Journal of the Indian Fisheries Association
30, 2003, 53-60

RESPONSES OF *CYPRINUS CARPIO* VAR. *COMMUNIS* TO CYHALOTHRIN, A PYRETHROID INSECTICIDE

A. Asia Begum, M. Ramesh, A. Noortheen, Hannah Sampth and B. Revathy

Unit of Pollution Biology, Department of Zoology, Bharathiar University Coimbatore – 641 046

ABSTRACT

Haematological and biochemical profiles in a freshwater teleost, *Cyprinus carpio* var. *communis*, exposed to sub-lethal toxicity of the insecticide cyhalothrin were studied. During the treatment, erythrocyte count, haemoglobin content and protein content decreased, whereas leucocyte count and glucose level increased. The data are discussed in relation to the significance of haematological and biochemical changes as non-specific biomarkers against anthropogenic stress.

Keywords: Cyhalothrin, erythrocytes, leucocytes, haemoglobin, protein, glucose, *Cyprinus carpio*

INTRODUCTION

Pesticides are one of the major xenobiotic substances that have been used in India for a long period for the management of pests in agricultural fields and control of vectors in public health operations (Dig and Wu, 1993). The growth of the Indian pesticide industry is one of the highest in the world and India is the second largest manufacturer of pesticides in Asia. The undue persistence, high mammalian toxicity, and developing resistance of the organochloride, organophosphate and carbamate insecticides led to a ban or restriction on their use in many developed and developing countries. Thus, attention is focused on less persistent, low mammalian toxicity compounds of pyrethrine.

Several studies have reported that these compounds are extremely toxic to fish and other aquatic organisms (Baradbury and Coats, 1989; Clark, 1995). In addition to their acute toxicity, many pyrethroids may have potentially deleterious effects at sub-lethal levels. Fish live in intimate contact with their environment and are, therefore, very susceptible to physical and chemical changes which may be reflected in their blood. Monitoring of blood parameters, both cellular and non-cellular, may have considerable diagnostic value in assessing the early warning signs of pesticide poisoning (Pant *et al.*, 1987). Alteration in the physiological and biochemical parameters of toxicant-treated fish has recently emerged as an important tool for water quality assessment in the field of environmental toxicology.

Lambda-cyhalothrin is a synthetic pyrethroid insecticide used all over the world to control the pests of cotton, rice, brinjal and tomato. It is a contact, residual and stomach poison with repellency properties. Information on the toxic effect of the above insecticide to aquatic organisms, particularly fish, is scanty. Hence, in the present study, the sub-lethal toxicity of lambda-cyhalothrin on blood chemistry and biochemical parameters of an economically important fish, *Cyprinus carpio* var. *communis,* was investigated.

MATERIAL AND METHODS

C. carpio var. *communis* were collected from the Aliyar Fish Farm of the Tamil Nadu Fisheries Development Corporation Limited. Fish were acclimatised to the laboratory conditions for about 15 days before the commencement of the experiment. During the above period, fish were fed *ad libitum* with rice bran and oil cake in the form of dough daily. Water was replaced every 24 hours after feeding in order to maintain a healthy environment with enough oxygen. The tap water used for the experiment was analysed for physicochemical characteristics following APHA (1974).

Fish (500 no.) were stocked in a large cement tank (1.2 x 1.8 x 0.9 m) previously cleaned and disinfected with potassium permanganate. Fish with an average weight of 6 g and length about 7-8 cm were selected for the experiment. The LC_{50} value of cyhalothrin to fish is 0.015 mg l^{-1} (Finney, 1978). One-tenth of this value (0.0015 mg l^{-1}) was taken for sub-lethal studies (Sprague, 1971). For the sub-lethal study, an aquarium of 100 l water capacity was taken and filled with water. Then, the sub-lethal concentration of the toxicant was added and mixed well; 100 fish were randomly selected from the stock and transferred into the aquarium. Four similar replicates were maintained. Fish were exposed to the sub-lethal concentration for 30 days. During this exposure period, fish were fed *ad libitum*. The water was changed periodically and the toxicant was renewed every 24 hours. The hydro-biological features of water were constantly monitored as these factors have a significant influence on the behaviour of toxicants. A common control was maintained. Fish from the experiment were sacrificed with respective controls at the intervals of five days up to the 30^{th} day. Blood was drawn from the heart through cardiac puncture using a hypodermic syringe. Whole blood was used for the estimation of erythrocytes, leucocytes and the haemoglobin content. The cells were counted by the method of Rusia and Sood (1992). Haemoglobin was estimated by the cyanmethemoglobin method (Drabkin, 1946). The remaining blood sample was centrifuged at 10,000 rev min^{-1} for 20 minutes to separate the plasma, which was used for glucose and protein estimations. Glucose was estimated by the *o*-toludine method of Hultmann (1959) and Dubowski (1962). Protein was estimated according to the method of Lowry *et al.* (1951). The significance between the sample means of control and experimental fish was tested using students 't' test at 5% level.

RESULTS

The physicochemical properties of the tap water used for maintaining the fish were: temperature 29 ± 1.0° C; pH 7.2 ± 0.2; dissolved oxygen 6.20 ± 0.01 mg l^{-1}; salinity 0.40 ± 0.02 ‰ and total hardness 19.00 ± 0.05 mg l^{-1}.

Table 1 shows the changes in erythrocytes, leucocytes and haemoglobin content of the fish exposed to sub-lethal concentration of cyhalothrin. During the above treatment period, the erythrocyte count decreased in the experimental fish when compared to control showing a maximum per cent decrease of 68.16 and a minimum of 29.25 at the end of the fifth and the 30th days, respectively, whereas, the leucocyte count increased during the above treatment period and it was directly proportional to the exposure period showing a minimum per cent increase of 30.23 at the end of the fifth day and a maximum per cent increase of 94.84 at the end of the 30th day. The haemoglobin content also decreased in the experimental fish when compared to the control showing a gradual decrease up to the 15th day (83.19%). But after the15th day, slight recovery was noted showing a minimum per cent decrease of 36.33 on the 30th day.

Table 1: Changes in the erythrocyte and leucocyte counts, and haemoglobin content of *Cyprinus carpio* var. *communis* treated with sub-lethal concentration of cyhalothrin

Exposure period (d)	Erythrocyte (no.x10^6 mm^{-3})		Leucocyte (no.x10^3 mm^{-3})		Haemoglobin (g dl^{-1})	
	Control	Experiment	Control	Experiment	Control	Experiment
5	4.049 ± 0.809	1.289 ± 0.257* (-68.16)	14.097 ± 0.337	18.359 ± 0.204* (+30.23)	5.244 ± 1.048	1.554 ± 0.310* (-70.36)
10	3.202 ± 0.640	1.031 ± 0.206* (-67.80)	14.565 ± 0.022	19.702 ± 0.229* (+35.26)	5.327 ± 1.065	1.136 ± 0.227* (-78.67)
15	3.512 ± 0.702	1.306 ± 0.261* (-62.81)	16.056 ± 0.301	22.086 ± 0.219* (+37.55)	5.933 ± 1.186	1.997 ± 0.399* (-83.19)
20	2.557 ± 0.511	1.479 ± 0.295* (-42.15)	16.953 ± 0.365	25.328 ± 0.178* (+49.40)	4.998 ± 1.006	1.053 ± 0.210* (-54.15)
25	2.689 ± 0.537	1.758 ± 0.351* (34.62)	16.511 ± 0.190	26.225 ± 0.316* (+58.83)	4.493 ± 0.898	2.060 ± 0.412* (-54.15)
30	2.304 ± 0.460	1.630 ± 0.326* (-29.25)	15.409 ± 0.267	30.023 ± 0.166* (+94.84)	3.440 ± 0.688	2.190 ± 0.438* (-36.33)

Values are mean ± SE of six individual observations.

Values in parentheses are per cent changes over control.

Degrees of freedom at 8t 0.05 = 2.306

* Values are significant at 5% level.

Changes in plasma glucose and protein levels of the fish exposed to the sub-lethal concentration of cyhalothrin are presented in Table 2. During the above treatment, plasma glucose level increased in the experimental fish when compared to control and was directly proportional to the exposure period showing a minimum per cent increase of 40.08 at the end of the fifth day and a maximum per cent increase of 75.76 at the end of the 30th day. On the other hand, plasma protein level decreased in the experimental fish when compared to control showing a minimum per cent decrease of 26.98 at the end of the 10th day and a maximum per cent decrease of 79.92 at the end of the 30th day. All the values are significant at 5% level.

DISCUSSION

Synthetic pyrethroids though effective at very low application rates are stable in the environment (Elliott, 1980). However, their rapid decomposition in soil through hydrolysis under aerobic conditions and restricted hydrolytic breakdown under aquatic environment are reported by Roberts and Standen (1977). Haematological changes may be used as an indicator of physiological stress in animals. Lowering in red blood corpuscles count in fish might be due the destructive action of pesticides on peripheral red cells as a result of which the viability of the cells is affected (Verma *et al.,* 1982). However, the authors are of the opinion that the damaging effects

Table 2: Changes in the glucose level and protein content of *C. carpio* var. *communis* treated with sub-lethal concentration of cyhalothrin

Exposure period	Glucose (mg %)		Protein (µg ml^{-1})	
(d)	Control	Experiment	Control	Experiment
5	89.20 ± 0.705	124.96 ± 0.999* (+40.08)	6.920 ± 1.384	4.978 ± 0.0995* (-28.06)
10	73.33 ± 0.586	109.36 ± 0.234* (+48.64)	6.730 ± 1.346	4.914 ± 0.9820* (-26.98)
15	63.86 ± 0.510	105.56 ± 0.0844* (+64.42)	6.689 ± 1.337	3.548 ± 0.7090* (-46.95)
20	61.78 ± 0.494	102.68 ± 0.821* (+66.20)	5.440 ± 1.088	2.410 ± 0.4820* (-55.69)
25	65.12 ± 0.520	110.23 ± 0.881* (+69.27)	5.374 ± 1.074	1.959 ± 0.3910* (-63.45)
30	61.14 ± 0.489	107.46 ± 0.859* (+75.76)	5.361 ± 1.072	1.0746 ± 0.2150* (-79.92)

Values are mean ± SE of five individual observations.
Values in parentheses are per cent changes over control.
Degrees of freedom at 8t 0.05 = 2.306
* Values are significant at 5% level.

on erythrocytes may be secondary, resulting from a primary action of the toxicant on the erythropoietic tissues due to which there exists a failure in red cell production. The severe anaemic state due to pesticide stress or haemolysing power of the toxicant, particularly on the red cell membrane, may be the reason for the decreased erythrocyte count in the present study. Christensen *et al.* (1972) established that any kind of stress not resulting in gross changes and mortality produce certain changes in the fish blood characteristics. McCarthy *et al.* (1973) reported that many factors alter the haematological parameters in fish; they include diet, strain, age, sex, method of capture and toxicants. This may be the reason for the decreased content of erythrocytes in control fish.

The variation in leucocyte count provides a more sensitive index of stress than do changes in erythrocyte abundance (McLeay and Gordon, 1977). Sen *et al.* (1992) observed a significant increase in leucocyte count in *Channa punctatus* and reported that the increase may be due to leucocytosis, which is an adaptation to meet the stressful condition by the animal. The leucocytosis observed in the present study indicates an immune system to protect the fish against infections that might have been caused by cyhalothrin, and also secondary infections, which may be contracted after the weakening of the condition of the fish and resistance to infections.

Hypoxia refers to any condition in which there is an inadequate supply of oxygen to the tissues. Matkovics *et al.* (1981) observed a quick decrease in *C. carpio* haemogolobin content in response to paraquat toxicity and the authors suggested that it might presumably be through methemoglobin formation and a direct response of the oxygen radical. The decrease in the haemoglobin content during sub-lethal treatment in the present study may be due to the rapid oxidation of haemoglobin to methemaglobin or the release of the oxygen radical brought about by the toxic stress of cyhalothrin. The significant recovery in erythrocyte count and haemoglobin level may be due to the triggering of erythrocytes from haemopoietic loci by hypoxemia, which may be a compensatory mechanism. This leads to survival of fish up to 30 days.

Carbohydrate metabolism is disturbed when animals are subjected to toxic stress and the immediate energy demand to the body is provided by glucose. When there is a physiological demand for energy, glucose may be oxidised completely to CO_2 and water, and energy is released (Ravichandran *et al.,* 1995). Stressful stimuli exhibit rapid secretion of both glucocorticoids and catecholamines from the adrenal tissue of fish, leading to hyperglycemia (Singh and Srivastava, 1981). Folmar *et al.* (1993) observed a significant increase in glucose associated with the exposure to both organic and inorganic chemicals. The authors further reported that the rapid rise in glucose results from glycogenolysis (release of glycogen reserves in muscles and liver) initiated by catecholamines, while sustained elevation of serum glucose is maintained by cortisol-stimulated gluconeogenesis.

In the present study, the elevation of glucose in plasma resulted from either decreased conversion of glucose into glycogen or glycogen breakdown was increased under the impact of cyhalothrin stress. The hyperglycemia induced by pesticide might be explained in part by the inhibition of cholinesterase at neuroeffector sites in the adrenal medulla leading to hypersecretion of adrenaline, which might have stimulated the breakdown of glycogen to glucose.

Florkin and Scheer (1970) reported that under conditions of stress, many organisms mobilise protein as an energy source via the oxidation of amino acids. The pesticide stress in fish resulted in serum protein degradation and the products of were fed to the tricarboxylic acid cycle through the aminotransferase system to meet the high energy demand (Sahib *et al.,* 1983). The depletion of protein under pollution stress may be due to the utilisation of proteins in glyconeogenesis to produce energy during stress or it may be due to the inhibition of protein synthesis (Mule and Lomte, 1995). On the other hand, Neff (1985) had opined that the decline in protein content might be related to impaired food intake, increased energy cost of homeostasis, tissue repair and detoxification mechanisms during stress. The inhibition of plasma protein during the sub-lethal treatments in the present study may suggest increased proteolysis or inhibition of protein synthesis. The liver cirrhosis or kidney nephrosis may form other reasons for the decreased level of plasma protein. The study on bioindicators in the present investigation may be employed to integrate the effect of pesticide toxicity and to assist in elucidating the mode of action of pesticides on living systems.

REFERENCES

APHA, 1974. Standard Methods for the Examination of Water and Wastewater (13th edition). American Public Health Association, New York, 874 pp.

Baradbury, S. P. and Coats, J. R., 1989. Comparative toxicology of the pyrethroid insecticides. *Rev. Environ. Contam. Toxicol.,* **108**: 134-177.

Christensen, G. M., McKim, J. M., Brungs, W. A. and **Hunt, H. A.,** 1972. Changes in the blood of the brown bullhead, *Ictalurus nebulosus* (Le Sver) following short and long term exposure to copper (II). *Toxicol. Appl. Pharmacol.,* **23**: 417-427.

Clark, J. M., 1995. Effects and mechanisms of action of pyrethrin and pyrethroid insecticides. *In*: Chang, L. W. (ed.), Handbook of Neurotoxicology. Marcel Dekker Inc., New York, pp. 511-546.

Dig, J. Y. and Wu, S., 1993. Laboratory studies on the effect of dissolved organic material on the absorption of organochloride pesticides by sediments and transport in rivers. *Water Sci. Technol.,* **28** (8-9): 199-208.

Drabkin, D. L., 1946. Spectrophotometric studies. XIV. The crystallographic and optical properties of the haemoglobin of man in comparison with those of other species. *J. Biol. Chem.,* **164**: 703-704.

Dubowski, K. M., 1962. An *o*-toluidine method for body-fluid glucose determination. *Clin. Chem.,* **8**:215-235.

Elliott, M., 1980. Established pyrethroid insecticides. *Pestic. Sci.,* **11**: 119-128.

Finney, D. J., 1978. Statistical Methods in Biological Assay (3rd edition). Griffin Press, London, 508 pp.

Florkin, M. and Scheer, B. T. (ed.), 1970. Chemical Zoology, Arthropoda. Vol. VI. Acadmic Press, Inc., New York, 460 pp.

Folmar, L. C., Bonomelli, S., Moddy, T. and Gibson, J., 1993. The effect of short-term exposure to three chemicals on the blood chemistry of the pinfish, *Lagodon rhomboides*. *Arch. Environ. Contam. Toxicol.,* **24**: 83-86.

Hultmann, E., 1959. Rapid specific method for determination of aldosaccharides in body fluids. *Nature,* **183**: 108-109.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. I., 1951. Protein measurement with folin phenol reagent. *J. Biol. Chem.,* **193**: 265-275.

Matkovics, B., Witas, H. O., Gabrielak, T. and Szabo, L., 1981. Paraquat as an agent affecting antioxidant enzymes of common carp erythrocytes. *Comp. Biochem. Physiol. C,* **87**: 217-219.

McCarthy, D. H., Stevenson, J. R. and **Roberts, M. S.,** 1973. Some blood parameters of the rainbow trout (*Salmo gairdneri*, Richardson). *J. Fish Biol.,* **5**: 1-8.

McLeay, D. J. and Gordon, M. R., 1977. Development of blood sugar bioassay for rapidly measuring stressful levels of pulpmill effluent to salmonid fish. *J. Fish. Res. Board Can.,* **34** : 477-485.

Mule, M. B. and Lomte, V. S., 1995. Copper sulphate induced alterations of protein in freshwater gastropod, *Thiara tuberculata*. *J. Ecobiol.,* **7** (3) : 177-180.

Neff, J. M., 1985. Use of biochemical measurements to detect pollutant-mediated damage to fish. *In*: Cardwell, R. D., Purdy, R. and Bahner, R. C. (ed.), Aquatic Toxicology and Hazard Assessment: 7th Symposium. American Society for Testing and Materials, Philadelphia, pp. 155-183.

Pant, J., Tewari, H. and Gill, T. S., 1987. Effects of aldicarb on the blood and tissues of a freshwater fish. *Bull. Environ. Contam. Toxicol.,* **38**: 36-41.

Ravichandran, S., Shanthi, K. M. and Indra, N., 1995. Effect of phenol on blood glucose level of freshwater fish, *Oreochromis mossambicus*. *Environ. Ecol.,* **13**: 129-131.

Roberts, T. R. and Standen, M. E., 1977. Degradation of pyrethroid insecticide WL 41706 (±) á-cyano-3-phenoxybenzyl 2,2,3,3-tetra-cyclopropane carboxylate in soils. *Pestic. Sci.,* **8**: 600-610.

Rusia, V. and Sood, S. K., 1992. Routine haematological tests. *In*: Mukherjee, K. L. (ed.), Medical Laboratory Technology, Vol. 1. Tata McGraw Hill Publishing Company Limited, New Delhi, pp. 256-258.

Sahib, K. A. I., Rao, K. S. P., Rao, K. R. S. S. and Rao, K. V. R., 1983. Regulation of acidic and basic types of proteins of a teleost, *Tilapia mossambica* under sublethal malathion impact. *Indian J. Fish.,* **30**: 314-319.

Sen, G., Behera, M. K. and Patel, P., 1992. Effect of zinc on haemato-biochemical parameters of *Channa punctatus*. *J. Ecotoxicol. Environ. Monit.,* **2** (2): 89-92.

Singh, N. N. and Srivastava, A. K., 1981. Effects of endosulfan on fish carbohydrate metabolism. *Ecotoxicol. Environ. Saf.,* **5**: 412-417.

Sprague, J. B., 1971. Measurement of pollution toxicity to fish – III. Sublethal effects and 'safe' concentration. *Water Res.,* **5**: 245-266.

Verma, S. R., Rani, S. and Dalela, R. C., 1982. Indicators of stress induced by pesticides in *Mystus vittatus:* Haematological parameters. *Indian J. Environ. Hlth.,* **24**: 5864.