Environment & Ecology 27 (1) : 127—130, 2009
 ISSN 0970-0420

Changes in Blood Profile of Fresh Water Fish *Rasbora daniconius* Exposed to Chloropyrifos

I. A. RAJA AND A. P. CHARJAN[1]

Department of Zoology, Shri Shivaji College of Arts, Commerce and Science Akola 444001 (MS), India
[1]Department of Zoology, G. S. Gawande College, Umarkhed (MS), India

Abstract

The effect of chloropyrifos was evaluated on the blood of exposed fish *Rasbora daniconius*.The acute toxicity test for 96 h was performed on the fresh water fish. Examination of erythrocyte and leucocyte was performed on control and exposed fish after 96 h of exposure to 0.05 mg/liter of chloropyrifos. The exposed group showed significantly lower values of erythrocyte count (RBC), hemoglobin content (Hb) and hematocrit (PCV) compared to the control fish. Values of MCV, MCH and MCHC were comparable in both groups of fish. There was a significant decrease in leucocyte count, the relative and absolute lymphocyte count and a significant increase in relative and absolute count of developmental forms of neutrophil granulocytes, myelocytes and metamyelocytes in the treated group. Relative and absolute count of monocytes and both the band and segmented neutrophil granulocytes were comparable in both groups.Changes in values of both the erythrocyte and leucocyte on exposure to chloropyrifos may be referred to alteration of hematopoiesis and to a decrease on non-specific immunity of the fish.

Key words : Chloropyrifos, *Rasbora daniconius*, Acute toxicity, Erythrocyte profile, Leucocyte profile.

Organophosphate pesticides have fully replaced the persistent chlorinated pesticides in the 1970 and in the beginning of 1980. The main advantage of the organophosphate pesticides was their low cumulative ability and short-term persistance in the environment. Although the organophosphate pesticides have been replacing by pyrethroid-based pesticides within the last 10—15 years, there is still an intensive utilization of organophosphates. Organophosphate pesticides are also utilized in fish culture (mainly those based on dichlorvos and trichlorfon) to suppress some parasitic diseases(1—3). Nevertheless, the pesticide preparations are considered harmful to fish in most cases (4). Chloropyrifos (durshan) is a common active organophosphate pesticides used against soil insects of field and vegetable crops throughout the Vidarbha region of Maharashtra. Not all of its effects to fish are known, despite of its intensive use. Therefore this study was carried out to determine the effect of chloropyrifos on fresh water fish *Rasbora daniconius* with reference to some blood parameters.

Methods

Adult experimental fish *Rasbora daniconius* (12 ± 0.8 cm) were collected from the local Kapsi water reservoir near Akola town and acclimatized to laboratory conditions in stocking aquarium for 2 weeks prior to the experiment. The aquarium was aerated, cleaned and water renewed daily. Fish were fed twice daily with insect larvae. In the acute toxicity experiment the fish were exposed to 0.05 mg/liter commercial grade chloropyrifos for 96 hours. The test was performed in 6 aquaria of 20-liter volume (two control aquaria, four aquaria with concentration of 0.05 mg/liter chloropyrifos.) Each aquarium was stocked with 10 fish. Basic physical and chemical indices of diluting water used in the test were maintained at standard levels, oxygen saturation of water ranged between 70 and 100%. Examination of erythrocyte and leucocyte profile was carried out on 15 control and 25 exposed fish after 96 h of exposure. Blood was sampled by cardiac puncture and stabilized with sodium heparin. Erythrocyte count (RBC), hematocrit (PCV), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), leucocyte count and differential leucocyte count were determined (5). Results were processed statistically by means of

Table 1. Derived blood parameters of *R. daniconius* exposed to chloropyrifos.

Indices	Units	Groups	N	Means	SD	Variance	Probability
		Control	13	252.07	28.56	815.67	
MCV	fl	Exposed	24	249.50	55.22	3049.24	0.88
		Control	13	60.69	7.00	49.00	
MCH	pg	Exposed	24	60.85	8.36	69.89	0.95
		Control	15	0.24	0.02	0.00	
MCHC	1.1^{-1}	Exposed	25	0.25	0.04	0.01	0.37

ANOVA.

Results and Discussion

Ninety six hour toxicity tests of chloropyrifos on fish *Rasbora daniconius* showed that there was no mortality of fish in the control aquarium. Oxygen saturation of water did not drop below 60% in any concentration tested, nor in the control group. Results of erythrocyte profile of both the control and exposed fish *Rasbora daniconius* are given in Table 1 and Figure 1. Compared to the control, fish after an acute exposure to chloropyrifos had lower erythrocyte count ($P<0.01$), hemoglobin content ($P<0.01$) and lower hematocrit value ($P<0.01$). The main hematological response of experimental fish to chloropyrifos was a significant decrease ($P<0.01$) of erythrocyte count, hematocrit value and hemoglobin content compared to the control fish. Decreased erythrocyte count and hemoglobin content in freshwater fish *Channa punctatus* after acute exposure to diazinon an organophosphate was also reported (6).

Results recorded for MCV, MCH and MCHC were comparable in both control and exposed fish. Results of leucocytes profile of both groups are given in Table 2 and Figure 2. The acute exposure to chloropyrifos resulted in lower leucocyte count ($P<0.01$), and both the relative and absolute lymphocyte count ($P<0.01$). There was an increase in both the relative and absolute count of developmental forms of neutrophil granulocytes, namely, myelocytes ($P<0.01$), metamyelocytes ($P<0.05$). Differences in relative and absolute count of monocytes and band and segmented neutrophil granulocytes in neither of groups were significant. In accordance with these results other organophosphate pesticides also induce changes, which give evidence for decreased hematopoiesis followed by anemia induction in fish. e.g. changes in erythrocyte profile induced by acute effect of dichlorvos in *Clarias batrachus* (7), trichlorphon in *Piaractus mesopotamicus* (8), malathion in *Cyprinion wabsoni* (9), formothion in *Heteropneustes fossilis* (10) and ekolux, an organophosphorus preparation in *Oreochromis mossambicus* (11). Another type of hematological response to the effect of organophosphorus compounds was a significant increment of mean corpuscular volume (MCV) associated with increment of hematocrit value and drop of MCHC. This response was registered in common carp after acute effect of phenitrothion, imidan and dichlorvos (12,13). Values of MCV, MCH and MCHC registered after 96 h exposure to chloropyrifos at 0.05 mg/liter concentration to *Rasbora daniconius* were comparable with the control group. Significant decrease of leucocyte count and significant relative and absolute lymphope-

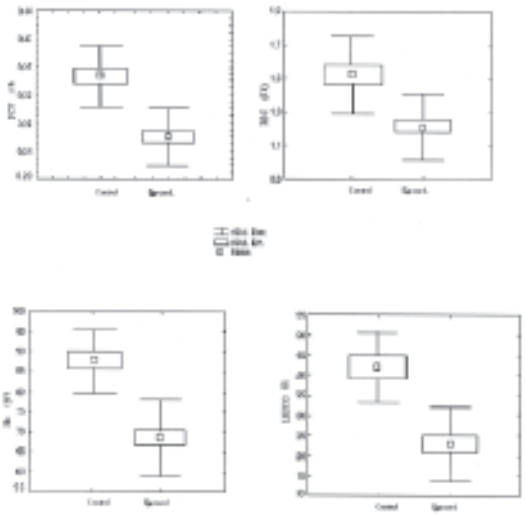

Figure 1. RBC, leucocytes, Hb and PCV in *R. daniconius* exposed to chloropyrifos.

Table 2. Leucocytes differential count in *R. daniconius* exposed to chloropyrifos. Marked effects are significant at $P<0.05$ (*) and $P<0.01$ (**).

Indices	Groups	N	Means	SD	Variance	Probability
	Control	9	45.33	8.33	72.76	
Lymphocytes	Exposed	20	19.36	5.33	28.41	0.00**
	Control	9	0.33	0.27	0.07	
Monocytes	Exposed	20	0.23	0.25	0.06	0.32
	Control	9	0.40	0.28	0.08	
Myelocytes	Exposed	20	5.55	3.85	14.82	0.00**
	Control	9	0.23	0.35	0.12	
Metamyelocytes	Exposed	20	1.44	1.39	1.93	0.02*
	Control	9	0.22	0.30	0.09	
Band neutrophils	Exposed	20	0.85	0.91	0.83	0.06
	Control	9	0.40	0.67	0.45	
Segmend neutrophils	Exposed	20	0.38	0.58	0.34	0.91

nia and granulocytosis characterize the leucocyte profile of *Rasbora daniconius* after the acute exposure to chloropyrifos. Lymphopenia as a consequence of methylparathion-based pesticide was reported by Nath and Banerjee (14) in *Heteropneustes fossilis* and by Siwicki et al. (15) in common carp after an acute effect of trichlorfon. Ghosh and Banerjee (16) report lymphopenia and both neutrophil and eosinophile granulocytosis in *Heteropneustes fossilis* after an effect of dimethoate in 96 h LC_{50} concentration. A decreased non-specific immunity in fish can be expected after acute exposure to organophosphate pesticides due to decreased leucocyte count, lymphopenia and granulocytosis. Several authors report lymphopenia and granulocytosis after exposure to many pollutants (4, 17—21).These changes in differential leucocyte count also give evidence for decreased level of non-specific immunity in fish after acute exposure to toxic substances. The mechanism of a toxic effect of all organophosphorus substances is the same. There is an inhibition of a whole series of enzymes and mainly of acetylcholinesterase (22—25). However specific sensitivity of fish to any organophosphate insecticide may be associated with different ability of absorption, acetylcholinesterase inhibition and detoxification, as reported by Oh et al. (26).

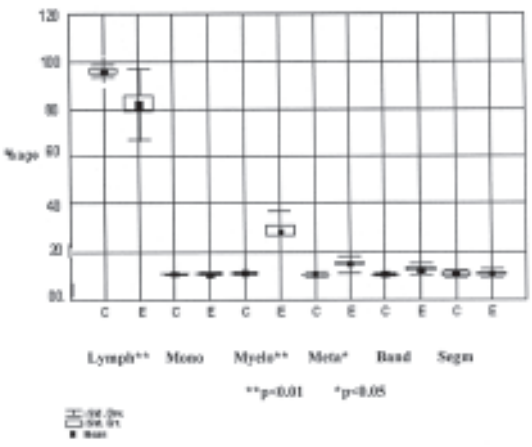

Figure 2. Differential count of leucocytes in *R. daniconius* exposed to chloropyrifos.

References

1. Noga J. E. 1995. Fish disease : Diagnosis and treatment. Mosby, St. Louis.
2. Schlotfeldt H. J. and D. J. Alderman. 1995.What should I do ? A practical guide for the freshwater fish farmer. Warwick Press, Weymouth.
3. Navratil S., Z. Svobodova and Z. Lucky. 2000. Chorobyryb. Edicni stredisko VFU, Brno.
4. Svobodov Z., J. Machova, J. Kolarova, B. Vykusova and V. Piacka. 1996. The effect of selected negative factors on hematological parameters of common carp, *Cyprinus carpio* L. and tench, *Tinca tinca* L. Proc. Sci. Papers to 75th Anniv. Found of the RIFCH Vodnany, pp. 95—105.
5. Svobodova Z., D. Pravda and J. Palackvoa. 1991. Unified methods of hematological examination of fish. Res. Inst. of Fish Cult and Hydrobiol., Vodnany.
6. Anees M. A. 1978. Hematological abnormalities in a freshwater teleost, *Channa punctatus* (Bloch), exposed to sublethal and chronic levels of three organophosphorus insecticides. Int. J. Ecol. Environ.

Sci. 4 : 53—60.
7. Benerjee G. and T. Ranindranath. 1990. Hematological changes induced by an organophosphorus insecticide in a freshwater fish *Clarias batrachus* (Linnaeus). Trop. Freshwat. Biol. 2 : 197—202.
8. Tavares D. M., M. L. Martins and K. S. Nascimento. 1999. Evaluation of the hematological parameters in *Piaractus mesopotamicus* Holmberg (Osteichthyes, Characidae) with *Argulus* sp. (Crustacea, Branchiura) infestation and treatment with organophosphate. Rev. Bras. Zool. 16 : 553—555.
9. Khattak I. U. D. and M. A. Hafeez. 1996. Effect of malathion on blood parameters of the fish, *Cyprinion watsoni.* Pak. J. Zool. 28 : 45—49.
10. Singh N. N. and A. K. Srivastava. 1994. Formothion induced hematological changes *in vivo* effect of the catfish *Heteropneustes fossilis*. J. Ecotox. Environ. Monit. 4 : 137—140.
11. Sampath K., S. Velammal, I. J. Kennedy and R. James. 1993. Hematological changes and their recovery in *Oreochromis mossambicus* as a function of exposure period and sublethal levels of ekalux. Acta Hydrobiol. 35 : 73—83.
12. Svobodov Z. 1971. Some hematological and metabolic changes in fish occurring after pesticide intoxication. Bull. V. Vodnany 7 : 29—36.
13. Svobodov Z. 1975. Changes in the blood picture of the carp intoxicated with organophosphate pesticides. Acta Vet. Brno. 44 : 49—102.
14. Nath R. and V. Banerjee. 1996. Effect of pesticides methylparathion and cypermethrin on the air-breathing fish *Heteropneustes fossilis*. Environ. Ecol. 14 : 163—165.
15. Siwicki A. K., M. Cossarinionier, M. Studnicka and A. Demael. 1990. In organophosphorus insecticide trichlorphon on immune response of carp (*Cyprinus carpio*) II. Effect of high doses of trichlorphon on nonspecific immune response. Ecotox. Environ. Saf. 19 : 99—105.
16. Ghosh K. and V. Banerjee. 1993. Alteration in blood parameters in the fish *Heteropneustes fossilis* exposed to dimethoate. Environ. Ecol. 11 : 979—981.
17. Wlasow T. 1985. The leucocyte system in rainbow trout, *Salmo gairdneri* Rich, affected by prolonged sub-acute phenol intoxication. Acta Ichthyol. Piscator. 15 : 83—84.
18. Murad A. and A. H. Hoiston. 1988. Leucocytes and leucopoietic capacity in goldfish, *Carassius auratus,* exposed to sub lethal levels of cadmium. Aquat. Toxicol. 13 : 141—154.
19. Schwaiger J., R. Hoffmann and R.D. Negele. 1993. Hematology in evaluation of experimental intoxication of fish. Ichthyohematology, 3rd Conf., Res. Inst. of Fish Cult. and Hydrobiol. Vodony, Czech Republic, Litomy, pp. 155—160.
20. Thakur N. and S. Sahai. 1993.Differential leucocyte counts of some fishes during malathion intoxication. Environ. Ecol. 11 : 875—878.
21. Alkahem H. F. 1994. The toxicity of nickel and the effects of sublethal levels on hematological parameters and behavior of the fish, *Oreochromis niloticus.* J. Univ. Kuwait. Sci. 21 : 243—252.
22. Goodman L. R., D. J. Hansen, D. L. Coppage, J. C. Moore and E. Matthews. 1979. Diazinon : Chronic toxicity and brain acetylcholinesterase inhibition in the sheepshead minnow, *Cyprinodon variegatus.* Trans. Am. Fish. Soc. 108 : 479—488.
23. Sastry K. V. and K. Sharma. 1980. Diazinon effect on the activities of brain enzymes from *Opiocephalus punctatus* (channa). Bull. Environ. Contam. Toxicol. 24 : 326—332.
24. Ansari B. A., M. Aslam and K. Kumar. 1987. Diazinon toxicity : Activities of acetylcholinesterase and phosphatases in the nervous tissue of zebra fish, *Brachydanio rerio* (Cyprinidae). Acta Hydrochim. Hydrobiol. 15 : 301—306.
25. Hamm J. T., B. W. Wilaon and D. E. Hinton. 1998. Organophosphate induced acetylcholinesterase inhibition and embryonic retinal cell necrosis *in vivo* in the teleost (*Oryzias latipes*).Neurotoxicology 19 : 853—870.
26. Oh H. S., S.K. Lee, Y. H. Kim and J. K. Roh. 1991. Mechanism of selective toxicity of diazinon to killifish (*Oryzias latipes*) and loach (*Misgurnus anguillicaudatus*). Aqua. Toxicol. and Risk Assess. 14 : 343—353.