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Effect of Sublethal Concentrations of Monocrotophos on Erythropoietic Activity and Certain Hematological Parameters of Fish *Anabas testudineus* (Bloch)

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The indiscriminate use of pesticides in agricultural operation adversely affects the aquatic environment to a very great extent. The biological and environment persistence of organochlorine pesticides have led to the extensive use of less persistent, easily biodegradable organophosphates (Ahamed *et al.* 1987; Siddiqui *et al.* 1991). This poses a great danger to freshwater organisms including fishes. Monocrotophos (phosphoric acid dimethyl (1-methyl-3(methylamino)-3-oxo-n-propenyl) ester), commonly known as Azodrin, is one of the organophosphates widely used for the control of agricultural pests in India (Ray *et al.* 1985). In order to determine the subtle, non-lethal effects induced by sublethal concentrations of pesticides on the physiology of fish, it is necessary to monitor certain clinical parameters. The use of hematological methods as indicators of sublethal stress, can provide valuable information concerning the physiological reaction of fish in a changing environment. The physical and chemical properties of fish blood are very sensitive to environmental changes (Huges and Nemcsok 1988). The objective of the present paper was to evaluate the effect of sublethal concentrations of monocrotophos on erythropoietic activity and hematology of *Anabas testudineus* (Bloch).

MATERIALS AND METHODS

Live specimens of *Anabas testudineus* (weighing 11± 2 g) were collected from a swamp located in Pondicherry, India. Fish were acchmatized in the laboratory for 15 days. Bioassays, to determine the 96-hr LC_{50}, were conducted (in Plexiglas aquarium) employing the technique as described by American Public Health Association (1981). Monocrotophos, technical grade (Kycer Agrochemical Ltd., Salem, India) was used in this study. The experiments were performed under natural light and ambient temperature. The quality of the test water (temperature 27.5±1.2°C, dissolved oxygen 6.2 ± 1.00 ppm, alkalinity 230±2.5 mg/L as $CaCo_3$, total hardness 356 ± 3.5 mg/L as $CaCO_3$ and pH 7.4 ± 0.10) was studied as per standard methods (APHA 1981).

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After acclimatization, the fish were divided into three main groups and further into three subgroups having six fish in each. These groups were exposed to 0, 1.9 and 9.5 mg/L (0, 1/10 and % of 96 hr LC_{50} = 19 mg/L) of monocrotophos for a period of 7, 14 and 21 days. Prior to the experiment and during experiment the fish were fed once daily with chopped beef liver *ad libitum.*

Feeding was stopped 24 hr prior to each sampling intervals. Six fish from each experimental and control aquarium were removed at each fixed exposure intervals. Blood samples were taken by direct heart puncture using a hypodermic syringe rinsed with heparin and transferred to individual sterilized glass vials (at 4°C) containing 100 µL 2% EDTA/2 mL blood. Hematological parameters were estimated by standard methods as described by Blaxhall and Daisley (1973). The RBC and WBC counts were made by Neubauer hemocytometer. Hemoglobin (g/100 mL) determination was performed by Sahli's hemometer. The hematocrit (Hct) was determined according to the method described by Snieszko (1960). Mean corpuscular hemoglobin concentration (MCHC) as the Hb in 100 mL blood/Hct X 100 and the mean corpuscular volume (MCV) as the Packed cell volume per liter of blood/RBC (Dacie and Lewis 1963). Statistical analysis was performed using students t-test (Fisher 1989). The significance level was taken as $P< 0.05$.

RESULTS AND DISCUSSION

The results of the present investigation showed various anomalies in the blood of *Anabas testudineus*, during prolonged exposure to monocrotophos (Tables 1 & 2). No significant changes were observed in the measured variables of fish maintained in uncontaminated water (controls). As a result of progressive exposure to 1.9 mg/L and 9.5 mg/L sublethal concentrations ofmonocrotophos for 7-21 days, *A. testudineus* showed significant decreases in the RBC, Hb and Hct (Tables 1 & 2). After 7days, the decrease or increase in the measured blood variables was greater in fish exposed to 9.5 mg/L monocrotophos.

Red blood cell indices such as MCV and MCHC exhibited alterations during prolonged exposure to monocrotophos. Mean cell volume increased significantly at 14 days to 9.5 mg/L and 21 days to 1.9 mg/L and 9.5 mg/L. However, MCHC was reduced significantly from the controls at 21 days to 9.5 mg/L (Tables 1 & 2).

The total RBC count showed a decreasing trend with increasing concentrations and exposure time to monocrotophos were attributed to decreased erythropoietic activity. In most vertebrates, including fishes, erythropoietic activity is regulated by

Table 1. Changes in hematological parameters in *Anabas testudineus*, during prolonged exposure to 1.9 mg/L monocrotphos.

Variable	Control	Exposure (days) 7	14	21
Hemoglobin (g/100ml)	14.53 ± 0.25	13.95 ± 0.18	13.53 ± 0.42	13.18[a] ± 0.39
Erythrocyte (10^6/mm^3)	4.09 ± 0.13	3.92 ± 0.10	3.78 ± 0.27	3.53[a] ± 0.17
Hematocrit (%)	36.12 ± 1.60	35.87 ± 0.62	35.37 ± 2.21	34.50 ± 1.05
MCV (μm^3)	89.24 ± 5.84	93.93 ± 4.75	102.10 ± 4.25	108.42[a] ± 5.75
MCHC (%)	40.03 ± 1.15	38.53 ± 2.10	37.85 ± 1.85	36.53 ± 1.21
WBC (10^4/mm^3)	4.71 ± 0.15	4.93 ± 0.10	5.15 ± 0.19	5.17[b] ± 0.14

Values are mean ± SE. [a]$p < 0.05$ [b]$p < 0.01$

erythropoietin produced in the kidney (Gordon *et al.* 1967). Erythropoietin promotes erythropoiesis by inducing hemopoietic stem cells to differentiate into erythroblasts (which form RBCs). Erythropoietin also activates pyridoxal phosphate in developing RBCs, inducing hemoglobin synthesis (Reddy *et al.* 1992). Hypoxia constitutes the fundamental stimulus for erythropoiesis with the kidney as the probable sensing organ for low blood oxygen tensions (Jacobsen and Krantz 1968).

Table 2. Changes in hematological parameters in *A. testudineus,* during prolonged exposure to 9.5 mg/L monocrotophos.

Variable	Exposure(days) Control	7	14	21
Hemoglobin (g/100ml)	14.53 ± 0.25	13.70 ± 0.26	13.29[b] ± 0.12	12.19[c] ± 0.39
Erythrocyte (10^6/mm^3)	4.09 ± 0.13	3.72 ± 0.22	3.30 ± 0.60	2.92[c] ± 0.13
Hematocrit (%)	36.312 ± 0.60	35.72 ± 0.87	35.12 ± 2.44	33.74[a] ± 0.80
MCV (μm^3)	89.21 ± 5.85	97.62 ± 3.86	108.42[a] ± 3.65	115.42[b] ± 5.12
MCHC (%)	40.03 ± 1.15	38.35 ± 2.10	37.58 ± 1.35	35.12[a] ± 1.28
WBC (10^4/mm^3)	4.71 ± 0.156	5.30 ± 0.201	5.35[b] ± 0.182	5.21[a] ± 0.087

Values are mean ± S.E. [a]P< 0.05 [b]p< 0.01 [c]p< 0.001

Monocrotophos reduces the ventilator-y movements and decreases the oxygen intake by impairing neuromuscular transmission through AchE inhibition (Murphy 1980). Thus, conditions conducive to erythropoietin production were present but conversely, *A. testudineus* showed a decrease in RBC count suggesting a decrease in erythropoietin activity. A structurally intact and normally functioning kidney is essential for erythropoietin production (Gordon *et al.* 1967). Histological observation revealed progressive dystrophic changes in the kidney tubules of *A. testudineus* exposed to monocrotophos (unpublished data). Kidney damages

usually cause a decrease in erythropoietin level, which in turn decreases RBC production and Hb synthesis even under hypoxic conditions (Reddy *et al.* 1992).

The decrease in Hct in fish exposed to pesticide was due to decreased RBC count, which in turn might be due to monocrotophos effects on blood forming organs (Srinivasan and Radhakrishnamurthy 1983). The increase in MCV of fish exposed to moncrotophos was due to beta adrenergic stimulation brought by pesticide-exposed stress conditions (Bulter *et al.* 1978). Thus anemia developed by *A. testudineus* in this study could be regarded as macrocytic anemia.

The probable mechanisms for developing anemia in *A. testudineus* could be due to the loss of erythrocytes as compensatory erythropoiesis could not be observed, which was reflected in the absence of immature erythrocytes in the peripheral blood. In the present study, protracted exposure to 1.9 mg/L and 9.5 mg/L concentrations of monocrotophos caused statistically significant increase ($P<0.01$ after 14 days) in WBC count. WBCs are inextricably involved in the regulation of immunological functions and a prolonged exposure of *A. testudineus* to monocrotophos may inflict immunological deficiency. These findings in *A. testudineus* are in partial agreement with the results of other researchers (Siddique *et al.* 1991; Ikhair-ud-din *et al.* 1996; Figar *et al.* 1995; Reddy *et al.* 1996; Dutta *et al.* 1992). The present study reveals that monocrotophos, an organophosphorus pesticide, has profound effect on the erythropoietic activity and other hematological parameters of fish.

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