SUMMARY AND CONCLUSIONS
SUMMARY AND CONCLUSION

Pesticidal pollution may be defined as the change in one or more components of the ecosystem by the pesticides or by their degradative products. (Sahai and Chouhan, 1977). In the last three decades, there has been a phenomenal increase in the usage of pesticides in agricultural and public health operations. Organophosphates (OP), the most versatile group of insecticides, are highly selective and are generally toxic to many non-target organisms including fish and other aquatic fauna (Murphy, 1980), posing a dual threat to mankind. Presence of organophosphorus pesticides has been detected even in naturally occurring water indicating the degree of environmental contamination by organophosphates (Boom et al. 1978). The pesticides though applied at different places, ultimately make their entry into the water bodies contaminating the aquatic ecosystem.

Phosalone, an organophosphorus insecticide used extensively for crop protection in this part of the country, was chosen as the toxicant in the present study. With the recognition that all pesticides are potentially lethal to fish even at relatively low concentrations in comparison with those commonly used in spray applications, it is now a normal practice to test all new chemicals for their toxicity to fish (Holden, 1973; Hayes, 1982). The question, therefore, is whether it is safe to use organophosphorus pesticides when fish are present in an aquatic system. Since fish occupy an important place in the food chain, it is necessary to know the effects of low concentrations of OP insecticides on non-target organisms, including fish.
Though much work has been done on the biochemical, behavioural, histochemical and histopathological parameters of different tissues and blood of different fishes (Murphy, 1980; Gerolt, 1983; Hayes, 1982; Ravi and Selvarajan, 1986), little information is available on the impact of organophosphorus pesticides on physiological functions of the blood and tissues of fish. Keeping this in view, the present work is designed to study the effects of sublethal concentrations of phosalone on certain physiological processes of the fresh water fish, *Tilapia mossambica* (peters).

In general in any study, involving pesticides, LC50/LD50 would be calculated before actually taking up further investigations with the pesticides. Accordingly, the concentration that kills 50% of the fish in 96h duration (LC50/96h) and its 95% confidence intervals are estimated as 0.27 (0.2138 to 0.3388) ppm. It was found that the LC50 of phosalone to fish *T. mossambica* obtained in the present study is well within the range reported by other workers under similar physiological conditions (Table 1.3).

Evidently, phosalone induced behavioural pathology in the fish. Muscular spasms, jerky movements, disorganised swimming and frequent surfacing activity have been observed. Increased surfacing activity and decreased opercular movements indicate prevalence respiratory distress. There was copius mucus exudation. Morphological observations revealed loss of intensity of the skin colour and appearance of black spots on the body. The colour of the gill was found to change from dark red to pale red indicating anaemic condition. Body weight of the fish increased implying imbalance in osmoregulation leading to edematous condition and finally to
Chronic toxic effects of phosalone on fish were studied at the end of days 1, 3, 7, 15 and 30 after exposing the fish daily to the sublethal concentration. The control fish received an equal volume of acetone only, instead of acetone + phosalone.

Total body weight, tissue and whole animal hydration levels and histosomatic indices (HSI) have been studied in fish, *T. mossambica* exposed for 1, 3, 7, 15 and 30 days to sublethal concentrations of phosalone. The results showed that there was progressive increase in all the above parameters with maximum significant (P<0.05) increases being observed on day 30. Several authors, who reported increases in these parameters in different non-target organisms exposed to different insecticides have attributed the increase to insecticide induced water gain (Vide Chapter-II).

It is well known that water constitutes about 80% of cell mass (Prosser, 1973) and hence an increase in total body weight of fish treated with sublethal concentrations of phosalone could be attributed to water gain. It has been reported that several OP compounds including phosalone cause permeability changes in gill lamellae leading to increased water influx (Houston, et al. 1970; Murphy, 1980; Gerolt, 1983). Further, when oxygen demand is high in animals exposed to sublethal concentrations of insecticides, as in the present study (Vide Chapter -V) large volumes of blood must pass through gill lamellae. As a result of this, diffusive uptake of water increases (Motais and Isia, 1972) causing an increasing in hydration levels of tissues and whole animal which in turn may increase HSI, since HSI represents the relationship between weight of a tissue and the weight of the rest of the
It is apparent from observations that multiple sublethal concentrations of phosalone might have altered the membrane permeability of the branchial epithelium of fish causing an increase in the rate of uptake of water and thus total body weight, hydration levels and HSI. Such changes, usually reflect the altered physiological status of fish under phosalone stress since it is known that variations in tissue hydration levels are an index of physiological status of the animal concerned.

Effect of sublethal concentrations of phosalone on haematological parameters of fish blood have been studied since these are a sensitive index to changes in ecological conditions and constitute an important diagnostic tool in toxicological studies (Dabrowska and Wlasow, 1986). There was a decrease in RBC, Hb and PCV in the fish exposed to sublethal concentrations of phosalone for 1, 3, 7, 15 and 30 days. It has been reported that a decline in the production of RBC results from decreased erythropoietic activity which is regulated by the hormone, erythropoietin (Jacobson and Krantz, 1968) produced by juxtaglomerular cells in the kidney (Prosser, 1984). Phosalone which produced kidney damage in fish (Janardan Reddy et al. 1990) might have produced kidney damage in T. mossambica also. As such there might be a decrease in the production of erythropoietin though there was a considerable decrease in the rate of oxygen uptake by the fish (Chapter V-A) treated with sublethal concentrations of phosalone, which in the normal course should have stimulated production of erythropoietin and thus of RBC (Prosser, 1984). However decreased erythropoietin levels might have suppressed erythropoietic activity resulting in reduced production of RBC and Hb in T. mossambica. Another factor that might have reduced RBC count is haemodilution. Phosalone, not only through its action on hypothalamus
(Gerolt, 1983) and consequent elevation in water retentivity of the blood, but also by its being capable of causing kidney damage and subsequent impairment of osmoregulatory mechanism, is supposably implicated in the manifestation of haemodilution. In the light of these observations, it is presumed that decreased erythopoietic activity and haemodilution were involved in decreased production of RBC and Hb, but the extent to which they are individually involved is open for discussion.

There was a gradual increase in MCV of the fish upon phosalone treatment. The increased MCV has been indicating the existence of endosmosis which involves the passage of a solvent from a less concentrated extracellular fluid to a more concentrated solution that is inside the cell. The increased blood volume and blood hydration level (chapter II) might have induced the endosmosis in RBC causing an increase in MCV.

The sublethal concentration of phosalone caused a decrease in MCH and the maximum and significant decrease was found on day 7 indicating the damage of red cell membrane and leakage of some sort of Hb into the plasma. Later the MCH showed a progressive and an insignificant increase till day 30, indicating the animals tendency toward normalcy as time passes.

MCHC showed a gradual decrease in fish throughout the exposure period. It is well known that MCHC represents the relationship between Hb and PCV (Thews and Hutton, 1983). Accordingly in the present study the decreased Hb and PCV lead to decrease in MCHC in fishes. There was a gradual and significant decrease in WBC count in **T. mossambica** exposed for 1, 3, 7, 15 and 30 days to a sublethal concentrations of phosalone. The
decreased WBC indicates the decrease of immunological set up of the fish under phosalone toxic stress.

Inorganic ions are powerful modulators of cellular activities (Bygrave, 1967) and play an important role in the metabolic adjustments accompanying stress conditions (Berish, 1973). Ionic composition of the blood and tissues plays an important role in regulating hydration levels of the cell and metabolic events of the cell system (Prosser, 1973). There was a progressive increase in sodium, chloride and potassium concentrations in the blood, liver, gill and muscle of *T. mossambica* exposed to phosalone for 1, 3, 7, 15 and 30 days. The ions are regulated by Na\(^+\)-K\(^+\) pump, located in the cell membrane, controlled by the enzyme Na\(^+\)-K\(^+\) ATPase (Katz and Epstein, 1968). Increased Na\(^+\), Cl\(^-\) and K\(^+\) concentrations in tissues and extracellular fluids have been indicating the disruption of Na\(^+\)-K\(^+\) pump in cell membrane by the inhibition of Na\(^+\)-K\(^+\) ATPase. It is well known fact that sodium always associated with chloride and bicarbonate, which are inseparable from the maintenance of acid-base balance (Tyler, 1977). This is probably the reason for accumulation of Na\(^+\), Cl\(^-\) and K\(^+\) in phosalone treated fish. This increase in K\(^+\) in concentration might lead to disturbances in the muscular activity (Chapter I-B) since K\(^+\) influences the muscular activity (Prosser, 1984).

Calcium (Ca\(^{2+}\)), magnesium (Mg\(^{2+}\)) and bicarbonate (HCO\(_3^-\)) concentrations were showed a gradual decrease in fishes exposed to phosalone for 1, 3, 7, 15 and 30 days, which might be attributed to the disruption of cell membrane permeability by altering Na\(^+\), K\(^+\) ATPase and increased blood and tissue hydration levels (Chapter-II).
The maintenance of cell functions and life is dependent upon continuous supply of adequate amounts of oxygen to the tissues. Oxygen consumption is considered as a measure of general metabolism of animals (Fry, 1957; Prosser, 1973; Wares and Igram, 1979) and a measurement of oxygen consumption reflects metabolic rate and thereby the energy output. There was a gradual decrease in whole animal oxygen consumption and respiration in tissues like muscle, liver, gill and kidney of fish exposed to phosalone for 1, 3, 7, 15 and 30 days. The decrease in whole animal oxygen consumption has been ascribed to inhibition of respiratory muscles by phosalone since several OP pesticides are known to inhibit muscles concerned with the ventilating movements (Prosser, 1973; Murphy, 1980; Hayes, 1982). Probably a decrease in whole animal oxygen consumption resulted a decrease in tissue respiration. It is also possible that phosalone might have caused some histopathological damage to the tissues of muscle, liver, gill and kidney, thereby decreasing the rate of oxygen uptake (Murphy, 1980; Webster and Webster, 1974; Hayes, 1982).

The apparent decreases in RBC, Hb, PCV, whole animal oxygen consumption and tissue respiration suggest diminished cellular oxidations and a corresponding decline in the energy output forcing the animal to resort to some other alternative mechanisms to get energy.

When hypoxia is prevalent and aerobic cellular oxidations are substantially lowered, the metabolism is stepped up to meet the energy demand. As such there were increases in LDH activity, lactic acid concentration followed by increases in phosphorylase "a" and blood glucose
and decreases in tissue glycogen content, SDH and ICDH in fish *T. mossambica* exposed to sublethal concentrations of phosalone.

When oxygen is in short supply, glycolysis is allowed to proceed through the generation of NAD by the reduction of pyruvate to lactate, a reaction catalized by LDH. This is essential because anaerobic glycolysis limits the amount of energy liberated per molecule of glucose oxidized. Consequently to provide a given amount of energy more glucose must undergo glycolysis under anaerobic conditions as compared with aerobic conditions. Apparently increased LDH activity might be providing necessary glucose for anaerobic glycolysis. Several OP insecticides are implicated in enhancing tissue LDH activity and lactic acid concentration (Chapter-V). It is not surprising, therefore, to suggest that increase in tissue LDH activity and lactic acid concentration reflect enhanced operational rate of anaerobic glycolysis to meet the energy demand. Moreover lactic acid may be utilized for resynthesis of glucose in the liver.

Glycogen phosphorylase is the enzyme that breaks glycogen and yields glucose. Eckner (1971) has reported that hypoxic conditions induce rapid glycogenolysis by activating phosphorylase to produce more glucose in response to the elevated energy demand. It has been observed that sublethal concentrations phosalone produced hypoxia in fish *T. mossambica* (see discussion - whole animal oxygen consumption and tissue respiration) which, in turn, might have increased glycogenolysis. This gains further support from increased phosphorylase activity reduced glycogen concentration and elevated blood glucose level (Vide Chapter-V).
The results of the present investigation also show decreased activity in oxidative enzymes like SDH and ICDH indicating a decrease in the rate of oxidative metabolism. The decreased levels of SDH and ICDH might also be due to disruption of structural integrity of mitochondria, since pesticides are known to cause changes in structure of mitochondria (Sastry and Siddiqui, 1983; Abidi, 1986).

By studying the chronic sublethal effects of phosalone on tissue gravimetry, haematology, inorganic ions, whole animal and tissue respiration and oxidative enzymes and a few aspects of metabolism of fresh water fish *T. mossambica*, certainly it is not the claim of the author that he has covered all aspects and answered every question in this field of study. While some questions are answered or at least clarified, new questions did arise forming a basis for further investigations under the limitations set both in the scope of this investigation and also the facilities available, these new questions could not be answered here, and hence, necessarily reserved for the future.