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, $10.5 \times 10^6$ tons; trichloroethylene, $10^6$ tons; PCBs, $10^4 - 10^5$ tons; chlorinated phenols, $1.5 \times 10^5$ 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 outweigh 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 quite 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 interpreting 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 \textit{V. cyprinoides} var. \textit{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 \textit{P. indica}. On the contrary LDO (WAF) was a respiratory stimulant in the case of \textit{V. cyprinoides} var. \textit{cochinensis}.

The presence of LDO (WAF) along with Ekalux, Aldrex and DDT resulted in conspicuous reduction in oxygen consumption of \textit{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 \textit{V. cyprinoides} var. \textit{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). Con­
siderable 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
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-destructive, 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 possess 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 *Mytilus 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, 1983a; 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 longevity 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 bioconcen-
tration 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 (NaTa).
Development of gill lesions have been noticed in *Lepomis microlophus*, when
this was exposed to 0.03 – 0.3 mg \textsuperscript{-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 physio-
logical 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 concentrations 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 concent-
tration 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 alkynaphthalenes. 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.