OnLine Journal of Biological Sciences 3 (8): 694-698, 2003
ISSN 1608-4217

Toxic Effect of a Synthetic Pyrethroid Insecticide (Cypermethrin) on Blood Cells of Rainbow Trout (*Oncorhynchus mykiss*, Walbaum)

M. Nuri Çakmak and Asiye Gırgın
Fisheries Faculty, Firat University, 23119 Elaziğ, Turkey

Abstract: In this study, haematological parameters of rainbow trout, ***Oncorhynchus mykiss*** were examined to determine if cypermethrin influenced the measurements. The results indicated that the haematological changes were more evident in fish exposed to cypermethrin. Levels of haematocrit, haemoglobin, leukocyte, red blood cells and MCHC decreased with increasing cypermethrin concentrations ($p<0.01$) but MCV level increased ($p<0.01$) and MCH level was not affected ($p>0.05$) with exposure of different cypermethrin concentration. There was also statistically significant differences in the mean levels of blood parameters of fish exposed to different cypermethrin concentrations ($p<0.05$).

Key words: Cypermethrin, rainbow trout, haematological parameters

Introduction

Cypermethrin is a synthetic pyrethroid insecticide and is used in control of many pests of cotton fruit and vegetable crops, for crach crevice and spot treatment to control insect pests in stores, warehouses, industrial buildings, houses, apartment buildings, greenhouses, laboratories and on ship, railcars, buses, trucks and aircraft. It is also used in schools, nursing homes, hospitals, restaurants and hotels (Eshleman and Murray, 1991). Wide use of this pesticide in crop dusting, orchards and forest spraying for mosquito control, means that some of it will to enter aquatic ecosystems. Thus it is necessary to know the effects of this broad spectrum pesticide on fish (Elliot, 1977; Casida ***et al.***, 1983).

Cypermethrin is very highly toxic to fish, aquatic invertebrates and other living organisms in water. Moreover, it is known that diseases, pollution and the presence of agricultural chemicals in water cause alterations in blood cells of fish resulting in losses of aquaculture (Dörücü and Girgin, 2001). Fish metabolize and eliminate cypermethrin more slowly than mammals or birds, perhaps explaining its higher toxicity in fish compared to other organisms (Bradbury and Coats, 1989).

Cypermethrin is commonly used for the control of insects in agriculture in the area where this study was carried out. Therefore it may reach into water in fish ponds and natural water bodies. Haematological parameters of fish are used as indicators of their physiological state and its study has become widespread in the control of pathologies and manipulation stress in fish farming (Martinez ***et al.***, 1994).

Previously, the effects of pyrethroid insecticides on survival (Shires, 1985), metabolism (Edwards ***et al.***, 1987), nervous system (Eshleman and Murray, 1991) and behaviour (Edwards ***et al.***,

1986) of rainbow trout have been investigated. Recently, Dörücü and Girgin (2001) have shown the exposure time related effects of a constant dose of cypermethrin on the haematological parameters of ***Cyprinus carpio***. Unfortunately, only limited information is available on the dose-related effects of cypermethrin on fish in laboratory conditions (Shires, 1985). Thus, the aim of this study was to investigate the effects of varied concentrations of cypermethrin on blood cells of ***Oncorhynchus mykiss***.

Material and Methods

A total of 50 rainbow trout obtained from Keban Trout Farm were immature <1 year old and weighing 30-35 g. The fish were divided into 5 groups (1 control and 4 experimental groups) and maintained in the 313 l aerated tanks at a constant water temperature (11±0.5°C) and ambient lighting for 2 weeks. Fish were fed ***ad libidum*** daily on a commercial dry trout diet. Cypermethrin [(R-S) Alphacyno 3- phenoxybenzyl (1R,1S) cis/trans-3 (2,2-dichlorovil)-dimethyl-cyclopropane carboxylate] Polytrin 200 EC, was from Novartis. After acclimation, the fish were exposed to 0.001, 0.003, 0.005, 0.007 ppm concentrations of cypermethrin for 96 h. Aged tap water was used in this study and test water was renewed everyday. Upon removal from the water, each individual in the sample was immobilized and 1 ml of blood extracted with a heparinized plastic syringe from the caudal vein.

The following determinations were then made: Haematocrit (Ht) by the microhaematocrit technique (13000 rpm for 5 min on Hettich micro rapid) using heparinized capillary tubes; Hb concentration (Hb g/100 ml) was determined spectrophotometrically using the cyanmethemoglobin method, read at 540 nm; red blood cell (RBC) and leucocytes using dilutions with Natt-herrich solution in Thoma Slide (Kocabatmaz and Ekingen, 1984). From the results of these parameters, mean corpuscular volume (MCV μ^3), mean corpuscular haemoglobin (MCH pg) and mean corpuscular haemoglobin concentration (MCHC%) were calculated as in (Dörücü and Girgin, 2001).

Differences in haematological parameters of rainbow trout exposed to varied concentrations of cypermethrin were tested by Analysis of Variance (One-way ANOVA) using Minitab Statistical Software Release 10. Relationships between levels of blood parameters and exposure concentrations were correlated by means of Product Moment Correlation Coefficient (Fowler and Cohen, 1992).

Results

No fish deaths occurred in the control group or the fish exposed to different concentrations of cypermethrin for 96 h.

The haematological changes were more evident in fish exposed to cypermethrin and were not observed in the control fish (Table 1). When fish were exposed to different cypermethrin concentrations, their food intake decreased and susceptibility to ambient movement increased. Moreover, after exposure to cypermethrin, an excessive amount of mucus was observed over the body surface of fish.

Table 1: Changes in haematological parameters of ***Oncorhynchus mykiss*** exposed to different concentrations of cypermethrin. Each value is mean±SE of 10 individual observations. (%) represents percent changes over the control.

Blood Parameters	Concentrations				
	Control	0.001 ppm	0.003 ppm	0.005 ppm	0.007 ppm
Haematocrit	32.30±0.63	31.00±1.70 (4.02)	30.40±0.47 (5.88)	29.40±1.30 (8.97)	28.80±2.00 (10.83)
Haemoglobin	7.07±0.13	6.80±0.46 (3.81)	6.14±0.18 (13.15)	5.35±0.32 (24.32)	4.80±0.20 (32.10)
Leucocyte	4.8±0.08	4.3±0.07 (10.41)	3.4±0.09 (29.16)	2.8±0.06 (41.66)	2.4±0.05 (50.00)
Erythrocyte	1.41±0.03	1.28±0.08 (9.21)	1.13±0.05 (19.85)	1.02±0.03 (27.65)	0.92±0.02 (34.75)
MCV	229.7±7.71	245.8±25.0 (7.01)	277.5±12.3 (20.80)	288.0±14.5 (25.38)	315.0±24.2 (37.13)
MCH	50.14±0.96	55.78±6.55 (11.33)	56.10±2.93 (11.97)	49.55±1.87 (1.09)	52.74±2.76 (5.26)
MCHC	21.94±0.49	22.66±2.20 (3.28)	20.22±0.62 (7.83)	17.71±1.30 (19.27)	17.56±1.48 (19.96)

Table 2: Simple correlation (r), determination (r^2) coefficients and levels of significance (p) between blood parameter values of rainbow trout and concentrations of cypermethrin

Blood parameters	r	r^2	p
Haematocrit	- 0.97	94	p<0.01
Haemoglobin	- 0.98	96	p<0.01
Leucocyte	- 0.94	88	p<0.01
Erythrocyte	- 0.96	92	p<0.01
MCV	+ 0.98	96	p<0.01
MCH	- 0.14	1.9	p>0.05
MCHC	- 0.95	90	p<0.01

Levels of haematocrit, haemoglobin, leukocyte, red blood cells and MCHC decreased with increasing cypermethrin concentrations, but MCV level increased and MCH level was not affected with exposure to different cypermethrin concentrations (Table 2). Exceptionally, the level of MCH at 0.001 ppm concentration was lower and MCHC at 0.005 ppm concentration was higher than in the control group (Table 1).

There were also statistically significant differences in the mean levels of blood cell numbers of fish exposed to different cypermethrin concentrations (One-way ANOVA, F=56.3, p<0.05).

Discussion

Studies carried out earlier (Anees, 1978; Coats and Jeffery, 1979; Khan, 1983; Edwards ***et al.***, 1986; Eells ***et al.***, 1993; Singh and Agarwal, 1993; Dörücü and Girgin, 2001) on the toxicity of synthetic pyrethroids to a number of animals showed that they were highly toxic to fish and other aquatic animals.

The results in this study also showed that cypermethrin caused notable changes in the blood status of rainbow trout. Similar findings have been reported by Dörücü and Girgin (2001) in carp, ***Cyprinus carpio***. Levels of blood parameters in the control group of this study were similar to the results in Kocabatmaz and Ekingen (1984) who reported the natural values of haematological parameters of some fish species, including rainbow trout. The mean corpuscular haemoglobin was the only measurement uninfluenced by cypermethrin concentration on 96 h exposure. However, cypermethrin had a negative effect on haematocrit, haemoglobin, leukocyte and red blood cells and a positive effect on MCHC. A decrease in red blood cells number during exposure to various pesticides was reported by Anees (1978) and by Krishan and Garg (1981) were similar to the findings in this study. The decrease in red blood cells and haemoglobin content may be due to the disruptive action of the pesticides on the erythropoietic tissue as a result of which the viability of the cells might be affected. Alterations in the haematological parameters were brought about by cypermethrin as an anemia due to decreased synthesis of red blood cells (Morgan ***et al.***, 1980). The decrease in haemoglobin concentration may be due to either an increase in the rate at which haemoglobin is destroyed or a decrease in the rate of haemoglobin synthesis (Moss ***et al.***, 1964).

In accordance with our findings, Cengiz and Ünlü (2002) have also observed increased mucus on the gills of fish exposed to endosulfan.

Most of the studies on the effects of pyrethroids are confined to reporting the biochemical and physiological alterations (Desaiah ***et al.***, 1975; Sivaprasad ***et al.***, 1984; Edwards ***et al.***, 1986; Edwards ***et al.***, 1987; Eells ***et al.***, 1993). Little attention has been paid to the haematological modulation induced by pyrethroids, especially by cypermethrin.

References

Anees, M.A., 1978. Haematological abnormalities in a fresh water teleost ***Channa punctatus*** exposed to sublethal and chronic levels of three organophosphorus insecticides. International J. Ecol. and Environ. Sci., 4: 53-60.

Bradbury, S.P., J.R. Coats, 1989. Comparative toxicology of the pyrethroid insecticides. Review of Environmental Contamination and Toxicol., 108: 133-177.

Casida, J.E., D.W. Gammon, A.H. Glickman and L.J. Lawrence, 1983. Mechanisms of selective action of pyrethroid insecticides. Ann. Review of Pharmacol. and Toxicol., 23: 413-438.

Cengiz, E.I., E. Ünlü, 2002. Histopathological changes in the gills of mosquitofish, ***Gambusia affinis*** exposed to endosulfan. Bull. Envir. Contamination and Toxicol., 68: 290-296.

Coats, J.R., N.L.O.D. Jeffery, 1979. Toxicity of four synthetic pyrethroid insecticides to Rainbow trout. Bulletin of Environmental Contamination and Toxicol., 23: 250-255.

Desaiah, D., K.L. Cutkomp, E.V. Vea, R.B. Koach, 1975. The effect of pyrethroids on ATPases of insects and fish. Gene Pharmacol., 6: 31-34.

Dörücü, M., A. Girgin, 2001. The effects of cypermethrin on some haematological parameters of ***Cyprinus carpio***. Aquaculture International, 9: 183-187.

Edwards, R., P. Millburn, D.H. Hudson, 1986. Comparative toxicology of ***cis***-cypermethrin in rainbow trout, frog, mouse and quail. Toxicology and Applied Pharmacol., 84: 512-522.

Edwards, R., P. Millburn, D.H. Hudson, 1987. The toxicity and metabolism of the pyrethroids ***cis***- and ***trans***-cypermethrin in rainbow trout, ***Salmo gairdneri***. Xenobiotica, 17: 1175-1193.

Eells, J.T., J.F. Rasmussen, P.A. Bandettini, J.F. Propp, 1993. Differences in the neuroexcitatory actions of pyrethroid insecticides and sodium channel-specific neurotoxins in rat and trout brain synaptosomes. Toxicology and Applied Pharmacol., 123: 107-119.

Elliott, M., 1977. Synthetic pyrethroids. American Chemical Society. Washington, pp: 1-28.

Eshleman, A.J., T.F. Murray, 1991. Pyrethroid insecticides indirectly inhibit GABA-dependent $^{36}Cl^-$ influx in synaptoneurosomes from the trout brain. Neuropharmacol., 30: 1333-1341.

Fowler, J., L. Cohen, 1992. Practical Statistics for Field Biology. Johan Willey and Sons. Chichester. England.

Khan, N.Y., 1983. An assessment of the hazard of synthetic pyrethroid, insecticides to fish and fish habitat. (In: Pesticide Chemistry; Human Walfare and the Environment. Miyamoto, J. and Kearney, P.C. Eds.). 3: 437-450.

Krishan, A.G., V. Garg, 1981. 2-3-4-triaminoazobenzene induced haematobiochemical anomalies in fish, ***Channa punctatus***. Bulletin of Environmental Contamination and Toxicol., 26: 136-141.

Kocabatmaz, M., G. Ekingen, 1984. The standardization of blood sampling and haematological methods in different species of fish. Doğa Bilim Dergisi. D_1, (In Turkish), pp: 149-157.

Martinez, F.J., M.P. Garcia-Riera, M. Canteras, Costa, Jde, S. Zamora, 1994. Blood parameters in Rainbow trout (***Oncorhynchus mykiss***): Simultaneous influence of various factors. Comparative Biochem. and Physiol., 107: 95-100.

Morgan, D.P., E.M. Stockdale, R.J. Roberts, H.W. Walter, 1980. Anemia associated with exposure to lindane. Archive of Environmental Health, 35: 307-310.

Moss, J.A., D.E. Hathway, 1964. Transport of organic compounds in the mammalian partition of dieldrin and telodrin between the cellular components and soluble proteins of blood. Biochem. J., 91: 383-393.

Shires, S.W., 1985. Toxicity of a new pyretroid insecticide, WL85871, to rainbow trout. Bulletin of Environmental Contamination and Toxicol., 34: 134-137.

Singh, A., R.A. Agarwal, 1993. Effect of Cypermethrin on lactate and succinic dehydrogenase and cytochrome oxidase of snail and fish. Bulletin of Environmental Contamination and Toxicol., 51: 445-452.

Sivaprasad, R.K., C.S. Chetty, D. Desaiah, 1984. ***In vitro*** effects of pyrethroids on rat brain and liver ATPase activities. Journal of Toxicology and Environmental Health, 14: 257-265.