CHAPTER-VI

DISCUSSION
Detergents and phenolic toxicants have become major environmental pollutants today. A critical survey of the effect of synthetic detergents to aquatic organisms has been carried out by Henderson et al., (1959), Abel (1974) and Marchlewksa (1983). Phenolic wastes are common water pollutants generated from a variety of industrial processes (Buikema et al., 1979). Literature survey revealed that pH, alkalinity and hardness may influence the toxicity of pollutants (Alabaster and Lloyd, 1980). In the present study a toxicological evaluations of detergents and phenolic toxicants on fresh water fishes viz., Oreochromis mossambicus and Cyprinus carpio were undertaken.

The literature concerning procedures and results of bioassay testing of fish and other organisms was reviewed by Maciorowski et al., (1980). Results of the toxicity tests on water quality parameters chemicals were included in the literature and comparison of various methods were discussed. The effects of pollution on fresh water fish from around the world were reviewed by Abel (1976), Stephan and Mount (1973), Spehar et al., (1980), Spehar et al., (1982), Phipps, et al., (1984), Roush et. al., (1985), Pashchenko and Kasumyan (1984), Gafa (1974), Herbert et al., (1957) and Holcombe et al., (1982).

Barbieri et al., (1998), reported the behavioural changes exposed to detergent SDS to fish C. carpio. The 96 h LC$_{50}$ value was 5ppm. The bioassay studies with SLS with reference to certain hematological parameters were carried out on the fish Rasbora daniconius (Ham) by Madhyastha and Nayak (1979). The 96 h LC$_{50}$ value was found out 6.30 mg/l.
Garasangi (Dissertation, 1997), reported that effects of some physico-chemical factors on fish *L. guntea*. The 96 h LC$_{50}$ of phenol observed was 26.0 mg/l at room temperature (26°C) and normal water pH (7.6). The 96 h LC$_{50}$ of phenol at 20°C was 21.0 mg/l and for 30°C temperature it was 21.0 mg/l. According to Razani et al., (1986) the 96 h LC$_{50}$ of phenol on Zebra fish *Brachydanio rerio* was 24 ppm. Whereas Korns et al., (1985), investigated the 96 h LC$_{50}$ of phenol for pink salmon was 3.7 ppm.

The 96 h LC$_{50}$ of phenol at pH 6.0 was 22.0 mg/l and at pH 9.0 it was 27.0 mg/l, reported by Garasangi (Dissertation, 1997). Norman Chagnon and Ihor Hlohowskyj (1989), studied the effects of phenol exposure on the thermal tolerance ability of centrol *Stoneroller minnow*. The 48 h LC$_{50}$ value for phenol was 17.9 mg/l. USEPA (1973), reported the proposed criteria for water quality. The 48 h LC$_{50}$ was 7.5 mg/l for rainbow trout for phenol. Angus (1983), studied Phenol tolerance in populations of mosquito fish from polluted and non-polluted water. The 48 h LC$_{50}$ was 47.4 mg/l for *Gambusia affinis*. Asheera Banu Sangli and Kanabur (2000), studied the acute toxicity of p-cresol to *Gambusia affinis* and *Lepidocephalichthys guntea*. The 96 h LC$_{50}$ values for cresol for *Gambusia affinis* was 33.0 mg/l and for *Lepidocephalichthys guntea* was 14.0 mg/l. Mattson et al., (1976), reported acute toxicity of organic compounds to fish *fathead minnow*. The 96 h LC$_{50}$ value for cresol for fish *fathead minnow* was 19.0 mg/l. Albersmeyer and von Erichsen (1959),
reported that 24 h LC50 for p-cresol was 4.0 mg/l for trout embryos, 16.0 mg/l for tench and 17.0 mg/l for roach.

Similarly the 96 h LC50 of different detergents are investigated. The acute toxicity of five synthetic detergents to two fishes viz., *Channa punctatus* and *Saccobranchus fossilis* has been worked out by Verma *et al.*, (1980). The 96 h LC50 values for *Channa punctatus* were 15.0 mg/l for Swanic 6L, 19.7 mg/l for Swaryl 10C, 19.5 mg/l for Swascol IP, 27.0 mg/l for Swascofix CD-38 and 35.0 mg/l for Idet 5L. For *Saccobranchus fossilis* were 6.4 mg/l for Swanic 6L, 11.2 mg/l for Swaryl 10C, 11.2 mg/l for Swascol IP, 17.4 mg/l for Swascofix CD-38 and 22.9 mg/l for Idet 5L. Cairns and Scheier (1962), studied the effect due to water hardness on fish *Lepiomis macrochirus* with ABS detergent. The 96 h LC50 value was 17.0 mg/l. Cairns and Scheier (1962), reported that testing in the soft water only by *Lepomis gibbosas* (L) fish for ABS detergent. The 96 h LC50 was 22.0 mg/l.

Paul *et al.*, (1995), reported that activity of acid phosphatase to *S. mossambicus* exposed to ‘wheel’ detergent. The 96 h LC50 was 66.1 mg/l.

Rajendra Nayak and Madhyastha (1987), concluded that the behavioural studies exposed to acute toxicity of ‘point’ detergent to fish *R. daniconius* (Ham). The 96 h LC50 value was found to be 160 mg/l. Similarly Mark *et al.*, (1991), studied the metabolism of Rainbow trout fish exposed to dioctyl sodium sulfosuccinate (DSS) detergent. The 96 h LC50 was 28.0 mg/l to the fish. Samson Raju *et al.*, (1994), reported the effect of ‘Ariel’ detergent as
oxidative enzymes and histology of fish *O. mossambicus*. The 48 h LC$_{50}$ was found to be 35 ppm to the fish.

All authors agreed that the death of the fish exposed to detergents and phenolic toxicants was mainly due to the coagulation and precipitation of the mucus secreted by the fish gills or damage to the gill respiratory epithelium.

Behavioural responses of the fishes were observed during acute and sublethal exposure to detergents and phenolic toxicants. The changes being more prominent at acute exposure. Surfacing, gulping of air and erratic opercular movements were observed in lethal concentrations of detergents and phenolic toxicants. At higher concentrations a thin layer of mucus was coated all over the body surface. Hemorrhaging from the pectoral fin and gills was observed due to the rupture of blood vessels within the gill filaments. The fishes died with their mouth wide open. During sublethal exposure the fishes were unable to feed regularly and exhibited aimless swimming. They become inactive and pale color.

Similar observations have been made by various workers on freshwater fishes, exposed to detergents and phenolic toxicants. Henderson *et al.* (1959), Abel (1974), Marchlewksa (1983), Schmid and Mann (1961), Cairns and Scheier (1962), Lemke and Mount (1963) and Foster *et al.* (1966), reported that changes in certain fish behaviours are sensitive indicators of sublethal exposures to detergents and phenolic toxicants.
In the present study the 96 h LC$_{50}$ values of SDS for fishes viz., $O$. mossambicus and $C$. carpio obtained in the present study were 15.0 mg/l and 24.60 mg/l respectively. Barbieri et al., (1998), reported the behavioural changes exposed to detergent SDS to fish $C$.carpio. The 96 h LC$_{50}$ value was 5ppm. The 96 h LC$_{50}$ values of SLS for fishes viz., $O$. mossambicus and $C$. carpio obtained in the present study were 8.5 mg/l and 10.5 mg/l respectively. Madhyastha and Nayak (1979), reported a 96 h LC$_{50}$ value for SLS to fish Rasbora daniconius (Ham) was 6.30 mg/l. Our results obtained here are well in agreement with those reported else where.

Similarly with phenol in the present study the 96 h LC$_{50}$ values for fish $O$. mossambicus and $C$. carpio were found to be 35.0 mg/l and 30.40 mg/l respectively. It was observed that in the present study phenol was found to be the least toxic compound to both $O$.mossambicus and $C$.carpio as compared to p-cresol, SDS, Triton X-100 and SLS.

Garasangi (Dissertation, 1997), reported the 96 h LC$_{50}$ value for phenol to fish $L$.guntea was 26.0 mg/l. Razani et al., (1986), reported a value of 24 ppm, for phenol (96 h LC$_{50}$) to Zebra fish. Whereas Korns et al., (1985), reported the 96 h LC$_{50}$ value for phenol to fish Pink salmon was 3.7 ppm. Norma Chagnon and Ihor Hlohowskyj1 (1989), reported the value of 17.9 mg/l for phenol (48 h LC$_{50}$) for Stoneroller minnow. USEPA (1973), reported that proposed criteria for water quality. The 48 h LC$_{50}$ was 7.5 mg/l for rainbow trout for phenol. Angus (1983), studied Phenol tolerance in populations of
mosquito fish from polluted and non-polluted water. The 48 LC₅₀ was 47.4 mg/l for *Gambusia affinis*. The results obtained here are well in agreement with the reported values of earlier workers. In the present investigation in case of para-cresol the 96 h LC₅₀ values for fish *O. mossambicus* and *C. carpio* were 28.0 mg/l and 26.0 mg/l respectively. Asheera Banu Sangli and Kanabur (2000), reported that the acute toxicity of cresol to fish *G. affinis* and *L. guntea* were 33.0 mg/l (96 h LC₅₀) and 14.0 mg/l (96 h LC₅₀) respectively.

Mattson *et al.*, (1976), reported a value of 19.0 mg/l (96 h LC₅₀) of p-cresol for fish *fathead minnow*. Albersmeyer and von Erichsen (1959), reported that 24 h LC₅₀ for p-cresol was 4.0 mg/l for trout embryos, 16.0 mg/l for tench and 17.0 mg/l for roach. The results obtained here are well in agreement with those reported elsewhere.

In the present investigation the 96 h LC₅₀ values for fish *O. mossambicus* and *C. carpio* to detergent Triton X-100 were 14.40 mg/l and 4.90 mg/l respectively. There is not much work has been carried out in acute toxicity of fish to detergent Triton X-100.

From the available literature it is evident that, in bioassay tests more than one detergent and phenolic compound were investigated on fresh water fishes. Detergents like SDS, SLS, and Triton X-100 and phenolic toxicants like phenol and para-cresol finds various applications in different industries. Hence an attempt has been made to compare the degree of toxicity of these detergents and phenolic toxicants tested as freshwater fishes.
The potency ratio and significance of difference between toxicity of SDS, SLS, Triton X-100, phenol and p-cresol of the exposed animals is presented in Table 3.4. In case of SDS Vs SLS, against *O. mossambicus* it was found that SLS was 1.76 times more toxic than SDS. However in SDS Vs Triton X-100 the potency ratio was declined to 1.04 and it was not significant. In case of SDS Vs Phenol, SDS Vs p-cresol, it was observed that SDS was 2.33 times significantly more toxic than phenol; and SDS was 1.97 times significantly more toxic than p-cresol. Similarly in case of SLS Vs Triton X-100, SLS Vs Phenol and SLS Vs p-cresol, it was found that SLS was 1.69 and 4.11 times significantly more toxic than Triton X-100; and phenol respectively and SLS was 3.40 times significantly more toxic than p-cresol. In case of Triton X-100 Vs phenol, Triton X-100 Vs p-cresol and Phenol Vs p-cresol, it was observed that, Triton X-100 was 2.43 and 2.00 times significantly more toxic than phenol and p-cresol respectively; and p-cresol was 1.21 times significantly more toxic than phenol.

Similarly, the potency ratio and significance of difference between toxicity of SDS, SLS, Triton X-100, Phenol and p-cresol of the exposed animals is presented in Table 3.5. In case of SDS Vs SLS, SDS Vs Triton X-100 and SDS Vs phenol for *C. carpio*, it was found that SLS was 2.34 times significantly more toxic than SDS, Triton X-100 was 5.02 times significantly more toxic than SDS and SDS was 1.26 times significantly more toxic than phenol. However in case of SDS Vs p-cresol. It was not significant and the
potency ratio was declined to 1.05. In case of SLS Vs Triton X-100, SLS Vs phenol and SLS Vs p-cresol, it was observed that Triton X- 100 was 2.14 times significantly more toxic than SLS and SLS was 2.89 and 2.47 times significantly more toxic than phenol and p-cresol respectively. Similarly in case of Triton X-100 Vs Phenol, Triton X-100 Vs p-cresol and phenol Vs p-cresol; it was found that Triton X-100 was 6.20 and 5.30 times significantly more toxic than phenol and p-cresol respectively and p-cresol was 1.16 times significantly more toxic than phenol.

A comparison of the slope function of dose mortality curves of SDS, SLS, Triton X-100, phenol and p-cresol and their deviation from parallelism of O.mossambicus presented in Table 3.8. In case of SDS Vs SLS, SDS Vs Triton X-100, SDS Vs Phenol and SDS Vs p-cresol, it was observed that deviation from parallelism as regards O.mossambicus was not significant indicating similar mode of action of the toxicants on O. mossambicus. Similarly in case of SLS Vs Triton X-100 the deviation from parallelism was not significant indicating similar mode of action like that of other toxicants on O. mossambicus. Whereas in SLS Vs Phenol the deviation from parallelism was found to be significant at 95% probability indicating that the curves may be considered parallel within the experimental error. In case of SLS Vs p-cresol the deviation from parallelism was not significant indicating similar mode of action like that of the other toxicants on O. mossambicus. Similarly in case of Triton X-100 Vs Phenol, Triton X-100 Vs p-cresol and Phenol Vs p-cresol the
deviation from parallelism was not significant indicating similar mode of action of the toxicants on *O. mossambicus*.

A comparison of slope functions of dose mortality curves of SDS, SLS, Triton X-100, phenol and p-cresol and their deviations from parallelism of *C. carpio* is presented in Table 3.9. In case of SDS Vs SLS, SDS Vs Triton X-100, SDS Vs Phenol and SDS Vs p-cresol the deviation from parallelism was not significant indicating similar mode of action of the toxicants on *C. carpio*. Similarly in case of SLS Vs Triton X-100, SLS Vs Phenol and SLS Vs p-cresol the deviation from parallelism was not significant indicating similar mode of action like that of other toxicants on *C. carpio*. In case of Triton X-100 Vs Phenol, Triton X-100 Vs p-cresol and Phenol Vs p-cresol the deviation from parallelism was not significant indicating similar mode of action of the toxicants on *C. carpio*.

Comparative account of acute toxicity of different detergents and phenolic toxicants tested in the present study.

A comparative account of the 96 h LC$_{50}$ values, Slope function and their 95% confidence limits values of the toxicity’s of SDS, SLS, Triton X-100, Phenol and p-cresol for *O. mossambicus* and *C. carpio* has been presented in Table 3.10.

By examining the 96 h LC$_{50}$ values for different toxicants tested, it was observed that the toxicity of the detergent and phenolic compounds for fish *O. mossambicus* was following magnitude:
SLS > Triton X-100 > SDS > p-cresol > Phenol.

In case of *C. carpio* was of the following magnitude:

Triton X-100 > SLS > SDS > p-cresol > Phenol

Safe concentrations of toxicants to *O.mossambicus* and *C.carpio* has been presented in Table 3.11.

In the present study alterations in the oxygen consumption rates of *O.mossambicus* and *C.carpio* exposed to lethal concentrations of SDS, SLS, Triton X-100, phenol and p-cresol were studied. The results are presented in Table 3.12 and 3.13.

The respiratory activity of the fish *O.mossambicus* was studied by exposing them to different concentrations of detergents and phenolic toxicants. The uptake of oxygen increased in fish up to 2nd day in both concentrations of SDS (11.0 and 13.0 mg/l). Depleted oxygen consumption was noticed on 3rd day and it showed an increase in oxygen uptake after 96 h at both the concentrations (97.91% at 11.0 mg/l and 93.75% at 13.0 mg/l). With two SLS concentrations (4.0 and 5.0 mg/l), the oxygen uptake during first day increased in fish at 4.0 mg/l, 2nd day depleted and gradually increased up to 4th day (73.33%). With 5.0 mg/l, declined oxygen uptake was observed during first day. And it increased up to 96 h (80.00%). Similarly the respiratorv activity of the fish exposed to two Triton X-100 concentrations (10.0 and 13.0 mg/l), the consumption of oxygen decreased from 1st day to 4th day (95.71% and 86.00%) at 10.0 mg/l. Increased oxygen uptake up to 2nd day decreased on 3rd day and it
showed an increased oxygen uptake after 96 h at 13.0 mg/l. In case of phenol the uptake of oxygen during first day increased, decreased on 2nd day again slight increased on 3rd day and oxygen uptake reduced after 96 h (81.81%) at 31.0 mg/l. Suppressed oxygen uptake was noticed up to 2nd day (76.92%). Increased oxygen consumption was showed after 72 h and it showed reduction after 96 h (74.54%) at 33.0 mg/l. Similarly with two concentrations of p-cresol (23.0 and 25.0 mg/l), uptake of oxygen suppressed during first day and then increased on 2nd day (83.95%) again reduced on 3rd day and it showed a slight increase after 96 h at 23.0 mg/l. With 25.0 mg/l, uptake of oxygen was increased up to 2nd day and suppressed on 3rd and 4th day (55.00% and 54.76%).

When C. carpio exposed to acutely toxic concentrations of SDS the respiratory activity showed depleted oxygen consumption in fish during first day (60.97%) and they were able to consume more oxygen up to 96 h (89.47%) at 20.0 mg/l. With 22.0 mg/l, oxygen consumption was significantly increased from 1st day to 4th day (48.78% and 92.10%). In case of SLS with two concentrations (8.0 and 9.0 mg/l), the consumption of oxygen increased up to 3rd day in both the concentrations (85.71% and 52.11%) and suppressed oxygen was noticed on 4th day in both concentrations. Similarly with two concentrations of Triton X-100 (3.0 and 4.0 mg/l), the respiratory activity was suppressed during first day and they were able to consume more oxygen up to 96 h (77.58%) at 3.0 mg/l. With 4.0 mg/l though the respiratory activity of the
fish decreased on 2nd day the uptake of oxygen increased on 3rd and 4th day (58.33% and 65.51%).

In case of phenol with two concentrations increased oxygen uptake was observed during first day (91.42%) and suppressed gradually up to 4th day (57.50%) at 20.0 mg/l. With 25.0 mg/l, the respiratory activity of the fish significantly increased from 1st day to 4th day (45.00% and 81.42%). Influence of p-cresol on the oxygen consumption was found to be quite suppressed in both the concentration of p-cresol (18.0 and 22.0 mg/l) up to 96 h.

In the present study significant reduction in oxygen uptake lower levels of detergents and phenolic toxicants might be due to the mucus secretion which clogs the gills. Higher concentrations of detergents and phenolic toxicants might have induced the fish to consume more oxygen particularly during the later part of the tests. This probably indicates that more oxygen is required by the fish to meet their metabolic demand under detergents and phenolic toxicants stress.

A number of workers have studied the alterations in oxygen consumption. Barbieri et al., (1998), studied increase in the oxygen consumption as increase in the lethal concentrations of SDS to fish C.carpio. In the present study it is evident that O2 consumption increases throughout the exposure period and in higher concentrations also the O2 consumption was increased till the end of the experiment in C.carpio exposed to two lethal concentrations of SDS. In fish O.mossambicus it is found that alterations in the
oxygen consumption. The present findings are consistent with the results of above authors. (Barbieri et al., 1998).

Garasangi (Dissertation, 1997), reported that *L. guntea* exposed to lethal concentrations of phenol showed reduced in oxygen consumption after 96 h. In the present investigation for fish *O. mossambicus* and *C. carpio* exposed to two lethal concentrations of phenol showed alterations (increase or decrease) in the oxygen consumption. At lower concentration (at 20.0 mg/l) of phenol for fish *C. carpio* showed significant reduction in the oxygen consumption throughout the experiment. The results obtained in the present findings are well in agreement with the above authors.

Asheera Banu Sangli and Kanabur (2000), studied the effect on oxygen uptake for fish *G. affinis* exposed to lethal concentrations of cresol. Alterations (increase or decrease) in the oxygen consumption was noticed throughout the exposure period. Kanabur and Asheera Banu Sangli (1998), reported acute toxicity of cresol to freshwater fish *L. guntea*. Here also found that increase or decrease in the oxygen consumption up to 96 h. Asheera Banu Sangli and Kanabur (2000), studied the variations in the oxygen consumption exposed to lower concentrations of cresol for fish *L. guntea*. In the present study alterations in the oxygen consumption was observed upto 96 h for fish *O. mossambicus* exposed to p-cresol. Where as in *C. carpio* the oxygen consumption was significantly reduced upto 96 h exposed to p-cresol. The results obtained in the present investigation are well in agreement with the above authors.
In the present study decrease in oxygen uptake of the fish under phenolic compounds may be attributed to the reduced gill permeability and which may cause respiratory distress finally led to the death of the fish (Lloyd, 1961).

Very little work has been carried out in oxygen consumption for fish to SLS and Triton X-100

During sublethal treatment the fishes viz., *O.mossambicus* (SDS, SLS and phenol) and *C.carpio* (SDS and phenol) were exposed to two different concentrations of detergents and phenolic toxicants (1/10th and 1/5th of 96 h LC50), for a period of 30 days and the alterations in the oxygen consumption rates were studied at intervals of 4th, 10th, 20th and 30th days of the treatment. The results are presented in Table 3.15 and 3.16.

The oxygen uptake rate by the fish *O.mossambicus* to SDS (1.5 mg/l), significantly declined and reached a value of 74.54% after 30th day of the treatment. On exposure to 3.0 mg/l, the oxygen consumption was decreased from 4th day to 30th day (125.00% and 76.29%). Similarly O2 consumption of the fish exposed to SLS (0.85 and 1.7 mg/l), the respiratory activity was increased up to 10th day (120.00%) and it showed reduction after 30th day (51.11%), at 0.85 mg/l. With 1.7 mg/l, the oxygen consumption values reached a maximum of 120.93% on 4th day and decreased gradually after 30th day of the experiment (60.00%). In case of phenol the oxygen uptake of exposed fish increased on 4th day and suppressed on 10th day. They were able to consume
more oxygen on 20th day (110.00%). Later it reduced to 33.33% (after 30th day) at 3.5 mg/l. With 7.0 mg/l oxygen consumption rate reached maximum of 104.00% on 4th day and decreased gradually after 30th day of the experiment to 45.00%.

Similarly for C. carpio the rate of oxygen uptake of the exposed fish to SDS (2.46 mg/l) significantly depleted and reached a value of 50.90% after 30th day of experiment. With 4.92 mg/l, it increased up to 10th day (127.77%) and it reduced to 60.00% after 30th day. With two sublethal concentrations of phenol (3.04 and 6.08 mg/l), a significant reduction was found from 4th day to 30th day (105.45% and 36.53%) at 3.04 mg/l. With 6.08 mg/l, the respiratory activity significantly suppressed from 4th day to 30th day (120.83% and 57.14%).

Exposure of animals to sublethal levels of detergents and phenolic toxicants may inflict stress on the mechanisms required for maintaining a healthy physiological state.

The rate of oxygen consumption in fishes has been identified as an indicator of intensity of metabolism Fry, 1957, 1971; Singh and Singh, 1979; in fishes any change from normal/control value might reflect on alteration in the respiratory epithelium of the gill. Gills are considered as the main osmoregulatory surface organs in fishes and are primary site of uptake of water borne pollutants (Evans, 1987). The intimate contact of the gill with water borne pollutants may lead to alterations in the respiratory surface area (Singh
and Singh, 1979), in turn lowering the diffusing of the gills (Katz, 1979; Hughes, 1980). Therefore gills may be the first site where the sublethal effects of pollutants would be observed (Lauren and McDonald, 1985). The most important aspect is the reduced oxygen consumption, which would create a physiological imbalance. These changes are related to metabolic rate, which is estimated indirectly by oxygen consumption.

Studies on metabolic rates therefore provides clues to the chemical mode of action in addition to revealing an important sublethal effect. Many workers have investigated, the effects of various substances on metabolic rate of fishes. (Calabrese et al., 1975; Hughes, 1980a; Watenpaugh and Beitinger, 1985). Lloyd (1960), suggested that the damage to the gill tissue was responsible for the death. Because of the breakdown of its vital function leading to decreased efficiency for gas exchange (Hughes, 1980b). Since where animal oxygen consumption may be altered at the gas exchange surface which is directly proportional to the activity of ectotherms (White, 1982). Many pollutants irrespective of type firstly cause damage to the gills (Skidmore and Tovell, 1972) and this might be expected to affect ion and water balance as well as respiration. It may be mentioned here that decrease in oxygen uptake in the fish exposed to detergents and phenolic toxicants may be attributed to atrophy of the respiratory epithelium enlargement of the water/blood barriers of the gill and reduced gill diffusing capacity.
Similar reports have been made by Samson Raju et al., (1994), reported that sublethal exposure to fish, *O. mossambicus* for ‘Ariel’ detergent, the respiratory lamellae showed drastic changes in separation of epithelial layer around respiratory lamellae and atrophy of respiratory lamellae. These pathological changes in respiratory gill might have resulted in such a shift from aerobic to anaerobic pathway in tissues of fish under aerial exposure.

Therefore the prime importance of the present investigation lies in the fact that, the results provide an insight into the metabolism especially the rate of O₂ uptake which is the index of sources of energy utilisation.

The results of the quantitative study of total glycogen and protein are presented in Table 4.1-4.18. Variations in the mean hepatic glycogen was observed in gill muscle and intestine exposed to two lethal concentrations of SDS. Decreased glycogen level was found in brain below the control level. Enhanced glycogen was noticed after 96 h in liver at 11.0 mg/l exposed to SDS for *O. mossambicus*.

Significant increase in the glycogen was found in liver at 4.0 and 5.0 mg/l in the early period of exposure, later decreased glycogen was observed (after 96 h), which was well above the control level. Variations in gill, muscle and brain glycogen was noticed. In intestine the glycogen level decreased after 96 h in both concentrations exposed to SLS for *O. mossambicus*.

The mean hepatic glycogen level decreased in liver after 96 h at 31.0 mg/l. Increased glycogen level observed after 96 h at 33.0 mg/l in liver.
Alterations (increase or decrease) in glycogen level was found in gill, muscle, brain and intestine, exposed to two lethal concentrations of phenol for fish *O. mossambicus*.

Increase or decrease of glycogen level was noticed in gill muscle brain and intestine. All the values remain well above the control level. But in liver at 20.0 mg/l, significant increase in the glycogen was observed in the early period of exposure, later it decreased (after 96 h). Which remain well above the control level. The liver glycogen level was increased upto 48 h at 22.0 mg/l, but decreased at the end of the exposure, after exposing them to lethal concentration of SDS for fish *C.carpio*.

The increased glycogen level was noticed at 20.0 mg/l in liver and brain after 96 h. At 25.0 mg/l, maximum increase of glycogen was found in the early period of exposure, later it significantly depleted (after 96 h) in liver gill and brain. Similar condition was noticed in intestine at 20.0 mg/l. Variations in glycogen content observed in muscle tissue exposed to two lethal concentrations of phenol for *C.carpio*.

In *O. mossambicus* exposed to two lethal concentrations of SDS, the total protein content was decreased in gill and intestine, below the control level. Alterations in the tissue protein content was noticed in liver, muscle, and brain throughout the exposure period.

Similarly in *O. mossambicus* treated with two lethal concentrations of SLS, decreased tissue protein was observed in gill and intestine from the
control level. Alterations of tissue protein content was indicated by fish in muscle. In liver at 4.0 mg/l, gradual increased protein content was noticed after 96 h. At 5.0 mg/l in brain enhanced protein level was observed after 96 h.

Whereas in phenol exposed to *O. mossambicus* of two lethal concentrations, increased protein level was found after 96 h in liver at both the concentrations. And depleted level of protein was observed in brain and intestine, below the control level. Alterations of protein was noticed in gill and muscle.

In fish *C. carpio* indicates alterations in the protein level in gill muscle and intestine. In liver tissue, protein content was increased after 96 h. Same trend was observed in brain tissue, exposed to two lethal concentrations of SDS.

Similarly in liver and gill protein was decreased after 96 h at 25.0 mg/l. Increase or decrease of protein was indicated in fish muscle brain and intestine exposed to two lethal concentrations of phenol for fish *C. carpio*.

A significant increase in the mean hepatic glycogen content in liver was found after end of the experiment at 1.5 mg/l exposed to sublethal concentrations of SDS. At 3.0 mg/l a maximum increase in the glycogen content was observed up to 20th day and later it was depleted. (after 30th day). Slight increase in the glycogen was noticed, in brain and intestine after 30th day treatment at 1.5 mg/l. Variations in glycogen was found in gill (which was
below the control level) muscle and brain exposed to two sublethal concentrations of SDS for fish *O. mossambicus*.

In brain the mean hepatic glycogen content showed increase after 30th day, at 7.0 mg/l. In liver, gill muscle and intestine, the glycogen level altered during the sublethal exposure of two phenol concentrations for *O. mossambicus*.

Similarly in *C. carpio* exposed to two sublethal concentrations of SDS, showed a significant increase in the liver (at 4.92 mg/l) gill, muscle and brain (at 2.46 mg/l) after 30th day of exposure. Glycogen content was depleted in liver (at 2.46 mg/l) muscle (at 4.92 mg/l) and in both concentrations of intestine.

In *C. carpio* exposed to two sublethal concentrations of phenol, the glycogen content decreased after the 30th day treatment in gill (at 3.04 mg/l) and in muscle (at 6.08 mg/l). Similarly a significant increase in the glycogen level was observed after 30th day in liver at 6.08 mg/l. With slight increase in the glycogen level was also observed in brain (at 6.08 mg/l) and intestine (at 3.04 mg/l). The glycogen level was altered in remaining tissues at different concentrations.

In fish *O. mossambicus* the liver protein increased at the end of the experiment (after the 30th day) at 1.5 mg/l, but the protein content was depleted after the 30th day of exposure in liver at 3.0 mg/l. In gill tissue protein content also decreased at the end of the treatment at 3.0 mg/l. Same trend was observed
in intestine at 1.5 mg/l. Alterations (increase or decrease) of protein. Content observed in muscle and brain, exposed to two sublethal concentrations of SDS for fish *O. mossambicus*.

Similarly in phenol for fish *O. mossambicus* increased protein was observed at the end of treatment at 3.5 mg/l in liver. At 7.0 mg/l depleted protein was observed after 30th day of exposure in liver. The similar condition was noticed in gill and brain tissue protein at 3.5 mg/l. Variations in protein was observed in muscle and intestine exposed to two sublethal concentrations of phenol.

Whereas in *C. carpio* exposed to two sublethal concentrations of SDS showed increased protein content after 30th day in liver at 2.46 mg/l. And depleted protein was found at the end of the exposure at 4.92 mg/l in liver. Similar trend was observed in gill protein at both concentrations and also in muscle protein at 4.92 mg/l. In brain, at both concentrations slight increased protein was observed after 30th day exposure. Variations in the protein content was noticed in intestine.

The gill tissue protein depleted at the end of the experiment in both concentrations. And slight depleted protein was also found after end of exposure in intestine at 3.04 mg/l. In liver muscle and brain alterations in tissue protein content was found, after exposing them to two sub lethal concentrations of phenol.
In the present study an increase in the liver glycogen and decrease in the muscle glycogen was observed in fishes. The decrease in the tissue glycogen content due to an imbalance in the endocrine control of the carbohydrate metabolism (Haux and Larsson, 1984). A rise in the synthesis of glycogen from a non carbohydrate source to carbohydrate mediated during the stress by adrenocorticoids (Larsson et al., 1976). A fall in the content of muscle glycogen may be attributed to the impaired secretion of insulin from the exposed fish (Shaffi, 1978; Lowe-Jinde and Niimi, 1984).

In the present study, a decrease/increase in the glycogen was noticed in the gill tissue at lethal and sublethal levels of exposure. Van Den Thillart, (1982) and Tort et al., (1984), reported that glycogen is used at higher rates in the glycolytic pathway when hypoxic conditions are present which need extra energy to restore normally.

In the brain tissue a rise and fall in glycogen content after detergent and phenolic toxicants intoxication at lethal and sublethal levels may be due to the accumulation of toxicants in the organ. It is possible that increased glycogenolytic activity and/or gluconeogenesis may be mediated through harmonal or neuromuscular changes.

Similar observations have been made by other fishes exposed to phenols. Ravichandran et al., (1994), investigated in the exposed fish O.mossambicus the protein content increased throughout the exposure period in liver tissue. In muscle it was increased up to 72 h and depleted after 96 h. In
the present investigation the liver protein was increased up to 96 h in both lethal concentrations (31.0 and 33.0 mg/l) of phenol exposed to *O. mossambicus*. In case of *C. carpio* the liver protein enhanced till the end of the experiment (after 96 h) at 20.0 mg/l, but in 25.0 mg/l, the tissue protein level decreased gradually up to 96 h. The present findings are consistent with the results of above author (Ravichandran *et al.*, 1994).

Indra *et al.*, (1999), reported that sublethal exposure of *O. mossambicus* to phenol toxicity, the protein content in the liver tissue decreased after 30 days of exposure. In the present study exposed to sublethal toxicity of phenol, the tissue liver protein increased gradually up to 30th day at 3.5 mg/l. With 7.0 mg/l, the liver protein content significantly declined after 30th day in *O. mossambicus*. Whereas in *C. carpio* exposed to sublethal toxicity of phenol found that alterations in the liver protein throughout the exposure period. The present findings are well in agreement with the above authors (Indra *et al.*, 1999).

Hazari Lal *et al.*, (1984), studied the sublethal exposure of *Cirrhina mrigala* to detergent linear alkyl benzene sulphonate toxicity. The protein content increased after 30 days of exposure.

It was suggested that the retardation in protein content indicate the tissue protein undergoes proteolysis resulting in the production of free aminoacids. Decrease in the protein level may indicate a reduced rate in their synthesis or enhanced mobilization to other needy tissues (Murthy and Devi, 1982).
Palanichamy et al., (1989), have suggested that the decline in the protein content in the tissue may be due to intensive proteolysis in all tissues. Bakthavathsalam (1980), have concluded that increasing trend of protein content in the liver might be due to greater concentration of the enzymes and decrease in proteins content may be due to lesser activity of enzymes.

There is not much work has been carried out on total glycogen and protein content estimation. But a standard work (Polyacrylamide gel electrophoresis SDS-PAGE and separation of protein from SDS) has been reported on biochemical aspects to the detergents SDS and SLS.

Behaviour has long been recognized as an excellent way to assess the condition and well being of a particular organism. Behavioural and characters may be associated with normality and well being while other may be associated with illness, injury, and fatigue. In fish behavioural indications of substandard performances or lack of general well being might include difficulty in maintaining equilibrium inability to keep pace with school and loss of swimming speed. As a consequence, behaviour is recognized as an integrator of physiological condition. Behavioural studies of individual detergents and phenol toxicants were studied (Chapter- III).

At lethal exposure the symptoms of poisoning in fish were observed for individual exposure to SDS, SLS phenol and para-cresol to O. mossambicus and C. carpio. The fish were restless and aggressive swimming was observed with attempts to jump out of the media. Loss of sensitivity was also observed with
reduced excitation and loss of equilibrium. There was excessive mucus secretion and fish died with mouth wide open and haemorrhage beneath the pectoral fin. Fish rolled over and died with bent bodies.

Rajendra Nayak and Madhyastha (1987), reported that changes in the behaviour of fish *R. daniconius* exposed to detergents toxicants of SLS and ‘Point’ to lethal and sublethal concentrations. The fish in the lethal concentrations of detergents exhibited many changes in their behaviour. The effect of detergent on the lateral fins sense organ was evident when they started rolling along their sides. The activation of mucus cells of the body as well as gills was evident from the presence of mucus balls at the bottom of the aquarium. The dead fish were slippery to touch exhibiting hyper secretion of mucus. The gills exhibited ruptured epithelial cells. When exposed to sublethal toxicity of 3.2 mg/l of detergent ‘Point’ and 0.127 mg/l of SLS stray incidents of hyper activity was observed. In the lethal (acute) level ranges of the commercial detergent ‘Point’ and SLS, the fish abdomen enlarged due to the accumulation of air bubbles. Rossini *et al.*, (1996), reported the acute toxicity bioassay using fish and *Daphnia obtusa* (Cladocerons) as a test organism to SDS, phenol and other chemicals. They observed that at 24 and 48 h of lethal exposures shows sensitivity within the range.

Cairns and Scheier (1962), observed excessive secretion of mucus in *L. gibbosus* and *L. macrochirus* exposed to acute and chronic levels of detergent, standard alkyl benzene sulfonate. The secretion of the mucus might be due to
possess a protective layer around the body and gills. Schmid and Mann (1961), suggested that the increase in the number of opercular movements due to an impairment of gaseous exchange on the gill surface, when exposed to 20.0 mg/l of detergent dodecyl benzene sulfonate on *L. gibbosus*.

Isomaa and Paatero (1981), reported changes in the shape and volume of erythrocytes incubated with surface active detergents of SDS and alkyl trimethyl ammonium salts. Bolis and Rankin (1978), reported the concentration vasodelation in isolated perfused gills of pink salmon, *O. gorbascha*, coho salmon, *O. Kisutch* and chum salmon, *O. Keta*, which were exposed to SLS. Dose dependant cytotoxic effects like the degeneration of the living cells exposed to anionic active detergent have also been reported by Poplar-Gubo and Hans (1982). Mann (1955), observed action of surface active washing agents to fish.

The decrease in the feeding rate, spitting out the feed after ingestion, and inability to recognize the feed in fish exposed to sublethal concentration of SLS and ‘Point’ detergent have also been reported by Bardach *et al.*, (1965) and Cairns and Loos (1967). In fish exposed to detergents Foster *et al.*, (1966), observed a decrease in the feeding rate of the fish *J. floibae* exposed to detergent ABS. Barbieri *et al.*, (1998), reported behavioural changes exposed to detergent SDS to fish *C. carpio* as the SDS concentration increases swimming capacity decreases for different lethal concentrations (1ppm, 5ppm, 10 ppm). Hazari Lal *et al.*, (1984), reported the behavioural pattern of fish fingerlings
(Cirrhina mrigala) exposed to synthetic detergents. Anderson (1971), reported behavioural features provide useful measures of sublethal toxicity because they represent the integrated results of any biochemical process. Jones (1951), reported the reactions of minnows to phenolic toxicant.

Chemical agents may be behaviourally toxic even though symptoms of structural or biochemical toxicity are not detectable (Thompson and Lilja 1964). The introduction of a chemical substance may (1) increase the time required to learn to escape or avoid noxious stimuli (2) decrease the animals sensitivity to subtle changes in the environment or (3) interfere with animal ability to retain previously learned behaviour. In the present investigation undertaken by us efforts were made to study the effect of detergents and phenolic toxicants to O.mossambicus and C.carpio fishes.

Fishes avoided the lethal concentrations of individual toxicants. However it was observed that at sublethal exposure, fishes preferred the polluted zone, relatively over the zone with food adour O. mossambicus and C.carpio. It is evident that the toxicant (detergent and phenols) lured the fishes to the toxicant zone inspite of the food odour to which they had been previously conditioned. The results are presented in Table 5.1-5.9.

Lethal concentrations of (Toxicant Zone), SDS, SLS, phenol and p-cresol was avoided by both O.mossambicus and C.carpio and preferred the fresh water zone.
Similarly fishes exposed to sublethal (with food odour) toxicity to *O.mossambicus* avoided toxicant zone and preferred food odour region of SDS, SLS and p-cresol of lower concentrations and both concentrations of phenol. Where as in *C.carpio* preferred toxicant zone when exposed to SDS. Similar trend was observed when they exposed to SLS and in phenol at higher concentration.

Fishes exposed to sublethal doses without food odour in *O.mossambicus* preferred the fresh water zone of SDS, phenol and p-cresol. Similar condition was noticed in *C.carpio* exposed to phenol and p-cresol. But when the fish *O.mossambicus* exposed to SLS, preferred equally to both toxicant and freshwater region, but *C.carpio* preferred toxicant zone of SLS.

In a mixture of sublethal doses of SDS and SLS both fishes preferred unpolluted region. In case of Phenol and p-cresol mixture, exposed to both fishes preferred toxicant zone. The same trend was observed when both fishes exposed to SDS and phenol mixture.

Study of preference and avoidance of sublethal doses of three detergent mixtures was undertaken to evaluate the effect of increase in mixture concentrations and it was found that higher concentrations of detergents and phenolic compounds preferred by the fish to the polluted region.

Exposed to three mixture concentrations of sublethal doses to *O.mossambicus* and *C.carpio* preferred the toxicant zone, but they avoided the fresh water region.
However fishes preferred SLS over SDS when given a choice in a *O. mossambicus*. but *C. carpio* preferred SDS over SLS at the sublethal concentration. Thus it is understood that SDS for *O. mossambicus* and SLS for *C. carpio* were irritant and repellent agent.

In case of phenol and p-cresol for *O. mossambicus* and *C. carpio* preferred phenol over p-cresol. p-cresol thus understood that it is irritant and repellent agent.

The fish *O. mossambicus* preferred the phenol over SDS in both sublethal concentrations, but *C. carpio* preferred SDS (at 1/10).

The ability of fishes to discriminate between changes in concentration of detergents and phenolic toxicants was verified. The higher concentration of SDS, phenol and p-cresol was preferred by *O. mossambicus*. Whereas in *C. carpio*, avoided the higher concentration and preferred lower concentration of SDS, phenol and p-cresol. In the observation the preference to mixing zone is considered to be the most adverse response, since the toxicant mixture and concentration increases before draining (Cherry and Cairns, 1982). In a mixture of three detergents and phenolic toxicants higher concentrations of such mixture was preferred in general.

Avoidance behaviour of carp to pesticides and decrease of the avoidance threshold by addition of sodium lauryl sulfate (SLS) observed by Ishida and Kobayashi (1995). They examined the behavioural avoidance of anionic surfactant sodium lauryl sulfate (SLS) was the most effective repellant in carp.
and the threshold was 10-2 μg/l. The addition of 1% SLS to the concentration series of fenitrothion (pesticide) decreased the avoidance threshold. The threshold for the mixture of fenitrothion and SLS was 1 μg/l.

In the present study *O. mossambicus* was avoided SDS and preferred SLS. Whereas in *C. carpio* SLS was avoided and preferred SDS. Thus it is understood to be SLS for *C. carpio* were irritant and repellent agent. The present findings are consistent with the results of above authors. (Ishida and Kobayashi, 1995).

There is not much work has been carried out on preference and avoidance reactions of fish to SDS, phenol and p-cresol.

Shelton (1971), concluded that the muscular expansion and contraction of buccal and opercular activities maintains a flow of water over gill surfaces in fishes. Mc Leay and Brown (1975), have shown that muscular exertion in fish is accompanied by a marked increase in blood lactate. Mazeaud *et al.*, (1977), studied increase in circulating catacolamines and plasmatic carticosteroids due to stress in fish. Exposure of fish to elevated level of surfactant cause multiple hepatomos to develop on the gill tissue resulting in diminished oxygen consumption and impairment of electrolyte balance (Schmid and Mann 1961). Schooling pattern of fish have been reviewed by Partridge (1982). Avoidance behaviour of carp to pesticides and decrease of the avoidance threshold by addition of sodium lauryl sulfate (SLS) observed by Ishida and Kobayashi (1995). Detergents cause impairment of chemoreceptor organs (Bardach *et al.*, 186).
1965) and damage to epidermis and pharyngeal wall (Brown et al., 1968). Behavioural changes have been used successfully as rapid and sensitive indicators of toxic stress in fish (Sprague et al., 1965; Sprague and Drury, 1969; Sprague, 1973; Bengtsson 1974; Thatcher, 1966 and Besh et al., 1977).

The results obtained support the concepts proposed by Thompson and Schuster (1968), that first those chemical agents that produce only behavioural changes that have serious and possibly irreversible deleterious effects on the animals ability to adopt may be identified and controlled. Second the identification of behavioural toxic effects of chemical agents may provide an early warning system which may allow the chemical toxicity before irreversible, structural and biochemical damage has occurred.

The present findings are consistent with the results of several other investigators on the mixture toxicity of various types of toxic substances as fish and other aquatic organisms. Thus it is concluded that whenever the experimental data on the toxicity of a specific mixture are not available, concentration additivity may be a reasonable assumption it hazard assessment procedures.