Research Article **Pathology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

TOXIC EFFECTS OF PHORATE ON BLOOD CELL PROFILES OF COMMON CARP *Cyprinus carpio* ON EXPOSURE TO LETHAL AND SUBLETHAL CONCENTRATIONS

DR. G. LAKSHMAIAH*

Department of Zoology, Sri Krishnadevaraya University, Anantapuramu-515003, A.P, India.

ABSTRACT

Phorate is an organophosphorus insecticide and miticide which is widely used throughout the world and also in India and Andhra Pradesh as a broad-spectrum insecticide for numerous crops. Fingerlings of *Cyprinus carpio* were exposed to acute lethal toxicity of Phorate for one day and 4 days and chronic sublethal toxicity of Phorate for 1, 7, 15 and 30 days. Blood cell profiles like red blood cell (RBC) and white blood cell (WBC) counts were studied in the fish exposed to acute and chronic toxicity of Phorate. Significant alterations were observed in the blood cell profiles of both acute and chronic toxicity exposed fish. The present study enhanced the knowledge of haematological alterations in fish due to acute lethal and chronic sublethal exposure of Phorate.

KEY WORDS: **Phorate, *Cyprinus carpio,* acute lethal toxicity, chronic sublethal toxicity.**

DR. G. LAKSHMAIAH
Department of Zoology, Sri Krishnadevaraya University,
Anantapuramu-515003, A.P, India.

***Corresponding author**

INTRODUCTION

The enormous growth in human population demanded more space to live and increase in the production of agriculture. In increasing the food production pesticides have become an indispensable part of modern agricultural practices. Pesticides play a significant role by keeping many dreadful diseases. Continuous use of pesticides results in their accumulation in various components of the environment. Presence of these pesticides above safe level in the environment will pose serious threat to non-target organisms.

The organophosphate compounds are used for the eradication of pests and insects and this was first demonstrated by Gerhard and Schrader in Germany [1]. For a variety of agricultural and domestic purposes organophosphates (OPs) are widely used as an important group of pesticides and insecticides throughout the world because of their high insecticidal property, low mammalian toxicity, less persistence and rapid biodegradability in the environment [2].

Economically, fishes constitute a very important group of animals and provide a rich source of food, liver oil and a number of other bi-products. A variety of fish species show uptake and accumulation of many contaminants such as pesticides [3]. Pesticides have been found to be highly toxic not only to fish but also to fish food organisms, thus threatening the life of fish [4]. Thus, pesticides can act both indirectly to decrease the food supply, and directly by increasing concentration in the food chain itself on the ultimate predator, human beings. Our understanding of their effects on fish may enable us to limit such harmful effects by monitoring and limiting their release in to the aquatic environment.

Blood is the fluid of life, growth and health, transporting oxygen from the lungs to body tissue and carbon dioxide from body tissue to the lungs, transporting nourishment from digestion, hormones from the glands throughout the body and transporting disease fighting immunoglobulins to the tissues and waste to the kidneys. The changes in the haematological parameters of fish are a helpful biomarkers for evaluating their health status [5]. For many years the study of haematological parameters has been used as diagnostic tool to investigate diseases and physiological and metabolic alterations [6].

The present study is aimed to elucidating the changes in blood cell profiles in the edible fresh water teleost fish, *Cyprinus carpio* exposed to acute lethal and chronic sublethal toxicity of phorate, a commonly used organophosphate insecticide.

MATERIALS AND METHODS

Test Species

The fresh water edible fish, *Cyprinus carpio*, a representative of the aquatic environment has been selected as test species for the present investigation. Fish *Cyprinus carpio* were collected from the department of fisheries, Anantapur, Andhra Pradesh and were immediately transported in big 20 Lts fish containers each with 50 fish to the laboratory. Then they were released into large cement tanks with sufficient dechlorinated tap water and allowed to acclimatize for 15 days. The fish were fed with commercial fish pellets daily. Then the fish were separated into the batch of having the size 10 ± 2 gms and were maintained in static water without any flow [7]. The animals were starved for 24 hours prior to each estimation to avoid any influence of differential feeding. The size of the animals selected was also maintained strictly throughout the investigation.

Test Chemical

Pesticide selected for this study is phorate (O,O-diethyl S-ethylthiomethyl phosphorodithioate), an organophosphorus insecticide which is widely used throughout the world and also in India and Andhra Pradesh as a broad-spectrum insecticide on numerous crops such as carrots, maize, sorgum, sugarcane, beetroot, potatoes, chillies, onion, sunflower, cotton, tomatoes, wheat and coffee including groundnut and

paddy. According to the US Environment Protection Agency (EPA), 2-3 million pounds of phorate, active compound was used in the USA alone during 1999 [8]. The pesticide was obtained from the local market, which was manufactured by Hyderabad Chemical Products Ltd. Commercial grade of phorate (10% CG) was selected to study its effect on fish *Cyprinus carpio* because of only commercial preparations are used in agriculture.

Acute and Chronic toxicity procedures

Lethal concentration (LC_{50}) of phorate to *Cyprinus carpio* was determined by Probit method of Finney [9]. LC_{50}/96 hours (0.71 ppm/lit) of phorate was taken as lethal concentration to study acute toxicity and one-tenth of the LC_{50}/96 hours (0.071 ppm/lit) concentration of phorate was taken as the sublethal concentration for chronic toxicity study.

Experimental Design

160 fishes were divided into two batches, again batch I was divided into 3 groups and batch II was divided into 5 groups comprising of 20 fishes each. Batch I was exposed for acute toxicity of Phorate (exposed to lethal concentration (LC_{50}) of Phorate - 0.71 ppm/lit) and batch II was exposed for Chronic toxicity of Phorate (exposed to sub lethal concentration = $1/10^{th}$ of LC_{50} - 0.071 ppm/lit). In batch I, group 1 was considered as normal control, group 2 and 3 were experimental groups. The fishes of group 2 were exposed for one day and group 3 for 4 days. In batch II**,** group 1 was considered as a normal control group, group 2, 3, 4 and 5 were experimental groups. The fishes of group 2 were exposed for one day, group 3 for 7 days, group 4 for 15 days and group 5 for 30 days.

Haematological profiles

After the completion of stipulated exposure period, the healthy *Cyprinus carpio* fishes were taken out and body surface was cleaned and blotted dry with adsorbent paper and the blood from the control and treated fingerlings was collected from incision at the caudal vein region of the fish into the heparinzed capillary tubes for hematological studies. The treated and control blood samples were used to estimate the total erythrocytic count and total leucocytic count. Red Blood Cell (RBC) and White Blood Cell (WBC) counts were made with a Neubauer crystalline counting chamber as described by Davidson and Henry [10].

Statistical analysis

DMR (Duncan's Multiple Range) test had been employed for the statistical analysis of the haematological data. P value (level of significance) is significant at < 0.05.

RESULTS AND DISCUSSION

In the present investigation marked alterations were observed in the levels of RBC and WBC count in the blood of the fish exposed to acute and chronic toxicity of phorate, over control fish. The data on the levels of RBC and WBC count (millions/ cubic mm) in the blood of the fish at one and 4 days on exposure to acute toxicity of phorate and 1, 7, 15 and 30 days on exposure to chronic toxicity of phorate, besides controls are presented in the Table-I. For comparative assessment, the differences obtained in relation to controls in each parameter at the said exposure periods were converted as mean values and percentages of the corresponding controls, the mean values and percent values were also given in the respective table and the graphs of percent changes against exposure periods were plotted in Graph I (RBC count) and II (WBC count).

It is seen that, relative to controls, the number of RBC was significantly decreased at day 1 and day 4 in the fish exposed to the acute toxicity of phorate in the order of 1>4 whereas in the fish exposed to chronic toxicity of phorate, a significant increase in the RBC number was observed at day 1 and significant decrease at day 7 and day 15 followed by a significant increase at day 30 in the order of 1>7>15<30. Relative to controls, the number of WBC was significantly increased at day 1 and decreased at day 4 in the fish exposed to acute toxicity of phorate

in the order of 1>4 whereas in the fish exposed to chronic toxicity of phorate, a significant decrease in the WBC number was observed at day 1 and gradually increased at day 7 and 15 followed by a significant decrease at day 30 in the order of 1<7<15>30.

Haematological investigations on fishes have confirmed that the variations in the blood will give indication of health condition and environmental changes [11, 12]. Haematological values are widely used to determine the systemic relationship and physiological adaptations, including the assessment of general health conditions [13]. In the present study the phorate induced reduction in RBC count at day 1 and day 4, WBC count at day 4 in the fish exposed to the acute toxicity of phorate may be due to haemolysis and/or haemorrhage caused actions [14] of phorate in the fish. This also indicated anemia in the pesticide exposed fish may be due to inhibition of erythropoiesis, haemosynthesis and osmoregulatory dysfunction or due to an increase in the rate of erythrocyte destruction in haematopoietic organs [15, 16, 17]. The reduction in erythrocyte count of *Cyprinus carpio* in the present study can be attributed to the haemodilution of blood due to the damage of fish organs [18]. Erythrocyte level was found to be depressed in fishes subjected to stressful conditions. Changes in the erythrocyte profile suggest a compensation of oxygen deficit in the body due to gill damage and the nature of the changes shows a release of erythrocytes from the blood depots [19].

Changes in the leucocyte system manifest in the form of leucocytosis with heterophilia and lymphopenia which are characteristics of leucocytic response in animals exhibiting stress. The increase in WBC count at day 1 can be correlated with an increase in antibody production, which helps in survival and recovery of the fish [20]. In the present study, the significant increase in WBC count at day 1 indicate hypersensitivity of leucocytes to phorate and these changes may be due to immunological reactions to produce antibodies to cope up with stress induced by phorate.

Table – I

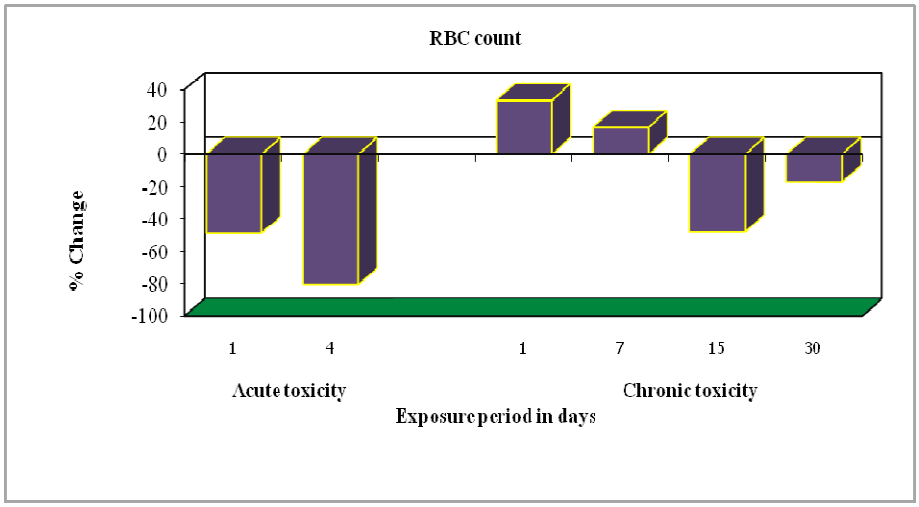
RBC and WBC Count (Millions/mm^3) in the blood of the fish Cyprinus carpio at different periods of exposure to acute and chronic toxicity of phorate (n=6).

NAME		EXPOSURE PERIOD IN DAYS							
		ACUTE TOXICITY			CHRONIC TOXICITY				
		CONTROL	1	4	CONTROL	1	7	15	30
RBC count Millions/mm^3	**Mean** **S.D. ±** **% change**	2.896c 0.128	1.480b 0.066 -48.90	0.555a 0.0435 -80.84	2.896c 0.128	3.860e 0.111 +33.25	3.380d 0.199 +16.68	1.505a 0.103 -48.04	2.395b 0.091 -17.31
WBC count Millions/mm^3	**Mean** **S.D. ±** **% change**	1.763b 0.014	1.775b 0.033 +0.66	1.606a 0.043 -8.88	1.763c 0.014	1.051a 0.025 -40.35	2.468d 0.044 +39.98	3.588e 0.038 +103.50	1.6450b 0.022 -6.70

Values with different superscripts with in the column are significantly different from each other at P<0.05 according to Duncan's Multiple Range Test (DMR) test. The values below the mean are percent changes over the control.

Graph - I

RBC Count (Millions/mm^3) in the blood of the fish Cyprinus carpio at different periods of exposure to acute and chronic toxicity of phorate.

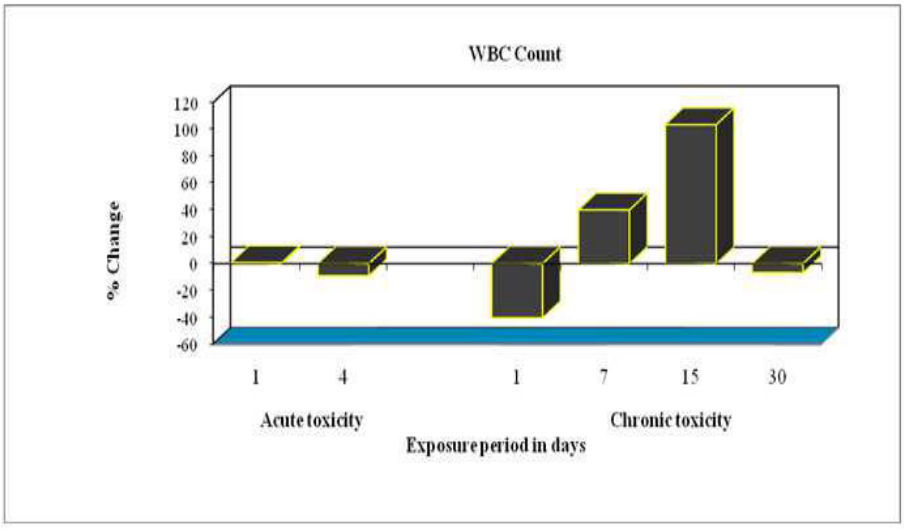


All the values are mean ± SD of six individual observations.

In the fish exposed to chronic toxicity of phorate, the initial increase in RBC count at day 1 may indicate the enhanced erythropoiesis which is triggered as initial typical toxic stress of phorate and gradual decrease at day 7 and day 15 may be due to a reduction in the rate of production and/or increase in the destruction of RBC as suggested by Larson [21]. And later an increase in RBC at day 30 is due to restoration near to normalcy and regained the erythropoiesis in the organs. The increase in the number of WBC at day 7 and 15 may be an adaptive response to the new environment and as a potent defense mechanism of the fish against the toxic entities [22]. Studies on such increased WBC number due to effect of chemical pollution have been reported in a number of fish populations [23, 24, 25]. And later, the decrease in WBC number at day 30 is due to restoration near to normalcy in the fish.

Graph - II

WBC Count (Millions/mm^3) in the blood of the fish Cyprinus carpio at different periods of exposure to acute and chronic toxicity of phorate.



All the values are mean ± SD of six individual observations.

CONCLUSION

The results obtained in the present investigation shows that this pesticide, phorate seems to exert significant effect on blood cell profiles of the fish *cyprinus carpio* at different exposure periods in acute and chronic toxicity. The findings of the present study clearly indicate that phorate is highly toxic to the fish *Cyprinus carpio* at lethal and sub lethal levels. Further, the lethal concentration is more toxic that caused severe damage to the blood cell profiles.

REFERENCES

1. O'Brien RD, In: Insecticides action and metabolism - Academic press, New York, London, (1967).
2. Singh RN, Pandey PK, Singh NN and Dass VK, Acute toxicity and behavioral responses of common carp *Cyprinus carprio* (Linn.) to an Organophosphate (Dimethoate). World Journal of Zoology, 5(3):183-188, (2010).
3. Heger W, Jung SJ, Martin S and Peter H., Acute and prolonged toxicity to aquatic organisms of new and existing chemicals and pesticides. Chemosphere, 31:2707-2726, (1995).
4. Grande M, Andersen S and Berge D., Effects of pesticides on fish. Norw J Agric Sci Suppl, 13:195-209, (1994).
5. Rehulka J, Minarik B and Rehulkova E., Red blood cell indices of rainbow trout, *Oncorhynchus mykiss* (Walbaum) in aquaculture. Aqua Res,35: 529-546, (2004).
6. Bansal SK, Verma SR, Gupta AK and Dalela RC., Physiological dysfunction of the haemopoietic system in a fresh water teleost, *Labeo rohita* following chronic chlordane exposure part I. Alteration in certain haematological parameters. Bulletin of Environmental Contamination and Toxicology, 22: 666-673, (1979).
7. Doudoroff P, Anderson BG, Bordwic GE, Galtsolf BS, Hart NB, Patrick R, Strong ER, Surber EW and Vanfforn WH., Bio-assay method for evaluation of acute toxicity of industrial wastes to fish sewage. Indi Wastes,23: 1380-1397, (1951).
8. EPA, Croplife America annual survey. http://www.usda.gov/nass (1999).
9. Finney DJ, Probit Analysis, 3rd edition, Cambridge University press, Cambridge: pp.333 (1971).
10. Davidson J and Henry, Laboratory methods , 14 Edn, 139-143, (1969).
11. Scharperclaus W., Haematological and serological techniques. In: Fish diseases. Vol. I. Ed: Gulab Primlani, Oxonian Pvt. Ltd, New Delhi, p71-90 (1986).
12. Nath R and Pani Prasad K., Effect of vibro toxin on haematology of *Clarias batrachus.* J Ecobiol, 19(2):101-104, (2006).
13. Alkinson J and Judd FW., Comparative haematology of *Lepomis microlophus* and *Chicchladoma cyanoguttaum*. Copeia, 12: 1230-1237, (1978).
14. Simonato JD, Guedes CL and Martinez CB., Biochemical, physiological and histological changes in the neotropical fish *Prochilodus lineatus* exposed to diesel oil. Ecotoxicol Environ Saf, 69: 112-120, (2008).
15. Jenkins F, Smith J, Rajanna B, Shameem U, Umadevi K, Sandhya V et al., Effect of sub-lethal concentrations of endosulfan on hematological and serum biochemical parameters in the carp (*Cyprinus carpio*). Bull Environ Contam Toxicol 70: 993-997, (2003).
16. Seth N and Saxena KK., Hematological responses in a fresh water fish *Channa punctatus* due to fenvalerate. Bull Environ Contam Toxicol, 71:1192-1199, (2003).
17. Ogundiran MA, Fawole OO, Adewoy, SO and Ayandiran TA., Pathologic lesions in the gills of *Clarias gariepinus* exposed to sublethal concentrations of soap and detergent effluents. J Cell Anim Bio, 3(5): 078-082, (2009).
18. Sweilum MA., Effect of sublethal toxicity of some pesticides on growth parameters, haematological properties and total production of Nile tilapia

(*Oreochromis niloticus* L.) and water quality of ponds. Aquaculture Research, 37: 1079–1089, (2006).
19. Drastichova J, Svobodova Z, Luskova V and Machova J., Effect of cadmium on hematological indices off common carp, *Cyprinus carpio* L. Bull Environ ContamToxicol, 72:725-732, (2004).
20. Joshi P and Deep H., Effect of lindane and malathion exposure to certain blood parameters in a freshwater teleost fish *Clarias batrachus*. Poll Res, 21:55-57, (2002).
21. Larson A., Some biochemical effects of cadmium on fish. In: Koeman, J.h., Stirk, J.J.T.W.A. (Eds). Sublethal effect of toxic chemicals on aquatic animals. Elsevier, Amsterdam, pp 3-13, (1975).
22. Sabitha Borah., Haematological and respiratory anomalies in the fresh water fish *Heteropneustes fossilis* exposed to crude oil. J Ecotoxicol Environ Monit, 16(3): 285-289, (2006).
23. Srivastava AK and Mishra J., Effects of Lindane on blood chloride in the Indian cat fish, *Heteropneustes fossilis,* Acta Hydrobiol, 24:175-192, (1982).
24. Gill TS and Pant JC., Cadmium toxicity inducement of changes in blood and tissue metabolites in fish. Toxicol Lett, 18: 195-200, (1983).
25. Suban Baskey, Mouri Ghosh, Panchali Tarafder, Kaushik Sarkar And Goutam Paul., Chlorpyrifos impairs haematological and hepatic tissue functions by producing oxidative stress. Int J Pharm Bio Sci, 5 (2): (B) 829 – 834 (2014).