Bull. Environ. Contam. Toxicol. (2006) 76:607–613

DOI: 10.1007/s00128-006-0963-5

Bulletin of Environmental Contamination and Toxicology

Effect of Monocrotophos on Erythropoietic Activity and Hematological Parameters of the Freshwater Fish *Channa punctatus* (Bloch)

S. Agrahari,[1] K. C. Pandey,[1] K. Gopal[2]

[1] Department of Zoology, University of Lucknow, Lucknow 226007, India
[2] Aquatic Toxicology, Industrial Toxicology Research Center (CSIR), 80, MG Marg, Lucknow 226 001, India

Received: 8 August 2005/Accepted: 8 March 2006

Monocrotophos is a commonly used organophosphate pesticide for pest control of crops in India (Ray et al. 1985). It is a systemic insecticide and acaricide of the vinyl phosphate group. Its wide use provides many routes of entry into aquatic environments and adversely affects many non-target species. Fishes form an important class of organisms on the basis of their use as nutritive food and are also a useful indicator of pollution. It is necessary to monitor certain clinical parameters in order to determine the sublethal concentrations of pesticides or pollutants on the physiology of fish. Use of haematological parameters as indicators of sublethal stress can provide valuable information concerning the physiological reaction of fish in a changing environment. The physical and chemical properties in fish blood are very sensitive to environmental changes (Hughes and Nemcsok 1988). Blood is a pathophysiological indicator of the body as it is highly susceptible to internal and external environmental fluctuations. The present study evaluates the effect of sublethal concentrations of monocrotophos on erythropoietic activity of the freshwater fish *Channa punctatus.*

MATERIALS AND METHODS

Disease-free fish, *Channa punctatus* (10 to 12 cm length and 18 to 20 gm weight) collected from a local resource, were bathed in 0.1% $KMnO_4$ solution and acclimatized under laboratory conditions for 15 days. They were kept in large holding tanks of 1000 liters capacity during the acclimatization period. Water was changed daily and fish were fed dry prawn twice a day.

Technical grade monocrotophos (36% E. C.) manufactured by Devidayal (Sales) Limited, Mumbai, India was used. A stock solution of the pesticide was prepared in acetone by dilution. Prior to exposure the fish were examined carefully for pathological signs.

Bioassays were conducted to determine acute toxicity (LC_{50}) employing the technique as described by APHA (1998). Median tolerance limit (LC_{50}) of monocrotophos to fish was estimated by exposing five groups of fish (6 fish per group) to different concentrations of the pesticide for the period of 96 hours. The recorded data was analyzed for LC_{50} and upper/lower confidence limit using the Trimmed Spearman- Karber method (Hamilton et al. 1977).

Correspondence to: K. Gopal

After acclimatization, fish were transferred to 150-Lglass aquaria containing chlorine free water. The physico-chemical properties of the test water (temperature 25 ± 0.5^{0}C, pH 6.9 ± 0.4, DO 8.5 ± 2.0 mg/L, hardness (as $CaCo_3$) 120 ± 1.9 mg/L, alkalinity (as $CaCo_3$) 360 ± 1.4 mg/L) were studied (APHA 1998). For sublethal exposure to the pesticide, acclimated fish were divided into three groups having eight fish in each. Two groups were exposed to 0.96 and 1.86 mg/L (1/20 and 1/10 of 96 hr LC_{50} = 18.56 mg/L) of monocrotophos for periods of 15 and 60 days. The third group served as the control. The experiment was repeated thrice.

Three fish from each experimental and control group were bled from the dorsal aorta into sterilized glass vials at 4^{0}C containing the anticoagulant 1% dipotassium ethylenediamine tetra acetate (EDTA). The erythrocyte and leukocytes counts were made by Neubauer hemocytometer. Packed cell volume (PCV) was measured by using 75 x 1.0 – 1.25 mm capillary tubes and erythrocyte sedimentation rate (ESR) as mm/hr was measured by the Micro-Wintrobe method of Blaxhall and Daisley (1973). Clotting time (CT) of the blood was determined by the method described by Srivastava (1969) and haemoglobin (g/100 mL) determination was performed by Sahil's hemometer. Mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were also determined (Dacie and Lewis 1977). Statistical analysis of the data was conducted using Student's "t" test.

RESULTS AND DISCUSSION

Monocrotophos induced significant decreases in erythrocyte count, haemoglobin content, PCV, MCH, MCV, MCHC and increases in ESR, leukocytes and CT in fish exposed to both concentrations. Nath and Banerjee (1995) observed significant decreases in erythrocyte count, haemoglobin content, PCV, MCV, MCH and increases in MCHC, leukocyte and ESR in *Heteropneustes fossilis* after 15 days treatment with divithion. The total erythrocyte count showed a decreasing trend with increasing concentration and exposure time to monocrotophos attributed to decreased erythropoietic activity. Gordon et al. (1967) observed that erythropoietic activity in fish is regulated by erythropoietin produced in the kidney. Erythropoietin promotes erythropoiesis by inducing hemopoietic stem cells to differentiate into erythroblasts, which form erythrocytes. Reddy et al. (1992) noticed that erythropoietin also activates pyridoxal phosphate in developing erythrocytes including haemoglobin synthesis. Hypoxia constitutes the fundamental stimulus for erythropoiesis with the kidney as the probable sensing organ for low blood oxygen tensions (Jacobson and Krautz 1968). Thus, conditions conducive to erythropoietin production were present but conversely, *Channa punctatus* showed an inhibition of erythrocyte count suggesting a decrease in erythropoietin activity.

Erythropoietin is a glycoprotein hormone that play a crucial role in ensuring adequate supply of oxygen to tissues by regulating the production of erythrocytes. The production of erythropoietin in the kidney, liver and the central nervous

Table 1. Alterations in haematological parameters of *Channa punctatus* to 0.96 and 1.86 mg/L of monocrotophos for 15 days.

Parameters	Control	0.96 mg/L	1.86 mg/L
Haemoglobin (g/100mL)	11.6 ± 0.02	11.18 ± 0.03	10.45 ± 0.23 [c]
Erythrocytes (10^6/mm^3)	3.15 ± 0.03	3.46 ± 0.02	3.01 ± 0.02
ESR (mm/hr)	5.97 ± 0.04	7.0 ± 0.05	7.33 ± 0.27 [c]
Clotting Time (Sec)	89.12 ± 0.08	97.09 ± 2.9[a]	121.34 ± 6.7 [c]
PCV (%)	32.48 ± 1.4	35.61 ± 0.92	48.29 ± 2.44 [c]
Thrombocyte count (10^3/mm^3)	58.14 ± 2.34	49.37 ± 0.26 [a]	33.09 ± 4.0 [c]
MCH (gm%)	36.83 ± 0.49	32.31 ± 0.05	34.72 ± 0.26 [b]
MCHC (g/dl)	35.71 ± 2.41	31.39 ± 0.80	21.64 ± 1.32 [c]
MCV (μm^3)	103.11 ± 0.42	102.92 ± 1.30	160.43 ± 2.19
Leukocyte count (10^3/mm^3)	15.35 ± 0.06	20.51 ± 0.19	24.19 ± 1.82 [c]
Lymphocytes (%)	62.25 ± 2.23	70.64 ± 0.99 [a]	72.91 ± 0.03 [c]
Eosinophils (%)	1.12 ± 0.06	1.33 ± 0.10	1.91 ± 0.15 [c]
Monocytes (%)	6.51 ± 0.13	5.68 ± 0.16 [b]	5.08 ± 0.21 [d]
Basophils (%)	2.5 ± 0.12	2.63 ± 0.20	2.93 ± 0.04 [a]
Neutrophils (%)	27.30 ± 1.29	20.41 ± 1.37 [a]	19.60 ± 0.16 [d]

Each value represent the mean ± SE of three observations, significance [a]$p< 0.05$ [b]$p<0.02$ [c]$p<0.01$ [d]$p<0.005$

system is greatly induced by hypoxic conditions (Masuda et al. 1994; Marti et al. 1996). Since the kidney of the teleost was found to contain a higher level of the immunoreactive erythropoietin than other tissues, it is suggested that the kidney is the major erythropoietic, as well as erythropoietin-producing, organ (Wickramasinghe 1993). Rahman et al. (2002) noted degeneration of kidney tubules and haematopoietic cells due to decreases in erythrocyte levels in both *Channa punctatus* and *Barbodes gonionotus* after diazinon exposure. It is known that erythrocyte and haemoglobin concentrations often follow a direct and

Table 2. Alterations in haematological parameters of *Channa punctatus* to 0.96 and 1.86 mg/L of monocrotophos for 60 days.

Parameters	Control	0.96 mg/L	1.86 mg/L
Haemoglobin (g/100mL)	11.72 ± 0.26	5.47 ± 0.69 [b]	3.46 ± 0.75 [c]
Erythrocytes (10^6/mm^3)	3.10 ± 0.06	2.50 ± 0.05 [b]	2.01 ± 0.09 [c]
ESR (mm/hr)	6.15 ± 0.16	9.39 ± 0.51 [b]	10.67 ± 0.45 [c]
Clotting Time (Sec)	91.45 ± 1.42	148.38 ± 3.55	156.16 ± 5.28 [c]
PCV (%)	32.73 ± 0.90	36.30 ± 0.04 [a]	15.02 ± 1.40 [c]
Thrombocyte count (10^3/mm^3)	56.62 ± 1.39	21.33 ± 4.6 [b]	13.65 ± 3.9 [c]
MCH (gm%)	37.81 ± 0.23	21.88 ± 2.0 [b]	17.20 ± 2.20 [c]
MCHC (g/dl)	34.92 ± 0.49	15.06 ± 3.12 [b]	23.03 ± 1.22 [c]
MCV (μm^3)	93.03 ± 1.80	145.20 ± 8.05 [b]	74.73 ± 1.0 [c]
Leukocyte count (10^3/mm^3)	15.27 ± 0.15	9.45 ± 1.04 [b]	5.84 ± 0.91 [c]
Lymphocytes (%)	64.62 ± 0.49	41.28 ± 1.02	39.50 ± 2.45 [c]
Eosinophils (%)	1.69 ± 0.05	3.13 ± 0.33 [b]	5.10 ± 0.37 [c]
Monocytes (%)	6.77 ± 0.14	6.61 ± 0.05	8.13 ± 0.04 [c]
Basophils (%)	2.24 ± 0.14	1.47 ± 0.04 [b]	0.78 ± 0.09 [c]
Neutrophils (%)	28.10 ± 0.42	36.15 ± 1.40 [b]	48.26 ± 2.00 [c]

Each value represent the mean ± SE of three observations, significance [a] $p<0.02$ [b] $p<0.005$ [c] $p<0.001$

physiological interrelationship (Chatterjee and Ganguli 1993). However, this study suggests that kidney damage usually causes a decrease in erythropoietin levels, which in turn decreases erythrocyte production and haemoglobin synthesis, even under hypoxic conditions (Table 1 and 2).

A decrease in haemoglobin level and PCV was recorded at both concentrations of monocrotophos after 60 days, while ESR was observed to increase. The reason may be the release of immature cells from haemopoietic tissues into the blood stream. The decrease in PCV in fish exposed to the pesticide was due to decreased erythrocyte numbers, which in turn might be due to monocrotophos exposure. The

decrease in PCV indicates anaemia or oligohanaemia (Wepener et al. 1992). The anaemia associated with erythropenia has also been reported by Srivastava and Mishra (1979) in *Coliso fasiciatus*. The PCV appears to be positively correlated with erythrocyte count. Significant decreases in the number of circulating thromobocytes in a teleost leads to increases in clotting time (CT), loss of haemoglobin content and immature erythrocytes (Table 1 and 2). However, Dacie and Lewis (1977) suggested that increased clotting time exhibited an impairment of kidney and liver, resulting in the loss of blood clotting proteins. Thus, it may be suggested that pesticides trigger a rapid mobilization of the haemopoietic system and fish normally appear to deal with it by adjusting blood clotting time with an abundance of circulating thrombocytes.

A reduction in leukocyte counts (i.e., leukopenia) was observed in *Channa punctatus* after chronic exposure of monocrotophos. Leukopenia after monocrotophos exposure in another freshwater teleost has been reported (Singh et al. 1992; Singh and Srivastava 1994). The observed leukopenia may arise owing to increased activity of the pituitary internal stress axis. Therefore, reduction in the number of circulating leukocytes is a response of the fish to increased levels of circulating ACTH and corticoid stress hormone (Srivastava and Agarwal 1977). But in contrast, the number of leukocytes increased after 15 days exposure to both concentrations. The increase in leukocyte count can be correlated with an increase in antibody production that helps in survival and recovery of the fish exposed to a sublethal concentration of pesticide (Joshi et al. 2002). The present observation elucidates hypersensitivity of leukocytes for monocrotophos. These changes may be due to immunological reactions to produce antibodies to cope with stress induced by monocrotophos (Table 1 and 2).

Differential leukocyte count was also found to be altered in *Channa punctatus* exposed to subacute and chronic concentrations of monocrotophos (Table 1 and 2). Lymphocyte, basophil and eosinophil counts were found to be increased during acute exposure, in contrast monocyte and neutrophil counts were decreased. Increases in the number of lymphocytes, basophils and neutrophils have been reported in freshwater fishes treated with fenvalerate and malathion (Mukhopadhyay and Dehadrai 1980; Sharma and Gupta 1982). Eosinophil, monocyte and neutrophil counts increased while the lymphocyte and basophil counts decreased after 60 days. A rise in neutrophil count escalates phagocytic action. Srivastava and Mishra (1985) observed a marked decline in lymphocytes and monocytes exposed to lindane. Neutrophil count was observed to decrease in *Clarias batrachus* (Thakur and Pandey 1990) exposed to benzenehexachloride (BHC). Fish blood is a pathophysiological indicator of the whole body function and therefore blood parameters are important in diagnosing the structural and functional status of fish exposed to a toxicant (Sampath et al. 1998).

The results of the present investigation reveal that the entire physiology of fish was affected under stress due to monocrotophos exposure. Monocrotophos induced haematological disturbances followed by metabolic disorders in *Channa punctatus,* which ultimately leads to the deterioration of general health of the fish.

Acknowledgements. The authors thank the Director, Industrial Toxicology Research Centre, Lucknow for providing the facilities and constant encouragement. We wish to acknowledge Mr. Sachin Behari Srivastava, Mr. Atul Srivastava and Mr. Kartikeya Shukla for their healthy criticism and support.

REFERENCES

APHA (1998) Standard methods for the examination of water and wastewater. American Public Health Association, Washington, DC

Blaxhall PC, Daisley KW (1973) Routine hematological methods for use with fish blood. J Fish Biol 5: 771-781

Chatterjee S, Ganguli S (1993) Effect of mahua oils cake on the blood of the fish *Clarias batrachus.* Environ Ecol 11: 888-891

Dacie JA, Lewis LM (1977) Practical haematology. J and A Churchill Ltd London

Gordon AS, Goper GN, Zaryani ED (1967) The kidney and erythropoiesis. Sem Haemat 4: 337-343

Hamilton MA, Russo RC, Thruston RV (1977) Trimmed Spearman-Karber method for estimating median lethal concentration toxicity bioassay. Environ Sci Technol 11: 714-719

Hughes GM, Nemcsok J (1988) Effects of low pH alone and combined with copper sulphate on blood parameters of rainbow trout. Environ Pollut 55: 89-95

Jacobson LO, Krautz SB (1968) Summary on erythropoietn. Ann New York Acad Sci 149: 578-583

Joshi P, Deep H, Bose M (2002) Effect of lindane and malathion exposure to certain blood parameters in a freshwater teleost fish *Clarias batrachus.* Pollut Res 21: 55-57

Marti HH, Wenger RH, Rivas LA (1996) Erythropoietin gene expression in human, monkey and murine brain. European J Neurosci 8: 666-676

Masuda S, Okano M, Yamagishi K, Nagao M, Ueda M, Sasaki R (1994) A novel site of erythropoietin production. J Biol Chem 269: 19488-19493

Mukhopadhyay PK, Dehadrai PV (1980) Biochemical changes in the air-breathing catfish *Clarias batrachus* (Linn.) exposed to malathion. Environ Pollut (Ser. A.) 22: 149-158

Nath R, Banerjee V (1995) Sublethal effects of devithion on the haematological parameters in *Heteropneustes fossilis* (Bl.). J Freshwater Biol 7: 261-264

Rahman ZM, Hossain Z, Mollah MFA, Ahmed GU (2002) Effect of diazinon 60 EC on *Anabas testudineus, Channa punctatus* and *Barbodes gonionotus.* International Center for Living Aquatic Resources Management Quart 25: 8-12

Ray PK, Prasad AK, Nandan R (1985) Pesticides: Major environmental problem. Sci Cult 51: 363-371

Reddy DC, Vijayakumari P, Kalarani V, Davies RW (1992) Changes in erythropoietic activity of *Sarotherodon mossambicus* exposed to sublethal concentrations of the herbicide diuron. Bull Environ Contam Toxicol 49: 730-737

Sampath K, James R, Albar Ali KM (1998) Effect of copper and zinc on blood parameters and prediction of their recovery in *Oreochromis mossambicus* (Pisces: Cichlidae). Indian J Fisheries 45: 129-139

Sharma RC, Gupta N (1982) Carbon tetrachloride induced haematological alterations in *Clarias batrachus* (L.). J Environ Biol 3: 127-131

Singh NN, Srivastava AK (1994) Formothion induced haematological changes in the freshwater Indian catfish *Heteropneustes fossilis.* J Ecotoxicol Environ Monit 4: 137-140

Singh NN, Srivastava AK, Srivastava AK (1992) Blood sorasia in the freshwater Indian catfish *Heteropneustes fossilis* after acute exposure to a sublethal concentration of propoxur. Acta Hydrobiol 34: 189-195

Srivastava AK (1969) Studies on the haematology of certain freshwater teleost *Mystus vittatus* thrombocytes and clotting of blood. Analytical Anzizar 123: 233-249

Srivastava AK, Agarwal V (1977) Involvement of pituitary interference axis and cholinergic medenish during the coldshock leukocyte sequence in a freshwater teleost, *Colisa fasciatus.* Arch Anat Mikn Morphol Expn 66: 97-108

Srivastava AK, Mishra S (1979) Blood dyscrasia in a teleost *Colisa fasciatus* following exposure to sublethal concentrations of lead. J Fish Biol 14: 199-203

Srivastava AK, Mishra J (1985) Lindane induced haematological changes in the catfish *Heteropneustes fossilis.* Wat Acad Sci Lett 8: 391-392

Thakur GK, Pandey PK (1990) BHC (Gammaxene) poisoning effect on leukocytes of an air breathing fish *Clarias batrachus* (Linn.). J Environ Biol 11: 105-110

Wepener V, Van Vuren JHJ, Dupreez HH (1992) The effect of hexavalent chromium at different pH values on the haematology of *Tilapia sparrmanii* (Cichloidae). Comp Biochem Physiol 161: 375-381

Wickramasinghe SN (1993) Erythropoietin and the human kidney: Evidence for an evolutionary link from studies of *Salmo gairdneri.* Comp Biochem Physiol 104: 63-65