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Toxicity of Chlorpyrifos-Based Pesticide Formulation Termicot® on Non-target Organism (*Clarias gariepinus*)

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Abstract: Chlorpyrifos is a highly toxic organophosphate pesticide used in agriculture. The surface run-offs from the use of this insecticide enter the aquatic ecosystem where it adversely affects fish and other aquatic organisms. This study was carried out to investigate the toxicity of Termicot® in the African cat fish, *Clarias gariepinus*. Before the 96 h acute toxicity testing, the physicochemical parameters of the water were analyzed. 250 juveniles of *C. gariepinus* with 2-4 cm length were exposed to varying concentrations of Termicot®. Range finding test was followed by 96 h acute toxicity test. The test fishes were exposed to sub-lethal concentrations representing one quarter and one eighth of the LC₅₀ for fifteen days. Blood samples were collected from exposed fish on the 5th, 10th and 15th days and evaluated for the presence of micronuclei. After various trials in the range finding test, it was observed that 2.00 mg/l killed 75% of the test fish species while 0.50mg/l killed 25%. The LC₅₀ determined was 1.2811 mg/l. The frequency of induction of micronuclei significantly increased ($P < 0.05$) with increase of concentration and decreased with increase of the exposure period. It is recommended that Termicot® should be used where it is the only option, and it should be in controlled concentration.

Keywords: Pesticide formulation, Toxicity, Termicot®, *Clarias gariepinus*, Chlorpyrifos

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Introduction

One of the major health problems ravaging developed and developing countries is poisoning from pesticides. Organophosphate pesticides are a leading group of compounds

implicated in acute and chronic poisoning cases from exposure to pesticides (Kadam and Patil, 2016). Chlorpyrifos is an organophosphate insecticide with solubility of

2 mg/l in water. Chlorpyrifos, also known as 0,0-diethyl-0-3,5,6-trichlor-2-pyridyl phosphorothioate, is used to control insects both in the field and at home. It has also been used to kill mosquito larva in water bodies (WHO, 2014) and as a barrier against termites in, around or under buildings. It is one of the fastest selling organophosphate pesticides but its domestic use was restricted due to its toxicity. Notwithstanding, chlorpyrifos still remains one of the most widely used organophosphate insecticide in the world (Ambaili *et al.*, 2011). In Europe, it is one of the organophosphate insecticides that is still in use (Kralj *et al.*, 2007).

Chlorpyrifos degrade in the soil through the activities of microbes (WHO, 2012) and can persist in the soil for 30 – 60 days depending on the pH of the soil. Chlorpyrifos is very toxic to aquatic organisms, mobile in the environment and is among the most detected pesticides in streams, rivers, ponds and reservoirs (Phillips *et al.*, 2007; Ensminger *et al.*, 2011). Concentrations up to 0.4 µg/l chlorpyrifos has been detected in drinking water (USEPA, 1998). As a result of constant application of chlorpyrifos for control of insect pests, large quantities of the insecticides are released into the water bodies. The random use of this pesticide coupled with accidental spillage of untreated effluents into the natural water ways have deleterious effects on fish population and other aquatic organisms and may contribute to long term effects in the environment.

The routes of entry into the body include inhalation from the air, ingestion or dermal contact. Chlorpyrifos is transformed within the body of an animal into chlorpyrifos-oxon and 3,5,6-trichloro-2-pyridinol. These

transformation compounds are more toxic than chlorpyrifos itself (Siddiqua *et al.*, 2016). Chlorpyrifos is a nervous disruptor which acts by inhibiting the activity of acetylcholinesterase (AChE) by the active metabolite chlorpyrifos oxon. This is a reversible reaction in which Acetylcholinesterase, an enzyme that breaks down the neurotransmitter acetylcholine so that subsequent impulses can be transmitted across the synapse. Inhibiting the AChE therefore results in repetitive firing of neurons leading to death by asphyxiation as respiratory control is lost (Sparling and Fellers, 2007). AChE can be recovered when exposure is sub-lethal doses/concentrations (Giesy *et al.*, 1999; Ecobichon, 1991). The other transformation product of chlorpyrifos trichloropyridinol is less toxic than chlorpyrifos oxon (USEPA, 2008). Studies have shown the effects of chlorpyrifos in developing organisms to include persistent neurobehavioral dysfunction. Some of these effects are carried on to the adulthood (Levin *et al.*, 2004; Levin *et al.*, 2003; Richendrerfer *et al.*, 2012; Braquenier *et al.*, 2010; Risher *et al.*, 2010). Data on the neurotoxic effects of chlorpyrifos on fish is limited. Results from genotoxic studies of chlorpyrifos in fish are contradictory. Exposure of *Daphnia magna* to 0.05 g/l of chlorpyrifos resulted in reproductive impairment.

In *Channa punctatus*, exposure to chlorpyrifos caused single-strand breaks on DNA and it was reported to be concentration dependent (EPA, 1985; Poriccha *et al.*, 1998; Ali *et al.*, 2009). Several studies have also shown that chlorpyrifos and its formulations can be genotoxic to fish (Ali *et al.*, 2008; Yin *et*

al., 2009; Yong *et al.*, 2011; Sandal and Yilmaz, 2011). There is dearth of scientific information on the toxicity of chlorpyrifos on fish species of Nigeria.

Fish, especially *Clarias gariepinus* is a good source of protein for many communities in Nigeria and exposure to pesticide has far reaching effects on them and the food chain in general. Hence this present study was designed to investigate the acute toxicity of commercial formulation of chlorpyrifos (Termicot®) and its genotoxic effects on *Clarias gariepinus* in vivo.

Materials and Methods

Test Substance:

Commercial formulation of the test substance, Termicot® was purchased from Apo Agro Stores at New Market, Owerri, Nigeria. The pesticide contains a percentage active ingredient (chlorpyrifos) of 20%, emulsifier 6%, and solvent 76%. The product was formulated for AFCOTT® Nigeria Limited, Lagos, Nigeria, and manufactured by Heramba Industries Limited, Gujrat, India.

Test Animals:

The test animals were procured from Magnificat fish farms, Owerri, Nigeria. A total number of 250 post-fingerlings of *Clarias gariepinus* ranging from 2-4 cm long. Sexes were not biased in the course of collection. Likewise, the necessary physical observations were ensured to avoid selection of an infected fish. They were then transported to the fishery and aquaculture technology farm at FUT0 in open gallons.

The fish were introduced into a concrete pond in the farm and acclimatized for 10 days before commencement of the bioassays. In the

holding pond, the fish were fed 2, 3, and 4 mm of Coppens® and Skretting® pellets two times/day for the period of exposure. The water in the pond was changed every alternate day to ensure constant availability of oxygen in the water.

Study Design:

The study was completely randomized, divided into two phases; the first phase was the acute toxicity tests, while the second phase was the fifteen day sub-lethal exposure.

Determination of Physicochemical Parameters of the Test Water:

Determination of pH:

Determination of pH was done by dipping the electrode of pH meter into the pond of water and reading was observed from the indicator after a few minutes and recorded accordingly (Table 1).

Determination of Dissolved oxygen:

A graduated titration bottle was filled to 20 ml line with the sample water and titrated against sodium thiosulphate using starch as indicator. The solution was titrated until a clean colorless solution was obtained. The values were then recorded accordingly (Table 1).

Determination of Temperature:

Temperature was determined using a mercury-in-glass thermometer. This was done by inserting the thermometer into the pond and held for about five minutes, and its value read in the water and recorded in degrees Celsius (C) (Table 1).

Acute Toxicity Testing:

Prior to the acute toxicity testing, a range-finding test was done to determine which concentrations of the chemical would kill

Table 1: Physicochemical parameters of the test water

| Parameters | Control | | T1 (0.4 mg/l) | T2 (0.8 mg/l) | T3 (1.2 mg/l) | T4 (1.6 mg/l) | T5 (2.0 mg/l) |
|------------------|----------------|--------------|---------------|---------------|---------------|---------------|---------------|
| | Initial Values | Final Values | | | | | |
| Temperature | 27.0 C | 27.0 C | 27.2 C | 27.0 C | 27.4 | 27.3 C | 27.7 C |
| Dissolved oxygen | 7.6 ppm | 8.1 ppm | 6.2 ppm | 5.1 ppm | 4.2 ppm | 3.7 ppm | 2.8 ppm |
| pH | 6.9 | 6.7 | 6.5 | 6.8 | 6.8 | 6.9 | 6.9 |

Table 2: The distribution of the fish during acute toxicity testing using the independent variables experimental design

| | T ₁ | T ₂ | T ₃ | T ₄ | T ₅ | Control | Total |
|---|----------------|----------------|----------------|----------------|----------------|---------|-------|
| | 0.40 mg/l | 0.80 mg/l | 1.20 mg/l | 1.60 mg/l | 2.00 mg/l | 0 mg/l | |
| REPLICATES | | | | | | | |
| R1 | 10 | 10 | 10 | 10 | 10 | 10 | 60 |
| R2 | 10 | 10 | 10 | 10 | 10 | 10 | 60 |
| R3 | 10 | 10 | 10 | 10 | 10 | 10 | 60 |
| Total No of fish per concentration | 30 | 30 | 30 | 30 | 30 | 30 | 180 |

100% and 0% of the test species. In performing this experiment, two different bowls each containing 10 litres of water were used, with four fish introduced into each. The various concentrations of the pesticide were introduced last. Based on the outcome of the range finding test, five uniformly spaced concentrations were chosen for the acute toxicity testing. The different groups were labeled T₁, T₂, T₃, T₄, and T₅ representing 0.4, 0.8, 1.2, 1.6, and 2.0 mg/l of the chlorpyrifos formulation, respectively. This is shown in the Table 2.

The experiment lasted for 96 h. The solution for each concentration was renewed every alternate day with 10 litres of fresh water each into which the chlorpyrifos formulation in different concentrations were introduced. The fish were not fed during the period of exposure. After 96 h, the mean mortality from each dose and its replicates were calculated and used in further calculations. Dead fish were removed from the bowls on a daily basis to avoid fouling of the test media. The median lethal concentration (LC₅₀) of the test pesticide

formulation was obtained from the Acute Toxicity bioassays, following the Probit Analysis Method as described by Finney (1971). The safe level estimate was based on Sprague (1971).

Estimation of Micronucleus:

Based on the result of the LC₅₀, two sub-lethal concentrations representing one-tenth and one-twentieth of the LC₅₀ were chosen. Each group contained 10 fish in 10 litres of water; there were three replicates per concentration.

Blood was taken with fresh syringes containing heparin from the caudal vein on days 5, 10 and 15, from one representative fish per specimen. Thin smear of heparinized blood was made on a clean grease-free glass slide and air dried for 10 min. Four drops of Giemsa stain were placed on the smear and allowed to stand for 2 min followed by four drops of distilled water, and allowed to stand for 7 min, after which the slides were washed off with distilled water, and then ordinary water. The slides were allowed to air-dry 1 h and viewed under oil immersion using ×1000 magnification, and the result was expressed in percentage. Also, cell lesions of different shapes were also checked and recorded.

$$\text{Percentage induction of micronuclei} = \frac{\text{Number of micronucleated cells}}{\text{Total number of cells count}} \times 100$$

Statistical Analysis:

Statistical analysis of the data was carried using IBM SPSS statistics 20. ANOVA was employed to compare mean difference between different concentrations with time duration and between durations within concentration. Statistical significance was settled at probability value of $P < 0.05$.

Results

Acute toxicity:

After various trials in the range-finding test, it was determined that 2.0 mg/l killed 75% of the test species, and 0.5 mg/l killed 25%. The range of concentrations for the acute toxicity was then determined at 5 uniformly spaced concentration with a maximum concentration of 2.0 mg/l (Table 3, Figure 1).

Micronucleus:

The effects of chlopyrifos on micronucleus have been depicted in Table 4 and Figures 2 and 3.

Discussion

Pollution from natural sources can cause hazard to a large extent in aquatic organisms which are part of the food chain. In addition, anthropogenic activities pose more severe damage than is intended by man. There is a global concern on the environmental pollution from agricultural activities as well as from industrial effluents because of great damage it has caused to the aquatic environment and the disruption of the food chain.

This study which evaluated the acute toxicity and genotoxicity of Termifos® on *Clarias gariepinus* revealed that Termifos® is a very toxic formulation, with 96h LC₅₀ of 1.281 mg/l (1281.1µg/l). This value obtained is higher than 0.862 mg/l and 0.92 mg/l reported earlier by Nwani *et al.* (2013) and Ogueji *et al.* (2007), respectively; but lower than the 1.57 mg/l reported by Gul (2005) when *Clarias gariepinus* and *Oreochromis nilotica* were exposed to commercial formulation of chlopyrifos ethyl and chlopyrifos methyl. Bernabo *et al.* (2011) and Sparling and Fellers (2007) also obtained higher LC₅₀ values when they

Table 3: Cumulative mortality of fish during the 96 h Acute Toxicity Testing

| Number of specimen exposed | Concentration (mg/l) | Number of Deaths | | | | % survival | % mortality |
|----------------------------|----------------------|------------------|------|------|------|------------|-------------|
| | | 24 h | 48 h | 72 h | 96 h | | |
| 30 | 0.40 | 1 | 1 | 2 | 2 | 80.00 | 20.00 |
| 30 | 0.80 | 1 | 1 | 2 | 4 | 73.33 | 26.67 |
| 30 | 1.20 | 1 | 2 | 1 | 6 | 66.67 | 33.33 |
| 30 | 1.60 | 4 | 4 | 4 | 6 | 40.00 | 60.00 |
| 30 | 2.00 | 5 | 5 | 6 | 7 | 23.33 | 76.67 |

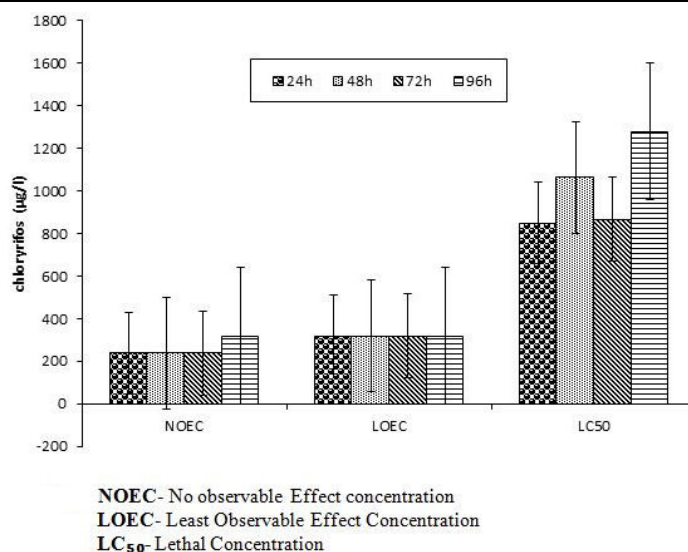


Figure 1: Statistical endpoints of acute toxicity testing for *C. gariepinus* exposed to chlorpyrifos for different durations (24 h, 48 h, 72 h, and 96 h)

Table 4: Induction of micronuclei in the erythrocyte of *C. gariepinus* exposed to chlorpyrifos for 15 days

| Pesticide Concentration (mg/l) | Micronuclei Induced | | | |
|--------------------------------|---------------------|--------------------------|--------------------------|--------------------------|
| | Day 0 | Day 5 | Day 10 | Day 15 |
| 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.16 | 0.00 | 4.07 ± 0.60 ^a | 3.57 ± 0.81 ^b | 3.33 ± 0.58 ^b |
| 0.32 | 0.00 | 4.57 ± 0.60 ^a | 4.37 ± 0.60 ^a | 4.07 ± 0.51 ^a |

Values are given as mean ± SD; superscripts with different alphabets are significantly different.



Figure 2: Pictograph showing non-micronucleated erythrocytes on Day 1: A) control; B) 0.16 mg/l concentration cell; C) 0.32 mg/l concentration cell.

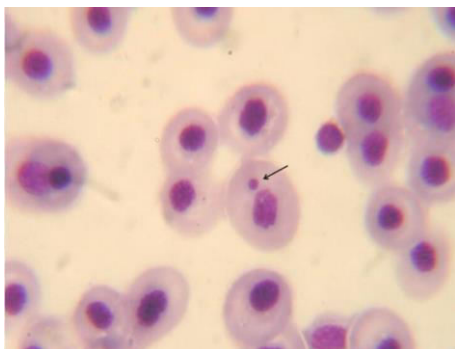


Figure 3: Pictograph showing cell with micronucleus

exposed the tadpoles of *Rana dalmatina* and *Rana boylei* to chlorpyrifos. These results show that different species can respond in completely different ways to a given xenobiotic. Another factor that may be responsible for the differences observed in LC₅₀ could be differences in the

physicochemical parameters in the various locations.

It was also observed in this study that the mortality rate as well as toxicity level was high at high concentration of chlorpyrifos formulation (Termifos®). Many behavioral and morphological changes were observed. On the addition of the pesticide into the test water, the fish were still, presumably trying to adjust to the xenobiotic introduced. Afterwards, the fish were seen to swim uncontrollably with some coming up to the surface, supposedly to gulp fresh oxygen. Increase in gill activity was also observed. There was an inverse relation between dissolved oxygen and increasing concentration of chlorpyrifos formulation. The average dissolved oxygen was higher in the lowest concentration; pH range was acid, tending to neutral.

The erythrocyte micronucleus assay (Mn) is one of the tests used to evaluate genetic damage. The sub-acute concentrations used in this study induced the formation of micronuclei in the erythrocyte of the test fish (*Clarias gariepinus*). Induction of micronuclei in this study was concentration dependent. A time – dependent decrease was observed. These same phenomena had been earlier reported by Bahari *et al.* (1994) in the same species. However, the findings of Utulu and Bakare (2010) showed a no dose-dependent response in rats treated with caffeine. Abara *et al.* (2014, 2018) recorded Mn induction which was both dose- and time-dependent when wistar rat was exposed to detergent and *Clarias gariepinus* to commercial formulation of organophosphate pesticide Best®, respectively.

The increase in the frequency of Mn in the erythrocyte of the exposed fish may be as a result of the disruption of the DNA repair process. The action of the genotoxic agents may give rise to increase in Mn frequency (Abara *et al.*, 2018). The Mn induction is a well-known biomarker for assessing the toxicity of exposure of organisms to xenobiotic. Some pesticides such as carbonate and dithiocarbamate have been reported to induce Mn formation in animals (Pacheco and Santo, 2002; Schmid, 1975).

Conclusion

It can be concluded from this study that commercial formulation of chlorpyrifos (Termifos®) is highly toxic to fish. Also, this pesticide has the capability of impairing behavioral, morphological and genetic activities of organisms. Hence, the use of Termifos® should be controlled and monitored to avoid deleterious effects on aquatic organisms.

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