Asian Fisheries Science **13**(2000): 225-233
Asian Fisheries Society, Manila, Philippines

https://doi.org/10.33997/j.afs.2000.13.3.004

Sublethal Effect of Quinalphos on Selected Blood Parameters of *Labeo rohita* (Ham.) Fingerlings

B.K. DAS and S.C. MUKHERJEE

Aquatic Animal Health Division
Central Institute of Freshwater Aquaculture
Kausalyaganga, Bhubaneswar 751 002
India

Abstract

The blood parameters, total erythrocyte count (TEC), total leucocyte count (TLC), hemoglobin (Hb), blood glucose and serum protein, and size and surface area of erythrocytes of *Labeo rohita* exposed to two sublethal concentrations i.e 1.12 mg·l and 0.22 mg·l of quinalphos were studied. There was a decrease in the serum protein level (-20.42 to -53.03%) over control ($p < 0.05$) in both concentrations from at least day 15 to day 45. The blood glucose level and TLC were elevated compared to control (13.20 to 33.56% and 10.88 to 41.68%, respectively) in two sublethal concentrations from day 15 to day 45. The Hb% decreased from -23.79 to -38.33% and TEC decreased from -2.87 to -11.32% in both sublethal concentrations.

Introduction

In aquaculture, organophosphates are widely used to control a variety of agricultural pests as well as ectoparasites in fish. Fish accumulate these xenobiotic compounds through their gills. Several workers have investigated the toxicity, uptake and tissue distribution, and hematological changes of pesticides in the fish (Tilak et al. 1980; Abidi and Srivastava 1988; Omoregie et al. 1990; Kumar and Nelson 1997; Das 1998). There are reports on the changes on serum protein (Abidi 1990; Gill et al. 1990); blood glucose level (Bhattacharya et al. 1987; Ghosh 1989); total erythrocyte count (TEC) (Mukhophdhayay and Dehadrai 1980; Sastry and Sharma 1981; Kumar and Nelson 1997), and hemoglobin percentage (Sastry et al. 1982; Pandey et al. 1980) due to various types of pesticides *e.g.* malathion in *Clarias batrachus,* ekalux in *Etroplus maculatus,* and quinalphos in *Channa punctatus.*

Literature on the effect of quinalphos ($C_{12}H_{15}N_2O_2PS$) on carp biochemical, histopathological, and hematological parameters are scanty. Since

quinalphos is extensively applied in agriculture for pest eradication in India, it is pertinent to study its hazardous effect on the aquatic system as it is assumed that the residue might affect the fish. Due to its food value, rohu, *Labeo rohita* is in high demand in India. It is also a candidate species in carp polyculture systems. Thus, it is necessary to study the deleterious effects of quinalphos on the physiological as well as hematological parameters of this important species. This study investigated the effect of quinalphos on its serum protein, on some blood parameters (*e.g.* glucose, Hb, TEC, TLC), and morphometric changes in the erythrocytes of rohu.

Materials and Methods

Test fish

Fingerlings of rohu, *Labeo rohita* (Ham.) weighing 8.52 ± 2.54 g with a mean body length of 6.11 ± 2.52 cm were collected from culture ponds of the Central Institute of Freshwater Aquaculture. Fish were brought to the wet laboratory and acclimatized for two weeks prior to experimentation. Chlorine free tap water was used throughout the course of the experiment. The physico-chemical characteristics of the test water were: temperature 26.5 ± 1.0°C; pH 7.4, hardness 78 mg·l (as $CaCO_3$), alkalinity 82 mg·l (as $CaCO_3$) and dissolved oxygen concentration 6.0 mg·l. The 96 h LC_{50} value of quinalphos was determined as per Reish and Oshida (1987). One tenth and 1/50th of 96 h LC_{50} were determined as 1.12 mg·l and 0.22 mg·l respectively.

Quinalphos

Quinalphos, which is a trade name of Basuquin 25 EC (O,O-diethyl O-quinoxalin-2-yl phosphorothiate) supplied by M/s Bhaskar Agrochemicals Limited, India was used throughout the study. For each experiment the required concentrations were prepared from fresh stock solutions.

Sublethal toxicity

Two sublethal treatments (1.12 mg·l and 0.22 mg·l) of quinalphos and an untreated (negative) control were tested for 45 days in a static system (40 l glass fibertank). Three hundred rohu fingerlings were selected for the present study and were divided into 15 groups. The first six groups were exposed to 1.12 mg·l and the second six groups were exposed to 0.22 mg·l of quinalphos for 45 days respectively, while the remaining three groups were maintained as control in quinalphos free tap water for the same period of time. The pesticide-exposed fish and the control groups were fed twice daily with pelleted feed (rice bran and fish meal) in an equal ration 1:1. Water in the enclosures was renewed every 24 hours to maintain constant concentration of quinalphos. During the period of exposure, aeration was provided to each tank. No mortalities occurred in any group during the experimental period.

Hematological study

One hundred fishes (one third of the fish from each group) were randomly sacrificed on the 15th, 30th, and 45th days. Slime and water present on the body surface of the fingerlings were removed by using blotting paper. Blood was collected from the heart with the help of a 2 ml glass syringe using anticoagulant EDTA (1%) for the estimation of blood glucose, hemoglobin percentage, total erythrocyte count (TEC) and total leucocyte count (TLC). Similarly, blood was collected without anticoagulants for serum protein analysis.

Serum protein was estimated as per the methods of Lowry et al. (1951). Ten ml of serum was taken and to it, 2 ml of alkaline copper sulphate solution was added. The whole mixture was allowed to stand for 10 minutes and to it, 0.5 ml of Folin-Wu reagent was added and mixed in a vortex mixture. The mixture was kept for 30 minutes and final optical density was measured at 740 nm in a spectrophotometer. Bovine serum albumin was used as a standard. TEC and TLC of fish blood were determined using Neubauer's hemocytometer with Toission's solution as diluting fluid for TEC and Turk's solution for TLC while Hb% was determined by using Sahli's hemometer. Blood glucose level was estimated following the method of Folin-Wu (1926). Folin-Wu protein free filterate was prepared by adding nine volumes of the mixture containing eight parts N/12 H_2SO_4 and one part of sodium tungstate directly to the blood. The medium was filtered and the filterate was taken in a tube. Two ml of alkaline copper sulphate solution was added in all the tubes. The optical density was measured at 420 nm. Simultaneously, standard glucose solution containing 1 mg·ml and a blank containing 2 ml distilled water was taken for the observations.

Blood smears were prepared on glass slides, air dried and were subsequently stained with Wrights Giemsa (Hesser 1960). Prepared slides were studied for measuring the cell size and surface area of the erythrocytes adopting the method of Singh and Singh (1982).

Statistical analysis

Students' t-test was employed to calculate the significance of the differences between control and experimental means. P values of 0.05 or less were considered statistically significant (Fisher 1950).

Results

Serum protein

Total serum protein in the control group of fishes ranged from 1.28 g·dl to 1.62 g·dl. The values of the total serum protein in the treated and control fish are represented in figure 1, while changes in terms of percentage of the control values are given in table 1. Treated fish showed low values of serum

protein levels than the control. Changes in the serum protein levels are statistically significant ($p < 0.05$) in both concentrations after 45 days of exposure. The highest percentage of reduction (53.03%) was recorded after 45 days in the case of 1.12 mg·l quinalphos treatment. In comparison, the 0.22 mg·l quinalphos treated fish showed lower reduction rate than the 1.12 mg·l treated fish.

Blood glucose

Blood glucose ranged from 73.94 mg·100 ml to 79.83 mg·100 ml in the control fish. The values of the blood glucose level in the treated fish exposed to 1.12 mg·l and 0.22 mg·l quinalphos for 15, 30 and 45 days are graphically represented in figure 2 while the percentage variations of blood glucose in treated groups are indicated in table 2. The changes in the blood glucose are statistically significant ($p < 0.05$) in both treatments after 15, 30 and 45 days. The maximum elevation of blood glucose was found after 45 days (33.56%) in the 0.22 mg·l quinalphos treatment. This was higher than the elevation observed after 45 days in the 1.12 mg·l quinalphos treatment.

Hemoglobin

Hemoglobin percentage varied from 4.6 gm% to 6.2 gm% in the control group of fingerlings. The percentage variation of hemoglobin from control is presented in table 3 while the variations are represented in figure 3. The values are statistically significant ($p < 0.05$) in both treatments after 15, 30,

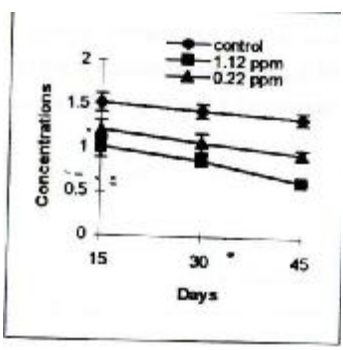

Fig. 1. Variation of serum protein (g·dl) exposed to quinalphos.

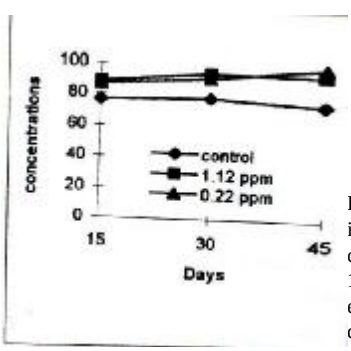

Fig. 2. Changes in blood glucose level (mg·100 ml) in rohu exposed to quinalphos.

Table 1. Percentage variation of serum protein of *Labeo rohita* exposed to quinalphos (a = $p < 0.05$) compared to control.

Days	1.12 mg·l	0.22 mg·l
15	-33.44[a]	-20.42[a]
30	-39.51[a]	-25.66[a]
45	-53.03[a]	-30.21[a]

Table 2. Percentage variation of blood glucose of *Labeo rohita* exposed to quinalphos (a = $p < 0.05$) compared to control.

Days	1.12 mg·l	0.22 mg·l
15	15.56[a]	13.20[a]
30	20.26[a]	15.74[a]
45	25.18[a]	33.56[a]

and 45 days. The maximum reduction percentage (-38.33) was recorded in the 0.22 mg·l treatment after 45 days.

Total erythrocyte count (TEC)

Figure 4 shows the pattern of change in TEC upon exposure to two different concentrations of quinalphos for 45 days. The percentage variations over control are listed in table 4. The variations of TEC are not significant ($p > 0.05$) on 15 days exposure in both treatments, but are statistically significant ($p < 0.05$) after 30 and 45 days of exposure. The amount of reduction in TEC was higher in 1.12 mg·l than 0.22 mg·l quinalphos treatment.

Total leucocyte count (TLC)

In both treatments, there was a rise in TEC on 15 days exposure which continued up to 45 days. The results are represented in figure 5 and the percentage elevation are depicted in table 5. The variations were significant ($p < 0.05$) in both treatments after 15, 30 and 45 days.

Morphometric changes in erythrocytes

Mean length, breadth, and surface area of erythrocytes of rohu exposed to 1.12 mg·l and 0.22 mg·l quinalphos are shown in table 6. The treated fish showed no statistically significant variation ($p > 0.05$) after 15 days exposure

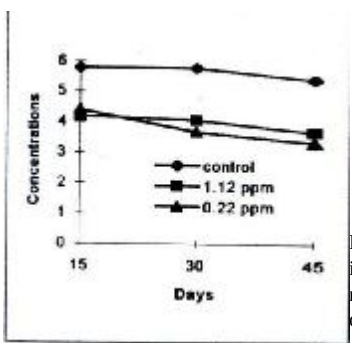

Fig. 3. Changes in Hb (gm%) in rohu exposed to quinalphos.

Table 3. Percentage variation of hemoglobin of *Labeo rohita* exposed to quinalphos (a = $p < 0.05$) compared to control.

Days	1.12 mg·l	0.22 mg·l
15	-27.73[a]	-23.79[a]
30	-29.46[a]	-36.40[a]
45	-32.03[a]	-38.33[a]

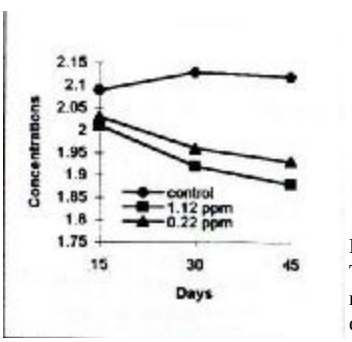

Fig. 4. Variation of TEC ($10^6 \cdot mm^3$) in rohu exposed to quinalphos.

Table 4. Percentage variation of TEC of *Labeo rohita* exposed to quinalphos (a = $p < 0.05$) compared to control.

Days	1.12 mg·l	0.22 mg·l
15	-3.86	-2.87
30	-9.86[a]	-7.98[a]
45	-11.32[a]	-8.96[a]

whereas after 30 days the variation in length, breadth, and mean surface area was statistically significant ($p < 0.05$) in the 1.12 mg·l and treatments are highly significant ($p < 0.01$ and $P < 0.05$) after 45 days. The quinalphos exposed erythrocytes became enlarged, the cell wall was crenated, showed distortion, and the nuclei showed hypertrophy.

Discussion

It is obvious that exposure of fish for a long time to most toxicants including pesticides interferes with protein metabolism. The present work also supports the observations by Sastry et al. (1982) in this regard who opined that such an interference results in the depletion of total protein in the plasma of fish when exposed to quinalphos. Similarly, histopathological changes in the kidney are due to organophosphs and organochlorin compounds. These toxicants lead to a considerable loss of blood proteins by renal excretion further augmenting its depletion in the blood (Verma et al. 1979; Sastry and Sharma 1981). It is therefore logical to presume that in the case of prolonged and continued exposure to the pesticides the deleterious effects of these substances on protein synthesis and kidney function account for the progressive reduction in the concentration of total serum protein.

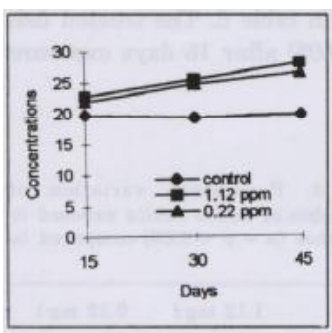

Fig. 5. Variation of TLC ($10^3 \cdot mm^3$) of rohu exposed to quinalphos.

Table 5. Percentage variation of TLC of *Labeo rohita* exposed to quinalphos (a = $p < 0.05$) compared to control.

Days	1.12 mg·l	0.22 mg·l
15	15.37^a	10.88^a
30	31.91^a	28.07^a
45	41.68^a	33.67^a

Table 6. Changes in mean size and surface area of erythrocytes (m) exposed to quinalphos (Mean ± SE, a = $p < 0.01$, ab = $p < 0.01,0.05$ and b = $p < 0.05$).

Days	Treatment (mm.)	Length (mm.)	Breadth (mm^2)	Surface area
15	Control	8.75 ± 0.22	6.50 ± 0.25	57.0 ± 3.18
	1.12 mg·l	8.25 ± 0.22^b	6.0 ± 0.35^b	49.75 ± 4.16^b
	0.22 mg·l	8.75 ± 0.22^b	6.75 ± 0.22^b	59.25 ± 3.25^b
30	Control	8.25 ± 0.22	6.5 ± 0.25	53.75 ± 3.13
	1.12 mg·l	7.25 ± 0.22^a	5.25 ± 0.22^a	38.25 ± 2.82^a
	0.22 mg·l	7.50 ± 0.43^b	5.25 ± 0.22^a	39.75 ± 4.11^b
45	Control	8.50 ± 0.25	6.50 ± 0.25	55.5 ± 3.75
	1.12 mg·l	6.50 ± 0.25^{ab}	4.50 ± 0.25^{ab}	29.5 ± 2.75^{ab}
	0.22 mg·l	7.25 ± 0.22^a	5.25 ± 0.22^a	38.25 ± 2.82^a

Stressors induce some changes that alter the homeostasis of the animal (Schreck 1981). The stressors first activate the chromaffin cells present in the walls of the cardinal veins and in some cases the heart and kidney of the teleost (Mazeaund and Mazeaund 1981). Chromaffin cells release adrenalin and a small amount of noradrenalin that stimulates the conversion of liver glycogen into blood glucose and the utilization of glucose by muscle. The adrenergic effects may result in the increase of blood sugar within minutes after the onset of stress due to pesticides. Umingel (1977) reported that blood sugar has a direct correlation to metabolism. The increase in blood sugar noticed in the present study could be attributed to differences in respiration and activity as pointed out by Ghosh (1987). The progressive accumulation of blood glucose reported in this investigation revealed that rohu exposed to sublethal concentration of quinalphos became hyperglycaemic. Omoregie et al. (1990) reported that tilapia showed marked hyperglyceamic response to stressed environmental conditions as a result of incomplete metabolism of the blood sugar due to impaired osmoregulation. Increased blood glucose concentration results from an imbalance between the hepatic output of glucose and the peripheral uptake of the sugar (Coles 1980). In the present study, exposure to different concentrations of pesticides caused an increase in the blood glucose levels leading to lethargy.

Abidi and Srivastava (1988) reported that an increase in the Hb% may be due to the catalysing actions of pesticides on the incorporation of body iron stored into hemoglobin. *Channa punctatus* exposed to subtle concentrations of quinalphos for 15 and 45 days had lower hemoglobin content (Sastry et al. 1982). Our present findings are consistent with the above study as a significant decrease in Hb% ($p < 0.05$) was recorded in 1.12 mg·l and 0.22 mg·l quinalphos.

Percentage reduction in total erythrocytes noticed in the present study revealed that rohu exposed to quinalphos became anemic, possibly due to hemodilution resulting from impaired osmoregulation across the gill epithelium as reported by Wedemeyer et al. (1984). Similar reduction in the erythrocytes of Nile tilapia exposed to Gammalin 20 and Actellic 25 EC was reported by Omoregie et al. (1990) and malathion in freshwater catfish (Mukhopadhayay and Dehadrai 1980).

Increase in the total leucocyte count has been attributed to several factors like increase in thrombocytes, lymphocyte or sqeezing of WBC in peripheral blood (Mahajan and Juneja 1979; Agrawal and Srivastava 1980). Increase in the TLC could be due to stimulated lymphopoiesis and/or enhanced release of lymphocytes from lymphomyeloid tissues as has been opined by Meenakala (1978). Such lymphocyte response might be due to the presence of toxic substances or may be associated with the pollutant induced tissue damage as was also opined by Haniffa (1990).

The physiological function of blood is to transport oxygen and nutrients to cells and to remove cell metabolites. When assessing the physiological effect of water toxicants on fish life, it becomes necessary to take into account the morphological changes occuring in the blood cells simply because

changes in erythrocytes may cause an imbalance in the respiratory physiology of the fish. The surface area reduction of erythrocytes noticed in the present study is suggestive of hypoxic effect prevailing over the body tissues as has been reported by Das (1998) in rohu fingerlings due to the effect of quinalphos on gill tissue.

Reduction of TEC and Hb% may be suggestive of an appreciable decline in the hematopoiesis leading to various types of anemia e.g. poikilocythemic, microcytic and hemolytic anemia. Increase in TLC is recorded probably due to thrombocytosis, lymphocytosis or leucopoiesis and/or enhanced release of lymphocytes from the lymphoid tissue under the effect of toxic compounds.

Conclusion

Quinalphos, a commonly used organophosphate by the agricultural sector at sublethal concentrations can reduce the blood protein, hemoglobin, and total erythrocyte count of fish exposed to it for a long period of time. However, it is important to evaluate the residual effects of this pesticide in different body tissues of fish as they are ultimately consumed by the human beings. Nevertheless it is obvious that the pesticide has deleterious effects on fish as observed through the present study.

Acknowledgment

The authors are thankful to Dr. S. Ayyappan, Director, Central Institute of Freshwater Aquaculture for the facilities provided.

References

Abidi, R. and U.S Srivastava 1988. Effect of endosulfan on certain aspects of haematology of the fish. *Channa punctatus*. Proceedings of National Academy of Science India 58: B (1):55-65

Abidi, R. 1986. D. Phil Thesis, University of Allahabad.

Abidi, R. 1990. Endosulfan induced changes in the total serum proteins of *Channa punctatus* (Bloch). Biol. Fisiol. Anim. Sao Paulo 14:41-48.

Agrawal, S. J. and A. K. Srivastava 1980. In effect of endosulfan on certain aspects of haematology of the fish, *Channa punctatus* Toxicology 17:97.

Bhattacharya, T. A.K.Ray and S.Bhattacharya. 1987. Histopathology of the kidney of *Channa punctatus* (Bloch) exposed to sublethal and chronic levels of industrial effluents. 11 Zoology Journal of Anatomy 115:263-272.

Coles, E.H.1980. Veterinary clinical pathology (3rd ed.) W.B.Saunders Co. Philadelphia London-Toronto 225 pp.

Das, B. K. 1998. Studies on the effect of some pesticides and commonly used chemicals on Indian major carps and their ecosystem *D. Phil Thesis*, Orissa University of Agriculture and Technology, Bhubaneswar (India) 139-162 pp.

Fisher, R.A.1950.Statistical methods for research workers. 11 th edition. Oliver and Boyd, London 75 pp.

Folin and Wu. 1926. Determination of blood glucose level. Journal of Biological Chemistry 38:81

Ghosh, T. K. 1987 Effect of organophosphorous compound on some metabolite levels of the fish, *Barbus stigma*, Environmental Biology 5(4):638-642.

Ghosh, T. K. 1989, Toxic impact of three organophosphates on carbohydrate metabolism in a freshwater Indian catfish, *Clarias batrachus*, Proceedings of Indian National Science Congress. 135-142pp.

Gill, T.S., J . Pandey and H. Tewari 1990. Enzyme modulation by sublethal concentrations of adicarb, Phosphamidon, and endosulfan in fish tissues. Pesticide Biochemistry and Physiology 38 (3):231-244.

Haniffa, M. A 1990 In Haematological effects of textile mills effluents on freshwater fish. *Oreochromic mossambicus*. Environmental Research 17:191.

Hesser, E. P 1960. Methods for routine fish haematologies. Progressive Fish Culturist 22 (4):164-171.

Kumar,G.S. and D.J. Nelson.1997. Effect of Ekalux on haematology of the freshwater fish *Etroplus maculatus*. Journal of Ecotoxicological and Environmental Monitoring 7:113-116.

Lowry, O. H , N. J. Rosebrough , A. L. Farr and R. J. Randall 1951. Protein measurement with folin phenol reagent, Journal of Biological Chemistry 193:265.

Mahajan , C. L. and C. J. Juneja 1979. Ind. J. Environ. Health 21:162. In Proceedings of the National Academy of Science India. 58 B (1):55-65.

Mazeaund,M.M. and I.Mazeaund.1981. Adrengic response to stress in fish In Stress and Fish . Pickering A.D. Eds. Academic Press.Newyork. Chap.3:150 pp

Meenakala 1978. Toxicity of textile mill effluents to a chosen fish. M. Phil thesis. Madurai Kamaraj University, Madurai, India.

Mukhopadhyay, P. K. and P. V. Dehadrai 1980. Biochemical changes in the air breathing catfish, *Clarias batrachus* (L) exposed to malathion. Journal of Environmental Biology 5:221-229.

Omoregie, E., E. B.C Ofodike and R. I. Keke. 1990. Tissue chemistry of *Oreochromis niloticus* exposed to sublethal concentration of gammalin-20 and acetellic -25EC. Journal of Aquatic Science 5 : 33-36.

Pandey, P. K., N. K. Singh and B. P Chaudhary 1980 Effect of organophoshphrous insecticide, malathion on the haematology of *Channa punctatus*. Proceedings of 6th. Indian Science Congress Part II. 213 pp.

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 1-62 pp.

Sastry, K. V., A.A Siddiqui and S. K. Singh 1982. Alterations in some biochemical and enzymological parameters in the snake head fish, *Channa punctatus* exposed clinically to quinalphos. Chemosphere 11:1211.

Sastry, K.V and K. Sharma 1981. Diazinon induced histopathogical and haematological alterations in a freshwater teleost, *Ophiocephalus punctatus*. Ecotoxicology Environment Safety, 5:329-340.

Schreck,C.B.1981. Stress and compensation in teleostean fishes response to social and physical factors in stress and fish packaging Chap.18. A.D.Bel. Academic press. New York.

Singh, R. R and B. R. Singh 1982 Effect of copper and zinc sulphate on the blood paramethers of *Mistus vittatus*. Matsya 8:1-6

Tilak, K. S , D. M. R. Rao, A. P. Devi and A. S. Murty 1980. Toxicity of carbaryl and 1-naphthal to the freshwater fish *Labeo rohita* , Indian Journal of Experimental Biology 18 (1):75.

Ummingel, B. L. 1977. Relation of whole blood sugar concentrations in vertebrates to standard metabolic rate. Comparative Biochemistry and Physiology 55:457-460.

Verma, S.R., S. K. Bansal, A. K. Gupta, R. C Dalela 1979. Pesticide induced haematological alterations in a freshwater fish., *Sarotheradon fossilis*. Bulletin of Environmental Contamination Toxicology 22:461-474.

Wedemeyer, G., D. J. Mcleay, and C. P. Good year. 1984. Assessing the tolerance of fish and fish population to environmental stress. The problems and methods of monitoring. In contaminant effects on fisheries Eds. W. V Cairns, P. V. Hodson and J.O Nriagu164-195 pp. John Wiley and son Inc. Newyork.

Manuscript received 08 July 1999; Accepted 01 March 2000.