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Changes in Erythropoietic Activity of *Sarotherodon mossambicus* Exposed to Sublethal Concentrations of the Herbicide Diuron

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Numerous biochemical indices of stress have been proposed to assess the health of non-target organisms exposed to toxic chemicals in aquatic ecosystems (Niimi 1990). The physical and chemical properties of fish blood are very sensitive to environmental changes (Hughes and Nemcsok 1988) and are commonly used (Wedemeyer and Yasutake 1977). Numerous laboratory studies have reported changes in red blood cells (RBC), hemoglobin (Hb) and packed cell volume (PCV) of fish exposed to pesticides, some reporting significant increases (Koundinya and Ramamurthi 1980; Sastry and Sharma 1981; Rao and Murthy 1983) and some significant decreases (Bansal et al. 1979; Mishra and Srivastava 1982; Srivastava and Mishra 1985). However, the factors responsible for such changes have not been determined.

Herbicides are among the most common contaminants of aquatic ecosystems since they are used both on the riparian vegetation and also directly in the water for aquatic weed control (Simon et al. 1984). Diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea], a phenylurea derivative is frequently used as a pre- and/or post-emergence herbicide (Murphy 1980) and it may secondarily contaminate freshwater ecosystems and non-target organisms. The effects of short-term exposure to relatively high concentrations of diuron on RBC, Hb and PCV of fish have been examined (Komarovski and Popovitch 1971; Braginskii et al. 1972). The objective of this study was to examine the effects of exposure for periods of 7-90 days, simulating periodic or episodic exposure of Sarotherodon (Tilapia) mossambicus (Peters) (Cichlidae) to sublethal concentrations of diuron on RBC, Hb and PCV and to explain how diuron produces changes in erythropoietic activity.

MATERIALS AND METHODS

Male Sarotherodon mossambicus of approximately equal size (10 ± 1 g; 8-12 cm total length) were transferred to large flow through laboratory holding tanks filled with uncontaminated water (Table 1) and provided with ad libitum food (1:1 ground nut cake/rice bran) for at least 7 days. After acclimatization, the fish were divided

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into groups and each group (n=18) exposed for 7, 15, 30, 60 or 90 days to one of two sublethal concentrations (0.22 $mg \cdot L^{-1}$ and 0.55 $mg \cdot L^{-1}$) of diuron (96 h LC50 = 2.2 $mg \cdot L^{-1}$; Vijayakumari 1988) or 0.1 $ml \cdot L^{-1}$ acetone (the dosing vehicle) and another group (n=18) maintained in uncontaminated water (Table 1) for 90 days. The fish mass/water volume ratio never exceeded 1 $g \cdot L^{-1}$. The fish were fed ad libitum during the experiments but starved for 1 day prior to experimental variables being measured.

Table 1. Physical and chemical parameters of uncontaminated water used during acclimatization and exposure of Sarotherodon mossambicus to diuron.

Variable	Range
Light regime	12 light : 12 h dark
Temperature	27 ± 1°C
Dissolved oxygen	7.8 to 8.0 $mg \cdot L^{-1}$
Salinity	0.19 $g \cdot L^{-1}$
Chlorinity	0.11 $g \cdot L^{-1}$
Sodium	128 m $mol \cdot L^{-1}$
Potassium	30.2 m $mol \cdot L^{-1}$
Calcium	4.28 m $mol \cdot L^{-1}$
Carbon dioxide	2.08 $mg \cdot L^{-1}$
Specific conductivity	212 μS
Alkalinity	102.0 $mg \cdot L^{-1}$ (as $CaCO_3$)
Hardness	112 $mg \cdot L^{-1}$ (as $CaCO_3$)

From each group of 18 fish exposed to diuron, acetone or uncontaminated water, six were used to collect blood samples, six to determine kidney oxygen consumption (KOC) and six to determine whole animal oxygen consumption (WOC). 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. The blood was maintained for 2 h at 2-4°C and serum obtained by centrifuging at 3000 rpm for 10 min. The kidneys were quickly dissected from the fish, weighed, transferred to cold fish Ringer and KOC measured using a constant volume respirometer (Umbriet et al. 1972). WOC of individual fish was measured by a modified Winkler's iodometric technique (Mackereth et al. 1978).

RBC, Hb and PCV were measured following Schreck and Moyle (1990); serum glutamic oxaloacetic transaminase (SGOT) and glutamic pyruvic transaminase (SGPT) following the UV-assay of Bergmeyer and Bernt (1974); serum alkaline phosphatase (SALP) following the continuous

assay of Walter and Schutt (1974) and protein following Bradford (1976). Blood water content (BWC) was determined as the difference in weight before and after drying the blood to a constant weight at 100°C. One-way ANOVA was used to analyse the data and significance at $P < 0.05$.

The behavior of each fish in each group was also qualitatively assessed daily. Normal behavior was defined as: swimming well coordinated with normal opercular movements and escape behavior. Observed changes in behavior include: erratic swimming rhythm, reduced opercular movements, mucus secretion and a change in gill color from pink-red to dark-brown.

RESULTS AND DISCUSSION

No significant changes occurred in the measured variables of fish maintained in uncontaminated water (controls) (Tables 2-4) or in fish exposed to 0.1 $ml \cdot L^{-1}$ of acetone for 7-90 days. As a result of progressive exposure to 0.22 $mg \cdot L^{-1}$ and 0.55 $mg \cdot L^{-1}$ sublethal concentrations of diuron for 7-90 days, S. mossambicus showed differential decreases in RBC, Hb, PCV (Table 2), WOC and KOC (Table 3); increases in SGOT, SGPT and SALP activities (Table 4) and no changes in BWC (Table 2). After 15 days except for PCV the decrease or increase in the measured blood variables was greater in fish exposed to 0.55 $mg \cdot L^{-1}$ diuron.

The behavior of fish maintained in uncontaminated water, 0.1 $ml \cdot L^{-1}$ of acetone, and 0.22 $mg \cdot L^{-1}$ of diuron did not change over 90 days and was normal. However, in fish exposed to 0.55 $mg \cdot L^{-1}$ of diuron progressive changes in behavior occurred with uncoordinated swimming, slow opercular movements, a reduction and eventual absence of escape behavior, increases in mucus secretion and a change in gill color from pink-red to dark-brown occurring after 60 day exposure.

The decreases in RBC in fish exposed to 0.55 $mg \cdot L^{-1}$ of diuron (Table 2) were attributed to decreased erythropoietic activity rather than hemodilution because of the absence of significant changes in BWC. Similar decreases in RBC and Hb in fish exposed to pesticides (Bansal et al. 1979; Mishra and Srivastava 1982) have not been adequately explained. In most vertebrates, including fish, erythropoietic activity is regulated by erythropoietin produced in the kidney (Gordon et al. 1967). Erythropoietin, besides promoting erythropoiesis by inducing hemopoietic stem cells to differentiate into erythroblasts which form RBCs, also activates pyridoxal phosphate in developing RBCs inducing Hb synthesis. Hypoxia constitutes the fundamental stimulus for erythropoiesis with the kidney as the probable sensing organ for low blood oxygen tensions (Jacobsen and Krantz 1968).

Diuron, like several other pesticides, impairs neuromuscular transmission through AchE inhibition (Murphy 1980) resulting in a reduction or cessation of respiratory ventilation movements and a

Table 2. Effects of exposure for 7-90 days to low (0.22 mg.L^{-1}) and high (0.55 mg.L^{-1}) sublethal concentrations of diuron on red blood cell (RBC) count (millions.mm^{-1}), hemoglobin (HB) concentration (g/100 ml), packed cell volume (PCV) (vol %) and blood water content (BWC) (% water) of Sarotherodon mossambicus. Each value is mean ± SD of 6 observations. Percentage decreases or increases compared to controls shown in parentheses. * significant at $p<0.05$, NS not significant.

Variable	Conc.	Control	Exposure (days) 7	15	30	60	90	
RBC	Low	2.05 (0.13)	1.81 (0.02)	1.53 (0.04)	1.69 (0.04)	1.73 (0.04)	1.78 (0.03)	NS
			(-11.7%)	(-25.4%)	(-17.6%)	(-15.6%)	(-13.2%)	
	High	2.24 (0.21)	1.91 (0.11)	1.65 (0.11)	1.59 (0.09)	1.49 (0.08)	1.43 (0.12)	*
			(-14.7%)	(-26.3%)	(-29.0%)	(-33.5%)	(-36.2%)	
Hb	Low	8.16 (0.18)	7.12 (0.07)	6.25 (0.09)	6.76 (0.04)	6.88 (0.03)	6.97 (0.02)	NS
			(-12.7%)	(-23.4%)	(-17.2%)	(-15.7%)	(-14.6%)	
	High	7.91 (0.12)	6.15 (0.13)	5.63 (0.12)	5.43 (0.12)	5.32 (0.09)	5.12 (0.10)	*
			(-22.2%)	(-28.8%)	(-31.3%)	(-32.7%)	(-35.3%)	
PCV	Low	32.4 (1.22)	29.18 (0.36)	26.13 (0.21)	26.50 (0.25)	26.88 (0.13)	27.04 (0.10)	NS
			(-10.0%)	(-19.4%)	(-18.2%)	(-17.1%)	(-16.6%)	
	High	30.96 (0.90)	26.82 (0.40)	25.86 (0.19)	24.03 (0.24)	22.94 (0.12)	22.25 (0.28)	*
			(-13.4%)	(-16.5%)	(-22.4%)	(-25.9%)	(-28.1%)	
BWC	Low	82.37 (5.85)	83.93 (7.50)	85.13 (6.45)	84.66 (4.32)	84.01 (5.26)	83.97 (6.27)	NS
			(+1.9%)	(+3.3%)	(+2.8%)	(+2.0%)	(+1.9%)	
	High	83.45 (5.55)	85.67 (6.12)	86.96 (6.22)	87.84 (8.28)	88.78 (6.88)	89.10 (7.84)	NS
			(+2.7%)	(+4.2%)	(+5.3%)	(+6.4%)	(+6.8%)	

decrease in oxygen uptake. Thus, conditions conducive to erythropoietin production occur which might promote erythropoiesis and RBC production. Conversely, S. mossambicus showed a decrease in RBC count suggesting a decrease in erythropoietic activity.

Table 3. Effects of exposure for 7-90 days to low (0.22 $mg.L^{-1}$) and high (0.55 $mg.L^{-1}$) sublethal concentrations of diuron on whole animal oxygen consumption (WOC) ($mlO_2.h^{-1}$) and kidney oxygen consumption (KOC) ($\mu lO_2.g.h^{-1}$) of Sarotherodon mossambicus. Each value is mean ± SD of 6 observations. Percentage decreases compared to controls shown in parentheses. * significant at $p<0.05$, NS not significant.

Variable	Conc.	Control	Exposure (days) 7	15	30	60	90	
WOC	Low	2.17 (0.05)	1.61 (0.17)	1.47 (0.01)	1.32 (0.03)	1.39 (0.02)	1.43 (0.02)	NS
			(25.7%)	(32.1%)	(39.0%)	(35.1%)	(33.9%)	
	High	2.17 (0.02)	1.55 (0.02)	1.35 (0.02)	1.27 (0.01)	1.24 (0.02)	1.22 (0.01)	*
			(28.6%)	(37.9%)	(41.5%)	(42.8%)	(43.8%)	
KOC	Low	21.84 (1.78)	20.26 (1.67)	19.47 (1.83)	20.72 (1.56)	21.07 (1.93)	21.69 (1.72)	NS
			(7.2%)	(10.8%)	(5.1%)	(3.5%)	(0.7%)	
	High	21.65 (1.85)	18.12 (1.45)	16.01 (1.12)	14.53 (1.21)	12.89 (1.10)	10.87 (0.95)	*
			(16.3%)	(26.0%)	(32.9%)	(40.5%)	(49.8%)	

A structurally intact and normally functioning kidney is essential for erythropoietin production (Gordon et al. 1967) but Vijayakumari (1988) observed progressive dystrophic changes in the kidney tubules of S. mossambicus exposed to 0.55 $mg \cdot L^{-1}$ of diuron. Kidney damage usually causes a decrease in erythropoietin levels which in turn decrease RBC production and Hb synthesis even under hypoxic conditions (Table 2). S. mossambicus exposed for 7-90 days to 0.55 $mg \cdot L^{-1}$ of diuron also showed progressive increases in SGOT, SGPT and SALP activities indicating liver damage and alterations in liver function. Liver damage is usually associated with increased erythropoietic activity in vertebrates (Tibor 1981). However, a negative association between liver damage and erythropoietic activity in this study suggests kidney damage and a consequent decrease in erythropoietic activity and thus in RBC and HB.

S. mossambicus exposed to 0.22 $mg \cdot L^{-1}$ of diuron showed initial (15-30 d) decreases in RBC, Hb, PCV, WOC and KOC and increases in SGOT, SGPT and SALP followed by a tendency to return to the control levels suggesting a restoration of erythropoietic activity. This did not occur in fish exposed to 0.55 $mg \cdot L^{-1}$ of diuron indicating a possible loss of adaptive and/or detoxifying capabilities.

In the field non-target species are often exposed to periodic

Table 4. Effects of exposure for 7-90 days to low (0.22 $mg.L^{-1}$) and high (0.55 $mg.L^{-1}$) sublethal concentrations of diuron on serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) ($\mu moles.mg^{-1}protein.h^{-1}$) and serum alkaline phosphatase (SALP) ($\mu moles\ pi.mg\ protein.h^{-1}$) activities of Sarotherodon mossambicus. Each value is mean ± SD of 6 observations. Percentage increases compared to controls shown in parentheses. * significant at $p<0.05$; NS not significant.

Variable	Conc.	Control	Exposure (days) 7	15	30	60	90	
SGOT	Low	2.45 (0.15)	2.64 (0.19)	3.10 (0.25)	2.92 (0.17)	2.77 (0.10)	2.70 (0.15)	NS
			(7.8%)	(26.5%)	(19.2%)	(10.6%)	(10.2%)	
	High	2.34 (0.10)	2.89 (0.08)	3.07 (0.15)	3.19 (0.14)	3.40 (0.08)	3.60 (0.06)	*
			(23.5%)	(31.1%)	(36.3%)	(45.0%)	(53.5%)	
SGPT	Low	1.59 (0.05)	1.82 (0.07)	2.20 (0.06)	2.11 (0.06)	2.0 (0.06)	1.95 (0.05)	NS
			(14.8%)	(38.7%)	(33.2%)	(26.3%)	(23.2%)	
	High	1.42 (0.12)	1.81 (0.10)	2.05 (0.10)	2.30 (0.14)	2.32 (0.14)	2.34 (0.14)	*
			(27.4%)	(44.0%)	(61.3%)	(62.5%)	(63.9%)	
SALP	Low	5.43 (0.32)	6.41 (0.28)	7.39 (0.22)	7.15 (0.23)	6.93 (0.15)	6.68 (0.17)	NS
			(18.0%)	(36.1%)	(31.7%)	(27.6%)	(23.0%)	
	High	6.18 (0.26)	7.63 (0.24)	8.01 (0.21)	8.46 (0.26)	8.94 (0.20)	9.14 (0.23)	*
			(23.5%)	(29.4%)	(36.9%)	(44.7%)	(47.9%)	

immersion in sublethal concentrations of pesticides. These experiments show that exposure for 7-90 days to 25% of the 96 h LC50 (0.55 $mg \cdot L^{-1}$) of diuron significantly decreased erythropoietic activity while exposure to 10% 96 h LC50 (0.22 $mg \cdot L^{-1}$) resulted in initial decreases and recovery. S. mossambicus exposed to 0.55 $mg \cdot L^{-1}$ of diuron also showed behavioral changes.

These results show that higher concentrations (0.55 $mg \cdot L^{-1}$) of diuron decrease erythropoetic activity and thus RBC, Hb and PCV, while lower concentrations (0.22 $mg \cdot L^{-1}$) enhance erythropoetic activity. This may be the general explanation for changes in erythropoietic activity of fish exposed to sublethal concentrations of pesticides.

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