

## Investigation of Biochemical Effects of Acute Concentrations of Lambda-Cyhalothrin on African Catfish *Clarias gariepinus*-Teugels

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**Abstract:** The impact of short-term exposure to waterborne lambda-cyhalothrin on *Clarias gariepinus* was evaluated through changes of selected biochemical parameters. Fish was exposed to 0.008, 0.009, 0.010, 0.011 and 0.012 mg L<sup>-1</sup> and control for 96h. The parameters measured were serum glucose, protein, cholesterol, triglyceride, Glutamic Pyruvic acid Transaminase (GPT), Glutamic Oxaloacetic acid Transaminase (GOT) and Alkaline Phosphatase (ALP). There was significant ( $p < 0.05$ ) alterations between the control values and the exposed groups on all parameters. The alterations in all parameters was significantly ( $p < 0.05$ ) dose dependent.

**Key words:** Lambda-cyhalothrin, biochemical parameters, *Clarias gariepinus*, acute toxicity

### INTRODUCTION

Pyrethroids insecticides, including lambda-cyhalothrin are widely used for the control of insect pests all over the world to increase the production of food grain and other agricultural products. It may also be used in public health applications to control insects such as cockroaches, mosquitoes, ticks and flies which may act as a disease vector (EXTOXET, 1993). Pyrethroids are several orders of magnitude more toxic to fish than the organophosphate pesticides they are replacing in many agricultural, commercial and residential applications (Oros and Werner, 2005). All pyrethroids are potent neurotoxins that interfere with nerve cell function by interacting with voltage-dependent sodium channels as well as other ion channels, resulting in repetitive firing of neurons and eventually causing paralysis (Shafer and Meyer, 2004). Due to the lipophilic nature of pyrethroids, biological membranes and tissues readily take up pyrethroids (Oros and Werner, 2005). Modifications in enzyme activity occur by cell death, increase or decrease enzyme production, obstruction of normal excretory route, increased cell membrane permeability, or impair circulation (Kaneko, 1989). Biochemical characteristics of blood are among the important indices of the status of internal environment of the fish organism (Edsall, 1999). Liver is the metabolic centre for detoxification of chemicals. Liver damage was confirmed by changes in the activities of Glutamate-Oxaloacetate Transaminase (GOT) and Glutamate-Pyruvate Transaminase (GPT) activities (Asztalos and Nemesok, 1985). Increase in blood glucose level is a general response of fish to acute pollutant effects including organophosphates and pyrethroids (Luskova *et al.*, 2002). The quantity of protein is

dependent on the rate of protein synthesis, or on the rate of its degradation. The quantity of protein may also be affected due to impaired incorporation of amino acids in the poly peptide chains (Singh *et al.*, 1996).

The aim of this study was to investigate the serum activities of GOT, GPT, ALP, protein and carbohydrate metabolisms after exposure of *Clarias gariepinus* juveniles to nominal acute concentrations of lambda-cyhalothrin (a commonly used insecticide with a view to accessing the possible mechanism of its toxicity).

### MATERIALS AND METHODS

Juveniles of *Clarias gariepinus* was purchased from Maigana fish farm in Zaria, Kaduna State Nigeria. The *Clarias* species averaging 14.33±0.50 cm standard length and body weight of 20.38±1.25 g were used for the study. The fish were conveyed to fisheries laboratory in a portable well-aerated white polythene bag containing water from the fish farm. They were held in large water baths of 160 L capacity at 24.5-25.5°C and acclimatized for two weeks in dechlorinated municipal water. During this period, the fishes were fed with pelleted diet containing 35% crude protein twice per day at 5% body weight. Also, the water in the glass aquaria was changed once every two days. The fishes were accepted as well as adapted to laboratory conditions when less than 5% death was recorded for the 14 days period and feeding was discontinued 24 h before the start of the experimental run (Reish and Oshida, 1987).

**Acute bioassay:** Acute 96 h static bioassays were conducted in the laboratory following the methods of

Sprague (1975) and APHA (1992). The nominal concentration for Lambda-cyhalothrin was 0.008, 0.009, 0.010, 0.011 and 0.012 mg L<sup>-1</sup> and a control with no toxicant. Each concentration was replicated three times. The desired Lambda-cyhalothrin concentration was measured and introduced into 25 L of dechlorinated tap water in the glass aquaria. The mixture was allowed to stand for 30 min before introducing test fishes. A total of 180 fish were stocked to give a loading rate of 10 fish per tank. Survival and mortality were recorded from 1 to 6, 8, 16, 24, 72 and 96 h. Fishes were considered dead when the opercular movement ceased and there was no response to gentle probing.

**Biochemical measurements:** For biochemical investigations, The caudal peduncle of fish was cut, blood was collected in non-heparinized tubes. The blood was immediately centrifuged at 1500 rpm for 10 min. Serum was then removed and stored at 4°C prior to immediate determination of biochemical parameters, glucose, cholesterol, triglycerides, total protein, Glutamic Pyruvic acid Trasaminase (GPT), Glutamic Oxaloacetic acid Trasaminase (GOT) and Alkaline Phosphatase (ALP). Blood glucose was estimated using the method of Trinder (1969). Blood cholesterol was measured according to the procedure of Pearson *et al.* (1953). Blood triglyceride was determined using the method of Rice (1970). The method of Lowry *et al.* (1951) was carried out to determine the value of total protein. The activities of blood GPT and GOT were estimated according to the methods of Reitman and Frankel (1957). To determine the activity of blood ALP, Bessey *et al.* (1946) method was used.

**Statistical analysis:** For the various biochemical parameters, the GenStat statistical analysis software was used to run analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT) was used to test for differences between different level of treatment and to separate means respectively, were applicable (Duncan 1955). Test of significance was at the 5% level of significance.

## RESULT

Analysis of variance (ANOVA) results of acute exposure to lambda-cyhalothrin indicated significant

( $p < 0.05$ ) dose dependent elevations in glucose, cholesterol, triglyceride levels in the serum (Table 1). On the other hand, there was a significant ( $p < 0.05$ ) dose dependent inhibition in GOT, GPT, ALP and protein. The control values for glucose, cholesterol and triglyceride were significantly lower, ( $p < 0.05$ ) than in the exposed groups (Table 1). However the control values for protein, GOT, GPT and ALP were significantly ( $p < 0.05$ ) higher than in the exposed groups.

## DISCUSSION

The significant ( $p < 0.05$ ) increase in glucose which was dose dependent may be considered to be manifestation of stress induced by lambda-cyhalothrin. Glucose increase is a general response of fish to acute and sublethal pollutant effects (Verma *et al.*, 1983; Ghazaly, 1994; Ceron *et al.*, 1997; Luskova *et al.*, 2002). Wedemeyer and Mcleay (1981) stated that high levels of blood glucose are caused by disorders in carbohydrate metabolism appearing in the condition of physical and chemical stresses. A variety of stressors stimulate the adrenal tissue, resulting in increased level of circulating glucocorticoids (Hontela *et al.*, 1996) and catecholamines (Nakano and Tomlinson, 1967). Both of these groups of hormones produce hyperglycaemia.

The inhibition of protein as reported in this investigation agrees with that of some other workers. Reeta *et al.* (1993), reported inhibition in the total serum protein of an air breathing fish *Heteropneustes fossilis* after exposure to different pesticides (DDT, YBHC and Malathion). Ravichandran *et al.* (1994) reported depletion of protein from 7. 9-45.0% due to proteolysis after exposing *Oreochromis mossambicus* to nominal concentrations of phenol. Several drugs have been known to decrease serum protein levels in humans, e.g Rifampin (Rifamycin) reduces serum protein by impairing protein synthesis; Trimethadione decreases protein levels by urinary loss and long-term use of laxatives results in fecal malabsorption and thus decreases serum protein levels (Gregor and John, 1995). Bradbury *et al.* (1987), pointed out that the decreased protein content might also be attributed to the destruction or necrosis of cells and consequent impairment in protein synthesis machinery. The quantity of protein is dependent on the rate of

Table 1: Means for *C. gariepinus* biochemical parameters after exposure to acute nominal concentrations of lambda-cyhalothrin

Conc (mg L <sup>-1</sup> )	Glucose (mg dL <sup>-1</sup> )	Protein (g dL <sup>-1</sup> )	Cholesterol (mg dL <sup>-1</sup> )	Triglyceride (mg dL <sup>-1</sup> )	GOT (iu L <sup>-1</sup> )	GPT (iu L <sup>-1</sup> )	ALP (iu L <sup>-1</sup> )
0.000	51.50 <sup>f</sup>	3.79 <sup>a</sup>	133.50 <sup>e</sup>	70.50 <sup>d</sup>	69.50 <sup>a</sup>	67.00 <sup>a</sup>	41.00 <sup>a</sup>
0.008	57.50 <sup>e</sup>	3.62 <sup>bc</sup>	146.50 <sup>d</sup>	82.00 <sup>b</sup>	63.50 <sup>b</sup>	56.50 <sup>b</sup>	29.00 <sup>b</sup>
0.009	60.50 <sup>d</sup>	3.67 <sup>b</sup>	159.00 <sup>c</sup>	78.50 <sup>c</sup>	61.50 <sup>b</sup>	53.50 <sup>c</sup>	26.50 <sup>c</sup>
0.010	62.50 <sup>c</sup>	3.59 <sup>bc</sup>	178.50 <sup>b</sup>	80.50 <sup>bc</sup>	59.00 <sup>c</sup>	51.50 <sup>c</sup>	22.50 <sup>d</sup>
0.011	64.50 <sup>b</sup>	3.53 <sup>c</sup>	180.50 <sup>b</sup>	83.50 <sup>ab</sup>	53.50 <sup>d</sup>	47.00 <sup>d</sup>	20.50 <sup>de</sup>
0.012	67.00 <sup>a</sup>	3.52 <sup>c</sup>	187.00 <sup>a</sup>	85.50 <sup>a</sup>	51.50 <sup>d</sup>	35.50 <sup>e</sup>	19.50 <sup>e</sup>

Means with the same superscript along columns are not significantly different ( $p < 0.05$ )

protein synthesis, or on rate of its degradation. The quantity of protein may also be affected due to impaired incorporation of amino acids into polypeptide chains (Ram *et al.*, 2003). Serum protein inhibition in the current investigation may be due to impairments of protein synthesis by lambda-cyhalothrin due necrosis of hepatocyte cells.

ALP is mainly localized at the cell membrane. Any damage in hepatic cells may result in alteration in ALP activity. The dose-dependent inhibition observed in this investigation is in agreement with the report of many other workers. Sastry and Sharma (1980) reported ALP inhibition after 96 h exposure to diazinon, however the normal control values resumed after 96 h but later increased due to prolonged exposure time (Sastry and Sharma, 1980). Goel *et al.* (1982), reported serum alkaline and acid phosphatase inhibition by 15% in *Heteropneustes fossilis* resulting from the effect of malathion. Similarly, Das and Mukherjee (2003), reported depletion of ALP due to sub-lethal exposure of *Labeo rohita* fingerlings to cypermethrin. Due to the resulting activity values of ALP, it may be assumed that the liver tissue of the experimental fish was not markedly affected by Lambda-cyhalothrin. The inhibition in protein level may also be due to the decrease in ALP activity as it plays an important role in protein synthesis (Pilo *et al.*, 1972). Triglyceride accumulation occurs in fatty liver due effect of toxicants. The accumulation occurs as a result of an imbalance between the rate of synthesis and the rate of release of triglyceride by the parenchymal cells into the systemic circulation (Gabeiel, 1986). The significant ( $p < 0.05$ ) dose dependent serum elevations of triglyceride observed, agrees with that of some other workers. Khurshid (2003) reported that cholesterol and total lipids of young chick embryo exposed to cypermethrin increased at 200 ppm and decreased at 400 ppm concentrations. He suggested that increase in cholesterol content at 200 ppm may indicate slow metabolism that resulted also in total lipid content and that the decrease at 400 ppm seems to have caused cell death in the embryonic tissue. Krishna *et al.* (1994) also reported increased levels of phospholipids and cholesterol contents in the tissues of *Tilapia mossambica* subjected to acclimation in sub lethal acidic water (pH 4.0). These results could possibly be correlated to the higher energy demands and impairments in the membrane organization induced by the acclimation to acidic water in order to get the positive survival value under the imposed acidic stress. In this investigation the dose dependent elevation in triglyceride may be due to higher energy demands and impairments in the membrane organization of *C. gariepinus* exposed to acute concentrations of lambda-cyhalothrin.

Transaminases are intracellular enzymes which exist in only a small amount of the serum. Therefore damage to the liver cell may result in leakage of the enzymes into the plasma/serum due to a large concentration gradient (Wroblewski and LA Due, 1955). In normal fish, the activity of liver GOT and GPT is extremely variable (Moon and Foster, 1995). Asztalos, have reported elevation in serum GOT activity of *Cyprinus carpio* which have hepatic cellular damage caused by methidation + paraquat. Conversely, Oruc and Uner (1999), reported inhibition in serral GPT and GOT enzyme activity following 2 and 30 days of exposure to 2, 4-Diamin. Similarly, Sadhu *et al.* (1985) have decreased GOT and GPT activities in the serum of *Channa striatus* following exposure to 0.1 ppm malathion for 10 days. In the present investigation a significant ( $p < 0.05$ ) dose dependent serum GOT and GPT inhibition was recorded and this finding is in agreement with that Oruc and Uner (1999), Sadhu *et al.* (1985). GOT and GPT are important in the diagnosis of heart and liver damage (Dere and Polat, 2001). The observed changes could be due to generalized organ/system failure as the fish approached death due to the effect of lambda-cyhalothrin.

## CONCLUSION

Synthetic pyrethroids to which lambda-cyhalothrin belongs are widely used in agriculture in Nigeria. They are increasingly being used in veterinary applications on farm and pet animals, for the protection of stored foodstuffs, for the control endemics and parasites in public health programmes as well as for household applications in kitchens and bedrooms. Chronic exposures of aquatic biota and individuals may result through run-off and adsorption of pyrethroids to small dust particles and various other surfaces respectively. The results of our study suggest that sub lethal exposure of *C. gariepinus* to lambda-cyhalothrin could lead to alterations in carbohydrate and lipid metabolism and possible organ damage. In the light of the above observations, it is recommended that lambda-cyhalothrin should be used with caution and in a sustainable manner, as it could be hazardous to aquatic biota, domestic animals and human beings as well.

## REFERENCES

- American Public Health Association (APHA), 1992.
- American Water Works Association (AWWA) and Water Environment Federation (WEF), (1992). Standard Methods for the Examination of Water and Wastewater. 18th Edn., Washington, D.C.

- Asztalos, B. and J. Nemcsok, 1985. Effects of pesticides on the LDH activity and isoenzyme pattern of Carp (*Cyprinus carpio*) sera. *Comparative Biochem. Physiol.*, 82: 217-219.
- Bassey, O.A., O.H. Lowery and M.J. Brock, 1946. A method for the rapid determination of alkaline phosphatase with 5 mm<sup>3</sup> of serum. *J. Biol. Chem.*, 164: 321-329.
- Bradbury, S.P., D.M. Symonic, J.R. Coats and G.J. Atchison, 1987. Toxicology of fenvalerate and its constituents isomers to the fathead minnow (*Pimephales promelos*) and blue gill (*Lepomis macrochirus*). *Bill. Environ. Cont. Toxicol.*, 38: 727-735.
- Ceron, J.J., E. Sancho, M.D. Ferrando, C. Gutierrez, and E. Andreu, 1997. Changes in carbohydrate metabolism in the eel *Anguilla anguilla*, during short term exposure to diazinon. *Toxicol. Environ. Chem.*, 60: 201-210.
- Das, B.K. and S.C. Mukherjee, 2003. Toxicity of Cypermethrin in *Labeo rohita* fingerlings: Biochemical enzymatic and haematological consequences. *Comp. Biochem. Physiol. C. Toxicol. Pharmacol.*, 134: 109-21.
- Dere, E. and F. Polat, 2001. The effect of paraquate on the activity of some enzymes in different tissues of mice (*Mus musculus*). *Turk. J. Biol.*, 25: 323-332.
- Duncan, D.B., 1955. Multiple range tests. *Biometric*, No. 11: 1-42.
- Edsall, C.C., 1999. A blood chemistry profile for lake trout. *J. Aquatic. Anim. Health*, 11: 81-86.
- EXTOXNET, 1995. Lambda-cyhalothrin. Pesticide Information Profile. USDA.
- Gabriel, L.P., 1986. Toxicology of the Liver. In: Casarett and Doull's Toxicology; The Basic Science of Poisons. 3rd Edn., Macmillan publishing Co. New York.
- Ghazaly, K.S., 1995. Carbohydrate metabolism in *Clarias lazara* exposed to three different pesticides. *J. Egypt Ger. Soc. Zool.*, 16A: 235-251.
- Goel, K.A., S.K. Tyagi and A.K. Awasthi, 1982. Effect of malathion on some haematological values in *Heteropneutes fossilis*. *Comp. Physiol. Ecol.*, 7: 259-261.
- Gregor, H.G. and F.K. John, 1995. Plasma and Serum Proteins In: Fundamentals of Clinical Chemistry. Norbert, W.T. (Ed.), Saunders, London.
- Hontela, A., C. Daniel and A.C. Ricard, 1996. Effect of acute and sublethal exposure to Cadmium on the interregal and thyroid function in rainbow trout, *Oncorhynchus mykiss*. *Aquat. Toxicol.*, 35: 171-182.
- Kaneko, J.J., 1989. Clinical Biochemistry of Domestic Animals. 4th Edn., Academic Press, San Diego, pp: 823.
- Khurshid, A., 2003. Cypermethrin, A pyrethroid Insecticides induces Teratological and Biochemical changes in young chick embryos. *Pak. J. Biol. Sci.*, 6: 1698-1705.
- Krishna, M.V., M. Bhaskar and S. Govindappa, 1994. Studies on lipid profiles of fish liver on acclimation to acidic medium. *J. Environ. Biol.*, 15: 269-273.
- Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurements with Folin Phenol reagent. *J. Biol. Chem.*, 193: 265-275.
- Luskova, V., M. Svoboda and J. Kolarova, 2002. The effects of Diazinon on blood plasma Biochemistry in Carp (*Cyprinus carpio* L.). *Acta Vet. Brno.*, 71: 117-123.
- Moon, T.W. and G.P. Foster, 1995. Tissue Carbohydrate Metabolism, Gluconeogenesis and Hormonal and Environmental Influences. In: Hochachka, P.W. and T.P. Mommsen (Eds.), *Metabolic Biochemistry*. Elsevier, Amsterdam, pp: 65-100.
- Nakano, T. and N. Tomlinson, 1967. Catecholamines and carbohydrate concentration in rainbow trout *Salmo gairdneri* in physical disturbance. *J. Fish. Res. Bd. Can.*, 24: 1701-1715.
- Oros, D.R., D. Hoover, F. Rodigari, D. Crane and J. Sericano, 2005. Levels and distribution of polybrominated diphenyl ethers in water, surface sediments and bivalves from the San Francisco Estuary. *Environ. Sci. Tech.*, 39: 33-41.
- Oruc, E.O. and N. Uner, 1999. Effects of 2,4-Diamin on some parameters of protein and carbohydrate metabolisms in the serum, muscle and liver of *Cyprinus carpio*. *Environ. Pollu.*, 105: 267-272.
- Pearson, S.S. Stern and T.H. Mogavack, 1953. A rapid procedure for the determination of serum cholesterol. *J. Clin. Endocrinol.*, pp: 666.
- Pilo, B., M.V. Asnani and R.V. Shah, 1972. Studies on wound healing and repair in pigeon 111. Histochemical studies on acid alkaline phosphatase activity during the process. *J. Anim. Phys.*, 19: 205-212.
- Ram, P.Y., S. Digvijay, S.K. Singh and A. Singh, 2003. Metabolic changes in freshwater fish *Channa punctatus* due to stem-barck extract of *Crton tiglium*. *Pak. J. Biol. Sci.*, 6: 1223-1228.
- Ravichandran, S., K. Midhunashanthi and N. Indira, 1994. Impact of phenol on protein metabolism in the freshwater fish *Oreochromis mossambicus*. *J. Ecotoxicol. Environ. Monit.*, 4: 33-37.

- Reeta, P., S. Bhargava and D.K. Saraf, 1993. Toxic effects of some biocides on total serum protein in *Heteropneustes fossilis*. *Ind. J. Environ. Toxicol.* 3: 5-6.
- Reish, D.L. and P.S. Oshida, 1987. Manual of methods in aquatic environment research Part 10. Short term static bioassay. FAO. fisheries. Technical paper 247. FAO Rome, pp: 1-62.
- Retiman, S., and F. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic acid transaminase. *Am. J. Clin. Pathol.*, pp: 2856.
- Rice, E., 1970. Triglycerides (Natural fats) in serum. *Stand. Meth. Clin. Chem.* 6, Academic Press, New York, pp: 215.
- Sadhu, K.A., D.K. Chowdhury and P.K. Mukhopadhyay, 1985. Relationship between serum enzymes, histological features and enzymes in hepatopancreas after sub lethal exposure to malathion and phophamidon in the murrel *Channa striatus* (B.L.). *Int. Environ. Studies*, 24: 35-41.
- Sasty, K.V. and K. Sharma, 1950. Diazinon effect on the activities of brain enzymes from *Opicephalus princtatus* (Channa). *Bull. Environ. Contam. Toxicol.*, 24: 326-332.
- Shafer, T.G. and D.A. Meyer, 2004. Effects of pyrethroids on voltage-sensitive calcium channels: A critical evaluation of strengths, weaknesses, data needs and relationship to assessment of cumulative neurotoxicity. *Toxicol. Applied Pharmacol.*, 196: 303-318.
- Singh, A., D.K. Singh, T.N. Mishra and R.A. Agarwal, 1996. Mulluscicides of plant origin. *Biol. Agric. Hortic.*, 13: 205-252.
- Spragne, J.B., 1975. Measurement of pollution toxicity to fish: In *Bioassay methods for acute toxicity*. *Water Resour.*, 3: 346-349.
- Trinder, P., 1969. Determination of blood glucose using 4-aminophenazone. *J. Clin. Pathol.*, 22: 246.
- Verma, S.R., S. Rani, P.I. Tonk and R.C. Dalela, 1983. Pesticide induced dysfunction in carbohydrate metabolism in three freshwater fishes. *Environ. Res.*, 32: 127-133.
- Wedemeyer, G.A. and D.J. Mcleay, 1981. Methods for Determining the Tolerance of Fishes to Environmental Stressors. In: *Stress and Fish*. Pickering, A.D. (Ed.), Academic Press, London, pp: 247-275.
- Wroblewski, F. and J.S. La Due, 1955. Serum glutamic oxaloacetic transaminase activity as an index of liver cell injury. A preliminary report. *Ann. Int. Med.*, 43: 345-360.