



Poisonous Effects of Carbamate Pesticide Sevin on Histopathological Changes of *Channa striata* (Bloch, 1793)

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ABSTRACT

The study was conducted to assess the histopathological impairment of gill and liver of freshwater snakehead murrel, *Channa striata*. The fish were exposed to sublethal concentrations (1.1 ppm) of insecticide Sevin for 30 days and a parallel control was run simultaneously. No histopathological effects were observed in control. Gill and liver of the exposed fish exhibited some remarkable alterations in their histology. Prominent changes include vacuolation, necrosis, epithelial lifting, shortening of lamellae, the fusion of adjacent lamellae, blood congestion, architectural distortion and degeneration of gills, lamellar fusion, hypertrophy, clubbing, few lamellar missing and shrinkage of blood vessels were observed in treated fishes. Hypertrophy of hepatocytes, necrosis, blood congestion, vacuolation, cellular degeneration, damage of nuclei was observed in the liver of exposed fishes. Duration of exposure of Sevin appears to have a reflective effect on gill and liver as with the increasing duration of exposure histopathological damages become more severe.

INTRODUCTION

The aquatic ecosystems are continuously being contaminated with toxic chemicals from industrial, agricultural and domestic activities. Pesticides are one of the major classes of toxic substances used for management of pests in agricultural lands. It controls pests in crops, but when it reaches the aquatic habitat, affect the nontarget organisms especially fishes. Fishes are particularly sensitive to the pesticidal contamination of water. Hence, these chemical pollutants, when entering into the body of fishes may significantly damage certain physiological and biochemical process and produce serious abnormalities in its organ systems and leads to the extinction of species (Banaee et al. 2011). The carbamate pesticide Sevin is also toxic to non-target aquatic organisms, especially fishes. Carbamates are reversible inhibitors of the enzyme acetylcholinesterase and they interfere with the cholinergic nervous system and cause death because the effects of the neurotransmitter acetylcholine cannot be terminated by carbamylated acetylcholinesterase (Wang et al. 2012). It is also found to be a neurotoxin (Branch & Jacqz 1986). The carbamate pesticide, Sevin is known to affect growth, metabolism and development of fish. According to Rani et al. (1990), Sevin is extremely toxic to aquatic and estuarine invertebrates.

The evaluation of pollution effects on various organisms including fish can be studied by using biomarker response.

But most biomarkers are narrow in their expression whereas histopathology is broad in its evaluation (Medja & Golemi 2011). Histopathological assessments are a suitable tool to evaluate fish stress since they can provide information about chronic and sub-lethal effects of xenobiotics (Raskovic et al. 2013, Traversi et al. 2014). Determination of adverse effects of chemical environmental stressors can be performed by using histopathological analysis in fish, as shown by previous studies (Nunes et al. 2015a,b, Rodrigues et al. 2017a, Limbu et al. 2018). Several types of histological changes have already been reported in fish, due to exposure to various environmental stressors (Lauriano et al. 2012, Mekkawy et al. 2012). Histopathology not only gives an early indication of pollution hazard but also provide useful data on nature and degree of damage to cells and tissues (Shaikh et al. 2010, Malik et al. 2012).

Toxicants are taken up through different organs of fishes because of the affinity between them. Chemical pollutants adversely affect different organs and organ systems. Gills and liver are the common target organs in fish. Gills are the first organ to respond to environmental changes and are involved in respiration, osmoregulation, acid-base regulation, and excretion functions (Raskovic et al. 2010, Rodrigues et al. 2017a). The gills are among the most vulnerable structure of the teleost fish because of their external location and close

contact with the water. So, they are liable to be damaged by any irritant materials whether dissolved or suspended in the water. According to Takashima & Hibiya (1995), gills and gastrointestinal tract in fishes considered the main passage for the entrance of pollutants to the internal body.

The liver is a detoxification organ and it is essential for both, the metabolism and the excretion of toxic substances from the fish body (Salamat & Zarie 2012). 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 in regards to the toxic effects of different xenobiotics (van Dyk et al. 2007, Simonato et al. 2008). The liver is a key organ involved in uptake and accumulation and the defence against toxicants, through metabolism and excretion of toxic substances (Casarett et al. 2008). Also, the incidence of lesions in gills and liver can lead to functional damage, interfering thus directly with fundamental processes for the maintenance of homeostasis in fish. Gills and liver are among the most sensitive organs frequently evaluated as indicators in assessing the health status of fish (Raskovic et al. 2010, Yancheva et al. 2016).

Fishes play significant roles in assessing the potentially toxic effects of aquatic environmental contaminants (Yancheva et al. 2016). A commercially and medicinal important freshwater fish *Channa striatus* was selected for the present study. *Channa striatus* (Bloch, 1793), is also known as snakehead murrel. This fish is well-known for its palate, high nutrition, curative and medicinal qualities. According to Mat Jais (1991), murrel flesh has high levels of arachidonic acid which is a precursor for prostaglandin and thromboxin, chemicals that affect blood clotting and the fusion of endothelial tissue in the process of wound healing. In the present study, an effort was made to assess the exposure effect of carbamate pesticide Sevin on the histology of gills and liver of the freshwater fish *Channa striatus*.

MATERIALS AND METHODS

The healthy freshwater fish *Channa striatus* of the weight (42.26 ± 0.93 g) and length (17 ± 2 cm) were selected for the experiment. These fish were acclimatized under the laboratory conditions for 20 days in an FRP tank filled with dechlorinated water. Water quality characteristics were determined by water quality analyser EUTECH (Cyberscan series 600, Singapore), and are as follows: temperature $27.5 \pm 2.37^\circ\text{C}$, pH 7.1 ± 0.02 , dissolved oxygen 6.2 ± 0.2 mg/L, alkalinity 252 ± 2.5 mg/L as CaCO_3 , total hardness 453 ± 4.1 mg/L. The fishes were fed daily with minced fish. The test solution was prepared from Sevin, Manufactured

by Parikh Enterprises Ltd. The Sevin itself was used as a stock solution. After the preparation of the stock solution, the different concentrations of the test solution were prepared by serially diluting the stock solution. The toxicity tests to calculate LC50 for 96 hours is carried out by the desired concentration of Sevin, prepared by adding the distilled water (1000 mL). Series of different concentrations (2, 4, 6, 8, 10 and 12 ppm) were prepared. The healthy fishes were selected and tested for 96 hrs. The experiment was started in the morning and behavioural changes were noted. The mortality of fishes was recorded 96 hr LC 50. The LC50 values for different periods were calculated by the method of probit analysis (Finney 1964). Then, fishes exposed in a sub-lethal concentration of Sevin is 1.1 ppm. At the end of the experiment, the gill and liver tissue was isolated from control and experimental fishes. The tissue was fixed in aqueous Bouin's solution, processed through graded series of alcohols, cleared in xylene and embedded in paraffin wax. Sections were cut at 3-5 μ thickness by using Yorco Rotary Microtome, stained with Ehrlich Haematoxylin and Eosin. Slides were observed under the light microscope (Labomed, CXR111) at varying magnifications (10, 40 and 100X) and photographs were taken under a research microscope supported with Q-win software (Leica).

RESULTS AND DISCUSSION

Gills under Control Condition

Histological observation of gills from control fish showed normal architecture. The structural particulars of the gill of control *C. striatus* are shown in Figs. 1A & 1B. Structurally, the gills are situated in the branchial chamber on either side of the body in fishes. The gill has a gill arch with a double row of elongated laterally projecting gill filaments. These filaments are flat and leaf-like and join at the base on gill rakers by a gill septum. Numerous semi-circular, leaf-like projections are lined up along both sides of the primary gill lamellae called as secondary gill lamellae, which consist of centrally placed rod-like supporting axis with blood vessels on either side. Each of these primary lamellae was flat leaf-like structure and has a series of secondary lamellae located to the primary lamellae with the central supporting axis. The gill is composed of many gill filaments which have primary gill lamellae and secondary gill lamellae that run perpendicular to each filament (Fig. 1A). The secondary lamellae also term as respiratory lamellae are highly vascularized and covered with a thin layer of epithelial cells. Blood vessels are extended into each of the secondary gill filaments provided with pillar and chloride cells. The secondary lamella is supplied with marginal blood sinus lined by endothelium. In between the secondary gill

lamellae and the primary filament, lined by a thick stratified epithelium. This region between the two adjacent secondary gill lamellae is known as the interlamellar region. The gill arch is a bony structure from which radiate double rows of paired filaments. The lamellae are lined by a squamous epithelium composed by pavement and non-differentiated epithelial cells. Each lamella is made up of two sheets of epithelium bordered by many pillar cells which are contractile and separate the blood channels in which erythrocytes are usually recognized within each capillary lumen. Between the lamellae, the filament is lined by a thick stratified epithelium constituted by several cellular forms, such as chloride cell, mucous or goblet cells.

Mucous cells and chloride cells are also existing in the epithelium of the filament and at the base of lamellae. Gills have an extensive surface area and minimal diffusion distance between dissolved oxygen and blood capillaries for efficient gaseous exchange. However, fish gills are equipped with a defence mechanism working against the environmental irritants which essentially is the mucus cell. The mucus cells react instantaneously to head is covered over by a thick tissue composed of mucus glands, taste buds, and connective tissue. The gill head contains bony part of gill arch and gill rakers. The epithelium surface of the gill arch showing the parallel arrangement of epithelial cells having mucus pores (Fig. 1B)

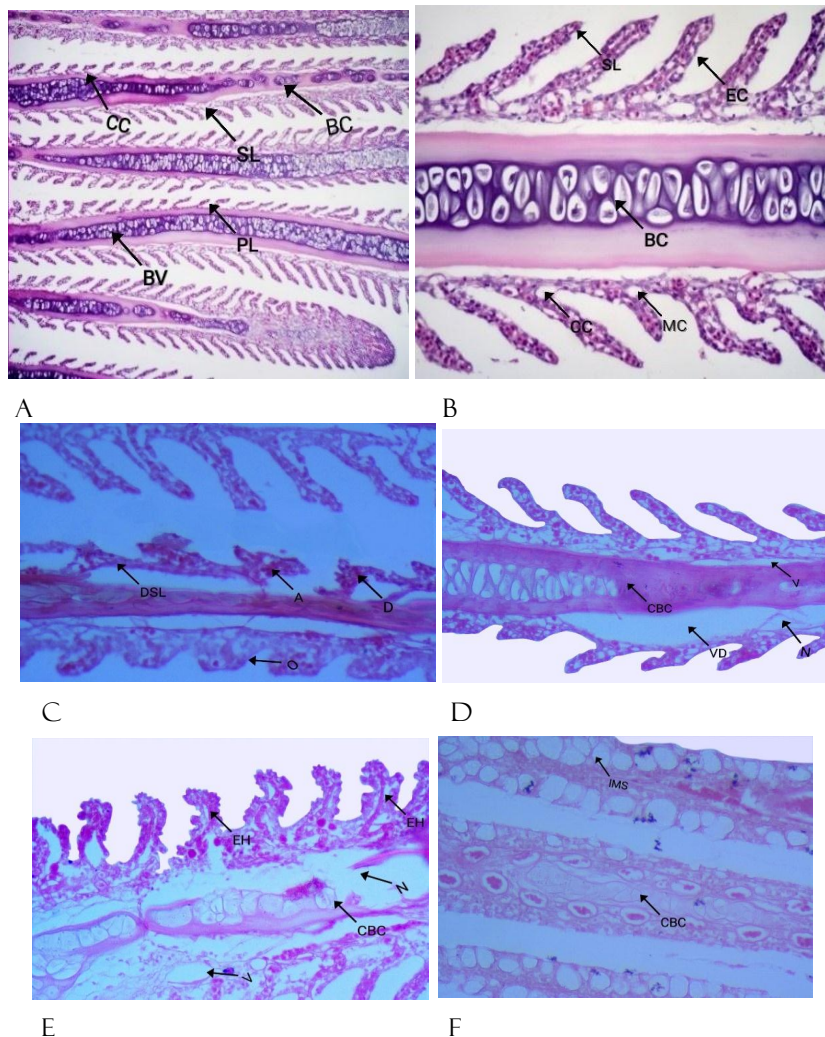


Fig. 1: Gill tissue of *C. striata*: A & B Control- PL- Primary Lamellae, SL- Secondary Lamellae, Chloride Cell, Mucous Cell, Blood Cells, Blood Vessels, Epithelial Cells (H&E, 10 and 40X). (B, C, D, E and F) exposed to 1.1 ppm of Sevin V- Vacuoles, H- Hypertrophy, DSL- Distorted Secondary Lamellae, Aneurism, O- Oedema, CBC- Congested Blood Cells, VD- Vasodilation, D- Desquamation, EH- Epithelial Hypertrophy, IMC- Increased Mucous Cells (H&E, 40X).

Histopathology of Gills Treated with Sevin

At a sublethal concentration of Sevin 1.1 ppm for 30 days, the gill tissues of *Channa striatus* revealed large inter-lamellar space, necrosis, lamellar fusions enlarged secondary gill lamellae, curved secondary gill filaments resulting in distension of the lamellae. Distorted, swelling of the tip of secondary gill lamellae and the erosion was observed (Fig. 1C and D). A large number of blood cells accumulated in secondary gill lamellae and resulted in the enlargement of gill lamellae, the fusion of secondary gill lamellae was also observed in some areas, epithelial hyperplasia, swelling of epithelial cells and bronchial epithelium disorganization were evident. Furthermore, the epithelial layer detached completely from the central portion of the gill lamellae. epithelial lifting, cell swellings congestions, the bending of secondary lamellae, the formation of edematous space between the layers of epithelium which may become infiltrated with red blood cells. Gill hyperplasia was observed in the primary epithelial cells. Then, the whole lamellar epithelium was found to be degeneration (Fig. 1. E and F).

Sevin is considered as a highly toxic pesticide to freshwater organisms. The present study was in agreement with similar observations reported under Sevin toxicity at 96 hr in the freshwater fish, *Channa punctatus* (Deepasree & Nair 2015). Degenerative epithelium of gill filaments and secondary lamellae accompanied by separation of their epithelium from the lamellar supporting cells was also demonstrated in the experiment of Bhuvaneshwari et al. (2015) on Zebrafish exposed to organochlorine pesticide and heavy metals. Dilation of blood capillaries, abnormal swellings epithelium was observed by Banae et al. (2013) in Rainbow trout exposed to pesticide diazinon.

There are several reports on various histological changes in fish gills, acute or chronic exposure with sublethal toxicant concentrations (Velcheva et al. 2010a&b, Muthukumaravel et al. 2013, Sousa et al. 2013 a&b, Khatun et al. 2016). According to Camargo & Martinez (2007), Ayandiran et al. (2009) and Vigario & Saboia-Morais (2014) the toxicants could induce gill histological alterations such as epithelium degeneration and necrosis, which means fish can develop numerous defence mechanisms, which could prevent the toxicant negative effects. These mechanisms are expressed in different morphological changes including oedema, the proliferation of epithelium and fusion.

Epithelium lifting was observed in the Sevin treated gills. Epithelial lifting increases the distance between the blood and epithelial layer and thus decrease the velocity of penetration of the molecules of Sevin into the blood. So, the epithelial lifting can be considered as a defence mechanism

adopted by fishes to slow down the penetration of pesticide through the lamellar epithelium. The epithelial lifting in gills decelerate oxygen uptake and produce dysfunctional gills, and eventually asphyxiate the fish (Kumar et al. 2010).

Gill lesions associated with the lamellar aneurysm were observed in prevalence in all treatment groups. The lesions may be due to the disturbance of blood flow in the blood channels. Similar histopathological lesions have been reported by (Devi & Mishra 2013) chlorpyrifos exposed in *Channa punctatus*.

Liver under Control Condition

The liver is the organ which is most associated with the detoxification and biomarker process. Due to its function, position and blood supply, it is also one of the most affected organs by contaminants in water (Camargo & Martinez 2007). Histological observation of liver from control fish in the present study showed normal architecture. The liver of untreated fish was exhibited parenchymatous appearance and mainly consisted of polygonal-shaped hepatocytes with their central nuclei. Sinusoids are irregularly distributed between the polygonal hepatocytes. Hepatopancreatic alveoli of the exocrine pancreas were seen to be placed in the parenchyma and the melanomacrophages centre stained light brown were located close to the hepatopancreas. Some of the hepatocyte nearby the hepatopancreas was slightly vacuolated it could be due to storage of glycogen and lipid (Fig. 2A & B).

Histopathology of the Liver Treated with Sevin

In sublethal concentration, 1.1 ppm of Sevin to *Channa striatus*, showed congestion of central vein, degeneration of hepatocytes, cytoplasmic vacuolization and a large number of distorted hepatocytes. The liver treated with a high dose of Sevin showed centrilobular necrosis characterized by necrosis of hepatocytes around the congested central vein. Hepatocytes showed increased granularity of cytoplasm with nuclear pyknosis leading to necrosis and complete loss of hepatic parenchyma (Fig. 2C). The liver of fishes treated with a low concentration of Sevin was reflected by disorganization of hepatic cords, degeneration, hepatic hypertrophy, focal necrosis with complete loss of hepatocytes at many places (Fig. 2D).

In fish, the liver is the main organ that plays an important role in the detoxification of chemicals and pesticides. During metabolism, the liver helps to break down the harmful substances, but beyond a certain limit, these toxic compounds distract the regulating mechanism of the liver and cause morphological alteration (Brusle et al. 1996). The fatty infiltration, hemosiderosis and congested central vein, se-

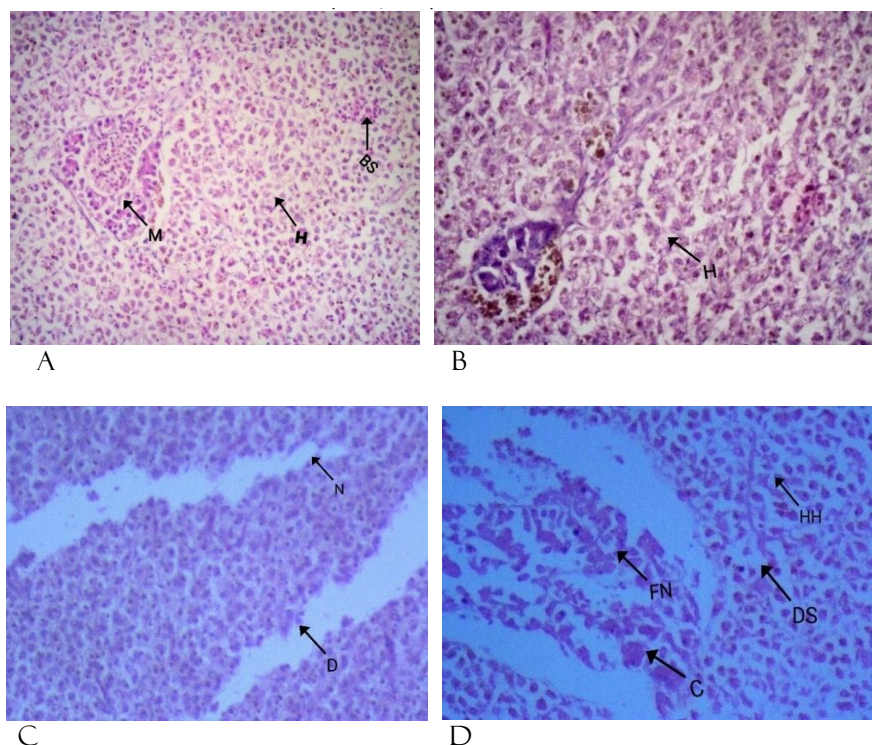


Fig. 2: Photomicrograph of liver control. H- Hepatocytes, N- Nucleus (5 μ m thickness; H&E staining 10 & 40 X) C and D: Photomicrograph of liver of fish exposed to Sevin. DH- Degradation of cellular hepatocytes, DS-Distortion, H- hypertrophy, N-Necrosis, C- Congestion, FN- Focal necrosis, HH- Hypertrophy of Hepatocytes (5 μ m thick; H&E staining; 10X and 40X).

vere necrosis, haemorrhage, vacuolation, severe infiltration of leukocytes, pyknotic and hepatic necrosis in the liver of the fish *Clarias gariepinus* after exposing to lethal concentrations of cypermethrin were observed by Ayoola & Ajani (2008); present result agrees with this result. The infiltration of fatty molecules and vacuolization in the liver specify the accumulation of fat and disparity between the rate of synthesis and release of a substance in hepatocytes (Gingerich 1982), whereas necrosis in some part of liver may appear due to extra workload on hepatocyte during detoxification (Petal & Bahadur 2011). Focal necrosis is probably due to the involvement of liver cells in the metabolic transformation of the insecticide, causing functional and structural changes to the cells (De Melo et al. 2008). Histological changes associated with different pesticides in freshwater fish have been studied by many authors *Clarius gariepinus* (Ayoola & Ajani 2008); *Oreochromis mossambicus* (Navaraj & Yasmin 2012); *Channa punctatus* (Magar & Shaikh 2013); *Labeo rohita* (Nagaraju & Rathnamma 2014); *Cyprinus carpio* (Chamarthi et al. 2014); *Channa gachua* (Rai & Mishra 2014, Kadam & Patil 2018); *Heteropneustes fossilis* (Pandey & Dubey, 2015); *Cyprinus carpio* (Lakshmaiah 2016) and *Trichogaster fasciata* (Mukti 2018).

Thus, the histological changes that were taking place in the present study, as the initial period of exposure in the organs of the fish on exposure to Sevin toxicity might be a part of the defence mechanism. The further accumulation of Sevin in the organs of the fish on continued exposure destroyed the organ structures. The slight structural reorganization of the gill and liver of the fish observed at day 30 of exposure to Sevin toxicity gives support to some extent that the ability of the fish to resist the sublethal stress and in the repair of the damage caused to the organ by enhancing the protein synthetic potentials and other associated activities of the cell.

CONCLUSION

Pesticide toxicant leads to many pathological changes in different tissues of fish exposed to Sevin. The increasing amounts of carbamate pesticide Sevin incoming to aquatic bodies can result in the amount of accumulation of contaminants increased in the fish and their consumers, which create a serious hazard to ecosystems. In the last decades, the fast growth of industry and agriculture has resulted in increased pesticides pollution, which is a significant environmental hazard for invertebrates, fish, and humans.

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