

# Histopathological Effects of Acutely Toxic Levels of Dizensate (glyphosate herbicide) on Gill and Liver of *Clarias gariepinus* Adult

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**Abstract** --- The toxicity of Dizensate herbicide on the was investigated with emphasis on histopathological effects of African catfish *Clarias gariepinus* Adult. Static bioassay was conducted to determine the  $LC_{50}$  of Dizensate herbicide to African catfish Adult. The fishes were exposed to 0, 19.2, 28.8, 38.4, 48.0 and 57.6mg/l of Dizensate herbicide. Histopathological examinations were performed on the gill, skin, liver and heart of test organisms exposed to *Dizensate* glyphosate under standard laboratory condition. 144 live and apparently healthy *C. gariepinus* Adult measuring 29.3-32.6cm standard length and weighed between 180g and 250g were randomly distributed into twelve (48.2cm x 33cm x 34cm) glass tanks of 80 litres capacity each were filled with 40litres aerated unchlorinated well water at twelve fish/tank for the experiment. The toxicant was introduced at the different concentrations stated above in duplicate per treatment. The lethal concentration ( $LC_{50}$ ) value of Dizensate herbicide was 43.65mg/l for 96h of exposure. Mean mortality was 0, 17, 33, 50 and 83% in the concentration of 19.2, 28.8, 38.4, 48.0 and 57.6mg/l respectively, while there was no mortality in the control treatment. Toxic reactions exhibited by the fish include erratic movement, air gulping, loss of reflex, molting, barbell deformation, hemorrhage and excessive mucus secretion in fish exposed to higher concentration of Dizensate glyphosate. Histopathology of the organs after 96 hr exposure revealed cell proliferation, lamellar fusion, lamellar cell hyperplasia, and epithelial lifting. In the liver, there was vacuolation of hepatocytes and necrosis. The changes in these tissues occur predominantly in the 96 hr exposure. Respiratory stress, erratic swimming and instant death of fish were observed in exposed fish, which varied with the concentration of the toxicant. Histopathological examination of the gill, skin, liver and heart of *C. gariepinus* Adult showed varied degrees of degenerative changes including vacuolation and necrosis which worsened with increasing concentration of the toxicant. Observations on the bioassay test indicated hyper exetability and the eagerness of the test fish to jump out of the pollutant. Dizensate glyphosate is highly toxic to *C. gariepinus*, therefore it's use directly in water bodies, near fish farms or in areas close to aquatic bodies should be moderated and regulated.

**Index Terms** --- Dizensate glyphosate, toxicity, African catfish (*Clarias gariepinus*), histopathological, necrosis,

## INTRODUCTION

The impact of chemical environmental contamination on fish health, consequently fish productivity is of economical relevance for fishes as well as aquaculture. Environmental pollutants have been reported to accumulate in fish [2] and have threatened human health either directly or indirectly through the food chain. These herbicides and pesticides when applied in restricted areas are washed and carried away by rains and floods to nearby aquatic system, thereby affecting aquatic biota, especially fish, which serves as a rich protein supplement for man [5]; [8] and [15]. The herbicides affect not only the physiology and survival of aquatic organisms including fish but also interact with their genetic make-up leading to mutations and/or carcinogenesis [20]; [6] and Nwani *et al.*, 2010).

However, the proper handling and use of herbicides in aquatic areas are especially critical. Accidental spills or over dose can kill fish or cause other damage to its habitats that may lead to reduction in the fish population.

Aquatic bioassays are necessary in water pollution control to determine whether a potential toxicant is dangerous to aquatic life and if so, to find the relationship between the toxicant concentration and its effect on aquatic animals [18]. Histopathological changes of gills such as hyperplasia and hypertrophy, epithelial lifting, aneurysm and increase in mucus secretion have been reported after the exposure of fish to a variety of noxious agents in the water, such as pesticides, phenol and heavy metal [16]. Also the liver is a very important organ which breaks down chemicals and as a result, liver cells are often among those that are damaged by toxic chemicals.

In view of the need for knowledge of the aquatic side-effects of glyphosate herbicide, the objective of this study is to determine the lethal concentration and the acute toxic effect of glyphosate herbicide with emphasis on the histopathology on *Clarias gariepinus*.

## Research Methodology

A 96-hour short-term static bioassay was conducted using the Adult of *Clarias gariepinus* as test organisms. This was done in order to study the toxicity of glyphosate herbicide on fish, and determine allowable levels or concentrations of glyphosate herbicide for very short exposures.

#### Sources and collection

The choice of *Clarias gariepinus* was informed by its ability to withstand stress and its high commercial value in Nigeria. *Clarias gariepinus* averaging  $215 \pm 35$ g and length range of 29.3cm and 32.6cm obtained from Akin Sateru Farms (a private hatchery) in Akute- Lagos State and transported in plastic bowl at 007Hr were used for the experiment. The bioassay experiment was carried out using the laboratory of Federal College of Fisheries and Marine Technology, Victoria Island, Lagos. Histological analysis was done at Histology Unit, Department of Anatomy, College of Medicine, University of Lagos.

#### Acclimatization of fish

The fish were held in 48.2cm by 33cm by 34cm, aquarium containing non-chlorinated water. The fish were allowed to acclimatize for more than one week under laboratory conditions to allow them adapt to experimental conditions ( $27 \pm 2$  °C). The period of acclimatization was extended beyond one week to ascertain the condition of the fish. The fish were inspected for disease conditions and general fitness. The fish were fed during the period of acclimatization and the water was changed every two days in order to remove faecal and unconsumed feeds. Feeding was discontinued during the 96-hour test period.

#### The determination of the physico-chemical parameters of the water

The physico-chemical parameters of the water used were examined. These parameters included temperature, dissolved oxygen (DO<sub>2</sub>) and the hydrogen ion concentration (pH). The temperature was measured with a clinical thermometer and the dissolved oxygen of the water was measured with a digital meter (Jenway9071), while the pH was measured using the HANNA HI 9813 GRO CHEK meter.

#### General bioassay techniques

The bioassay was carried out in a rectangular glass tank. The top was covered with mesh net aided by elastic rubber band to prevent the fish from escaping. Each tank size of 48.2cm X 33cm X 34cm of 80 liters capacity, filled with 40 litres unchlorinated well water contained twelve fish. After a range – finding test, the concentrations prepared for the experiment were 0, 19.2, 28.8, 38.4, 48.0 and 57.6mg/l, with two

replicates. The amount of herbicide which contained the require milligram of Dizensate herbicide was determined from the 480 g/L of Dizensate herbicide formulation.

The behavioural pattern of the fish and other external changes in the body of fish were observed accordingly. Dead fish were identified by an absolute lack of movement. They were removed as soon as this was noticed, and disposed. The LC<sub>50</sub> value of the *Clarias gariepinus* for 96 hrs was calculated using the probit analysis.

**Table 1: Table shows the lethal concentrations (96-h LC<sub>50</sub>) value of Dizensate herbicide to C. gariepinus adult after several hours.**

TIME (Hours)	Log C value	LC <sub>50</sub>
24	1.755	56.89mg/l
48	1.716	52.00mg/l
72	1.707	50.93mg/l
96	1.640	43.65mg/l

Key: LC50=Lethal Concentrations

#### HISTOPATHOLOGY STUDIES

At the end of the experiments, one fish per treatment were sampled after 96hour of exposure to glyphosate herbicide for histological analysis. The fish was sacrificed with a blow on the head, using a mallet and was dissected to remove the liver and the gill. The organs were fixed in 10 % formalin for 3 days after which the tissue was dehydrated in periodic acid Schiff's reagent (PAS) following the method of [12] in graded levels of 50%,70%,90% and 100% alcohol for 3 days, to allow paraffin wax to penetrate the tissue during embedding. The organs were then embedded in molten wax. Tissue were sectioned into a thin sections (5-7µm) by means of a rotator microtome and were dehydrated and stained with Harris haematoxylin-Eosin (H&E) stain. [4] using a microtone and each section was cleared by placing in warm water (38°C) where it was picked with clean slide and oven dried at 58°C for 30 minutes to melt the wax. Slides containing sectioned materials/tissue was cleared using xylene and graded levels of (50%, 70%, 90% and 100%) of alcohol for 2 minutes each.

The section was stained in haematoxyline Eosin for ten minutes. The stained slides were observed under a light microscope. At varying X100 magnification, sections were examined and photographed using an Olympus BH2 microscope fitted with photographic attachment (Olympus PM C35 AD4) a camera (OlympusC40 AB -4)

**Statistical analysis:** The dose response of mortality were analysed by probit analysis (Finney, 1971) based on a computer programme by Ge Le PaHoure, Imperial College, London and adopted by Don-Pedro (1989), This was used to derive the  $LC_{50}$ .

$LC_{50}$  = Median lethal concentration that causes 50% mortality of exposed animals.

## RESULTS

### The physico-chemical characteristics of the water

Tables 10-13 showed the results of the water parameters after Dizensate herbicide exposure of *C.gariepinus* adult. The pH, temperature and dissolved oxygen were determined at different time interval. The results obtained before the test were found to be close to the water quality parameters of the control experiment.

The pH value obtained shows that Dizensate herbicide has slight effect on the pH of water. The pH reduced slightly from  $6.9 \pm 0.2$  in control to  $6.1 \pm 0.1$  in test treatment of concentration 48.0mg/l after the whole experiment as shown in tables 11. Temperature varies between  $25 \pm 0.2$  to  $27 \pm 0.1$  in the 19.2mg/l concentration. The  $DO_2$  decrease from  $5.9 \pm 0.1$  in control experiment to  $3.5 \pm 0.1$  in test treatment of concentration of 57.6mg/l

### ACUTE TOXICITY

The results of the acute toxicity test are presented in Table 1. The  $LC_{50}$  value based on probit analysis was found to be 43.65 mg/L for 96 hrs of exposure to the glyphosate herbicide (Fig.1). The results obtained showed that there was no mortality of fish in the control experiment throughout the 96 hrs. There was 17% mortality of the fish exposed to 28.8 mg/L while at 57.6 mg/L, 83% mortality was observed. During this study the behaviour of the control fish was normal, while the fish introduced into the different concentrates of the herbicides showed different abnormal behaviour. Abnormal behaviour such as erratic swimming, sudden quick movements and restlessness were observed in fish exposed to the chemical. At high concentration of 48.0 and 57.6mg/L, the fish became very weak and settled at the bottom. Normal colour and behavioural response was observed in the control experiment.

## HISTOPATHOLOGICAL EFFECT

### Liver

Transverse section through the liver showed normal cellular pattern, normal central vein, biliary epithelium, hepatic plate and hepatocytes. No lesion, necrosis, pigments, malignancy, inflammation or

inclusion bodies were seen in the control. There were areas of slight lesion, necrosis, malignancy, pigment, inclusion bodies and inflammation in the livers exposed to the glyphosate herbicide. Vacuolation and disarrangement of tissue was seen in concentration of 28.8mg/l of glyphosate herbicide treated fish. Shrinkage of cell and hyperplasia of cell was observed. Complete degenerated tissue was observed in this highest concentration of 57.6mg/l within 96 hours.

### Gill

Sections through the gill showed normal cellular pattern, ranging from gill arch, gill rakers, filament, venus, sinus, cartilaginous support, pseudo-brachial lamella, ceratobrachial bone of the arch, mucous epithelium lining on the membrane and branches of the afferent and efferent arterioles, and nucleous. No lesion, necrosis, pigments, malignancy, inflammation or inclusion bodies were seen. Moderate and severe areas of lesion, necrosis, malignancy, pigment and inclusion bodies were observed in fish exposed to glyphosate herbicide in concentration of 19.2mg/l. Degeneration of lamellar and hypertrophy of cell occurred in concentration of 19.2mg/l of glyphosate herbicide treated fish while fish exposed to concentration of 48mg/l also shows hypertrophy of gill arch and complete degeneration of filament. These were evident in highest concentration of 57.6mg/l of glyphosate herbicide treated fish within 96 hours period.

Mortality (%) of *C. gariepinus* Adult exposed to different concentrations of glyphosate herbicide (bioassay test) (Table 3) showed that the fish was sensitive to concentrations from 28.8 – 57.6mg  $L^{-1}$ . The table indicated that within 96h, about 33% of the fish died in concentration of 38.4mg  $L^{-1}$ , while 58% died in concentration of 48mg  $L^{-1}$ , suggesting that the 96h  $LC_{50}$  of glyphosate herbicide might lie between 38.4 and 48mg  $L^{-1}$ . The concentration values were converted to Logit, while the mortality (%) was converted to Probit values according to methods of [11], and the transformed values were used to determine the 96h  $LC_{50}$  graphically. Figure 1 presents the  $LC_{50}$  graph with the regression equation  $Y = 6.0025X - 4.8416$ , where Y= probit response and X= logit (log-dose). From the equation, the 96h  $LC_{50}$  was calculated as 43.65 mg  $L^{-1}$ .

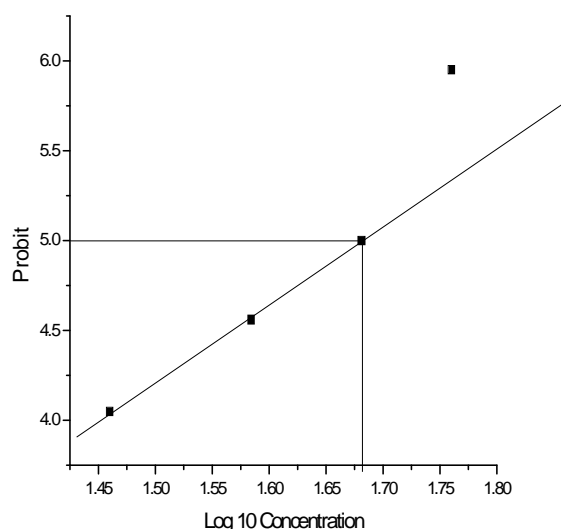
**Table 3: Mortality(%) of *O. niloticus* Adult exposed to different concentrations of glyphosate herbicide.**

Concentration(mg)	Time (Hours)			
	0	24	48	72
				96

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T0 = 0	0.0	0	0	0	0
T1 = 19.2	0.0	0	0	0	0
T2 = 28.8	0.0	0	0	17	17
T3 = 38.4	0.0	17	17	33	33
T4 = 48.0	0.0	17	33	33	58
T5 = 57.6	0.0	50	67	67	83

96 hours LC<sub>50</sub> value exposed Adult to dizensate herbicide



**Fig.1 : Shows the LC<sub>50</sub> for Adult of *C. gariepinus* at 96h exposure to Dizensate Herbicide**

## DISCUSSION

The glyphosate herbicide exerted toxic effect on the fish in the present study and toxicity increased with increased concentration. The physico-chemical properties of glyphosate herbicide clearly indicated that it is a pollutant, as its presence in water changed the physical and chemical qualities of water to critical levels that could hardly support aquatic productivity. The maximum safe concentration (96h LC<sub>50</sub>) range for adult fish samples 43.65mg/l compared favourable with those of Omitoyin et.al (2006) that reported the effect of gramoxone (paraquat) juvenile *Clarias gariepinus* with LC<sub>50</sub> value of 18mg/l for 96h exposure. As expected, high concentration of glyphosate herbicide in the areas resulted in increased water temperature with corresponding reduction in dissolved oxygen concentrations.

Abnormal behaviours such as incessant jumping and gulping of air, restlessness, loss of equilibrium, increase opercular activities, surface to

bottom movement, sudden quick movement and resting at the bottom observed in this study were similar to the observations of Ajani et. al [1] and [7]. The fish were stressed progressively with time before eventually dying. The stressful ailment of respiratory impairment due to the toxic effect of glyphosate herbicide on the gills was similar to the report of [19]. The observed increasing state of inactivity with both increasing concentrations and exposure period agree with the report of [3]. Water quality parameters had little variation, physicochemical parameter measured seemed to be within optimum range for fish culture as reported by [19] and [18]. Accumulation of mucus in the gills of fish exposed to the different concentrations of Dizensate herbicide in this study might be responsible for the mortality recorded. This report was similar to the work of [14] who worked on the effect of insecticide ethofenprox on Nile Tilapia. [13] reported that the accumulation of mucus on the gills reduces respiratory activities in fish. The inability of the gill surface to actively carry out gaseous exchange might be responsible for the observed mortalities. The mortality pattern recorded corroborates with that reported by [20] which stated that there should be less than 35% mortality in one of the concentration and at least more than 65% mortality in the highest concentration.

## CONCLUSION

The results of this study revealed that glyphosate herbicide is toxic to fish organs and causes histopathological changes in different vital organs such as Liver and Gills. Glyphosate is widely used in the mistaken belief that it is harmless, safe and readily breaks down leaving no residues. Consequently, it is sprayed in public areas while people are present and by operators without protective clothing. These affirmations are based on studies examining the active ingredient only. The facts revealed in this study shows that Glyphosate causes a range of defects and health problems to the test organism; *Clarias gariepinus*. The herbicide has caused a host of acute and chronic effects ranging from inflammation and necrosis of tissues.

It could be concluded that Glyphosate herbicide, has harmful effects on the physiology, and histology structure of fish which in turn affect the growth rate and reproduction and leads to deterioration of meat quality of the exposed fish to the point that it can be hazardous to humans at certain levels in water. It is time that the widespread use of this toxic chemical on ponds area, water ways, drains, streams, roadsides, footpaths, parks, gardens,

schools, farms, forestry, national parks etc is stopped or highly restricted.

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