Chapter 7

Effects of chlorpyrifos on morphology of tissues in *Oreochromis mossambicus*

Contents

7.1 Introduction
7.2 Materials and methods
7.3 Results
7.4 Discussion
7.1 Introduction

The study of structural damage of organs or tissues is an integral part of pollution toxicology. Apart from haematological and biochemical effects, pathoanatomical and histopathological changes in fish tissues and pollutant accumulation in fish are also investigated as part of the toxicity test procedure. Histopathology is an effective tool to visualize the stress-induced structural changes in cells and tissues.

Organisms have tremendous capacity to overcome the environmental stress conditions and thus to maintain the homeostasis. Cells, which have reached their limit of adaptability, begin to show structural changes, which indicate their failure to withstand the changed environment. If adverse conditions persist or if the initial pathological stimulus is severe, then these processes continue and progress into a sequence of events leading to cell death (Varanasi et al, 1989; Jehosheba, 2004). The extent of severity of tissue damage of a particular compound as toxicant depends on its toxic potentiality in the tissues of organisms (Murthy, 1986). Various chemicals with their varied mode of action in different tissues bring about certain architectural changes ultimately culminating in either death of the organism or making the organism less viable for survival.

The methods of established value in the evaluation of environmental impact at the organ and cellular levels are histology and cytology (Braunbeck et al, 1990). Histopathological techniques are rapid, sensitive, reliable and comparatively inexpensive tools for the assessment of stress-response to pollutants. Hence an attempt has been made to observe the possible structural changes in gill, liver and brain tissues of the teleost fish.

Gill is the major route of entry of any pollutant by virtue of its immediate contact with the medium. Uptake of xenobiotics from water by fish is determined by numerous factors, the most important of which are the transfer capacity of the gills and the physico-chemical properties of the compound. According to Murthy (1986)
respiratory distress is one of the early symptoms of pesticide poisoning and gills take part in metabolism and elimination of xenobiotics.

Liver, the first organ to face any foreign molecule through portal circulation is subjected to more damage (Jayantha Rao, 1982). The parenchymatous hepatic tissue has many important physiological functions and also detoxication of endogenous waste products as well as externally derived toxins, drugs, heavy metals and pesticides (Roberts, 2001a). Fish liver is particularly susceptible to chemical damage.

In the teleost nervous system, pathological changes related to neurons and their processes are only occasionally observed, and it is in the meninges and blood vessels, and the microglia, that the principal changes associated with neural dysfunction are normally observed. Brain plays a regulatory role in fish physiology and it is the most important organ in fish toxicology especially when pesticides are involved in their mode of action in the nervous system (Ware, 1983).

Hence the sublethal effects of chlorpyrifos on morphology of gill, liver and brain tissues from *O. mossambicus* were studied

### 7.2 Materials and methods

Collection, maintenance of fish and feeding were done as described before in the section 3.2.B.

A group of 6 fishes without sex determination, in duplicate were exposed to 27.3 ppb (1/3 sublethal concentration of LC50 value) for observing the histopathological changes. The test solution and the water in which fishes were maintained, were renewed daily. Fishes were randomly selected from control groups and treated groups, for histopathological observations by sampling after 21 days of pesticide exposure with the sampling after 7 days also.

The tissues were collected by dissecting the fishes and transferred to 10% neutral formol saline immediately and kept for fixation for 24 hours. These tissues
were dehydrated in ascending graded series of alcohol and were cleared in xylene until they became translucent. Then the tissues were transferred to molten paraffin wax for 1 hour to remove xylene completely and impregnated with wax. Then the blocks were cut in a microtome to prepare sections of thickness 3 to 5 microns. Wax was removed in xylene and then cleared in descending graded series of alcohol and then brought to water. The sections were stained with haematoxylin and eosin and mounted in DPX (Roberts, 2001b) and observed under light microscope.

7.3 Results

The results of histological studies of gill, liver and brain tissues of *O. mossambicus* of control and actone control, and those exposed to sublethal concentration of chlorpyrifos are given in plates 3-23. The structural alterations were observed under light microscope in the sections of gill, liver and brain tissues of fish from treated group. The tissues of fishes from chlorpyrifos-treated groups appeared in a structure different from those of control group fishes.

The gill tissue of the control was observed as shown in plate 3. Generally, the gills of *O. mossambicus* comprised two sets of four holobranchs, forming the sides of the pharynx. Each holobranch consisted of two hemibranchs projecting from the posterior edge of the branchial arch or gill arch in such a way that the free edges diverged and touched those of the adjacent holobranchs. Close examination of the hemibranchs of a fresh gill shows that they consist of a row of long thin filaments, the primary lamellae, which project from the arch like the teeth of a comb. The surface area of each primary lamellae is increased further by the formation of regular semi lunar folds across its dorsal and ventral surface-secondary lamellae. The dorsal and ventral rows of secondary lamellae on each primary lamellae are staggered so that they complement the spaces in the rows of lamellae of adjacent filaments.

Gill tissue of fish exposed to acetone control is given in plate 21. It appeared in its intact architecture, similar to that of control. The changes observed in the gill tissue on exposure to chlorpyrifos for 7 days (Plate 4&5) included hyperplasia of
Light photo micrograph of cross-section of gill tissue of *Oreochromis mossambicus* showing histopathological effects of technical grade chlorpyrifos

Plate 3: Control (20x)
Plate 4: exposed for 7 days showing desquamated and necrosed areas (arrowed) (20x)
Plate 5: exposed for 7 days showing secondary lamellae clubbed (arrowed) (20x)
Light photo micrograph of cross-section of gill tissue of *Oreochromis mossambicus* showing histopathological effects of technical grade chlorpyrifos exposed for 21 days

Plate 6: hy-hyperemia (20x)
Plate 7: loss of architecture (20x)
Plate 8: completely necrosed and desquamated gill filaments (20x)
Light photo micrograph of cross-section of liver tissue of *Oreochromis mossambicus* showing histopathological effects of technical grade chlorpyrifos

Plate 9: Control (40x)
Plate 10: Control showing hepatopancreas (arrowed)(40x)
Plate 11: exposed for 7 days showing swelling, rounding off and detachment of cells; pa-abnormally structured acinar cells showing necrosis of pancreas (40x)
Light photomicrograph of cross-section of liver tissue of *Oreochromis mossambicus* showing histopathological effects of technical grade chlorpyrifos
Plate 12: exposed for 7 days showing rounding off and detachment of cells (40x)
Plate 13: exposed for 21 days showing regenerating cells (arrowed) (40x)
Plate 14: exposed for 21 days showing vacuolated areas with fat deposition (40x)
Light photo micrograph of cross-section of brain tissue of *Oreochromis mossambicus* showing histopathological effects of technical grade chlorpyrifos

Plate 15: control (20x)

Plate 16: exposed for 7 days showing vacant areas of degenerating neurons (arrowed)(40x)

Plate 17: exposed for 7 days showing areas of degenerating neurons (arrowed)(40x)
Light photo micrograph of cross-section of brain tissue of *Oreochromis mossambicus* showing histopathological effects of technical grade chlorpyrifos

Plate 18: exposed for 21 days showing encephalomalacia (marked) (40x)

Plate 19: exposed for 21 days showing demyelinated areas and pycnotic nuclei (arrowed) (40x)

Plate 20: exposed for 21 days showing demyelinated areas and pycnotic nuclei (arrowed) (40x)
Light photo micrograph of cross-section of tissues of *Oreochromis mossambicus* showing histopathological observation
Plate 21: gill tissue exposed to acetone for 21 days (20x)
Plate 22: liver tissue exposed for 21 days (40x)
Plate 23: brain tissue exposed for 21 days (40x)
epithelial lining of secondary lamellae leading to their fusion and clubbing. Many epithelial cells appeared necrosed and desquamated from the mucosa.

The changes observed in the gill tissue on exposure to chorpyrifos for 21 days (Plate 6, 7&8) included hyperemia indicated by engorged capillaries. Complete loss of architecture of gill filaments was observed. Completely necrosed and desquamated gill epithelial cells and increased mucus production were also seen on prolonged exposure to chlorpyrifos.

The liver tissues of pesticide treated fishes showed structural alteration unlike those from control group (Plate 9 &10). Generally, the liver in teleost fish is a compound organ in the form of hepatopancreas. Sinusoids, which are irregularly distributed between the polygonal hepatocytes, are fewer in number and are lined by endothelial cells with very prominent nuclei. Hepatocytes are polygonal and have a distinctive central nucleus with densely staining chromatin margins and a prominent nucleolus.

The liver tissue of fish exposed to acetone (Plate 22) appeared in regular network pattern of functional cells. The changes observed in the liver tissue on exposure to chlorpyrifos for 7 days are shown in Plates 11&12. The changes included swelling and rounding off of hepatocytes, detachment of cells from each other. Pancreatic acini appeared to have lost its architecture. Cytoplasm of hepatocytes became more basophilic.

The changes observed in the gill tissue on exposure to chorpyrifos for 21 days are shown in Plate 13&14. The cells had distinct vacuoles in cytoplasm indicating fatty change. Necrotic areas appeared prominent. Hyperplasia or regenerating hepatic cells at certain regions were observed.

Brain tissue from tilapia is generally as shown in Plate 15. In the brain of fishes, five major regions are distinguished. They are telencephalon, diencephalon, mesencephalon, metencephalon and myelencephalon. In fishes, the roof of the telencephalon is covered with membranous tissue and lateral ventricle does not exist.
The diencephalon is the region that contains the third ventricle and is composed of the distinct components- epithalamus, thalamus and hypothalamus. The epithalamus consists of pineal body and the habenular nuclei, which connects with thalamus. The hypothalamus is more readily defined and usually relatively large in fishes. It appears to comprise mainly nuclei responsible for coordination of forebrain stimuli and lateral line impulses. The mesencephalon is relatively large and anatomically subdivides into the optic tectum, which provides the roof of the third ventricle, and the tegmentum, which is its floor. It contains the center of the visual sense, as well as the integration center between this sense and the other senses of locomotion.

The cerebellum or metencephalon occupies the interior portion of the dorsal wall of the fourth ventricle and is composed of a cortex and medulla. The metencephalon is the integration center between the auditory sense and the sense of the lateral line.

The brain tissue of fish exposed to acetone (Plate 23) did not show any deterioration in its structure and appeared in a structure similar to that of control. Exposure On exposure to chlorpyrifos for 7days, the major change observed in brain tissue was degenerating neurons, vacant areas (Plate 16&17). Prolonged exposure to chlorpyrifos (Plate 18, 19&20) resulted in encephalomalacia. Presence of pycnotic nuclei with demyelinating neurons was also observed.

7.4 Discussion

Cellular responses to pollutant-induced sublethal injury provided highly sensitive indicators of environmental impact (Hose et al., 1996).

The gills are among the most delicate structures of the teleost body. Gill is a multifunctional organ involved in gaseous exchange, acid base balance, transport of \( \text{Na}^+ \), \( \text{Ca}^{2+} \), \( \text{Cl}^- \) and nitrogenous secretion (Perry, 1997). Morphological studies have provided further support for the idea that opacular membranes and skin are excellent models for branchial salt secretion (Foskett et al, 1982). Their external location and intimate contact with the water make them liable to damage by any irritant materials,
whether dissolved or suspended, in the water. The most frequently observed changes when the gill filaments come in contact with chlorpyrifos—an irritant—is swelling of lamellar epithelial cells or edema of the subepithelial space.

Lamellar edema is most frequent following the exposure to chemical pollutants such as heavy metals, red tides and certain pesticides (Roberts, 2001a). Increased mucus secretion renders a defense mechanism to the stress by the pesticide but the same fails when it is exposed for a prolonged time.

Hyperplasia was generally more pronounced towards the distal tip of the lamellar filament resulted in clubbing. As the time of exposure to chlorpyrifos increased, the different lamellar filaments became desquamated and completely lost their architecture indicating its failure to overcome the stress. Hyperemia with excess blood in the vessels supplying gill filament was observed with increased number of RBCs. This observation can be correlated with the increased RBC count in haematological examination, after 21 days of exposure of fish to chlorpyrifos.

Gill is the first organ that comes in direct contact with the insecticide. Binding of hydrophobic organophosphate with various lipid and protein groups of gill epithelial cells might be the reason for altered structure of gill filaments. In the present study, on exposure to chlorpyrifos, morphological observation can be correlated with the inhibition of ATPase enzymes involved in ion transport mechanism in microsomal preparation of the gill epithelial cells. Loss of structural integrity of the gills may easily lead to a drop in the concentration of blood electrolytes, such as sodium, chloride and calcium. Mallatt and Stinson (1990) have described that electrolyte loss occurs after exposure of freshwater fish to pesticides.

These findings are in good agreement with the reports of Rao et al (2003) and Jauch (1979). Rao et al. (2003) observed the bulging of secondary lamellae at the terminal ends, lesions and erosions at the base of lamellae on 12th day of exposure of *O. mossambicus* to chlorpyrifos. A thick coat of mucus on the gill filaments was found to be persisting on 18th day of exposure. According to Jauch (1979), fenthion
upon 96-hr exposure induced gill lesions, including hyperplasia and desquamation of the epithelium and thrombosis in the secondary gill lamellae.

A study by Gopalakrishnan (1990) elucidates the effect of organophosphorus pesticide Dimecron on the gill and liver of the fish *Etroplus*. Dimecron induced branchial congestion in the gill filaments. Edematous fluid lifted the respiratory epithelium in a few secondary lamellae, which were found thickened. The cells between the secondary lamellae were thickened to such an extent that the inter-lamellar spaces occluded, which gave the filament a compact appearance.

Epithelial hyperplasia with lamellar fusion, epithelial hypertrophy, telangiectasia, edema with epithelial separation from basement membranes, general necrosis and/or epithelial desquamation have reported following exposure to DDT and malathion (Walsh and Ribelin, 1975) and exposure to paraquat dichloride (Hendricks, 1979).

Liver tissue after 7 days of exposure showed swelling and rounding off of hepatocytes and the starting of detachment of cells from each other. The pancreatic acinar cells appeared to lose the normal architecture. They found to be detached from the surrounding hepatic cells. The cytoplasm of the cells became more basophilic indicating the protein precipitation leading to the non-functional condition of hepatic tissue. On prolonged exposure to chlorpyrifos, vacuolated areas seen might be indicating fatty changes. This may subsequently lead to fibrotic changes. Hyperplasia observed at certain regions might be the feature of regenerating hepatic tissue. This might be the reason for slightly elevated ALT and ALP levels in hepatic tissue after 21 days of exposure to chlorpyrifos. These damages can result from a wide range of stimuli, from long-standing biliary obstruction, heavy metal or pesticide poisoning. In aquatic system, fish liver ultrastructure has proved to be particularly susceptible to low levels of environmental contaminants (Braunbeck et al, 1990).

Sudha Singh et al. and Tilak et al have also reported these changes. A Study by Sudha Singh et al. (1998) reported clubbing, vacuolation and also necrosis of...
pancreatic tissue by the exposure of endosulfan and carbaryl on *Nandus nandus*. Intercellular spaces and spaces around pancreatic mass were also seen. They observed that after a long-term exposure of both endosulfan and carbaryl, the liver tissues were converted into spongy mass. The pancreatic tissues were seen shrunken and scattered due to heavy necrosis of hepatic mass.

Tilak et al. (2005) observed the similar changes in liver of *Catla catla*. The pathological changes included degeneration of cytoplasm in hepatocytes, atrophy, formation of vacuoles, and rupture in blood vessels, necrosis and disappearance of hepatocyte cell membrane disposition. Hepatic cords are found to be decreased in size and nucleus became pyknotic.

Radhaiah and Jayantha Rao (1992) reported moderate cytoplasmic degeneration in hepatocytes, formation of vacuoles, rupture in blood vessels and appearance of blood vessels among hepatocytes and pyknotic nuclei in the hepatic tissue of *T.mossambica* exposed to fenvalerate.

In the present study, brain tissue, after short-term exposure to chlorpyrifos, the vacant areas appeared due to the degenerating neuron as shown in Plate 16 &17. Encephalomalacia observed on prolonged exposure to chlorpyrifos, might be because of these degenerating and demyelinating neurons in the damaged brain tissue under toxic stress induced by chlorpyrifos. The present observations are in consonance with those from the studies by Tilak et al (2005). Since chlorpyrifos is an organophosphorus compound and organophosphates are neurotoxins, the chlorpyrifos intoxication caused chromatolysis, i.e. Dissolution of the nissel bodies.

The organophosphorus compounds are hydrophobic in nature; they have more affinity towards membrane lipids. The reactive species of xenobiotics produced by metabolism, bind to cell macromolecules including DNA, RNA and protein (Robert, 1990). In the present study, affinity of chlorpyrifos to the membrane lipid might have favoured it to pass across the blood-brain barrier. Further interaction of the insecticide with the neuronal cell membrane and cellular organelles might have led to their degeneration. Chlorpyrifos might have bound with various
lipid and protein groups making their metabolism impaired. Myelin sheath was seen as damaged. The degenerated neuronal cells might have been replaced by vacant areas resulting in encephalomalacia in brain tissue. Recent investigations have provided further evidence that organophosphorus insecticides, besides their typical action as inhibitors on AChE, interfere with the allosteric behaviour of the enzyme through interaction with the membrane lipids (Domenech et al., 1977).

Tilak et al (2001a) observed similar changes in *Labeo rohita* under chlorpyrifos toxicity. (Yacobu, 1999) reported swelling of axon, atrophy, necrosis and pyknotic nuclei in the brain tissue of the fish *Ctenopharyngodon idellus* under fenvalerate toxicity and severity of damage is more in lethal exposures than in sublethal exposure. Chlorpyrifos technical grade caused more degenerative changes in brain than in 20%EC exposure.

The present study revealed a visual evidence of sterical derangement in gill, liver and brain tissues of *O. mossambicus* on sublethal exposure to chlorpyrifos. The impairment of the cellular antioxidant defense or increases in the production of highly reactive free radical species may be the primary cause of cellular injury.