



Toxicity of Endosulfan on Biochemical of some vital organs of snake head Fish *Channa punctatus* (Bloch.)

1- Mohan Kumar (Research scholar), 2-Dr. Rishikesh Kumar
Department of Zoology, MLSM college, LNM University, Darbhanga.

Abstract

The current study includes the alterations induced by chronic (30 days) exposure of the snake head fish *Channa punctatus* to a sublethal concentrations (0.01 ppm conc.) of Endosulfan on the profile of total protein and lipid in the liver, kidney, testis and ovary. The liver, kidney, testis and ovary showed significant depletion of glycogen, total protein and lipid content amounting. The present study therefore points towards a severe metabolic dysfunction in response to Endosulfan toxicity in the fish *Channa punctatus* (Bloch.)

Keywords: Endosulfan, *Channa punctatus*, Toxicity, Protein contents, Lipid contents.

INTRODUCTION:

Endosulfan is widely known as toxic insecticide acting as a contact poison for variety of insects and mites. Various negative effects have been described as a result of fish exposure to endosulfan. Chronic effects on fish include oxidative damage (Ballesteros *et al.*, 2009), genotoxicity (Neuparth *et al.*, 2006), damage to testes, changes in circulating thyroid hormones and alteration of acetylcholinesterase activity in the brain (Dutta *et al.*, 2006). Depressed levels of testosterone and estradiol have also been found in fish with elevated residues of endosulfan (Singh *et al.*, 2008). Other effects are reduced feeding behaviour, alterations in development, sexual and escape behaviour, and reproductive physiology (Balasubramani & Pandian, 2008). Studies on endosulfan toxicity also showed that it is acutely toxic to most fishes with LC₅₀ ranging from 0.1 to 41 µg/L (Datta, *et al.* 2003; Beyger *et al.* 2012). Although currently banned in many countries due to its adverse effects on human health and the environment, endosulfan is still continuously employed in

several developing countries in Asia for high commercial value crops. Therefore, methods for the fast and low cost assessment of the adverse effects of endosulfan in aquatic ecosystems need to be developed. Accurate and precise techniques have elucidated the unknown toxic effects of compounds, including endosulfan (Park DS, *et.al.*, 2015). Its toxicity to fish has been reported at LC₅₀ values lower than those of terrestrial animals(Chow *et.al.*, 2013).

The fish, *Channa punctatus* (Bloch), locally known as “Garai”, having the presence of suprabranchial accessory respiratory organs, an air-breathing teleost and endosulfan were selected for present study.

MATERIALS & METHODS

The air-breathing teleost *Channa punctatus* procured live from the local fish market, Darbhanga were washed with 0.1% KMnO₄ solution to remove dermal infection if any. Healthy fish of average length (9–12cm) and weight (21–25 g) were acclimated for 15 days to laboratory conditions. The fish were fed with chopped goat liver every day adlibitum. Running tap water was used in all the experiments and the fish were adjusted to natural photoperiod and ambient temperature. No aeration was done.

Static acute bioassays were performed to determine LC₅₀ values of endosulfan for 24, 48, 72 and 96 hours following the methods of APHA, AWWA & WPCF (1985). The LC₅₀ values for these periods were 8.25 ppm, 6.25 ppm, 4.25 ppm and 3.25 ppm respectively. The sub-lethal concentration was determined following the formula of Hart *et al.* (1945). Twenty acclimated fish were exposed to a sub-lethal concentration (0.01 ppm) of endosulfan for 30 days. Side by side same number of fish as that of experimental one was maintained as the control group. At the end of exposure period the fish were anaesthetized with 1:4000 MS 222 (tricane, methane, sulfonate, sandoz) for two minutes. On dayth of 30 fish taken out, the liver, kidney, testis and ovary were quickly dissected out, weighed to nearest mg and processed for the quantitative estimation of glycogen content by method of Carroll *et al.* (1956), total protein by the methods of Varley *et al.* (1980) and total lipid extraction was done by the method Folch *et. al.* (1957).

RESULTS:

BIOCHEMICAL OBSERVATIONS IN ORGAN TISSUE:

GLYCOGEN:

Glycogen in wet tissues of liver, kidney, testis and ovary in control fishes was estimated to be 25.66 ± 2.38 mg/g in liver, 21.5 ± 0.06 mg/g in kidney, 18.72 ± 0.05 mg/g in testis and 21.37 ± 1.89 mg/g in ovary. Under endosulfan experimental exposure the glycogen content of the wet tissues of undertaken organs was recorded decreased significantly in comparison to control and was recorded 16.32 ± 0.01 mg/g in liver, 12.8 ± 0.01 mg/g in kidney, 10.08 ± 0.01 mg/g in testis and 14.08 ± 0.01 mg/g in ovary in comparison to control (Table-1).

PROTEIN:

The protein profiles of liver, muscle, testis and ovary in response to endosulfan exposure showed a significant decline. The liver and kidney showed statistically more significant decline. The liver and kidney showed statistically more significant ($P < 0.001$) decline i.e. 31% in , while 30% in liver. The testis showed significant ($P < 0.05$) while ovary showed significant at ($P < 0.01$). The testis showed decline 19% while ovary 17%. Total protein in the control liver, kidney, testis and ovary was estimated to be 102.19 ± 1.81 , 75.006 ± 1.04 , 80.48 ± 1.41 , and 121.01 ± 1.89 respectively. As against there, the total protein profiles in the experimental lots were 50.08 ± 1.96 , 45.08 ± 0.01 , 60.16 ± 0.98 and 70.08 ± 0.01 respectively (Table-2).

TOTAL LIPID:

The estimation of total lipid in the wet tissue of liver, kidney, testis and ovary in control fish *Channa punctatus* recorded under the control condition showed 28.10 ± 1.56 mg/g in liver, 20.68 ± 1.17 mg/g in kidney, 16.74 ± 0.72 mg/g in testis and 21.19 ± 0.97 mg/g in ovary.

The estimation of total lipid was recorded significantly decreases under the experimental concentration and duration of endosulfan exposure, and was to be 15.40 ± 0.56 mg/g in liver, 18.49 ± 0.83 mg/g in kidney, 12.54 ± 0.77 mg/g in testis and 16.05 ± 0.66 mg/g in ovary (Table-3).

TABLE – 2

Profiles of tissue glycogen (mg/g wet tissue) in different organ of *Channa punctatus* chronically exposed to endosulfan for 30 days. Values are mean \pm SE Of 5 observations.

Tissue	Control	Endosulfan treated
Liver	25.66 \pm 2.38	16.32 \pm 0.01
Kedney	21.5 \pm 0.06	12.8 \pm 0.01
Testis	18.72 \pm 0.05	10.08 \pm 0.01
Ovary	21.37 \pm 1.89	14.08 \pm 0.01

Value are mean \pm SE of 5 observations, Significant level =P<0.05

TABLE – 2

Profiles of total protein (mg/g wet tissue) in tissue of *Channa punctatus* chronically exposed to endosulfan for 30 days. Values are mean \pm SE Of 5 observations.

Tissue	Control	Endosulfan treated
Liver	102.19 \pm 1.81	50.08 \pm 1.96
Kedney	75.006 \pm 1.04	45.08 \pm 0.01
Testis	80.48 \pm 1.41	60.08 \pm 0.01
Ovary	121.01 \pm 1.89	70.08 \pm 0.01

Value are mean \pm SE of 5 observations, Significant level =P<0.05

TABLE – 3

Profiles of total lipid (mg/g wet tissue) in tissue of *Channa punctatus* chronically exposed to endosulfan for 30 days. Values are mean \pm SE of 5 observations.

Tissue	Control	Endosulfan treated
Liver	28.10 \pm 1.56	15.40 \pm .56
Kedney	20.68 \pm 1.17	18.49 \pm 0.83
Testis	16.74 \pm 0.72	12.54 \pm 0.77
Ovary	21.19 \pm 0.97	16.05 \pm 0.66

Value are mean \pm SE of 5 observations, Significant level =P<0.05

DISCUSSION

The responses recorded for the fish in this study are similar to those reported by other authors under various stress conditions (Paul and Banerjee, 1996; Rani *et al.*, 1997; Palanivelu *et al.*, 2005; Ufodike and Onusiriuka, 2008; Lata *et al.*, 2008). Behavioural responses of fish to most toxicants are the most sensitive indicators of potential toxic effects (EIFAC, 1983). Acute toxic effect mercuric chloride was observed on zebrafish by Vutukuru SS, Basani K. (2013). The toxic effects of surfactant, dodecyl dimethyl benzyl ammonium chloride (1227) on larval locomotors of zebrafish was observed by Yanan, W. *et al.* (2015). It is, therefore, conclude that the toxicity of the pesticide endosulfan depend upon a number of physical, chemical and biological factors. Each of which may be used as a tool for pesticide toxicity to fish.

Glycogen

Under endosulfan experimental exposure the glycogen content of the wet tissues of undertaken organs was recorded decreased significantly in comparison to control fishes, four organs, liver, kidney, testis and ovary. The result of the present findings shows conformity with the result of Lakshmanan *et al.* (2013) who studied the impact of decrease in glycogen content in liver and kidney. The present finding is also in conformity with the result of Shankar and Kulkarni (2007), who observed the same trend in *Notopterus* under toxicant stress. Tripathy and Singh (2003); Rita and Milton (2006); Rani *et al.* (2008) also reported decrease in glycogen content in various tissues under pesticides stress.

Protein:

Under endosulfan experimental exposure the total protein of the wet tissues of undertaken organs was recorded decreased significantly in comparison to control fishes, four organs, liver, kidney, testis and ovary. Previous workers have also reported decline in tissue protein profiles in a number of fish species exposed to various pesticides and endosulfan. Ramalingam and Ramalingam (1982) noted a steady decline in the total protein of liver and muscle after 7 and 15 days exposure of the fish, *Sarotherodon mossambicus* to malathion and mercury and correlated it with an intensive proteolysis. Similarly, a significant decrease in the protein content was recorded by Kumar and Ansari (1984) in the Zebra fish, *Brachydanio rerio*, exposed to malathion and suggested inhibition of protein synthesis by the toxicant. Proteins being involved in the architecture and physiology of the cell seem to occupy a key role in the cell metabolism. The observed significant depletion of tissue protein in the present case denotes high catabolic potency of those organs and may be attributed to the intensive proteolysis and utilization of their degradation products for metabolism under the toxic influence of Herbiclon. They might have been fed into TCA cycle through aminotransferase system to cope with the excess demand of energy during stressful situations as suggested by Jha (1991). The loss of gonadal proteins may also be associated to the direct action of pyrethroids leading to arrest of vitellogenesis in ovary and loss of germ cells in testis (Jha and Jha, 1995). Moreover, the decreased protein contents might also be attributed to the tissue destruction, necrosis, or disturbance of cellular function and consequent impairment in protein synthetic machinery (Srivastava, *et al.*, 1995). Deshmukh, D. R. (2015) has observed toxicity of endosulfan on protein level in a freshwater catfish, *Wallago attu*. The liver of *Clarias gariepinus* exposed to the cypermethrin showed hyperplastic hepatic and necrosis of hepatic cells (Andem A. B. *et al.* 2016). The toxicity was found to increase with endosulfan concentration, various structural changes were already induced on the morphology of the vital organs, i.e. gill, liver and kidney even with exposure to low, sublethal endosulfan concentration.

Lipid:

The test fish *Channa punctatus* when exposed to sub lethal concentration of endosulfan (0.01 ppm) for 30 days, significant depletion in total lipid content in the tissue of all four organs, liver, kidney, testis and ovary. The estimation of total lipid was recorded significantly decreases under the experimental concentration and duration of endosulfan exposure.

Previous workers have also reported similar decline in total lipid of different tissue profiles in a number of fish species exposed to various pesticides and endosulfan as reported by Rani (2008), Shankar and Kulkarni (2007). The decrease might have occurred mainly due to altered lipid metabolism and energy demand in fishes under stress of toxicants.

CONCLUSION

The test fish *Channa punctatus* when exposed to sub lethal concentration of endosulfan (0.01 ppm) for 30 days, significant depletion in glycogen, total lipid and total protein content in the tissue of all four organs, liver, kidney, testis and ovary. The decrease might have occurred mainly due to altered glycogen, lipid and protein metabolism and energy demand in fishes under stress of toxicants.

ACKNOWLEDGEMENT

The authors are thankful to the Department of Zoology, MLSM College, LNM University, Darbhanga, for the provision of laboratory facilities used in this study.

REFERENCES

- Andem A. B. Ibor O. R., Joseph A. P., Eyo V. O., Edet A. A., 2016 : Toxicological Evaluation and Histopathological Changes of Synthetic Pyrethroid Pesticide (Cypermethrin) Exposed to African Clariid Mud Catfish (*Clarias gariepinus*) Fingerlings. International Journal of Toxicological and Pharmacological Research; 8(5); 360-367.
- APHA, 1985 : Standard methods for the examination of water and waste water (16th Ed). American Public Health Assoc., Washington D.C.
- Balasubramani A., T.J. Pandian, 2008: Curr. Sci. **94**, 883
- Ballesteros M.L., D.A. Wunderlin, M.A. Bistoni, 2009: Ecotoxicol. Environ. Saf. **72**, 199.
- Carroll, N. V., Longley, R. W., and Roe, J. H., 1955: Abstracts, American Chemical Society, 127th meeting, Cincinnati, 23C.
- Chow WS, Chan WK, Chan KM, 2013 : Toxicity assessment and vitellogenin expression in zebrafish (*Danio rerio*) embryos and larvae acutely exposed to bisphenol A, endosulfan, heptachlor, methoxychlor and tetrabromobisphenol A. J. Appl. Toxicol. 33:670–678.10.1002/jat.v33.7
- Deshmukh, D. R. 2015: Toxicity of endosulfan on protein level in a freshwater fish *Wallago attu*. J. Trends. Life Sci.Res., 3(2):15-18.
- Dutta H.M., D.A. Arends, 2003: Environ. Res. **91**, 157.

- Dutta HM, Misquitta D, Khan S., 2006: The effects of endosulfan on the testes of bluegill fish, *Lepomis macrochirus*: a histopathological study. *Archives of Environmental Contamination and Toxicology*. 51:149-156
- EIFAC (European Inland Fisheries Advisory Commission).1973: Water quality criteria for European freshwaterfish report on ammonia and inland fisheries. *Water Research*, 7: 1011-1022.
- Folch, J., Lees, M and Sloan S, G.H., 1957: A simple method for the isolation and purification of lipids from animal tissues. *J. Biol. Chem.*, 226:497-507.
- Hart, W.B., Dondoroff, P. and Greenbank, J., 1945: The evaluation of toxicity of industrial wastes, chemicals and other substances to freshwater fishes. Atlantic Refining Company. *Phil. Part (1)* : 317-326.
- Jha, B.S., 1991 : Alterations in the protein and lipid contents of intestine, liver and gonads in the lead exposed freshwater murrel, *Channa punctatus* (Bloch). *J. Ecobiol.* 3(1) : 29-34.
- Jha, B.S. and Jha, M.M., 1995 : Biochemical effects of nickel chloride on the liver and gonads of the freshwater climbing perch, *Anabas testudineus*, (Bloch). *Proc. Nat. Acad. Sci. (India)* 65B(1) : 39-46.
- Kumar, K. and Ansari, B.A., 1984 : Malathion toxicity effect on the liver of the fish, *Brachydanic reno* (Cyprinidae). *Ecotoxicol Environ. Sal.* 23: 199-205.
- Lakshman S.A., Rajendran, C. and Sivasubramaniyan 2013. Impact of dichloros on tissue glycogen and protein content in fresh water finger lings, *O. mossambicus*. *International Journal of research environmental science and Tech.* 3(1):19-25.
- Lata, S., Sriwastwa, V.M.S., Maurya, J.P. and Chaudhary, S.K. 2008: Urea induced testicular changes in *Mystus vittatus*. *J. Eco. Biol.*, 23:11- 17.
- Neuparth T., J.W. Bickham, C.W. Theodorakis, F.O. Costa, M.H. Costa, 2006: *Bull. Environ. Contam. Toxicol.* **76**, 242
- Palanivelu, V., Vijayavel, K., Ezhilarasi Balasubramanian, S. and Balasubramanian, M.P. 2005: Impact of fertilizer (urea) on oxygen consumption and feeding the freshwater fish *Oreochromis mossambicus*. *Environmental Toxicology and Pharmacology*, 19: 351–355.
- Park DS, Jeon HJ, Park ES, 2015: Highly selective biomarkers for pesticides developed in *Eisenia fetida* using SELDI-TOF MS. *Environ. Toxicol. Pharmacol.* 39:635–642.10.1016.
- Paul, V.I. and Banerjee, T.K. 1996: Ammonium sulphate induced stress related alterations in the respiratory epithelium of the air breathing organ of the catfish (*Heteropneustes fossilis*). *Journal of Biosciences*, 21: 519-526.
- Ramalingam, K. and Ramalingam, K., 1982 : Effects of sublethal levels of DDT, malathion and mercury on tissue proteins of *Sarotherodon mossambicus* (Peters). *Proc. Ind. Acad. Sci. (Anim. Sci.)* 91(6) : 501–505.
- Rani, E.F, M. Elumalai, M.P. Balasubramanian 1997: The toxicity of mixtures of monocrotophos and ammonium chloride to a freshwater fish *Oreochromis mossambicus*. *Biomedical Lett.*, **55** : 193-198.

- Rani, R. Gautam,R. & Kumar,S. 2008: Toxicity of Nuvan on kidney cholesterol on *Labeo rohita*. Ind. J. Environ. & Eco-plan 15(1-2), 115-118.
- Rira, J.J., Arockia & Milton, M.C., John2006: Effect of Carbamate pesticide (methonil) on the bio-chemical components of the fresh water *Oreochromis mossambicus* Pteva) Ind. J. Eniron & Eco-planing 12(1), 1-8.
- Shankar,D S. and Kulkarni,RS.2007: Tissue cholesterol and serum cortisol level during different reproductive phases of the female freshwater fish *Notopterus notopterus* (Pallas) Journalof Environmental Biology, 28(1):137-139.
- Singh P.B., V. Singh, P.K. Nayak, 2008: Food Chem. Toxicol. **46**, 2533.
- Srivastava, A.K., Singh, N.N. and Srivastava, A.K., 1995 : Bio-chemical change in freshwater. Indian Cat. Fish. Following exposure to sublethal concentration of propoxur J. Freshwater Biol. 7(4) 257-260.
- Tripathi, P.K., Srivastav, V.K.& Singh, A, 2003: Toxic effect of dimethoate (organophosphate) on metabolism and enzyme system of freshwater teleost fish *Channa punctatus*.Asian Fisheries Science, 16:349-359.
- Ufodike, E.B.C. and Onusiriuka, B.C. 2008: Acute toxicity of inorganic fertilizers to African catfish, *Clarias gariepinus* (Teugals). Aquaculture Research, 21: 181186.
- Vutukuru S.S., Basani K. 2013: Acute effects of mercuric chloride on glycogen and protein content of Zebra fish ,Daniorerio. J. Environ Biol. 34:277–281.
- Varley, H. Gowenlock, A.H. and Bell, M., 1980 : Practical clinical Bio-chemistry, Vol. I, General topics and commoner tests. William Heinemann Medical Books Ltd., London.
- Yanan, W., Yuan Z., Sun M., and Zhu W. 2015: Exploring the effects of different types of surfactants on zebrafish embryos and larvae.Springer Nature.Sc.Rep. article No. 10107.
