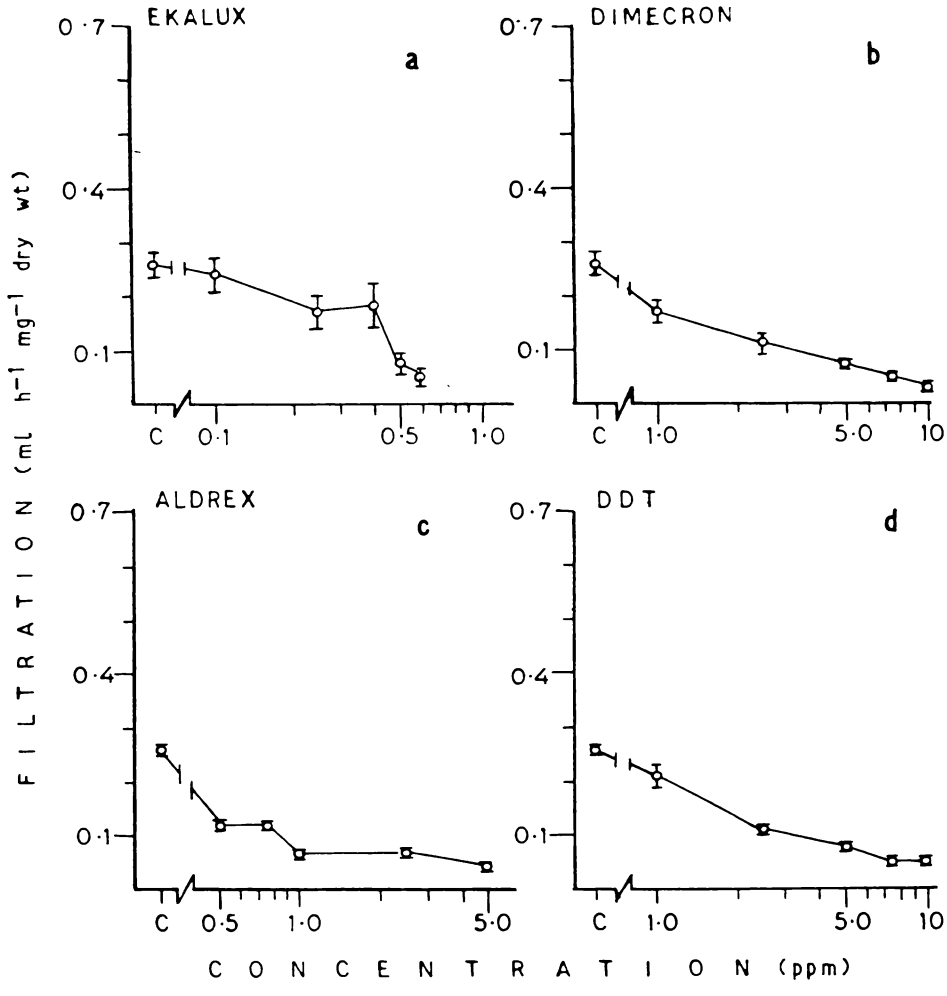


Fig. 61. Villorita cyprinoides var. cochinensis. Average filtration under different sub-lethal concentrations of pesticides, along with respective standard errors (vertical bars). a) Ekalux b) Dimecron c) Aldrex d) DDT.

4.2.1.3.2.5 Light Diesel Oil (WAF)

The concentrations of PHC in LDO (WAF) used were 0.75, 1.0, 1.25, 1.75 and 2.0 ppm. There was no significant reduction in the rate of filtration even at the higher PHC concentration in LDO (WAF). As a matter of fact even those animals retained in a test medium which contained 2.0 ppm PHC in LDO (WAF) filtered around 75% of water compared to that by control animals. The calculated EC50 was 2.6 ppm. Reduction in filtration at 1.75 and 2.0 ppm of PHC was significant (Table 58; Fig. 62a)

4.2.1.3.2.6 Persian Gulf Crude (WAF)

It was noticed that drastic reduction in filtration rate did not take place when V. cyprinoides var. cochinensis was exposed to PHC concentrations in P.G. Crude (WAF) from 0.50 to 2.50 ppm. Therefore, the EC50 was calculated and it was found as 8.2 ppm (Table 59; Fig. 62b).

4.2.1.4 Rate of Filtration: Under Toxicant Mixtures

As in the case of oxygen consumption the effect of pesticides and oil when present in concert have been worked out. It is assumed that the presence or absence, lower or higher concentrations of various toxicants can affect the rate of filtration of bivalves and thereby ultimately influence the scope for growth. It is known that variations in the rate of water filtered can directly affect the intake of food and hence the gain in energy. Reduction of filtration rate will thus exert a direct stress on the animal by reducing the quality of food available.

4.2.1.4.1 *Perna indica*

The rate of filtration of P. indica when encountered with varying and unvarying concentrations of different pesticides and oil fractions have been

Table 58. Villorita cyprinoides var. cochinensis. Average filtration (ml h^{-1} mg^{-1} dry wt.) under sublethal concentrations of LDO (WAF), along with respective standard error, percentage performance and EC50 level.

Concentration of PHC in LDO (WAF) (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.75	0.246	0.01	98.79	
1.00	0.249	0.01	100.00	
1.25	0.229	0.01	91.97	2.6
1.75	0.195**	0.01	78.31	
2.00	0.187**	0.01	75.10	
Control	0.249	0.01	-	

Table 59. Villorita cyprinoides var. cochinensis. Average filtration (ml h^{-1} mg^{-1} dry wt.) under sublethal concentrations of P.G. Crude (WAF), along with respective standard error, percentage performance and EC50 level.

Concentration of PHC in P.G. Crude (WAF) (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.5	0.259	0.01	97.74	
1.0	0.245	0.01	92.45	
1.5	0.220	0.01	83.02	8.2
2.0	0.229	0.01	86.42	
2.5	0.206	0.02	77.74	
Control	0.265	0.01	-	

** $P < 0.01$

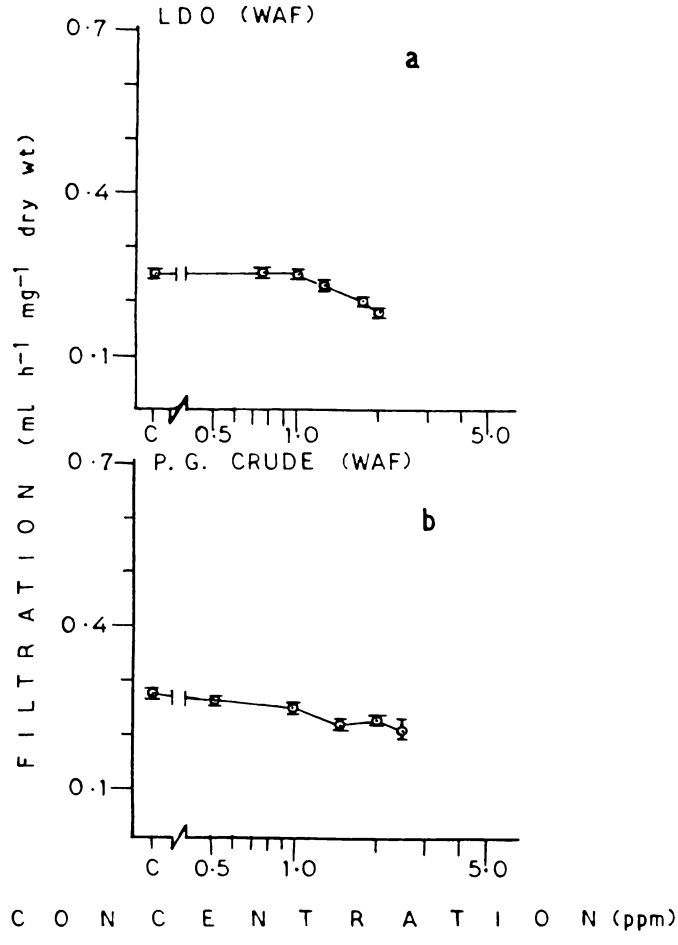


Fig. 62. Villorita cyprinoides var. cochinensis. Average filtration under different sub-lethal concentrations of petroleum hydrocarbons (PHC), along with respective standard errors (vertical bars). a) PHC in LDO (WAF) b) PHC in P.G.Crude (WAF).

worked out and the results are presented in the following section.

4.2.1.4.1.1 PHC in LDO (WAF) unvarying and Ekalux varying

Table 60 and Fig. 63 show the rte of filtration by P. indica, when exposed to 0.75 ppm PHC in LDO (WAF) and 0.50 to 1.50 ppm of Ekalux. Presence of 0.75 ppm of Ekalux, along with 0.75 ppm PHC in LDO (WAF) resulted in drastic reduction in the filtration rate. It is seen from the results that all the higher concentration of Ekalux, however, exerted more or less equal stress factor as evidenced by the filtration performance. To some extent, the result obtained with those animals exposed to 1.0 ppm of Ekalux was different from the trend shown by the representatives of this species exposed to the other concentrations.

The trend in the rate of filtration by P. indica exposed to an unvarying concentration of 2.0 ppm PHC in LDO (WAF) and varying concentrations of Ekalux was assessed. The result is presented in Table 61 and Fig. 63 b. Declension in the rate of filtration was the general trend. However, in one concentration ie, 1.25 ppm of Ekalux with 2.0 ppm PHC in LDO (WAF), there was an increase in filtration rate. The EC50 worked out was 1.1 ppm and the results were found to be statistically significant.

4.2.1.4.1.2 Ekalux unvarying and PHC in LDO (WAF) varying

In another series of experiment the concentration of Ekalux was maintained unvarying and that of PHC in LDO (WAF) subjected to variation from 0.75 to 2.0 ppm. In the first set of experiment, the constant concentration of Ekalux was 0.50 ppm. The presence of Ekalux, along with PHC in LDO (WAF) resulted in decrease in filtration rate. At the highest concentration ie;

Table 60. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 0.75 ppm of PHC in LDO-WAF (unvarying) and Ekalux (varying), along with respective standard errors, percentage performance and EC50 level.

Ekalux Concentration (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.50	0.441**	0.01	68.16	
0.75	0.346**	0.04	53.48	
1.00	0.243**	0.03	37.56	1.3
1.25	0.310**	0.02	47.91	
1.50	0.347**	0.04	53.63	
Control	0.647	0.03	-	

Table 61. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 2.0 ppm of PHC in LDO-WAF (unvarying) and Ekalux (varying), along with respective standard errors, percentage performance and EC50 level.

Ekalux Concentration (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.50	0.447**	0.05	69.09	
0.75	0.324**	0.02	50.08	
1.00	0.343**	0.05	53.01	1.1
1.25	0.420**	0.06	64.92	
1.50	0.301**	0.05	46.52	
Control	0.647	0.03	-	

** $P \leq 0.01$

2.0 ppm PHC in LDO (WAF), the animals filtered only 0.30 ml of water h^{-1} mg^{-1} dry wt. This was found to be around 46% of the water filtered by control animals. Giving statistically significant indication, it was found that the EC50 was 2.0 ppm of PHC with 0.5 ppm of Ekalux (Table 62; Fig. 63c).

The presence of 1.50 ppm of Ekalux with the same concentrations of PHC in LDO (WAF) as used previously also resulted in reduction in filtration. The only variation being, very low performance levels in all the concentration components. The variation noticed was statistically significant. EC50 recorded was 1.2 ppm (Table 63; Fig. 63d).

4.2.1.4.1.3 PHC in LDO (WAF) unvarying and Dimecron varying

Dimecron in combination with PHC in LDO (WAF) was also employed to analyse the effect on filtration in P. indica. Table 64 presents the data obtained from a series of experiment where the Dimecron concentration was altered from 10.0 to 50.0 ppm in the medium which contained a uniform quantity (0.75 ppm) of PHC in LDO (WAF). Dimecron concentration above 40.0 ppm in the presence of PHC drastically reduced filtration rate. The EC50 was 44.7 ppm. Dimecron, though relatively less toxic, at a concentration of 50.0 ppm with 0.75 ppm PHC in LDO (WAF), resulted in plunging down of filtration rate to 35% to that of the control animals (Table 64; Fig. 64a).

In another series of experiment the unvarying concentration of PHC was raised to 2.0 ppm. The resultant decrease in the filtration rate was not very conspicuous even at the highest level and as a matter of fact, EC50 concentration was increased to 47.3 ppm of Dimecron (Table 65; Fig. 64b).

Table 62. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 0.5 ppm Ekalux (unvarying) and PHC in LDO-WAF (varying), along with respective standard errors, percentage performance and EC50 level.

Concentration of PHC in LDO (WAF) (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.75	0.441**	0.01	68.16	
1.00	0.479**	0.04	74.03	
1.25	0.398**	0.06	61.51	2.0
1.75	0.432**	0.06	66.77	
2.00	0.301**	0.05	46.52	
Control	0.647	0.03	-	

Table 63. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 1.5 ppm Ekalux (unvarying) and PHC in LDO-WAF (varying), along with respective standard errors, percentage performance and EC50 level.

Concentration of PHC in LDO (WAF) (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.75	0.347**	0.04	53.63	
1.00	0.269**	0.03	41.58	
1.25	0.298**	0.06	46.06	1.2
1.75	0.325**	0.03	50.23	
2.00	0.301**	0.05	46.52	
Control	0.647	0.03	-	

** $P < 0.01$

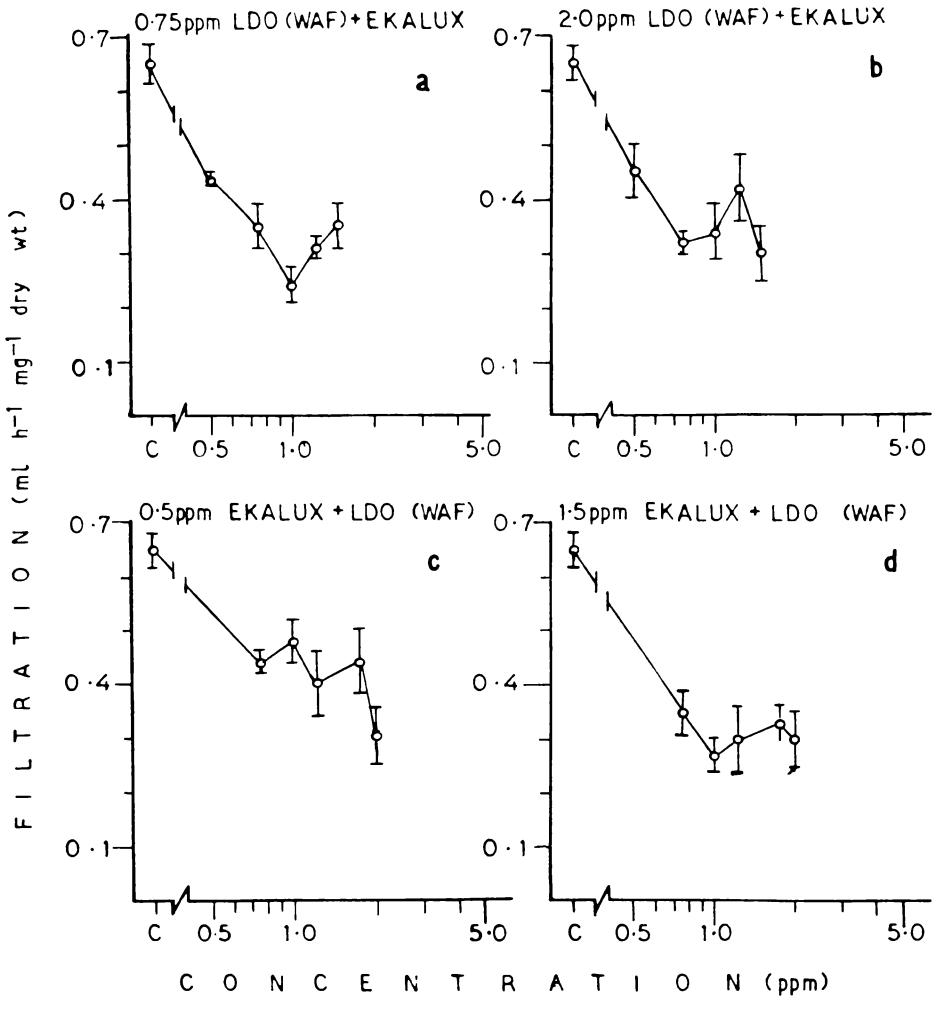


Fig. 63. Perna indica. Average filtration under different sublethal concentrations of pesticide and petroleum hydrocarbon (PHC) in combinations, along with respective standard errors (vertical bars) a&b. PHC in LDO-WAF- (unvarying) and Ekalux (varying) c&d. Ekalux (unvarying) and PHC in LDO-WAF- (varying)

Table 64. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 0.75 ppm of PHC in LDO-WAF (unvarying) and Dimecron (varying), along with respective standard errors, percentage performance and EC50 level.

Dimecron Concentration (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
10.0	0.585	0.02	97.50	
15.0	0.445**	0.02	74.17	
25.0	0.483*	0.04	80.50	
40.0	0.403**	0.02	67.17	44.7
50.0	0.212**	0.02	35.33	
Control	0.600	0.02	-	

Table 65. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 2.0 ppm of PHC in LDO-WAF (unvarying) and Dimecron (varying), along with respective standard errors, percentage performance and EC50 level.

Dimecron Concentration (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
10.0	0.449*	0.05	74.83	
15.0	0.479**	0.02	79.84	
25.0	0.348**	0.02	58.00	
40.0	0.333**	0.02	55.50	47.3
50.0	0.286**	0.02	47.67	
Control	0.600	0.02	-	

* $P < 0.05$

** $P < 0.01$

4.2.1.4.1.4 Dimecron unvarying and PHC in LDO (WAF) varying

In a reciprocal series of experiment the concentration of Dimecron was retained unvarying and that of PHC in LDO (WAF) was subjected to variation from 0.75 to 2.0 ppm. When the concentration of Dimecron was 10.0 ppm and that of PHC 0.75 to 2.0 ppm, it was noticed that the animals filtered in an erratic manner. The resultant variations were, however statistically significant, The EC50 was 3.0 ppm (Table 66; Fig. 64c).

The fact that increase in the Dimecron concentration to 50.0 ppm drastically affected the rate of filtration of P. indica could be seen from the Table 67 and Fig. 64d. The EC50 was 2.1 ppm PHC in LDO (WAF). Though some of the results obtained were erratic and did not show any pattern, the variations were statistically significant.

4.2.1.4.1.5 PHC in LDO (WAF) unvarying and Aldrex varying

Aldrex in combination with LDO (WAF) was used to study the rate of filtration in P. indica. In one series of experiment an unvarying concentration of 0.50 ppm of PHC in LDO (WAF) with varying concentration of Aldrex was used in the test medium. The presence of 0.50 ppm of PHC with 1.0 to 4.0 ppm of Aldrex, resulted in erratic rate of filtration. Although the EC50 was 5.4 ppm the animals were found to filter less and more quantities of water, irrespective of variation in the Aldrex concentration in the medium (Table 68 and Fig. 65a). The variations observed were statistically significant.

Table 69 and Fig. 65b present the data on the rate of filtration by P. indica exposed to combinations of 2.0 ppm PHC in LDO (WAF) with 1.0 to 4.0 ppm of Aldrex in five concentration gradients. Here also, the rate of filtration was erratic. The EC50 was 3.8 ppm of Aldrex.

Table 66. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 10.0 ppm Dimecron (unvarying), PHC in LDO-WAF (varying), along with respective standard errors, percentage performance and EC50 level.

PHC in LDO-WAF Concentration (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.75	0.585	0.02	97.50	
1.00	0.457**	0.01	76.17	
1.25	0.488**	0.02	81.33	3.0
1.75	0.408**	0.05	68.00	
2.00	0.449*	0.05	74.83	
Control	0.600	0.02	-	

Table 67. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 50.0 ppm Dimecron (unvarying) and PHC in LDO-WAF (varying), along with respective standard errors, percentage performance and EC50 level.

PHC in LDO-WAF Concentration (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.75	0.212**	0.02	35.33	
1.00	0.413**	0.02	68.83	
1.25	0.350**	0.04	58.33	2.1
1.75	0.354**	0.03	59.00	
2.00	0.286**	0.04	47.67	
Control	0.600	0.02	-	

* $P<0.05$

** $P<0.01$

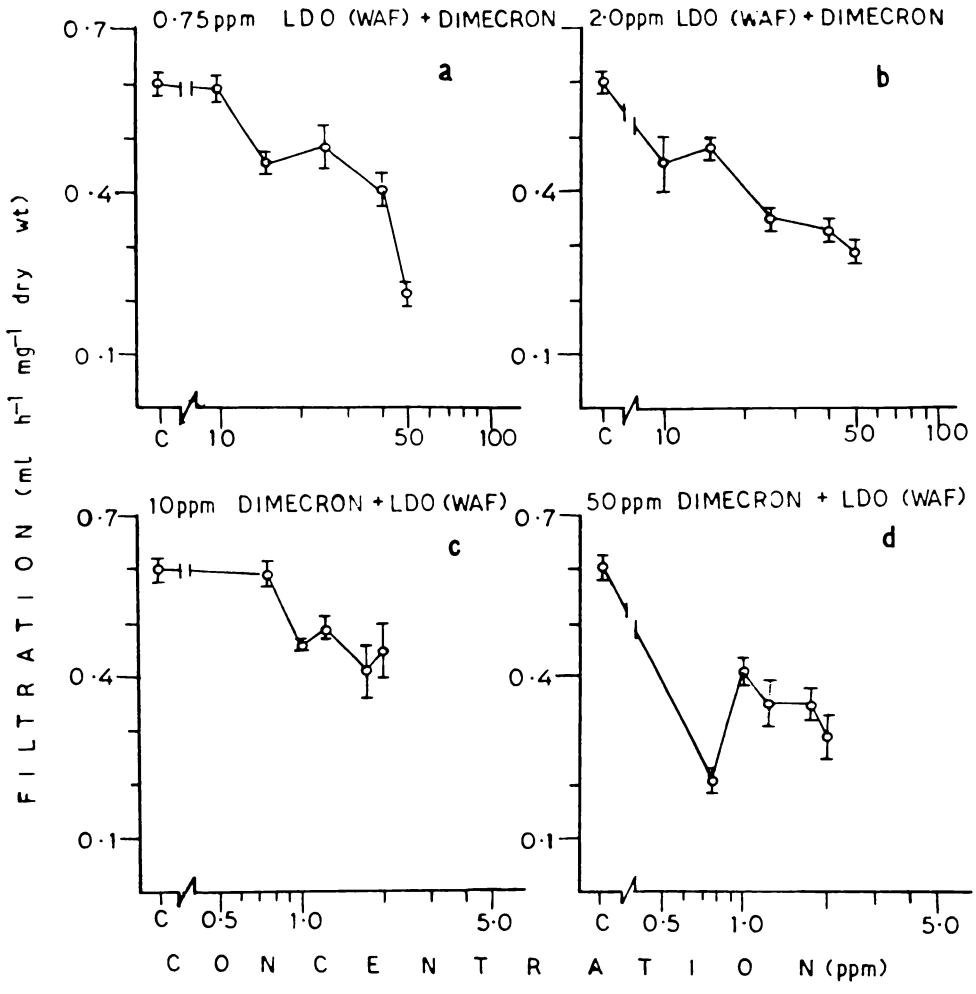


Fig. 64. Perna indica. Average filtration under different sublethal concentrations of pesticide and petroleum hydrocarbon (PHC) in combinations, along with respective standard errors. (vertical bars). a&b. PHC in LDO-WAF- (unvarying) and Dimecron (varying) c&d. Dimecron (unvarying) and PHC in LDO-WAF (varying).

Table 68. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 0.5 ppm of PHC in LDO-WAF (unvarying) and Aldrex (varying), along with respective standard errors, percentage performance and EC50 level.

Aldrex Concentration (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
1.0	0.547**	0.03	79.85	
1.5	0.363**	0.03	52.99	
2.0	0.515**	0.04	75.18	
3.0	0.523**	0.04	76.35	5.4
4.0	0.294**	0.03	42.92	
Control	0.685	0.02	-	

Table 69. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 2.0 ppm of PHC in LDO-WAF (unvarying) and Aldrex (varying), along with respective standard errors, percentage performance and EC50 level.

Aldrex Concentration (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
1.0	0.399**	0.02	58.25	
1.5	0.502**	0.03	73.28	
2.0	0.540**	0.04	78.83	
3.0	0.364**	0.05	53.14	3.8
4.0	0.515**	0.02	75.18	
Control	0.685	0.02	-	

** $P < 0.01$

4.2.1.4.1.6 Aldrex unvarying and PHC in LDO (WAF) varying

A combination of 1.0 ppm of Aldrex with 0.50 to 2.0 ppm PHC in LDO (WAF) in the test media also resulted in erratic trend in filtration rate. The EC50 was 2.9 ppm, however, the variations noticed were statistically highly significant (Table 70; Fig. 65c).

In another series of experiment the concentration of Aldrex was enhanced to 4.0 ppm keeping the PHC concentration in LDO (WAF) varying as in the previous case. The toxicity of PHC in LDO (WAF) was found to increase in the presence of Aldrex. The EC50 was 1.3 ppm and the results were statistically significant (Table 71; Fig. 65d).

4.2.1.4.1.7 PHC in LDO (WAF) unvarying and DDT varying

DDT in the presence of LDO (WAF) formed the toxicant combination to assess the rate of filtration in P. indica in another series of experiment. The presence of 0.15 ppm of PHC in LDO (WAF) with 0.25 to 1.0 ppm of DDT resulted in erratic rate of filtration. Beyond 0.40 ppm of DDT in the presence 0.15 ppm PHC in LDO (WAF), the animals started filtering more water and when the combination concentration in the medium was 1.0 ppm of DDT with 0.15 ppm PHC in LDO (WAF), P. indica filtered more water than their counterparts in the control (Table 72; Fig. 66a).

Table 73 and Fig. 66b depict the rate of filtration in P. indica, when the animals were subjected to a combined concentration of 0.75 ppm PHC in LDO with 0.25 to 1.0 ppm of DDT. The reduction in filtration was minimal. EC50 registered was 2.4 ppm of DDT.

Table 70. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 1.0 ppm Aldrex (unvarying) and PHC in LDO-WAF (varying), along with respective standard errors, percentage performance and EC50 level.

Concentration of PHC in LDO (WAF) (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.50	0.547**	0.03	79.85	
0.75	0.299**	0.03	43.65	
1.00	0.372**	0.02	54.31	2.9
1.50	0.448**	0.05	65.40	
2.00	0.399**	0.03	58.48	
Control	0.685	0.02	-	

Table 71. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 4.0 ppm Aldrex (unvarying) and PHC in LDO-WAF (varying), along with respective standard errors, percentage performance and EC50 level.

Concentration of PHC in LDO(WAF) (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.50	0.294**	0.03	42.92	
0.75	0.245**	0.01	35.77	
1.00	0.259**	0.05	37.81	1.3
1.50	0.282**	0.01	41.17	
2.00	0.515**	0.02	75.18	
Control	0.685	0.02	-	

** $P < 0.01$

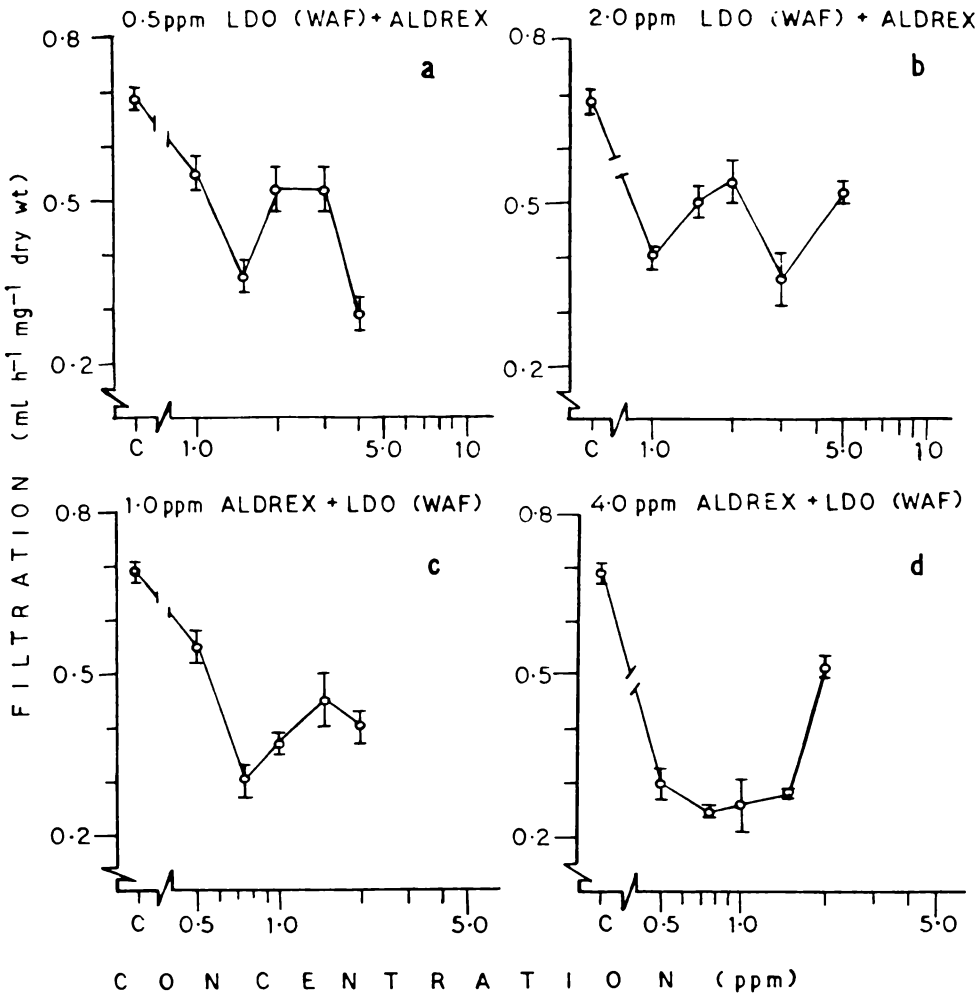


Fig. 65. Perna indica. Average filtration under varying sublethal concentrations of pesticide and petroleum hydrocarbon (PHC) in combinations, along with respective standard errors. a&b. PHC in LDO-WAF-(unvarying) and Aldrex (varying) c&d. Aldrex (unvarying) and PHC in LDO-WAF-(varying).

Table 72. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 0.15 ppm PHC in LDO-WAF (unvarying) and DDT (varying), along with respective standard errors and percentage performance.

DDT Concentration (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
0.25	0.590	0.03	99.33
0.40	0.387**	0.04	65.15
0.50	0.477**	0.01	80.30
0.75	0.531	0.04	89.39
1.00	0.602	0.04	101.35
Control	0.594	0.03	-

Table 73. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 0.75 ppm PHC in LDO-WAF (unvarying) and DDT (varying), along with respective standard errors, percentage performance and EC50 level.

DDT Concentration (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.25	0.552	0.02	92.93	
0.40	0.557	0.02	93.77	
0.50	0.477*	0.03	80.30	2.4
0.75	0.464**	0.03	78.11	
1.00	0.440**	0.03	74.07	
Control	0.594	0.03	-	

* $P<0.05$

** $P<0.01$

4.2.1.4.1.8 DDT unvarying and PHC in LDO (WAF) varying

The presence of 0.25 ppm of DDT at a constant level and 0.15 to 0.75 ppm PHC in LDO (WAF) did not exert any conspicuous effect in the rate of filtration in P. indica (Table 74; Fig. 66c).

Increase in the concentration of DDT to 1.0 ppm generally resulted in reduction in the rate of filtration. However, at one instance, when the test medium contained 1.0 ppm of DDT with 0.75 ppm PHC in LDO (WAF) animals were found to filter more water. The EC50 was 1.2 ppm (Table 75; Fig. 66d).

4.2.1.4.2 *Villorita cyprinoides var. cochinensis*

V. cyprinoides var. cochinensis was also used to assess the resultant toxic effects when representative of this animal was exposed to combinations of PHC in LDO (WAF) with Ekalux and Aldrex. Here also in one series of experiment, the concentration of PHC in LDO (WAF) was maintained unvarying and that of pesticides made to vary. In another series, the concentrations of the pesticides were retained unvarying and that of PHC concentrations made to fluctuate. The results obtained are presented below.

4.2.1.4.2.1 PHC in LDO (WAF) unvarying and Ekalux varying

Presence of 0.50 ppm PHC in LDO (WAF) with 0.25 to 1.5 ppm of Ekalux, did not conspicuously affect the rate of filtration in V. cyprinoides var. cochinensis. However, there was slight reduction in the rate of filtration when compared to that of control animals. But this reduction was more or less uniform in all the concentration combinations (Table 76; Fig. 67a).

Table 74. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 0.25 ppm DDT (unvarying) and PHC in LDO-WAF (varying), along with respective standard errors and percentage performance.

Concentration of PHC in LDO-WAF (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
0.15	0.590	0.03	99.33
0.25	0.563	0.02	94.78
0.40	0.563	0.01	94.78
0.50	0.587	0.03	98.82
0.75	0.552	0.02	92.93
Control	0.594	0.03	-

Table 75. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 1.0 ppm DDT (unvarying) and PHC in LDO-WAF (varying), along with respective standard errors, percentage performance, and EC50 level.

Concentration of PHC in LDO-WAF (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.15	0.602	0.04	101.34	
0.25	0.456**	0.02	76.77	
0.40	0.636	0.04	107.07	1.2
0.50	0.459**	0.02	77.27	
0.75	0.440**	0.03	74.07	
Control	0.594	0.03	-	

** $P < 0.01$

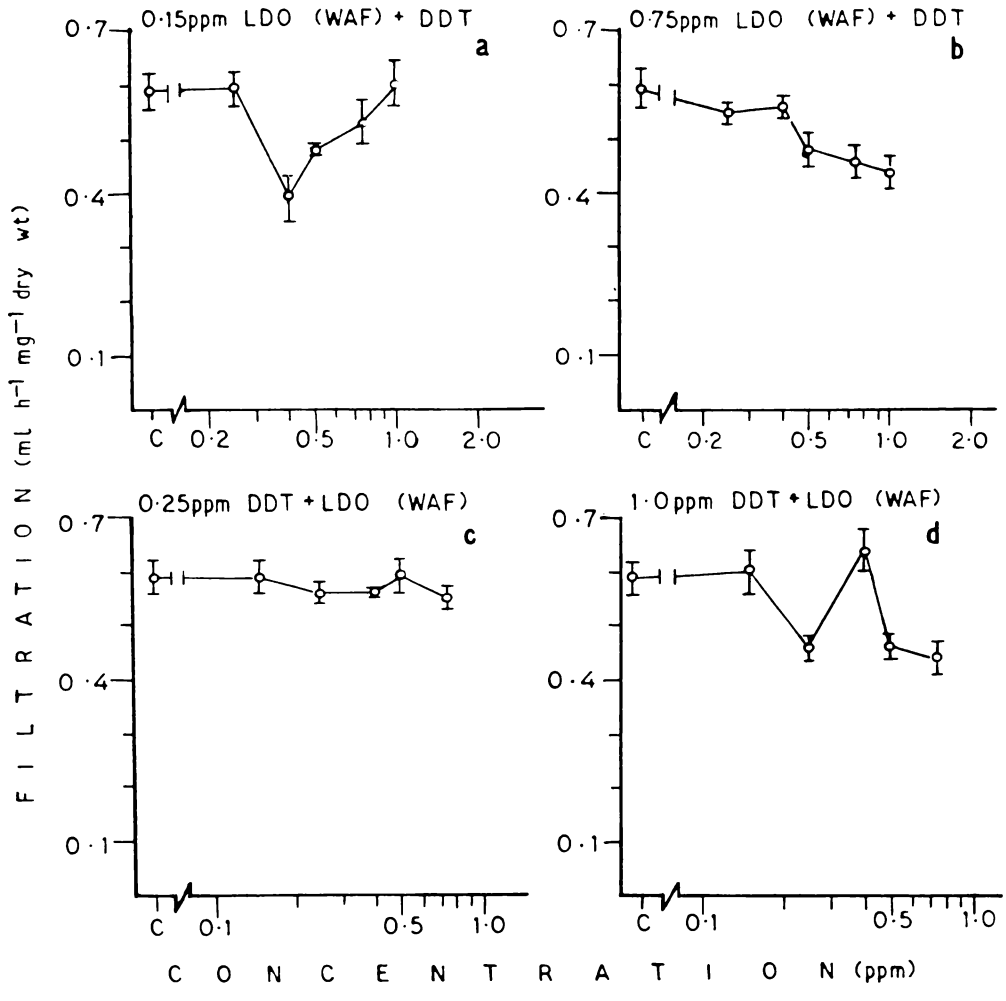


Fig. 66. Perna indica. Average filtration under varying sublethal concentrations pesticide and petroleum hydrocarbon (PHC) in combinations, along with respective standard errors. a&b. PHC in LDO-WAF (unvarying) and DDT (varying) c&d. DDT (unvarying) and PHC in LDO-WAF (varying).

The presence of 2.0 ppm PHC in LDO (WAF) along with 0.25 to 1.50 ppm of Ekalux, however, drastically reduced the rate of filtration of V. cyprinoides var. cochinensis. But interconcentration variations were minimal. The EC50 was 2.6 ppm. The results obtained were statistically significant (Table 77; Fig. 67b).

4.2.1.4.2.2 Ekalux unvarying and PHC in LDO (WAF) varying

Table 78 and Fig. 68a present the data on the average rate of filtration by V. cyprinoides var. cochinensis, when exposed to a combined concentration of 0.25 ppm of Ekalux with 0.50 to 2.0 ppm PHC in LDO (WAF). The rate of reduction in filtration was less even when the medium contained 2.0 ppm PHC with 0.25 ppm of Ekalux. The reduction in performance was only around 25% to that of control. The calculated EC50 was 3.7 ppm.

In another series of experiment the concentration of Ekalux was increased to 1.50 ppm and that of PHC in LDO (WAF) varied from 0.50 to 2.0 ppm. The animals exposed beyond 0.75 ppm PHC in LDO (WAF) filtered less quantities of water. The EC50 was 2.2 ppm. The results obtained were statistically significant (Table 79; Fig. 68b).

4.2.1.4.2.3 PHC in LDO (WAF) unvarying and Aldrex varying

Table 80 gives the data obtained on the influence of 0.50 ppm of PHC in LDO and 1.0 to 4.0 ppm of Aldrex on the rate of filtration by V. cyprinoides var. cochinensis. None of the concentrations employed could bring about more than 20% reduction in filtration rate. The worked out EC50 was 9.4 ppm of Aldrex (Fig. 69a).

Table 76. Villorita cyprinoides var. cochinensis. Average filtration (ml h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 0.5 ppm of PHC in LDO-WAF (unvarying) and Ekalux (varying), along with respective standard errors and percentage performance.

Ekalux Concentration (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
0.25	0.238	0.01	96.75
0.50	0.220	0.01	89.43
0.75	0.222	0.02	90.24
1.00	0.220	0.01	89.44
1.50	0.218	0.01	88.62
Control	0.246	0.01	-

Table 77. Villorita cyprinoides var. cochinensis. Average filtration (ml h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 2.0 ppm of PHC in LDO-WAF (unvarying) and Ekalux (varying), along with respective standard errors, percentage performance and EC50 level.

Ekalux Concentration (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.25	0.180**	0.01	73.17	
0.50	0.175**	0.01	71.38	
0.75	0.167**	0.02	67.89	2.6
1.00	0.172**	0.02	69.92	
1.50	0.131**	0.01	53.25	
Control	0.246	0.01	-	

** $P < 0.01$

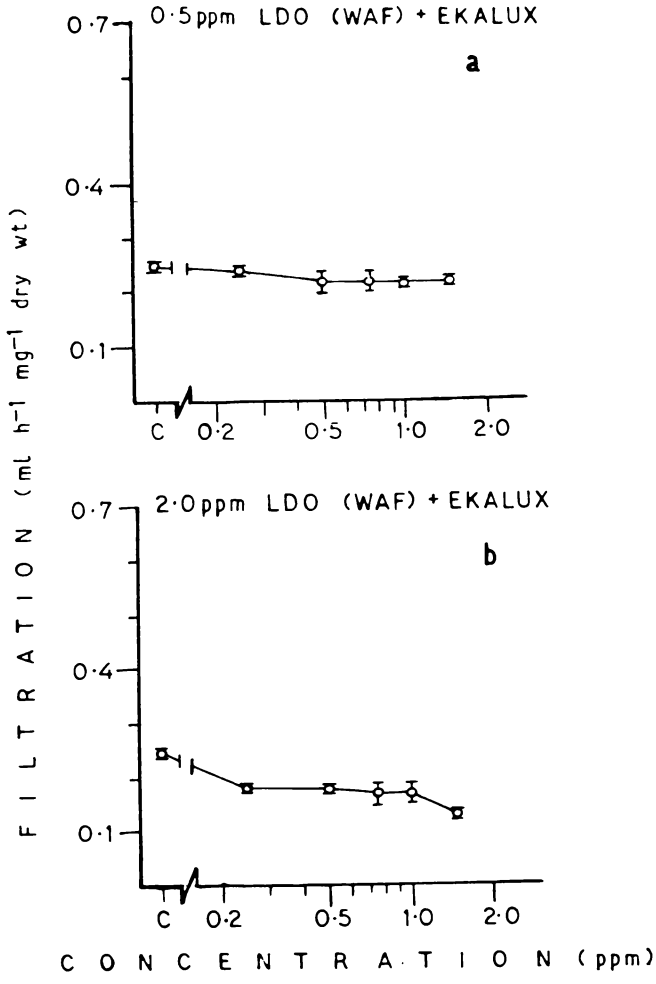


Fig. 67. Villorita cyprinoides var. cochinensis. Average filtration under varying sublethal concentrations of pesticide and petroleum hydrocarbon (PHC) in combinations, along with respective standard errors. a&b. PHC in LDO-WAF- (unvarying) and Ekalux (varying).

Table 78. Villorita cyprinoides var. cochinensis. Average filtration (ml h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 0.25 ppm of Ekalux (unvarying) and PHC in LDO-WAF (varying), along with respective standard errors, percentage performance and EC50 level.

Concentration of PHC in LDO-WAF (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.50	0.238	0.01	96.75	
0.75	0.215	0.01	87.40	
1.00	0.201*	0.01	81.71	3.7
1.50	0.195*	0.02	79.27	
2.00	0.180**	0.01	73.17	
Control	0.246	0.01	-	

Table 79. Villorita cyprinoides var. cochinensis. Average filtration (ml h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 1.5 ppm Ekalux (unvarying) and PHC in LDO-WAF (varying), along with respective standard errors, percentage performance and EC50 level.

Concentration of PHC in LDO-WAF (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.50	0.218	0.01	88.62	
0.75	0.172**	0.01	69.92	
1.00	0.173**	0.01	70.33	2.2
1.50	0.157**	0.01	63.82	
2.00	0.131**	0.01	53.25	
Control	0.246	0.01	-	

* $P < 0.05$

** $P < 0.01$

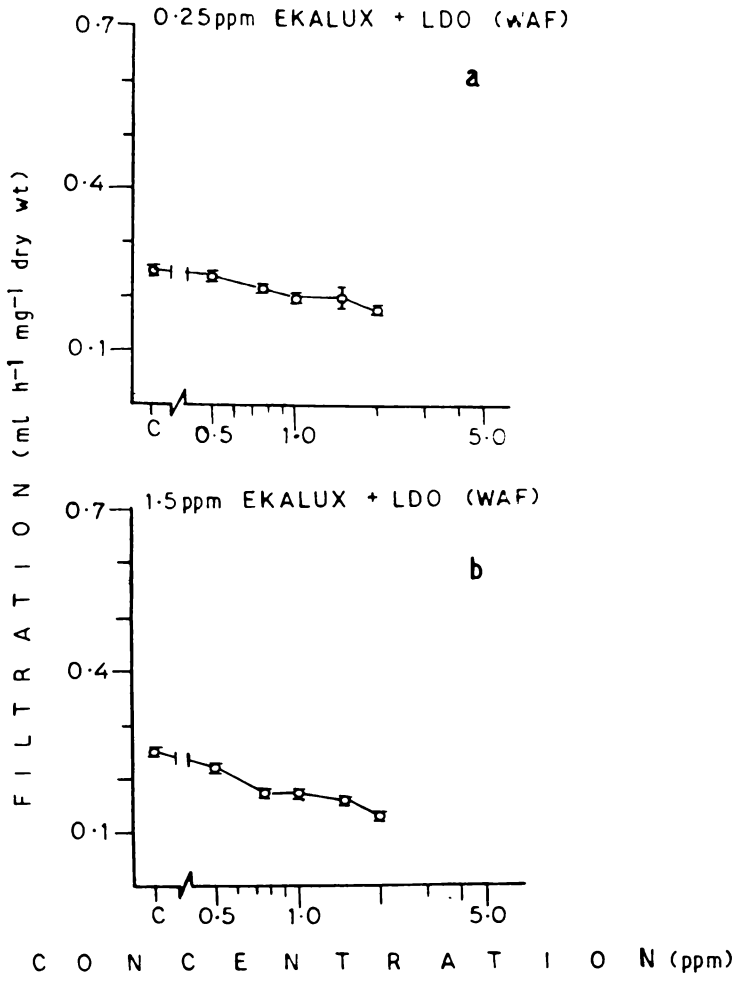


Fig. 68. Villorita cyprinoides var. cochinensis. Average filtration under varying sublethal concentrations of pesticide and petroleum hydrocarbon (PHC) in combinations, along with respective standard errors. a&b. Ekalux (unvarying) and PHC in LDO-WAF- (varying).

Table 81 and Fig. 69b give the data obtained on the average rate of filtration by V. cyprinoides var. cochinensis, when exposed to 2.0 ppm of PHC in LDO (WAF) and 1.0 to 4.0 ppm of Aldrex. A concentration-bound decrease in the rate of filtration was clearly evidenced in the rate of filtration. The EC50 was 5.1 ppm. The reduction in filtration performance by the animals was found to be statistically significant.

4.2.1.4.2.4 Aldrex unvarying and PHC in LDO (WAF) varying

In a reciprocal series of experiment 1.0 ppm or 4.0 ppm of Aldrex was added to 0.50 to 2.0 ppm of PHC in LDO (WAF) in the medium to assess the rate of filtration by V. cyprinoides var. cochinensis. The data is presented in Table 82 and 83 and Fig. 69 c-d.

The presence of 1.0 ppm of Aldrex with 0.50 to 2.0 ppm PHC in LDO (WAF) did not drastically affect the rate of filtration. EC50 was 3.5 ppm of PHC. Increase in the Aldrex concentration to 4.0 ppm clearly reduced the rate of filtration by this animal. The results obtained were found to be statistically significant. The EC50 was 2.2 ppm of PHC. Increase in the concentration of PHC in LDO (WAF) above 1.0 ppm was found to have clear cut toxic effect on performance (Table 83; Fig. 69d).

4.2.1.5 Rate of Byssogenesis: Under Individual Toxicant Stress

The production of byssus threads is an important response of mytilids which has been found to get affected by stress. The number of threads produced, the nature of threads and the ability of the adhesive discs to make the threads efficient mooring lines have been shown to get influenced by the presence of toxicants. Therefore, the process of byssus production was

Table 80. Villorita cyprinoides var. cochinensis. Average filtration (ml h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 0.5 ppm of PHC in LDO-WAF (unvarying) and Aldrex (varying), along with respective standard errors, percentage performance and EC50 level.

Aldrex Concentration (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
1.00	0.261	0.01	98.49	
1.50	0.239*	0.004	90.19	
2.00	0.236*	0.01	89.06	9.4
3.00	0.236*	0.01	89.06	
4.00	0.210**	0.01	79.25	
Control	0.265	0.01	-	

Table 81. Villorita cyprinoides var. cochinensis. Average filtration (ml h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 2.0 ppm of PHC in LDO-WAF (unvarying) and Aldrex (varying), along with respective standard errors, percentage performance and EC50 level.

Aldrex Concentration (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
1.00	0.231*	0.01	87.17	
1.50	0.223**	0.01	84.15	
2.00	0.204**	0.02	76.97	5.1
3.00	0.177**	0.02	66.79	
4.00	0.144**	0.01	54.34	
Control	0.265	0.01	-	

* $P < 0.05$

** $P < 0.01$

Table 82. Villorita cyprinoides var. cochinensis. Average filtration (ml h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 1.0 ppm Aldrex (unvarying) and PHC in LDO-WAF (varying), along with respective standard errors, percentage performance and EC50 level.

Concentration of PHC in LDO-WAF (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.50	0.261	0.01	98.49	
0.75	0.231*	0.01	87.17	
1.00	0.232*	0.01	87.55	3.5
1.50	0.217*	0.02	81.89	
2.00	0.231*	0.01	87.17	
Control	0.265	0.01	-	

Table 83. Villorita cyprinoides var. cochinensis. Average filtration (ml h^{-1} mg^{-1} dry wt.) under the sublethal concentrations of 4.0 ppm Aldrex (unvarying) and PHC in LDO-WAF (varying), along with respective standard errors, percentage performance and EC50 level.

Concentration of PHC in LDO-WAF (ppm)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)	EC50 (ppm)
	Mean	SE		
0.50	0.210**	0.01	79.25	
0.75	0.146**	0.01	55.09	
1.00	0.160**	0.01	60.38	2.2
1.50	0.157**	0.01	59.25	
2.00	0.144**	0.01	54.34	
Control	0.265	0.01	-	

* $P<0.05$

** $P<0.01$

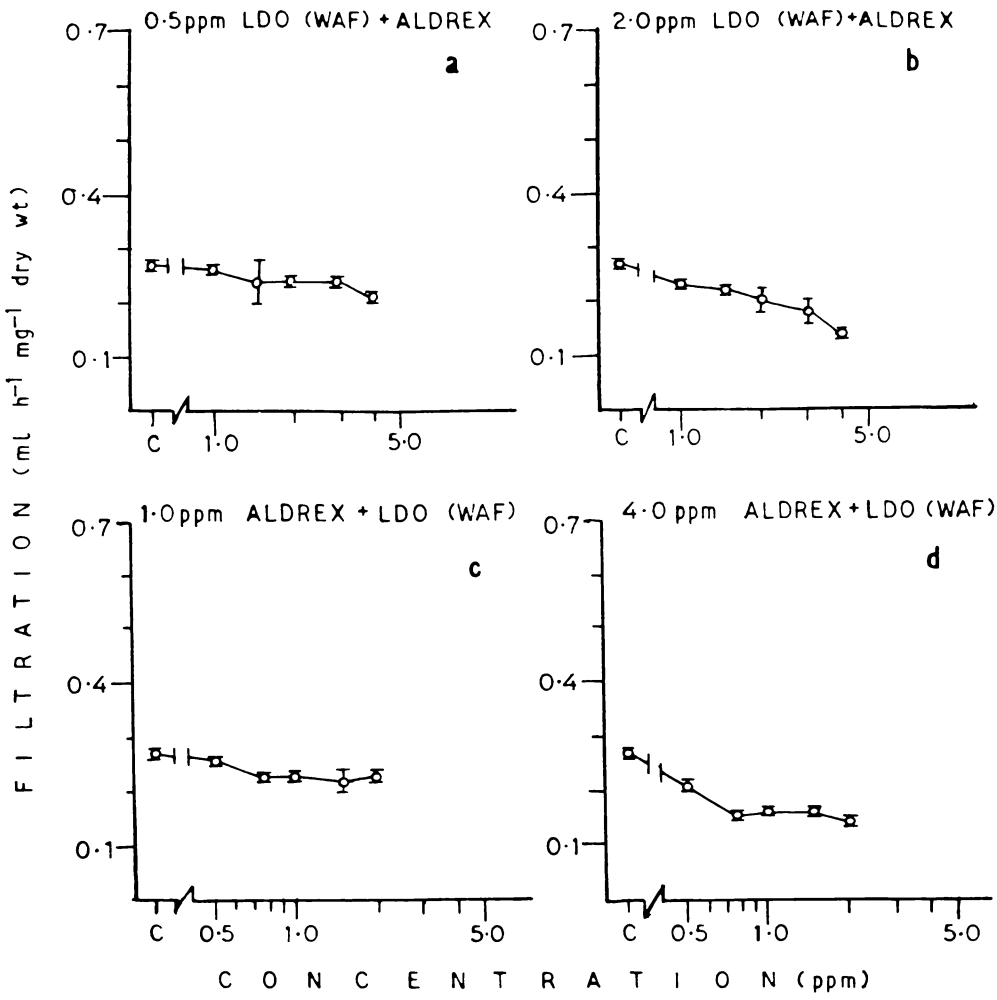


Fig. 69. Villorita cyprinoides var. cochinensis. Average filtration under varying sublethal concentrations of pesticide and petroleum hydrocarbon (PHC) in combinations, along with respective standard errors. a&b. PHC in LDO-WAF- (unvarying) and Aldrex (varying) c&d. Aldrex (unvarying) and PHC in LDO-WAF (varying).

studied mainly by counting the number of threads secreted by the animals exposed to various concentrations of pesticides and petroleum hydrocarbon present in the water accommodated fractions. Ekalux, Dimecron, Aldrex, DDT, LDO (WAF) and P.G. Crude (WAF) were utilized as toxicants to assess the rate of byssus production by P. indica. Since the sublethal concentrations vary depending on the nature of the toxicants, the concentrations employed have been selected accordingly.

4.2.1.5.1 Ekalux

The data obtained on byssogenesis by P. indica exposed to 0.05 to 0.30 ppm of Ekalux is presented in Table 84 and Fig. 70a. A very clear cut concentration dependent decrease was noticed. Even when the test medium contained 0.20 ppm of Ekalux, the animals dropped the rate of byssogenesis by 50%. The EC50 was 0.20 ppm. The variation noticed in the rate of production of byssus by those animals maintained in concentrations beyond 0.10 ppm were statistically highly significant.

4.2.1.5.2 Dimecron

This pesticide was used in slightly higher concentrations considering its low toxicity. The five concentrations employed varied between 2.5(to 12.0 ppm. In all the concentrations the number of byssus produce showed decrease. This was especially so when the animals encounter 10.0 or 12.0 ppm of Dimecron in the test medium. The reduction in t number of byssus produced, when the animals were maintained in a medi containing 2.50 ppm of Dimecron was not statistically significant. results obtained in the rest of the concentrations were statistically si

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4.2.1.5.1 Ekalux

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4.2.1.5.2 Dimecron

This pesticide was used in slightly higher concentrations considering its low toxicity. The five concentrations employed varied between 2.50 to 12.0 ppm. In all the concentrations the number of byssus produced showed decrease. This was especially so when the animals encountered 10.0 or 12.0 ppm of Dimecron in the test medium. The reduction in the number of byssus produced, when the animals were maintained in a medium containing 2.50 ppm of Dimecron was not statistically significant. The results obtained in the rest of the concentrations were statistically signi-

Table 84. Perna indica. Byssogenesis: Average number of threads produced (individual^{-1} 10h^{-1}) under sublethal concentrations of Ekalux, along with respective standard deviation, percentage performance and EC50 level.

Ekalux Concentration (ppm)	Byssogenesis (individual^{-1} 10h^{-1})		Performance (% of control)	EC50 (ppm)
	Mean	SD		
0.050	9.65	1.67	86.94	
0.075	8.20*	1.97	73.87	
0.100	7.15**	1.23	64.41	0.20
0.200	5.50**	1.16	49.55	
0.300	4.45**	0.91	40.09	
Control	11.10	1.18	-	

Table 85. Perna indica. Byssogenesis: Average number of threads produced (individual^{-1} 10h^{-1}) under sublethal concentrations of Dimecron along with respective standard deviation, percentage performance and EC50 level.

Dimecron Concentration (ppm)	Byssogenesis (individual^{-1} 10h^{-1})		Performance (% of control)	EC50 (ppm)
	Mean	SD		
2.5	9.35	0.75	83.85	
5.0	7.40**	0.78	66.37	
7.5	8.03*	1.01	72.02	12.4
10.0	6.05**	1.08	54.26	
12.0	5.20**	0.94	46.64	
Control	11.15	1.53	-	

* $P<0.05$

** $P<0.01$

ficant. The concentration that caused 50% reduction in byssogenesis was 12.4 ppm (Table 85; Fig. 70b).

4.2.1.5.3 Aldrex

Aldrex was proved to be a very high toxic chemical with reference to byssogenesis in P. indica. The concentrations used to expose the animals ranged from 0.10 to 1.0 ppm. Even in the test medium which contained only 0.10 ppm of Aldrex the rate of byssus production was reduced to nearly 50%. In the highest concentration used ie; 1.0 ppm, the animals produced only an average of two threads/individual, which was only around 21% of that produced by the control animals. The EC50 was 0.20 ppm. All the readings obtained were statistically significant (Table 86; Fig. 70c).

4.2.1.5.4 DDT

Table 87 and Fig. 71a depicts the results obtained on the average number of byssus threads produced by P. indica, under sublethal concentrations of DDT. The test media contained 0.25 to 2.50 ppm of DDT in five different steps. Increase in the concentration of DDT to 0.75 ppm and above resulted in 50% or more reduction in byssus production. The EC50 was 1.20 ppm and the results were found to be statistically significant.

4.2.1.5.5 Light Diesel Oil (WAF)

Five concentrations of PHC in LDO (WAF) ranging between 0.075 to 0.75 ppm were added to the media to assess the rate of byssus production by P. indica. 0.075 ppm of PHC in LDO (WAF) did not produce any drastic reduction in the secretion of byssus. In those animals maintained in the highest concentration ie; 0.75 ppm of PHC, production of byssus threads was

Table 86. Perna indica. Byssogenesis: Average number of threads produced (individual^{-1} 10h^{-1}) under sublethal concentrations of Aldrex, along with respective standard deviation, percentage performance and EC50 level.

Aldrex Concentration (ppm)	Byssogenesis (individual^{-1} 10h^{-1})		Performance (% of control)	EC50 (ppm)
	Mean	SD		
0.10	5.45**	0.70	52.91	
0.25	5.55**	0.81	53.88	
0.50	3.65**	1.06	35.44	0.20
0.75	3.35**	0.82	32.52	
1.00	2.20**	0.69	21.36	
Control	10.30	0.42	-	

Table 87. Perna indica. Byssogenesis: Average number of threads produced (individual^{-1} 10h^{-1}) under sublethal concentrations of DDT, along with respective standard deviation, percentage performance and EC50 level.

DDT Concentration (ppm)	Byssogenesis (individual^{-1} 10h^{-1})		Performance (% of control)	EC50 (ppm)
	Mean	SD		
0.25	8.55*	1.12	83.01	
0.50	6.70**	0.96	65.04	
0.75	5.40**	0.94	52.43	1.2
1.00	3.95**	0.55	38.35	
2.50	4.90**	0.96	47.57	
Control	10.30	0.42	-	

* $P \leq 0.05$

** $P < 0.01$

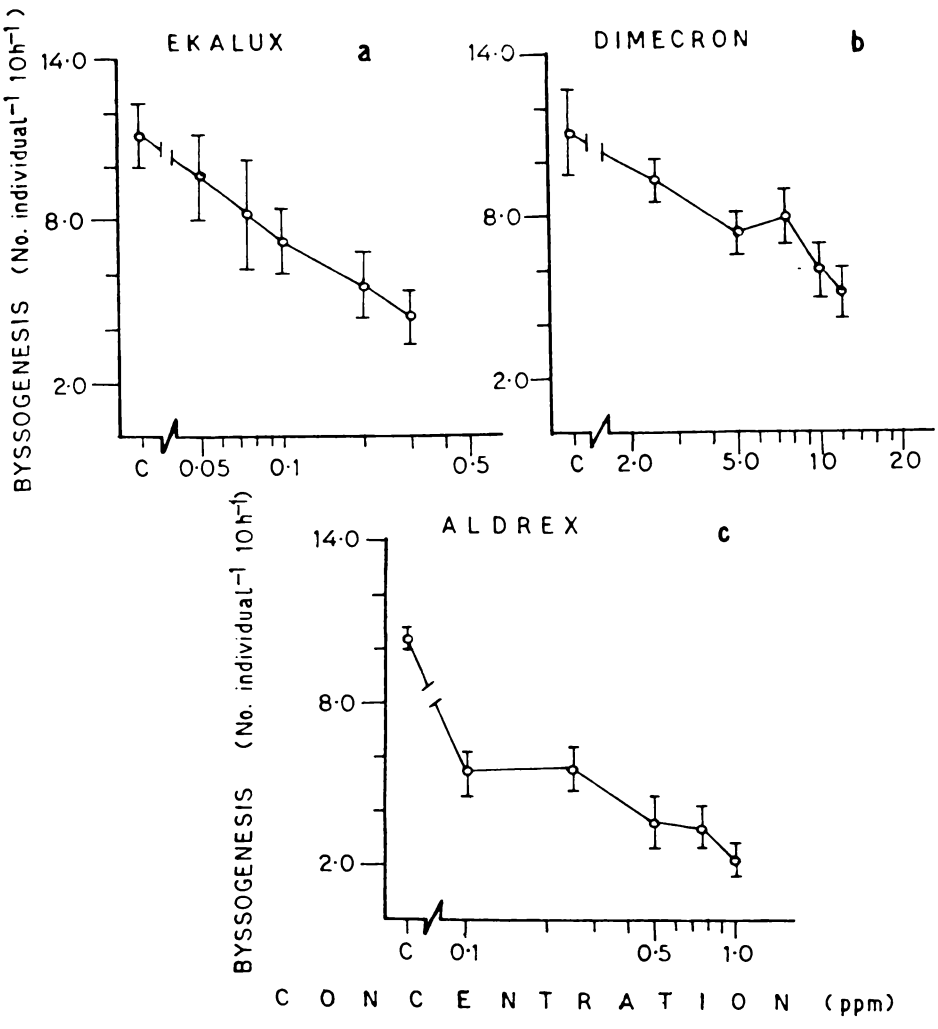

Fig. 70. Perna indica. Average byssogenesis under different sublethal concentrations of pesticides, along with respective standard errors (vertical bars) a) Ekalux b) Dimecron c) Aldrex.

around six threads/individuals, which was around 57% of the number produced by the control animals. The EC50 was 0.9 ppm and the results were statistically significant (Table 88; Fig. 71b).

4.2.1.5.6 Persian Gulf Crude (WAF)

The water accommodated fraction of P.G. Crude, at PHC concentrations varying from 0.75 to 1.75 ppm were added to the test media to analyse the effect of this on byssogenesis of P. indica. The presence of 0.75 ppm of PHC was found to marginally increase the production of byssus. However, increase in the PHC concentration to 1.0 ppm reduced the number of byssus threads produced. The results obtained gave statistically significant variation between concentrations. The EC50 was 2.8 ppm (Table 89; Fig. 71c)

4.2.1.6 Rate of Byssogenesis: Under Toxicant Mixtures

Variations in the number of byssus threads produced in the presence of different PHC concentrations in LDO (WAF) in combination with Ekalux or Aldrex was taken into consideration to assess the combined toxicity of these components with reference to byssogenesis in P. indica. Thus PHC in LDO (WAF) in unvarying concentration along with varying concentrations of Ekalux or Aldrex and Ekalux or Aldrex at unvarying levels with varying PHC in LDO (WAF) concentrations, were added to the test medium to analyse the combined toxicity with reference to byssogenesis.

4.2.1.6.1 PHC in LDO (WAF) unvarying and Ekalux varying

Table 90 and Fig. 72a show the results obtained on the combined effect of PHC in LDO (WAF) unvarying along with varying concentrations of Ekalux

Table 88. Perna indica. Byssogenesis: Average number of threads produced (individual^{-1} 10h^{-1}) under sublethal concentrations of PHC in LDO (WAF), along with respective standard deviation, percentage performance and EC50 level.

Concentration of PHC in LDO-WAF (ppm)	Byssogenesis (individual^{-1} 10h^{-1})		Performance (% of control)	EC50 (ppm)
	Mean	SD		
0.075	9.50	1.65	86.36	
0.150	7.35**	0.86	66.82	
0.300	7.50**	0.93	68.18	0.9
0.500	6.20**	0.59	56.36	
0.750	6.30**	1.10	57.27	
Control	11.0	0.28	-	

Table 89. Perna indica. Byssogenesis: Average number of threads produced (individual^{-1} 10h^{-1}) under sublethal concentrations of P.G.Crude (WAF) along with respective standard deviation, percentage performance and EC50 level.

Concentration of PHC in P.G. Crude (WAF) (ppm)	Byssogenesis (individual^{-1} 10h^{-1})		Performance (% of control)	EC50 (ppm)
	Mean	SD		
0.75	11.3	0.62	102.73	
1.00	8.3**	1.29	75.45	
1.25	7.4**	1.14	67.27	2.8
1.50	6.55**	1.31	59.55	
1.75	7.2**	0.82	65.45	
Control	11.0	0.28	-	

* $P < 0.05$

** $P < 0.01$

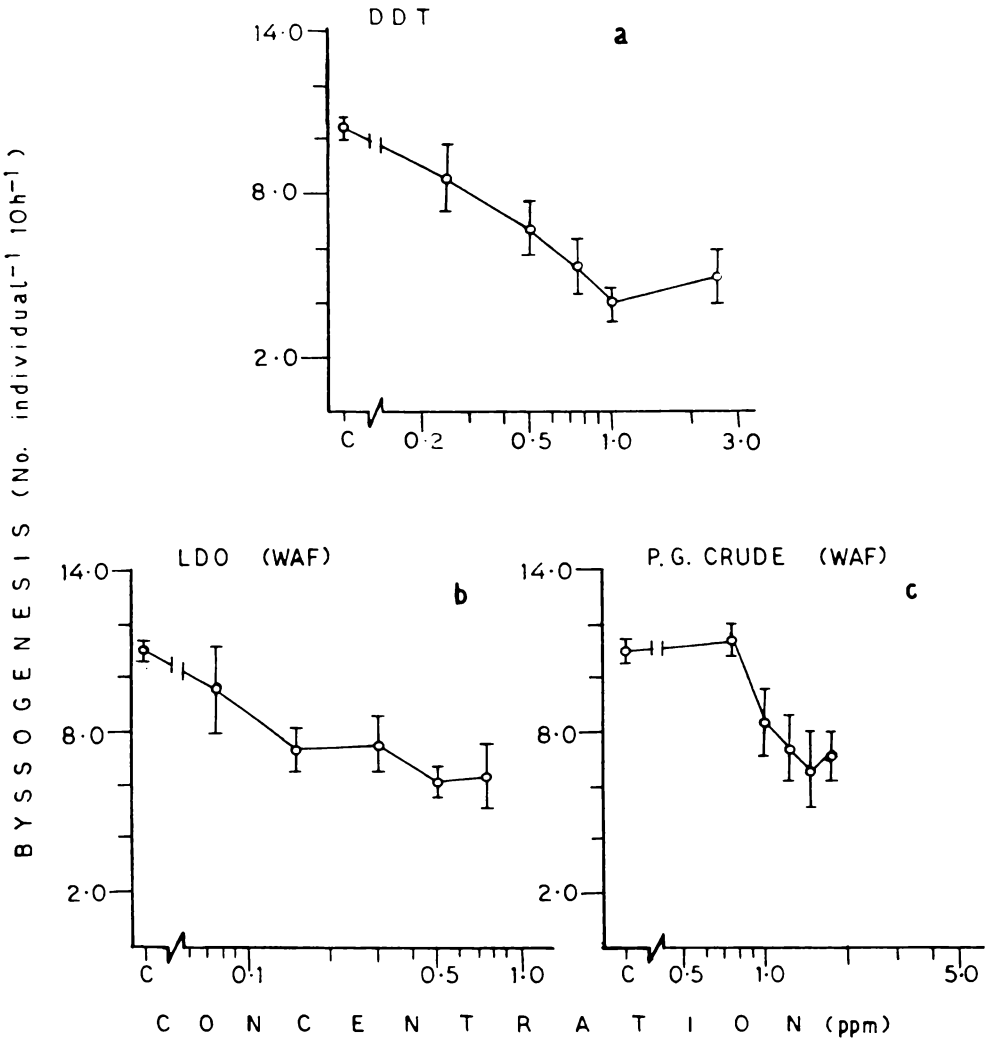

Fig. 71. Perna indica. Average byssogenesis under different sublethal concentrations of pesticide and petroleum hydrocarbon (PHC) along with respective standard errors (vertical bars) a) DDT b) PHC in LDO-WAF- c) PHC in P.G.Crude-WAF-

in the test media. The PHC concentration was 0.75 ppm and that of Ekalux varied from 0.50 to 1.50 ppm in five steps. The presence of 0.75 ppm of PHC and 0.75 ppm of Ekalux resulted in nearly 50% reduction in the number of byssus produced by P. indica. The EC50 was 0.9 ppm of Ekalux. The results obtained were statistically significant. It can be seen from the results that the combined presence of these two components proved to be highly toxic to P. indica.

In another series of experiment the PHC concentration in LDO (WAF) was increased to 2.0 ppm along with 0.50 to 1.50 ppm of Ekalux. The animals produced only negligible number of byssus threads in all the combinations. It is evident from the result that even 0.50 ppm of Ekalux in the presence of 2.0 ppm of PHC clearly tampered with the rate of production of byssus threads. The results were statistically significant and the EC50 was 0.3 ppm (Table 91; Fig. 72b).

4.2.1.6.2 Ekalux unvarying and PHC in LDO (WAF) varying

In a reciprocal series of experiments the concentration of Ekalux in the test medium was maintained unvarying and that of PHC in LDO (WAF) was subjected to variations. Test media contained 0.50 ppm of Ekalux with 0.75 to 2.0 ppm of PHC in five steps. Only when the test medium contained 1.75 and 2.0 ppm of PHC with 0.50 ppm of Ekalux, reduction in byssogenesis around 50% occurred. The effective concentration to reduce the number of byssus threads produced by 50% was 1.8 ppm of PHC. All the results were statistically significant (Table 92; Fig. 72c).

Table 93 and Fig. 72d show the effects of 1.50 ppm of Ekalux and 0.75 to 2.0 ppm PHC in LDO (WAF) in the test medium on byssogenesis in P.indica.

Table 90. Perna indica. Byssogenesis: Average number of threads produced (individual^{-1} 10h^{-1}) under sublethal concentrations of PHC in LDO (WAF)-0.75 ppm-(unvarying) and Ekalux (varying), along with standard deviation, percentage performance and EC50 level.

Concentration of Ekalux (ppm)	Byssogenesis (individual^{-1} 10h^{-1})		Performance (% of control)	EC50 (ppm)
	Mean	SD		
0.50	7.9**	1.10	69.00	
0.75	6.2**	0.69	54.15	
1.00	4.8**	0.82	41.92	
1.25	5.85**	0.96	51.09	0.9
1.50	4.0**	0.91	34.93	
Control	11.45	0.44	-	

Table 91. Perna indica. Byssogenesis: Average number of threads produced (individual^{-1} 10h^{-1}) under sublethal concentrations of PHC in LDO (WAF)-2.0 ppm-(unvarying) and Ekalux (varying) along with standard deviation, percentage performance and EC50 level.

Concentration of Ekalux (ppm)	Byssogenesis (individual^{-1} 10h^{-1})		Performance (% of control)	EC50 (ppm)
	Mean	SD		
0.50	4.35**	0.47	22.06	
0.75	4.25**	0.53	20.90	
1.00	5.35**	0.34	21.70	0.3
1.25	3.4**	0.43	26.17	
1.50	2.35**	0.38	31.30	
Control	11.45	0.44	-	

** $P < 0.01$

Table 92. Perna indica. Byssogenesis: Average number of threads produced (individual^{-1} 10h^{-1}) under sublethal concentrations of 0.5 ppm Ekalux (unvarying) and PHC in LDO-WAF (varying) along with standard deviation, percentage performance and EC50 level.

Concentration of PHC in LDO-WAF (ppm)	Byssogenesis (individual^{-1} 10h^{-1})		Performance (% of control)	EC50 (ppm)
	Mean	SD		
0.75	7.90**	1.10	69.00	
1.00	8.10**	0.62	70.74	
1.25	7.70**	1.27	67.25	1.8
1.75	6.15**	0.50	53.71	
2.00	4.35**	0.47	37.99	
Control	11.45	0.44	-	

Table 93. Perna indica. Byssogenesis: Average number of threads produced (individual^{-1} 10h^{-1}) under sublethal concentrations of 1.5 ppm Ekalux (unvarying) and PHC in LDO-WAF (varying) along with standard deviation, percentage performance and EC50 level.

Concentration of PHC in LDO-WAF (ppm)	Byssogenesis (individual^{-1} 10h^{-1})		Performance (% of control)	EC50 (ppm)
	Mean	SD		
0.75	4.00**	0.91	34.93	
1.00	4.95**	0.87	43.23	
1.25	3.65**	0.34	31.88	0.5
1.75	2.1**	0.62	18.34	
2.00	2.35**	0.38	20.52	
Control	11.45	0.44	-	

** $P < 0.01$

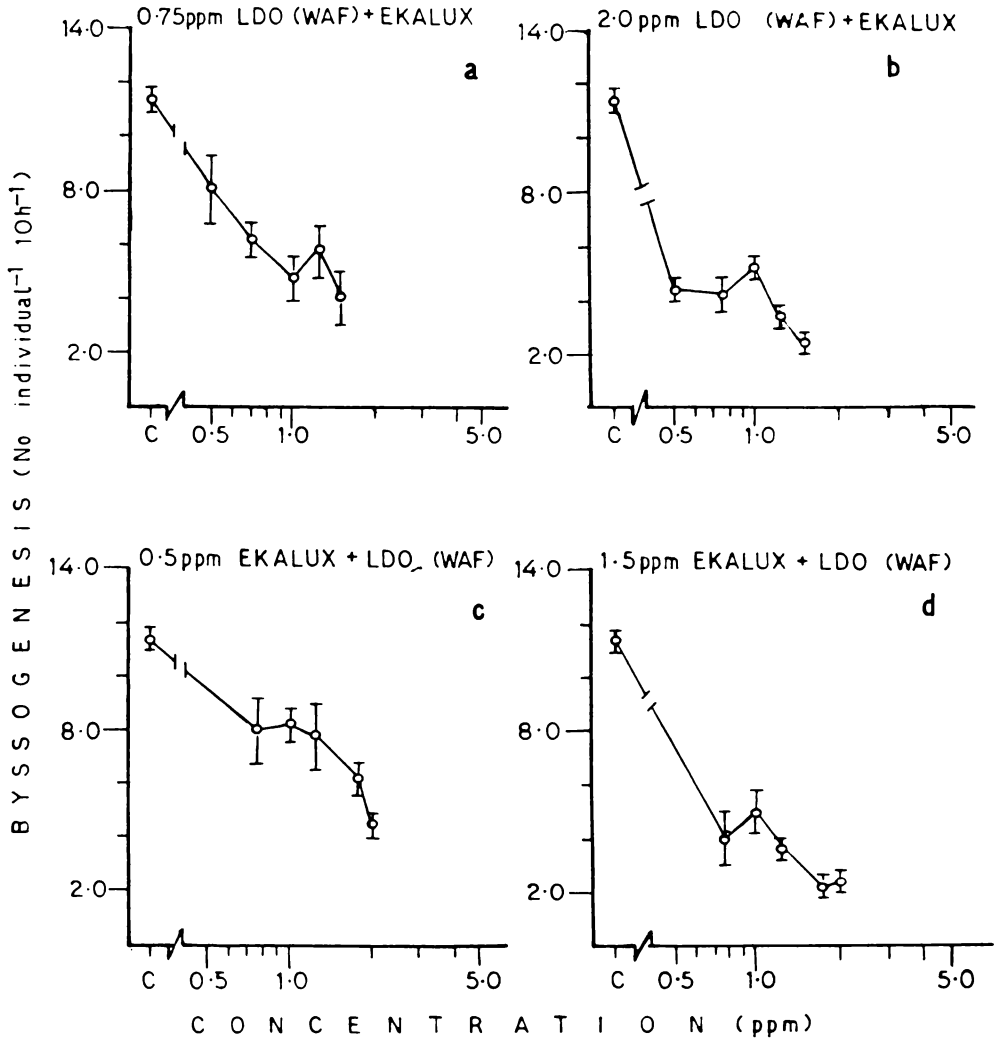

Fig. 72. Perna indica. Average byssogenesis under different sublethal concentrations of pesticide and petroleum hydrocarbon (PHC) in combinations, along with respective standard errors (vertical bars). a&b. PHC in LDO-WAF- (unvarying) and Ekalux (varying) c&d. Ekalux (unvarying) and PHC in LDO-WAF- (varying).

All the combination concentration employed were highly toxic to the animals. The presence of 1.50 ppm of Ekalux with 0.50 ppm of PHC resulted in 50% reduction in byssogenesis. This value was less than even the lowest experimental concentration.

4.2.1.6.3 PHC in LDO (WAF) unvarying and Aldrex varying

The concentration of PHC in LDO (WAF) was 0.50 ppm and that of Aldrex was 1.0 to 4.0 ppm in this series of experiments conducted. All the combinations were proved to be highly toxic, amply justified by the EC50 obtained (1.30 ppm of Aldrex). The presence of 1.0, 1.50 and 2.0 ppm of Aldrex did not show clear cut interconcentration differences. However, the presence of 4.0 ppm of Aldrex along with 0.50 ppm PHC in LDO (WAF) resulted in nearly a total reduction in byssogenesis. All the results were statistically significant (Table 94; Fig. 73c).

In another series of experiment 2.0 ppm of PHC in LDO (WAF) was employed along with 1.0 to 4.0 ppm of Aldrex in five steps. There was only negligible production of byssus threads by all the animals retained in all the combinations. Producing statistically significant results the calculated EC50 was 0.5 ppm of Aldrex (Table 95; Fig. 73d).

4.2.1.6.4 Aldrex unvarying and PHC in LDO (WAF) varying

In a reciprocal series of experiment unvarying concentration of Aldrex along with varying concentration of PHC in LDO (WAF) were used to assess byssogenesis. In one set, the Aldrex concentration was 1.0 ppm and that of PHC was 0.50 to 2.0 ppm. The presence of even 0.50 ppm of PHC along

Table 94. Perna indica. Byssogenesis: Average number of threads produced (individual^{-1} 10h^{-1}) under sublethal concentrations of 0.5 ppm PHC in LDO-WAF (unvarying) and Aldrex (varying) along with standard deviation, percentage performance and EC50 level.

Aldrex Concentration (ppm)	Byssogenesis (individual^{-1} 10h^{-1})		Performance (% of control)	EC50 (ppm)
	Mean	SD		
1.0	6.35**	1.02	55.70	
1.5	4.60**	0.52	40.35	
2.0	4.75**	0.25	41.67	1.30
3.0	3.55**	0.57	31.14	
4.0	1.85**	0.50	16.23	
Control	11.4	0.63	-	

Table 95. Perna indica. Byssogenesis: Average number of threads produced (individual^{-1} 10h^{-1}) under sublethal concentrations of 2.0 ppm PHC in LDO-WAF (unvarying) and Aldrex (varying) along with standard deviation, percentage performance and EC50 level.

Aldrex Concentration (ppm)	Byssogenesis (individual^{-1} 10h^{-1})		Performance (% of control)	EC50 (ppm)
	Mean	SD		
1.0	3.30**	0.53	28.95	
1.5	4.00**	0.16	35.09	
2.0	3.05**	0.34	26.75	0.50
3.0	2.45**	0.73	21.49	
4.0	1.20**	0.63	10.53	
Control	11.40	0.63	-	

** $P < 0.01$

with 1.0 ppm of Aldrex reduced byssus production by nearly 50% to that of control. The EC50 was 0.60 ppm (Table 96; Fig. 73a). Table 97 and Fig. 73b present the data obtained on byssogenesis by P. indica exposed to 4.0 ppm of Aldrex in combination with 0.50 to 2.0 ppm of PHC in LDO (WAF). All the concentration combination employed were highly toxic and the number of threads produced by the group of animals maintained in different steps were negligible. The EC50 was 0.50 ppm. The results obtained were statistically significant.

4.2.2 LONG-TERM SUBLETHAL TOXIC RESPONSE

Efforts were made to find out the long-term effects of exposure to realistic concentrations of pesticides and water accommodated fractions of petroleum hydrocarbons, on the activity of Perna indica. For this, these animals were retained in very low concentrations of Ekalux, Dimecron, Aldrex DDT and WAFs of LDO and P.G. Crude for periods ranging from one to fourteen days (in the case of pesticides) or one to twenty one days (in the case of WAFs of oil). Subsequently they were transferred to raw sea water and maintained for a uniform period of seven days. The representative samples were taken after seven days duration and the rate of oxygen consumption and filtration were assessed. Therefore, the results obtained show the rate of oxygen consumption or filtration of P. indica subjected to exposure of very low levels of pesticides for seven and fourteen days and to WAFs for seven, fourteen and twenty one days. It is assumed that the oxygen consumption and filtration pattern of animals which have an internal load of pesticides or oil could be elicited by this method. Further, transferring such animals to raw sea water and maintaining them for seven days and subsequent measurement of oxygen consumption and filtration would give an idea on the

Table 96. Perna indica. Byssogenesis: Average number of threads produced (individual^{-1} 10h^{-1}) under sublethal concentrations of 1.0 ppm Aldrex (unvarying) and PHC in LDO-WAF (varying) along with standard deviation, percentage performance and EC50 level.

PHC in LDO-WAF Concentration (ppm)	Byssogenesis (individual^{-1} 10h^{-1})		Performance (% of control)	EC50 (ppm)
	Mean	SD		
0.50	6.35**	1.02	55.70	
0.75	4.8**	0.43	42.11	
1.00	4.75**	0.55	41.67	0.60
1.50	2.9**	0.53	25.44	
2.00	3.3**	0.53	28.95	
Control	11.4	0.63	-	

Table 97. Perna indica. Byssogenesis: Average number of threads produced (individual^{-1} 10h^{-1}) under sublethal concentrations of 4.0 ppm Aldrex (unvarying) and PHC in LDO-WAF (varying) along with standard deviation, percentage performance and EC50 level.

PHC in LDO-WAF Concentration (ppm)	Byssogenesis (individual^{-1} 10h^{-1})		Performance (% of control)	EC50 (ppm)
	Mean	SD		
0.50	1.85**	0.50	16.23	
0.75	2.50**	0.82	21.93	
1.00	1.70**	0.23	14.91	0.50
1.50	1.55**	0.66	13.60	
2.00	1.20**	0.63	10.53	
Control	11.40	0.63	-	

** $P < 0.01$

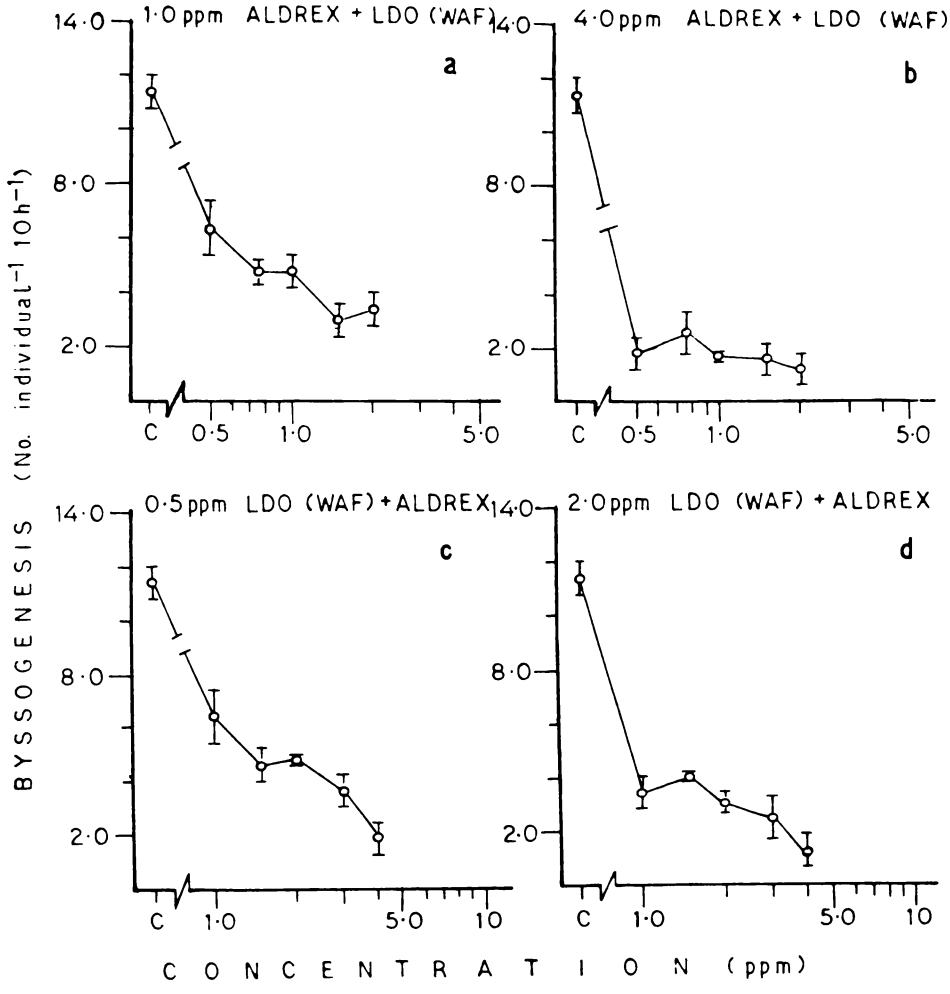

Fig. 73. Perna indica. Average byssogenesis under different sublethal concentrations of pesticide and petroleum hydrocarbon (PHC) in combinations, along with respective standard errors (vertical bars). a&b. Aldrex (unvarying) and PHC in LDO-WAF (varying) c&d. PHC in LDO-WAF (unvarying) and Aldrex (varying).

trend in these activities after a limited period of depuration. The results obtained on these aspects are presented in this section.

4.2.2.1 Rate of Oxygen Consumption

Analysis of the oxygen consumption pattern of P. indica exposed to pesticides or oil at realistic concentrations for periods ranging from one to twentyone days as the case may be and subsequent withdrawal from the test media and exposure to raw sea water give an opportunity to assess the reaction of the animal to temporary changes in stress factor. Alterations in the oxygen consumption pattern are controlled by the behaviour of bivalves. Giving a stress at a low level for prolonged period does help in analysing variations in the basic metabolic rate. Therefore, the present series of experiment give information on the influence of a stress and subsequent release of the stress. The data obtained from the experiments are presented below.

4.2.2.1.1 Ekalux

Table 98 a&b give the data obtained on the pattern of oxygen consumption by P. indica exposed to 5.0 ppb of Ekalux for fourteen days and subsequent transfer to raw sea water for seven days. The oxygen consumption was reduced considerably after fourteen days. The percentage performance of the animal to that of control was 67. When the animals were transferred to raw sea water and the oxygen consumption measured after seven days, indicated slight regaining of normalcy evidenced by the increase in oxygen consumption. The animals were consuming oxygen at a level around 85% to that of control (Fig. 74b).

Table 98 a&b. Perna indica: Average oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.) under a sublethal concentration of 5.0 ppb Ekalux, for periods upto 14 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 98 a.

Duration of Exposure (days)	Oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	1.66	0.09	97.5
	(1.70)	(0.11)	
14	1.01**	0.08	67.0
	(1.51)	(0.09)	

Table 98 b.

Duration of Exposure (days)	Oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	1.22*	0.06	85.4
	(1.43)	(0.07)	

* $P < 0.05$

** $P < 0.01$

Control values in parenthesis.

Another set of individuals of P. indica was exposed to 10.0 ppb of Ekalux. It is clear from the data obtained that an increase in the concentration of Ekalux had effects only after fourteen days, when the animals were respiring at a reduced rate. However, the capacity of the animals to regain normalcy was evidenced by increase in the oxygen consumption after seven days of exposure to raw sea water. Only at one instance the results obtained were found statistically significant (Table 99 a&b; Fig. 74c).

A further increase in the Ekalux concentration to 30.0 ppb, however did not create very drastic difference even after fourteen days of exposure to the toxicant, as the reduction in the rate of oxygen consumption from control animals was only around 44 percent. The only conspicuous feature of the results obtained was that the animals even after transferring to raw sea water respired at a reduced rate, consuming 1.09 $\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt. However, this value shows the animals' capacity to regain normalcy (Table 100 a&b; Fig. 74d).

4.2.2.1.2 Dimecron

P. indica was exposed for fourteen days to low levels of Dimecron concentrations such as 250, 750 and 1200 ppb. Subsequently they were transferred to raw sea water and maintained for seven days. The results obtained on the oxygen consumption of such animals are presented in Table 101a to 103b and Fig. 75 a-d.

Table 101 a&b show the trend in oxygen consumption by P. indica exposed to 250 ppb of Dimecron. The decrease in the oxygen consumption after seven days was not conspicuous. However, when they were retained

Table 99 a&b. Perna indica. Average oxygen consumption (µg O_2 h^{-1} mg^{-1} dry wt.) under a sublethal concentrations of 10.0 ppb Ekalux, for a periods upto 14 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 99 a.

Duration of Exposure (days)	Oxygen consumption (µg O_2 h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	1.57	0.08	92.7
	(1.70)	(0.11)	
14	0.90**	0.10	59.9
	(1.50)	(0.09)	

Table 99 b.

Duration of Exposure (days)	Oxygen consumption (µg O_2 h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	1.14	0.13	79.4
	(1.43)	(0.07)	

** $P < 0.01$ Control values in parenthesis

Table 100 a&b. Perna indica. Average oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.) under a sublethal concentration of 30 ppb Ekalux, for periods upto 14 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 100 a.

Duration of Exposure (days)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.)		Performance (% of control)
	Mean	SE	
7	1.27**	0.07	74.8
	(1.70)	(0.11)	
14	0.84**	0.09	55.9
	(1.50)	(0.09)	

Table 100 b.

Duration of Exposure (days)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.)		Performance (% of control)
	Mean	SE	
7	1.09**	0.07	76.3
	(1.43)	(0.07)	

** $P < 0.01$ Control values in parenthesis

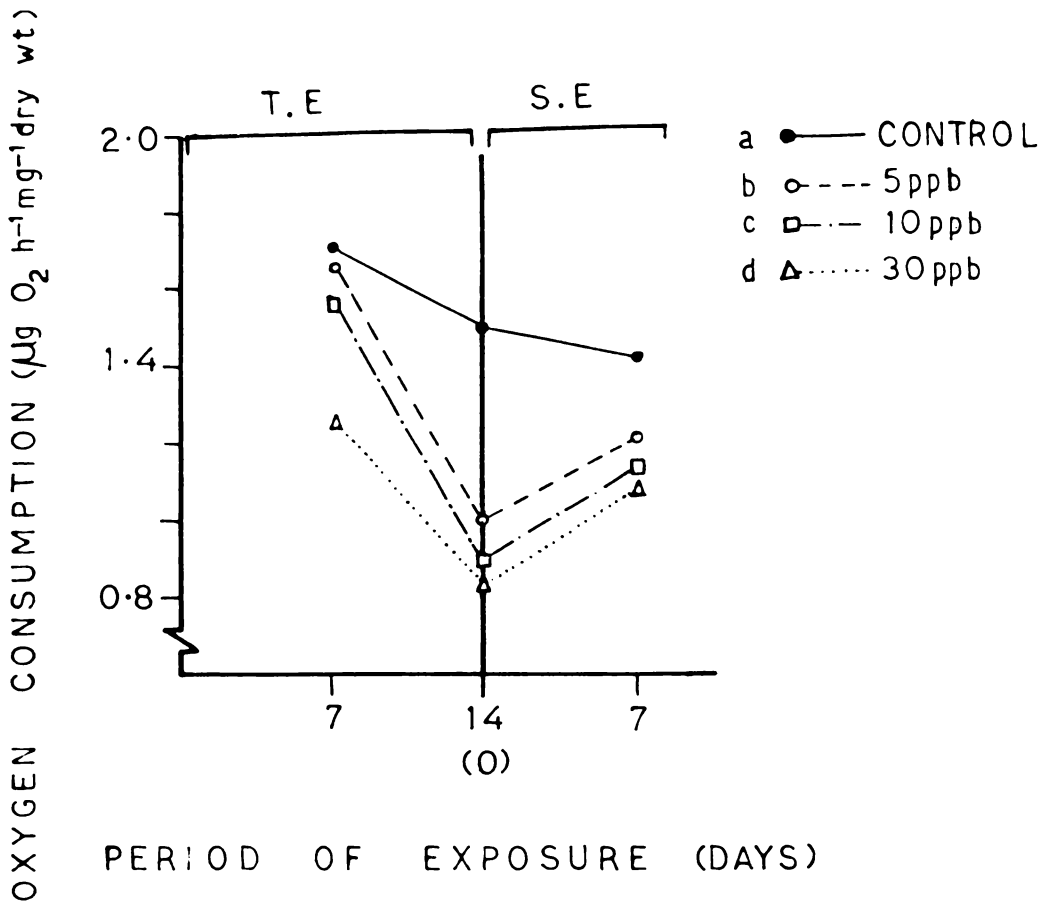

Fig. 74. Perna indica. Average oxygen consumption on chronic exposure to different sublethal concentrations of Ekalux, for periods upto 14 days (TE) and subsequently exposed to raw sea water upto 7 days (S.E) - For standard errors, see Table 98 a&b, 99 a&b and 100 a&b. (T.E- Toxicant exposure; S.E- Sea water exposure).

for longer duration ie; fourteen days the animals reduced consumption considerably. This reduction was found to be statistically significant. From the data on the oxygen consumption after seven days exposure to raw sea water it is evident that the animals are capable of switching back to normalcy. The animals consumed 1.26 µg oxygen h^{-1} mg^{-1} dry wt. which was 88 percent of consumption of the animals retained in control (Fig. 75-b).

The Dimecron concentration was increased to 750 ppb in another set of experiments. The animals decidedly consumed less oxygen after seven and fourteen days. The rate of consumption was only around 50 percent to that of control. The capacity to regain normal activity was slightly disrupted evidenced by reduced oxygen consumption even after exposure of the animals to raw sea water (Table 102 a&b; Fig. 75-c).

A four fold increase in the Dimecron concentration did not have a concomitant effect on the oxygen consumption pattern in P. indica. However, after fourteen days exposure, animals were respiring at a level less than 50 percent, that their counterparts in the controlled condition did. Even after reducing oxygen consumption to such low levels the animals regained normalcy after seven days of exposure to raw seawater and they were performing at a level similar to those animals exposed to Dimecron for seven days (Table 103 a&b; Fig. 75d).

4.2.2.1.3 Aldrex

Perna indica was maintained for fourteen days in test media containing three concentrations namely, 10, 50 and 100 ppb of Aldrex. Representatives from the culture tanks were sampled on the seventh and fourteenth day, to analyse the trend in oxygen consumption. Further, these experimental

Table 101 a&b. Perna indica. Average oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.) under a sublethal concentration of 250 ppb Dimecron, for periods upto 14 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 101 a.

Duration of Exposure (days)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.)		Performance (% of control)
	Mean	SE	
7	1.55 (1.70)	0.09 (0.11)	91.2
14	0.93** (1.50)	0.13 (0.09)	61.8

Table 101 b.

Duration of Exposure (days)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.)		Performance (% of control)
	Mean	SE	
7	1.26 (1.43)	0.07 (0.07)	88.2

** $P < 0.01$ control values in parenthesis

Table 102 a&b. Perna indica. Average oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.) under a sublethal concentration of 750 ppb Dimecron for periods upto 14 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 102 a.

Duration of Exposure (days)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.)		Performance (% of control)
	Mean	SE	
7	1.25**	0.07	73.7
	(1.70)	(0.11)	
14	0.78**	0.10	51.9
	(1.50)	(0.09)	

Table 102 b.

Duration of Exposure (days)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.)		Performance (% of control)
	Mean	SE	
7	1.04**	0.10	72.9
	(1.43)	(0.07)	

** $P < 0.01$ Control values in parenthesis

Table 103 a&b. Perna indica. Average oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.) under a sublethal concentration of 1200 ppb Dimecron for periods upto 14 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 103 a.

Duration of Exposure (days)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.)		Performance (% of control)
	Mean	SE	
7	1.18**	0.08	69.7
	(1.70)	(0.11)	
14	0.65**	0.04	42.9
	(1.50)	(0.09)	

Table 103 b.

Duration of Exposure (days)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.)		Performance (% of control)
	Mean	SE	
7	1.08*	0.10	75.5
	(1.43)	(0.07)	

* $P < 0.05$

** $P < 0.01$

Control values in parenthesis

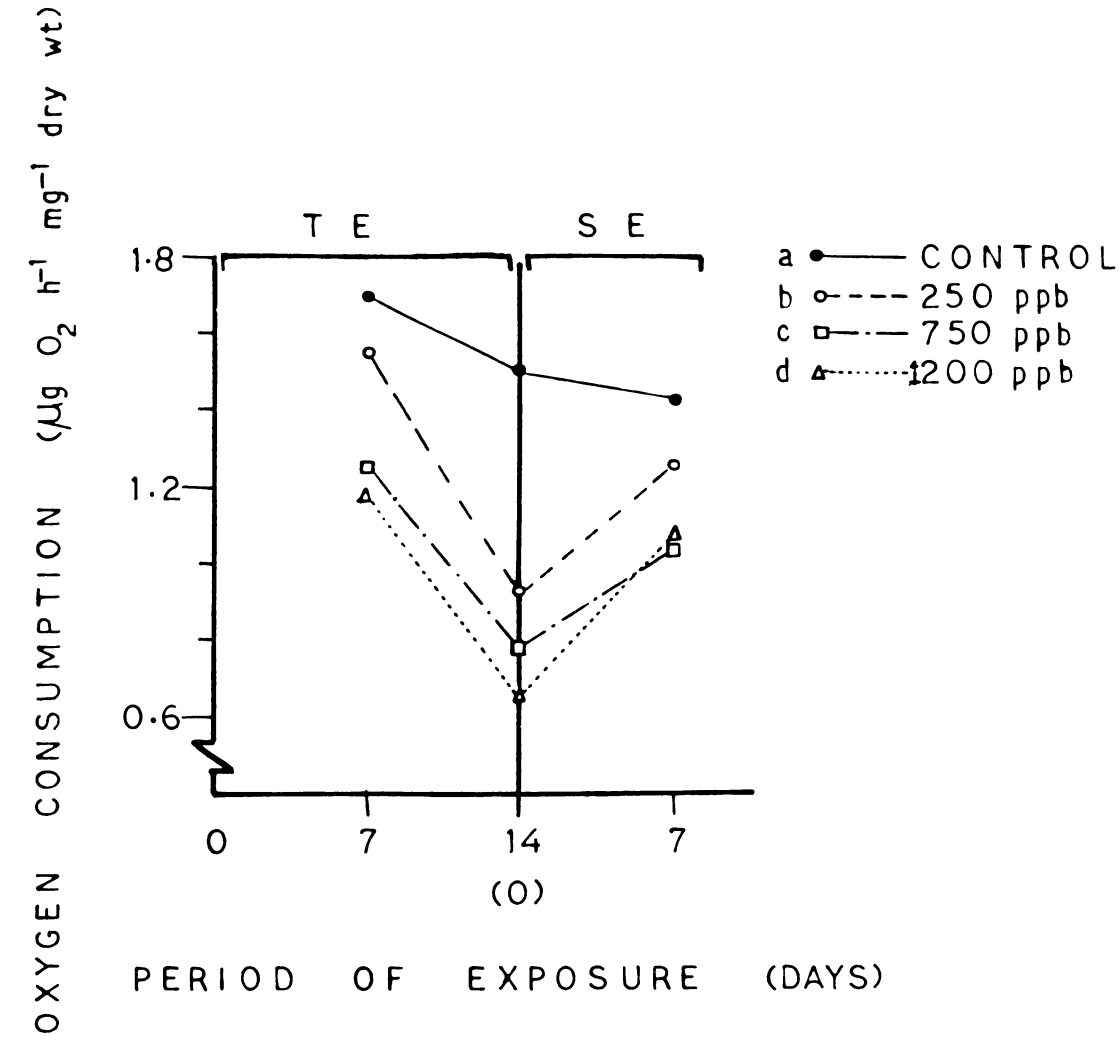

Fig. 75 Perna indica. Average oxygen consumption on chronic exposure to different sublethal concentrations of Dimecron, for periods upto 14 days (T.E) and subsequently exposed to raw sea water upto 7 days (S.E - For standard errors, see Table 101 a&b, 102 a&b and 103 a&b. (T.E-Toxicant exposure, S.E - Sea waater exposure)

animals were transferred to raw sea water and maintained for seven days and the oxygen consumption recorded.

Table 104 a&b and Fig. 76 b depict the rate of oxygen consumption by 10.0 ppb Aldrex exposed P. indica, during exposure and recovery periods. It is clear from the results that the rate of consumption showed minimal deviation from that obtained with controlled animals.

Table 105 a&b and Fig. 76 c indicate the results obtained with animals exposed to 50.0 ppb of Aldrex. Conspicuous feature was increased rate of oxygen consumption after fourteen days of exposure. But for this reading, reduction in oxygen consumption was minimal. In the case of those animals exposed to 100 ppb of Aldrex, exposure upto seven days brought about clear cut decrease in oxygen consumption. On the other hand after fourteen days, animals were found to respire more, than their counterparts maintained under controlled condition for fourteen days. A recovery period of seven days helped the animal to regain normalcy, evidenced by the trend in oxygen consumption (Table 106 a&b; Fig. 76d).

4.2.2.1.4 DDT

Table 107 a to 109 b and Fig. 77 a-d give the data obtained on the oxygen consumption of P. indica subjected to prolonged exposure to 25, 75 and 250 ppb of DDT.

The presence of 25 ppb of DDT brought about reduction in oxygen consumption after seven days. However, when the same animals continued to encounter this concentration for fourteen days, the rate of oxygen uptake increased and they were consuming more oxygen than what their counterparts

Table 104 a&b. Perna indica. Average oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.) under a sublethal concentration of 10.0 ppb Aldrex for periods upto 14 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 104 a.

Duration of Exposure (days)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.)		Performance (% of control)
	Mean	SE	
7	1.84	0.19	94.4
	(1.95)	(0.09)	
14	1.41	0.11	97.8
	(1.44)	(0.08)	

Table 104 b.

Duration of Exposure (days)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.)		Performance (% of control)
	Mean	SE	
7	1.40	0.09	93.8
	(1.50)		

Control values in parenthesis

Table 105 a&b. Perna indica. Average oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.) under a sublethal concentration of 50.0 ppb Aldrex, for periods upto 14 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 105 a.

Duration of Exposure (days)	Oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	1.76	0.17	90.4
	(1.95)	(0.09)	
14	1.75*	0.09	121.1
	(1.44)	(0.08)	

Table 105 b.

Duration of Exposure (days)	Oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	1.45	0.17	97.3
	(1.49)	(0.09)	

* $P<0.05$ Control values in parenthesis

Table 106 a&b. Perna indica. Average oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.) under a sublethal concentration of 100 ppb Aldrex, for periods upto 14 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 106 a.

Duration of Exposure (days)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.)		Performance (% of control)
	Mean	SE	
7	1.53	0.18	78.4
	(1.95)	(0.09)	
14	1.68	0.15	116.7
	(1.44)	(0.08)	

Table 106 b.

Duration of Exposure (days)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.)		Performance (% of control)
	Mean	SE	
7	1.42	0.08	94.7
	(1.50)	(0.09)	

Control values in parenthesis

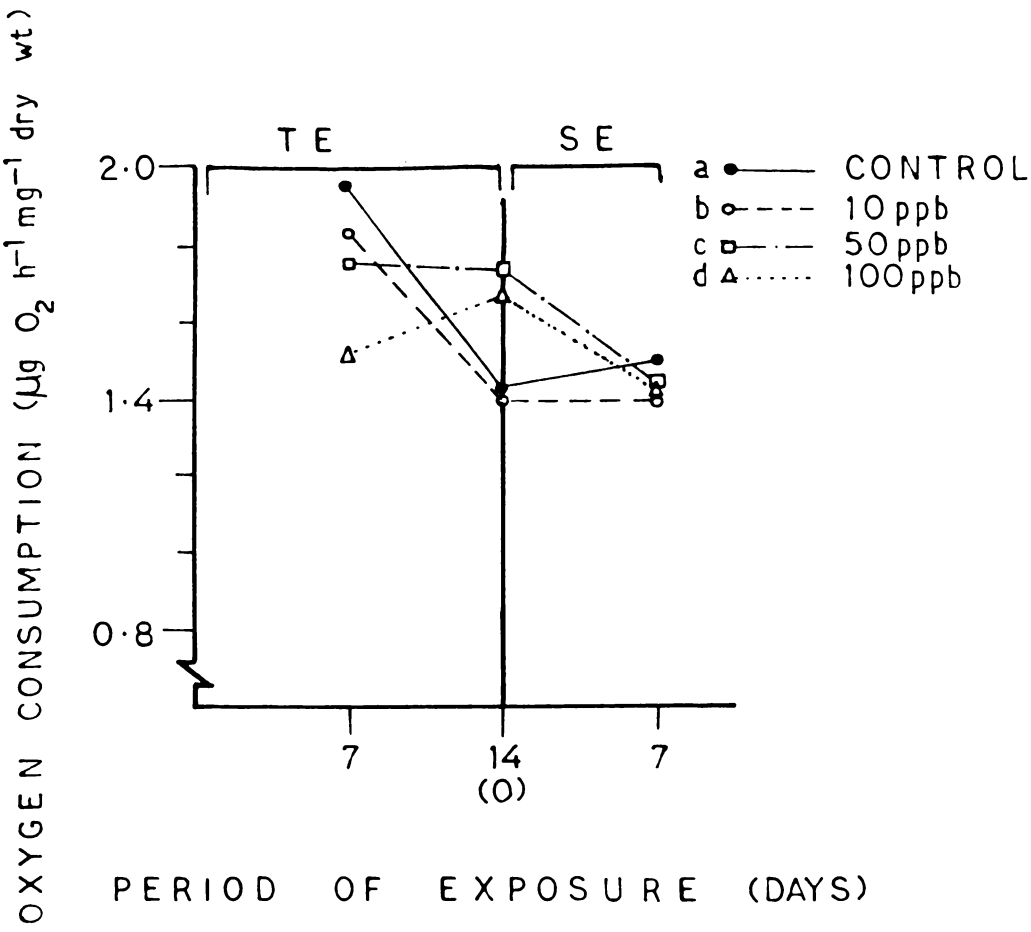

Fig. 76. Perna indica. Average oxygen consumption on chronic exposure to different sublethal concentrations of Aldrex, for periods upto 14 days (T.E) and subsequently exposed to raw sea water upto 7 days (S.E)- For standard errors, see Table 104 a&b, 105 a&b and 106 a&b (T.E- Toxicant exposure; S.E- Sea water exposure).

in controlled condition consumed. These animals when brought back to raw sea water, consumed quantities of oxygen similar to what the control animals did (Table 107 a&b; Fig. 77-b).

Enhancement in the concentration of DDT to 75.0 ppb also gave comparable results (Table 108 a&b; Fig. 77 c). The only variation was that, even seven days' exposure of toxicant exposed animals to raw sea water, did not help in bringing back the rate of oxygen consumption to 100 percent, as against that observed in the case of 25.0 ppb exposed animals.

In the case of those animals which encountered 250 ppb of DDT in the culture media, the quantity of oxygen consumed after seven days was drastically less, but continued maintenance in the same medium for fourteen days resulted in an overshoot in oxygen consumption. The animals' performance rate was 23 percent more than that of control. Curiously enough, when these animals were brought to raw sea water and maintained for seven days they were found to consume less oxygen than the control animals (Table 109 a&b; Fig. 77 d).

4.2.2.1.5 Light Diesel Oil (WAF)

In another series of experiment PHC in the water accommodated fraction of LDO was used as a toxicant to study the oxygen consumption pattern in P. indica subjected to prolonged exposure. Continued exposure to 75.0 ppb of PHC in LDO (WAF) resulted in gradual reduction in oxygen consumption. Thus after twenty one days the average consumption was 1.14 $\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt, which was around 25 percent less than the rate consumed by controlled animals maintained for twenty one days. Exposure to raw sea

Table 107 a&b. Perna indica. Average oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.) under a sublethal concentration of 25 ppb DDT for periods upto 14 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 107 a.

Duration of Exposure (days)	Oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	1.54**	0.09	78.8
	(1.95)	(0.09)	
14	1.55	0.10	107.6
	(1.44)	(0.08)	

Table 107 b.

Duration of Exposure (days)	Oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	1.51	0.09	100.0
	(1.50)	(0.09)	

** $P < 0.01$ Control values in parenthesis

Table 108 a&b. Perna indica. Average oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.) under a sublethal concentration of 75 ppb DDT, for periods upto 14 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 108 a.

Duration of Exposure (days)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.)		Performance (% of control)
	Mean	SE	
7	1.51**	0.09	77.5
	(1.95)	(0.09)	
14	1.77	0.14	122.7
	(1.44)	(0.08)	

Table 108 b.

Duration of Exposure (days)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.)		Performance (% of control)
	Mean	SE	
7	1.36	0.16	90.9
	(1.50)	(0.09)	

** $P < 0.01$ Control values in parenthesis

Table 109 a&b. Perna indica. Average oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.) under a sublethal concentration of 250 ppb DDT, for periods upto 14 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 109 a.

Duration of Exposure (days)	Oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	1.19**	0.15	60.8
	(1.95)	(0.09)	
14	1.78**	0.07	123.2
	(1.44)	(0.08)	

Table 109 b.

Duration of Exposure (days)	Oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	1.30	0.12	87.2
	(1.50)	(0.09)	

** $P < 0.01$ Control values in parenthesis

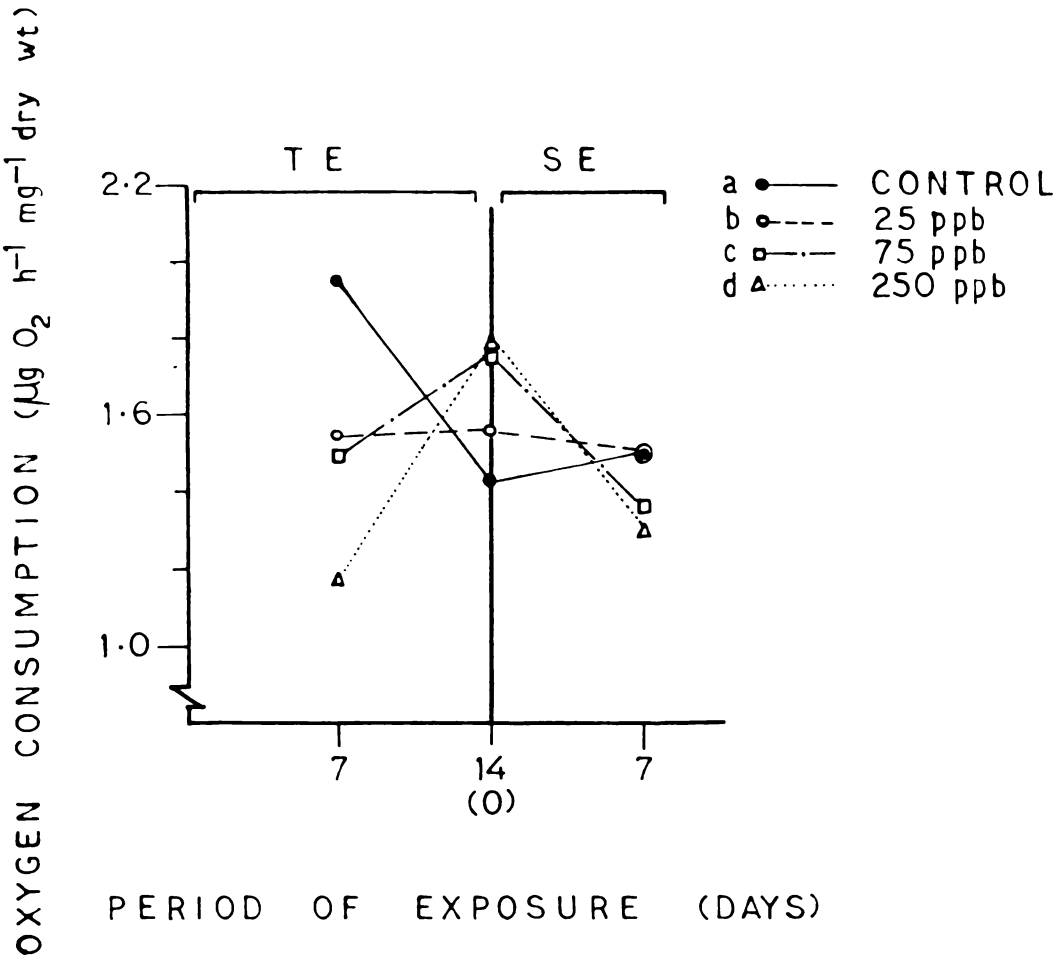

Fig. 77. Perna indica. Average oxygen consumption on chronic exposure to different sublethal concentrations of DDT for periods upto 14 days (T.E) and subsequently exposed to raw sea water upto 7 days (S.E)- For standard errors, see Table 107 a&b, 108 a&b, and 109 a&b - (T.E- Toxicant exposure; S.E - Sea water exposure.

water for seven days helped in regaining the rate of oxygen consumption (Table 110 a&b; Fig. 78-b).

Another set of animals were maintained in a media charged with 300 ppb of PHC in LDO (WAF) for twentyone days. The data obtained by assessing the oxygen consumption pattern by these animals after every seven days are presented in Table 111 a&b and Fig. 78 c. Here also continued exposure reduced the rate of consumption of oxygen by the animals. After twentyone days of exposure the average oxygen consumption was 0.94 $\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt which was around 63 percent of the amount consumed by control animals. When these animals were brought back to raw sea water and maintained for seven days the rate of consumption, although increased, did not record normal levels.

The presence of 750 ppb PHC in LDO (WAF) did not produce results markedly different from that obtained for animals exposed to 300 ppb PHC in LDO (WAF). Even after twenty one days of exposure to 750 ppb of PHC, the reduction was only around 50 percent. Bringing back these animals to raw sea water, resulted in enhancement of oxygen consumption, although normal levels of consumption was not reached (Table 112 a&b; Fig. 78 d).

4.2.2.1.6 Persian Gulf Crude (WAF)

P. indica was exposed to three PHC concentrations contained in P.G. Crude (WAF) for twentyone days before transferring to raw sea water. The results obtained on the oxygen consumption of animals with such exposure history are presented in Table 113 a to 117 b and Fig. 79 a-d.

Table 110 a&b. Perna indica. Average oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.) under a sublethal concentration of 75 ppb PHC in LDO (WAF), for periods upto 21 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 110 a.

Duration of Exposure (days)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.)		Performance (% of control)
	Mean	SE	
7	1.48	0.17	95.9
	(1.54)	(0.11)	
14	1.50	0.09	91.1
	(1.64)	(0.16)	
21	1.14*	0.11	76.1
	(1.50)	(0.07)	

Table 110 b.

Duration of Exposure (days)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.)		Performance (% of control)
	Mean	SE	
7	1.54	0.08	94.3
	(1.63)	(0.10)	

* $P < 0.05$ Control values in the parenthesis

Table 111 a&b. Perna indica. Average oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.) under a sublethal concentration of 300 ppb PHC in LDO (WAF), for periods upto 21 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 111 a.

Duration of Exposure (days)	Oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	1.55	0.21	100.6
	(1.54)	(0.11)	
14	1.43	0.13	87.2
	(1.64)	(0.16)	
21	0.94**	0.11	62.6
	(1.50)	(0.07)	

Table 111 b.

Duration of Exposure (days)	Oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	1.40	0.12	85.9
	(1.63)	(0.10)	

** $P < 0.01$ Control values in parenthesis

Table 112 a&b. Perna indica. Average oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.) under a sublethal concentration of 750 ppb PHC in LDO (WAF), for periods upto 21 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 112 a.

Duration of Exposure (days)	Oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	1.36	0.18	88.2
	(1.54)	(0.11)	
14	1.39	0.19	84.7
	(1.64)	(0.16)	
21	0.76**	0.14	50.8
	(1.50)	(0.07)	

Table 112 b.

Duration of Exposure (days)	Oxygen consumption (μg O_2 h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	1.34*	0.09	82.0
	(1.63)	(0.10)	

* $P < 0.05$

** $P < 0.01$

Control values in parenthesis

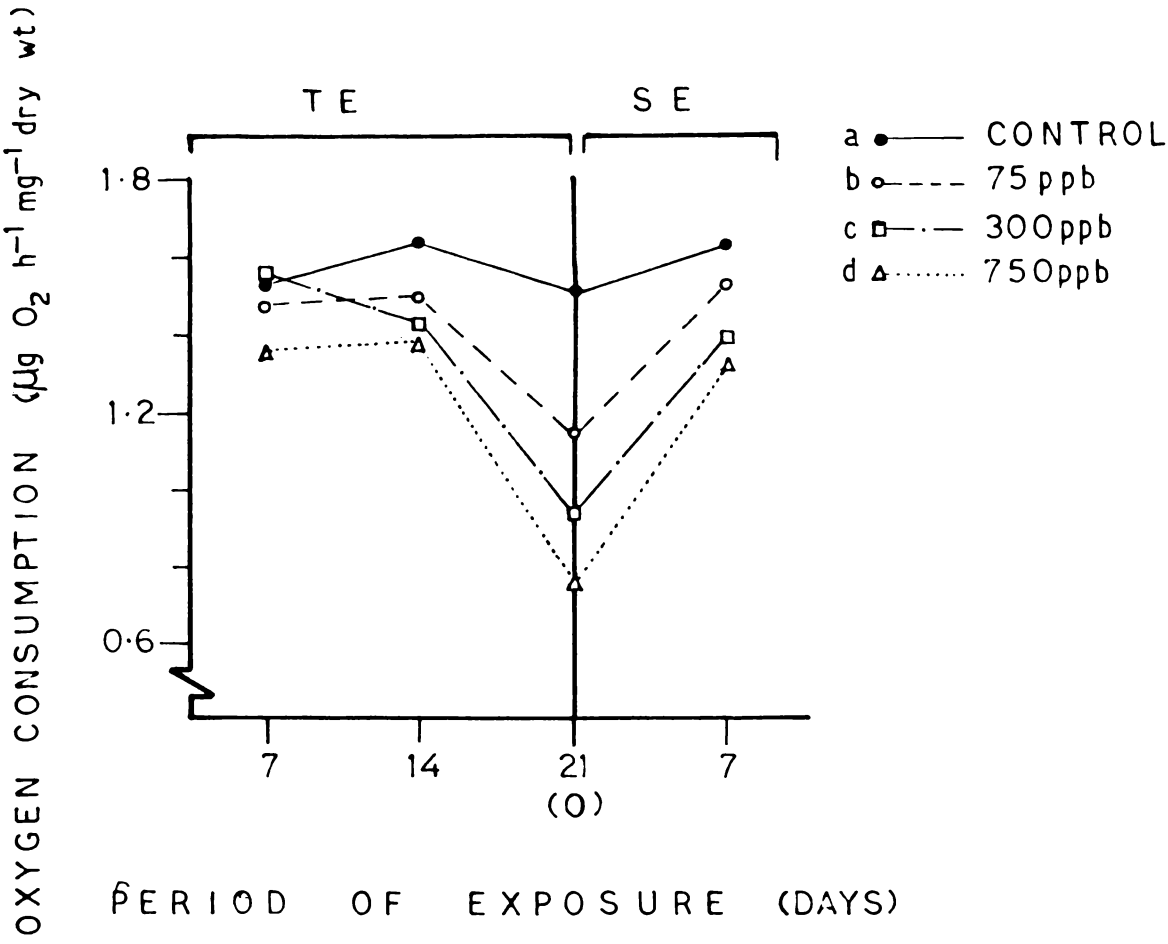

Fig. 78. Perna indica. Average oxygen consumption on chronic exposure to different sublethal concentrations of petroleum hydrocarbon in LDO-WAF- for periods upto 21 days (T.E) and subsequently exposed to raw sea water upto 7 days (S.E) - For standard errors, see Table 110 a&b 111 a&b and 112 a&b- (T.E- Toxicant exposure; S.E sea water exposure)

Here also, prolonged exposure to 750 ppb PHC in P.G. Crude (WAF) resulted in gradual decrease in oxygen consumption. On an average the animals were consuming 1.47 $\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt. after seven days, which was brought down to 1.07 $\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt after twenty one days. The animals increased their oxygen consumption when maintained in raw sea water condition for seven days (Table 113 a&b; Fig. 79-b).

Table 114 a&b and Fig. 79c indicate the data obtained on oxygen consumption of P. indica exposed to 1250 ppb PHC in P.G. Crude (WAF). Gradually reducing the rate consumption the animals utilized low quantities of oxygen after twentyone days. Subsequently, when these animals were removed to raw sea water and retained for seven days, the rate of consumption increased to 82 percent.

Enhancing the concentration of PHC in P.G. Crude (WAF) to 1750 ppb did not decidedly change the performance rate of P. indica in comparison to those exposed to 1250 ppb. However, here also reduction in consumption was noticed and regaining normalcy was evidenced after seven days' exposure to raw sea water. Reduction in consumption after twenty one days was statistically significant (Table 115 a&b; Fig. 79-d).

4.2.2.2 Rate of Filtration

As in the case of the experiments conducted for oxygen consumption, representatives of animals, sampled from the same lot of test were employed to assess the rate of filtration. It is known that rate of oxygen consumption and filtration are interconnected behaviour, with considerable physiological significance. Analysing the rate of filtration gives an insight into the quantity of water circulated within the mantle cavity of the bivalve. Understand-

Table 113 a&b. Perna indica. Average oxygen consumption (µg O_2 h^{-1} mg^{-1} dry wt.) under a sublethal concentration of 750 ppb PHC in P.G. Crude (WAF) for periods upto 21 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 113 a.

Duration of Exposure (days)	Oxygen consumption (µg O_2 h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	1.47	0.13	95.2
	(1.54)	(0.11)	
14	1.24*	0.08	75.3
	(1.64)	(0.16)	
21	1.07**	0.07	71.3
	(1.50)	(0.07)	

Table 113 b.

Duration of Exposure (days)	Oxygen consumption (µg O_2 h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	1.39	0.09	85.4
	(1.63)	(0.10)	

* $P<0.05$

** $P<0.01$

Control values in parenthesis

Table 114 a&b. Perna indica. Average oxygen consumption (µg O_2 h^{-1} mg^{-1} dry wt.) under a sublethal concentration of 1250 ppb PHC in P.G. Crude (WAF), for periods upto 21 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 114 a.

Duration of Exposure (days)	Oxygen consumption (µg O_2 h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	1.47	0.2	95.6
	(1.54)	(0.11)	
14	1.29	0.11	78.7
	(1.64)	(0.16)	
21	0.88**	0.11	58.8
	(1.50)	(0.07)	

Table b.

Duration of Exposure (days)	Oxygen consumption (µg O_2 h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	1.35*	0.09	82.4
	(1.63)	(0.10)	

* $P < 0.05$

** $P < 0.01$

Control values in parenthesis

Table 115 a&b. Perna indica. Average oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.) under a sublethal concentration of 1750 ppb PHC in P.G.Crude (WAF), for periods upto 21 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 115 a.

Duration of Exposure (days)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.)		Performance (% of control)
	Mean	SE	
7	1.44	0.15	93.3
	(1.54)	(0.11)	
14	1.26	0.09	76.9
	(1.64)	(0.16)	
21	0.90**	0.15	60.4
	(1.50)	(0.07)	

Table 115 b.

Duration of Exposure (days)	Oxygen consumption ($\mu g\ O_2\ h^{-1}\ mg^{-1}$ dry wt.)		Performance (% of control)
	Mean	SE	
7	1.34*	0.06	81.7
	(1.63)	(0.10)	

* $P < 0.05$

** $P < 0.01$

Control values in parenthesis

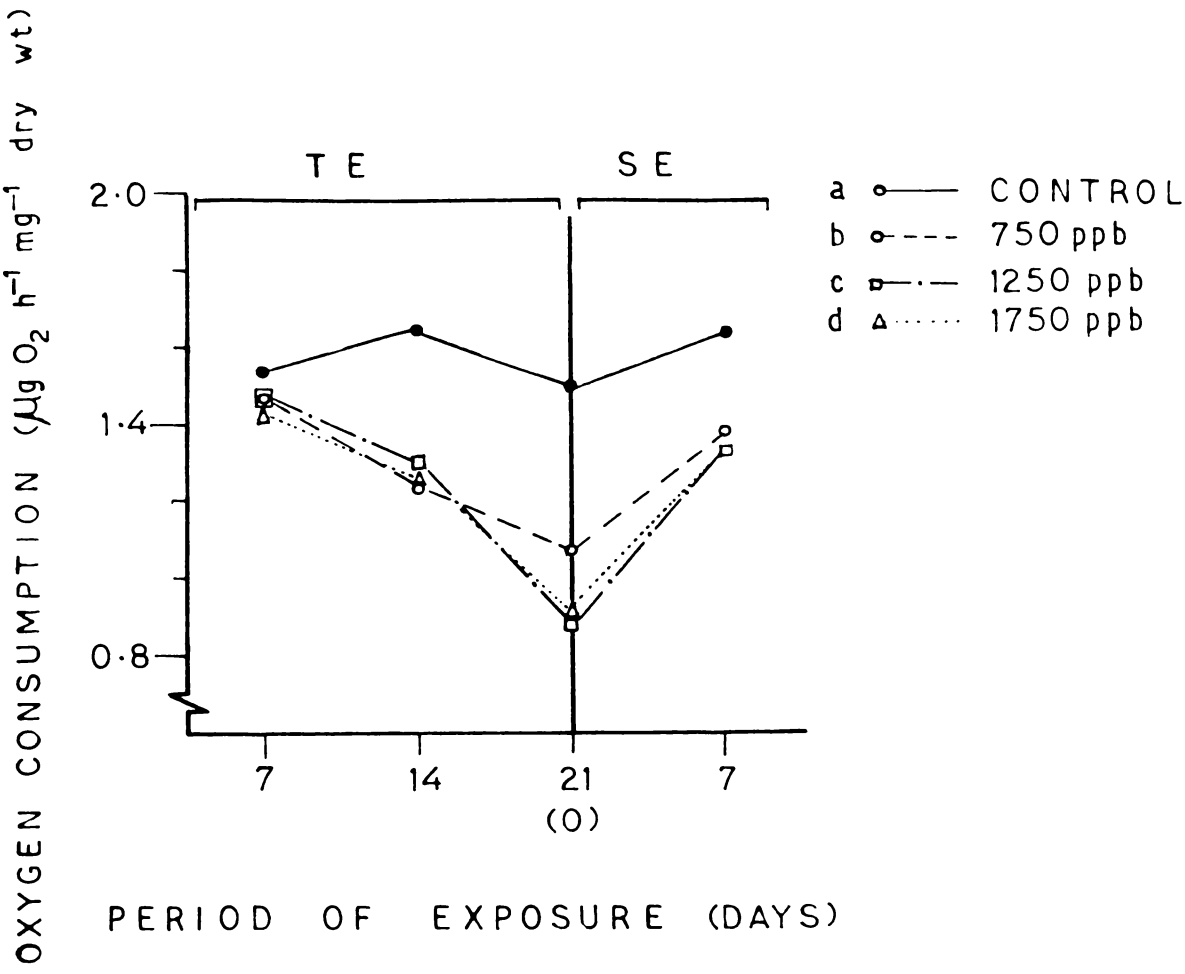

Fig 79. Perna indica. Average oxygen consumption on chronic exposure to different sublethal concentrations of petroleum hydrocarbon in P.G. Crude-WAF- for periods upto 21 days (T.E) and subsequently exposed to raw sea water upto 7 days (S.E) -For standard errors, see Table 113 a&b, 114 a&b and 115 a&b - (T.E-Toxicant exposure; S.E- Sea water exposure).

ably, this gives information on the capacity of the animal to use the dissolved oxygen in water as well as removal of food particles. Therefore, the results obtained give direct information on the energy expenditure and the energy gain of a bivalve put under stress. The following results highlight the data obtained on the rate of filtration by P. indica subjected to prolonged exposure to pesticides and oil fractions, and subsequently maintained in raw sea water.

4.2.2.2.1 Ekalux

Three concentrations of Ekalux ie; 5.0, 10.0 and 30.0 ppb were added to the media to assess the filtration rate of P. indica, during prolonged exposure. Table 116 a&b and Fig. 80 b detail the data obtained from the experiments, where the test concentration was 5.0 ppb. Soon after exposure to this concentration, the rate of filtration was reduced. This trend was continued till seven days. The reading obtained after fourteen days shows that the animals have become more active and filtered more water, when compared to their counterparts in control. When the animals were transferred to raw sea water and maintained for seven days the rate of filtration reduced.

The results obtained when the test concentration was 10.0 ppb are presented in Table 117 a&b and Fig. 80c. Here also, prolonged exposure for fourteen days resulted in an increase in the rate of filtration. The percentage performance was around 120, 100 being the performance level of control animals. When the animals were changed to raw sea water the rate of filtration was reduced and compared very well with those animals maintained under controlled condition.

Presence of 30 ppb of Ekalux, as a matter of fact resulted only in increase in the rate of filtration by P. indica. Subsequent exposure to raw sea

Table 116 a&b. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under a sublethal concentration of 5.0 ppb Ekalux, for periods upto 14 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 116 a.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt)		Performance (% of control)
	Mean	SE	
7	0.439**	0.03	80.9
	(0.543)	(0.02)	
14	0.504	0.02	106.6
	(0.473)	(0.01)	

Table 116 b.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	0.417	0.02	91.9
	(0.454)	(0.01)	

** $P < 0.01$ Control values in parenthesis

Table 117 a&b. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under a sublethal concentration of 10.0 ppb Ekalux, for periods upto 14 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 117 a.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	0.476	0.03	87.7
	(0.543)	(0.02)	
14	0.570**	0.02	120.51
	(0.473)	(0.01)	

Table 117 b.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	0.439	0.03	96.7
	(0.454)	(0.01)	

** $P < 0.01$

Control values in parenthesis

Table 118 a&b. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under a sublethal concentration of 30.0 ppb Ekalux, for periods upto 14 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors percentage performance.

Table 118 a.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	0.539	0.03	99.3
	(0.543)	(0.02)	
14	0.541**	0.01	114.4
	(0.473)	(0.01)	

Table 118 b.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	0.418	0.02	92.1
	(0.454)	(0.01)	

** $P < 0.01$ Control values in parenthesis

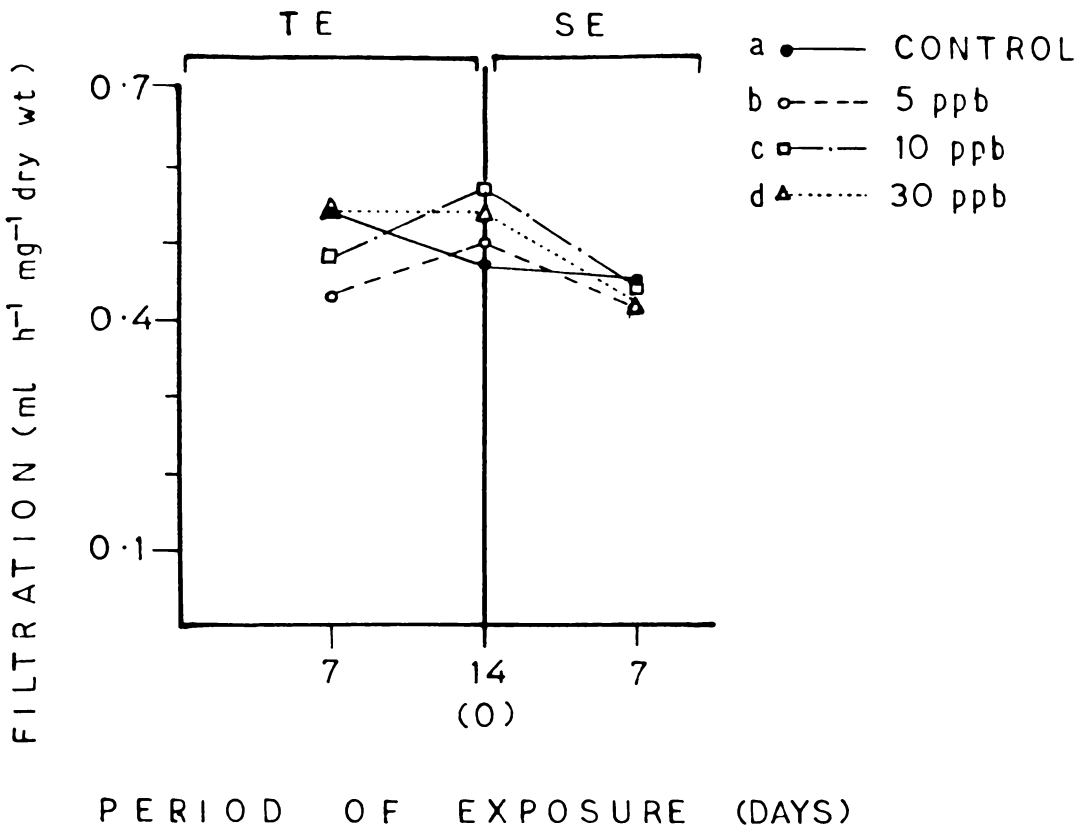

Fig. 80. Perna indica. Average filtration on chronic exposure to different sublethal concentrations of Ekalux for periods upto 14 days (T.E) and subsequently exposed to raw sea water upto 7 days (S.E) - For standard errors, see Table 116 a&b, 117 a&b and 118 a&b - (T.E- Toxicant exposure; S.E - sea water exposure).

water however, slightly reduced the rate of filtration and the results are inconclusive (Table 118 a&b; Fig. 80 d).

4.2.2.2.2 Dimecron

Table 119 a to 121 b and Fig. 81 a-d present the data obtained on the average filtration by P. indica, under sublethal concentrations of Dimecron viz. 250, 750 and 1200 ppb.

From the data it is clear that presence of Dimecron, irrespective of variation in concentration resulted in increased filtration rate, when the duration of exposure exceeded seven days. However, animals maintained in all the three concentrations filtered considerably less quantity of water during the initial seven days of exposure. Even when the animals from all the concentration were taken to raw sea water and maintained for seven days they continued to filter more water, as evidenced by the results.

4.2.2.2.3 Aldrex

Aldrex was added so as to derive concentrations such as 10, 50 and 100 ppb in the medium and the animals were maintained for fourteen days in these concentrations. Table 122 a&b and Fig. 82 b show the readings obtained on filtration. Initial elevation and subsequent reduction was the main feature of the results obtained after fourteen days of exposure to 10 ppb of Aldrex. The animals' performance level was around 70 percent to that of control animals. Increase in the rate of filtration after transferring the animals to raw sea water shows that they slowly regained normalcy.

Both in the case of 50 ppb and 100 ppb exposed animals, the trend in filtration rate was comparable during the exposure phase. However, the capa-

Table 119 a&b. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under a sublethal concentration of 250 ppb Dimecron, for periods upto 14 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 119 a.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	0.345**	0.01	63.5
	(0.543)	(0.02)	
14	0.571**	0.02	120.7
	(0.473)	(0.01)	

Table 119 b.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	0.496	0.02	109.3
	(0.454)	(0.01)	

** $P < 0.01$ Control values in parenthesis

Table 120 a&b. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under a sublethal concentration of 750 ppb Dimecron, for periods upto 14 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 120 a.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	0.344**	0.02	63.4
	(0.543)	(0.01)	
14	0.553**	0.02	116.9
	(0.473)	(0.01)	

Table 120 b.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	0.529*	0.03	116.5
	(0.454)	(0.01)	

* $P < 0.05$

** $P < 0.01$

Control values in parenthesis

Table 121 a&b. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under a sublethal concentration of 1200 ppb Dimecron, for periods upto 14 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 121 a.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	0.337**	0.01	62.1
	(0.543)	(0.02)	
14	0.568**	0.01	120.1
	(0.473)	(0.01)	

Table 121 b.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance
	Mean	SE	
7	0.537**	0.03	118.3
	(0.454)	(0.01)	

** $P<0.01$ Control values in parenthesis

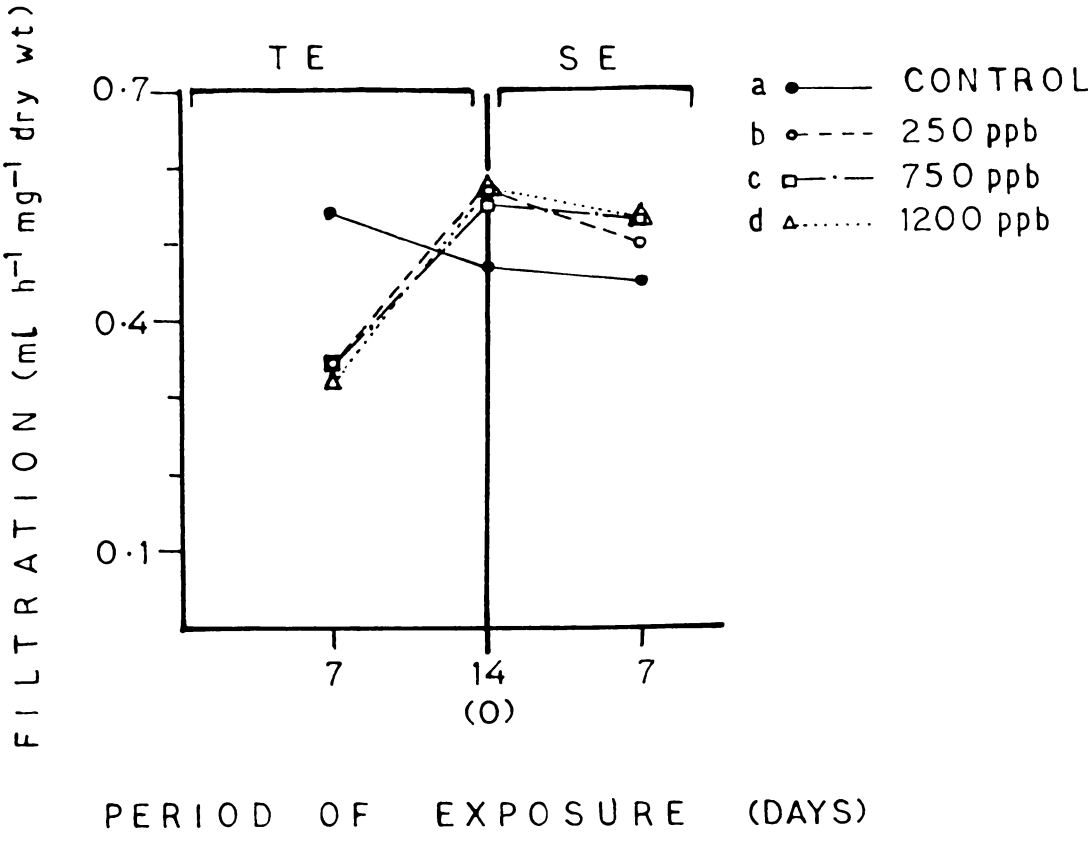

Fig. 81. Perna indica. Average filtration on chronic exposure to different sublethal concentration of Dimecron for periods upto 14 days (T.E) and subsequently exposed to raw sea water upto 7 days (S.E)- For standard errors, see Table 119 a&b, 120 a&b and 121 a&b - (T.E- Toxicant exposure; S.E- Sea water exposure).

Table 122 a&b. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under a sublethal concentration of 10 ppb Aldrex, for periods upto 14 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 122 a.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	0.540	0.03	104.9
	(0.515)	(0.04)	
14	0.445**	0.06	70.9
	(0.627)	(0.01)	

Table 122 b.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance
	Mean	SE	
7	0.475	0.02	92.4
	(0.514)	(0.02)	

** $P < 0.01$ Control values in parenthesis

Table 123 a&b. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under a sublethal concentration of 50.0 ppb Aldrex, for periods upto 14 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 123 a.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	0.574	0.04	111.5
	(0.515)	(0.04)	
14	0.439**	0.02	70.0
	(0.627)	(0.01)	

Table 123 b.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	0.398**	0.02	77.4
	(0.514)	(0.02)	

** $P < 0.01$ Control values in parenthesis

Table 124 a&b. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under a sublethal concentration of 100 ppb Aldrex, for periods upto 14 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 124 a.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	0.518	0.04	100.58
	(0.515)	(0.04)	
14	0.394**	0.02	62.84
	(0.627)	(0.01)	

Table 124 b.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	0.427**	0.02	83.07
	(0.514)	(0.02)	

** $P < 0.01$ Control values in parenthesis

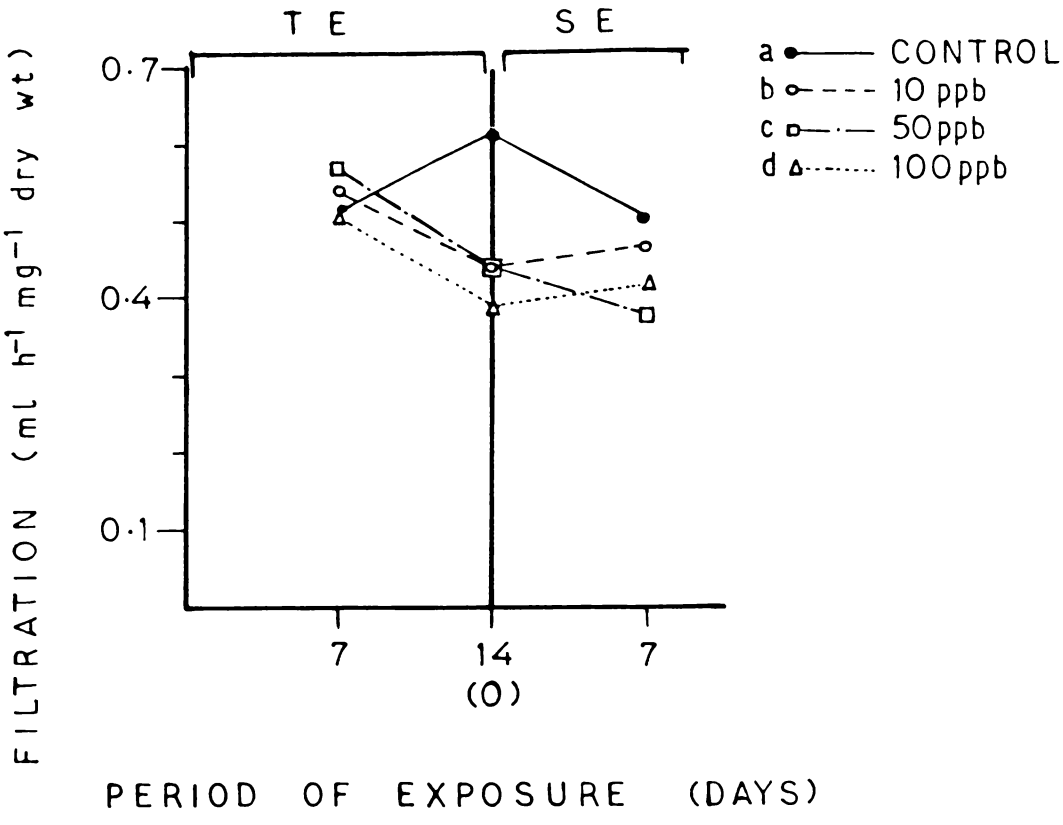

Fig. 82. Perna indica. Average filtration on chronic exposure to different sublethal concentrations of Aldrex for periods upto 14 days (T.E) and subsequently exposed to raw sea water upto 7 days (S.E) - For standard errors, see Table 122 a&b, 123 a&b and 124 a&b - (T.E- Toxicant exposure; S.E- sea water exposure).

city to regain normalcy, by the animals exposed to these concentrations was relatively slow as indicated by the results (Table 123 a&b, 124 a&b and Fig. 82 c&d).

4.2.2.2.4 DDT

Three concentrations of DDT were employed in this experiment. They were 25, 75 and 250 ppb. The presence of 25 ppb of DDT in the culture medium, resulted an increase in the rate of filtration during the initial period of seven days. Continued exposure to this concentration brought down the rate of filtration by around 25 percent to that of control. On exposure to raw sea water, animals did not show any drastic change in the filtration rate (Table 125 a&b; Fig. 83 b).

The presence of 75 ppb also resulted in initial increase in the rate of filtration. However, animals exposed continuously for fourteen days, brought down their rate of filtration by around 50 percent. When these animals were exposed to raw sea water the rate of filtration increased after seven days, indicating their capacity to regain normalcy (Table 126 a&b; Fig. 83 c).

The presence of 250 ppb of DDT in the culture media reduced rate of filtration after seven and fourteen days. After fourteen days animals were filtering only less than 50 percent of the quantity of water filtered by animals maintained in controlled conditions and this variation was very significant statistically. These animals were transferred to raw sea water and when their filtration was assessed after seven days, the rate showed marginal increase (Table 127 a&b; Fig. 83 d).

Table 125 a&b. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under a sublethal concentration of 25 ppb DDT, for periods upto 14 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 125 a.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	0.604	0.06	117.3
	(0.515)	(0.04)	
14	0.479**	0.07	76.4
	(0.627)	(0.01)	

Table 125 b.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	0.409**	0.02	79.6
	(0.514)	(0.02)	

** $P < 0.01$ Control values in parenthesis

Table 126 a&b. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under a sublethal concentration of 75 ppb DDT, for periods upto 14 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 126 a.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	0.614	0.06	119.2
	(0.515)	(0.04)	
14	0.290**	0.02	46.3
	(0.627)	(0.01)	

Table 126 b.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	0.412**	0.02	80.2
	(0.514)	(0.02)	

** $P < 0.01$ Control values in parenthesis

Table 127 a&b. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under a sublethal concentration of 250 ppb DDT, for periods upto 14 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 127 a.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	0.408*	0.03	79.2
	(0.515)	(0.04)	
14	0.310**	0.03	49.4
	(0.627)	(0.01)	

Table 127 b.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	0.397**	0.02	77.2
	(0.514)	(0.02)	

* $P < 0.05$

** $P < 0.01$

Control values in parenthesis

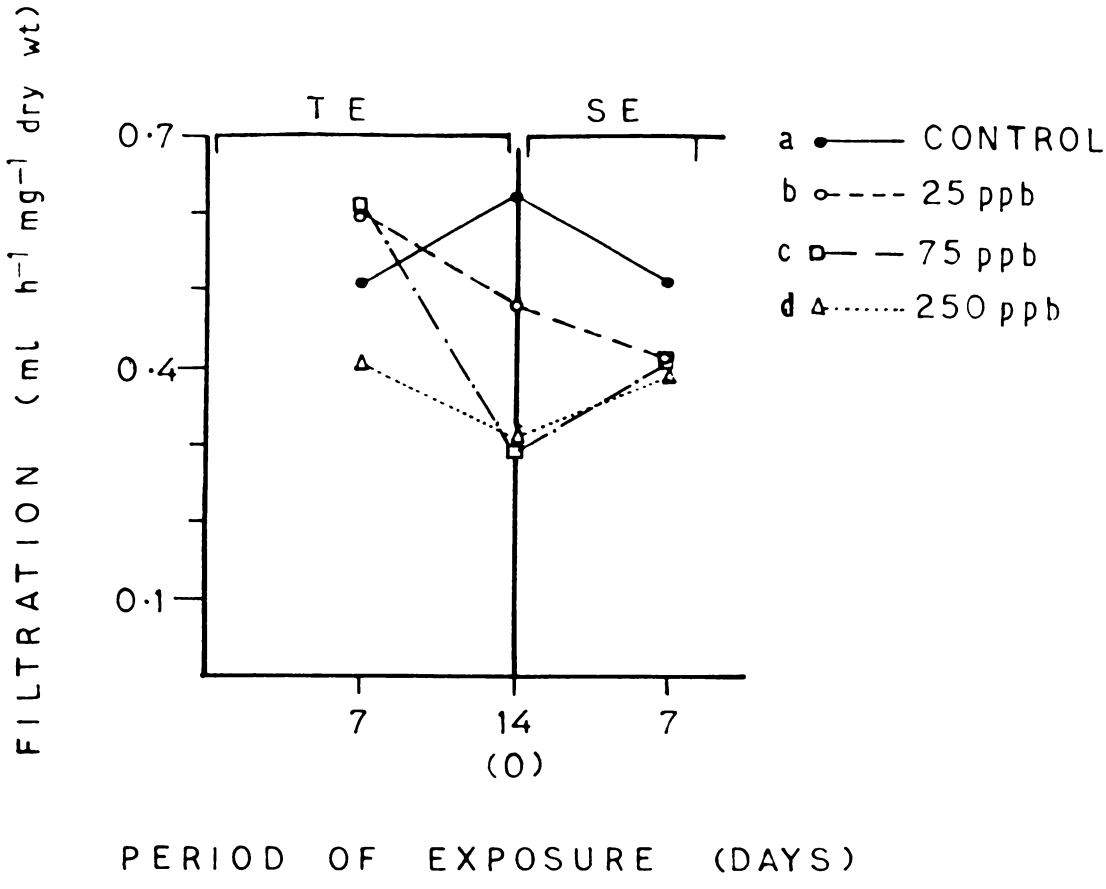

Fig. 83. Perna indica. Average filtration on chronic exposure to different sublethal concentrations of DDT for periods upto 14 days (T.E) and subsequently exposed to raw sea water upto 7 days (S.E) - For standard errors see, Table 125 a&b, 126 a&b and 127 a&b - (T.E- Toxicant exposure; S.E- sea water exposure).

4.2.2.2.5 Light Diesel Oil (WAF)

Table 128 a to 130 b and Fig. 84 present the data obtained on the rate of filtration by P. indica, exposed to various PHC concentrations in LDO (WAF) for periods upto twentyone days and subsequent withdrawal and maintenance in raw sea water for seven days.

75 ppb PHC in LDO (WAF) did not bring about any clear cut variation in the rate of filtration of the animals exposed for twentyone days continuously in the toxicant medium or after maintaining them in raw sea water for seven days (Table 128 a&b; Fig. 84 b).

The presence of 300 ppb of PHC also affected only in a uniform manner. Thus the variation in the rate of filtration between 7, 14 and 21 days were comparable. Exposure to raw sea water for 7 days brought back the animal to normalcy (Table 129 a&b; Fig. 84 c).

In the case of 750 ppb exposed animals, there was not much inter-period variations, although the animals maintained in this concentration uniformly filtered sea water. Quick recovery was evinced when they were exposed to raw sea water (Table 130 a&b; Fig. 84-d).

4.2.2.2.6 Persian Gulf Crude (WAF)

Table 131 a to 133 b and Fig. 85 present the data gathered on the rate of filtration by P. indica exposed to 750, 1250 and 1750 ppb of PHC in P.G. Crude (WAF). Only in the case of those animals exposed for twentyone days to 1750 ppb PHC, reduction in filtration was conspicuous. However, all the animals maintained in all these concentrations uniformly filtered less water,

Table 128 a&b. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under a sublethal concentration of 75.0 ppb PHC in LDO (WAF), for periods upto 21 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 128 a.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	0.490	0.02	94.1
	(0.521)	(0.02)	
14	0.491**	0.01	89.1
	(0.551)	(0.01)	
21	0.544	0.02	97.5
	(0.558)	(0.01)	

Table 128 b.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	0.547	0.01	95.8
	(0.571)	(0.01)	

** $P < 0.01$ Control values in parenthesis

Table 129 a&b. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under a sublethal concentration of 300 ppb PHC in LDO (WAF), for periods upto 21 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 129 a.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	0.466*	0.02	89.4
	(0.521)	(0.02)	
14	0.467**	0.02	84.8
	(0.551)	(0.01)	
21	0.493**	0.01	88.4
	(0.558)	(0.01)	

Table 129 b.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	0.557	0.02	97.6
	(0.571)	(0.01)	

* $P < 0.05$

** $P < 0.01$

Control values in parenthesis

Table 130 a&b. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under a sublethal concentration of 750 ppb PHC in LDO (WAF), for periods upto 21 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 130 a.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	0.386**	0.03	74.1
	(0.521)	(0.02)	
14	0.412**	0.02	74.8
	(0.551)	(0.02)	
21	0.473*	0.03	84.8
	(0.558)	(0.01)	

Table 130 b.

Duration fo Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	0.556	0.02	97.4
	(0.571)	(0.01)	

* $P < 0.05$ Control values in parenthesis

** $P < 0.01$

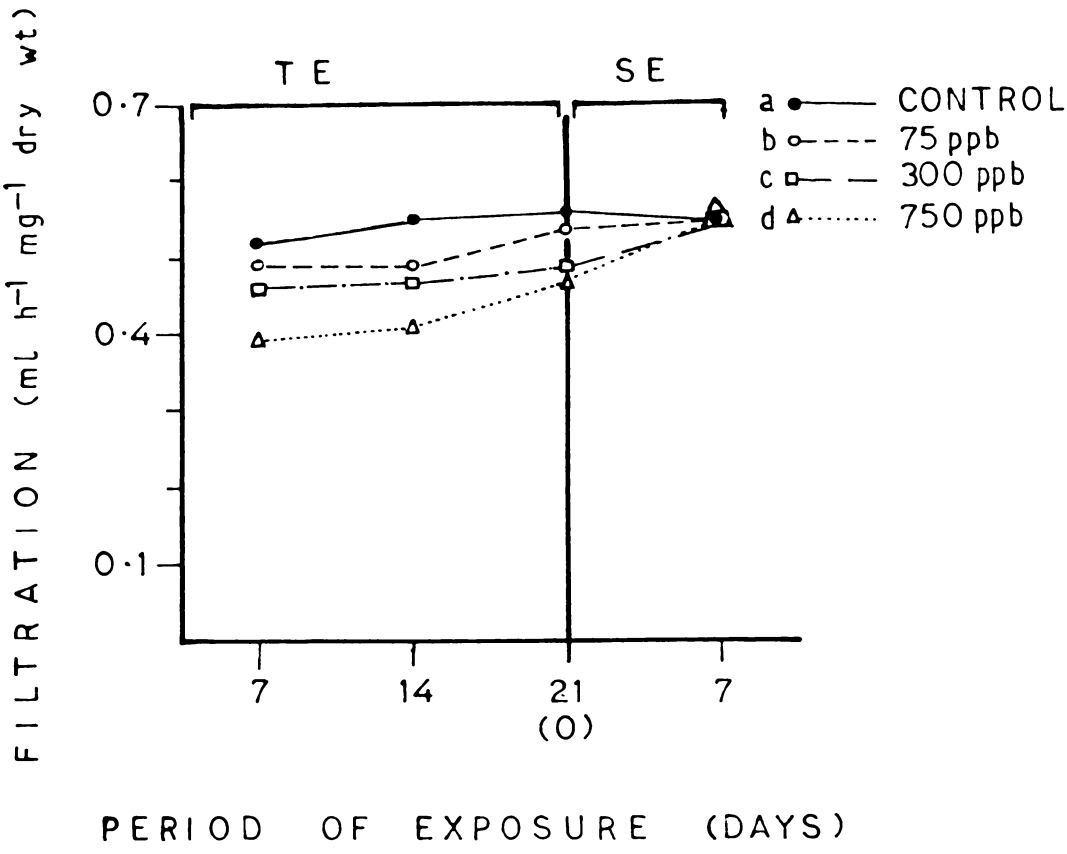

Fig. 84. Perna indica. Average filtration on chronic exposure to different sublethal concentrations of petroleum hydrocarbons in LDO-WAF- for periods upto 21 days (T.E) and subsequently exposed to raw sea water upto 7 days (S.E)- For standard errors see, Table 128 a&b, 129 a&b and 130 a&b- (T.E- Toxicant exposure; S.E- sea water exposure).

Table 131 a&b. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under a sublethal concentration of 750 ppb PHC in P.G. Crude (WAF), for periods upto 21 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 131 a.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	0.507	0.01	97.3
	(0.521)	(0.02)	
14	0.467*	0.02	86.6
	(0.539)	(0.01)	
21	0.482**	0.01	86.3
	(0.558)	(0.01)	

Table 131 b.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	0.586	0.01	102.6
	(0 571)	(0.01)	

* $P < 0.05$ Control values in parenthesis

** $P < 0.01$

Table 132 a&b. Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under a sublethal concentration of 1250 ppb PHC in P.G. Crude (WAF), for periods upto 21 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b) along with respective errors and percentage performance.

Table 132 a.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	0.477	0.02	91.6
	(0.521)	(0.02)	
14	0.463**	0.02	85.9
	(0.539)	(0.01)	
21	0.509**	0.02	91.2
	(0.558)	(0.01)	

Table 132 b.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	0.532	0.02	93.2
	(0.571)	(0.01)	

** $P < 0.01$ Control values in parenthesis

Table 133 a&b Perna indica. Average filtration (ml h^{-1} mg^{-1} dry wt.) under a sublethal concentration of 1750 ppb PHC in P.G. Crude (WAF), for periods upto 21 days (Table a) and subsequently exposed to raw sea water for 7 days (Table b), along with respective standard errors and percentage performance.

Table 133 a.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	0.473	0.03	90.8
	(0.521)	(0.02)	
14	0.423**	0.03	78.5
	(0.539)	(0.01)	
21	0.432**	0.02	77.4
	(0.558)	(0.01)	

Table 133 b.

Duration of Exposure (days)	Filtration (ml h^{-1} mg^{-1} dry wt.)		Performance (% of control)
	Mean	SE	
7	0.588	0.03	102.9
	(0.571)	(0.01)	

** $P < 0.01$ Control values in parenthesis

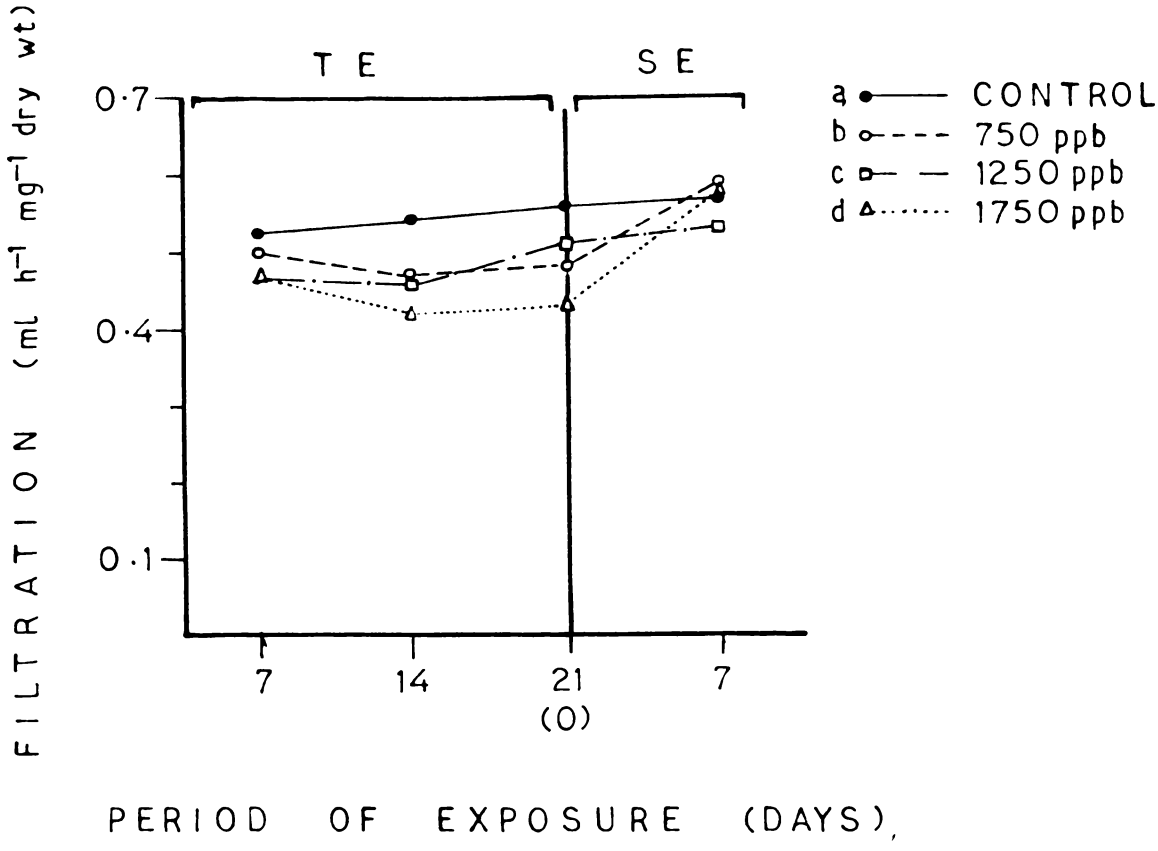

Fig. 85. Perna indica. Average filtration on chronic exposure to different sublethal concentrations of petroleum hydrocarbons in P.G.Crude-WAF- for periods upto 21 days (T.E) and subsequently exposed to raw sea water upto 7 days (S.E) - For standard errors see, Table 131 a&b 132 a&b and 133 a&b - (T.E- Toxicant exposure; S.E- sea water exposure).

the variations being not very conspicuous. However, the variations in filtration were found to be statistically significant. In general the animals' capacity to regain normalcy was found to be considerable when they were kept in raw sea water for seven days.

4.3 PETROLEUM HYDROCARBON LOAD OF WHOLE TISSUE OF *PERNA INDICA*

P. indica was maintained in culture media which contained water accommodated fractions (WAFs) of Light Diesel Oil (LDO) and Persian Gulf Crude (P.G. Crude) for twentyone days. Subsequently a set of selected animals were brought back to raw sea water and maintained for seven days. The whole tissue of these animals were analysed to find out the petroleum hydrocarbon (PHC) load after 21 days exposure to WAF and after 7 days of exposure to raw sea water as the case may be. The results obtained are presented in Table 134 and 135 and Fig. 86 and 87.

4.3.1 Light Diesel Oil (WAF)

The concentrations of PHC in LDO (WAF) used were 75, 300 and 750 ppb and time dependent increase in the body load of petroleum hydrocarbon was noticed. The maxium increase in the load occurred between fourteenth and twentyfirst days of exposure in all the concentrations. The increase in the concentrations can be summarised as follows. Relatively rapid uptake in the first seven days, magnified accumulation during seventh to fourteenth day and slow accumulation from fourteenth to twentyfirst day. This was true of all the concentrations. The rate of depuration was found to be influenced by the internal load. Maximum quantity was depurated from the tissues of those animals which were maintained in 750 ppb for twentyone days. Relati-

Table 134. Perna indica. Petroleum hydrocarbon concentration in the whole animal tissue ($\mu g\ g^{-1}$ dry wt.), when exposed to different sublethal concentrations of LDO (WAF), for periods upto 21 days (accumulation) and subsequently exposed to raw sea water for 7 days (depuration), along with standard deviation.

Duration of Exposure (days)	75 ppb		300 ppb		750 ppb	
	Mean ($\mu g\ g^{-1}$ dry wt.)	SD	Mean ($\mu g\ g^{-1}$ dry wt.)	SD	Mean ($\mu g\ g^{-1}$ dry wt.)	SD
0	ND	-	ND	-	ND	-
7 Acc.	98.04	1.37	174.47	15.91	294.32	7.65
14 Acc.	336.57	13.16	605.06	41.78	777.85	9.69
21 Acc.	403.18	38.64	687.60	23.58	975.64	25.15
7 Dep.	135.71	14.01	195.91	10.34	343.85	13.61

Acc. : Accumulation; Dep. Depuration ND. Not Detectable

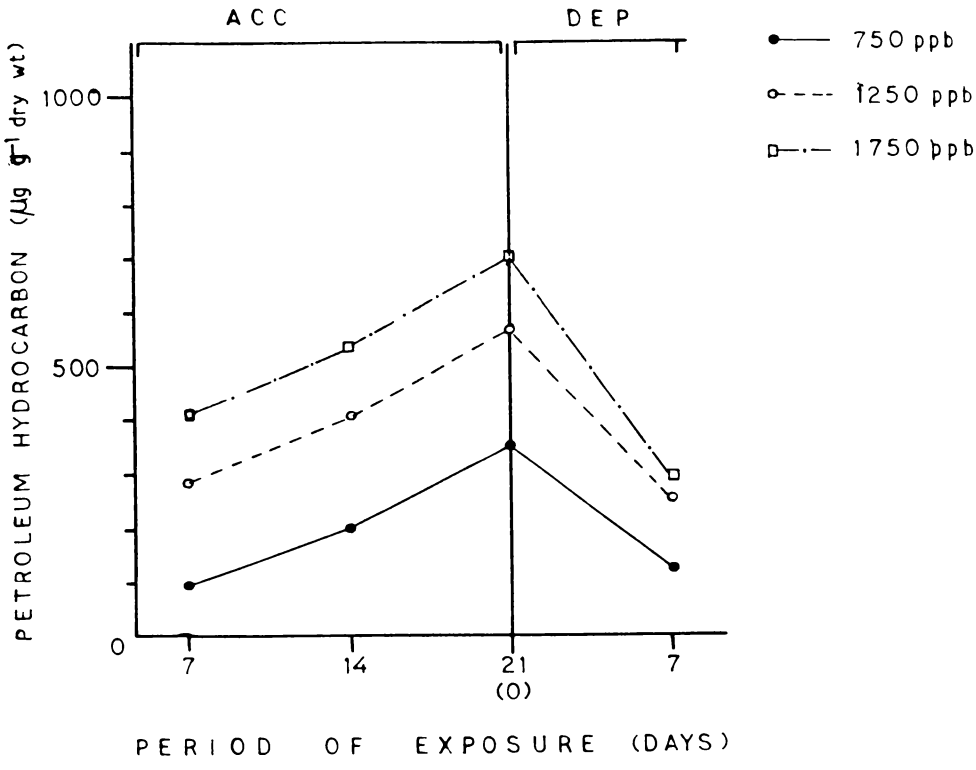

Fig. 86. Perna indica. Petroleum hydrocarbon (PHC) concentration in the whole tissue, on chronic exposure to three different concentrations of PHC in LDO-WAF- for a period upto 21 days (Accumulation-ACC.) and subsequently exposed to raw sea water upto 7 days (Depuration-Dep.).

vely, these animals accumulated the maximum after twenty one days, when compared with the external concentrations. The level of petroleum hydrocarbon reached in the tissues after seven days of depuration was, however, higher than that those recorded after seven days of accumulation (Table 134; Fig. 86).

4.3.2 Persian Gulf Crude (WAF)

P. indica was maintained in culture media which contained three different concentrations of P.G. Crude (WAF) viz. 750, 1250 and 1750 ppb, for twenty one days. The quantity of hydrocarbon got accumulated in the whole tissue of this animal was analysed at seven days' intervals.

After twenty one days a representative sample of these animals were transferred to raw sea water and maintained for seven days. The quantity of petroleum hydrocarbon removed from the tissue was also analysed. The results are presented in Table 135 and Fig. 87. Concentration dependent increase in the uptake, controlled by duration of exposure was the cardinal feature of the results obtained. Unlike in the case of LDO (WAF) the trend in uptake was not uniform by the animals maintained in the three concentrations. Maximum uptake of petroleum hydrocarbons occurred between seven and fourteen days in 750 ppb maintained animals, whereas animals exposed to 1250 and 1750 ppb the period 0-7 days recorded the maximum uptake. Between seventh and fourteenth day the uptake was minimal in both these concentrations. The rate at which the animals depurated petroleum hydrocarbons was more or less uniform, although the quantity thrown out was different. Here also, with reference to quantity, the rate of depuration was controlled by internal concentration.

Table 135. Perna indica. Petroleum hydrocarbon concentration in the whole animal tissue ($\mu g\ g^{-1}$ dry wt.), when exposed to different sublethal concentrations of P.G. Crude (WAF), for periods upto 21 days (accumulation) and subsequently exposed to raw seawater for 7 days (depuration), along with standard deviation.

Duration of Exposure (days)	750 ppb		1250 ppb		1750 ppb	
	Mean ($\mu g\ g^{-1}$ dry wt.)	SD	Mean ($\mu g\ g^{-1}$ dry wt.)	SD	Mean ($\mu g\ g^{-1}$ dry wt.)	SD
0	ND	-	ND	-	ND	-
7 Acc.	99.71	27.22	284.10	52.41	416.51	31.31
14 Acc.	205.70	11.36	409.24	8.85	538.95	11.36
21 Acc.	358.54	18.35	569.57	13.75	704.20	18.19
7 Dep.	127.41	9.86	252.01	12.28	295.34	14.32

Acc. : Accumulation Dep. Depuration ND. : Not Detectable

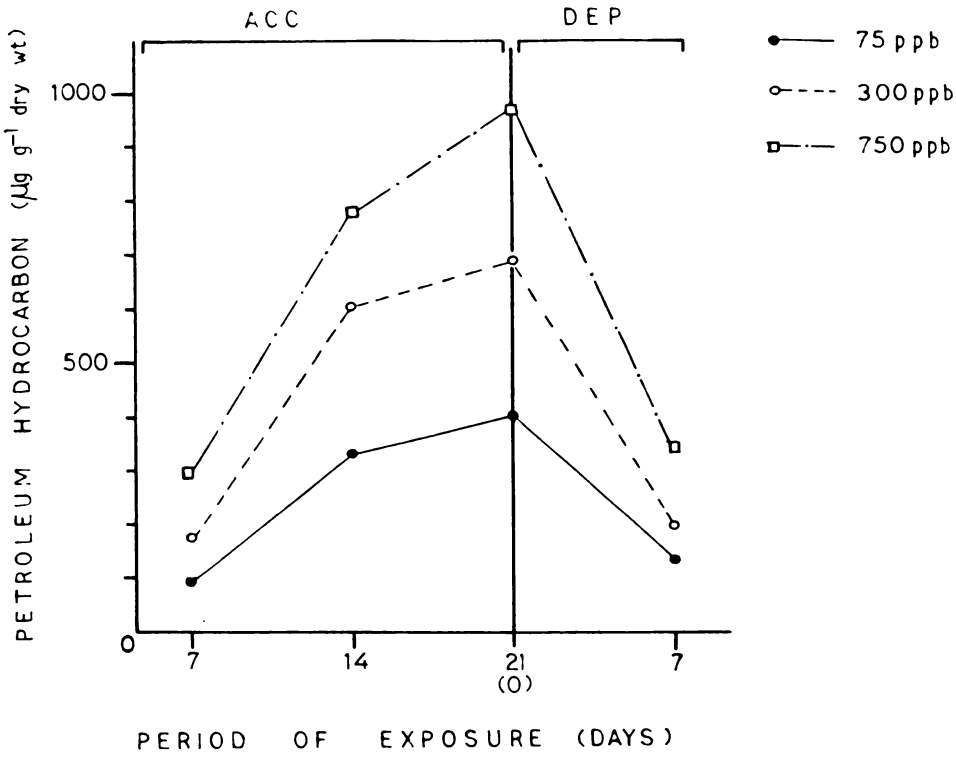

Fig. 87. Perna indica. Petroleum hydrocarbon (PHC) concentration in the whole tissue, on chronic exposure to three different concentrations of PHC in P.G.Crude-WAF-for a period upto 21 days (Accumulation-ACC.) and subsequently exposed to raw sea water upto 7 days (Depuration-Dep.).

DISCUSSION

V. DISCUSSION

The main ecological concern about the damages that could be caused by pesticides, arose in the finding that marine organisms collected far off from the coasts contained traces of DDT. There was an overestimation regarding the capacity of the marine organisms to degrade and detoxify man-made chemicals. 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) suggested that the ecological consequences of these chemicals must be recorded and evaluated in long-term, extensive monitoring programmes. It is estimated that, vinyl chloride, 10.5×10^6 tons; trichloroethylene, 10^6 tons; PCBs, $10^4 - 10^5$ tons; chlorinated phenols, 1.5×10^5 tons; synthetic pesticides, more than 10^5 tons etc. are the annual output of toxic chemicals. A part of these are bound to reach the oceans in due course. Bioconcentration and biomagnification will necessarily increase the load of these pesticides in marine biota and this can affect life and activity of the marine animals. It is known that the capacity to accumulate due to both bioconcentration and biomagnification tends to outweight elimination and the resulting dynamic equilibrium level depends on the properties and ambient concentration of the pesticides concerned, the potential of the animals to counteract 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 quite limited. Further, a number of degradation pathways have intermediate products which have very drastic deleterious effects on marine organisms.

Oil refers to diverse, separable liquids produced by plants and animals. Crude oil or petroleum is a complex mixture of hydrocarbons. It is known that crude oil consists of many individual components. Evidences show that except for areas directly affected by heavy spills or major industrial activities, detrimental effects of oil have remained marginal. In the case of oil pollution assessment, use of indicator organisms have yielded encouraging results. Hydrocarbon determination of Mytilus edulis corresponded broadly the known history of local oil inputs and provided insights into the natural cleansing capacity of biotopes subsequent to an oil spill. Further, the responses of mussels to toxic petroleum hydrocarbons (PHC) opened the door, to sound evaluation of oil product's toxicities.

The combined toxicity of oil and pesticides on marine and estuarine organisms is relatively a virgin field of studies. Investigations on the combined toxicity of these two components have got practical value since, both these components, soon after discharge, remain in the upper water column of estuarine and coastal waters. Further, the lipophilic action of pesticides, enhances the possibility of these chemicals getting concentrated in areas which are chronically polluted by oil. Therefore, the studies on these lines have 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, the time factor is usually compensated by employing unrealistic high concentrations, on the assumption that external concentrations has a direct bearing on the rate of intake and thereby the resultant mode of activity of the animals.

Four pesticides and two oil components have been used to study lethal toxicity on P. indica and V. cyprinoides var. cochinensis. Among the pesticides used, Ekalux an organophosphate was the most toxic, although another organophosphate Dimecron was least toxic for both the animals.

The lethal concentrations of pesticides reduced as a function of time. In the case of the hermit crab, Eisler (1969) found that the LC50 values of Heptachlor, DDT and Malathion decreases as a function of time. Commenting on the sensitivity of organophosphorous insecticides Negherbon (1959) and O'Brien (1966) stated that organophosphorous insecticides inhibit competitively and irreversibly the action of several enzymes, especially choline esterases, which are the chemical mediators of transmission between nerve and effector, resulting in central nervous system stimulation. Organochlorine compounds are distinctly hazardous to the areas which are the sites of entry in the animal body, including ingestion and contact. Any organochlorine insecticide, after entering the body acts as a central nervous system stimulant with the resultant hyper-irritability, convulsions and coma.

Armstrong and Millemann (1974b) found that histopathological evidence of death due to acute toxicity of Sevin in the clam, Macoma nasuta was necrosis of epithelial tissue of the gill mantle, siphon and suprabranchial gland. The severity of damage was found to be directly related to the test concentrations. They found that the gills were the most severely affected organs. It is quiet likely that death was caused by anoxia, brought about by the loss of lateral cilia of the gills or cessation of their movement, stopping circulation of water through the gill filaments and the disjunction of the filament cells disrupts flow of oxygen and the blood.

The change in the lethal concentrations as a function of time indicates that the quantity of the pesticides that enters the body could be dose dependent and that prolonged exposure brings about irreparable damage at high concentrations.

Time dependent mortality has been reported in fish exposed to Dieldrin (Adema and Vink, 1981). Similar time dependent toxic effects have been reported by Laughlin and co-authors (1977). Findings, employing Heptachlor on estuarine organisms by Schimmel et al., (1976a) show that it is possible that significant mortality can occur in pink shrimp population without the compounds being detected in tissues. Variations in acute toxicity levels and toxicity upon chronic exposure have been proved to control the toxicity of Methoxychlor in Cancer magister. The variations are so conspicuous and could be 2600 times more than what is observed under chronic exposure conditions. Further, differences are noticed between developmental stages, juveniles and adult crabs (Armstrong et al., 1976).

The toxicity data presented here in the case of P. indica, give an insight into chemical dependent variation in pesticide toxicity. The maximum deviation in lethal concentration occurred during the period of 48 and 72 h in the case of Dimecron (only 20% died during the first 48 h, even in the highest concentration), DDT and Aldrex whereas the period between 72 and 96 h the deviation was less, around 15% in the case of Dimecron and 30% in the case of DDT and Aldrex. On the contrary in the case of Ekalux, maximum deviation occurred between 72 and 96 h. This is an aspect which has not been highlighted in any paper on acute toxicity of pesticides on marine bivalves.

A more or less similar trend is exemplified in the toxicity doses of the various pesticides in the case of V. cyprinoides var. cochinensis also. Therefore, it may be concluded that during the early phase of exposure to acute doses of pesticides, these two bivalves tend to have the capacity to tolerate higher doses and slight increase in time results in drastic increase in the mortality rate, although the animals were maintained in the same external concentrations throughout the period of exposure. In this connection, it may be noted that during the early phase of exposure to acute levels of pesticides, considerable damage of external tissue occurred in both the bivalves which would likely to affect deleteriously the basic physiological functions of the animals. After such a rate of external damage, probably the animal succumbs to the toxic effects owing to continued exposure in higher concentrations.

In the case of the Water Accommodated Fractions (WAFs) of LDO and P.G. Crude, both the bivalves employed in the studies have shown a

very high capacity of tolerance to petroleum hydrocarbons in the culture medium. However, P. indica was comparatively more sensitive than V.cyprinoides var. cochinensis.

A combination of Ekalux or Dimecron, along with the WAF of LDO did not bring about death in P. indica, within the concentration range employed. The concentrations used had combinations of Ekalux or Dimecron and LDO (WAF) at a level of 1/10 of LC50 values as the highest concentrations. The fact that no death occurred clearly indicate that animals were capable of handling such concentrations effectively so as not to produce any immediate effect to result in death within 96 h. No information is available on the combined effects of organophosphates and petroleum hydrocarbon on any species of Perna or Mytilids for that matter, from a lethal toxicity standpoint. Therefore, comparison with evidences obtained under such situations are not possible.

In the case of DDT, Aldrex and LDO (WAF) combinations, clear cut mortality occurred and the reaction between the combinations and the animals were more than additive. This indicates that, the presence of these components at comparatively lower concentrations can be hazardous to P. indica. It may be noted here that, simple additivity at some instances indicates, the components involved affecting the animal independently. The reasons are unknown. It is not clear whether the animal has the capacity in warding off of one of the components in the combinations. However, the information available on combined toxicity of other group of toxicants does not support this hypothesis. A major drawback in interpretting con-

trasting results is owing to the lack of information on the physiological stage of animals, although the animal used in this experiments came from the same lot.

Sprague (1970) remarked "probably the most exciting and potentially useful recent development in pollution biology has been a method of predicting toxicity of mixtures of toxicants". Methodology developed aids in measuring the simultaneous effects of several pollutants which can be expressed in numbers. Combined toxicity could be used at any level, lethal or sublethal, at organismic (in the case of acute toxicity) or organic level (in the case of sublethal toxicity). Sprague and Ramsey (1965) used the toxic unit method to predict the toxicity of copper and silver to the Atlantic salmon (Salmo salar). Other techniques for evaluating the toxicity of mixtures of chemicals have also been advanced. Most of these follow mathematical models for additive joint toxicity that yields their harmonic mean of the LC50s of the components (Finney, 1971). This model tests the hypothesis that the toxicity of chemical mixtures is simply additive. Smyth et al., (1969) normalised the values obtained from Finney's equation with a frequency distribution curve and adjusted the values to indicate additive toxicity with zero. The study of mixtures of toxic chemicals in sea water and the resultant benefits or hazard is fairly new and only a few methods have been investigated.

An analysis of slope functions on the combined toxicity of DDT with LDO (WAF) and Aldrex with LDO (WAF), on P. indica indicates that the trend in mortality between concentration combinations could decidedly vary.

This probably shows that the death of P. indica was caused not only by increase or decrease in the concentrations employed in a combination but also in a particular series of experiment, the death could have occurred either at concentrations with less variability or more variability. The reason for this variation within a combination is unknown. However, it could be assumed that the rate of uptake coupled with damage of external tissues may also play a cardinal role in causing mortality. Another interesting feature of the result obtained was that, Ekalux was the most toxic pesticide for P. indica and Dimecron, the least toxic. But when supplied in combination with oil fraction, both these pesticides became relatively less toxic even when the highest concentration combination contained nearly 50% of the lethal dose. If it is to be assumed that presence of oil component in the medium makes the pesticides more available for voluntary uptake, the animals probably avoided uptake. Further, the lipophilic nature of the pesticides would have resulted in the production of more complex mixtures of pesticides and oil in the medium, resulting in reduced uptake. The reduction in toxicity of organophosphates in the presence of petroleum hydrocarbon is a very important finding in the present investigation on the combined toxicity on P. indica.

The results obtained on the combined toxicity of V. cyprinoides var. cochinensis, however give a different picture. Ekalux, was found to become highly toxic in the presence of higher concentrations of LDO (WAF). The result obtained on individual toxicity shows that, both Ekalux and LDO (WAF) became toxic at 96 h level only at concentrations much higher than

those obtained under combination conditions. This indicates that the toxicity of these two toxicants increased, when they are present in combination. Similar results were obtained also in the case of the organochlorine pesticide Aldrex. In this connection it may be pointed out that although, V. cyprinoides var. cochinensis is relatively a hardy species when compared to P. indica, it need not necessarily mean that this species can tolerate higher concentrations of certain pesticides, when present in combination with LDO (WAF), as evidenced by the present result.

Information on combined acute toxicity of pesticides and petroleum hydrocarbon is uniformly lacking. More information is available on the combined toxicity of metals and metal oil mixtures. Commenting on the increased toxicity of metal combinations, Moulder (1980) remarked that the reduction in the rate of excretion, alterations in distribution within the tissue and inhibition of detoxification may result in increased toxicity. Mohan et al., (1986a) commenting on the accentuation of toxic effects at comparatively low concentrations by two metal ions in the test medium, noted that the depurative processes of mussels were deleteriously affected when they were forced to ward off two metals. Considerable decrease in the LC50 values in majority of cases when the components were supplied in concert indicate that at low concentrations these components exerted higher toxic action on the bivalves. A variation from this will be found only when the combined action is simple additivity. Interspecies variations in combined toxicity of Mytilids have been demonstrated by Menon et al., (1987). They reported that, to combinations of silver and copper and copper and silver, P. viridis responded in a less than additive manner, whereas for

P. indica, it was more than additive. These authors attributed this to the capacity of P. indica and P. viridis to selectively block the binding sites of such metals to which they have high resistance. Baby (1987) found that the toxicity of individual metals enhances in the presence of other. Inter-metal variation in toxicity could occur as evidenced by the results that zinc shows the maximum variability in toxicity in the presence of other metals or petroleum hydrocarbons. Mercury proved to be highly toxic in presence of other metals. Phillips (1976) and Bryan (1984) suggested that it is necessary to delineate the reasons for variation in uptake and thereby manifestation of toxicity by marine mussels exposed to toxicant combinations. It is evident that homeostasis and the comparative capabilities of bivalves get narrowed down when subjected to exposure to a combination of pollutants.

5.2 SUBLETHAL TOXICITY: SHORT-TERM EXPOSURE

Sublethal effects of pollutants are carried out essentially to delineate responses of an organism that would be exemplified by alterations in the physiology, biochemistry and cell structure, behaviour and neurophysiology and reproduction. Waldichuk (1979) aptly remarked that "a response is not linear with pollutant concentration".

Sublethal responses can usually delineate linear and non-linear reactions. However, under laboratory conditions this will be decidedly 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 insipient lethal threshold. During the present study the concentrations used were 1/10 of the LC50 recorded and those below this levels. Rates of oxygen consumption and filtration were the parameters tested for both the bivalves and byssogenesis was an additional parameter for P. indica.

5.2.1 OXYGEN CONSUMPTION

It has become apparent from the results obtained that the trend in oxygen consumption can vary from linear to non-linear pattern. This was especially so, when the toxicants were used in combinations. Ekalux, even at the highest concentration used, did not bring about any drastic change in oxygen consumption. Rate of oxygen uptake is a useful tool to assess stress as it indicates the energy expenditure required to meet the demands of an environmental alteration (Thurberg et al., 1975). In the case of heavy metals it is proved that their presence can either elevate or depress the rate of oxygen uptake in marine bivalves. Normally, heavy metals are respiratory depressants (Mathew and Menon, 1983; Baby and Menon, 1986). Though the pattern of oxygen consumption by P. indica and V. cyprinoides var. cochinensis did not indicate a clear cut changes, depending on the nature of the pesticides distinct variations were observed. Elevation in oxygen consumption occurred when P. indica was exposed to Aldrex, whereas, in the case of V. cyprinoides var. cochinensis there was marginal depression in oxygen consumption. In the case of P. indica a reduction in oxygen consumption, although occurred in the case of other pesticides, the variations were marginal. Dimecron, while functioning as a respiratory depressant for P. indica, was a stimulant in the case of V. cyprinoides var. cochinensis.

Same was the trend recorded in the case of Ekalux. In the case of V. cyprinoides var. cochinensis, it was found that increase in the concentration of all the four pesticides, irrespective of their variations in composition, in general, the rate of oxygen consumption increased at higher concentrations. The presence of petroleum hydrocarbons reduced the rate of oxygen consumption in the case of P. indica. On the contrary LDO (WAF) was a respiratory stimulant in the case of V. cyprinoides var. cochinensis.

The presence of LDO (WAF) along with Ekalux, Aldrex and DDT resulted in conspicuous reduction in oxygen consumption of P. indica, although Dimecron in combination with petroleum hydrocarbon registered an elevation in the trend while Dimecron alone induced reduction in oxygen consumption. This shows that the presence of pesticides along with the water accommodated fractions induced a stress. The presence of Aldrex alone, conspicuously increased oxygen consumption. However, the fact that the reduction in oxygen consumption occurred when the test medium contained very low concentrations of Aldrex and petroleum hydrocarbon clearly show that a more than additive response occurred. It is possible that the lipophilic nature of Aldrex, probably increased the rate of entry of this organochlorine pesticide. Lipophilic nature of the pesticide clearly result in increased uptake, evidenced by oxygen consumption. This is proved in the case of DDT also. The finding that, in the presence of water accommodated fraction of oil, all the pesticides used, except Dimecron, became respiratory depressants is a very significant result. In the case of V. cyprinoides var. cochinensis all the four pesticides used, stimulated respiration when they were present alone. On the other hand when these pesticides were supplied along with the WAF of LDO, there was drastic reduction

in respiratory rate. It has to be assumed that in the presence of petroleum hydrocarbon, toxicity of the organophosphate and organochlorine, increased. Or it is possible that, the animals avoided oil pesticide contact by reducing filtration. It has to be assumed that increase or decrease in oxygen consumption is brought about by stress, but there is a reversal in the reaction of the animal to overcome the stress effect by either increased rate of oxygen consumption as recorded in the experiments conducted with pesticides or oil fractions alone, or decrease in oxygen consumption when the toxicants were employed in combinations. This is uniformly applicable in the case of V.cyprinoides var. cochinensis.

Enhanced rate of oxygen consumption or energy expenditure appears to be the common response of molluscs to low or moderate concentrations of petroleum hydrocarbons - 30 to 600 ppb - (Gilfillan, 1975; Widdows et al., 1982). High petroleum hydrocarbon concentration may result in depression in oxygen uptake mainly as a result of partial valve closure or by the narcotizing effect on ciliary activity. Both of these will reduce ventilation rate and thus oxygen availability. However, fundamental cause for an enhanced rate of oxygen consumption as a response to low hydrocarbon concentration remains unknown. It is quite likely that it is a direct effect and not mediated through behavioural changes.

In the present context, the presence of pesticides along with petroleum hydrocarbon resulted in a decrease in oxygen consumption. Various explanations have been put forward by different authors to explain the reasons for decrease in oxygen consumption by bivalves, especially in the presence of heavy metals. The reduction in oxygen consumption obtained from whole body respiratory rate indicate the overall performance of the animal, qualified by

behavioural responses like, shell valve closure, siphonal activity and the rate of gill irrigation. Therefore, it has to be assumed that the recorded reduction in oxygen consumption is a compensation for modified or altered behavioural response. Supression in ciliary activity rather than by the direct inhibition of the respiratory rate may result in the reduction of oxygen consumption (Brown and Newell, 1972). Decrease in the oxygen tension of the mantle fluid has been quoted as a reason for reduction in oxygen consumption in the case of Mytilus edulis exposed to copper contaminated water (Manley, 1983). It has been proved that behavioural aspects like shell-closure frequency and rate of ciliary beat can affect the oxygen tension of the mantle fluid which will directly influence the rate of oxygen uptake since it bathes the tissues, mainly the ctenedia. Therefore, the finding that the presence of organophosphates, organochlorines and petroleum hydrocarbons, individually increases oxygen consumption and reduces when in combination, opens up an important aspect of oxygen consumption mechanisms in bivalves. The oxygen budget of the mantle fluid is controlled by the oxygen that diffuse into the fluid from the inhaled water and the quantity removed by respiration. Increase in the rate of respiration indicates enhanced ventilation and therefore supractivity on the part of gill, the adductor muscles and the muscles that control the inhalent and exhalent apertures. Reduced respiratory rate shows partial suppression in the above type of activity on the part of the bivalve.

Studies have shown that at relatively low concentrations pesticides can kill or immobilize fishes, crustaceans or molluscs (Eisler, 1969, 1970 a&b); kill eggs and larvae of bivalve molluscs (Davis, 1961); induce deleterious changes in tissue composition of molluscs (Eisler and Weinstein, 1967). Further,

patterns induced by Methoxychlor or Malathion could be similar in the case of Mercenaria mercenaria. Disproportionate changes in calcium and zinc levels in the mantle of quahaug clams was an important change occurring when these clams were exposed to Methoxychlor and Malathion. Very low concentrations of these two pesticides in the external medium may not induce changes in the pumping rate of the quahaug clam, M. mercenaria (Eisler, 1972). Considerable increase in oxygen consumption and hypersensitivity to stimuli in bluntnose minnows in sublethal concentrations of Endrin was reported by Mount (1962) and Cairns and Scheier (1964) found increased oxygen consumption in Lepomis gibbosus exposed for 12 weeks to very low concentration of Dieldrin. However, information on the effects of pesticides in combination with water accommodated fractions of oil on the respiratory behaviour of molluscs is generally lacking. It is felt that more information should be made available which will help in establishing ranges of pesticide concentrations within which obvious physiological, morphological or behavioural changes can take place. Only this will help in constructing profiles for one or several pollutants singly or in combination.

Effects of pesticides on the embryonic development, survival and growth of larvae is available in the literature (Davis and Hidu, 1969; Buchanan et al., 1970; Epifanio, 1971; Calabrese, 1972; Bookhout and costlow, 1975). Upward shift in the metabolic rate evidenced by oxygen consumption of fishes exposed to low concentrations of DDT, Ethylparathion and Pentachlorophenol have been demonstrated by Peer Mohamed and Gupta (1984). Moore (1985) discussing on the cellular responses of xenobiotics, suggested that many toxic substances or their metabolites result in cell injury by reacting primarily with

biological membranes. Membrane damage include changes in cellular compartmentalization such as injury to lysosomes or mitochondria, changes in the content or activity of enzymes or other membrane components. Increase in activity to meet a particular environmental challenge, according to Moore (loc cit.) is an index of detoxification. The major difficulties in interpreting the data on the combined toxicity of pesticides and oil is that remarkably little is known of the mechanism of toxicity of even main classes of chemical contaminants (Malins and Collier, 1981; Moore et al., 1984). Concrete evidence of contaminant related pathological changes in cell structure has been obtained from investigations of the hepatopancreas or digestive gland of marine molluscs (Lowe et al., 1981; Tripp et al., 1984).

Enhanced rate of oxygen consumption or energy expenditure of molluscs to low and moderate concentrations of petroleum hydrocarbons is available (Gilfillan, 1975; Fong, 1976; Gilfillan et al., 1976; Gilfillan et al., 1977; Widdows et al., 1982). However, at higher concentrations respiration may be depressed, mainly as a result of partial valve closure or narcotizing effect on ciliary activity, both of which will reduce ventilation rate and thus oxygen availability (Gilfillan, 1975; Johnson, 1977; Stainken, 1978; Sabourin and Tullis, 1981). The reasons for enhanced rate of oxygen consumption in response to lower hydrocarbon concentrations remain unknown. But it appears to be a direct effect of oil on metabolism and not mediated through behavioural changes. Hydrocarbons may increase respiration rate as a result of the uncoupling of oxidative phosphorylation or an increased flux through the glycolytic pathway. Anderson (1977) concluded that there was little agreement between sublethal responses of marine organisms and the level of hydrocarbon contamination in the tissue. This was further confirmed by Widdows et al., (1982).

5.2.2 RATE OF FILTRATION

Assessment of the rate of filtration is a very useful index to analyse toxicant stress in bivalves. In nature, the rate of filtration is an index of the energy that will be available for growth. Therefore, experiments designed to delineate filtration rate of bivalves, when exposed to sublethal concentrations of pollutants have got direct applicability. Abel (1976) advocated the use of filtration rate measurements to determine the effect of pollutants. This method has advantages in that, that it is non-distructive, can be carried out with the minimum of equipments and provides more accurate results on sublethal toxicity. The mechanism involved in the variation of filtration rate by marine mussels, when exposed to pollutant is not properly understood. It is known that the rate of filtration can vary depending on factors other than the presence of pollutants. Widdows et al., (1982) working on the responses of Mytilus edulis to the water accommodated fractions of North Sea Oil demonstrated that the presence or absence of algal food in the polluted medium can result in variations in the filtration rate irrespective of the pollutant concentrations. Invariably, the animals exposed to media which contained algal food, filtered more water. Many endogenous and exogenous factors may be expected to influence the rate of filtration in the case of bivalves.

The results obtained during the present investigations give the following evidences. In the presence of pesticides or water accommodated fraction of oil, P. indica reduced the rate of filtration, although the reduction was not concomitant with external concentrations of the pollutants. Dimecron or LDO (WAF), when present in combination the pesticide oil fraction

resulted in drastic reduction in the rate of filtration than when these components were present individually. However, in those experiments performed with DDT and LDO (WAF) or LDO (WAF) and DDT, the reduction in the rate of filtration was minimal or marginal when compared with the rate of filtration of animals exposed to DDT concentrations alone. The results obtained from combination studies show that the trend in filtration was more comparable to that obtained in the presence of LDO (WAF) alone.

Mohan et al., (1986b), opined that unlike many parameters of potential value as an indication of sublethal stress, filtration is an important one since this gives a direct idea on the quantity of water propelled through the gills. A few papers which have dealt with filtration under stress are of Cole and Hepper (1954), Eknath and Menon (1979), Palmer (1980), Reddy and Menon (1980), Widdows et al., (1982), Mathew and Menon (1983), Stickle et al., (1984), Prabhudeva and Menon (1985), Jacob and Menon (1987a).

To understand the various factors that affect the rate of filtration, it is necessary to delineate the processes involved in filtration. The water enters the pallial cavity of a bivalve through the inhalent siphon before passing through the gill ostia or into the supra-branchial chamber. Such circulated water is expelled through the exhalent siphon, which is narrower than the inhalent siphon, lying at the posterior end of the mantle. Both the siphons posses velum, which can regulate the current flow. Usually the bivalves reduce the effective area of lamellar contact with the water by means of mucus. Mytilus edulis does not filter in very dilute suspensions. Filtration is initiated at a critical particle concentration. Davids (1964) found that the pumping rate of M. edulis was reduced when the cell concentration increased within a particular level. It has been proved that the

scope for activity for mussel varies with environmental parameters. Bayne et al., (1973) found a close agreement between scope for activity and filtration rate. Reduction in feeding rate or energy acquisition by molluscs exposed to petroleum hydrocarbons has been reviewed by Moore et al., (1987). The inhibition of feeding rate in the presence of petroleum hydrocarbon according to various authors is due to the narcotic effect of hydrocarbon (Johnson, 1977; Widdows et al., 1982; Stickle et al., 1984).

The present finding on filtration in the case of P. indica, when compared with that of oxygen consumption gives some interesting information. In general, in the presence of pesticides alone, the rate of oxygen consumption did not show any drastic difference from the control, while the rate of filtration reduced, except in the case of Dimecron, which is comparatively less toxic. On the other hand, these pesticides when employed in combination with LDO (WAF) the rate of filtration and oxygen consumption drastically deviated from that of control animals. However, in the case of the estuarine bivalve V. cyprinoides var. cochinensis though the rate of oxygen consumption increased marginally, the rate of filtration drastically reduced from that of control animals, when exposed to pesticides. The decrease in the quantity of water filtered and the increase in oxygen consumption show that the animals probably retain the water that enter the pallial cavity for a longer duration, before being expelled leading to repeated circulation of water within the pallial cavity. This might have resulted in the reduction of the dye concentration, which get entangled in the gills which have considerable coating of mucus. Stepping down of particle number below a threshold level would have essentially resulted in the cessation of filtration and the animals would have used only the oxygen dissolved in the water trapped in the pallial cavity.

Among the two species of bivalves studied here, V. cyprinoides var. cochinensis, seems to be in a position to virtually suspend filtration at least at some instances. Another notable feature of the filtration performance of this animal was that interconcentration variation in filtration rate could be clearly insignificant, indicating that the animal is capable of performing at reduced pace, irrespective of increase in the concentration of the toxicants. Further, considerable interconcentration variabilities in filtration occurred, indicating erratic behaviour of the bivalve, when subjected to stress. It has not been conclusively proved that, the ciliary beat is neurocontrolled. However, other organ systems which are involved in filtration such as the mantle, the siphon and the adductor muscles could be influenced by disruption in nervous control. The pesticides employed in the present study were all neurotoxins and would have resulted inhibitions of Acetylcholine esterase (AChE) activity. At sublethal levels, depending on the period of exposure AChE inhibition has been proved to occur in the case of fishes (Coppage, 1972; Coppage et al., 1975). Therefore, slow inhibition of AChE in sublethal exposed animals could have eventually resulted in changes in the rate of filtration. These aspects have to be looked into greater detail to arrive at a clear cut conclusion.

5.2.3 BYSSOGENESIS

The byssal apparatus of a bivalve consists of various organs used for attachment and locomotion in post-larval and adult life (Brown, 1952; Roberts 1976b). The byssus threads anchor the bivalve to the substratum while the foot ensures locomotion in post-larval and adult life.

The byssus consists of a cylindrical stem, made up of many tightly-packed laminae; many fine threads, attached by means of short rings. The thread is known to have four distinct portions; the ring, by which each thread is attached to the stem; a short proximal flat portion with a corrugated surface; a hard distal portion and an adhesive disc (Brown, 1952). The detailed structure of the byssus thread of Mytillus galloprovincialis, using electron microscope has been worked out by Bairati and Vitellaro-Zuccarello (1974). Their findings substantiated that a byssus contains six different structures, i. the laminae of the root and the stem core, ii. the laminae of the outer portion, iii. the surface, iv. the central portion of the threads, v. the distal portion of the threads and vi. the adhesive plate of the disc. Regarding the thread generating process, several factors are known to contribute the formation. The most important of them are the production and secretion from the foot glands (Brown, 1952; Pujol, 1967) a rapid moulding in the longitudinal groove, their subsequent tanning and the effect of the foot's muscular contractions. The mechanical action of the foot is the most important factor, involved in the attachment process of byssus threads. It is known that the foot allow the adhesive disc to attach itself to the substratum and would quickly and completely retract squeezing the accumulated secretion from the terminal gland into the groove and at the level of the terminal disc it would be moulded into a cylindrical filament which will be connected to the adhesive disc. The distal portion of the thread thus would be spun out, by the way silk is produced (Bairati and Vittellaro-Zuccarello, 1974). It is known that the saline environment would probably contribute the hardening and tanning of the secreted material (Field, 1922). Hawkins and Bayne (1985) clearly indicated the physiological significance of byssus production.

These authors estimated that in the case of M. edulis, during the production of byssus, 44% of carbon and 21% of nitrogen have been utilized. This is one of the reasons why production of the byssus is used as an important parameter to assess stress in Mytilidae. Investigations on this line are available in the literature (Van Winkle, 1970; Roberts, 1975; Reddy and Menon, 1979, 1980; Mathew and Menon, 1983 a; Mohan et al., 1986a). In all these investigations production of byssus, nature of the secretion and the structure of threads have been assessed as an important and useful criteria to assess the activity of Mytilidae. Stress induced reduction in byssogenesis have been demonstrated by the above authors.

A general pattern on byssus production by P. indica, exposed to Ekalux, Dimecron, Aldrex, DDT and to the WAFs of LDO and P.G. Crude was declension in the production with increase in concentration. In test media which contained a combination of these toxicants, the drop in byssogenesis was drastic and was concentration dependent. Among the four pesticides, in comparison to their lethal toxic doses, Aldrex was proved to be the most toxic with reference to byssogenesis. A combination of oil fraction alongwith Ekalux and Aldrex did not produce an increase in the toxicity of pesticides. However, presence of these pesticides resulted in a reduced effective concentration value of LDO (WAF).

Reports available on byssogenesis show that low levels of pesticides can deleteriously affect byssus production. Robert (1975) found that 50% reduction in byssal attachment occurs in the presence of 0.45 mg/l of Endosulfan. According to him the reduction in byssal attachment is owing to

impairment of pedal activity. Menon et al., (1983) found that 50% reduction of byssus attachment occurred when Perna viridis was exposed to 0.1 µl/l of Heptachlor. Reddy and Menon (1980) found that P. viridis reduced byssus production by around 50% when the medium contained 2.5% of water soluble fraction of LDO. There is a general dearth of literature which has gone to the detail of byssus secretion by bivalves under pesticide or oil stress. A notable feature of the present study was that even at toxicant levels, where the animals survived normally, the capacity to produce byssus thread was impaired with. It may be assumed that the neurotoxic capacity of the pesticide can clearly affect the functioning of the muscles of the foot, which is an important organ involved in the secretion of byssus in the Mytilidae. Further, the narcotic behaviour of the WAF of oil has already been proved (Widdows, et al., 1982; Moore, et al., 1987). It is not clear, to what an extent, low concentrations of pesticides and oil will directly interfere with synthesis or impair with the other processes involved in byssus production.

5.3 SUBLETHAL TOXICITY : LONG-TERM EXPOSURE

Long-term sublethal toxicity tests are most commonly designed to provide information on the effects of various concentrations of toxicants on survival, growth and reproductive success of an organism. The period involved with long-term toxicity studies will have relevance depending on the longivity of the animal concerned. Periods 96 - 240 h will be of high significance when bacteria are used as test organism. On the other hand, higher invertebrates which live for a few months to 3 to 4 years, the periods used will correspondingly be longer. Longer duration of experiments, employing aquatic animals would involve feeding also. Therefore, the tissue con-

centration of the toxicant will be the combined effects of both bioconcentration and bioaccumulation. In the present experiment the period for which the animals were kept for observation varied from 14 to 21 days, 14 in the case of pesticides and 21 in the case of WAFs of oil. Only Perna indica was used for the studies because this species has been proved to be a very sensitive marine bivalve to toxicant stress.

Parrish (1974) kept Crassostrea virginica exposed to Aroclor 1254, DDT, DDT and Dieldrin for 56 weeks to study the effects on accumulation and loss. He found that prolonged exposure reduces the residue within the tissue. Further, the rate of growth of exposed oysters was not different from that of controlled ones. This happens when the exposure concentration was very low. Lowe (1964) found that the gill lamellae of fish thickened when exposed to sublethal concentrations of Toxaphene. Schultz (1970) reported necrotic changes in epithelial cells and increase of mucus cells in the gills of carp exposed to different concentrations of Natrium trichlor acetate (Na Ta). Development of gill lesions have been noticed in Lepomis microlophus, when this was exposed to 0.03 – 0.3 mg^{-1} of Hydrothol 191 (Eller, 1969). However, he found that the lesions in gills began to disappear after 14 days. The pathological changes induced by pesticides were reduction of haemoglobin without a change in the erythrocyte count after a prolonged exposure to DDT (Rudd and Genelly, 1956). During the present investigation the physiological processes like oxygen consumption and filtration rate of Perna indica, exposed to very low concentrations of pesticides for 14 days and WAFs of LDO and P.G. Crude for 21 days were assessed. Prolonged exposure resulted in clear cut changes in activity only in the case of those animals

exposed to Ekalux and Dimecron. On transfer to raw sea water the revival was very quick. On the other hand, though the animals showed only slight variation in reaction on exposure to Aldrex and DDT, their revival was very slow. This shows that the damage of tissues and processes involved in respiration in P. indica exposed to Ekalux and Dimecron was long standing. In the case of those animals exposed to WAFs of LDO and P.G. Crude also, the revival was rather quick. It becomes clear that the animals are capable of regaining normalcy and it is possible that repairs of affected organ can occur enabling the animals to perform normally, when the external concen trations are considerably very low.

The rate of filtration of P. indica, subjected to continuous exposure to very low concentrations of Ekalux and Dimecron for 14 days, resulted in an increase. However, in the case of Dimecron exposed animals the interconcentration variations in the rate of filtration were negligible. The probably indicates that the effect or damage on the concerned organ system is comparable. It may be noticed that Dimecron was less toxic to P. indica. In the case of Aldrex and DDT exposed animals the rate of filtration decreased after prolonged exposure. Here is an instance where distinct differences in behaviour was noticed by the same animal exposed to organophosphates and organochlorines, the former resulting in increased activity while the latter proving to depress the activity. Similar observations on marine bivalve is not available in the literature.

Edwards (1978) while studying the effects of water soluble oil fractions (WSF) on the shrimp, Crangon crangon proved that increased heart rate, coincides with an increase in respiration in this animal which was maintained

in very low concentration of water soluble fraction (WSF) for longer duration upto 20 days. He suggested that there is a possibility, that an increase in respiration and heart beat rate were associated with increased metabolic rate, necessary for the excretion of aromatic hydrocarbons from the body. This finding when compared with the present results does not support the above assumption. Here, the rate of oxygen consumption of P. indica was found to decrease appreciably as a result of prolonged exposure to low levels of petroleum hydrocarbons. Similarly, the rate of filtration also was found to decrease on exposure to WAFs of both LDO and P. G. Crude although, the declension was not as conspicuous with the declension in oxygen consumption Another aspect of the finding is that these bivalves have the capacity to regain normalcy when transferred to raw sea water and the duration for this ranged between 0–7 days. Although mussels are known to accumulate petroleum hydrocarbons, when exposed to sublethal concentrations, they are known to have remarkable capacity to depurate 80-90% of the accumulated hydrocarbon on transfer to unpolluted water (Lee et al., 1972a). Discussing the physiological responses of a marine snail during long-term exposure, Stickle et al., (1984) found that the caloric consumption rate of Thias lima declined in an inverse manner with increasing aromatic hydrocarbon concentrations. Bivalves exposed to WAF of crude oil have established reduced feeding rate (Gilfillan, 1975). Widdows et al., (1982); Stickle et al., (loc. cit) found that the variation in total energy expenditure of snails was controlled by variation in oxygen consumption rate as a result of exposure to aromatic hydrocarbon concentrations and duration of exposure. However, conflicting results have been reported for the effects of WAF of crude oil or its compounds on oxygen consumption rate of marine inverte-

brates, subjected to chronic exposure. It is understood that lethal concentration of WAF of crude oil depress oxygen consumption rate in bivalves (Dunning and Major, 1974; Widdows et al., 1982). It seems necessary to understand the relationship between the petroleum hydrocarbon concentration to which a molluscan species is exposed and its long-term LC50 in order to understand the change of oxygen consumption or filtration during exposure, into a predictable response pattern. The petroleum hydrocarbon concentration of test media employed during the present investigation was decidedly below the long-term LC50 levels evidenced by normal activity by the animals when they were shifted to raw sea water. It may be pointed out in this context that chronic exposure of higher invertebrates, to very low levels of toxicants, especially petroleum hydrocarbons and pesticides may result in damage and repair of tissues which directly come into contact with the contaminated water as reported by Eller (1969).

5.4 PETROLEUM HYDROCARBON LOAD OF WHOLE TISSUE OF *PERNA INDICA*

Mixed function oxidases (MFO) are important in the metabolism of foreign organic compounds by marine organisms and our information on the uptake of oil is mainly confined to the reaction of MFO system. If it can be established that hydrocarbons are the dominant cause of MFO induction for sites remote from land influence, the quantity of MFO present in target organism might be useful as a measure of prolonged exposure to increased background concentrations of oil (Johnston, 1984). The tissue load, consequent to exposure to low levels of petroleum hydrocarbon derived from LDO (WAF) and P.G. Crude (WAF) by P. indica, on chronic exposure clearly showed that

there is a time dependent increase in the load. However, maximum uptake was found to occur during the first seven days of accumulation, especially in the case of animals exposed to higher ranges of PHC derived from P.G Crude. On the other hand in the case of PHC derived from LDO (WAF) this happened during the period of 7–14 days.

Anderson et al., (1974) have shown that although alkanes and aromatic hydrocarbons were both accumulated, the aromatic hydrocarbons were accumulated more rapidly to high concentrations and were retained in the tissue for longer periods of time than were the alkanes. Regarding release, they found that naphthalene was usually released from the tissues more rapidly than were alkylnaphthalenes. Therefore, they opined that the PHC composition in the tissues of oil exposed marine animals would be substantially different from that of the oil to which they were exposed. Among the animals they found that, crustaceans absorbed PHCs more quickly than other group of invertebrates. Neff and Anderson (1981) opined that in the case of molluscs, the rate of uptake is slow and complete depurative process will take around two months. In the present study, even after seven days of depuration, Perna indica did show considerable load of PHC in the tissue. Trend in depuration was however, a sudden release of major quantity in seven days time. Blumer et al., (1970) reported that when oysters were exposed to oil water mixture, they non-selectively accumulated in their tissues, a wide variety of PHCs in direct proportion with the concentration in the exposure water and they suggested that it is possible that the aromatic hydrocarbons are retained indefinitely. Lee et al., (1972a) found that 90% of alkanes and aromatic hydrocarbons were released by Mytilus edulis within

two weeks of return to isotope free sea water. Similar results were presented by Stegeman and Teal (1973). The remaining hydrocarbons were released much more slowly.

The physiological responses of marine organisms to the stress induced by exposure to sublethal concentrations of pollutants are only poorly understood. It is likely that the nature and magnitude of sublethal responses could be species dependent. The information on oxygen consumption, filtration rate and tissue load indicates that in the case of PHC derived from both LDO (WAF) and P.G. Crude (WAF) the decrease in oxygen consumption was drastic between the 14th and 21st day. When this is compared with the tissue load, it becomes clear that this has nothing to do with the increase in tissue concentration, that occurred in 14th and 21st days. The maximum increase in the tissue load concentration occurred between 7th and 14th day in the case of LDO (WAF) exposed animals and between 0-7 days in P.G. Crude (WAF) exposed animals. Anderson et al., (1974) have aptly remarked that respiratory responses of marine bivalves exposed to low levels of PHC seems to be transitory.

The present finding on the lethal and sublethal effects of pesticides and oil alone and in combination provide a clear cut insight into the possible effects of exposure of marine and estuarine bivalves into realistic concentrations of the above toxicants. Although, no attempt was made to work out the load of organophosphates and organochlorines in the tissues, the results presented here form an important documentation on a field of study which has not been investigated extensively. Further, experimentation will be necessary to explain certain unsolved responses detailed here in. Chronic ex-

posure studies should be given more attention so that the probable effects especially on the 'scope of growth' of subtidal and benthic bivalves, faced with chronic realistic concentrations of pesticides and oil could be properly understood. Experiments on the effects of pesticides on target and non-target organisms have amply proved that the chemicals designed and developed to kill pests will kill any animal either terrestrial or aquatic. The recent trend is to develop pesticides which do not chemically interfere with body metabolism or the nervous system. The problem of pest control has been the accumulation of toxic substances in men by repeated exposure, while the short lived pests develop resistance to the pesticides over a few generations leading to usage of more quantity of pesticides to have side-effects. Stronger pesticides were used to overcome this resistance and this made more dangerous the accumulation of toxicity in men. During the introduction of many of the pesticides sufficient knowledge was not available on the probable side-effects on other living forms. Recently development of less harmful and more effective pesticides has attracted the attention of scientists. The trend is to develop pesticides termed as 'designer pesticides' which will affect only the process of egg laying in insects. It may be hoped that in the coming years we will have pesticides which don't belong to the 'broad spectrum' category, but will be developed to disrupt only specific responses of the insects.

SUMMARY

VI: SUMMARY

The work presented here centres around the toxicity of two organochlorines and two organophosphates pesticides and the WAFs of LDO and P.G. Crude on a typical marine bivalve, Perna indica and a common estuarine bivalve, Villorita cyprinoides var. cochinensis. Aspects like individual toxicity, combined toxicity, changes in rate functions consequent to exposure to sublethal levels of pesticides singly and in combinations, sublethal effects on chronic exposure and uptake and depuration of oil have been documented.

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

In general, information on the toxic effects of pesticides and oil on marine and estuarine bivalves are detailed out in the Review of Literature. In this chapter, available papers on lethal and sublethal toxicity of organochlorines and organophosphates, crude and refined oils are critically reviewed. Work which explain the various causative factors that govern the sublethal toxicity of pesticides and oil are also documented. Information available on combined toxicity of pesticides and oil although limited, is also provided in this chapter.

The chapter on Material and Methods details out, the animals used for the present study, areas and methods of collection, instrumentation employed, chemical methods followed and the experimental designs to evaluate lethal toxicity, oxygen consumption, filtration, byssogenesis, accumulation and depuration of PHC. 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.

Among the pesticides used, Ekalux, an organophosphate was the most toxic, although Dimecron another organophosphate was the least toxic for both P. indica and V. cyprinoides var. cochinensis. It was noticed that lethal concentrations of pesticides reduced as a function of time. In the case of Dimecron, V. cyprinoides var. cochinensis was found to be more sensitive than P. indica. With the passage of time, oil fraction was found to exert a certain degree of lethal effect on V. cyprinoides var. cochinensis. Regarding the combined lethal toxicity, the following generalizations were arrived at. Ekalux and Dimecron, in combination with LDO (WAF) was not highly lethal to P. indica whereas, DDT and Aldrex was found to be more toxic. Ekalux and Aldrex resulted in more than additive toxic reaction when supplied in combination with LDO (WAF) in the case of V. cyprinoides var. cochinensis.

The oxygen consumption has shown both linear and non-linear patterns. Ekalux did not bring about conspicuous reduction in oxygen consumption in P. indica. While there was elevation in oxygen consumption by Aldrex exposed P. indica, there was depression in oxygen consumption by V. cyprinoides var. cochinensis. Dimecron functioned as a respiratory depressant for P. indica, while it was a stimulant in the case of V. cyprinoides var. cochinensis.

Ekalux, Dimecron, Aldrex and DDT in combination with LDO (WAF) brought about a conspicuous reduction in the oxygen consumption of P. indica. Similarly, in the case of the same combinations, resultant effect was depression in oxygen consumption in V. cyprinoides var. cochinensis.

In the presence of pesticides or WAF of oil P. indica reduced the rate of filtration although the reduction was not concomitant with the external concentration of pollutants. Ekalux or Dimecron and LDO (WAF) when present in combination resulted in a drastic reduction in the rate of filtration, whereas DDT in combination with LDO (WAF) brought about only marginal variation in the filtration rate of P. indica. The decrease in the quantity of water filtered and the increase in oxygen consumption indicate that the animals retain the water that enters the pallial cavity for a longer duration before expulsion. On a unit weight basis P. indica was found to filter more water than V. cyprinoides var. cochinensis. The reason may be that P.indica is incapable of complete closure of the shell valve because of interference by the byssal mass. Among the two species of bivalves, V. cyprinoides var. cochinensis seems to be in a position to virtually suspend filtration, atleast at some instances. Further, interconcentration variations in filtration rate could be insignificant.

Rate of byssogenesis by P. indica under individual toxic responses was worked out. The presence of all the four pesticides used and oil in the test media resulted in the reduction of byssus production. When employed alone, Aldrex was the most toxic with reference to byssogenesis. LDO (WAF) in combination with Ekalux proved to be highly toxic, to interfere with byssogenesis. Similarly, Aldrex in combination with LDO (WAF) also resulted in conspicuous reduction in byssogenesis although interconcentration variability were negligible.

Chronic exposure of P. indica to Ekalux, Dimecron, Aldrex, DDT and the WAFs of LDO and P.G. Crude and subsequent effect on oxygen con-

sumption and filtration rate were assessed. Further, such chronically exposed animals were transferred to raw sea water, maintained for seven days and the rate of oxygen consumption and filtration were assessed. Ekalux and Dimecron could bring in variation in oxygen consumption only after prolonged exposure. Further, a fourfold increase in Dimecron concentration did not have a concomitant effect on the oxygen consumption pattern in P. indica. In the case of Aldrex and DDT exposed animals there was an increase in oxygen consumption after fourteen days. In the case of animals exposed to WAFs of LDO and P.G. Crude, the rate of oxygen consumption was not very significant even after twenty one days.

During the period of revival the pesticide exposed animals were found to gain normalcy steadily. In the case of P. indica which had a pre-exposure history to low levels of petroleum hydrocarbons, regaining of normalcy was quicker.

The load of petroleum hydrocarbons in the tissues of P. indica, when exposed to low levels of LDO (WAF) and P.G. Crude (WAF) for twenty one days and subsequently maintained in raw sea water for seven days was also worked out. Relatively rapid uptake for seven days, magnified accumulation from seventh–fourteenth day and slow accumulation from fourteenth to twenty first day was the main trend. The rate of depuration was also found to be influenced by internal load. Concentration dependent increase in the uptake controlled by duration of exposure was the cardinal feature of the result obtained. The rate at which animals depurated PHC was more or less uniform, although the quantities thrown out were different in each case.

The chapter on Discussion enlightens the results obtained in light of the available literature. It becomes clear that more investigations are warranted on the chronic exposure of molluscs to low levels of pesticides and petroleum hydrocarbons and related effects, so that 'scope for growth' of animals encountering similar situations in nature can be worked out.

List of References is given at the end of the thesis.

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