ISSN 2349-7750

INDO AMERICAN JOURNAL OF

PHARMACEUTICAL SCIENCES

Available online at: http://www.iajps.com *Review Article*

THE CYPERMETHRIN TOXICITY EFFECT OF *POECILIA LATIPINNA* BIOCHEMICAS CHANGES

S Senthilmurugan

Department of Zoology, Annamalai University, Tamilnadu,608 002, India

Email Id: senthilmuruganphd@yahoo.co.in

Abstract:

Fishes exposed to 0.2, 0.4, 0.6, 0.8 and 1 ppm of cypermethrin noticed many changes in the behavioral response loss of balancing, increased opercula beats, erratic swimming, swimming at the water surface and gulping for air noticed in the experimental fish. The 96 hr LC_{50} value of the fish exposed to various concentrations of cypermethrin ranging from 0.2 to 1 ppm was plotted and the LC_{50} values found to be 0.7 ppm .The biochemical changes observed in the liver of *Poecilia latipinna* exposed to 0.175 ppm concentration at 24 hours, 96 hours, 240 hours and 360 hours includes organization of the hepatic cell, completely vacuolated nuclei, more number of vacuolation in heptocytes, decrease in size of nucleus (Pycnotic).The histomorphology of the intestine in the control fish exhibited simple columnar epithelium, lamina propria, sub mucosa, tunica muscular is and serosa. A few goblet cells containing lymphocytes were located between the epithelial cells. The mucosa was highly branched and folded. The intestine of the experimental fish revealed damage to the appearance of few goblet cells, disintegration of cell wall, and shrinkage of columnar epithelial cells, Damage of columnar epithelial cells and hyperactivity and appearance of more number of goblet cells. The biochemical changes of the liver and intestine were studied in control and experimental fishes using Haematoxylin and Eosin based on the observation, the effect of cypermethrin was discussed with light of the available literature.

Keyword: biochemical**,** intestine**,** Behavioral responses**,** *Poecilia latipinna, cypermethrin*

Address for Correspondence:

S Senthilmurugan

Department of Zoology, Annamalai University,

Tamilnadu,608 002

Email Id: senthilmuruganphd@yahoo.co.in, India

INTRODUCTION:

Fish are particularly sensitive to a wide verity of pesticide chemicals and toxic concentration may rise not only from spillage of agricultural practical if their use is excessive but also from several other sources. The industrial development and rapid urbanization has led to development of polluted zones discharging potentially toxic compounds in the environment toxicology studies followed [1] Apart from causing death either directly or due to starvation by destruction of food organisms many pesticides have been shown to effect growth rate, reproduction and behavior with the evidence of tissue damage [2] .Owing to the excessive use of synthetic pyrethroids, the environment and water resources are being polluted, thus endangering aquatic life directly and human life indirectly [3].

The bivalves exposed to several envinmental factors may reflect adaptive metabolic mechanisms due to the challenges of the changing environment including the pollutants[4]. Domestic sewages are run off from agriculture fields loaded with pesticides and fertilizers, pollute the water bodies. Commonly used pesticides can be harmful living organisms, pets, and their environment [5] Bioassay of heavy metals in marine and coastal ecosystem is of great concern for decline or loss of fishery resources since past several decades[6, 7]. Heavy contamination of pesticides in water in turn leads to oxygen depletion and cases of poisoning and the mass mortality of fishes is not uncommon.

The recently introduced synthetic pyrethroids with multiple beneficiary qualities have attracted farmers to use these compounds in pest control. But these compounds have been found to be highly toxic to fish [8, 3]. Pesticides are generally used in contemporary agriculture to aid in the manufacture of high quality food [9]. A number of chemicals released into environment contaminate especially aquatic ecosystem through different ways [10].

Domestic sewages are sprint decomposed commencement agriculture field weighed down with pesticides and fertilizers contaminate the water body tissues or organisms at very low levels [11].Pesticides represent some of the most spread pollutants, raising difficult problems for the environmental production as the cause noxious effect upon certain useful organisms. The use of pesticides has increased with the growing awareness about their utility in agriculture production, animal husbandry and post harvest technology and in the public health and welfare of mankind.Fish is very sensitive to changes in water due to addition of effluents and toxicants[12] and changes occurring in the biochemical characteristics of fish provide a sensitive measure to know the health of fish fauna [13] Most heavy metals are release in to the aquatic phase as a result of direct input, atmospheric deposition and erosion due to rain water and therefore aquatic animals may be exposed to elevated levels of heavy metals due to their wide use of anthropogenic purpose [14].

The introduction of small amounts of many relative toxic materials in a aquatic environment cause multiple change is the internal dynamics of aquatic organism at sublethel levels: in aquatic toxicology, extensive literature is available on effects of various pollutants on the biochemical composition of tissue of different types in marine bivalve [15] from the available literature it appears that the study of biochemical composition is very useful in understanding the mechanism of metal toxicity to green mussels.Food is an important source of energy for all living organisms. Food energy is used for building up body tissue, which further signifies that a balanced diet is necessary for proper functioning of the body. Recent understanding of different biochemical processes has proved useful in determining the mechanism of toxicity in different toxicants also in unfolding the adaptive protective mechanisms of the body to ;combat the toxic effect of the pollutants, besides it is also observed that some biochemical alteration occurring in the body give the first indication of stress in the organism and hence the efforts, on the part of pollution biologists to explore the possibility of making use of the phenomenon to locate certain type of pollutants in nature.

In the present study an attempt has been made to observe the biochemical changes induced by a synthetic pyrethroid, cypermethrinon histology, of certain vital organs namely intestine and liverof an aquarium fish *Poecilia latipinna* commonly known as black molly.

MATERIAL AND METHODS:

Collection and maintenance of fishes

Live specimen of *Poecilia latipinna* commonly known as black molly is used for present study (Fig.2). A total number of 150 fishes procured from Department of Zoology, Annamalai University transported to the laboratory with sealed polythene bags filled with oxygen. The size of the fish was ranged between 5 cm and 7 cm in length. The fishes acclimatized in the laboratory for about 15 days. They were fed with formulated fish feed and boiled

egg and the water was exchanged every alternative days.

Fig No: 1 Experimental animal

Determination of lethal concentration (LC_{50})

Stock solution of Cypermethrin (10% EC) (Sicorin 10 Scientific fertilizer Co.Pvt., Ltd., Trichy) pesticide was prepared by diluting 1ml insecticide in 100ml of distilled water. From the stock solution different concentrations of Cypermethrin, 0.2, 0.4, 0.6, 0.8 and 1ppm were prepared by diluting the respective milliliter of the stock solution in one liter of test water. Healthy fishes (n=10) with an average weight of 2 gram and an average length of 5cm were maintained in each test solution for 4 days to determine the LC_{50} values. Feeding was stopped one day prior to the experiment and also during the experimental period. The LC_{50} values were found by arithmetic graph method. Graph was used in this type of analysis with percent survival on the ordinate against the concentration on the abscissa. Each data points is plotted and connected to form a graph. A horizontal line was drawn from the 50 percent survival point to intersect the plot. A vertical line from the intersection point was then dropped to the abscissa. This intersection point was then dropped to the abscissa. This intersection point on the abscissa corresponds to the 96 hours LC_{50} [16].

MATERIALS AND METHODS

A. Total Carbohydrate Estimation:

The total carbohydrate content was estimated by the technique of [17]. A 10% homogenate of tissue was prepared using 5% TCA and this was centrifuged at 3000 rpm for 10 minutes. Samples were cooled in the dark at room temperature for 30 minutes. The supernatant was collect and the optical density was measured in a spectrophotometer [17] at a wavelength of 620 nm a blank reading. Blank was prepared by mixing 1 ml of distilled water with 4 ml of Biuret reagent. The total carbohydrate content in mg/g of tissue.

B. Total Protein Estimation:

Protein was estimated by the method of [18]. 1% tissue homogenate were prepared in 10% TCA and centrifuged at 3000 rpm for 15 minutes. The gal set was dissolved in 1 ml of 1N NaOH to the above 5 ml of alkaline copper reagent was added and after 10 minutes, 0.5 ml of folin phenol reagent was measured after was added and rapidly The moisture content was estimated by subtracting the dry weight (dried in a hot air oven) of the muscle tissue from the known wet of the muscle tissue.

C. Lipid Estimation:

The total lipids were extracted by the method of[19] to find out total lipid, known volume of experiment samples were homogenized with 1 ml of methonal and 2 ml of chloroform to which again 2ml of chloroform : methanol (2:1 v/v) was added and mixed thoroughly. To this, 0.2 ml- 0.09 % sodium chloride solution was added. The above mixture was poured into separately funnel, mixed and allowed to stand for few hours.

The lower phase was separated and 0.5 ml of extract was measured and poured into a clean test tube. It was allowed to try in vacuum dedicators over silica gel, dissolved in 0.5 ml concentrated sulphuric acid and mixed well. The tube was plugged with non-absorbent cotton wool and placed in a boiling water bath for 10 minutes and the tubes were cooled at room temperature. 0.3 ml of this acid digest was taken for experimental analysis. 0.5 mg of cholesterol For stand and,0.5 ml of distilled water for blank separately. To each tube, 5ml of vanillin reagent was added. Mixed well and allowed to stand for half an hour and the developed color were measured at 250 nm.

RESULTS:

Observations on the behavioral responses of *Poecilia latipinna*, (Fig. 2) exposed to cypermethrin at different concentrations exhibited several pathological symptoms. The control group showed normal behavior during the test period.The loss of balancing, increased opercula beats, erratic swimming, swimming at the water surface and gulping for air noticed with the experimental fish of the present study. In 1 ppm concentrations, loss of balancing, frequent surfacing and rapid opercula movements were noticed. In 0.8 ppm concentrations, fishes were resting at bottom in crowds while in 0.6 ppm concentration, there was a reduction in swimming activity followed by mucous secretion from mouth and gills. In 0.4 ppm concentration, gills become pale and fishes were violent showing erratic swimming movements.

In contrast, the controls did not show any change in the behavioural symptoms with respect to swimming activity, opercula movements and mucous secretion. Controls responded very well to external stimulus. The LC_{50} value for 96 hours duration of *Poecilia latipinna* exposed to cypermethrin was found to be 0.7 ppm .The arithmetic graph plotted for LC_{50} value is presented in (Figure 1).The liver of the normal fish comprises a continuous mass of hepatic cells with cord like formation. Hepatic cells are roundish, polygonal, containing clear spherical nucleus. They are located among sinusoids forming cord like structure known as hepatic cell cords. Bile canaliculated is centrally located in each cord. A large number of blood sinusoids are found in the hepatic mass of these cords. Thin bile canaliculi are observed between the hepatic cells (Figure 3).

The biochemical changes noticed in the liver of the exposed fish at the same concentration with the 360 hours exposure showing degeneration of cytoplasm in hepatocytes (DCH) and disintegration of of hepatocytes (DNH). (Figure 6). The intestine of *Poecilia lattpinna* exhibit the simple columnar epithelium, lamina propria, submucosa, tunica muscularis and serosa. The long thin columnar epithelial cells of the mucosa showing surface microvilli. A few goblet cells containing lymphocytes were located between the epithelial cells. The mucosa was highly branched and folded. The observation of the experimental Fish exposed to 0.175 ppm of cypermethrin for 24 hours showed with a few numbers of goblet cells. Intestine of the exposed to 0.6 ppm at 4 days duration shows more goblet cells. The sublethal exposure of cypermethrin of 0.6 ppm at 10 days duration showed more goblet cells and disintegration of cell wall (Fig 8). The intestine of the fish exposed to 0.6 ppm concentration at 15 days duration shows appearance of more goblet cells, slight shrinkage of columnar epithelial cells (Fig 8).

Table 1. Total carbohydrate levels in muscles

Muscles Hours of Exposure				
Carbohydrate	**24 Hours**	**48 Hours**	**72 Hours**	**96 Hours**
Control	7.54 ± 0.19	6.83 ± 0.19	5.91 ± 0.04	4.77 ± 0.09
Treatment	6.27 ± 0.61	6.05 ± 0.02	4.86 ± 0.03	3.81 ± 0.02
% Changes	16.8	11.0	17.7	20.12

Table 2. Total proteins levels in muscles

Muscles Hours of Exposure				
Proteins	**24 Hours**	**48 Hours**	**72 Hours**	**96 Hours**
Control	7.24 ± 0.19	6.83 ± 0.19	5.94 ± 0.04	4.77 ± 0.09
Treatment	6.07 ± 0.61	4.05 ± 0.02	3.86 ± 0.03	3.22 ± 0.02
% Changes	13.8	10.0	12.7	17.12

Table 2. Total Lipids levels in muscles

Muscles Hours of Exposure				
Lipids	**24 Hours**	**48 Hours**	**72 Hours**	**96 Hours**
Control	3.24 ± 0.19	5.83 ± 0.19	4.94 ± 0.04	3.77 ± 0.09
Treatment	3.07 ± 0.61	2.05 ± 0.02	2.86 ± 0.03	2.22 ± 0.02
% Changes	13.8	10.0	12.7	17.12

DISCUSSION:

The Observations pertaining to behavioral responses of *Poecilia latipinna*, exposed to cypermethrin at different concentrations exhibited several pathological symptoms in physiochemical alteration[20]. The control group showed normal behavior during the test period. The loss of balancing, increased opercula beats, erratic swimming, swimming at the water surface and gulping for air noticed with the experimental fish of the present study. Fishes exposed to different pesticides and their behavioural changes in terms of toxic effect was reported by many workers [21]. Similar responses to those of*L.thermalis* such as irregular swimming, abnormal opercular movement, gulping of air and frequent surfacing were reported upon caldan insecticide exposed to *Poecilia reticulata* [22].

The observation of the experimental fish exposed to 0.175 ppm of cypermethrin at 24 hours shows less affected structure with hepatocytes showing

prominent nuclei (Figure 4). Fish exposed to 96 hours duration with same concentration of cypermethrin showed enlargement of hepatic cells (EHC) and formation of vacuoles (FV).(Figure 5). The fish exposed to cypermethrin at 0.175 ppm concentration with 240 hours, noticed damages in the hepatic cells, pycnotic nucleus (PC) and necrosis of hepatocytes (NH) (Figure 6). The biochemical changes noticed in the liver of the exposed fish at the same concentration with the 360 hours exposure showing degeneration of cytoplasm in hepatocytes (DCH) and disintegration of of hepatocytes (DNH).

Tilak *et al*.,[23] reported in *Ctenopharyngodon idellus* exposed to fenevalerate has induced discrete pathological changes in the liver tissues includes degeneration of cytoplasm in hepatocytes, atrophy formation of vacuoles, rupture in blood vessels, necrosis and disappearance of hepatocyte wall and disposition of hepatic cords, degenertion in hepatocyte wall and decrease in size of nucleus[24] reported similar changes noticed in the present study when *Gambusia affinis* exposed to a synthetic pyrethroid.

The observation of the experimental Fish exposed to 0.175 ppm of cypermethrin for 24 hours showed with a few numbers of goblet cells. Intestine of the exposed to 0.6 ppm at 4 days duration shows more goblet cells. The sublethal exposure of cypermethrin of 0.6 ppm at 10 days duration showed more goblet cells and disintegration of cell wall (Fig.11). Sumithra (2004) reported the effect of caldan on the histology of intestine in *Poecilia reticulate*. The changes include shrinkage of lamina propria, vaucolation of columnar epithelial cells, damage and disintegration of epithelial cells and goblet cells. Mandal and Kulshreshthra (1979) while studying the effect of Sumithion in the intestine of *clarius batrachus*, noticed lesion formation in villi, enlarged mucous cells, vacuolization of epithelial cells of mucosa and occurrence of cellular exudates in lumen.

The changes observed were degenerative villi, epithelial cells were degenerated and formed a syncytial mass. Mucous secreting goblet cells showed hyperactivity. Also seen were the oedematous epithelia and connective tissues, dilated blood vessels and lymphocyte migration. Among the three pesticides endosulfan toxicity was more severe as it caused degeneration of the villi besides damage of the epithelial cells, Malathion also showed insignificant damages in the fish *Barbus stigma*. As reported by [25] when fishes exposed to pollutants observed heperactivity of goblet cells also noticed in the present study as *P. reticulate* exposed to cypermethrin at 0.007ppm concentration for longer duration like 192 hrs noticed more number of goblet cells with hyperactivity.

REFERENCES:

1. Agnithothrudo V. 1988 Pyrethroids: their future and toxicity. In: P.K. Gupta and U.Ragivarkash. Advances in toxicology and environmental health. Proceedings of the VT Annual Conference of the Societh of Toxiocology, Guwahati, 65-69.
2. Anand Kumar, V, and Reddy S.L.N, 1997. Acetylcholinesterase in freshwater fish, Clarias batrachus (Linne) as a diagnosis of environmental pollution endosulfan and cypermethrin.J.Aquatic Bio.,**12**(1220:42-46 (16 Ref.)
3. Anita Kumari S, and Sree Ram Kumar N. 1997 Histopatholigical alterations induced by aquatic polluatants in Channa Punctatus from Hussainsagar Lake (A.P). J. Environ.Biol. **18 (1)**: 11-16.
4. Anita Susan T, Veeraiah K, Tilak K.S. 2003 Histopathological changes in the vital tissues of the fish Cirrhinus mrigala exposed o fenvalerate technical grade. Poll. Res., **22(2)**: 179-184.
5. Baile W, and Oberai S, 1991. Comparitive histopathology of methylparathion and endosulfan treated liver of the cat fish Clarias batrachus (Linn). Himalayan J. Environ. Zoo, **5(1)**: 23-28.
6. Bhatnagar M.C, Tyagi M, and Bana A.K, 1994. Malathion poisoning to Clarias batrachus: a Histological study. Port Acad. Environ.Bio., **3(1)**:119-123.
7. Bismuth C, Baud F.J, Conso F, Frejuville J.P, and Garner, R. 1987. Pyrethroids and potent allergens cause allergic rhinitis, asthmaand extrinsic allergic alveolitis. Toxicology clinque.Flammarion, Paris; 424.
8. Canli M. 1996. Effects of Mercury, Chromium and Nickel on glycogen reserves and protein level in tissues of cyprinus caryrio. Trukish Journal of Zoology 20: 161-168.
9. Dutta H.M, Roy P.K, Singh N.K, Adikari S, and Munshi J.D. 1998. Effect of sublethal levels of malathion on the gills of Heteropneustes fossilis; Scaning electron microscopic study. J. Envron. Pttol. Toxicol Oncol, **17(1)**: 51-63.
10. Ebele S, Oladimeji A.A, and Daramolla J.A. 1990.Molluscicidal and piscidal properties of copper (II) tetraxo sulfate (VI) on Bulinus globorus (Mordet) and Clarias anguillaris (L). Aquatic toxicology, **17**: 231-238.
11. Harf, W.B., Doudroff, P, and Greenbunk, J. 1945. The evalution of toxicity industrial waste chemical and other substances to freshwater fishes. The Atlantic printing co, Philadelphia, PA: 317.

12. Kumaraguru A.K, and Beamish F.W.H. 1982 Lethal toxicity of permethorin NRDC-143 to rainbow trout Salmo gairdner, in relation to body weight and water temperature Water Res. **15**: 503-505.
13. Lewis M. 1978 Acute toxicity of copper, Zinc and manganex in single and mixed salt solution to Juvenaile long fin dace, Agosia Chrysogaster.J. Fish Biol, **13**: 695-700.
14. Luther Das V, Jeewaporada P.N, Veeraiah. K, 1999.Toxicity and effects of cypermethrin on biochemical constituents of fresh water teleost, Channa Punctatos. J. Ecotoxicol. Environ. Monit.**9(3)**: 197-203.
15. Mulla, M.S. 1978.Biological activity and longevity of new synthetic pyrethroids against mosquitoes on some non target insect. Environ. Entomol. 4:428-430.
16. Murthy A.S, 1986. Toxicity of pesticides to fish.CRC Press inc., Boca Rocton, Florida.
17. Reddy M.Y, Venkatewadu P, Suimdrarurth K.R. 1986 Photophamidon and lindane induced changes in the hemolymph biochemistry of a penaeid prawn, Metapenaeus monoceros. Natl. Acad.Sci. Lett. 9.**5**:155-157.
18. Rodrigues E, Ranzani-Paiva M.J.T, Pacheco F.J, and Verga M, 2001. Histopathologic lesions in the liver *Prochilodus lineatus* exposed to a sublethal concentration of the organophosphate insecticide Dipterex 500 super R (Trichlorofon); Acta-Scientiarum Maringa, **23(2)**: 503-505.
19. Sandhya G. 1995. Determination of methyl parathion in the liver and kidney of fresh water fish *Channa puntatus* by high performance liquid chromatography. Env. Eco, **13(4)**: 824-826.
20. Thurberg F.P, Calabrease A, Gould E, Greig R.A, Dawson M.A, and Tucker R.K. 1977. Responce of lobster Homarus amercansus to sublethal levels of cadmium and mercury. In: Physiological Responses of Marine Biota to pollutants. F.J. Vernberg,A. Calebrese, F.P. Thurberg and W.B. Vernberg.(eds). Academic press, New York, 195-197.
21. Victor B. 1984. Reproductive biology of the freshwater prawn, *Caridina rajadhari*.Ph. D. thesis, Marathwadw University, Aurangabad.(M.S.)
22. Wagh S.B, and Khillare Y.K. 1989 Histopathological changes in alimentary canal in fresh water fish Barbus Stigma .10th AEB session and Int. Symp. Madras (Abst. Proc).
23. Waldichuk M, 1974. In: Pollution and physiology of Marine organisms, (eds) F.J. Vernberg and W.B. Vernberg, Acadamic press, New York. 1974.
24. Zitco V, Carson W.G, and Metcalfe C.D, 1977. Toxicity of pyrethroids to juvenile Atlantic *Salmon*. Bull. Environ Contam. Toxicol **18**:35-41.
25. Zitco V, Mc Lease D.W, Metcalfe C.D, and Crson L.G. 1970 Toxicity of cypermethrin, deltamethrin and related pyrethroids to *salmon* and *lobster*. Bull. Environ. Conta, Toxicol, **21**:336-343.