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Research Article

Impact of triclosan on histopathological changes in gills of Zebra fish, *Brachydanio rerio*

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Abstract

Triclosan (TCS) is commonly used as an antibacterial and antifungal agent in household cleaning products. It also used not only for human and veterinary medication, but also for the promotion of growth in livestock and aquaculture species. The present study was carried out to study the histopathology of gills in the zebra fish, *Brachydanio rerio* (Ham) which exposed to triclosan (0.32 mg/L) for 7 and 28 days in fresh water (pH 6.0). Several histopathological changes were observed in fish organs would serve useful purpose in evaluating the toxic effects of triclosan. Histopathological changes in gills, were observed microscopically showed increasing degrees of damage in the tissues in correlation with the concentration of triclosan, in the gills of control groups exhibited a normal architecture.

Keywords: Triclosan, Histopathology, gills, zebra fish, *Brachydanio rerio*

Introduction

The pollution of water bodies has become a universal phenomenon in the world due to the rapid industrialization and increase in human population. In the last five decades use of chemicals has increased sharply due to intensive agricultural practices (Fung and Mar, 2001). Among aquatic organisms pesticides may have many negative effects, one in particular being the potential to cause oxidative stress through enhanced generation of reactive oxygen species (ROS) or corruption of antioxidant defense systems (Livingstone, 1998). The uptake of chemicals by aquatic organisms may occur from the water, sediments, suspended particulate matter and from food. The final result of exposure to contaminants depends on the particular dietary and ecological life styles of the aquatic organisms (Livingstone, 1998). It is known that aquatic organisms are more sensitive to toxicants than terrestrial organisms, including mammals and in this respect they may provide experimental data for evaluation of oxidative stress induction, mutagenicity and other adverse effects of pollutants (Lackner, 1998). Triclosan is a commonly used antimicrobial that is incorporated into dish soap, detergent, toothpaste, mouthwash, hand soap, fabric, deodorant and shampoo, in addition to innumerable other personal care and household products (Dann and Hontela, 2011).

Triclosan (TCS) is a polychloro phenoxy phenol commonly used as an antibacterial and antifungal agent in household cleaning products. The usage

amount of TCS was more than 3 million tons per year and it was more than 3.5 million tons per year in Europe (Halden Ru Paull, 2005). TCS is used extensively from more than 30 years. It also used not only for human and veterinary medication, but also for the promotion of growth in livestock and aquaculture species (Daughton and Ternes, 1999). TCS can be bio-accumulated and create endocrine-disrupting and damaging the DNA in some exposed fish and mammals (Crofton *et al.*, 2007). The genotoxic chemical becomes more hazardous when it possesses bio-accumulative properties and enters in the food chain of the ecosystem. Fishes as bioindicators of pollutant effects are very sensitive to changes in their environment and play major roles in assessing potential risk associated with contaminations of new chemicals in aquatic environment (Lakra and Nagpure, 2009). Histopathological characteristics of specific organs can express condition and represents time-integrated impacts of both exogenous and endogenous orgin.

Gills are critical organs for respiratory and osmoregulatory functions of pesticide poisoning (Oritz *et al*., 2002). Therefore in the present study an attempt was made to investigate the effects triclosan (0.32 mg/L) 7 and 28 days on the histo-architecture of the gills of zebra fish *Brachydanio rerio*. Cellular assay techniques are employed to study pollutant induced injuries on the internal organ system of organisms. Such injuries serve as reliable biological indicators of pollution and are effectively used in assessing stress effects. Toxicants are the functional response of organisms which provide information on the nature of toxicant and also powerful tool for monitoring anthropogenic contamination.

Materials and Methods

The fish, Zebra fish, *Brachydanio rerio* (Ham) having mean weight 3 to 5 g and length 4 to 6 cm were collected from your friend's aquarium, Kolathur, Chennai at acclimatized to laboratory conditions. They were given the treatment of 0.1%KMNO4 solution and then kept in plastic pools for acclimatization for a period of nine days. They were fed on tubifex warm daily. The triclosan was used in this study and stock solutions were prepared. Triclosan LC_{50} values were (0.32 mg/L) taken as sub-lethal concentrations for this study. Thirty fish were selected and divided into 3 groups of 10 each in the experimental setup. The experiment was carried out for a period of 7 and 28 days. The gills were dissected out and fixed in Bouin's fixative, dehydrated using ethyl alcohol, cleared in xylene and embedded in paraffin wax (58 to 60^0C). Sections of 6 mm thickness were obtained, stained in haematoxylin and eosin are mounted in DPX (Dextrene Plasticizon Xylene) following standard methodology (Gurr, 1962).

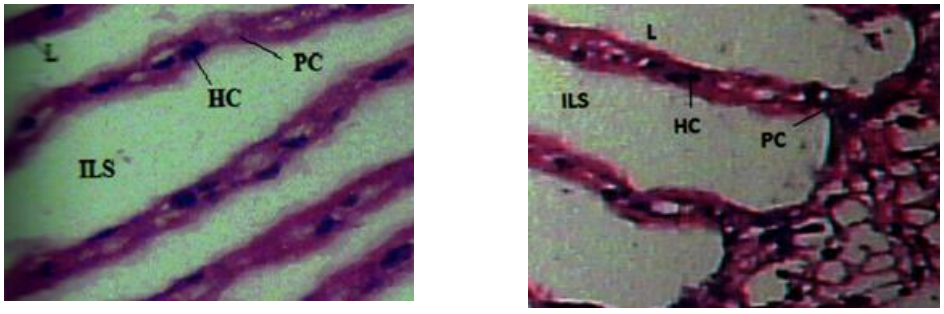
Results and Discussion

The gills zebra fish *Brachydanio rerio* measures about 3-5 mm in length The gills of control fish showed uniform arrangement of the lamellae (L) with uniform interlamellar spaces (ILS).

The surface of each lamella or filament was covered by a thin cuticle underline by a monolayer epithelium. Figures (1, 2, 3 and 4). Connective tissues were present in the septa of the filaments and in the axes of the gills. The gill lamellae consisted of epithelia and hemocytes (HC). The epithelium through the branches and filaments was generally thin. It was thicker near the nuclei and the tips of the filaments, which widened to form a distal lacuna. The hemocytes were rounded and rare, pillar cells (PC) which are specialized epithelial cells, extended into the lamellar sinus at internals and abutted similar cells that extended from the opposite surface. Apical microvilli and basal infoldings were generally very limited or absent. Similar morphology was exhibited between different filaments along the length of the gill axis. When the zebra fish *Brachydanio rerio* exposed to (0.32mg/L) of triclosan, swelling of gill lamellae (SL). Fusion of gill lamellae (FL) and accumulation of hemocytes (HC) were observed figures (5 and 6).

Formation of abnormal gill tip (AG), malformation at the tip of the gills (MF) and accumulation of hemocytes (HC) Figures (7 and 8) and malformation were noted due to the toxicity of

(1) (2)



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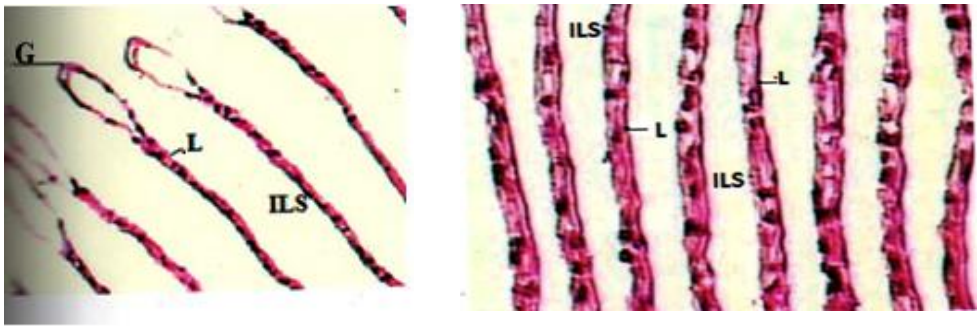

Figure.1, 2, 3 and 4. Control zebra fish *Brachydanio rerio* gill cells.

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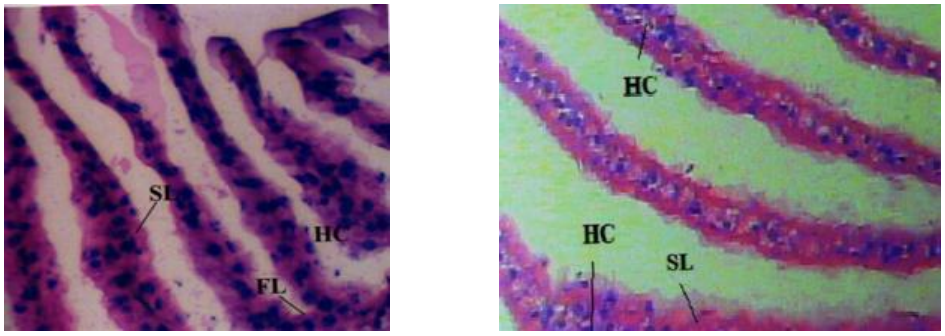

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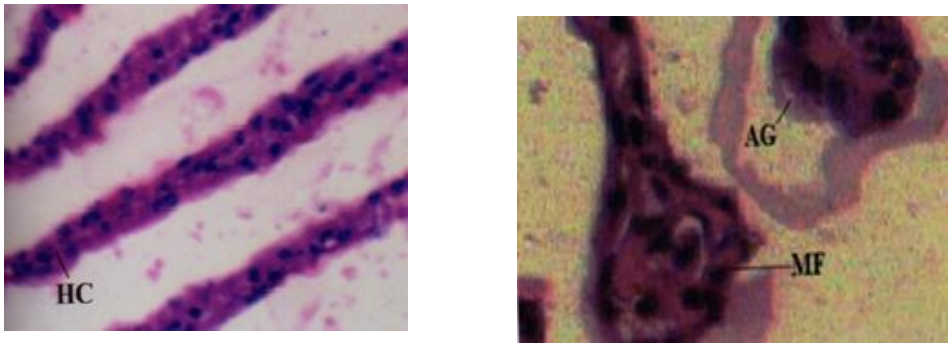
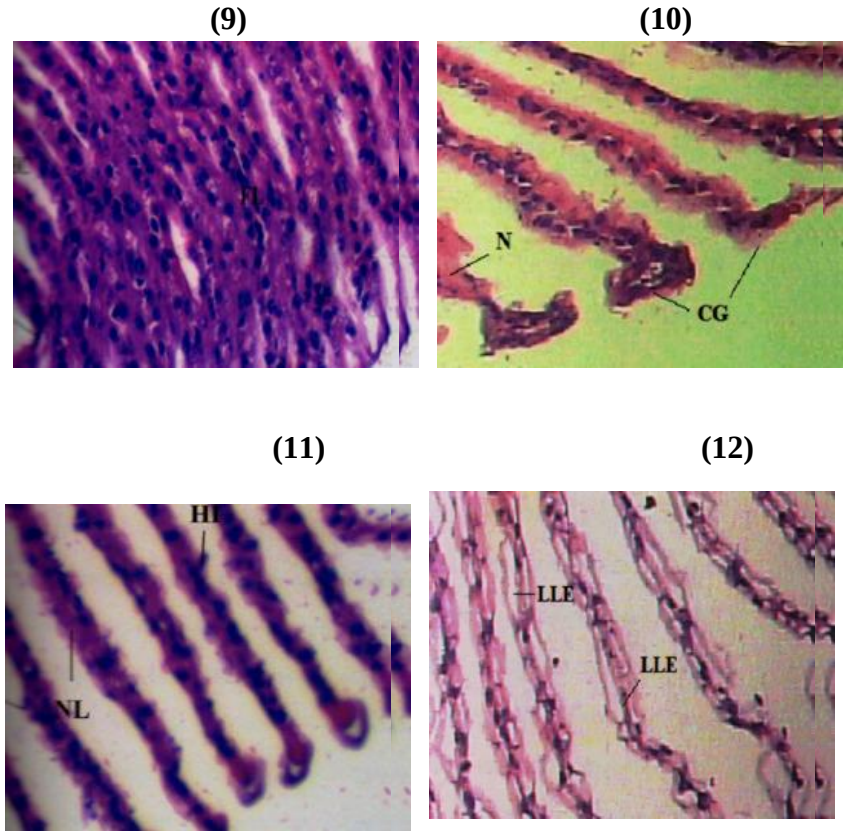


Figures. 5, 6, 7 and 8 Triclosan exposure in 7 days in the gills of zebra fish *Brachydanio rerio*



Figures. 9, 10, 11 and 12 Triclosan exposure in 28 days in the gills of zebra fish*Brachydanio rerio*

triclosan. In aquatic organisms, the gills represent a vital organ, since they play an important role in the transport of respiratory gases and regulate the osmotic and ionic balance. Toxic substances may cause damage to gill tissues, thereby reducing the oxygen consumption and disrupting the osmoregulatory function of aquatic organisms (Ghate and Mulherkar, 1979). In the present study, exposure of zebra fish to triclosan. The fish were exposed to 28 days (0.32 mg/L) subthethal concentration of triclosan resulted in fusion of gill lamellae (FL), accumulation of hemocytes (HC), necrotic (N) and formation of clavate-globate gill tip (CG) Figure (9 and 10). Exposure to triclosan in hemocytic infiltration (HI), hemocoelic space and necrotic gill lamellae (NL) Figure (11). The gills of the experimental groups exposed to triclosan exhibited a lifiting of the lamellar epithelium (LLE) Figure (12), but there were structural changes in the gill cells compared to those of the control prawns. Similar lesions have been reported to occur in the prawns *Macrobrachium kistensis* (Ghate and Mulherkar, 1979) and *Macrobrachium idea* (Victor *et al.,* 1990) following exposure to heavy metals.

Inflammatory changes, such as swelling lifting of lamellar epithelium and hyperplasia have also been noted in the gill lamellae of various species of fish following exposure to insecticides (Sunitha and Sahai, 1983; Roy and Munshi, 1991; Nowak and Barbara, 1992). In an exhaustive review of toxicant: irritant-induced changes in the gills stated that such inflammatory changes tend to be largely non-specific and such changes might simply reflect a physiological adaptation to stress (Mallatt, 1985). The observed inflammatory changes in the present (accumulation of hemocytes in the hemocoelic space, swelling and fusion of lamellae and lifting of lamellar epithelium) might be viewed simply as protective mechanism, since the vulnerable surface area of the gills is decreased in order to maintain the osmoregulatory functions. However, such reactions, while slowing down toxicant uptake, could conceivably lead to dysfunctional or even non-functional gills, eventually resulting in asphyxia (Tamse *et al*., 1995).

A loss of cellular ions and proteins could have further impaired gill functions in the test zebra fish, a sequence of events observed in the milkfish, *Chanos chanos* exposed to nifurpirinol (Tamse *et al.*,1995). Thus, the changes observed in test zebra fish exposed to triclosan in the present study were more likely to have represented a progressive loss of basic biological functions of the gills rather than protective mechanisms. In the present study, exposure of zebra fish to triclosan resulted in notable structural alterations in the lamellae of gills including necrotic, hyperplastic, and clavate-globate lamellae, as well as swelling, fusion, and increased mucus secretion. Similar lesions have been reported to have occurred in the prawns *Macrobrachium kistensis* (Ghate and Mulherkar, 1979) and *M. malcolmsonii* exposed to endosulfan (Bhavan and Geraldine, 2000). In juvenile giant freshwater prawn *Macrobrachium rosenbergii* exposed to water bone copper (Li *et al.,* 2008) in common carp *Labeo rohita* exposed to hexachlorocyclohexane (Dass and Subhas Chandra Mukherjee, 2000) in freshwater fish *Cyprinus carpio* exposed to bifenthrin (Velisek *et al.,* 2009).

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