International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 28-07-2022, Accepted: 14-08-2022, Published: 30-08-2022
Volume 7, Issue 8, 2022, Page No. 184-187

Effect of pesticide chlorpyrifos on the gill histology of *Labeo rohita*

G Shakila, R Rajakumar
Department of Zoology, A.V.V.M Sri Pushpam College, (Affiliated to Bharathidasan University), Poondi, Thanjavur, Tamil Nadu, India

Abstract
Acute toxicity (96 h LC_{50} – 0.7 ppm) of chlorpyrifos was evaluated in the freshwater fish *Labeo rohita* in static bioassay over a 96–hours exposure period using probit method. Histopathological investigations revealed various degrees of pathological lesions in gill. The gill showed the fusion, malformation of secondary lamellae at the tips, vaculation, and necrosis and detached of gill epithelium. The histological alterations in the fish could be used as an important tool for assessment of aquatic pollution.

Keywords: *Labeo rohita* - acute toxicity – chlorpyrifos – gill histology

Introduction
The high rate of explosion of human population, increase in urbanization and industrial activities as well as uncontrolled utilization of arable land have created problem of disposal of waste waters. The domestic sewages, untreated or partially treated effluents from industries are supplemented with pollutants such as heavy metals, pesticides and many toxic organic compounds have wholly contributed to massive fish death of aquatic ecosystem. These toxic chemicals and metals have altered the quality of water that affects the health fish and other aquatic organisms (Dhasarathan *et al*., 2000; Pazhanisamy and Indra, 2007) [5, 12]. Hence the present study was carried out to evaluate the chlorpyrifos toxicity in the Indian Major Carp *Labeo rohita.*

Materials and Methods
The fingerlings of the freshwater fish *Labeo rohita* the weight (50±5g) and length (15 ± 0.5 cm) was selected for the experiment and were collected from Kathirasan Aqua farm, Thanjavur. Then healthy fish were then transferred into the glass aquaria (40x20x20) containing de-chlorinated tap water (Temperature 28 ± 2^0C; total hardness 525 ± 31 mg/l; salinity 1.12 ± 0.18 ppt and pH 7.6 ± 0.05). Fish were acclimated to the laboratory conditions for 10 to 15 days before experimentation. Toxicity tests were conducted in accordance to the standard methods (APHA, 1992) [1]. Per cent mortality was calculated and the values were fitted into probit scale. Probit analysis was carried out as suggested by Finney (1971)[8]. Based on the acute toxicity test (96h LC_{50} – 0.7 ppm) sub lethal concentrations (10%) of chlorpyrifos were prepared and were used as the experimental concentration of the chlorpyrifos in the subsequent experiments.
After 30 days of subacute toxicity chlorpyrifos exposed fish *Labeo rohita* was dissected. The target organs of the present study gill was isolated and fixed in formal alcohol. After 24 hours of fixation, the tissues were passed through an ascending series of alcohol for dehydration and then cleared in xylene. The paraffin embedded wax block was sectioned at a thickness of 8 µm with the help of a rotary microtome. The sections were stained with Harri's haematoxylin and then counterstained with 70 % alcoholic eosin (Humason, 1967) [9]. After dehydration the tissue sections on the glass slide was mounted with cover slide using DPX mountant.

Results
Histology of gill in the control fish *Labeo rohita*
Histological organization of the gill of control fish *Labeo rohita* was composed of four pairs of gill arches, which are supported by a skeletal bone. The gill by itself was made up of double rows of gill filaments from which the lamellae arose perpendicularly. The gill filaments arose from the gill arches were supported by cartilage skeleton. They were the primary gill lamellae. The secondary gill lamellae were arose from the primary lamella. The primary lamellae were lined by squamous epithelium which was composed of pavement and undiffentiated cells. Below the epithelium lamellar blood sinuses were separated by pillar cells. Between the lamellae, the gill filament was lined by a thick stratified epithelium which was constituted by several cell types namely chloride, mucous and pavement cells. The secondary lamellae were composed of simple epithelium which were involved in gaseous exchange (Plate – 1 and fig. a).

Histopathology of the gill of 10% sub lethal chlorpyrifos treated *Labeo rohita*

Histopathological alterations of the gill of 10% sub lethal chlorpyrifos treated fish comprised of different histological changes which included the proliferation and thickening of epithelial cells and gill filaments which could ultimately led to the fusion of secondary lamellae (Plate – 1 and fig. b).

Histopathology of the gill of 30% sub lethal chlorpyrifos treated *Labeo rohita*

Significant histopathological abnormalities were observed in the 30% sub lethal chlorpyrifos treated fish constituted a number of uninterrupted lesions on the gill epithelium. Existence of edema throughout the lamellar epithelium was eloquent. Detachment of lamellar epithelium was conspicuous in the present study (Plate – 1 and fig. c).

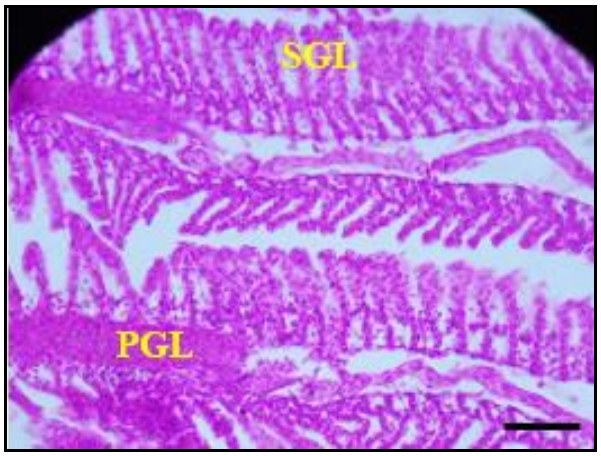

Fig. a. Gill Tissue of Control Fish
PGL – Primary Gill Lamellae
SGL – Secondary Gill Lamellae

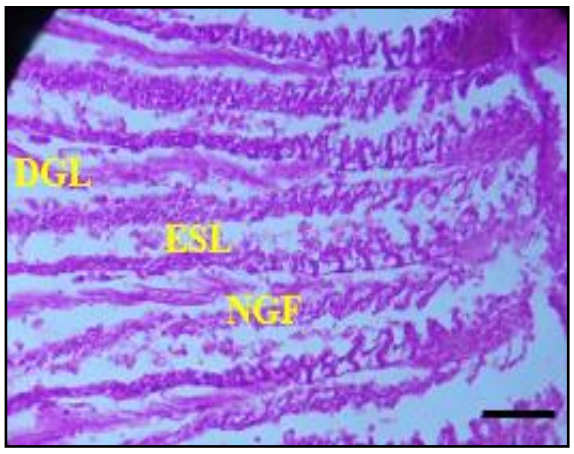

Fig. b. Gill Tissue of 10% SLC Chlorpyrifos treated fish
PGL – Primary Gill Lamellae
GTS – Globular Tips of Secondary Lamellae
FSL – Fusion of Secondary Gill Lamellae

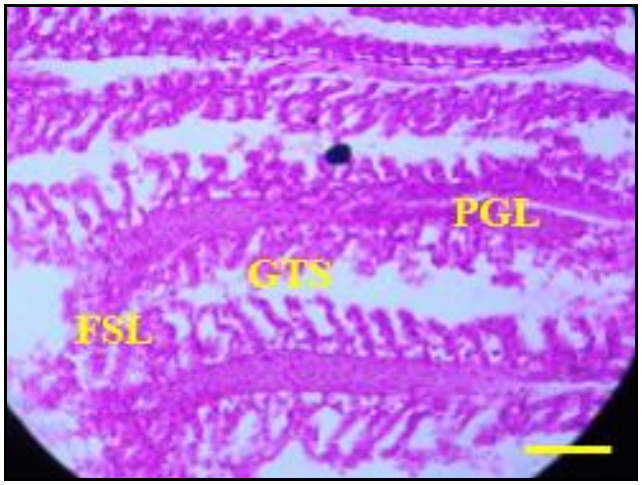

Fig. c. Gill Tissue of 30% SLC Chlorpyrifos treated fish
DGE – Detached Gill Epithelium
ESL – Erosion of Secondary Lamellae
NGF – Necrosis of Gill Filament

Fig 1: Histopathological Alterations in the Gill tissue of Chlorpyrifos treated *Labeo rohita*

Discussion

Rapid global industrialization and chemical pollutants have transformed the natural condition of the aquatic environment resulting in the alterations of structural organization of organs and functional asymmetry of the aquatic organisms. Toxicants has been indexed among the potent toxicant chemicals that adversely affecting the aquatic biota (Mai.D.Ibrahem,2012). Although the tissue processing for histopathological study is time consuming but the histopathology of organs appeared to be a promising biomarker for assessing the environmental contamination (Oliveira Ribeio *et al*.,2006) [11].

Fish gills are described as multifunctional organ and they perform gas exchange, ion transport, acid base regulation and waste excretion (Dang *et al.,* 2001; Evans *et al.,* 2005 and Vigliano *et al.*,2006) [3, 6, 17]. Gill

surface accounts for over 50% of the surface area of a fish hence it is not surprising to discern the gill as the prime target organ for waterborne toxicants (Playle, 1998) [13].

In the present investigation the gill tissue of 10% sub lethal chlorpyrifos treated fish exhibited eloquent structural deviations such as proliferation and thickening of epithelial cells and gill filaments. Which could ultimately led to the fusion of secondary lamellae. Proliferation with thickening of gill epithelium have been reported by Figueiredo-Fernades *et al. (*2007) [7] in Nile Tilapia. Rajini *et al.* (2015) [14] observed fusion of secondary lamellae in *Danio rerio* on exposure to combination of pesticides. They advocated that the proliferation of gill epithelium led to the fusion of secondary lamellae.

In the 30% Sub lethal toxicity exposed fish several lesions and formation of edema were seen. Detachment of lamellar epithelium was also noticed. Alexopoulos *et al*. (2003) [2] and Van Heerden *et al*. (2004) [16] supposed that the edema with detachment of lamellar epithelium could play a defense mechanism against the toxicant's virulence by increasing the distance across the waterborne toxicant and the blood stream inside the gill. Sub-acute toxicity of chlorpyrifos exposed *Labeo rohita* exemplified expressive histopathological aberrations, the degree of damage was found to be congruence with the concentration of chlorpyrifos. Similar observations have been contemplated by Das and Mukherjee (2003)[4] in *Labeo rohita;* Ramesh and Nagarajan, (2013) [15] in *Clarias batrachus*.

Conclusion

Under the light of this study, it is concluded that chlorpyrifos is highly toxic to the fish *Labeo rohita*. Histopathological studies revealed changes in the structural integrity of the cells of gill. These results of the present study indicate that *Labeo rohita* is a potential biological indicator of environmental pollution.

Acknowledgments

We would like to thank the President and Secretary of A.V.V.M Sri Pushpam College (Autonomous) Poondi for providing necessary facilities during the course of our studies.

References

1. APHA. American Public Health Association, American Waterwork Association, Water Pollution Control Federation, standard methods for the examination of water and waste water., 1992, 1193.
2. Alexopoulos E, McCrohan CR, Powell JJ, Jugdaohsingh R, White KN. Bioavailability and toxicity of freshly neutralized aluminium to the freshwater crayfish *Pacifastacus leniusculus*. - Archives of Environmental Contamination and Toxicology,2003:4(4):509-514.
3. Dang ZC, Berntssen MHG, Lundebye AK, Flika G, Wendelaar Bonga SE, Lock, RAC. Metallothionein and cortisol receptor expression in gills of Atlantic salmon, *Salmo salar*, exposed to dietary cadmium. - Aquatic Toxicology,2001:53(2):91-101.
4. Das BK, Mukherjee MD, Toxicity of cypermethrin in *Labeorohita* fingerlings, biochemical enzymatic and haematological consequences. *Comp. Biochem. Physiol. C. Toxicol. Pharmacol*.,2003:134:109-121.
5. Dhasarathan P, Palaniappan R, Ranjit Singh AJA. Effect of endosulfan and goldfish (*Carassius auratus*) exposed to a glyphosate formulation using the micronucleus test and the comet assay. *Mutagenesis*,2000:22(4):263-268.
6. Evans DH, Piermarini PM, Choe KP. The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. - Physiological Reviews, 2005:85:97-177.
7. Figueiredo-Fernandes A, Ferreira-Cardoso JV, Garcia-Santos S, Monteiro SM, Carrola J, Matos P, *et al*. Histopathological changes in liver and gill epithelium of Nile tilapia, *Oreochromis niloticus*, exposed to waterborne copper. - Pesquisa Veterinária Brasileira,2007:27(3):103-109.
8. Finney DJ. *Probit Analysis.* Cambridge Univ. Press, London., 1971.
9. Humason. Animal tissue techniques.Fourth Edition, W.H.Freeman and Company, San Franscisco. USA., 1967.
10. Mai D Ibrahem. Experimental exposure of African catfish Clarias Gariepinus (Burchell, 1822) to chlorpyrifos: Clinical evaluation, tissue alterations and residue assessment Journal of Advanced Research,2012:3:177-183i
11. Oliveira Ribeiro CA, Filipack F, Mela M, Silva PH, Randi MAF, Costa JRA, Pelletier E. Hematological findings in neotropical fish *Hoplias malabaricus* exposed to subchronic and dietary doses of methylmercury, inorganic lead and tributyltin chloride. - Environmental Research,2006:101:74-80.
12. Pazhanisamy K, Indra N. Toxic effects of arsenic on protein content in the fish, *Labeo rohita* (Hamilton). *Nature Environment and pollution Technology*.,2007:6(1):113-116.
13. Playle RC. Modelling metal interactions at fish gills. - *Science of the Total Environment*,1998:219(2-3): 147-163.
14. Rajini A, Revathy K, Selvam G. Histopathological Changes in Tissues of Danio rerio Exposed to Sub Lethal Concentration of Combination Pesticide. Indian Journal of Science and Technology,2015:8(18):1-12.
15. Ramesh F, Nagarajan K, Histopathological Changes in the Muscle Tissue of the Fish *Clariasbatrachus* Exposed to Untreated and Treated Sago Effluent. Advances in Bioscience and Bioengineering.,2013:1(2):74-80.

16. Van Heerden D, Vosloo A, Nikinmaa M. Effects of short-term copper exposure on gill structure, metallothionein and hypoxia-inducible factor-1á (HIF-1á) levels in rainbow trout (*Oncorhynchus mykiss*). - *Aquatic Toxicology*,2004:69:271-280.
17. Vigliano FA, Aleman N, Quiroga MI, Nieto JM. Ultrastructural characterization of gills in juveniles of the Argentinian silverside, *Odontesthes bonariensis* (Valenciennes, 1835) (Teleostei: Atheriniformes). - Anatomia, Histologia, Embryologia,2006:35:76-83.