

The effect of quinalphos on histopathological changes in the Gills of fresh water fish, *Anabas testudineus*

B.Aswin*¹, S.Binu kumari², S.Ravisankar³, M.Mohan kumar⁴,
A.P.Ambikadevi⁵ and M.K.Drishya⁶

¹²³⁴⁵⁶ PG and Research Department of Zoology, Kongunadu Arts and Science college, Coimbatore-641029, Tamilnadu, India.

Abstract: The quality of water is affected by human activities and is declining due to rise of urbanization, population growth, industrial production, climate change and other factors. The fish as a bioindicator of aquatic medium, it play an important role in the monitoring of water pollution because of the sudden death of fish indicates the heavy pollution and the effects of exposure to sublethal levels can be measured in histopathological responses of the fishes. Quinalphos is one of the organophosphate pesticides represent one of the most widely used classes of pesticide with high potential for human exposure in both rural and residential environments. The fresh water fish, *Anabas testudineus* was selected as the test animals. 1/10th of 96 hrs LC₅₀ was taken as sublethal concentration of Quinalphos pesticides. After the stipulated period of exposure (24, 48, 72 and 96 hrs) fishes were sacrificed and tissue viz, gill was isolated and used for histopathological studies. It was found that degeneration of epithelial lining in gills.

Key words: Quinalphos, *Anabas testudineus*, Sublethal, Histopathology

I. Introduction

Water pollution is the biggest threat of urbanization, industrialization and modern agricultural practices. It leads to variation in physical, chemical and biochemical properties of water bodies. The aquatic environment has always been subjected to different types of pollutants. The problems of environmental pollution and its harmful effect on aquatic biota, including fish is receiving focus during the last few decades [1]. Industrial discharges containing toxic and hazardous substances, including heavy metals and pesticides contribute hugely to aquatic ecosystem [2]. Man has attempted to increase the world's food production to solve the problem of malnutrition. The increased use of fertilizer to nourish the plant and by increased use of pesticides to protect them from pests. Recently a large quantity of pesticides and fertilizers are used to nourish the plants and food production. These chemical have entered into the aquatic system and create pollution, which pose a great threat to aquatic organisms.

II. Materials And Method

Fresh water fish, *Anabas testudineus* were exposed to 24 hrs, 48 hrs, 72 hrs and 96 hrs to a sublethal concentration of Quinalphos pesticide. At the end of exposure period, fish were randomly selected for histopathological examination. They were collected from the Aliyar fish farm, pollachi stocked and acclimatized for a time period of 10-15 days in the laboratory conditions in glass aquaria containing dechlorinated water. The water of the aquarium was aerated continuously through stone diffusers connected to a mechanical air compressor. Water temperature ranged between 26±50°C and the pH was maintained between 6.6 and 8.5. Fish were fed twice daily alternately with rice bran and oil cake. For the present study, matured adult fishes were exposed to 1/10th concentrations of LC₅₀ of quinalphos for 24, 48, 72 and 96 hrs continuously. Three replicates of ten fishes for each exposure of the pesticides were used. In these aquaria water was replaced daily with fresh treatment of pesticides. Each experiment was accompanied by its respective control.

Three groups of fishes were exposed to 1/10th of the pesticide 'quinalphos' for 24, 48, 72 and 96 hrs. Another group was maintained as control. All the groups received the same type of food and other conditions were maintained similarly. At the end of exposure period, fish were randomly selected for histopathological examination. Tissues of gills, were isolated from control and experimental fish. Physiological saline solution (0.85% NaCl) was used to rinse and clean the tissues. They were fixed in aqueous Bouin's solution for 48 hrs, processed through graded series of alcohols cleared in xylene and embedded in paraffin wax. Gills alone were processed by double embedding technique. Sections were cut at 6μ thickness stained with Haematoxylin Eosin, dissolved in 70% alcohol [3] and were mounted in Canada Balsam. The photographs at 200x magnification were taken with computer aided microscope (Intel play Qx3, Intel Corporation, Made in China).

III. Results And Discussion

Gill histology of control fish revealed the intact nature of both primary and secondary gill lamellae. The secondary lamellar surface was covered with simple squamous epithelial cells and capillaries separated by mucous cells. Each primary gill lamellae was flat leaf like in structure. It consisted of double rows of secondary lamellae with the central supporting axis. They were situated laterally on either side of the interbranchial septum. The secondary lamellae on both sides were highly vascularized and covered by a layer of cells with uniform interlamellar spaces.

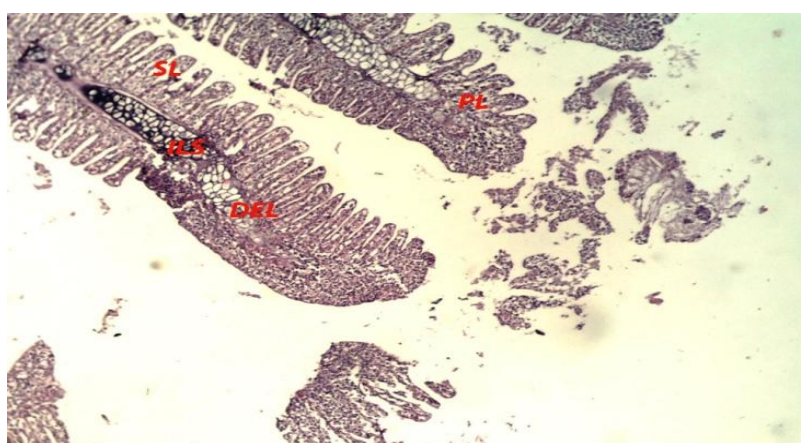
When the fish was exposed for 24 hours to the short term exposure of quinalphos, there was degeneration of epithelial lining. After 48 hours of exposure, degeneration changes in the secondary lamellar of gill was noted. After 72 hours of exposure, there was fusion of secondary lamellae with irregular lamellar spaces. After 96 hours of exposure, structural alterations such as epithelial proliferation, lamellar fusion and necrosis were observed. Edematous changes, characterized by epithelial detachment, were observed in gill filaments and secondary lamellae. Moreover, aggregations of inflammatory cells were noticed in gill filaments. Also dilation and congestion in blood vessels of gill filament were observed. Atrophy of secondary lamellae was seen.

HISTOPATHOLOGY OF THE GILL OF *Anabas testudineus*



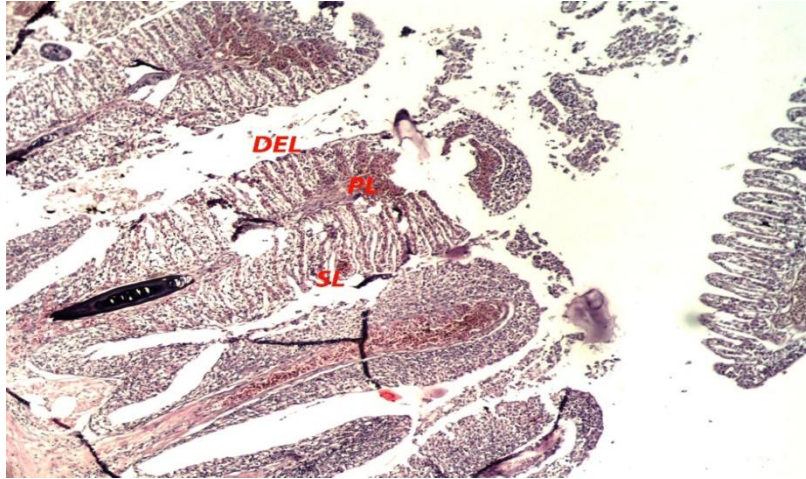
A. Control gill section of *Anabas testudineus*

- PL – Primary lamellae
- SL – Secondary lamellae
- LS – Lamellar space
- ILA -Inter lamellar space
- SA - Supporting axis

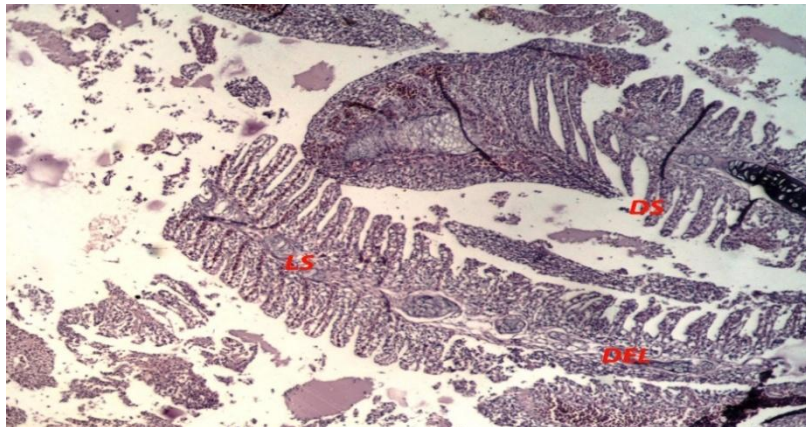


B. Gill section of fish exposed to 24 hours of quinalphos

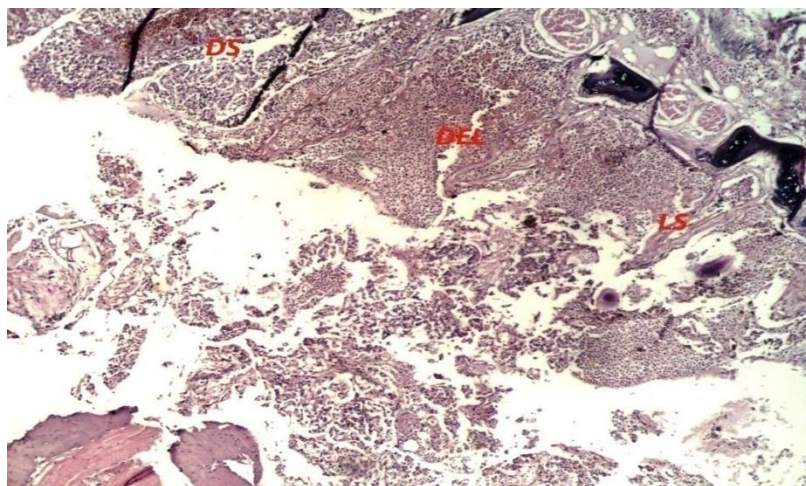
- SL – Secondary lamellae
- ILS – Inter lamellar space
- DEL-Degeneration of Epithelial lining



C. Gill section of fish exposed to 48 hours of quinalphos
PL - Primary lamellae
SL - Secondary lamellae
DEL- Degeneration of Epithelial lining



D. Gill section of fish exposed to 72 hours of quinalphos
LS - Lamellar space
DEL - Degeneration of Epithelial lining
DS - Degenerated secondary lamellae



E. Gill section of fish exposed to 96 hours of quinalphos
LS - Lamellar space
DEL - Degeneration of Epithelial lining
DS - Degenerated secondary lamellae

In gills degeneration of epithelial lining was observed in 24 hour observation, In 48 hour degeneration of secondary lamella will occur. In 72 hour fusion of secondary lamellae with irregular lamellar spaces will occur. In 96 hours structural alteration, epithelial proliferation, lamellar fusion and necrosis can be observed. Dilation and congestion in blood vessels of gill filament and atrophy of secondary lamellae also observed.

It is possible that the damage of the gills could be a direct result of the salts, heavy metals, pesticides, sewage and fertilizers, which are conveyed to the water [4] [5]. Reported on the tips of the swollen gills, the gill lamellae with turgidness and contraction with mucous exudation and reduction in the lamellar space due to the effect of pesticide [6] Studied the histopathological effects of Cyphenothrin on the gills of *Lebistes reticulates*.

The results showed necrosis, degeneration of secondary lamellae due to odema, shortening of secondary lamella. [7] Says that the lifting of lamellar epithelium is due to other histological change observed, probably induced by the incidence of severe edema. Cell proliferation with thickening of gill filament epithelium may lead to the lamellar fusion [8]. Alterations like epithelial lifting, hyperplasia and hypertrophy of the epithelial cells, besides partial fusion of some secondary lamellae are examples of defense mechanisms, since; in general, these result in the increase of the distance between the external environment and the blood and thus serve as a barrier to the entrance of contaminants. As a consequence of the increased distance between water and blood, the oxygen uptake is impaired [9]. Hypertrophy, hyperplasia, fusion of adjacent lamella and telengeastases of the gill were noticed, when fish exposed to LAS for chronic test [10], [11]. [12] have observed hyperplasia, lamellar fusion, curling and bulging of tips of primary gill lamellae, exudation of erythrocytes, when the fish *Cyprinus carpio* was exposed to sublethal concentration of endosulfan.

Proliferation of pavement cells, mucous cells and chloride cells seem to be protective, which limit the accesses of chemicals with the branchial surface, on other hand they may also block respiratory gas exchange and then lead to animal smothering. Uplifting of epithelium, necrosis, and increased density of the cells on secondary lamella hyperplasia and hypertrophy of the epithelial cells are common defense mechanisms. It result in the increase of the gap between the external environment and the blood and thus serve as a barrier to the entry of the contaminants These alterations, more commonly associated with chronic exposures than acute lethal exposures, are greatly increase the blood-to-water diffusion distance decrease interlamellar distance and lead to a total reduction in the diffusive conductance of the gills to respiratory gases [13].

IV. Conclusion

In gills degeneration of epithelial lining was observed in 24 hour observation. In 48, hour degeneration of secondary lamella will occur. In 72 hour fusion of secondary lamellae with irregular lamellar spaces will occur. In 96 hours structural alteration, epithelial proliferation, lamellar fusion and necrosis can be observed. Dilation and congestion in blood vessels of gill filament and atrophy of secondary lamellae also observed. From the present study, it is concluded that the above histopathological parameters are the one important and specific biomarkers with regard to effects of toxicants on organisms. So it is also suggested that adequate care should be taken to neutralize and detoxify the toxicants present in the agricultural effluent and follow the treatment procedure before let out into aquatic systems. And we should decrease the pesticide consumptions in agricultural fields. Indiscriminative usage of pesticide will leads into bioaccumulation and biomagnification in humans and other vertebrates it will leads to severe histological and physiological changes.

Acknowledgements

The authors are grateful to Department of Zoology, Kongunadu Arts and Science College for guiding and providing necessary help for conducting this research studies.

References

- [1]. Jagadeesan, G., A Jebanesan. and A Mathivanan,. (2001). In vivo recovery of organic constituents in gill tissue of *Labeo rohita* after exposure to sublethal concentrations of Mercury. J. Exp. Indelleriia., 3:22-29.
- [2]. Woodlings, J.D., S.F Brinkman. and B.J Horn. (2001). Non uniform accumulation of Cd and Cu in kidneys of wild brown trout *Salmo trutta* populations. Arch. Environ. Contam. Toxicol., 40:318-385.
- [3]. Humason, G,L (1962). Animal tissue technique III(ED) W.H.Freeman and Co., San Fransisco. International journal of Pharmaceutical and Biological Archives. 2(4):1215-1217.
- [4]. Temmink J., P.Bouweister, De Jong and J Van Den Berg. (1983). An ultrastructure of chromatin induced hyperplasia in the gill of rainbow trout, *Salmo gairdneri*. Aquatic toxicology, 4, 165-179.
- [5]. Rooj, C N, (1994). The behaviour and gill histology of an Indian hill-stream fish exposed to a plant toxin. J. Ecotoxicol,Monit, 4(3) : 217- 220.
- [6]. Erkmen, B. M, Caliskan. and S.V, Yeril (2000). Histopathological effects Cyphenothrin gills of the *Lepistes reticulates*. Vet.Hum. Toxicol., 42:5-7.

- [7]. Pane E.F., A Haque. and C.M Wood. (2004). Mechanistic analysis of acute, Niinduced respiratory toxicity in the rainbow trout, *Oncorhynchus mykiss*: an exclusively branchial phenomenon. *Aquatic Toxicology*, 69, 11-24.
- [8]. Figueiredo-Fernandes A., J. V Ferreira-Cardoso., Garcia-Santos., S M Monteiro, J Carrola., P Matos and A Fontainhas-Fernandes. (2007). Histopathological changes in liver and gill epithelium of Nile tilapia, *Oreochromis niloticus* exposed to water borne copper. *Pesq.Vet. Bras.*,27(3), 103-109.
- [9]. Fernandes M. N. and A. F, Mazon. (2003). Environmental pollution and fish gill morphology. In: Val, A. L. and B. G. Kapoor (Eds.). *Fish adaptations*. Enfield, Science Publishers., 203-231.
- [10]. Hampel, M., J.B Ortiz-Delgado, C Sarasquete. and J Blasco, (2008). Effects of sediment sorbed linear alkylbenzene sulphonate on juveniles of the Senegal sole, *Soleasene galensis*: toxicity and histological indicators. *Histology and histopathology*, 23, 87-100.
- [11]. Rejeki, S., D Desrina, and A.R Mulyana, (2008). Chronic affects of detergent surfactant (Linear Alkylbenzene Sulfonate LAS) on the growth and survival rate of sea bass (*Latescalifer Bloch*), larvae *Journal of Coastal Development.*, P.213
- [12]. Riji John, K. and N, Jayabalan., (2007). Sublethal effects of endosulfan on the histology and protein pattern of *Cyprinus carpio* gill. *J. Applied Ichthyology*. 9: 49-56.
- [13]. Reddy P.B. and S.S Rawat., (2013). Assessment of Aquatic Pollution Using Histopathology in Fish as a Protocol.