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# Acute Toxicity of Glyphosate-Based Herbicide Glycot on Juvenile African Cat Fish *Clarias gariepinus* (Burchell 1822)

**Ani LC[1], Nwamba HO[1], Ejilibe CO[1] and Nwani CD[2]***

[1]*Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology, Enugu, Nigeria*
[2]*Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, Nigeria*

## Abstract

The present study was designed to evaluate the acute toxicity of glyphosate on the juvenile of *Clarias gariepinus*. The acute toxicity bioassay was conducted to determine the 96h $LC_{50}$ values following the probit analysis method while the safe level of the test pesticide was estimated by multiplying the 96 h $LC_{50}$ with different application factors (AF). The 24, 48, 72 and 96h $LC_{50}$ values (with 95% confidence limits) estimated by probit analysis were 34.72(31.02-37.20), 31.90 (28.12-33.89), 27.40 (24.98-29.30), 24.60 (21.95-26.54) $mgL^{-1}$ respectively. There were significant differences ($p<0.05$) in the LC50 values obtained at different exposure time. The safe level for the herbicide varied from $2.46 \times 10^{-1}$ to $2.46 \times 10^{-4}$ $mgL^{-1}$. Exposed fish showed uncoordinated behavior such as erratic and jerky swimming, jumping out of water, gulping of air, loss of equilibrium status, hyperactivity, decreased opercula movement and subsequently death. Mortality increased with increase in concentration of glyphosate and time of exposure. The study indicated that glyphosate has toxic effects on the *Clarias gariepinus*. The herbicide should be prudently used in both terrestrial and aquatic eco systems to avoid ecotoxicological hazards.

**Keywords:** *Clarias gariepinus*; Glyphosate; Toxicity; $LC_{50}$; Behavioural changes; Safe level

## Introduction

Glyphosate, a broad-spectrum weedicide is one of the most frequently applied pesticides in agriculture for the control of great variety of annual biennial and perennial grasses, sedges, broad leaved weeds and woody shrubs [1]. It is also used for aquatic weed control in fish ponds, lakes, canals and slow running water [2].

Glyphosate is formulated as an isopropylamine salt and can be described as an organophosphorous compound. Glyphosate is described by the manufacturer as pesticide of low toxicity and environmental friendliness [3]. But research has shown that higher concentrations of the product can be toxic, producing a number of physiological changes in organisms and in some cases resulting to death depending on the level, duration and route of exposure [4]. Various concentrations of glyphosate have been shown to be toxic to juveniles of cat fishes, producing mortality, low survival and various abnormal behavioural changes such as loss of equilibrium status, air gulping, hyper activity, decreased opercula movement, erratic swimming and jerky movements which have been shown to be deleterious to the survival rate of the affected species[5].

In the world today, glyphosate is the most widely used herbicide and its consumption has increased to about 95% in the period from the year 2000 to 2004 [6]. Its high water solubility, extensive usage (especially in shallow water systems) and effects on non-target aquatic organism is a concern [7]. Studies have revealed that the responses of aquatic organisms to acute concentrations of glyphosate, its products and surfactants were variably possible due to differences in the formulation of the herbicide, level of mixture of component herbicide, species-specific sensitivity and condition of the media which are known to modify toxicity of the herbicide to exposed organisms [8].

Environmental factors such as pH, turbidity, alkalinity, dissolved oxygen, temperature and conductivity influence the rate of reaction of the pollutants entering the water or the lethal effects on the aquatic organisms. Due to increase in the use of glyphosate herbicide in the recent times, it becomes necessary to study the lethal toxicity and stress of the herbicide on local species which would help in formulating the strategies for safe guarding aquatic organisms. The present study thus aims at the determination of the acute toxicity, behavioural responses and possible safe levels of African catfish *Clarias gariepinus* exposed to glyphosate herbicide.

## Materials and Methods

Three hundred juveniles of *Clarias gariepinus* with mean weight of 32.16 ± 0.22 g and length of 17 ± 0.10 cm were procured from Sacen fish farm Enugu State, Nigeria and used for the investigation. The fish were acclimatized for fourteen days in four plastic aquaria tanks of 200 L capacity each. During the acclimation period, juveniles were fed twice daily with Copen feed (2 mm) at 3% body weight. Feeding was terminated 24 h before the experiment to empty their stomach and avoid pollution of the water with their faeces. For the present study, commercial formulation of glyphosate 41% SL with trade name "Glycot" manufactured by Sabero Organics Gujarat Ltd India and supplied by Afcott Nigeria plc were used.

Acute toxicity bioassay was conducted to determine the 96 h $LC_{50}$ values of commercial formulation of glyphosate (Glycot). A range finding test was carried out to determine the concentrations of the test solutions for the definitive test. This was determined by subjecting juveniles of *Clarias gariepinus* to different concentrations of glyphosate herbicide. In the definitive test, a set of 30 fish specimen in three replicates of 10 fish each were randomly exposed to each of the five

***Corresponding author:** Nwani CD, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, Nigeria, Tel: +234 806 360 7400; E-mail: chris.nwani@unn.edu.ng

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selected concentrations of glyphosate herbicide (18.5, 21.5, 24.4, 27.4, 30.4 and 33.4 $mgL^{-1}$). Another set of 10 juveniles were simultaneously maintained in water without test chemical and considered as control. The experiment was conducted in plastic aquaria (60 cm × 30 cm × 30 cm) containing 10 litres of water. The test solution was changed on every 24 h to counter-balance the decreasing pesticide concentration. During the experiment, fish behavior and other external changes in the body of fish were observed daily. Mortality of the fish due to glyphosate exposure was recorded up to 96 h at every 24 h interval to obtain $LC_{50}$ values of the test pesticides. The median lethal concentration ($LC_{50}$) value was determined following the probit analysis method described by Finney [9]. The safe level of the test pesticide was estimated by multiplying the 96 h $LC_{50}$ with different application factors (AF) and was based on Hart et al. [10], Sprague [11], Committee on Water Quality Criteria (CWQC) [12], National Academy of Science/National Academy of Engineering (NAS/NAE) [13], Canadian council of Resources and Environmental Ministry (CCREM) [14], and the International joint commission (IJC) [15]. The physicochemical properties of test water namely temperature, pH, total hardness and conductivity were analyzed using standard Procedures [16].

## Statistical Analysis

The data obtained were statistically analyzed by statistical package SPSS (Version 17). These data were subjected to one way Analysis of Variance (ANOVA) and Duncan's multiple range test to determine the significance difference at 5% probability level.

## Results

### Physico-chemical parameters of the test water

The results of physico-chemical characteristics of the test water are presented in Table 1. The pH of water ranged from 8.00-8.75. The water temperature ranged from 25.9-27.7°C. The conductivity varied from 100-260 ppm, whereas total hardness ranged from 0.160-0.395 during the experimental period.

### Behavioral response of fishes to different test concentrations

The behavioral responses of fish were observed in the exposed fish as well as in the control (Table 2). Normal swimming behaviour and natural colour were observed in the control throughout the exposure period and in the lowest (18.5 $mgL^{-1}$) concentration of the herbicide at 24 h exposure period. In tanks with higher concentration of the test chemical, the fish swam erratically with Jerky movements and hyperactivity while body pigmentation was greatly reduced. Faster opercula movement, surfacing and gulping of air were observed. With increase in duration of the exposure, swimming and body movements were retarded. Later, fish lost balance, became exhausted, lost consciousness owing to respiratory incumbency and finally settled down passively at the bottom of the tank with the operculum wide open and ultimately died.

| Parameters | Range | Mean S.E |
|---|---|---|
| PH | 8.00-8.75 | 8.375 ± 0.375 |
| Temperature (°C) | 25.9-27.7 | 26.80 ± 0.9 |
| Conductivity (ppm) | 100-260 | 180 ± 80 |
| Total hardness | 0.160-0.395 | 0.278 ± 0.118 |

**Table 1:** The physico-chemical parameters of the test water.

### Fish survival and mortality at different test concentration and time intervals in *Clarias gariepinus* exposed to glyphosate

The numbers of survived and dead fish were examined depending on the duration of exposure (24, 48, 72 and 96 h) in *Clarias gariepinus*. The herbicide concentration of 33.3 $mgL^{-1}$ showed the highest fish mortality of 100% and lowest survival of 0% while no mortality was recorded in the control throughout the experiment (Table 3). This shows that increase in mortality rate results to decrease in survival rate of fish.

### Median Lethal concentrations of glyphosate herbicide

The concentration of glyphosate herbicide that would bring about 50% mortality of test organism at different time interval is referred to as $LC_{50}$. The $LC_{50}$ values of different concentrations of glyphosate on *Clarias gariepinus* were found to be 34.72 (31.02-37.20), 31.90 (28.12-33.89), 27.40 (24.98-29.30) and 24.60 (21.95-26.54) $mgL^{-1}$ for 24, 48, 72 and 96 h exposure time, respectively (Table 4). Our results indicate that as the exposure time increases from 24 to 96 h, the median lethal concentration ($LC_{50}$) reduces.

### Estimation of safe level

The concentration of glyphosate herbicide that is harmless to fish is referred to as safe level. The safe level of glyphosate estimated by different methods at 96 h exposure are presented in Table 5. The values of safe level of glyphosate in *Clarias gariepinus* varied from $24.6 \times 10^{-1}$ to $2.46 \times 10^{-4}$.

## Discussion

The study was carried out to evaluate the acute toxicity of glyphosate on the juvenile of *Clarias gariepinus*. Acute toxicity studies are the very first step in determining the water quality requirements of fish. This present study showed that exposure of the juvenile catfish to glyphosate resulted in increased mortality rate and decreased survival rate at different level of concentrations. The result is in agreement with the

| Exposure time | 24 h | | | | | | | | 48 h | | | | | | | 72 h | | | | | | | 96 h | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc. ($mgL^{-1}$) | 0 | 18.5 | 21.5 | 24.4 | 27.4 | 30.4 | 33.3 | 0 | 18.5 | 21.5 | 24.4 | 27.4 | 30.4 | 33.3 | 0 | 18.5 | 21.5 | 24.4 | 27.4 | 30.4 | 33.3 | 0 | 18.5 | 21.5 | 24.4 | 27.4 | 30.4 | 33.3 |
| Behaviour | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Hyper activity | - | - | - | + | + | + | + | - | - | - | + | + | + | + | - | - | - | + | + | + | - | - | - | + | + | + | + | + |
| Equilibrium status | +++ | +++ | ++ | ++ | + | + | + | +++ | +++ | +++ | ++ | + | + | + | ++ | ++ | ++ | ++ | ++ | + | - | +++ | + | + | + | + | - | - |
| Swimming rate | +++ | +++ | +++ | +++ | + | + | +++ | +++ | +++ | +++ | ++ | ++ | + | + | ++ | ++ | ++ | ++ | + | + | - | +++ | + | + | + | + | - | - |
| Fin movt | +++ | +++ | +++ | +++ | + | + | +++ | +++ | +++ | +++ | ++ | ++ | + | + | ++ | ++ | ++ | ++ | + | + | - | +++ | + | + | + | + | - | - |
| Jerky Movt | - | - | - | + | ++ | +++ | +++ | +++ | - | - | - | - | ++ | ++ | ++ | - | + | + | ++ | ++ | ++ | - | + | + | + | + | - | - |

Key=none -, mild +, moderate ++, Strong +++

**Table 2:** Behavioral changes at different test concentration and time intervals observed in *Clarias gariepinus* exposed to glyphosate.

| Exposed concentration ($mgL^{-1}$) | Number exposed | Periods (Hours) | | | | % Survival | % Mortality |
|---|---|---|---|---|---|---|---|
| | | 24 | 48 | 72 | 96 | | |
| Control 0.00 | 30 | 0 | 0 | 0 | 0 | 100 | 0 |
| 18.5 | 30 | 0 | 3 | 3 | 3 | 90 | 10 |
| 21.5 | 30 | 0 | 6 | 6 | 9 | 70 | 30 |
| 24.4 | 30 | 3 | 6 | 9 | 15 | 50 | 50 |
| 27.4 | 30 | 6 | 6 | 12 | 18 | 40 | 60 |
| 30.4 | 30 | 9 | 12 | 18 | 24 | 20 | 80 |
| 33.3 | 30 | 12 | 21 | 27 | 30 | 0 | 100 |

**Table 3:** Fish survival and mortality at different test concentrations and time intervals in *Clarias gariepinus* exposed to glyphosate.

| Concentration | 24 | 48 | 72 | 96 |
|---|---|---|---|---|
| $LC_{10}$ | 25.57$^{a}$ (21.08-27.02) | 19.73$^{a}$ (17.24-22.06) | 19.60$^{c}$ (17.24-21.86) | 18.69$^{d}$ (16.20-20.24) |
| $LC_{20}$ | 28.40$^{a}$ (23.42-30.92) | 23.27$^{b}$ (20.89-26.12) | 21.99$^{c}$ (20.00-23.02) | 20.54$^{d}$ (17.72-23.01) |
| $LC_{30}$ | 30.60$^{a}$ (27.20-33.02) | 26.21$^{b}$ (23.14-28.90) | 23.89$^{c}$ (21.02-25.69) | 21.98$^{d}$ (19.02-23.98) |
| $LC_{40}$ | 32.68$^{a}$ (28.40-34.60) | 29.01$^{b}$ (25.15-33.02) | 25.65$^{c}$ (23.10-27.61) | 23.30$^{d}$ (21.02-25.20) |
| $LC_{50}$ | 34.72$^{a}$ (31.02-37.20) | 31.90$^{b}$ (28.12-33.89) | 27.40$^{c}$ (24.98-29.30) | 24.60$^{d}$ (21.95-26.54) |
| $LC_{60}$ | 36.88$^{a}$ (32.10-38.27) | 35.08$^{b}$ (33.20-40.28) | 29.28$^{c}$ (25.02-33.98) | 25.97$^{d}$(23.01-27.12) |
| $LC_{70}$ | 39.35$^{a}$ (35.08-41.95) | 38.84$^{b}$ (36.20-40.28) | 31.43$^{c}$ (27.10-35.28) | 27.53$^{d}$ (25.13-29.20) |
| $LC_{80}$ | 42.45$^{a}$ (38.17-44.01) | 43.74$^{b}$ (40.76-45.79) | 34.15$^{c}$(30.48-38.12) | 29.46$^{d}$ (26.55-32.64) |
| $LC_{90}$ | 47.15$^{a}$ (44.19-50.12) | 57.59$^{b}$ (48.92-59.27) | 38.32$^{c}$(35.12-41.27) | 32.38$^{d}$ (30.10-34.72 |

Values with different alphabetic superscripts differ significantly ($p<0.05$) between exposure time within lethal concentration.

**Table 4:** Lethal concentrations of glyphosate herbicide for *Clarias gariepinus* at different intervals.

| Chemical | 96 h $LC_{50}$ ($mgL^{-1}$) | Method | AF | Safe level ($mgL^{-1}$) |
|---|---|---|---|---|
| Glyphosate | 24.6 | Hart et al. (1948) | - | $8.08 \times 10^{-1}$ |
| | | Sprague (1971) | 0.1 | $24.59 \times 10^{-1}$ |
| | | CWQC (1972) | 0.01 | $24.59 \times 10^{-2}$ |
| | | NAS/NAF (1973) | 0.1-0.00001 | $24.60 \times 10^{-1}$-$2.60 \times 10^{-4}$ |
| | | CCREM (1991) | 0.05 | 1.23 |
| | | IJC (1977) | 5% of 96h$LC_{50}$ | 1.23 |

$C = 48\ hLC_{50} \times 0.03/S^2$, where C is the presumable harmless concentration and $S = 24\ hLC_{50}/48\ h\ LC_{50}$.

**Table 5:** Estimate of safe levels of glyphosate at 96h exposure time.

report of Olele and Zelibe [17] that fish and other aquatic organisms are harmed by herbicide contaminated water. Behavioural changes are the most sensitive indicators of potential toxic effects in fishes [18]. The observed behavioral alteration in the studied formulation of glyphosate are consistent with previous report on glyphosate based herbicides [19,20] and other pesticides like chloropyriphos [21], Profenofos [22], butachlor [23] and fenthion [24]. Fish exposed to higher concentrations of glyphosate exhibited faster swimming with jerky movement, gulping of air, faster opercula movement, loss of equilibrium, skin discoloration and subsequently death.

The median lethal concentration ($LC_{50}$) value reported in the present study for commercial formulation of glyphosate is higher than 1.05 $mgL^{-1}$ and 13.6 $mgL^{-1}$ reported by Ayoola [19] and Langiano and Martinez [25] when *Oreochromis niloticus and Prochilodus lineatus* were exposed to glyphosate and glyphosate-based herbicides respectively. The $LC_{50}$ obtained in the present study for glyphosate is lower than 108 $mgL^{-1}$ and higher than 3.74 $mgL^{-1}$ obtained by Clements et al. [26] and Shiogiri et al. [27] when bull frog tadpoles and neotropical fish (*Piarsctus mesopotamicus)* were exposed to glyphosate-based herbicide roundup for 48 h. The $LC_{50}$ value however is lower than 620 $mgL^{-1}$ and 975 $mgL^{-1}$ 96h $LC_{50}$ reported by Shiogiri et al. [28] when *Cyprinus carpio* and *Palloceroscaudi maculatus* were exposed to glyphosate and glyphosate commercial formulation (Rodeo) herbicides respectively. The $LC_{50}$ value of 24.60 $mgL^{-1}$ reported in the present study is also lower than the 32.54 $mgL^{-1}$ obtained by Nwani et al. [29] when the same fish species (*Clarias gariepinus*) was exposed to similar glyphosate based herbicide round up. Previous literature indicates that the toxicity of glyphosate-based herbicides varies from one species to another species and even in strains of the same species. Toxicity is both concentration and time dependent, this account for the differences in the values obtained at different concentration and exposure times. Toxicity of chemicals to fish have been reported to be affected by water quality parameter (such as pH, temperature etc.), size and age, type of species, water quality, concentration and formulation of test chemicals [30].

The safe level obtained for glyphosate in the present study varied from 2.460 to $2.460 \times 10^{-4}$. However, due to large variation in safe levels as determined by different methods, the estimates of safe levels cannot be guaranteed [31]. Extrapolation of laboratory data to field is not always meaningful value and hence, it is difficult to decide on acceptable concentration based on laboratory experiments that may be considered safe in the field [32]. From this experiment, it is evident that glyphosate is toxic to fish and its application should be monitored and controlled to avoid possible eco-toxicological hazards.

## Conclusion

From the present research it was evident that glyphosate is less toxic to *Clarias gariepinus* than other herbicide widely used in Nigeria. Most of the behavioral and physiological abnormalities were recorded mainly at higher concentrations. However, the herbicide should be prudently used in both terrestrial and aquatic eco-systems to avoid eco-toxicological hazards. More studies on the toxicity of glyphosate and its formulations on catfishes are necessary to understand the mechanisms of actions of the herbicide.

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## Conflict of Interest

The authors declare that there are no conflicts of interest.

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