Changes in gill structure induced by uptake of endosulfan in *Channa punctatus* followed by subsequent recovery

Garima Harit[1] and Neera Srivastava[2]

[1]Department of Zoology, IIS University, Mansarovar, Jaipur, India.

[2]Department of Zoology, University of Rajasthan, Jaipur, India.

E-mail: **haritgarima@live.com**

Article Received: 24 July 2017 *Article Accepted: 8 August 2017* *Article Published: 12 August 2017*

ABSTRACT

In present investigation pathological effects produced by sub-acute exposure of pesticide organo-chloride endosulfan on gills of freshwater fish *Channa punctatus* and its bioaccumulation was studied. The changes noticed in gills after sub-acute treatment were found to be degenerative. The sub-acute exposure of gills to endosulfan at two sub-lethal concentrations 0.01 ppm (Group II) and 0.02 ppm (Group III) for 120 hrs showed extensive hyperplasia, vacuolization, detachment of basement membrane from pillar cell system and enlarged blood capillaries. Group I served as control. Statistically significant increase was noted in endosulfan in treated groups. The effects were similar in both the sub-lethal concentrations and the intensity of damage was dose dependent. Post exposure recovery was noted after transferring the surviving fish to normal tap water for another 120 hrs. Remarkable recovery was noted after 72 hr to 120 hr interval in group II but only stray signs of recovery were seen till the end of recovery phase in group III. Thus, it can be concluded from the present work that the gills require more than 120 hr for complete histological recovery and normal functioning. Elimination of endosulfan was slow & concentrations of endosulfan remained significantly high till the end of recovery period.

Keywords: *Channa punctatus*, endosulfan, uptake, histopathology, gills, recovery.

1. INTRODUCTION

Fish are able to accumulate and retain pesticides and other pollutants from their environment. Pesticides affect specific vital organs such as liver, gill and kidney (Napit, 2013). Fish may accumulate pesticides by absorption through gills. The ability of fish to metabolize organochlorines is moderate; therefore, contaminant loading in fish is reflective of the state of pollution in the surrounding environment (Guo *et al.*, 2008). Endosulfan has been recognized as serious pollutant of aquatic environment. It affects fish directly by accumulation in their body (Srivastava *et al.*, 2002). It causes serious impairment in metabolic, physiological and structural changes in different organs and can cause potential damage of population (Harit & Srivastava, 2009a). Histopathological effects of endosulfan on gills have, however, not received much attention (Ghanbahadur *et al*., 2015). Research on the ability of fish to recover after removal of pesticides is also scanty (Berntssen *et al*., 2008 & Harit & Srivastava, 2009b). Therefore, the present paper is a contribution to the assessment of endosulfan toxicity on *C. punctatus*. The ability of endosulfan to bioaccumulate in fish may lead to various degenerative processes which will influence nutritive value of the fish, thereby posing health hazards to human beings on consumption. Since gills are important in respiration as well as osmoregulation of fish, bioaccumulation of endosulfan and histopathological changes in gills have been correlated. Subsequent recovery study was carried out to note the extent of reversibility of these effects.

2. MATERIALS & METHODS

Live specimens of fresh water fish *Channa punctatus* were collected from local water bodies & acclimatized for two weeks under laboratory conditions. Group I served as control, group II treated with 25% (0.01ppm) and group III treated with 50% (0.02 ppm) of LC_{50} value (0.04 ppm) of endosulfan. All groups were kept in 500L glass aquaria containing 70 fish in each. Ten fish from each group were autopsied at intervals of 72 hr, 96 hr & 120 hr. Remaining treated fishes were subsequently transferred to endosulfan free freshwater for another 120 hrs to study the recovery response of these fishes.

Samples of gills were taken from the same region in each fish; they were macerated with anhydrous sodium sulfate. The samples were then processed for extraction with acetone & hexane & estimated for endosulfan residue by the EPA method (1974). A glass column, packed with pre-washed glass wool, activated silica gel & anhydrous sodium sulfate was first rinsed with hexane, followed by the sample extracts. All elutes obtained from gills were completely evaporated with the help of a rotatory vacuum evaporator; the dried extracts were then dissolved in 2 ml hexane. Endosulfan residue was analysed with the help of a gas chromatograph. Bioaccumulation factor for pesticide was calculated as follows –

$$\text{Bioaccumulation factor} = \frac{\text{Concentration in Organism}}{\text{Concentration in Water}}$$

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For histopathological studies routine histological procedure was followed. Sections were cut at 5µ & stained with Harris' haematoxylin & eosin.

3. RESULT

Table 1: Bioaccumulation of endosulfan (ppm) in gills of *Channa punctatus* after sub-acute exposure to endosulfan

Groups	Parameters	72 Hr	96 Hr	120 Hr	% Accumulation
I	Endosulfan content	BDL	BDL	BDL	-
	B.F.	-	-	-	-
II	Endosulfan content	0.6869 ±0.00017	0.7212 ±0.00028	0.7763 ±0.00012	88%
	B.F.	68.69	72.12	77.63	-
III	Endosulfan content	1.7458 ±0.00015	1.777 ±0.00029	1.8107 ±0.00014	96.41%
	B.F.	87.29	88.85	90.53	-

Table 2: Recovery response in endosulfan content (ppm) in gills of *Channa punctatus* pre-exposed to endosulfan for 120 hr

Groups	Parameters	0 Hr	72 Hr	96 Hr	120 Hr	% Recovery
I	Endosulfan content	BDL	BDL	BDL	BDL	-
	B.F.	-	-	-	-	-
II	Endosulfan content	0.7763 ±0.00012	0.7592 ±0.00036	0.7345 ±0.0004	0.7230 ±0.00018	6.86%
	B.F.	77.63	75.22	73.45	72.3	-
III	Endosulfan content	1.8107 ±0.00014	1.806 ±0.00062	1.7976 ±0.00043	1.7748 ±0.00026	1.98%
	B.F.	90.53	90.3	89.88	88.74	-

B.F.: Bioaccumulation Factor; BDL: Below Detectable Limit

The concentration of endosulfan increases in groups II & III in comparison to control (Table 1). Significantly higher accumulation is noted in group III as compared to group II. Bioaccumulation factor & percent accumulation in treated groups show progressive increase from 72 hr onwards (Table 1). Results indicate a significant influence of dose & duration on endosulfan uptake by the gills.

In comparison to control (Fig.1) pathological conditions are observed to increase progressively in treated groups (Figs. 2 & 3). Vacuolization, hyperplasia, fusion of lamellae, enlargement of blood capillaries, disorganized pillar cell system & detachment of basement membrane from pillar cell system is clearly seen. Bud formation occurs prominently in the cartilaginous region along with blood cell accumulation. These pathological changes are more prominent in gills of group III showing telangiectasis in some lamellae (Fig.3). Cartilaginous core runs as a narrow streak throughout its length.

Recovery in group II is 6.86% while in group III it is just marginal i.e. 1.98% (Table 2). A decrease in endosulfan content & lowering of bioaccumulation factor depicts that the accumulation of endosulfan is reversible to some extent. Notable recovery is observed in histopathological features in group II (Fig. 4). In group III, however, only stray signs of recovery can be observed till the end of the recovery phase, though recovery is progressive from 0 hr to 120 hr interval (Fig. 5). Recovery of histopathological condition is inversely proportional to exposure & dose level.

Histology of Gills: Experimental Phase:

Fig.1 Photomicrograph of the gills of *Channa punctatus* showing normal secondary gill lamellae (sgl); normal pillar cell system (pcs); blood capillary (bc); epithelium (ep); normal cartilaginous core (cc). X200 (Group I, 72 hr)

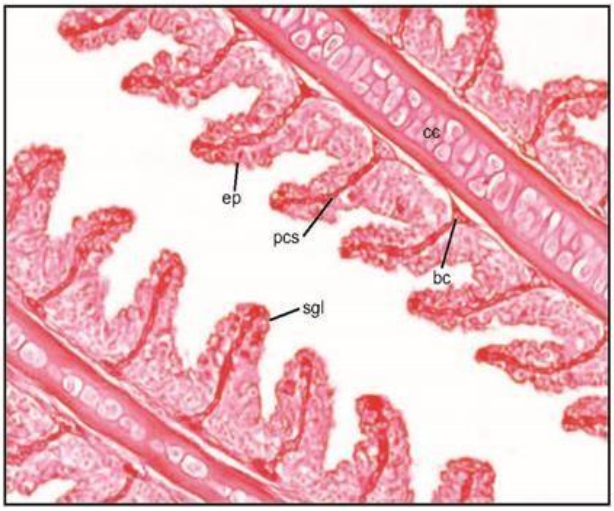

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Fig 2: Photomicrograph of the gills of *Channa punctatus* showing short, broad & blunt sec. gill lamellae (sgl), fusion (fu), hyperplasia (hp), vacuolization (vc), disorganized pillar cell system (D) & detachment of basement membrane (dt), blood cell accumulation (bc) in sgl, narrow cartilagenous core (th) shows bud formation (bf). X100 (Group II, 120 hr)

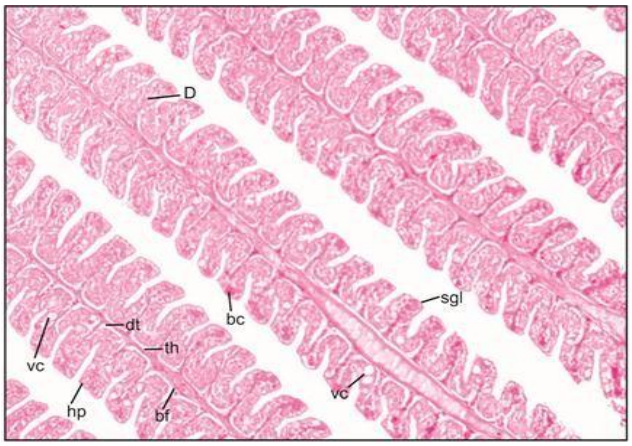
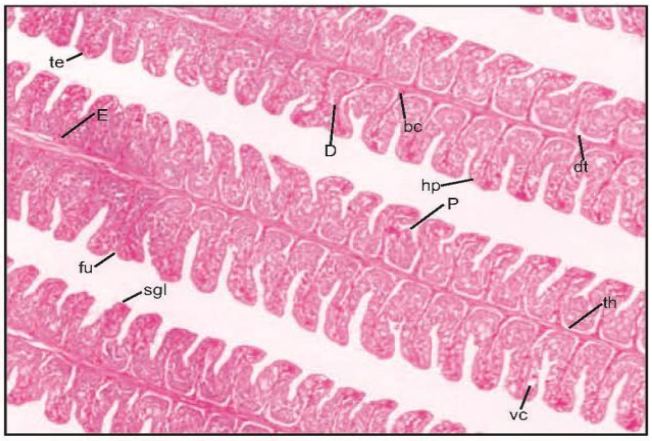


Fig. 3: Photomicrograph of the gills of *Channa punctatus* showing shortened sec. gill lamellae (sgl), fusion (fu) of sgl, extensive hyperplasia (hp), vacuolization (vc), enlarged blood capillaries (E), disorganized pillar cell system (D) can be clearly noticed & detachment of basement membrane (dt), blood cell accumulation (bc), proliferation (P) from the base of sec. gill lamellae & telangiectasis (te) in some sec. gill lamellae is also visible , narrow cartilagenous core (th) is observed. X100 (Group III, 120 hr).



Recovery Phase:

Fig. 4: Photomicrograph of the gills of *Channa punctatus* showing marked recovery; triangular shape of sec. gill lamellae (sgl), mild fusion (fu), hyperplasia (hp), vacuolization (vc), normal pillar cell system (pcs); blood capillary (bc); epithelium (ep); and cartilaginous core (cc). X100 (Group II, 120 hr)

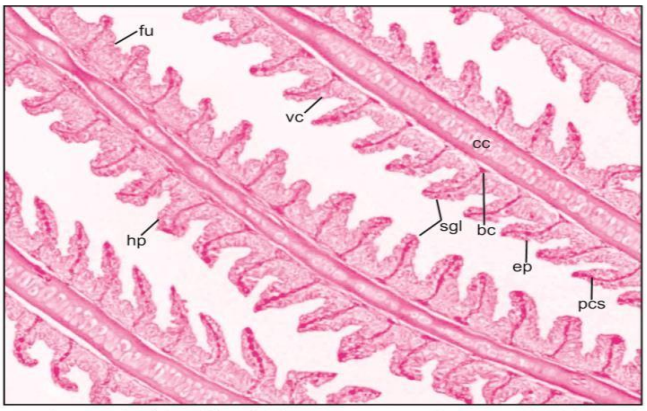
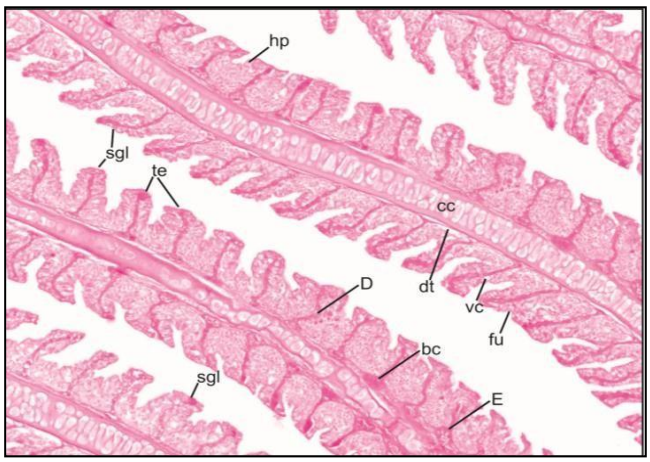


Fig 5: Photomicrograph of the recovery in gills of *Channa punctatus* showing blunt shape of sec. gill lamellae (sgl) mild fusion (fu) of sgl, hyperplasia (hp), vacuolization (vc), enlarged blood capillaries (E), disorganized pillar cell system (D) can be clearly noticed & detachment of basement membrane (dt) is seen only in few lamellae, blood cell accumulation (bc), telangiectasis (te) in some sec. gill lamellae is also visible and normal cartilaginous core (cc). X100 (Group III, 120 hr)



4. DISCUSSION:

Miranda *et al.* (2008) have suggested various factors (i.e. water solubility, degree of ionization, stability, molecule size or shape and lipid content of the species) to be responsible for endosulfan accumulation. Iyamu *et al.* (2007) have suggested lipophillic nature of OCPs to be an important factor. It has earlier been suggested that organochlorine residue accumulates in aquatic biota as a result of its low solubility in water and high solubility in fats (Matthiessen *et al*., 1982). In the present study, it is observed that exposure to endosulfan, even in minute quantities results in its accumulation in the gills of *C. punctatus* leading to tissue dysfunction. Significant residues of endosulfan have been detected in various tissues after acute exposures (Nowak, 1996; Srivastava & Kaushik, 2001 & Harit & Srivastava, 2007). An increase in endosulfan content has been observed with an increase in dose & duration of exposure of the kidney of *C. punctatus* (Verma, 1989) & ovary of *Mystus vittatus* (Verma, 1990). In the present study too, a dose & duration dependent elevation is recorded in endosulfan content of gills of *C. punctatus*.

Histopathological effects of endosulfan on gills of *Rarbora daniconius* was studied by Ghanbahadur *et al*., (2015) for 96 hrs. They also observed that the effects were although similar in treated groups but intensity of damage was dose dependent as observed in the present study. Similar observations were also recorded by Velmurugan *et.al* (2009). They concluded a direct correlation between pesticide exposure and histopathological disorders in gills and liver of *Cirrhinus mrigala* exposed to dichlorvos for 10 days. They also suggested that this could result in severe

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physiological problems, ultimately leading to the death of fish. Present results are also supported by a study conducted by John & Jayabalan (2007) who also observed hyperplasia, lamellar fusion and exudation of erythrocytes & bulging of the tips of primary lamellae on exposure of *Cyprinus carpio*, to endosulfan. Severity of histological changes in the gills was reported to be dependent on both the concentration of endosulfan & duration of exposure (John & Jayabalan, 2007). Kumari & Kumar (1997) suggested that hyperplasia & necrosis of secondary gill lamellae reduces the respiratory area thereby reducing the respiratory & osmoregulatory potential of the gills. Similar pathological changes observed in the present study, would definitely result in respiratory distress. High concentration of endosulfan in gills, as observed in the present study can be directly correlated with pathological changes noted in the gills of the treated fish. These results are supported by the work of Singh & Sahai (1990) who similarly recorded accumulation of endosulfan in the gills along with pathological changes in *Puntius ticto* exposed to endosulfan for 15 days.

Retention of endosulfan in gills is proportional to the level of endosulfan in the medium during treatment. On return of the fish to endosulfan free water, slow elimination of endosulfan is observed in gills of both treated groups. Present results are supported by the observations made by Johnson & Toledo (1993) & Ponmani & Dhanakkodi (1996) in gills of *B. rerio* & whole body of *C. carpio* respectively. They also noted gradual elimination of endosulfan on depuration.

Slow recovery in the pathological condition of gills & liver has been recorded by Studnicka *et al*. (1983) in carp fry (*Cyprinus carpio*) following depuration for 7, 14, 21 & 28 days; fry were pre-exposed to solutions of DDT, lindane & alpha HCH for 96 hr. It is probable that gills may show recovery if kept in endosulfan free water for longer duration.

5. CONCLUSION:

Fish pre-treated with lower dose show early signs of recovery than those pre-treated with higher dose; however, complete recovery is not achieved in either of the pre-treated groups. It can, therefore, be concluded that a longer period would be required for endosulfan levels to fall below detectable limits. Slow elimination indicates that the stress of pre-exposure probably alters certain basic biodegradation mechanisms. Retention of large amounts of endosulfan by the gills can also be correlated with histological damage observed in the gills. In comparison to control, these histopathological changes are persistent during recovery span.

This study is important from an ecological point of view, as it can be good indicator of the level of pollutant/pollutants that may be toxic/lethal to the fish. Low concentrations of toxicants in the water may not be detectable by ordinary water analysis techniques & water may be considered within safe limits; fish living in these waters are adversely influenced, gills being the early targets. Adverse influence on the nutritive value of fish cannot be ruled out. On consumption of such fishes, man will not be a beneficiary.

6. ACKNOWLEDGEMENT:

The authors are grateful to Head, Department of Zoology & CAS, Department of Zoology, University of Rajasthan, Jaipur, for providing necessary facilities.

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