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# Effect of Chlorpyrifos on Histology of Liver, Gills, and Brain of *Oreochromis Mossambicus* (Peters)

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***Abstract***

*Most of the river waters in Kerala are found to have several pollutants such as heavy metals, plasticizers, and pesticides. Chlorpyrifos, commonly known as Dursban is a broad-spectrum organophosphate insecticide widely used in Kerala to control insects in crops, termites and to control mosquito larvae. This pesticide is known to reach the aquatic ecosystem causing several damages to aquatic organisms. Fish being on top of the food chain bioaccumulation of these pesticide residues are of concern. This study was to find the histological changes in the liver, brain, and gills of Orechromis mossambicus, commonly called Tilapia, a common freshwater edible fish, on exposure to chlorpyrifos for 7, 14, and 21 days to evaluate the extent of toxicity. For this fish, Orechromis mossambicus of age one to three were procured from a local fish farm of Pathanamthitta district, Kerala, and were acclimatized in tanks in the laboratory and exposed to chlorpyrifos for 7, 14, and 21 days and histology of brain, liver, and gills were carried. The microphotographs of the sections show severe damage to these tissues as early as 14 days of exposure.*

**Keywords:** *Oreochromis mosambicus, chlorpyrifos toxicity, histology, liver, brain, gills*

## 1. Introduction

In recent decades the level of pollutants in aquatic ecosystems such as heavy metals, pesticides, and others have increased alarmingly because of domestic, industrial, and agricultural effluents (Ondarza et al., 2011). Contamination of water by insecticides is mainly due to intensive agriculture combined with surface runoff and subsurface drainage, usually within a few weeks after application (Banerjee et al., 2011). Chlorpyrifos (0,0-diethyl-0-(3,5,6-trichloro-2-pyridyilphosphorothioate, CPF) is commonly known as Dursban is a broad-spectrum organophosphate insecticide widely used to control foliar insects in crops (Rusyniak and Nanagas, 2004) and subterranean termites (Venkateswara Rao, 2005) and is an irreversible inhibitor of choline esterase in all animal species. It is the second-largest-selling organophosphate agrochemical in India (Mathur and Tannan,1999).

Among aquatic vertebrates, fishes have been largely used as bioindicators to evaluate the quality of aquatic ecosystems in general and environmental pollution through their structural, functional, and behavioral patterns. Many of the toxic contaminants at sub-lethal levels, in the aquatic ecosystem, can cause noticeable effects on the physiology of fish. Histological investigations of different tissues exposed to pollutants are useful for toxicological studies and monitoring water pollution. Tissue alterations in fish exposed to a different concentration of insecticides are a functional response of organisms which provides information on the nature of the toxicant. Thus, histology is a suitable biomarker in the evaluation of the health of an organism exposed to pollutants and can be used as bio monitoring tool for toxicity studies (Meyers and Hendricks, 1985). Histological changes are the result of the integration of a large number of interactive physiological processes, therefore, it helps to identify target organs of toxicity and mechanism of action (Ramalingam et al., 2000).

*Oreochromis mossambicus* has been selected as the test animal in this study because of its extraordinarily hardy nature to adapt to different aquatic systems, ease of reproduction, ability to adapt to different artificial culture systems, omnivorous nature, and general hardiness in the culture environment.

## 2. Materials And Methods

### Collection and maintenance of test animals

The euryhaline teleost cichlid fish, *Oreochromis mossambicus* (Tilapia) was selected for the present study. Fishes aged one to three were collected from the local fish farm, Pannivelichira fish farm, Pathanamthitta, Kerala, in an oxygenated polythene bag to the laboratory and acclimatized for 10 days in laboratory conditions in a glass tank before the start of the experiment.

### Experimental protocol

*Oreochromis mossambicus* was exposed to sub-lethal concentration (1/3 of LC50 value) of technical grade chlorpyrifos [$LC_{50}$ 82ppb = 82 µg/L] for a period of 7, 14, and 21 days.

Fishes were randomly selected from the control group and treated groups. Sampling was performed after 7, 14, and 21 days of exposure to the pesticide, for histological studies of the gill, brain, and liver.

### Histological studies

The histological investigations were carried out in the liver, brain, and gills of fishes treated with sub-lethal concentrations ($LC_{50}$) of chlorpyrifos after 7, 14, and 21 days of exposure. Histological sections of the liver, brain, and gills were taken according to standard procedure (Humason, 1972). The liver, brain, and gills were dissected out and fixed in 10% formalin. They were processed for histological examination adopting the standard method, such as fixation, dehydration, embedding, sectioning, and staining. Formalin was used for fixation and the tissue was dehydrated by treating them in various grades of alcohol ranging from 30% to 100% followed by clearing them with xylene until they become translucent. 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 rotary microtome to prepare sections. The sections were stained with hematoxylin and eosin and mounted in DPX. Histological sections were examined with the help of a compound microscope and the photomicrograph of the stained slide was taken, analyzed, and interpreted.

## 3. Results and Discussion

In the present study the histological sections of all the tissues- liver, brain, and gill, under study revealed several structural alterations under a light microscope. The tissues of fishes from chlorpyrifos-treated groups appeared in a structure different from those of the control group fishes.

The liver, the first organ to face any foreign molecule through portal circulation is subjected to more damage. The parenchymatous hepatic tissue in teleosts has many important physiological functions and also detoxifies endogenous waste products as well as externally derived toxins, drugs, heavy metals, and pesticides. 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 seen to be lined by endothelial cells with very prominent nuclei. Hepatocytes were often swollen with glycogen or neutral fat. In the present study, the changes observed in the liver tissue (Plate 1d) included swelling and rounding off of hepatocytes, and detachment of cells from each other. Pancreatic acini appeared to have lost its architecture.

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 the lateral ventricle does not exist. The diencephalon is the region that contains the third ventricle and is composed of distinct components- epithalamus, thalamus, and hypothalamus. The epithalamus consists of the pineal body and the habenular nuclei, which connect with the thalamus. The hypothalamus is more readily defined and usually relatively large in fish. It appears to comprise mainly nuclei responsible for the 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.

On exposure to chlorpyrifos for 7 days, the major change observed in brain tissue was degenerating neurons. Prolonged exposure to chlorpyrifos resulted in encephalomalacia. The presence of pycnotic nuclei with demyelinating neurons was also observed (Plate. 2b).

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 toughed 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 lamella is increased further by the formation of regular semilunar 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.

The diffusion of a xenobiotic across the gill epithelium will depend largely on lipid solubility. Generally, the teleost gill arch is a curved osseous structure from which radiates the bony supports of the primary lamellae of which the surface area is increased further by the formation of regular semilunar folds called the secondary lamellae.

On short-term exposure to pesticides, the changes observed in gills were hyperemia, clubbing, and edema. After 21 days of pesticide exposure, gills became edematous with prominent clubbing (Plate 3d). Separation of primary gill lamellae and hemorrhage in the blood vessels outside the secondary gill lamellae were observed. Hyperemia of gill filaments that engorged with blood vessels appeared. Hyperplasia was observed in secondary gill lamellae, which led to the fusion of adjacent primary and secondary gill filaments.

**Sections showing the histological effects of chlorpyrifos on the Liver, Brain, and Gill tissues of Orechromis *mossambicus* (Tilapia)**

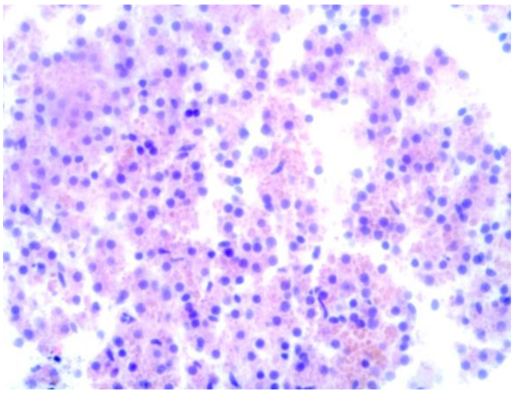

**Plate 1a shows the control liver**

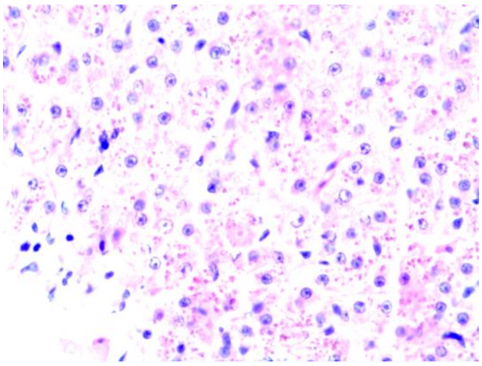

**Plate 1b shows the liver of Fish exposed to 7 days**

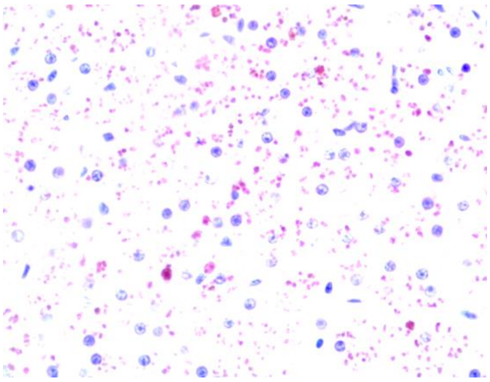

**Plate 1c showing the liver of Fish exposed to 14 days**

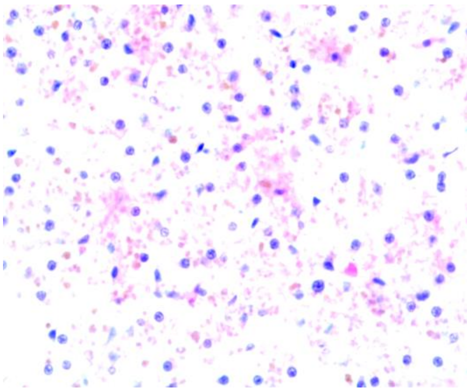

**Plate 1d showing the liver of Fish exposed to 21 days**

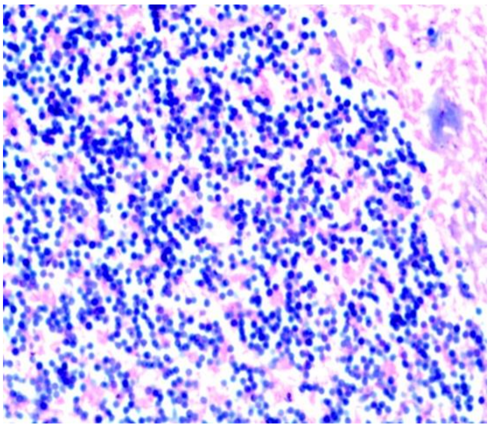

**Plate 2 shows the control brain**

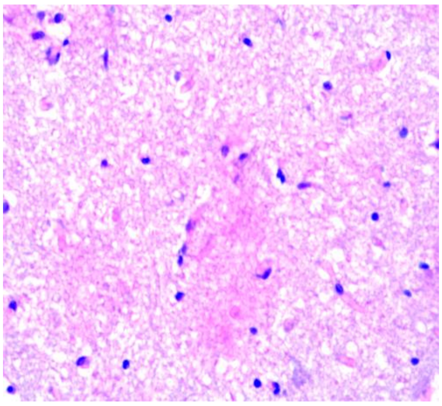

**Plate 2b shows the brain of Fish exposed to 7 days**

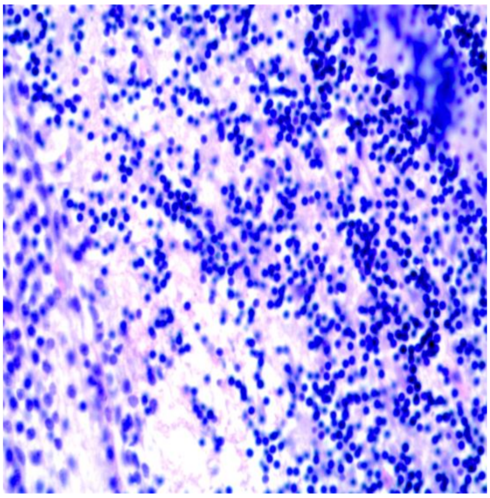

**Plate 2c showing the brain of Fish exposed to 14 days**

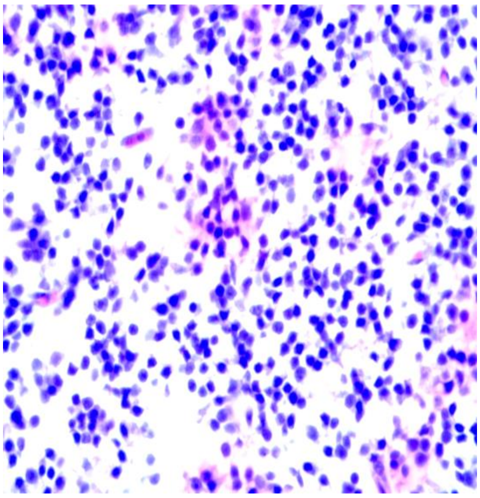

**Plate 2d showing the brain of Fish exposed to 21 days**

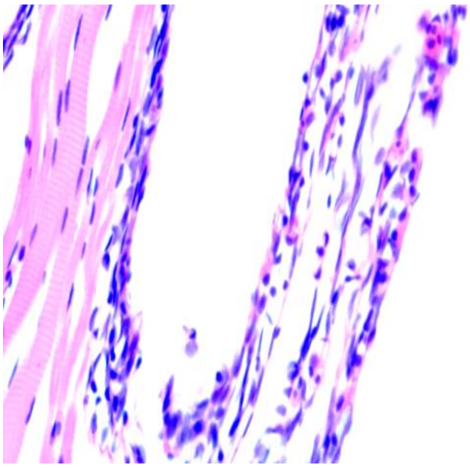

**Plate 3 shows the control gills**

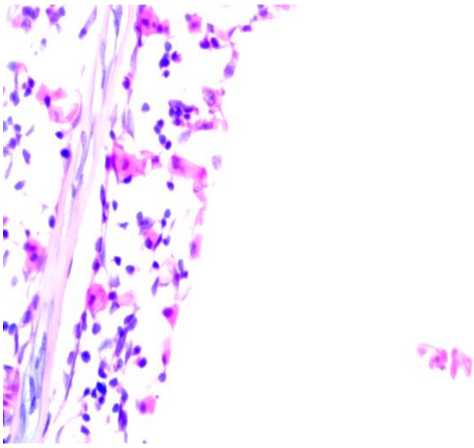

**Plate 3b shows the gills of Fish exposed to 7 days**

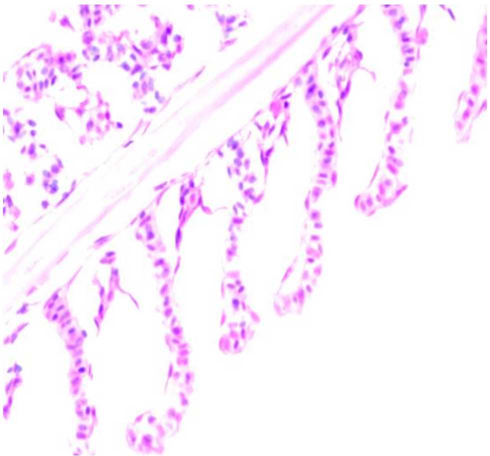

**Plate 3c showing the gills of Fish exposed to 14 days**

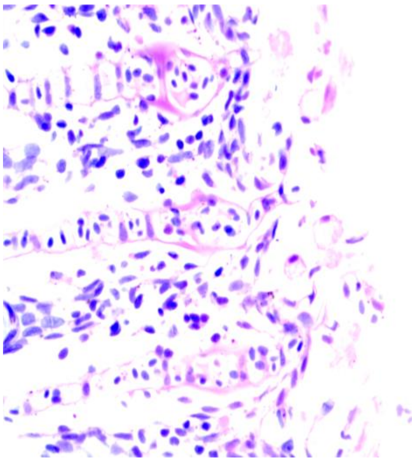

**Plate 3d showing the gills of Fish exposed to 21 days**

The present investigation aimed at delineating the toxic effect of chlorpyrifos on the histological changes in commercially important freshwater fish, Tilapia (*Oreochromis mossambicus*). It was confirmed that Chlorpyrifos, even in microgram quantities can induce toxicity to fish.

Histological observations revealed deleterious anatomical and morphological alterations in the gill, liver, and brain tissues on exposure to chlorpyrifos. The specific changes in each tissue reveal the incapability of these tissues to withstand the toxic effects induced by chlorpyrifos.

Cellular responses to pollutant-induced sub-lethal 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 the gaseous exchange, acid-base balance, and transport of $Na^{+}$, $Ca^{2+}$, $Cl^{-}$, and nitrogenous secretion (Perry, 1997).

Morphological studies have provided further support 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 gills filaments come in contact with chlorpyrifos an irritant is swelling of lamellar epithelial cells or edema of the subepithelial space. Hyperplasia was generally more pronounced towards the distal tip of the lamellar filament resulting 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.

Gill is the first organ that comes in direct contact with the insecticide. The binding of hydrophobic organophosphate with various lipid and protein groups of gill epithelial cells might be the reason for the altered structure of gill filaments.

Once the toxicants cross the biological barriers and enter the bloodstream, they will reach and accumulate in the fish's internal organs. Numerous studies have quantified contaminants in different fish organs to evaluate environmental quality, seeking causal relationships with fish health, and based on these, the liver is likely to be the best choice. According to Van der Oost et al., 2003, the liver is a detoxification organ and it is essential for both, the metabolism and the excretion of toxic substances in the fish body. According to Mohamed (2009), the liver is also a target organ due to its large blood supply, which causes noticeable toxicant exposure. Moreover, the liver is reported to be the primary organ for bioaccumulation and thus, has been extensively studied regarding the toxic effects of different xenobiotics (van Dyk et al., 2007).

In the present study, brain tissue, after short-term exposure to chlorpyrifos, vacant areas appeared due to the degenerating neurons. 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. Since chlorpyrifos is an organophosphorus compound and organophosphates are neurotoxins, the chlorpyrifosintoxification caused chromatolysis, i.e. Dissolution of the vessel bodies. Recent investigations have provided further evidence that organophosphorus insecticides, besides their typical action as inhibitors on AChE, interfere with the allosteric behavior of the enzyme through interactions with the membrane lipids.

The present study revealed visual evidence of disorganization in the gill, liver, and brain tissues of *Oreochromis mossambicus* on sub-lethal exposure to chlorpyrifos. The present study reveals that the organophosphorus insecticide, chlorpyrifos has the potency to cause histological and biochemical alterations in fish. The observed lower $LC_{50}$ value proved that this pesticide is highly toxic and causes damage to aquatic organisms.

Concerted efforts to reduce the use of pesticides and the promotion of organic farming can help to resolve the problem of pesticide pollution. Regulations that limit the use of pesticides with alternative solutions that are safer and non-toxic to the environment and humans should be encouraged. One such alternative is the “natural pesticides”. These are not synthetically produced but are derived from nature such as botanical, biological agents, and inorganic minerals. These solutions are generally assumed to be less toxic for fish than synthetic pesticides and could represent an interesting alternative.

The present investigation suggests that the natural water bodies must be monitored regularly and precisely for the presence of toxicants like pesticides as they are known to affect aquatic systems including safe fish production.

## 4. Conclusion

Many pollutants exist in the aquatic environment for short or long periods, at sub-lethal levels. These levels are not noticed because they do not cause immediate fish mortality. These can cause morphological and physiological changes, causing illness and reducing fitness for life. The present investigation aimed at delineating the toxic effect of chlorpyrifos on histological and muscle protein in commercially important freshwater fish, *Oreochromis mossambicus*.

Chlorpyrifos present in aquatic ecosystems can affect aquatic fauna in different ways. Alterations in the physicochemical properties of water, destruction of the delicate balance in the environment, entry into the food chain, and physical damage to the vital tissues of aquatic fauna are the threatening issues of modern-day pesticides. Long-term exposure to these products causes countless abnormalities and reduces the lifespan of organisms.

In the present study, the toxic effects of chlorpyrifos on the liver, brain, and gills of the freshwater fish, *Oreochromis mossambicus* were studied. The experimental fishes were divided into control and chlorpyrifos exposed groups. All the experimental fish species were observed after an interval of 7, 14, and 21 days. It was observed that exposure to chlorpyrifos induced drastic changes in the tissues of *Oreochromis mossambicus.*

Based on the histological studies it is evident that chlorpyrifos is highly toxic to fish and imposes a life-threatening effect on fish at sub-lethal concentration and lethal concentrations. Altered histological responses can be used as tools in bio-assessment to monitor eco-toxicological risks associated with pesticides such as chlorpyrifos to various fish.

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