

## EFFECTS OF ACUTE AND SUB-LETHAL CONCENTRATIONS OF PHOSTOXIN ON WEIGHT CHANGES AND HAEMATOLOGY PARAMETERS OF *Clarias Gariepinus*

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### ABSTRACT

Fish are sensitive to a wide variety of chemicals and toxic conditions. In aquatic environment, organophosphate may cause several physiological and biochemical defects in fishes. *Clarias gariepinus* (mean weight  $10\pm 0.1g$ ) was exposed to acute and sub-lethal concentrations of phostoxin to evaluate the toxicity of phostoxin organophosphate. Effect of phostoxin on haematological parameters and weight changes of juvenile *Clarias gariepinus* was also investigated. The concentrations used during the acute toxicity test were 1.00, 2.0, 4.00, 6.00 mg/l with a control of 0.00mg/l; while the concentrations of the toxicant used during the 28-days sub-lethal exposure were 0.60, 0.40, 0.20 mg/l with a control of 0.00 mg/l. The lethal concentration (LC50) value of phostoxin was 2.20mg/l for 96h of exposure. The results obtained from the sub-lethal exposure showed that there was a progressive decrease in weight gain of fish as the concentration of the toxicant increased. Also, haematological indices indicated that the fish became hyperglycaemic and hypoproteinaemic and the severity of this condition were directly proportional to the phostoxin concentration. *C.gariepinus* is susceptible to phostoxin; therefore use of phostoxin in disinfection on/near fish farm or area close to aquatic environment should be discouraged.

**Keywords:** *Clarias gariepinus*, phostoxin, Toxicity test, Weight, Haematology

### INTRODUCTION

The effect of agricultural chemicals use and their residue on non- target organism have not been seriously considered in Nigeria (Ayoola, 2007). The indiscriminate use of pesticides, careless handling, accidental spillage, or discharges of untreated effluents into natural waterways have harmful effects on fish population and other forms of aquatic life and may contribute long term effects in the environment (Akhtar, 1986). Water pollution by pesticides is a serious problem to all aquatic fauna and flora and to a considerable extent man (Ayoola, 2008). In aquatic environment, pesticides may also cause several physiological and biochemical defects in fishes (Vasanthi *et al.*, 1989).

*Clarias* species is a widely distributed fish in Asia and Africa. In these areas, the fish is extremely popular on account of its tasty flesh, its unparalleled hardness, its rapid growth and high market price (FAO, 2003). African catfish (*C.gariepinus*) is the most cultured fish in Nigeria (Omitoyin,2004). *C.gariepinus* tolerates both well and poorly oxygenated waters and is widely cultivated and thus used as a biological indicator in ecotoxicology studies (Ayoola, 2008).It is also common preliminary practice in fish culture operations to use synthetic toxins including chlorinated hydrocarbons and organophosphates to eradicate predators and competing fish from nursery, rearing and production ponds prior to the stocking of preferred commercial fish species. However, the

application of synthetic toxins and organophosphates is not advisable due to their toxicity to other non-target aquatic species, even to the consumers of the fish and persistence in the environment (Marrs, 1993; Dementi, 1994; Davies, 1995).

Acute and sub-lethal bio-assays are carried out to check the short and long term effects of these organophosphates on animal life forms as regards blood biochemistry and weight gain (Sampath *et al.*, 1993). Among this organophosphate family is Phostoxin. Phostoxin, metal and phosphide fumigants are acted upon by atmospheric moisture to produce phosphine gas. Phostoxin tablets and pellets contain aluminum phosphide (AIP) as the active ingredient and will liberate phosphine .Mild inhalation exposure causes malaise (indefinite feeling of sickness), fatigue, nausea, and respiratory distress in man. Moderate poisoning causes weakness, vomiting, and pain just above the stomach, chest pain, diarrhoea and dyspnoea (difficulty in breathing) (ATSDR, 2000). Symptoms of severe poisoning may occur within a few hours to several days, resulting in pulmonary oedema (fluid in lungs) and may lead to dizziness, unconsciousness and death (ATSDR, 2000).There has been no report on the study of phostoxin effects on *Clarias gariepinus* which is very popular because of the price it commands. This study therefore investigates some haematological parameters, the acute and sub-lethal effects of phostoxin on *C.gariepinus*.

## MATERIALS AND METHODS

Five hundred (500) Juveniles (average weight  $10.0 \pm 1.0$ g) of pure *Clarias gariepinus* were procured from a reputable farm in Lagos state, Nigeria. The juvenile stages were used because they are more sensitive than adult in toxicity tests (Solbe, 1995). They were transported in plastic container, well aerated can to the Department of Marine Sciences, University of Lagos. The fish were acclimatized in laboratory conditions for two weeks during which they were fed with commercial floating pellets at 5% of body weight. Unconsumed feed and faeces were removed and water replenished regularly as recommended by Oyelese and Faturoti (1995).

A total of 24 glass aquaria were used for the entire experiment. The acclimatized juveniles were selected randomly and ten fish were placed in each of the aquaria containing 10 litres of water which was covered with nets to prevent the animals from jumping out and escaping. Dechlorinated tap water of physico-chemical characteristics; temperature, pH and Dissolved oxygen were used and they were monitored daily using the standard method of Boyd (1981) and APHA (1998).

A stock solution was prepared by taking 1ml of photoxin and mixing it with 19mls of distilled water. Pre-determined amounts of photoxin solution were measured out using 2ml Hypodermic syringe into aquaria containing 10 litres of water. The chemical was applied at the centre of the container to ensure even distribution within the test media.

### Acute toxicity tests

For each treatment, there were three replicates and a control. Ten fish were put into

$$\% \text{Weight change of fish} = \frac{\text{Average Final wt} - \text{Average Initial wt}}{\text{Average Initial wt}} \times 100$$

### Blood collection and Analysis

Haematological tests were conducted at the end of the 28 days experiment. Blood was collected from the fishes by cardiac puncture with the aid of a Hypodermic needle and syringe (2ml), put into EDTA vials and taken to laboratory for analysis using method described by Masson *et al.*, (2002). The haematological parameters analysed in this experiment were Glucose, Total proteins and Albumin. Glucose was measured in the laboratory using an electronic blood glucose meter. A relatively small drop of blood from each sample was placed on a disposable test strip which interfaces with a digital meter. Within several seconds, the level of blood glucose was shown on the digital display.

each small glass aquaria for the acute bio-assay test which lasted for 96 hours. The Phostoxin toxicant was applied at levels of 1.00, 2.00, 4.00 and 6.00 ml/l with a control of 0.00mg/l after the range finding test. During the exposure period, dead fish observed was removed and recorded. The lethal concentration that caused 50% mortality (96-h  $LC_{50}$ ) was estimated by probit analysis as described by (Wardlaw, 1985).

### Sub-lethal tests

From the  $LC_{50}$  of the acute tests, the sub-lethal tests were conducted. The toxicant was applied at lower levels of 0.60, 0.40, 0.20ml/l and a control of 0.00ml/l. The test chemical and test media were changed every 24hours and the experiment lasted for 28 days. The fishes were fed 3% of their body weight with 2mm Coppens feed twice everyday throughout the duration of the sub-lethal toxicity tests. Water quality parameters (Temperature, pH, and Dissolved Oxygen) of the test solution were monitored throughout the duration of the experiment.

### Weight Measurement

The weight of the test animals in treated and untreated (control) test media were recorded at the commencement and termination (after 28 days) of the sub-lethal test. Weight changes in the fishes were carried out at 7 day intervals of the experiments so as to reduce the introduction of handling stress in the test animals. This was done with the aid of a battery operated (Camry EK5055 Max. 5kg/11lb d=1g/0.05oz) weighing scale. Percentage weight gain of the fishes was calculated using the formula:

Total protein was measured in the laboratory using the Colorimetric Biuret method as described by Gornall *et al.*, (1970). Albumin was measured in the laboratory using the Colorimetric Bromocresol Green method (BCG). In buffered solution at pH=4.2, albumin binds with Bromocresol Green (BCG) to produce a blue-green complex. The change in absorbance at 628nm (618-638) correlated with the concentration of albumin in the specimen. The lethal concentration ( $LC_{50}$ ) at 96h was computed using the probit and logit analyses.

## RESULTS

The mean physico-chemical parameters of the test concentration (photoxin) on *C.gariepinus* are presented in table 1. The 96-h

LC<sub>50</sub> determined by probit analysis for the phostoxin organophosphate was estimated to be 2.20mg/l. (Table 2 and Fig 1). The exposure of the African catfish, *Clarias gariepinus* to sub-lethal concentrations of phostoxin organophosphate was found to result in weight decrease of about -25g to -28.4g during the commencement and termination of the experiment (Figs 2 & 3). Statistical comparisons of observed weight changes showed that there was significant ( $p < 0.05$ ) difference in the weight of animals exposed to sub-lethal concentrations of phostoxin compared to the control animals which had a weight gain of about +42.6g. The weight loss of the exposed animals increased as the concentration increased (Tables 3 & 4).

Glucose concentrations in blood of the *Clarias gariepinus* exposed to different sub-lethal concentrations of phostoxin showed that there was a decrease in the Glucose concentrations in the animals compared to the controls (Tables 5 and fig 4). The Glucose concentration in the exposed animals was 68.33mg/l as compared to the controls which had a mean Glucose concentration of 88mg/l. The Glucose concentration in animals exposed to sub-lethal concentrations of Actellic was significantly different ( $p > 0.5$ ) from the concentrations in control animals.

The mean total protein concentration in the exposed animals to phostoxin was 32.8g/l as compared to the controls which had a value of 23.7g/l. The increase could be attributed to the toxicity of the phostoxin organophosphate. The total protein concentration in animals exposed to sub-lethal concentrations of Actellic was significantly different ( $P > 0.5$ ) from the concentrations in control animals.

Albumin concentrations in blood of the *Clarias gariepinus* exposed to different sub-lethal concentrations of phostoxin showed that there was a slight significant increase in the concentrations in the exposed animals compared to the controls. The mean albumin concentration in the exposed animals was 8.33g/l as compared to the controls which had a concentration of 6.20g/l. The albumin concentration in animals exposed to sub-lethal concentrations of Actellic was not significantly different ( $p > 0.5$ ) from the concentrations in control animals.

## DISCUSSION

Grillitsch *et al.*, (1999) reported that organisms exhibit behavioural responses to chemical stress both at acute and sub-lethal toxicity. This elicits the potency and sensitivity of the fish – *Clarias gariepinus* to the test chemical. Similar findings were also recorded in

this study. The result of haematological parameters showed marked reduction between control fish and the fish exposed to different concentrations of Phostoxin, which is an indication of the deleterious effects of the chemical pollutant to the body fluid of *Clarias gariepinus*. In this study, the acute toxicity level of phostoxin organophosphate against *Clarias gariepinus* was found to be 2.50mg/l based on the 96hr LC<sub>50</sub> value. The exposure of the Catfish to sub-lethal concentrations of phostoxin organophosphate resulted in the fish becoming hyperglycaemic and hypoproteinaemic.

The haematological parameters showed marked reduction between control fish and the fish exposed to different concentrations of phostoxin which is an indication of the deleterious effects of the chemical pollutant to the body fluid of *Clarias gariepinus*. Omoregie *et al.*, (1990) reported that organophosphates and pollutants have significant effects, which can result in several physiological dysfunctions in fish.

Mucus was observed to have accumulated on the gills of the fish, which might be responsible for the mortalities recorded in this study. Ayoola (2007) reported that accumulation of mucus on the gills reduces respiratory activity in fish. The inability of the gill surface to actively carry out gaseous exchange might be responsible for the recorded mortalities. The observed restlessness and jumping of fish in bio-assay media might be due to the effect of the active ingredients pirimiphos-methyl in the phostoxin organophosphate. Solbe (1995) reported that these active ingredients could bind on to acetylcholine receptors in the nervous system thus causing the excitation and the resultant frequent jumping and restlessness.

During the sub-lethal exposure, a decrease in weight by the fish with increase in concentration was observed. This observation is similar to earlier report of Omoregie *et al.*, (1990) who reported that sub-lethal concentrations of toxicants often result in several physiological dysfunctions instead of an outright mortality of fish. The retardation of growth might be due to interactions of the pirimiphos-methyl with normal metabolism of the fish and to the under utilisation of the feeds due to the toxicity of the test chemicals. Exposed test species recorded a slight increase in weight. This finding also agrees with the reports of Onusiriuka and Ufodike (1998) on *Clarias gariepinus* and Omoregie *et al.*, (1998) on *Oreochromis niloticus*. The water quality parameters in the different treatments showed no significant difference hence the effects on this study could be negligible.

The statistically significant ( $p < 0.05$ ) decrease and increase in values of the glucose haematological parameters (Table 4) and significant ( $p < 0.05$ ) increase in total proteins and albumin studied was not uncommon in fish exposed to sub-lethal concentrations of toxicants. Similar reduction of haematological indices was reported by Musa and Omoregie (1999) when *Clarias gariepinus* was treated with sub-lethal doses of malachite green. Omoregie *et al.*, (1994) had earlier reported similar observations when they subjected *Oreochromis niloticus* to sub-lethal concentrations of formalin. The reduction and increase in these blood parameters is an indication of hyperglycaemia, hypoproteinemia and caused by exposure to the phostoxin concentrations.

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**Table 1: Mean physico-chemical parameters of the test concentrations (phostoxin) on *C. gariepinus* for 96-h period**

Concentration (mg/l)	Physico- chemical parameters		
	Do <sub>2</sub> (mg/l)	pH	Temp °C
0.00	5.4 ± 0.1 <sup>e</sup>	7.5 ± 0.1 <sup>b</sup>	27.0 ± 0.5 <sup>a</sup>
0.5	5.2 ± 0.4 <sup>b</sup>	7.0 ± 0.2 <sup>b</sup>	27.0 ± 1.3 <sup>b</sup>
1.0	5.1 ± 0.3 <sup>c</sup>	6.9 ± 0.3 <sup>a</sup>	26.9 ± 1.6 <sup>b</sup>
1.5	5.0 ± 0.1 <sup>d</sup>	6.9 ± 0.1 <sup>a</sup>	27.0 ± 0.2 <sup>b</sup>
2.0	5.0 ± 0.4 <sup>d</sup>	6.9 ± 0.2 <sup>a</sup>	27.2 ± 1.6 <sup>b</sup>

\*Mean values followed by the superscript in each column are not significantly different (p<0.05)

**Table 2: Probit analysis of Phostoxin**

C (= dose)	Log C	Affected (%)	Probit Responses
1.0	0	0	4.05
2.0	0.3	33.3	4.56
4.0	0.6	66.7	5.44
6.0	0.8	83.33	5.95
Control	0	0	0

**Table 3: Weight changes (g) of Phostoxin on *Clarias gariepinus* for 28 days.**

Concentration (ml/l)	Day 0	Day 7	Day 14	Day 21	Day 28
Control	20.33	23.00	25.00	27.33	29.00
0.2P	20.00	21.00	18.00	16.67	15.00
0.4P	19.67	19.33	17.67	16.33	14.67
0.6P	20.00	19.67	19.33	17.67	14.33

**Table 4: Percentage weight changes (%) of Phostoxin on *Clarias gariepinus*.**

Concentration Tested (ml/l)	Mean Initial weight (g)	Mean Final weight (g)	Percentage Weight changes (%)
Control	20.33	29.00	+42.60
0.2P	20.00	15.00	-25.00
0.4P	19.67	14.67	-25.00
0.6P	20.00	14.33	-28.40

**Table 5: Haematological parameters of Phostoxin on *Clarias gariepinus*.**

Blood Parameters	Control	0.2P	0.4P	0.6P
Glucose (mg/L)	88	61	97	47
Total Protein (g/L)	23.7	33	34.4	31
Albumin (g/L)	6.2	7.2	9.7	8.1

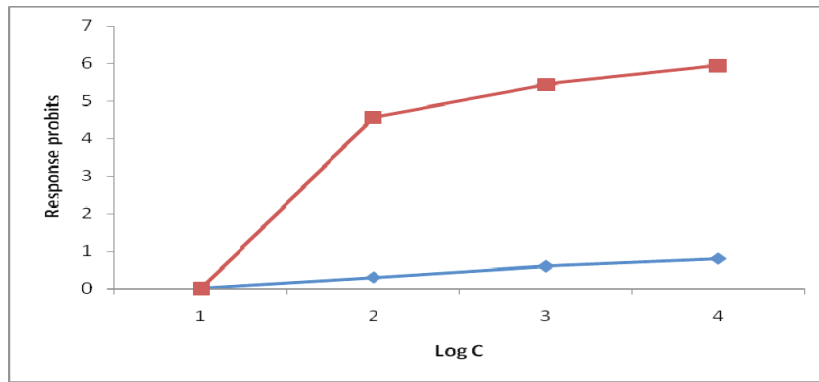


Figure1: An experimental example of a linear relationship between probit response and log-dose of Phostoxin

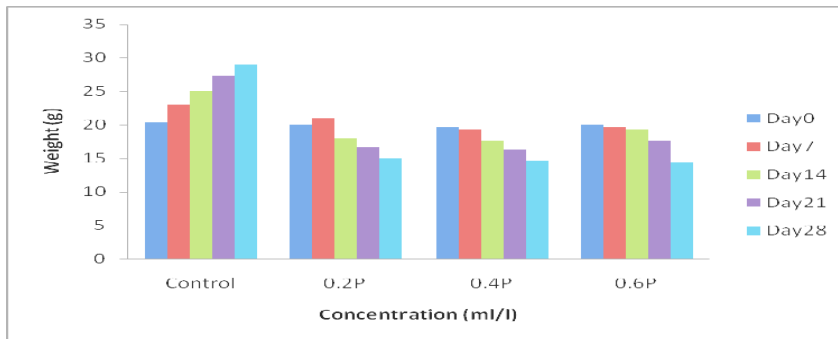


Figure2: Chart showing weight changes (g) of Phostoxin on *Clarias gariepinus* for 28days.

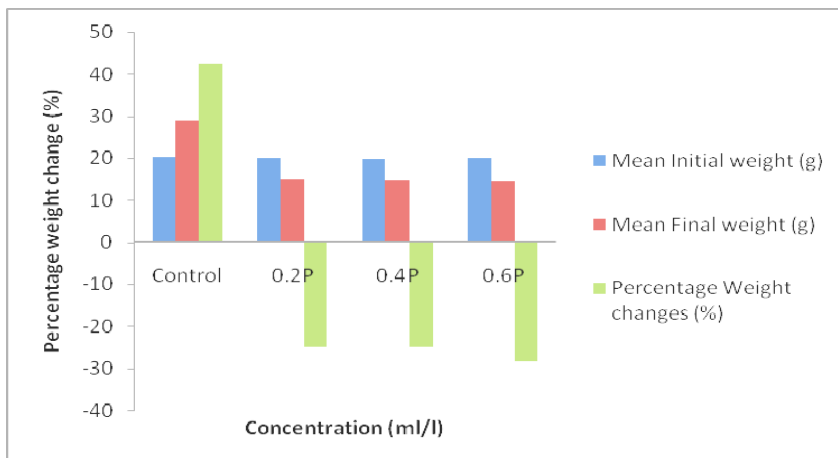


Figure 3: Chart showing percentage weight changes (%) of Phostoxin on *Clarias gariepinus*.

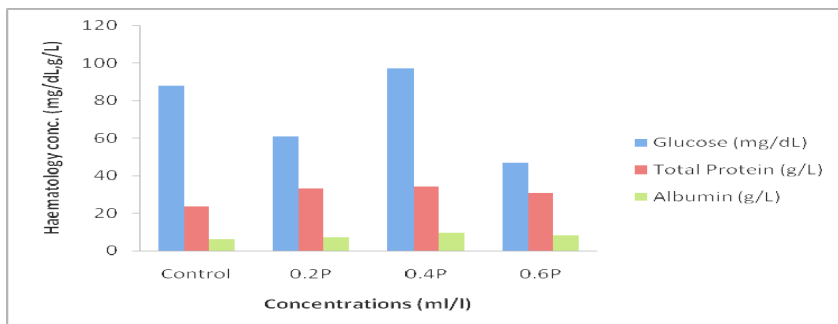


Figure4: Chart showing Haematology of Phostoxin on *Clarias gariepinus*.