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# Acute Toxicity of Paraquat Dichloride Based Herbicide against *Heterobranchus bidorsalis* Fingerlings

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## Abstract

Paraquat dichloride based herbicides is commonly used to control several weeds in both terrestrial and moist areas in the Niger Delta region of Nigeria. This study evaluated acute toxicity of *Heterobranchus bidorsalis* fingerlings exposed to paraquat dichloride. The fishes were allowed to acclimatize in the laboratory for 7 days, and range finding test was carried out. Based on the result of the range finding test the main experiment was carried out varying concentration of 0.00 ppm, 16.00 ppm, 18.00 ppm, 20.00 ppm, 22.00 ppm and 24 ppm. The study adopted 96 hours renewal bioassay approach. Results showed that as the concentration of the paraquat dichloride and exposure duration increased the mortality rate increased. At 0.00 ppm, 15.00 ppm, 18.00 ppm, 20.00 ppm, 22.00 ppm and 24.00 ppm the mortality rate was 0.00%, 23.33%, 43.33%, 66.67%, 80.00% and 93.33% respectively. There was significant increase ($p < 0.05$) in mortality rate among the various concentrations of the paraquat dichloride. The $LC_{50}$ values at 24 hours, 48 hours, 72 hours and 96 hours were 30.28 ppm, 22.72 ppm, 20.23 ppm and 18.44 ppm respectively. The findings of this study showed that there is the need to properly discharge the empty cans of paraquat dichloride so as to avoid possible contamination of aquatic ecosystem when the cans find their way into the aquatic ecosystem through runoff.

***Keywords:*** *Heterobranchus bidorsalis; Mortality; Surface Water Pollution; Paraquat Dichloride; Toxicity*

## Introduction

Paraquat is among the commonly brands of herbicides for the control of several broad leaves and grasses in plantations and other weeds in non-crop land/urban and household settings. Specifically, paraquat dichloride based herbicide is defoliant for some plants and it is a quaternary nitrogen based herbicides that has the tendency to destroy plant tissues through contacts were its then absorbed and then translocated by the xylem. Banaee., *et al.* [1] described paraquat as a contact and non-selective herbicide that can be used to control several terrestrial and aquatic weeds.

Over the last 6 decades paraquat based herbicides have been in the market in about 100 different countries in the world [2]. In many developing nations some of the individuals that use the paraquat do not have enough information about the toxicity of the herbicides. As such some use it without the use of protective equipment, while some other use leaking knap sack sprayer which then expose them to high risk of toxicity.

According to Arivu., *et al*. [2], paraquat is toxic to human health and enters the body through inhalation, ingestion and damaged skin integrity. Paraquat is corrosive and therefore can easily be absorbed by damaged skin tissues. Arivu., *et al.* [2] reported that a farmer died after 3 and an half hours of spraying diluted paraquat using leaking knapsack sprayer, and several others have suffered from severe acute and chronic effects result from occupational use of paraquat. The authors therefore considered paraquat to be toxic to human health despite the fact that the manufacturers have previously considered it safe for use. Low concentration of paraquat in human could lead to death probably because it has the tendency to cause multi-organ failure (viz: lungs, heart, kidneys, adrenal glands, central nervous system, liver, muscles and spleen) and it could also cause respiratory disorder [2].

Pesticides typically enter the aquatic ecosystem when they are sprayed in agricultural field close to the aquatic ecosystem and through runoff after rainfall. Accidental spills and carelessly discharged of empty cans could be another major means of pesticides contamination in the environment [3-13]. Like other pesticides, most herbicides when indiscriminately used could lead to pollution of the aquatic ecosystem where it could affect the health of aquatic organisms including fishes [14], zooplankton (main food source of young fishes) [13]. Banaee., *et al*. [1] reported that paraquat based herbicides is injurious to fishes and other non-target organisms over a long-term contact. Instances of herbicides affecting non-target organisms including humans have been reported in literatures [2].

Several studies on the effect of paraquat based herbicides on fishes have been reported in literature using fingerlings of *Labeo rohita* [2], juvenile African Catfish (*Clarias gariepinus*) [13], fingerlings of *Oreochromis niloticus* [15], adult ornamental gourami fish (*Trichogaster trichopterus*) [1], *Clarias batrachus* [16]. But information about acute toxicity of paraquat dichloride to fingerlings of *Heterobranchus bidorsalis* is scanty in literature. Hence this study assessed acute toxicity of *Heterobranchus bidorsalis* fingerlings exposed to paraquat dichloride.

## Materials and Methods

### Fish source and acclimation processes

*Heterobranchus bidorsalis* fingerlings of mean length 6.00 cm were purchased from a private fish farm in Yenagoa metropolis, Bayelsa state, Nigeria. The fish were transported to the laboratory in 20 liter plastic container. At the laboratory, the fish were allowed to acclimatize for 7 days in an aquarium. The fish was fed with their normal fish diet during acclimatization period.

### Trial Test

During the trial test, a concentration of 15 ppm, 30 ppm, 45 ppm and 60 ppm of paraquat dichloride were made in the aquarium. The aquarium water was renewed at every 24 hours and the trial period lasted for 48 hours. The trial experiment was carried out to determine the sub-lethal concentration for the main experiment.

### Main Experiment

From the trial test result, varying concentration of paraquat dichloride was made viz: 0.00 ppm, 16.00 ppm, 18.00 ppm, 20.00 ppm, 22.00 ppm and 24 ppm. This concentration was made by using the formula previously described by Inyang., et al. [4-6]:

mls x stock solution (mg/L) = aquarium water (ml) x desired concentration (ppm or mg/l).

In the main experimental set-up each aquarium contains 12 fishes and this was carried out in replicate. The aquarium water and the toxicant were renewed at every 24 hours of exposure. Mortality was confirmed when the fishes did not respond to repeated prodding [17]. Mortality rate of the fish samples were calculated as:

$$\text{Mortality rate} = \frac{\text{Number of dead fish}}{\text{Total number of fish exposed to the toxicant}} \times 100$$

The physicochemical characteristics of the water used were obtained based on the method previously described APHA [18]. The resultant values were Temperature 26.8°C, pH 6.13 - 6.43, conductivity 92.41 - 139.08 µ/cm, Turbidity 0.15 - 0.79 NTU and dissolved oxygen 5.21 - 7.32 mg/L.

### Statistical Analysis

SPSS was used to determine the mean, standard deviation and show significant variations for percentage mortality. The significance level was determined at p = 0.05 and Waller Duncan statistics was used to compare between mean concentration for each exposure period. The percentage mortality was transformed to probit using Finney's Table. Then regression analysis was carried out for probit values against logarithm of the concentration using Microsoft excel. The resultant x value and intercept value were substituted in the equation Y= b + ax in which variable x and b (intercept) were derived from the regression analysis. The $LC_{50}$ was then calculated by substituting the probit value of 50 in the equation Y= b + ax, and a values was determined through change of formula. The anti-logarithm value of "a" was taken as the $LC_{50}$.

## Results and Discussion

The percentage mortality of *Heterobranchus bidorsalis* exposed to paraquat dichloride based herbicides is presented in table 1. At 96 hours the mortality was 0.00%, 23.33%, 43.33%, 66.67%, 80.00% and 93.33% at 0.00 ppm, 16.00 ppm, 18.00 ppm, 20.00 ppm, 22.00 ppm, and 24.00 ppm respectively. There was significant increase ($p < 0.05$) in mortality as the concentration of the paraquat dichloride increased. Furthermore, the mortality rate also increased as the period of bioassay increased (viz: 24 hours, 48 hours, 72 hours and 96 hours). This suggests that paraquat dichloride is exerting toxic effect on the fish as toxicity increased with increased concentration [13]. This trend have been reported in *Heterobranchus bidorsalis, Clarias gariepenus* exposed to detergent by Oyoroko and Ogamba [17], *Heterobranchus bidorsalis* exposed to cassava mill effluents by Seiyaboh and Izah [19].

| Concentration, ppm | Hours | | | | |
|---|---|---|---|---|---|
| | 0 | 24 | 48 | 72 | 96 |
| 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00a | 0.00 ± 0.00a | 0.00 ± 0.00a | 0.00 ± 0.00a |
| 16.00 | 0.00 ± 0.00 | 6.67 ± 5.77ab | 10.00 ± 0.00ab | 13.33 ± 5.77b | 23.33 ± 5.77b |
| 18.00 | 0.00 ± 0.00 | 10.67 ± 9.02abc | 13.33 ± 5.77b | 30.00 ± 10.00c | 43.33 ± 5.77c |
| 20.00 | 0.00 ± 0.00 | 20.00 ± 10.00bcd | 43.33 ± 5.77c | 56.67 ± 5.77d | 66.67 ± 5.77d |
| 22.00 | 0.00 ± 0.00 | 23.33 ± 5.77cd | 50.00 ± 10.00cd | 60.00 ± 0.0d | 80.00 ± 10.00e |
| 24.00 | 0.00 ± 0.00 | 30.00 ± 10.00bd | 56.67 ± 5.77d | 76.67 ± 5.77e | 93.33 ± 5.77f |

***Table 1:*** *Percentage mortality of Heterobranchus bidorsalis exposed to paraquat dichloride based herbicides.*

*Each data is expressed as mean ± standard deviation (n = 3); Different letters along the column indicate significant difference (P < 0.05) according to Waller Duncan test statistics.*

The $LC_{50}$ values of *Heterobranchus bidorsalis* exposed to paraquat dichloride based herbicides is presented in table 2. At 24 hours, 48 hours, 72 hours and 96 hours the $LC_{50}$ was 30.28 ppm, 22.72 ppm, 20.23 ppm and 18.44 ppm respectively. The $LC_{50}$ values were in a decreasing trend as the exposure period increased. The $LC_{50}$ values in this study has some similarity to the work of Arivu., *et al.* [2] that reported $LC_{50}$ value of 28.13, 27.61, 26.22 and 25.71 mg/l freshwater fingerlings of *Labeo rohita* (with weight of 6 - 8g and length of 8 - 10 cm) exposed to paraquat based herbicides for 24, 48, 72 and 96 hours respectively. The values of this study were also higher than the findings of Ladipo [13] that reported $LC_{50}$ value of 1.75 mg/l after 96 hours of exposing juvenile African Catfish (*Clarias gariepinus*) with mean weight of 47.97g and length of 20.04 cm to Paraquat dichloride. Babatunde and Oladimeji [15] reported $LC_{50}$ value of 12.25 mg/l in fingerlings of *Oreochromis niloticus* with mean weight of 7.35 exposed to paraquat (1,1-dimethyl-4,4-bipyridinium dichloride). Banaee., *et al*. [1] reported LC50 values of 7.16 mg/l, 4.45 mg/l, 2.19 mg/l and 1.41 mg/l for 24 hours, 48 hours, 72 hours and 96 hours in adult ornamental gourami fish (*Trichogaster trichopterus*) with body weight of 7.15g and length 8.45 cm exposed to paraquat. Furthermore, Wast., *et al.* [16] reported $LC_{50}$ value of 251.641 mg/l and 325.425 mg/l at pH 5.6 and 7.6 respectively in *Clarias batrachus* with mean length of 10.80 cm exposed to Paraquat for 96 hours. The variation could be associated to the fish species, age, length and weight, and biochemical characteristics of the used for the study as well as the chemical nature of the herbicides.

The decrease in $LC_{50}$ and increased mortality as the exposure period and concentration increased could be associated to stress and/or alteration on the various organs/systems. Typically, when fishes come in contact with paraquat it could lead to disorders in the activity level of several enzymes involved in cellular and biochemical activities, change of blood biochemical factors, damage tissue and cause oxidative stress [1]. Martin-Rubi., *et al.* [20], Banaee., *et al.* [1] reported that photochemical disintegration and auto-oxidation of paraquat could enhance the production of hydrogen peroxide, super oxide radical, oxygen and hydroxyl radical which could cause oxidative stress and toxicity at cellular level in both animal and plant cells. Therefore paraquat may have affects the metabolic and several response systems in the fishes hence leading to death. Hashemi., *et al.* [21] also reported that paraquat could alter haematological indices in *Mesopotamichthys sharpeyi*. Seiyaboh., *et al.* [22] also reported that paraquat dichloride could alter plasma indices in *Clarias gariepinus*. Ogamba., *et al.* [23] also reported that paraquat dichloride could cause an alteration in many metabolic and enzymatic characteristics of *Clarias gariepinus* at certain concentration. Generally, Izah., *et al*. [24] have reported that many toxicants have the tendency to alter behavioral response, electrolytes, haematological, histopathological, enzymatic and metabolites of the fishes.

| Hours | Equation from regression analysis | $LC_{50}$ ppm |
|---|---|---|
| 24 | Y = 4.908178x - 2.2700 | 30.28 |
| 48 | Y = 11.39314x -10.4545 | 22.72 |
| 72 | Y = 10.33125x - 8.49162 | 20.23 |
| 96 | Y = 12.60857x - 10.9584 | 18.44 |

***Table 2:*** *$LC_{50}$ values of Heterobranchus bidorsalis exposed to paraquat dichloride based herbicides.*

## Conclusion

Studies have indicated many herbicides is toxic to biota at varying concentrations. This study evaluated acute toxicity of paraquat dichloride to *Heterobranchus bidorsalis*. Results showed that the paraquat dichloride cause mortality which increased on increased exposure period and concentration. As such care should be taken during the use of paraquat dichloride based herbicides close to surface water especially in the coastal region that are prone to water flooding.

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