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HISTOPATHOLOGICAL EFFECT OF PESTICIDE PROFENOFOS ON THE SKIN OF AIR BREATHING TELEOST *Channa gachua*

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The effect of pesticide profenofos (organophosphate), on the skin of air breathing fish *Channa gachua* were studied. The histopathological alteration in the skin have been observed in Laboratory Condition after exposing to sub-lethal concentration of Profenofos (0.06 ppm) during different time period (10 days, 15 days and 20 days). The gross pathological changes after profenofos exposed includes:- The epithelial cells start separating, dermal tissues disorganised. The thickness of skin decreased, reduction in number and size of epidermal cells also occur. On the basis of the above finding it is concluded that widely used profenofos in agriculture has produced significant effects on skin of air breathing fish *Channa gachua*.

Keywords: Profenofos; histopathology; skin; *Channa gachua*.

1. INTRODUCTION

Channa gachua is one of the important fresh water fish in the tropical region of India belonging to the group of an air breathing teleost. It is commonly called 'Chenga'. It can survive out of water for a considerably long period if the skin remains moist. Pesticides in sub-lethal concentrations present in the aquatic environment are too low to cause rapid death directly but may affect functioning of the organisms, disrupt normal behaviour and reduce the fitness of natural population. In the aquatic environment, one of the most important manifestation of the toxic action of chemical is the over stimulation or depression of respiratory activity. The oxygen consumption of an animal is the important physiological parameters to assess the toxic stress, because it is a valuable indicator of energy expenditure in particular and metabolism in general (Prosser and Brown, 1977). Pesticides enter into the

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fish mainly through gills and with the onset of symptoms of poisoning, the rate of oxygen consumption increases (Premdas and Anderson, 1963). In air breathing fishes, gaseous exchange is bimodal with water via buccopharynx, gill and air breathing organs in respiration varies from species to species. Hence, any change in normal respiratory epithelium would ultimately affect the rate of oxygen uptake. Organochlorines, organophosphates, organo-sulphate, carbamate and pyrethroids are the major groups of pesticides.

These are persistent their chemical stability gives the products long live in aquatic media and in animal and plant tissue. In case of inland water contamination pesticides are known commonly, closer to areas of their applications which affect growth, reproduction and nutritional value of fish, when their concentration in water exceeds the critical maximum limit [1]. Among different classes of pesticide organophosphate are more frequently used, because of their high insecticidal property, low mammalian toxicity, less persistence and rapid biodegradability in the environment. Alterations in the cellular morphology of pesticide treated fish [2] and their physiological functions upon exposure to different pesticide concentrations have been observed by Gupta and Saxena [3]. The investigation of histopathology of various organs may therefore, prove it is cost effective tool to determine health of fish population and reflecting the health of entire aquatic ecosystem Vinodhini and Narayanan (2009). The present study also showed that this type of pesticide are more toxic in the fish in comparison to other pesticides, such as LC_{50} of endosulfan [4], chlorpyrifos [5] carbosulfan, glyphosate, and atrazine [6]. Contamination by pesticides in aquatic ecosystem is a serious problem and fishes are more frequently exposed to these pollutants and may be taken in through skin and contaminated foods. Acute and chronic toxic effects of Organophosphate in different fish species were extensively studied [7,8].

Padmini and Rajaram [9] studied the effect of different concentrations of organophosphate chlorpyrifos on Protein, glycogen and lipid in Liver and Kidney of *channa gachua* for 96h and reported decreased levels in both the tissues in comparison with control. Profenofos, an organophosphate pesticide is extremely toxic to fishes. The toxic action of profenofos is the inhibition of the acetylcholine esterase activity resulting is neurotoxicity to aquatic vertebrate and also humans.

The Organophosphate insecticides have been shown to exert Lethal effect on some species of fishes and aquatic fauna [10], Shrafeldin et al., 2015, [11]. Monocrotophos (Organophosphate) pesticide has been found to cause reproductive toxicity in fishes [12]. The sublethal concentration of organophosphate pesticide Chloropyrifos were investigated on fresh water air breathing fish *Channa punctatus* [13].

Profenofos is commonly used in India for pest control in mango, banana, cotton, and pineapple agriculture (Das et al., 2006; Reddy and Rao, 2008; Kavitha and Rao [14]; US EPA, 2015). The toxicology of Profenofos was re-evaluated by the 2007 JMPR which estimated an ADI of 00.03 mg/kg bw and an ARFD of 1mg/kg bw. Commercial Name – Celcron, Brand Name – Excel Crop Care Limited, Chemical Name - Profenofos (%EC), Molecular Weight – 373.63, Molecular Formula – C_{11} H_{15} $BrC1O_3PS$. These pesticides may cause two types of poisoning:-First, Acute poisoning, resulting from short term exposure to toxic materials and Second, Chronic poisoning, resulting from long term exposure to small quantities of toxic materials from their ingestion. Acute toxicity is of more importance to people engaged in manufacturing and formulating pesticides and to those responsible for their application. Chronic toxicity, however are of much greater public interest, because of their potential effect on the consumer of agricultural products including fishes. Many of the species bear the ability to breath on atmospheric oxygen for quit a long time (*Clarias batrachus, Heteropneuster fossilis, Channa punctatus, Channa gachua, Anabas testudineus* itc). While few need high concentration of ambient oxygen for normal life (*Labeo rohita, Catla catla, Cirrhana mrigala*). The freshwater air breathing fishes snakeheads are fishes of family channidae, to which two genera (Channa, 26 Species and Parachanna, 3 species) belong [15]. Mortality of fish or decline in a population is at present indicating that the affects of environmental stress factors are exceeding the acclimation tolerance limit of fish. Present work will provide sufficient information on the pollutional effects of profenofos contamination in the aquatic environment on fish biology, Histology, fresh water as well as derelict swamps leading to a better understanding for talking safety measures for improved aquatic organisms and mankind health.

2. MATERIALS AND METHODS

Fish were washed with potassium permanganate ($KMnO_4$) at very low concentration of 0.05mg/l of water to remove dermal infections. After proper washing, fish were acclimated for 20 days in the laboratory conditions, before being subjected to various experiments. During the bio-assay tests fishes did not receive any food. The physiochemical properties of water used for the fish bio-assy test were determined according to the procedure described in standard methods [16]. The water quality parameters were as follow: pH=4.2, Temperature=27^0C, Dissolved Oxygen =6.8mg/l (In test water), CO_2 = 8.0 mg/l, Hardness in $CaCO_3$ = 56 mg/1. Alkalinity as HCO_3 = 130 mg/l. For the preparation of stock solution, 1 ml of profenofos was dissolved in 1 litter of distilled water. The bio-assay test to determine the toxicity of profenofos was done following the method described by APHA [16]. For experimentation, laboratory acclimatized fishes were exposed to different concentration of profenofos. Water in test aquaria was changed every 24 hours and was supplied with full aeration. A batch of 10 fishes was also maintained along with experimental fishes as control group. The fish were then exposed to 0.2ppm,0.4 ppm, 0.6 ppm and 0.8 ppm concentration of profenofos and their percent mortality at different time intervals (24 hours, 48 hours, 72 hours and 96 hours) were recorded (Table 1) as measure of acute toxicity and future statistical calculations. Kaber method, Probit analysis method. The safe concentration of the toxicant was determined by adopting the formula of Hart et.al. [17]. At different exposure periods, skin of treated specimen and control specimen of *Channa gachua* were dissected out and kept in normal saline to remove traces of blood. The small pieces of these specimens were fixed in Bouins fluid and 10% Formaline for 24 hours Tissue were dehydrated in different graded series of alcohol, they were cleared in xylene and embedded in paraffin. Further the sagittal sections (5µm) were cut using a rotary microtome and mounted on glass slides. Sections were deparaffinised in xylene, hydrated in ethanol and stained with haematoxylin and alcoholic eosin (H&E) for general histological evaluation [18]. The photomicrographs of stained sections were made using Nikon upright microscope on 400x magnification.

3. RESULTS

The histopathological examination of the skin of *Channa gachua* revealed three principal layers - the epidermis, dermis and sub cutis in control fish. For the histopathological analysis the epidermis of fish can be divided into three layers: basal layer (innermost layer), middle layer and outer layer. The cellular components identified were epithelial cells or polygonal cells, mucous cells or goblet cells and club cells (Fig.1, Table 2). The basal layer consists of a single layer of columnar epithelial cells, the middle layer consist of large sized spherical or oval club cells and outer layer consist of polygonal epithelial cells with small sized mucous cells. The dermis was divisible into two layers, the outer stratum laxum and inner stratum compactum. The stratum laxum was composed of comparatively loosely arranged connective tissue fibres, richly supplied with blood capillaries and nerves. Stratum compactum was composed of compactly arranged connective tissue fibres, branches of main blood capillaries and nerves from sub cutis. The sub cutis layer was innermost layer situated between the dermis and muscles. The sub cutis layer was composed of loose connective tissue and was richly infiltrated with the fat cells. The skin of *Channa gachua* exposed to profenofos showed epithelial cells start separating and dermal tissues were disorganised. The thickness of skin decreased, reduction in number and size of epidermal cells also occurred (Fig.2, Table 2). The epidermis and dermis collagen appearing loose and bursting of blood capillaries in dermis were obtained. The degenerated epithelial cells and club cells, scar tissue with bundle of collagen fibres were also seen (Fig.3, Table 2).. In the later stage of profenofos exposure skin showed epidermis and dermis recognised. The hyperplasia of the middle layer epidermis shows many club cells The degeneration of club cells gets occupied by haphazardly arranged epithelial cells.

Table 1. Mortality of *Channa gachua* exposed to Profenofos for different period (For determination of LC_{50} value)

Aquarium No.	No. of Fish	Conc. of Profenofos	Mortality Time							
			24 hours		48 hours		72 hours		96 hours	
			Number	%	Number	%	Number	%	Number	%
1	10	Control	0	0	0	0	0	0	0	0
2	10	0.2ppm	0	0	1	10	3	30	4	40
3	10	0.4ppm	1	10	4	40	5	50	6	60
4	10	0.6ppm	2	20	7	70	7	70	9	90
5	10	0.8ppm	6	60	8	80	9	90	10	100

Table 2. Histological effect of Profenofos (0.06ppm) on skin when exposed to different period

Control Fish (Fig 1)	Profenofos treated fish		
	For 10 days (Fig 2)	For 15 days (Fig 3)	For 20 days (Fig 4)
Epithelial Cell – Normal Dermal Tissue – Organized Skin Thickness – Normal Epidermal Cell - Normal	Epithelial Cell – Start separating Dermal Tissue – Dis-organized Skin Thickness – Decreased Epidermal Cell – Decrease in number	Epidermal cell + Dermal collagen – Loose Blood Capillaries - Bursting	Middle Layer of Epidermis – Show Hyperplasia Club cell – Degenerate Epidermis and Dermis – Dis-organized

Table 3. Profenofos (0.06ppm) effect on different experimental control and treated fish

Effects on Skin	Control (10-20 Days)		Profenofos Treated					
			10 Days		15 Days		20 Days	
	1st Fish	2nd Fish	3rd Fish	4th Fish	5th Fish	6th Fish	7th Fish	8th Fish
Effect on Epidermis	-	-	++	++	+++	+++	++++	++++
Effect on Dermis	-	-	+	+	++	++	+++	+++

[+] Intensity of Pesticide; [-] No Effect

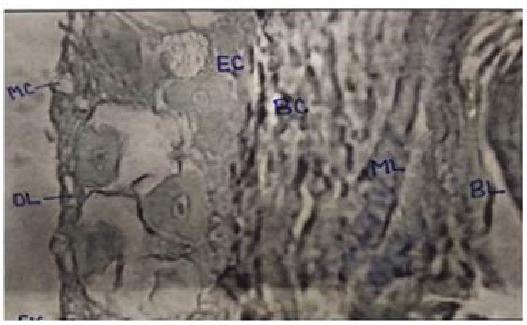

Fig. 1. T. S. of Normal Skin (H/E 400)
BC= Blood Cells, BL= Basal Layer, EC= Epithelial Cells, ML= Middle Layer OL= Outer Layer

Fig. 2. T. S. of Profenofos Treated Skin (after 10 days) (H/E 400)
EC= Epithelial Eells, DT= Dermal Tissue

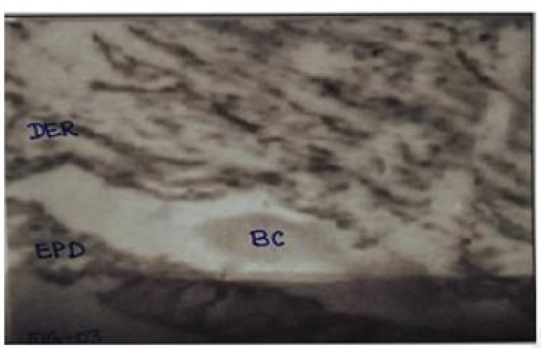

Fig. 3. T. S. of Profenofos Treated Skin (after 15 days) (H/E 400)
BC= Blood Capillaries, DER= Dermis, EPD= Epidermis

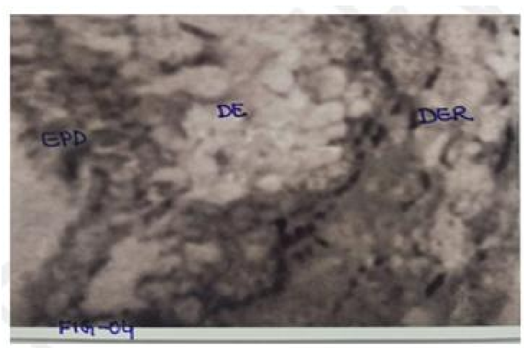

Fig. 4. 2 T. S. of Profenofos Treated Skin (after 20 days) (H/E400)
DE= Disorganised Epidermis, DER= Dermis, EPD= Epidermis

(Fig.4, Table 2). The effect of profonofos occur more on Epidermis in comparison to Dermis. The control fish showed no effect like profenofos treated fish up to 20 days (Table 3).

4. DISCUSSION

Channa gachua as an air breathing fish raises its snout above the water surface to engulf air for aerial respiration. Forced air exposure prevents normal branchial respiration. Occurrence of pesticides in high concentrations in agriculture waste waters and their toxicity to aquatic organisms especially fish species have been reported by many researchers [19,20].

At lethal concentrations, profenofos toxicity like other organophosphate is rapidly reflected in behavioural alterations of exposed fishes. Increased mucous secretion probably helps in countering irritating effect of profenofos in skin and mucous membrane. Excitement, hyperactivity and abnormal jerky swimming observed in exposed fisher may be caused by accumulation of neurotransmitter in neuromuscular junctions. Similar results were observed by (Srivastava et.al., 2007), when *Labeo rohita* and *Channa punctatus* exposed to paper mill effluent. Loss of balance and drowning reflect the progression towards death as fish succumbs to the continued high exposure of profenofos. Similar alterations in behaviour of dimethoate exposed fish have been reported earlier in *Heteropneustes fossilis* [21], and *Cyprinus carpio* [22], Stalin *et. al.,* 2008). Behavioural manifestation of acute toxicity in *Channa gachua* were more or less similar to those reported in other fishes exposed to cadmium . (Nagaraju etal,. 2011). Behavioural manifestations have been established as sensitive indicator of chemically induced stress in aquatic organisms (Suedel et al., 1997; [23,24,25,26]. In the present investigation a gradual erosion in skin (Epidermis) with darkening have been observed .These pigment cells showed variation in shape and hyper melanism (Schliwa,1986). Banerjee (1993), Mittal and Munshi [27], reported darkening of the skin due passive dispersion of the pigment which are under neutral control. Melanophores play an important role in development of skin lesions. Profenofos treated fish are indicative of inflammatory responses. Increase in agranulocytes and granulocytes and macrophages occurs in different pathological conditions and is associated with immunological reaction of fish and play a significant role in local defence mechanism by facilitating transfer of antibody across the mucosa [27] Roberts and bullock, 1980.). The profenofos treated skin showed appearance of blood between outer layer and middle layer indicates trauma of blood vessels causing leaking of blood into abrasion cavity that coagulate to plug the wound gap. This change is accompanied with transduation and increase in interstitial fluid conductivity (Casley Smith and Vineet,1980). Similar to present investigation Agarwal et.al., (1979), noticed the appearance of a large intercellular space in the fish epidermis as a result of exposure to common salt solution and correlated them with requisite passage for the movement of nutrients required for increased metabolic activities of these cells when the fish is under stress.

5. CONCLUSION

The respiratory system provides the most extensive surface of a fish with the aquatic environment. Profenofos as a organop0hosphate pesticide used as its histopathological effect on the skin of *Channa gachua*. The pathological alteration in the skin of *Channa gachua* have been observed in laboratory condition after exposing to the sub-lethal concentration of profenofos (0.06ppm) during different time period (10 days, 15 days and 20 days). The skin of *Channa gachua* revealed three principal layers, the epidermis, dermis and sub cutis in control fish. For the histopathologtical analysis the epidermis of fish can be divided into three layers: basal layer (innermost layer), middle layer and outer layer. The cellular component identified were epithelial cells or polygonal cell, mucous cells or goblet cells and club cells. The skin of *Channa gachua* exposed to profenofos showed epithelial cells start separating, dermal tissues disorganised. The thickness of skin decreased, reduction in number and size of epidermal cells also occur. It can be concluded that the most commonly used organophosphate pesticide, profenofos is also toxic to aquatic environment as even the doses as low as $1/5^{th}$ of 96 hour LC_{50} concentration. It is also Sound harmful even on air breathing fish *Channa gachua*. It is advised to use very low (less than0.06ppm)concentration of pesticides for conservation of environment. Thus it is concluded that agrochemical pesticide profenofos is toxic in nature and almost care is needed while using it over crop-field so as to save the fishes and other aquatic organism.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Moore A, Waring CP. The effect of a synthetic pyrethroid pesticide on some aspects of

reproduction in Atlantic salmon Salmo salar I., Aqu. Toxicol. 2001;52(1):12.
2. Abhilash R, Prakasam VR. Toxic, physic-morphological and behavioural responses Oreochromis mossambicus exposed to commercial grade endosulfan. Envirn. Ecol. 2005;54(2):234-238.
3. Gupta P, Saxena PG. Biochemical and haematological studies in freshwater fish Channa Punctatus exposed to synthetic pyrethroids, Poll. Res. 2006;25(3):499-502.
4. Pandey RK, Singh RN, Singh S, Singh NN, Das VK. Acute toxicity bioassay of dimethoate on fresh water air breathing catfish *Heteropneustes fossilis* (Block). J. Environ. Biol. 2009;30:437-440.
5. Ali D, Nagpure NS, Kumar S, Kumar R, Kushwaha B. Genotoxicity assessment of acute exposure of chlorpyrifos to acute toxicity in fish due to profenofos; 2008.
6. Nwani CD, Nagpure NS, Kumar R, Kushwaha B, Kumar P, Lakra WS. Lethal concentration and toxicity stress of Carbosulfan, glyphosate, and Atrazine to freshwater air breathing toxicity stress of Carbosulfan, glyphosate, and Atrazine to freshwater air breathing fish *Channa punctatus* (Bloch). Int Aquat Res. 2010;2:105-11.
7. Anita B, Yadav AS, Cheema N, Genotoxic effects of chlorpyrifos in freshwater fish cirrhinus mrigala using micronucleus assy, Advances in Biology. 2016;1-6.
8. Arjmandi R, Tavakol M, Shayeghi M. Determination of organophosphorus insectivide residues in the rice paddies. Intl, J. Environ. Sci. Technol. 2010;7:175-182.
9. Padmini K, Rajaram P, Effect of chlorpyrifos on some biochemical constituents in lever and kidney of freshwater fish, *Channa gachua* (F. Hamilton), International Journal of Science and Reserch (USR). 2016;5(4):1975-1979.
10. Bacchetta C, Rossi A, Ale A, Campana M, Parma MJ, Cazevave J. Combined toxicological effects of pesticides:- A fish multi-biomarker approach. Ecological Indicators. 2014;36:532-538.
11. Singh CB, Ansari BA. Bioassay of profenofos and cypermethrin on Zebrafish, Danio rerio. Scholar Academic Journal of Bioscience. 2016;4(7):578-582.
12. Ru S, Tian H, Wang W, Bing X. Effect of monocrotophes on the reproductive axis in the female goldfish (Carassius auratus) Comp. Bischem. Physiol.Part C. 2010;152:107-113.
13. Yogita D, Mishra A. Histopathalogical Alterations in Gill and Liver Anatomy of fresh water, Air Breathing Fish Channa punctatus after pestiside Hilban (chlorpyrifos)Treatment.Adv. Biores. 2013;4(2): 57-62.
14. Kavitha P, Rao VJ. Sub lethal effects of profenofos on tissue specific antioxidative responses in a Euryhyaline fish, Oreochromis mossambicus. Ecotoxicol Environ Saf. 2009;72:1727-1733.
15. Nelson JS. Fishes of the world. 4th ed. Hoboken (New Jersey, USA); 2006.
16. APHA. Standard method for the examination of water and waste water 20th edn. American public health association Washington, DC. USA; 1998.
17. Hart WB, Doudoroff P, Greenbank J. The evaluation of the toxicity of industrial wastes, chemical and other substances to freshwater fishes .l Atlant. Refinding Co. (Phill). 1945;317.
18. Bernet D, Schmidt H, Meir W, Burkhardt-Holm P, Wahli T. Histopatholog*y in fish:* Proposal for a protocol to assess aquatic pollution. J. Fish Dis. 1999;22:25-34.
19. Capel PD, Larson SJ, Winterstein TA. The behaviour of thirty-nine pesticides in surface waters as a function of scale. Hydrol. Process. 2001;15:1251-1269.
20. Galloway T, Handy R. Immunotoxicity of organo-phosphorous pesticides. Ecotoxicol. 2003;12:345-63.
21. Pandey S, Nagpure NS, Kumar R, Sharma S, Srevastava SK, Verma MS. Genotoxicity evaluation of acute doses of endosulfan to freshwater teleost *Cahanna punctatus* (Bloch) by alkaline single-gel electrophoresis. Ecotoxicol Environ Saf. 2006;65:56-61.
22. Singh RN, Pandey RK, Singh NN, Das VK. Acute toxicity and behavioural responses of common carp *Cyprinus carpio* (linn.) to an organo phosphate (Dimethoate). WJZ. 2009;70-75.
23. Baltova S, Velcheva I. Some Morphological modification of the Blood Cells from Alburnus alburnus in Intoxication with Heavy Metals (Pb, Zn and Cb). *Proceedings of the Balkan Scientific Conference of Biology in Plovdiv* (Bulgaria). 2005;461-467.
24. Vutukuru SS. Acute effect of hexavalent Chromium on survival, Oxygen consumption, Haematologiacal parameters and some biochemical profiles of the Indian major carps *Labeo rohita* Int. J. Environ. Res. Publ. Hlth. 2005;2(3):456-462.
25. Askari Hesni M, Dadolahi-Sohrab A, Savari A, Mortazav MS. Study the Acute Toxicity of Lead Nitrate Metal Salt on Behavioural Changes of the Milkfish (*Chanos chanos*).

World J. Fish & Marine Sci. 2011;3(6):496-501.

26. Remyla SR, Mathan R, Kenneth SS, Karunthchalam SK. Influence of Zink on Cadmium induced responces in a freshwater Teleost fish *Catla catla*. *fish* Physiol. Biochem. 2008;34:169-174.
27. Mittal AK, Munshi JSD. A comparative study of the structure of the skin of certain air breathing fresh water teleosts. J. Zool. Lond. 1971;163:515–532.
