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Haematological Assessment of *Oreochromis Niloticus* (Linn, 1787) Exposed To Acute Toxicity of the Herbicide Propanil

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Abstract: **The acute toxicity of herbicide propanil to juveniles of *Oreochromis niloticus* of mean standard length of 6.23±0.41 cm and 9.20±1.10 gm body weight were investigated under laboratory condition at the concentrations of 10, 15, 20 and 25 uL^{-1}. At the cellular level, acute exposure to the toxicant resulted in significant increase in the leucocyte count when compared with those of the control specimens. The mean Haemoglobin, Haemotocrit and TEC of the exposed fish were lower than values of the control group of fish. Statistical analysis showed that the differences were significant ($p<0.05$). The haemoglobin, haematocrit and TEC values decreased with increased concentration, while total leucocytes count increased with increase in concentration. At Organ level *Oreochromis niloticus* exposed to acute concentrations of herbicide propanil exhibited agitated swimming, loss of equilibrum, air gulping, period of quiescence, and the fish turned on its flank and swarm in circles and finally died In conclusion, acute concentrations of propanil are harmful to *O. niloticus*, a non target organism. It is recommended that manufacturers should be compelled to state categorically the effect of propanil to non target organisms (or aquatic organisms).**

Keywords: **Acute toxicity, propanil, haematology, *Oreochromis niloticus.***

1. INTRODUCTION

Pollution brings undesirable changes in the environment, which affects the biotic composition of the ecosystem. Most of the pollutants are either emitted to the atmosphere through gases which serve as medium, or as discharges to water bodies, or directly through the introduction of the chemical to attack particular organisms such as in pest control programs (Holden, 1977). According to West and Biney (1991), besides habitat loss and over exploitation, pollution ranked third as the main cause of fish species loss, and that there are three main sources of aquatic pollution in Africa, Urban development, industrial waste and the use of pesticides. Akpata (1986) also observed that Pollution and contamination of aquatic environment is increasing in scope and magnitude, and this could be attributed to increasing agricultural practices, such as use of agro- chemicals. Industries, increase in human population, especially in urban centers and the inadequate considerations to environmental impact analysis of the various developmental projects also contribute immensely to pollution. In recent years, serious concern has been voiced about the rapid deteriorating state of freshwater bodies with respect to pesticide (Akpata, 1986). It is recognized that in fresh water systems, pesticides have high pollution potential that could be measured through the use of fish, as fish are prone to effects of contaminated water bodies (Tariq *et al.*, 1996).

Over 5.5 billion litres of pesticides are sold around the world each year which consist of herbicides, insecticides, fungicides and rodenticides, but herbicides are sold in largest quantities followed by insecticides, fungicides and rodenticides (McEwen and Stephenson, 1975). Organophosphorus pesticides (OPs) are the most commonly used pesticides in the world due to their quick degradation (Eto, 1974

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Propanil is a broad spectrum herbicide (Kellogg *et al.*, 2000). (It is biodegradable). According to Manson (1981) it is a selective broad spectrum herbicide used for the control of weeds in rice field. It decomposes within a short period after treatment which allows crops to be grown on the land in future seasons. According to Worthing (1987), it hydrolyses in extremely acidic or basic condition, but it is stable under normal temperature and pressure and may pose a slight fire hazard if exposed to heat, strong oxiders and flame. The thermal decomposition of Propanil will also release toxic oxides of Nitrogen, Carbon and Corrosive fumes of Chlorides. The choice of herbicide Propanil in this study is not out of place based on the assessment above.

Most studies on the effects of pesticides on fish involved acute exposure and the determination of LC_{50} (Mayer and Hamelink, 1988). The determination of the toxicity of any substance in the laboratory serves as a guide to the probable direct effect of the product when it is used on the field. This assessment in the laboratory is mostly in the form of toxicity test to determine the concentration of such compound that would kill 50% of the experimental animal. The results from such bioassay (or test) provide information about the acute or short-term toxicity which can be used to determine the "safe level" of such toxicant (Auta, 2000).

2. MATERIALS AND METHODS

Two hundred Fingerlings of *Oreochromis niloticus* were obtained from Amina Fish Farm, Ltd., Yola, Adamawa State, Nigeria. The *Oreochromis niloticus* averaging 6.23±0.41 cm (total length) and 9.20±1.10 gm body weight were used for this study. The fishes were held in the laboratory in large water baths of 160 l capacity at 24.5-26.5°C and acclimated for two weeks prior to the experiment. The fishes were fed Pfizer pelted diet during acclimation and tests. A daily photoperiod of 16:8 h light:dark was maintained during acclimation and tests. One hundred and twenty fish of the *O. niloticus* were exposed to 10, 15, 20 and 25 uL^{-1}, respectively, in water bowls of 20L.. There were two replicates and a control in each treatment.The water quality was monitored using (APHA, 1981). Ten fish were randomly distributed into each bowl of toxicants for the 96 hours acute toxicity.. At the end of the 96 hours the caudal artery was punctured from the caudal peduncle and blood samples collected by use of heparinized microcapillary and sampling tubes. Blood samples were taken at least 40 seconds after collecting the fish from the water bowl. Haematocrit (Ht) was analyzed using capillary tubes filled with blood and centrifuged at 11,000 r/min for six minutes. The mean values of haematocrit (%) were measured with a microcapillary reader. Haemoglobin (Hb) levels were obtained by means of Boehringer-Mannheim commercial kits, based on colorimetric determinations. Total Erythrocyte Count (TEC) count was performed with a Neubauer count chambers diluting

the blood (200 times) in Toisson's solution. Similarly, Total Leucocyte (TLC) was performed with microscope Neubauer count chamber diluting the blood) in Turk's solution.

Acute toxicity:

Acute 96 h static bioassays was conducted in the laboratory as described by Sprague (1973) and APHA (1985) to determine the toxicity of herbicide propanil to *O. niloticus*. A total of sixteen (16) glass tanks of size 30.5 x 30.5 x 92.5cm, fifteen of them containing nominal concentration of the herbicide propanil and one control (without toxicant) was used for the experiment. Also the physic-chemical parameters of the diluting water (temperature, pH, Dissolved oxygen, total alkalinity, conductivity, etc) during the acute test were measured by methods described by APHA (1985). The desired herbicide propanil concentrations were measured and introduced into 50L of dechlorinated and aerated water in the glass tanks. The mixtures were allowed to stand for 30 minutes before introducing test organisms *O.niloticus*. Thereafter the tanks were stocked at 10 fish per tank for the experimental run.

Water Analysis (Physico-chemical Properties of Water:

Temperature:

Temperature values in Degree Centigrade of water from surface to bottom were measured with the mercury bulb thermometer. This was done by immersing in the test water sample, and the reading was immediately taken after allowing about 2 minutes reaching equilibrium.

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Dissolved Oxygen:

Dissolved oxygen was determined by the use of Digital Dissolved Oxygen Meter.

pH (measure of acidity or alkalinity):

The hydrogen ion concentration (pH) was determined by direct potentionmetry using a pH meter.

Total alkalinity:

Total alkalinity was determined by using standard method (APHA, 1985). 100ml of sample was pipetted into a conical flask and 3 drop of phenolphthalein indicator were added to it. 3 drops of methyl orange indicator was added. As the sample turned yellow, 0.02N sulphuric acid was added from the burette until the colour changed to orange. The quantity of the acid used was then recorded.

Calculation: - The following formula was used.

a). Phenolphthalein alkalinity

$= \frac{B \times N \times 50{,}000}{\text{ml of sample}}$ (as mg/l $CaCo_3$)

Where B = ml of titration for sample to reach methyl orange and point.

N = normality of acid (0.02N).

b). Total alkalinity/acidity of $CaCo_3$

$= \frac{B \times N \times 50{,}000}{\text{Ml of sample}}$ (as mg/l $CaCo_3$)

Behavioural response:

The behavior and general condition of the fish were observed prior to, during and after bioassay. Observations of the behavior were carried out at intervals of 24, 48, 72, and 96 hours.

Respiration:

The parameters under respiration were keenly observed and recorded. These parameters included opercular and tailfin movements.

Hematology:

Two fish were randomly sampled at 24 hours interval during the 96 hours test with a small hand net from each tank and then the fishes were immediately anaesthetized in MS 222 (Tricaine methane sulphonate) for blood collection.

Collection of blood samples:

Blood was sampled as described by Blaxhall and Diasely (1973). Blood was collected by severance (2cm) of the caudal peduncle. Blood was collected with a 5mm syringe.

Determination of pack cell volume:

Pack Cell Volume (PCV) was carried out by micro-westegreen method as described by Blaxhall and Diasely (1973). The blood sampled from the severed caudal peduncle was drawn into micro-haematocrit tube. The tubes were sealed with wax and centrifuged for five minutes. The PCV was measured with the aid of a microhaematocrit reader and expressed as the volume of the erythrocytes per $100cm^3$.

Hemoglobin:

Hemoglobin determination is the quickest method for detecting anemia. The salili-Hellige hemoglobin determination was performed as follows: The sallied pipette was filled slightly above the $20mm^3$ mark, the pipette was wiped with a soft absorbent tissue to remove excess blood and the volume was adjusted to exactly $20mm^3$ by blotting the tip. The blood was expelled into a calibrated (transmission) test tube containing 10.0 milliliters of 0.1N hydrochloric acid, and the pipette was raised several times in the acid solution. The sample was allowed to stand for not less than 3 minutes before reading the value in the colorimeter. The intensity of color was measured at 530 to 540µm and was recorded either as percent transmission.

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Red blood cell count or total erythrocyte count:

The techniques of red blood counts of fish blood are similar in most respects to those used in mammalian counts. However, the diluting fluids normally used for mammalian counts were not applicable to fish bloods. Gorder's and Hayme's diluting solutions were distorted after a few minutes. Refer to appendix II.
The standard RBCC diluting pipette and a 1:200 dilution were used for the red blood cell count. Blood was drawn just beyond the 0.5 mark on the pipette. The tip of the pipette was wiped with a soft absorbent tissue to adjust the volume to exactly the 0.5 mark. The pipette was immediately filled to the 101 mark with Hendricks diluting fluid (Appendix II). Partial rotation of the pipette while being filled assured the complete mixing of the blood and diluting fluid, and prevented clotting. With its ends griped between the thumb and second finger, the pipette was then shaken for 30 to 60 seconds. After the pipette had been shaken, a few drops of the diluted blood were expelled from it. Control of over flow of fluid was maintained by replacing the index finger over the bulb end of the pipette. The haemocytometer (counting chamber) was a Neubauer, the pipette was held to the edge between the cover slip and the chamber, and capillary action drew the diluted suspension of cells into the chamber. The haemocytometer was then placed under the light microscope, and the cells were counted. The haemocytometer is divided into ruled areas $1mm^2$, with the centre square millimeter divided into 25 groups of 16 small squares. The cells within the boundaries of five of these small squares were counted. Each corner plus the center group were counted when the red blood cell count was computed, the number of cell counted in all five squares was multiplied by 10^6, this gave the total number of cells per cubic millimeter (mm^3) of blood (Hesser, 1960).

Total leucocytes count:

Shaw's solutions A and B allowed differentiation between leucocytes, erythrocytes and thrombocytes. Both solutions were filtered just prior to use. Solution A was made fresh each day, solution B was stable for several days. Leucocytes were counted using Shaw's solution A and B. The blood was drawn up to the 0.5 mark, solution A was added to fill the bulb of the pipette approximately half filled, and mixed. Then, the pipette was removed from solution A and filled to the mark 101 with solution B. The pipette was then shaken as in the erythrocyte count. A few drops were expelled and the haemocytometer was filled in the manner described previously. For comparison of the total number of leucocytes, the cells in the four large squares noted by the large cycle were counted. The total number of cells counted multiplied by 500, determined the total number of leucocytes per cubic millimeter (mm^3) of blood (Hesser, 1960).

Analysis of the blood sample:

Blood samples were analysed immediately after collection. The parameters analysed for were the Pack Cell Volume (PCV) also referred to as Haematocrit, Hemoglobin, Red Blood Cell (RBC),White Blood Cell (WBC). The values for Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were calculated according to the formulae below.

$$MCV\ (um3) = \frac{Ht}{RBC\ (Cells\ mm^{-}3)} x10$$

$$MCH\ (pg\ cell^{-l}) = \frac{Hb(g100ml)^{l}}{RBC\ (cells\ mm^{-}3} \times 10$$

$$MCHC\ (g\ 100ml^{-l}) = \frac{Hb\ (g\ 100ml)}{Ht\ (\%)} \times 10$$

Mortality of the juveniles:

Observations to determine the mortality of *O. niloticus* were carried out at 24, 48, 72, and 96 hours.

Statistical analysis:

Analysis of variance (ANOVA) and Duncan multiple range tests were employed to test for differences between treatments (CRD). Correlation coefficient (r) were used to determine the relationship between the various parameters. Regression coefficient between the probit kill and log concentration of the toxicant were determined after the acute toxicity bioassay.

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3. RESULTS

Behavioral Responses:

The behavior and general condition of the fish was observed prior to, during and after the bioassay. Observation of behavior was carried out at intervals of 24, 48, 72 and 96 hours.

The behavioral reactions in order of their appearance were agitated swimming, loss of equilibrium, air gulping, periods of quiescence and death. Hyperactivity was the most sensitive response of the toxicant effect on fish. Blood was observed around the gill coverings of the dead fishes. These reactions were more pronounced in tanks containing higher level of toxicants. Also, copious accumulation of mucus was observed on the gill filaments and body surfaces of the fish. However, the intensity of mucus production was pronounced in which case the body become sloughed off.

As can be seen in Table 1, 1-6 represents different behavioral reactions in order of their appearances. Agitated movement occurs across all the concentrations from 10uL^{-1} to 25.00uL^{-1}. Loss of equilibrium is dose dependent as it is the next behavioral reaction after Agitated movement. It occurred from 15.00 to 25.00uL^{-1}.

Table 1: Behavioral Responses to different doses of propanil to *O. niloticus*

Behavioral Responses	Doses of Propanil in uL^{-1}				
	0.00	10.00	15.00	20.00	25.00
Agitated movement	**1**	**1**	**1**	**1**	
Loss of equilibrium		**2**	**2**	**2**	
Air gulping			**3**	**3**	
Period of quiescence			**4**	**4**	
Death			**5**	**5**	
Copious accumulation of mucus and blood on gill filaments				**6**	

1-6 = Behavioral responses at different doses of propanil to *O. niloticus*

Physico-Chemical Parameters of Test Water Monitored during the Experiment:

The Physico-chemical parameters of the test water monitored during the tests are given in Table 2. The Physico chemical parameters of the test water fluctuated only slightly during the bioassays, but were not thought to have affected fish mortality.

Table 2. Physico-chemical parameters of test water monitored during the experiment for herbicide Propanil on *Oreochromis niloticus*

Conc.(uL/l)	Temp	Dissolved oxygen	pH	Conductivity	Alkalinity (mg/l) CaCo3
0.00	27.75	10.00	6.88	0.14	34
10.00	26.20	9.80	6.60	0.15	34
15.00	26.10	9.60	6.71	0.16	36
20.00	25.80	8.80	7.50	0.30	36
25.00	25.50	8.60	7.52	0.32	38

Respiratory Rates:

In Table 3 below, Opercular and Tail fin beats per minute were highest at 0 hrs in the specimens exposed to the propanil. These values were dose-dependent. In *O.niloticus* exposed to various concentration of Propanil, the values at 25.00uL^{-1} were greater than 20.00uL^{-1} > 15.00uL^{-1} > 10.00uL^{-1}. Tthe values decreased with duration of exposures in different toxicants as seen at 24, 48, 72 and 96 hours. At 72 and 96 hours the values of the tail fin beats of *O. niloticus* to the toxicants were lower than that of the 0.00uL^{-1}.

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Table: 3. Mean Opercular and Tail Fin Beat of *Oreochromis niloticus* exposed to acute level of propanil

Parameters	Exposure Periods (Hours)				
	0	24	48	72	96
Opercular Beat	67.33[a]	63.33[b]	61.67[c]	52.67[d]	53.33[d]
Tail Fin Beat	64.67[a]	62.67[b]	60.66[c]	60.67[d]	61.00[d]

Means with the same superscript are not significantly different.(P>0.05)''

Mortality:

The result of toxicity test showing mean mortality of each fish at various concentrations of Propanil are presented in Table 4. The first mortality of 3 number specimen occurred at the lowest concentration of 10.00uL^{-1} while at the highest concentration of 25.00uL^{-1}, 8 No. Mortality was recorded.

Table 4: Mortality of *O. niloticus* juveniles exposed to acute concentration of propanil 96 Hours

	Conc./uL^{-1}				
	0.00	10.00	15.00	20.00	25.00
Exposure period (hrs)	R_1R_2	R_1R_2	R_1R_2	R_1R_2	R_1R_2
24 Hours	- -	- 3	2 2	1 2	2 1
48 Hours	- -	- -	1 -	3 3	3 6
72 Hours	- -	- 2	2 2	3 -	2 1
96 Hours	- -	3 -	1 2	2 2	- 1
Mortality	$^0/_{10}$ $^0/_{10}$	$^3/_{10}$ $^5/_{10}$	$^6/_{10}$ $^6/_{10}$	$^9/_{10}$ $^7/_{10}$	$^9/_{10}$ $^8/_{10}$
Mean Mortality (%)	0	4	6	8	9

Haematological Parameters:

The result of *O. niloticus* to acute concentrations of herbicide propanil is summarized in Tables 5 which provide the comparative data on the estimated blood parameters for each group of fish. The blood indexes in each treatment varied significantly and were dose – dependent.

Table 5: Effects of different concentration of propanil on haematological parameters of *O. niloticus*

Concentration (mgl^{-1})	Parameters			
	Hb	Haematocrit (%)	Total Erythrocyte count (TEC) x 10^6mm^3	Total leucocytes count (TLC) x 500mm^3
0.00	15.00[a]±2.34	48.00 ± 1.72[a]	285.00 ± 2.21[a]	3,746.00± 80.01[a]
10.00	13.00[b]±1.05	38.00 ± 2.56[b]	280.00 ± 1.52[b]	4,584.00±70.02[b]
15.00	11.30[c]±2.25	35.23 ± 1.33[c]	276.00 ± 2.80[c]	5,028.00±30.09[c]
20.00	9.00[d]±2.35	28.00 ± 2.51[d]	273.00 ± 1.50[d]	6,000.00±25.15[d]
25.00	8.30[ed]±1.13	25.00 ± 1.15[e]	271.00 ± 4.60[d]	6,850.00±28.00[e]

Values with same superscript in the same column are not significantly different at p>0.05

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4. DISCUSSION

Oreochromis niloticus exposed to acute concentrations of herbicide propanil exhibited agitated swimming, loss of equilibrum, air gulping, period of quiescence, and the fish turned on its flank and swarm in circles and finally died. Hyper activities were the most common responses effects on *O niloticus* and were dose dependent. Such activity was also reported by Matsumura (1975) to be the primary and principal sign of nervous system failure due to pesticide poisoning which affects physiological and biochemical activities in non target organisms. Pal and Koner (1987) opined that disruption of the functioning of the nervous system of fish might be the cause of slow and agitated swimming, erratic movement and loss of equilibrum.

Accumulation of mucus also was observed on the gill filaments and body surface of the dead fish after their exposure to the lethal concentration of Propanil. Hossain *et al.* (1987) stated that increase in production of mucus over the body as a result of toxicant may interfere with the gaseous exchange, secretion and waste products and osmoregulation. Similar observations were made by Shafiel and Costa (1990); Babatunde (1997) and Auta (2001), who studied the effects of pesticides on different species of fish. The accumulation of mucus may result from an increase in the activity of mucus cells subsequent to exposure to pesticides. This resulted in increase in the production of mucus over the body of the fish. It seems that the solution of the pesticides tend to precipitate or coagulate muco protein on the gill epithelium. This may interfere with the gaseous exchange, secretion of waste products and osmoregulation. The toxic action of the toxicants appeared combined effects of precipitation of mucus on the gills and osmoregulatory stress with death resulting from suffocation. Blood was also observed around the gill coverings of the dead fishes. This suggests that the fishes might have suffered from gill haemorrhage. Similar findings were reported by Shafiel and Costa (1990) when fry and fingerlings of *Oreochromis mossambicus* (Peters) were exposed to the following pesticides, Ronstar, Elsan, Endosulfan, Basfapon, Rogor 40 (dimethoate) and Azodrin 60.

The results of the opercular and tail fin beats also showed that increase in time of exposure resulted in decrease of opercular and tail fin beats in *O. niloticus*, this suggests decreased oxygen consumption and reduced energy. The reduction of respiratory rate implies that the fish had become fatigued due to several attempts to escape from the toxic medium to facilitate more oxygen intake. These behavioural patterns are indicative of respiratory impairment, due to the effect of the toxicant on the gills and general metabolism. (Chindah *et al.,* 2004)

The examination of haematological parameters in *O.niloticus* indicated that the herbicide propanil at acute levels elicited response which involved a decrease in the percentages of Packed Cells Volume (PCV), Haemaglobin, Red Blood Cells (RBC), Neutrophils, Monocytes and Lymphocytes, indicating severe anaemia in the exposed fish as seen in Tables 5 below. The anaemic effect could be due to destruction of or inhibition in erythrocyte production. Eisler (1967) reported that erythropenia (deficiency in the number of red blood cells) in fish exposed to methoxychlor and methylparathion had low haemoglobin and haematocrit content value as well as low erythrocyte sedimentation rate (ESR). Similarly, anaemia associated with erythropenia was reported by Srivastava and Mishra (1979) in *Colisa fasciatus* after acute exposure to lead. Leucocytosis was evidenced by the increase in Total Leucocytes Count (TLC) with increased concentration of the herbicide Propanil.

In the case of mortality, the acute toxicity of the herbicide Propanil to *O. niloticus* showed that Juvenile *O. niloticus* was sensitive to propanil. The results of this study clearly showed that the mortality of this freshwater fish species were dose dependant. These findings are in line with that of Tripathi, (1992) and Alam and Maughan (1993). Time of mortality for *O. niloticus* exposed to acute concentrations of Propanil varied.Similar observations were made by Lloyd (1992). Several workers have reported that fish have a broad range of sensitivities to pesticides toxicity (Ufodike and Omoregie, 1990; Tripathi, 1992; and Alam and Maughan, 1993).

5. RECOMMENDATIONS

a. It is recommended that attempts should be made to monitor and control the usage of Propanil.
b. Manufacturing industries should look into ways of reducing the potency of Propanil to non-target organisms such as fish, while maintaining its effectiveness as rice herbicide.
c. Manufacturers should also be compelled to state categorically the effect of Propanil and other chemicals, to non-targeted (or aquatic organisms).
d. Proper education of farmers on the danger of Propanil to the environment is urgently required.

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