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Acute Toxicity of Glyphosate-based Isopropylamine formulation to Juvenile African catfish (*Clarias gariepinus*)

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ABSTRACT
The formulation of glyphosate composed of Isopropylamine salt (41 % wt) is widely used as herbicide both in the terrestrial and aquatic environment. This study examined the toxicity of the surfactant Isopropylamine salt of glyphosate herbicide to post juvenile African catfish (*Clarias gariepinus*).. This experimental design was based on the standard Total Residual Chlorine (TRC) method with interval time of 24, 48, 72 and 96 hrs. Gulping of air, restlessness, surface to bottom movement, sudden quick movement and excessive resting at the bottom were abnormal behaviour observed in the exposed fish. Mortality of *C. gariepinus* increased ($p<0.05$) with an increase in glyphosate concentration. The results showed that the 96h LC_{50} value of glyphosate was 300 mg/L while the maximum allowable concentration (MAC) of this toxicant is 30.0 mg/L. The effect of the toxicant on *C. gariepinus* was both time and concentration-dependent.
Keywords: Acute toxicity, *Clarias gariepinus*, Glyphosate, LC_{50}, Mortality.

INTRODUCTION
Increase in amount of industrial, agricultural and commercial chemicals discharged into the aquatic environment cause various deleterious effects on aquatic organisms (McGlashan and Hughies, 2001).Pollutants accumulate in aquatic organisms directly from contaminated water and indirectly via the food chain (Sasaki *et al*., 1997). The effects of Organophosphorus and Chlorinated pesticides have been extensively studied and detected in fish (Capkin *et al.,* 2006; Pandey *et al.,* 2005; Rao, 2006a; Rao, 2006b). Herbicides are widely used for the control of water plants, which may impede the flow of water during the summer when sudden heavy rain can cause flooding (Annune *et al.,* 1994). The constant flow of agricultural effluents into fresh water often leads to accumulation of pollutant in such water bodies (Mason, 1991).

Glyphosate is a broad spectrum herbicide used primarily in agricultural applications for the control of variety of annual, biennial and perennial grasses, sedges, broad-leaved weeds and woody shrubs. It is also used in fruits orchards, vineyards, conifer plantations and many plantation crops (Ayoola 2008). It is also used for aquatic weed control in fish ponds, lakes, canals and slow running water (Tsui and Chu 2003). Glyphosate is perhaps the most important herbicide ever developed (WHO, 1994). The annual report by NRAAVC, 1997 revealed a 96-hour LC_{50} of technical grade glyphosate for bluegill sunfish and rainbow trout to be 120 mg/L and 86 mg/L, respectively. The technical grade of glyphosate is of moderate toxicity to aquatic species, and the toxicity of different glyphosate formulations can vary considerably. Touchdown 4-LC® and Bronco® have low LC_{50}s for aquatic species (<13 mg/L) and are not registered for aquatic use. On the other hand, Rodeo® has relatively high LC_{50}s (>900 mg/L) for aquatic species and is permitted for use in aquatic systems (NRAAVC, 1997).

The report of NRAAVC (1997) on glyphosate toxicity to aquatic fishes indicated high LC_{50} for Rainbow trout (600mg/L), Common Carp (385 mg/L) and Bluegill sunfish (300 mg/L). Direct or indirect contamination of water by pesticide can lead to fish kills, reduced fish productivity or elevated concentrations of undesirable chemical in edible fish tissue, which poses health riskes to consumers (Adedeji *et al.,* 2009).The aim of this study was to determine the acute toxicity of glyphosate (IPA 360 g/L) on post-juvenile Africa Catfish (*Clarias gariepinus*)

MATERIALS AND METHODS

Experimental fish and chemicals

One hundred and twenty healthy post juvenile *Clarias gariepinus* of mixed sexes with a mean weight of 135.44±1.99 g and mean length of 28.32±0.844 cm were procured from Osayi farms in Benin City, Edo state. They were kept in 60 L aquaria at 27.5±0.4 ºC, pH 6.8, with 12:12 h photoperiod. They were left unfed for 24 hrs to adapt to a change in the environment before feeding commenced with the fish diet. Fish were normally fed once a day with pelleted commercial food (Durante Aquaculture fish concentration-2mm). Laboratory aquaria were well aerated and provided with external filtration and a layer of gravel on the bottom. They were allowed to acclimatize to experimental conditions for two weeks prior to the start of the experiment. Careful netting and handling were implemented to minimize stress. The commercial formulations of glyphosate (360 g/l-41 w.wt IPA) at five nominal concentrations mg/L were used. The concentrations used for the acute test was drawn from a range finding test.

Determination of physicochemical parameters of water

The water quality parameters of tests aquaria were determined by standard methods (APHA, 1998).

The acute toxicity

The acute toxicity test was performed according to the OECD (1992) procedure for the static non-renewal technique. Feeding was discontinued 24 h prior to testing. Preliminary screening was carried out to determine the appropriate concentration range for testing the chemical. The tests consisted of a control and five concentration groups, three replicates per group, with eight fish in each replicate. At the beginning of the tests and every 24 hours, the symptoms and the number of dead fish were recorded. The results of the median lethal concentration (LC_{50}) at 24 h, 48 h, 72 h and 96 hrs were computed using the USEPA (1999) probit analysis computer program.

RESULTS

Acute toxicity tests

The results of the 24-h, 48-h, 72-h and 96-h LC50 values for glyphosate (360g/L) for post juvenile catfish were calculated by the probit method and presented in Table 2. Endpoints used to assess acute toxicity were Mortality, Behavioural and Morphological alterations. No mortality or morphological changes were observed in the controls for the 96hrs acute toxicity test. Fish in the control experiment appeared active and healthy throughout the test period.

Table 1: Physicochemical characteristics of water used in the experiment

Parameters	Range
Temperature (^{0}C)	25.70-26.40
PH	6.60-6.90
Conductivity (mm/cm)	263-298
Dissolved Oxygen (mg/l)	6.10-6.70
Total Hardness	200-300
Salinity (mg/l)	0.09-0.16

DISCUSSION

Glyphosate herbicide is one of the widely used herbicides that could be persistent and mobile in soil and water. It is known to be one of the most common terrestrial and aquatic contaminants (Cox, 1998; Ayoola, 2008).The LC_{50} value of glyphosate varies widely and depends on fish species and the test conditions (WHO, 1994) alongside the active ingredients present in the herbicide. Table 1 above shows that the water quality was within the recommended standard for test water (OECD, 1992). The present study showed that the 96 h LC_{50} of glyphosate herbicide on the juvenile was 300 mg/L. This was quite different from the works of Ayoola, 2008 who reported 96h LC_{50} of 0.063mg/l for glyphosate on post juvenile catfish and the study by Nwani, *et al*., (2010) reported LC_{50} of 32.54 mg/L for glyphosate on freshwater air-breathing fish *Channa punctatus* (Bloch). The LC_{50} for this experiment was higher than the value of 2-55 mg/L range reported by Hildebrand *et al*., (1982), Jiraungkoorskul *et al*., (2003) and Glusczak *et al.,* (2007) for other fish species exposed to glyphosate. This value was also lower than the concentration ranges reported in the previous study carried out by Nesković (1993) for *Cyprinus carpio* with LC_{50} 620 mg/l. The LC_{50} value for the study was quite higher than some previous studies and could be attributed to the difference in species, the weight of fish and the type of glyphosate herbicide used. Furthermore, the technical report presented by NRAAVC, 1997 revealed a range (300-600 mg/L) of LC_{50} of glyphosate to fish. Again the effect of temperature on modifying toxicity and the test organisms and chemicals was observed by Tsui and Chu, (2003). Non-photosynthetic organisms should be more tolerant to the toxicity of IPA salt of glyphosate (Folmar *et al*., (1979); Tsui and Chu, 2003) since the test organisms do not rely on the pathway of aromatic amino acids synthesis.

Table 2: Mortality rate of post Juvenile Catfish (*C. gariepinus*) exposed to glyphosate herbicide for 96 hrs

Concentrations (mg/L)	No. of Fishes	24hrs	48hrs	72hrs	96hrs	Total Mortality
Control	8	0	0	0	0	0
420	8	8	0	0	0	8
360	8	0	4	2	0	6
300	8	0	4	0	0	4
180	8	0	0	0	0	0
30	8	0	0	0	0	0

Glyphosate toxicity increased with increased concentration (Ayoola, 2008) aggravating several abnormal behaviours such as incessant jumping and gulping of air, restlessness, surface to bottom movement, sudden quick movement as well as resting at the bottom..These observations were absent in the control group. Similar observations were reported by Ayoola, (2008), Omoniyi *et al*., (2002), Rahman *et al.,* (2002) and Aguigwo (2002). Roundup used by farmers in the fields may leach into freshwater ponds as aerial drift or as run-offs. The toxic effect of glyphosate (360g/L) on post juvenile catfish in this experiment was both dose and time-dependent and agrees with the study carried out by Okonkwo *et al.,* 2010.

CONCLUSION

Findings in the present study showed glyphosate herbicide toxicity to Africa catfish (*C. gariepinus*) is both dose and time-dependent. Acute toxicity studies is recognized as an important step in determining water quality requirements of fish but also reveal toxicant concentrations (LC_{50}) that cause fish mortality. Therefore, further studies should be carried out to ascertain the toxicity of glyphosate on *C. Gariepinus* as determination of toxicant concentration in water may not provide information on the severity of contamination..

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