DISCUSSION
Pesticide exposure - Determination of LC<sub>50</sub> values

The most generally accepted technique of evaluating the toxicity of pesticides is the determination of LC<sub>50</sub> values where the toxicity of the pesticide to aquatic organisms is assessed by the concentration of the toxic compound in water that will kill half of the animals exposed for a specified period of time. In the present study the % mortality in different concentrations of malathion showed a straight line when log concentration of malathion was plotted against probit mortality in this fish (Figs. 1 and 2). Thus the mean LC<sub>50</sub> values obtained through % mortality, probit mortality and Dragstedt-Behrens methods were found to be 5.433 ppm for normal (25°C), 4.830 ppm for higher (33°C) and 6.117 ppm for lower (15°C) temperatures. Thus the toxicity of pesticide to a particular aquatic organism is usually expressed in terms of LC<sub>50</sub> values which represents the amount of poison required (mg/litre) to kill 50% of the test population, namely fishes in the present study. The LC<sub>50</sub> values (ppm) of commercial grade malathion (50%) in Labeo rohita of the present investigation at all the temperatures relatively lower when compared to the LC<sub>50</sub> values of technical grade malathion 93% in the very same species of carp reported earlier (Bashamohideen and
Thus the data on LC_{50} values of malathion in carps reveals that technical grade malathion is less toxic than the commercial grade malathion to fishes and the relatively higher toxicity of commercial grade samples could be due to other ingredients present as emulsifiers which act as synergists and in turn may enhance the toxicity of the actual malathion. The present finding also agrees with the previous report that the commercial grade pesticides are relatively more toxic than the technical grade pesticides (Pickering et al., 1962; Sallatha et al., 1961). Studies involving the determination of LC_{50} values as in the present study are highly useful in determining the sub-lethal concentration of pesticides in view of the fact that the sub-lethal concentration was reported to be taken approximately one third of the LC_{50} value (Reed and Muench, 1938; Konar, 1969). Further the period for which the LC_{50} is determined is of considerable importance, because LC_{50} values are much higher at 24-hr than for example at 96-hr exposure (Pickering et al., 1962; Haider Ahmed, 1979; Koundinya and Ramamurthy, 1980; Bashamohideen and Subbarao, 1982 a). In this respect, most of the investigations in the above studies including the present study on the toxic effects of pesticides on fish have preferred 48-hr exposure, because
its relatively longer period than 24-hrs where the effects may not be consistent on one hand and because of it relatively lower period than 72 or 96 hrs where variations in oxygen and malathion may be more on the other hand. In this type of study involving the evaluation of toxicity of malathion in different species of fish, symptoms of poisoning in the form of increased irritability, hyper sensitivity and tremors of the whole body of this carp have been observed at LC$_{30}$ level of exposure, indicating the lethal effects developed by this carp leading ultimately to mortality and in this context it is interesting to note that similar symptoms of poisoning of organophosphorus pesticides in fishes were reported earlier (Crealy, 1974) and more specifically malathion poisoned blue gills extend their pectoral fins forward to the limit before dying (Eaton, 1970). Moreover, examination of the gills of dead fish in the present study indicated the formation of 'coagulation' film (appearance of the mucus covering over the gill) and the colour of the gill lamellae was changed from red to brown colour indicating impairment of blood circulation and the intestine was found to be completely filled with water and occupied a major part inside the body, presumably failure of osmoregulatory machinery would have resulted in the storage of water in the carp. Thus the data on the LC$_{30}$ values of an organophosphate insecticide like malathion will be
highly useful in the final evaluation of extent of pollution of aquatic environment by agricultural chemicals. Also, pesticides were found to be highly toxic not only to fishes, but also organisms which constitute food for fishes (Anderson, 1960; Loosanoff, 1960), hence it would be possible to establish limits and levels of susceptibility of toxic agents by the biotic components, mostly, fishes of the aquatic environment. It is known that LC$_{50}$ values cannot be considered safe for prolonged exposure because continued action of less toxic chemicals may eliminate fish species indirectly.

**Time course of pesticide exposure:**

LC$_{50}$ values cannot be considered safe for prolonged exposure as continued action of less toxic chemicals may eliminate fish species indirectly. Further, chronic and sub-lethal effects of pesticide, involving time course of studies which are conspicuous by their absence in fish population have considerable importance in that they may indicate the sequences of events in physiological systems providing information on the nature and completion of compensatory mechanisms likely to operate during pollutant stress. The time course in the rate of oxygen consumption in the individuals of *Labeo robinius* during the sub-lethal exposure period of malathion in the rate of O$_2$ consumption
initially increased at 24-hr exposure period than the inhibition in \( O_2 \) consumption of the fish in subsequent exposure was progressive and reached a maximum at the 15-day exposure period. After the 15-day of suppression in the rate of \( O_2 \) consumption the increased inhibition of \( O_2 \) consumption in the initial days was probably due to continuous initial exposure of fish to the pesticide and these variations could be attributed with fast that insecticides gain entry largely through the gills of fish (Helden, 1967; Premdas and Anderson, 1963; Ferguson and Good Year, 1967) and therefore, the first physiological activity to be affected is \( O_2 \) consumption in aquatic animals. During the later period of the 30-day sub-lethal exposure of malathion was raised near to the control levels, indicating that the fish is in the process of compensation to pollutional stress resulting due to pesticide exposure. Thus, in the present investigation sub-lethal concentration of pesticides could cause a physiological system to oscillate outside its normal range of variations, mostly suppressive at with time, the system could show indications of its return to the nearing normal state without suffering lasting effects. A possible example of such compensation for the effect of a pesticide such as malathion in fish population was suggested by (Bashamchideen and Subbarao, 1980; Bashamchideen and Parvati, 1982), where a
fairly good amount of recovery of $O_2$ consumption was noticed within 30-days in this carp exposed to sub-lethal concentrations (1 ppm) of malathion. In this context, the results of this investigation on time course of $O_2$ consumption during pesticide exposure reveals that malathion is more toxic to fish population.

**Pesticide exposure - $O_2$ consumption in whole fish:**

Respiratory activity ($O_2$ consumption) has been used as sensitive and good indicator of stress in aquatic animals exposed to pollutants in general (Sellers et al., 1975; Bayne et al., 1980; Mahajan and Dheer, 1980; Bashamohideen and Subbarao, 1982; Bashamohideen and Parvati, 1984; Bashamohideen, 1982, 1984). It is also reported interestingly by Mukhopadhayay and Dehadri (1978) that in air breathing cat fish *Clarias batrachus* where the microsomal drug metabolism enzymes which bring about the biodegradation of the pollutant from liver and gills were enhanced under sub-lethal exposure of malathion toxicity after 30-days indicating that the organs are on the way of adapting to pollutional stress. Thus, this removal of inhibition in $O_2$ consumption due to pesticides could be explained due to the activation and enhancement of detoxifying enzymes which bring about the biodegradation of the pesticide to reduce its toxicity.
in the way of recovery from earlier suppression as seen in this fish *Labeo rohita* of the present investigation. Hence $O_2$ consumption as involved in this investigation could serve as good indicator of pollutional stress at sub-lethal level and ultimately might contribute including pollution at a stage where remedial measures are possible as also suggested by Mahajan and Dheer, 1980, Bashamohideen and Subbarao, 1982 in fish population.

**Pesticide exposure - RBC Number**

Generally RBC will be elliptical in shape with nucleus in fish (Raizada and Singh, 1982) an average number of RBC would be $1.85 \times 10^6/mm^3$ in male and $1.08 \times 10^6/mm^3$ in female fish of *C. auriga*. Similar studies were made in *Labeo rohita* (Ragchi and Ibrahim, 1974) carp (Siddiqui *et al.*, 1970), *teleost* (Srivastava, 1968 a) and *Catla catla* (Rao and Behera, 1973). The survey of literature on the effects of chemicals on RBC count in fish highlighted the following trends. Pandey *et al.*, (1976) observed the decline in RBC count in *Channa punctatus* due to malathion. Similarly Panigrahi (1977) reported reduction in RBC count in *Anabas* due to mercury. Raizada and Gupta (1982) studied the effect of fungicide on RBC reduction in *trichogaster*. Jayanta Rao (1982) found that phosphomidon reduced the RBC content in *Tilapia*. Madhu (1984) studied the effect of Lindane on
RBC count in *Tilapia*. Subbaramaiah (1985) reported that atrazine reduced the RBC number in *Tilapia*. Further, Mahajan and Dheer (1980) have studied that changes in relative populations of neutrophils, thrombocytes and RBC when considered together could serve as good indicators of pollutional stress at sub-lethal levels even though there may be no mortality.

The above literature and the present study indicate that pesticides are reducing the RBC number in the fish. This clearly indicates that haemopoietic tissue is damaged and hence degeneration in RBC number. Because of RBC reduction one can suspect that pesticides are inducing anaerobic condition in fish. The reduction in RBC can also be accounted for reduction in gaseous transport as evidence through variations in O₂ and CO₂ equilibrium curves under pesticide toxicity (Rangaswamy, 1984).

**Pesticide exposure - Symptoms of poisoning**

Investigation on the symptoms of poisoning including the behaviour of the fish with other clinical manifestations is necessary and make it possible to diagnose and differentiate poisoning from disease in fish exposed to pesticides. The most important behavioral change in the form of a symptom which could be easily observed in an aquaria during pesticide
exposure is the opercular movement of the fish as suggested by (Chandy 1970). In the present study there is a slight increase in the rate of opercular movement of *Labeo rohita* when it is transferred immediately into the sub-lethal exposure of malathion at 24-hrs period at all the temperatures studied. This is due to increased locomotory activity arising out of the animals tendency to escape from the stress medium and this situation is called as escape reaction of the animal, as suggested by (Potts, 1954; Gross, 1977; Bashamohideen and Parvateeswarrao, 1972). After 24-hrs there was a progressive decrease in the ventilatory activity (percular movements) through, 7-day exposure period and maximum % suppression was observed at the 15-day sub-lethal exposure period. During this decreased activity the dissolved pollutant malathion may not only restrict gas transfer but their irritant effect can also interfere with ventilation. Such an effect is seen in the form of 'coughs' which are a regular feature of ventilatory rhythm of many fish and in polluted water their frequency tends to increase disturbing the normal rate of opercular movement, as suggested by Hughes (1981). In this decreased rate of opercular movement the time taken for 10 opercular movements obviously increase, this coincides with the decreased rate of O₂ consumption in the same sub-lethal exposure periods as noticed earlier.
Similar observations were made by (Jayantaraao, 1982 and Bashamohiddeen and Obileeu, 1985) in fish exposed to pesticides. The possible reasons for the decrease in opercular movements are (1) because of the dissolved pesticides the density of the water is increased. This density of water adds more on the inhibition of opercular movements in this carp and also as reported earlier by (Khan and Schmitzer, 1972). The other reason would be the active neurotropical link between the nervous system and opercular system (as, 1981) might have been impaired which ultimately resulted in the suppression of opercular activity. In the later half of the sub-lethal exposure period of malathion especially towards the end of the 30-day exposure period the suppression is removed to a greater extent and in fact this carp exhibited a fairly good amount of recovery in the opercular activity indicating that the fish is in the process of adaptation during malathion exposure in the 30-day exposure period. Thus opercular activity serves as a very good source of indicator in stress condition as also suggested by Thiedo (1963) where the opercular movements can be regarded as adequate indicator for environmental stresses in fishes.

The conspicuous symptom of poisoning is noticed in the change of colour of the fish from normal to black during sub-lethal exposure in the present study. The reason for
this reaction bringing about change in colour might be due to irritability of skin to chemicals in this carp and also as suggested in *Tilapia* by Jayantharao (1984). The other morphological changes were also noticed in the behavioral pattern of the fish i.e. in the form of increased irritability, hyper excitability, tremors of the whole body of the fish and ultimately loss of equilibrium of fish during malathion impact. Such type of behavioral changes were earlier reported in fishes Matelev et al. (1981). These changes may be correlated with the high degree of suppression in AchE activity in the brain of the fish O'Brien (1967), Cremllyn (1974), and more specifically malathion poisoned blue gills extended their pectoral fins forward to the limit before dying Eaton (1970) as also observed in the carp of this investigation. Moreover examination of the gills of dead fish in the present study indicated the formation of coagulation film (appearance of mucus covering over the gills) and the colour of the gill lamellae was changed from red to brown colour indicating impairment of blood circulation and the intestine was found to be completely filled with water and occupied by a major part inside the body, presumably failure of osmoregulatory machinery would have resulted in the storage of water in this fish. The surfacing phenomenon observed in this carp
during malathion exposure is an indication of hypoxic conditions in the malathion treated water.

Thus the above symptoms of poisoning observed in this carp mainly may be attributed to the behavioral changes. Behaviour is neuropsychically regulated and energy dependent phenomena. The pesticides are not only found to inhibit acetyl cholinesterase activity (AchE) but also favour the release of acetyl choline (Ach) in the neural and neuromotor regions, there by causing 'hyper excitability' ATPase system (Mg\(^2+\) + Ca\(^2+\) and Na\(^+\) – K\(^+\) ATPase and specifically Mg\(^2+\) ATPase (Matsumura et al., 1969 b; Cutkomp and Koch, 1981, ITRC Report 1980, CSIR News 1982). The double effect of pesticides namely (a) inhibition of AchE, release of Ach (b) inhibition of ATPase complex may be backbone for anomalies in the behaviour of fish.

The accumulation of Ach during pesticide stress was previously reported to be the prime factor for convulsion. But recently it is identified that ammonia plays an important role in this venture besides Ach (Martin and Agrawal, 1982). It is quite reasonable because pesticides induce ammonia toxicity in animals (Martin and Agrawal, 1982). Hence it is presumed that the inhibition of ATPase system and accumulation of Ach are not only the prime cause but the pesticides seem
to act upon other biochemical systems which have equal role to play either in the regulation of behavioral movements or indirectly support the nervous tissue function.

Since the abnormal behavior is an index of biochemical effects of toxicants, a model namely 'Behaviour Index Detector Model' (BIDM) can be designed taking fish as a test species in evaluating the quality of aquatic environment. A systematic approach by correlating the level of toxicant versus the magnitude of abnormality in behavior can be taken as an index, in formulating a 'Simple and Sensitive Biodetector Model' (SSBM) in analyzing the possible level of toxicants in the environment. The validity of this model can be assessed by correlating the abnormality in behavior with behavior regulating biofactors like enzymes under stress condition.

Influence of temperature on Malathion toxicity

Temperature as one of the most important environmental factors, is known to play a vital role in the ecophysiology of aquatic animals. But studies involving the influence of environmental temperature with reference to pollutional stress, are highly inadequate in general and particularly conspicuous by their absence in commercial fishes with reference to organophosphate pesticides. Temperature has
profound effects on the potency of various insecticides (Naraharhi, 1971) and organophosphates are in many cases more potent at higher ambient temperature than at lower temperature.

This fish at an elevated temperature is confronted with a much increased oxygen demand in an environment that is relatively hypoxic (Dewilde and Houston, 1967; Houston and Dewilde, 1968) hence the activity of the organism increases as reflected in its elevated rate of \( O_2 \) consumption whereas at lowered temperature, oxygen availability in the medium increases hence activity of the organism decreased as reflected in its depressed rate of oxygen consumption in this fish \textit{Labro robita}. In the present study under evaluation of pesticide toxicity, relatively greater % mortality has been observed in this carp at higher (35°C) temperature than at lower (15°C) temperature (Figs. 1 and 2, Table 1, 2 and 3). Comparative evaluation of the slope values of the probit lines at higher, normal and lower temperatures (Fig. 2) suggest that the slope of the straight line at higher temperature is higher than the slopes of the straight lines at lower temperature. Since the slope of the straight line indicates the degree of toxicity of pesticide (Chambers and Yarbrough, 1975), the relatively higher slope at higher temperature (35°C) suggest that malathion is more
toxic to the fish at higher temperature than at lower temperature, further the delayed mortality of this carp at lower temperature as compared to higher temperature may be due to changes in several physiological processes especially respiration and circulatory rate as also suggested by Cahnas et al. (1979). The LC$_{50}$ values of malathion in higher temperature adapted carp were found to be lower than in the lower temperature adapted ones (Figs. 1 and 2; and Tables 1, 2 and 3). This indicates greater malathion toxicity at higher temperature than at the lower temperature. These findings on LC$_{50}$ values based on temperatures in this carp are in excellent agreement with the finds of Wuyts (1964) on zinc toxicity in ramshorn snail, *Helisoma aspersulum* and of Gupta et al., 1981 on copper toxicity in fresh pond snail *Viviparus bengalensis*, where a raise in temperature increased the toxicity of these metals. The % suppression in O$_2$ consumption and in RBC number in this carp at different sub-lethal exposure periods of malathion are found to be significant in higher temperature (35°C) adapted carp than in lower temperature adapted fish. (Figs. 4, 5 and 6, 7 and Tables 7, 8, 9 and 11, 12, 13). The % maximal suppression in the above parameters at 15-day period was found to be higher but the % recovery in these parameters was found to be lower in higher temperature adapted fish.
than in the lower adapted ones, indicating that malathion is more effective and toxic at higher temperature. This differential toxicity of malathion based on temperature with reference to $O_2$ consumption and RBC number could be explained by the facts that in fishes, respiration and pesticide uptake usually via gill (Holden, 1962; Ferguson et al., 1966) will be more rapid at higher temperatures because $O_2$ demand will be greater and solubility of oxygen is lesser, hence increased metabolism and $O_2$ uptake would however result in lowering the dissolved $O_2$ concentration and subsequent accumulation of waste products, thus increasing susceptibility, hence the toxicity of the pesticide, increases with raise in ambient temperature as also reported earlier by Nasek et al. (1969).

Similar trend in malathion toxicity with references to temperature has been reported by Kabeer et al. (1979) where increased inhibition in the rate of ciliary activity of fresh water mussel at higher temperatures was observed, further it was also explained that this could be so due to rapid penetration of malathion into the ciliary cells at higher temperature, thereby leading to the inhibition of respiratory metabolism of the animal (Corbett, 1974). Thus the increased % suppression in $O_2$ consumption and in RBC number in this carp at higher temperature ($35^\circ$C) might be due to increased diffusion of the malathion into the interior
of the cells, resulting a decline in these factors and also this % suppression is less at lower temperature and greater at higher (35°C) temperature with reference to normal (25°C) temperature. Thus a raise in temperature from normal (25°C) to higher (35°C) also raises the toxicity of malathion, hence suppression of O₂ consumption and RBC number by malathion, is suggestive of thermal dependency. Further the enhanced toxicity of malathion in this carp at higher (35°C) temperature could be due to the greater accumulation of malason, a toxic metabolite of malathion in the liver of the carp as also suggested by Murphy et al. (1963). Thus unlike the organochloride pesticides which are more potent and toxic at lower temperature (Narashahi, 1971 a, Wang, et al. 1972) the organophosphate pesticide like malathion of the present investigation is found to be more potent effective and toxic at higher (35°C) temperature, in the case of carps. Hence it could be concluded that malathion toxicity in carps increased with a rise in ambient temperature.

The symptoms of poisons are also greatly affected by ambient temperature during malathion toxicity. The initially enhanced opercular activity during 24-hrs sub-lethal exposure of malathion is significantly much more in malathion exposed fishes at higher temperature than the lower. This
suggests that the rate of escape reaction as suggested by Gross, Petts exhibited due to increase in opercular activity is also correspondingly more pronounced at higher temperature than the lower in this carp. Further relatively higher suppression in opercular activity which has been observed at higher temperature compared to lower temperature and relatively greater % recovery in opercular activity noticed in lower temperature than the higher during malathion exposure indicate that the malathion toxicity increases with increase in ambient temperature. This is supported by a similar trend in malathion toxicity with reference to temperature in the fresh water mussel (Habib Ahmed et al. 1979). The other symptoms of poisoning like (1) opercular activity, (2) changes in the colour of the fish, (3) changes in pectoral fins, (4) change in gill colour, (5) secretion of mucus, (6) abdominal changes and (7) surfacing phenomenon, are greatly influenced by ambient temperature during malathion exposure. The intensities/rates/amounts of these symptoms mentioned above are much more pronounced in this carp during the sub-lethal exposure of malathion at higher temperature than the lower with a intermediate level of effect at normal temperature. Based on this evidence and because of CNS is thought to regulate changes in temperature selection
(Fischer, 1978; Anderson and Peterson, 1959; and Basamohideen and Parvati, 1985) argued that the sub-lethal amounts of DDT and malathion directly affect the CNS and thus alter the thermal acclimatory mechanisms during pesticide exposure as noticed in the present investigation.