CHAPTER-III
RESULTA AND OBSERVATIONS

Behavioral changes are the most sensitive indication of potential toxic effects. The behavior of an organism represents the final interrelated result of a diversity of biochemical and physiological processes. Thus, behavior is considered as promising tool in toxicology. The freshwater fish N. botia when exposed to different concentrations of tributyltin oxide showed behavioral changes. The test animals abnormal behavior varied according to the test solution concentration. Behavioral changes were observable within the initial hours. The control fish behaved in a natural manner, they were active and well coordinated movements. They were alert to the slightest disturbance. In the control, there were no detectable behavioral changes or deaths observed till the end of experiment. In 24h and 48h exposure of tributyltin oxide, the fish showed abnormal swimming and tended to gather at the surface. Fish exposed to 72h and 96h lethal concentrations of tributyltin oxide demonstrated abnormal behavior changes. They tried to avoid the toxic water with fast swimming, the fish were observed to have breathing difficulties and tried to breathe air from the surface water. They slowly become lethargic, restless and secreted excess mucus all over the bodies. Opercular movements increased initially in all exposure period but decreased later steadily. The increased cough rate, flaring of gills, increase in production of mucus from the gill and hitting against the wall of aquarium were noticed for all exposure period but the severity was observed more in 96hrs exposure period. Fish exposed to 96hrs concentrations tried to avoid the toxic water with fast swimming and jumping, and they showed jerky movements. Lastly, they settled on the bottom of aquarium, and after some time their bellies turned upward and the fish died. The major cause of mortality might be due to respiratory epithelium damage by oxygen culminating in the formation of a mucus film over the gills of fish and intervening in enzyme activity in respiratory metabolism.

TOXICITY:

For the experimentation and before each change of water animal behavior was recorded. After every 24h of the treatment, the mortality was recorded which is necessary for establishment of LC$_{10}$ and LC$_{50}$ concentrations for 24h, 48h, 72h and 96hrs exposure. Results for acute toxicity for tributyltin oxide to the freshwater fish
*N.botia* are summarized in (Table, 1-4 and Fig.1-4). The results are expressed in table and graphical form.

The LC\(_{50}\) values were calculated for 24h, 48h, 72h and 96hrs by Finney’s method (1971). The results of acute toxicity are summarized in (Table, 5). The LC\(_{50}\) values obtained for tributyltin oxide exposed for 24h, 48h, 72h and 96hrs exposures were 0.01852 ppm, 0.0153 ppm, 0.01311 ppm and 0.01099 ppm respectively.

In the present investigation, LC\(_{50}\) values up to 96hrs exposure period indicated high toxicity. The variance ‘V’ of LC\(_{50}\) for 24h, 48h, 72h and 96 hrs were recorded 0.00031, 0.00024, 0.00019 and 0.00039 ppm respectively (Table, 5).

The calculated minimum and maximum fiducial limits of tributyltin oxide for 24h, 48h, 72h and 96 hrs were 2.233 to 2.3026, 2.154 to 2.2152, 2.091 to 2.1447 and 2.002 to 2.0797 ppm respectively (Table, 5).

The Chi-square values were calculated, these values were used to test homogeneity of data, (Table, 5).

Lethal dose obtained for tributyltin oxide were 24h, 48h, 72h and 96 hrs were 0.4445 ppm, 0.7344 ppm, 0.9439 ppm and 1.055 ppm respectively, (Table, 5).

The safe concentration of tributyltin oxide 0.00253 ppm was recorded (Table, 5).

The results obtained for toxicity evaluation of tributyltin oxide on *N.botia* indicated that tributyltin oxide found to be toxic as exposure period increases.

**RESPIRATION:**

The rate of oxygen consumption in fish, *N.botia* exposed to tributyltin oxide is summarized in table No. 6 and fig 5. The rate of respiration showed in the control group 0 hr it was 0.139 ±0.04 mg/hr/gm body weight, at 24 hr was 0.130 ± 0.05 mg/hr/gm body weight, at 48 hr it was 0.131 ± 0.05 mg/hr/gm body weight, at 72 hr it was 0.120 ± 0.03 mg/hr/gm body weight and at 96 hr it was 0.101 ± 0.06 mg/hr/gm body weight. Upon exposure to LC\(_{10}\) concentration of TBTO, the rate of oxygen consumption at 0 hr was 0.137 ±0.03, after 24 hr was 0.093 ± 0.04 mg/hr/gm body weight, after 48 hr it was 0.088 ± 0.04 mg/hr/gm body weight, after 72 hr it was 0.077 ± 0.02 mg/hr/gm body weight and after 96 hr it was 0.0.068 ± 0.02 mg/hr/gm body weight. When exposed to LC\(_{50}\) concentration, the rate of oxygen consumption at 0 hr it was 0.138 ± 0.004, after 24 hr was 0.077 ± 0.005 mg/hr/gm body weight, after 48 hr it was 0.067 ± 0.05 mg/hr/gm body weight, after 72 hr it was 0.062± 0.04 mg/hr/gm body weight, after 96 hr it was 0.049 ± 0.03 mg/hr/gm body weight.
When compared with control values after 24 hr of exposure to LC_{10} concentration there was significant decrease in the rate of oxygen consumption 25\% (P<0.001). Similarly, after 48 hr of exposure to LC_{10} concentration the oxygen consumption decreased to 32.29\% (P<0.001). After 72 hr of exposure to LC_{10} concentration the oxygen consumption decreased to 47.23\% (P<0.001). While at 96 hrs of exposure to LC_{10} concentration there was an increase in the rate of oxygen consumption up to 58.88\% (P<0.001). Similarly, when compared with control values after 24 hr of exposure to LC_{50} concentration, there was significant decrease in the rate 39.09\% (P<0.001) and after 48 hr of exposure to LC_{50} concentration the rate was decreased to 45.79\% (P<0.001). After 72 hr of exposure to LC_{50} concentration the rate of oxygen consumption was decreased to 50.47\% (P<0.001) and after 96 hr of exposure the rate was decreased to 56.48\% (P<0.001).

**BIOCHEMICAL:**

Effects of different concentration of tributyltin oxide were studied to determine the biochemical constituents in gill, liver and kidney of fish, *N.botia*. The tissues were analyzed to observe the effect after 24h, 48h, 72h and 96hrs exposures for the lethal concentration (LC_{50}). The data were supported to various statistical analysis and the variance, standard deviation and standard error of the mean were calculated. Students ‘t’ test was used to find out significance. The level of significance was used in the present study (p<0.05, p< 0.01 and p<0.001), Mungikar, (1997).

Biochemical alterations in freshwater fish *N.botia*, due to TBTO acute toxicity were studied. The animals were exposed to acute concentration for duration of 96hrs.

In the gill of control fish, the total protein was observed (64.12 ± 1.602). The fish exposed to 0.01852 ppm, 0.0153 ppm, 0.01311 ppm and 0.01099 ppm induced significantly depletion in protein content (62.46 ± 2.776, 8.38\%), (57.73 ±0.016 ,14.86\%), (54.20 ±0.015, 19.97\%) and (50.21 ± 0.04, 30.11\%) mg at 24h, 48h, 72h and 96 hrs respectively, the results are summarized (Table-8 of Fig-7). In the liver of control, the observed protein content was (89.20±4.24). In the liver of experimental animals the protein content were significantly decreases (76.11±1.602, 17.04\%), (68.13±4.240, 24.42\%), (58.86±2.776, 33.66 \%) and (53.60±2.776, 42.85\%), mg at 24h, 48h, 72h and 96hrs respectively, the results showed in (Table, 8 of Fig-7). In the kidney of control fish, protein content found to be (72.53±1.602). In the kidney of experimental the protein content depletion were recorded and the observed values
were (69.26±4.240, 13.29%), (65.86±5.778, 20.50%), (54.73±3.205, 26.60%) and (49.60±2.76, 33.66%), at 24h, 48h, 72h and 96 hrs respectively, the results showed in (Table-8 of Fig-7).

Fresh water fish, N.botia exposed up to 96hrs tributyltin oxide acute concentration. The gill of control fish, the total lipid level was observed (1.87±0.04). The fish exposed 0.01852 ppm, 0.0153 ppm, 0.01311 ppm and 0.01099 ppm concentration showed significant decline in lipid content when compared to control. In the experimental fish gill lipid content recorded (1.68±0.04, 18.10%), (1.49±0.04, 26.01%), (1.20±0.04, 30.34%) and (0.98±0.04, 45.21%), % mg at 24h, 48h, 72h and 96hrs respectively, the results showed in (Table-9 of Fig-8). In the liver of control, the lipid content was (2.42±0.03). In the liver of experimental fish the lipid content significantly decreases (1.92±0.04, 21.82%), (1.69±0.05, 39.17%), (1.28±0.05, 46.88%) and (1.20±0.03, 53.22%), % mg at 24h, 48h, 72h and 96hrs respectively, the results showed in (Table-9). In the kidney of control fish, observed lipid content found to be (1.67±0.04). In the kidney of experimental animals decline in the lipid content were recorded and the observed values were (1.58±0.036, 7.62%), (1.49±0.03, 12.29%), (1.46±0.07, 26.23%) and (1.17±0.03, 30.25%), % mg at 24, 48, 72 and 96 hours respectively, the results showed in (Table, 9 of Fig-8).

For the acute concentration of tributyltin oxide exposed N. botia up to 96 h the total glycogen content in the gill was (9.15±0.08). The fish exposed to acute concentrations glycogen content was recorded (7.92±0.03, 6.93%), (7.24±0.04, 8.15%), (6.54±0.06, 16.82%) and (5.67±0.06, 27.52%), % mg at 24h, 48h, 72h and 96 hrs respectively, the results are summarized in (Table, 7 of Fig-6). In the liver of control fish, the glycogen content was (19.20±0.070). In the liver of experimental fish, decline in the glycogen content not found significant when compared with control. The values recorded (15.52±0.076, 15.20%), (14.82±0.034, 17.17%), (12.80±0.045, 35.79 %;) and (10.52±0.045, 47.71%), % mg at 24h, 48h, 72h and 96hours respectively, the results showed in (Table, 7 of Fig. 6). In the kidney of control glycogen content found to be (12.83±0.01). In the experimental glycogen were recorded (11.25±0.05, 2.58%), (10.32±0.04, 3.3%), (9.56±0.045, 4.73%) and (8.25±0.01, 47.55%), % mg at 24h, 48h, 72h and 96shrs respectively, the results showed in (Table,7 of Fig. 6).

In the present study results clearly indicate that biochemical constituents of protein, lipid and glycogen in the gill, liver and kidney of the test animal N.botia
decreases significantly as the exposure period increases and indicated exhaust or mobilization of organic constituents from the tissue for completing various metabolic activities under stress condition of tributyltin oxide.

**HISTOPATHOLOGY:**

Teleost fish have five pairs of gill arches. In the front four pairs the slender gill filament form two lines facing towards the back and these two lines fused to each other at the base by gill septum. Each gill arch supports one set of gill filaments. The gill reekers help to make sure that no foreign materials get in to filaments to clog them up. Each paired gill filament in turn supports numerous lamellae extending out from both side of the filament body. Lamellae are the actual organ where exchange of gases takes place. Numerous semicircular secondary gill lamellae are lined up along both sides of the primary gill lamellae. The primary gill lamellae consist of centrally placed rod like supporting axis with blood vessels on either side. The secondary gill lamellae also termed as respiratory lamellae, are highly vasulirised and covered with thin layer of epithelial cells. The region between the two secondary gill lamellae is known as inter lamellae space. The gill epithelium is the dominant site of gas exchange, ionic regulation, acid- base balance and nitrogenous waste secretion ammonia.

The gills being delicate structure and ever first organ comes in direct contact with pollutants in aquatic animals and rapidly exhibits histopathological changes. This is well reflected in the damage of respiratory epithelium and secondary gill lamellae of exposed fish in the present study. Marked histological changes observed in gills of fish *N. botia* exposed to lethal concentration. Observations of the sections of gills under the light microscope as well as in micro photographs made it clear that there have been drastic effect of TBTO at all exposure which has affected gills of test fish *N. botia*. In the present study the damages of gills exposed to 24h lethal concentration of TBTO were observed in primary and secondary gill lamellae. Gills of fishes were found to have histological lesions. The secondary lamellae which are the main sites of gas exchange in the gill breathing fishes were seen swollen and abnormal. The tips of primary as well as secondary lamellae were seen club-shaped by lamellar fusion (Plate-1, Fig B), and shortening of the length of secondary lamellae also observed. The fish *N. botia* exposed to 48h lethal concentration the symptoms observed were similar as observed in 24 h lethal concentration exposre but the severity of lesions were found more (Plate-1,Fig-C). Shortened and fusion of secondary gill lamellae,
vascular degeneration, bulging of tip of gill filament and the developments of vacuoles in the epithelium were noticed in the 72h and 96hrs lethal concentration exposure of TBTO. Large number of mucus cells in the gill heads and gill rakers were observed. At 96hrs exposure the damage of gills are severe. Shortened and clubbing of ends of secondary lamellae, fusion of adjacent secondary gill lamellae, and necrosis in primary gill lamellae were well noticed. Curling of secondary gill filaments, hyperplasia and hypertrophy of nuclei were seen in 72h and 96hrs exposer. Besides these changes pyktonic nuclei, vacuolization, degeneration of epithelial and pillar cells and lifting of secondary gill lamellae were common and significant in 24h,48h,72h and 96h exposers of TBTO.(Plate-1 Fig-A,B,C,D and E)

The liver is a central metabolic organ in fishes and has numerous anabolic and catabolic functions. The normal liver is a bilobed orange colored organ. The surface of liver is covered by a thin membrane and some connected tissues extending inward in to parenchyma. The parenchyma tissue is composed of parenchymal cells (hepatic cells) Hepatic cells are roundish polygonal with spherical nucleus and granular cytoplasm. Hepatic cells are located around the sinusoids forming cord like structure called hepatic cell cords, pancreatic tissue can be differentiated from hepatic tissue by its acinar arrangement.

Histological changes in the liver of fish _N. botia_ exposed to 24h lethal concentration of TBTO showed vacuolization in the cytoplasm, degeneration of hepatic cells and changes in the shape of hepatocytes (Plate -2, Fig-B). The decrease in the size of the hepatic cells was also observed due to shrinkage of the cell. The nuclei become piknotic and eccentric. In 48h exposer similar histological changes were noticed but in addition to these changes hypertrophic nuclei and hyperplasia of cells and degeneration of cell membranes commenced (Plate-2, Fig-C). Damage becomes more pronounced by 24h and 48h exposer and is more severe in 96hrs exposer of TBTO. Induced histological changes observed in this exposer period observed in drastic change in shape and more decrease in the diameter of the hepatic cell compare to 24h and 48h of exposer. Many of the hepatic cells ruptured, extensive degeneration of hepatocytes, vacuolisatin was prominent. Aggregation of hepatic cells, hyperplasia, disappearance of hepatic cell wall, more gaps between the cells due to aggregation and destruction of blood sinusoids were also noticed (Plate-2, Fig-D and E).

Kidney is a vital organ of excretion and osmoregulation in fish species. It is highly susceptible to toxic substances because of rich blood supply. In fish, as in
higher vertebrates, the kidney performs an important function to maintain homeostasis. The kidney being the only organ to remove toxic substances is affected by contaminated water. Histological alteration can be used as indicators of the effect of pollutants on the organisms including fish.

Kidney in teleost fish is elongated bodies extending along the whole length of visceral cavity. They are situated on the dorsal side of the body wall. Teleostean kidney consists of head and body. Head kidney is the anterior portion of the kidney and consists of lymphoid tissues. Body kidney consists of numerous functional excretory units, the nephrons. Each nephron consists of renal corpuscles and a long convoluted uriniferous tubule. The renal corpuscle is made up of a doubled walled cup the Bowmen’s capsule and a knout of arterioles glomerules. Renal tubules are thin and short in the neck segment. The renal tubules are composed of cuboidal epithelial cells, Cilia and microvilli are present in the tubular lumen while distal convoluted segment, epithelial cells have no microvilli (Plate-3, Fig-A).

On microscopic examination of kidney of the fish *N. botia* treated with 24h and 48h lethal concentration exposor of TBTO , necrosis of cell and renal tubules, swelling in renal tubules, degeneration of cytoplasm within nuclei, disorganisation of connective tissue (haemopoitic tissue) were observed. The disintegration of cell membrane, hypertrophy of nuclei, vacuoles and swelling of glomeruli were also seen (Plate-3, Fig-B and C). As the exposure period increases the severity of damage also observed in more intensity. At the 96hrs exposure vacuolization, degeneration of cell membrane, necrosis, damage of hematopoietic tissue and renal tubules and hypertrophy of nuclei were much pronounced. In addition to this aggregation of cells and disintegration of glomeruli were also seen at 96hrs exposure. (Plate-3, Fig-D and E)