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Toxicological effects of formaldehyde concentrations on African Cat fish, *Clarias gariepinus* fingerlings

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Abstract
The acute toxicity of formaldehyde on African catfish (*Clarias gariepinus)* fingerlings was studied using the static bioassay. The fishes were fed twice daily at 2.5% of their body weight for 21 days. The rate of fingerlings mortality increased with increasing concentration of formaldehyde. The first death was noticed two minutes after the introduction of toxicant in the bowl with the lowest concentration in (30.0 mg/L), other behavioural symptoms include restlessness, rapid body movement, and difficulty in respiration displayed by fishes moving to the surface to gulp air, intense opercula movement, accumulation of mucus on body and loss of equilibrium by swimming sideways. A log concentration probit regression analysis was significant ($p<0.05$) and yield a co-efficient of determination, $r^2 = 0.637$. The LC_{50} and 95% confidence limits to the concentration of formaldehyde was determined as 1.80 ± 0.25 ppm and LC_{50} interval of 0.706 – 2.094 ppm. The results showed the necessity to regulate the discharge of formaldehyde from domestic and industrial sources into aquatic systems, hence the need for caution on it uses even in fish harvesting.

Keywords: Toxicity, formaldehyde, *Clarias gariepinus*, probit, mortality.

1. Introduction
The use of synthetic chemicals such as formaldehyde as a means of increasing agricultural productivity has pose a serious treat and great consequences to the water bodies [4]. The application of formalin to fish pound to kill bacteria, fungi and others harmful micro-organism pose a great problem to human after the consumption of the affected fishes [11]. Owing to the increasing demand for fishes, the world market farmers continue to use Formalin and other chemicals to maximized harvest efficiency of the fish, although this may be detrimental to human health [15]. Ayuba *et al*., (2013) [3] showed that increase concentration of Formalin on aquatic water reduce the amount of oxygen circulated, causes respiratory distress among the fishes, loss of balance, gulping for air, vertical movement of fishes, excessive accumulation of mucus and death. Fish are a major test organism in ecotoxicological studies because of their link to man in the food chain [17]. Also, they are particularly useful for the assessment of waterborne and sediment-deposited toxins where they may provide advanced warning of the potential danger of new chemicals and the possibility of environmental pollution [21].
Clarias gariepinus are found throughout the world, especially in Africa and the Middle East (Froese and Pauly, 2014) [10] and live in fresh water lakes, rivers and swamps, as well as human made habitats such as oxidation ponds. The Africa catfish was introduced all over the world in the early 1980s for aquaculture purposes and is found in countries far outside its natural habitat such as Brazil, Vietnam Indonesians and Indians. In Africa the catfish has been reported as being second in size, although fish base suggest the African sharp-tooth catfish surpass that species in both maximum length and weight. It is very suitable for aquaculture as it grows fast and feed on a large variety of agricultural byproducts. It is hardy and can tolerate adverse water quality conditions [1]. It can be raise in high densities, resulting in high net yields (6-16 t/ha/yard). In most countries it fetches a higher price than tilapia as it can be sold live at the market. It matures and relatively reproduces in capacity; it tolerates the difficult situation in aquaculture [10]. Reports on the potential toxic effects of formaldehyde on fishes are limited, and none so far on the use of tropical fish as test organism, hence the need for this study.

2. Materials and Methods

2.1 Fish samples

Clarias gariepinus fingerlings were collected from hatchery complex of University of Calabar fish farm, Calabar, Cross River State with the aid of a scoop net in the early hours of the morning to avoid heat, high intensity and stress. They were transported to the laboratory in an open plastic bucket containing aerated habitat water. The *C. gariepinus* fingerlings were kept in a glass holding tanks for at last 21 days (three weeks) to allow them acclimatized to the prevailing laboratory conditions in one tank (30 x 30 x 50) cm 2/3 filled with water and continuously aerated with electrical air pumps. The fingerlings were fed with coppers feed containing 40% crude protein at 2.5% of body weight twice daily. *C. gariepinus* was selected because it is an ecologically and economically important group of the tropical inland waters.

2.2 Preparation of Test media

The formaldehyde was purchase from Victoria Equipment's Shop Calabar, Cross River State. Stocks of the test chemical were prepared by dissolving 0.2 ml of formaldehyde in 1000 ml of test water in a conical flask in accordance with Dede and Kagbo, (2001) [6]. The test concentrations were prepared by serial dilution. A wide range of concentrations were tested, including one which killed all the organisms within twenty (24) hours and another concentration which did not kill the organisms within 96 hours. Following this procedure, the appropriate test concentrations space at logarithmic intervals was selected for the bioassay.

2.3 Exposure of test organism

Appropriate graded test concentrations spaces at logarithms intervals to include one concentration showing 100 percent mortality, another with no mortality were used for the test. Tests were conducted in rectangular glass aquaria's with four litres of test solution. Twenty (25) fingerlings of *Clarias gariepinus* were exposed to formaldehyde as sub-lethal concentrations of 0.00 (control), 30.0, 50.0, 70.0 and 100.0 ppm of concentrations for four (4) days. All experiments were set up in three replicates. The volume of water to the weight of fishes was calculated in accordance with Reish and Oshida (1987) [22]. Careful observations were made to note the number of mortalities of the test organisms during the four days of exposure. *C. gariepinus* were considered dead if it remains motionless when touched with a glass rod.

2.4 Statistical Analysis

The mortality-concentrations data were subjected to probit transformation, regression analysis and LC_{50} values were computed using Predictive Analytical Software (PASW). The significant of the slope were tested using Chi-square. Graphs were drawn using Microsoft excel (version 2013).

3. Results

3.1 Manifestation time

Time–concentrations such as manifestation, overturning and survival times were carefully monitored and recorded. Manifestation time (the time interval between the initial exposure of fish to toxicant and the first appearance of symptoms of toxicity in fish) was observed to decrease with increase in concentration of formaldehyde (Table 1 and Fig.1).

Table 1: Manifestation, Overturning and Survival Time of *Clarias gariepinus* Exposed to concentrations of Formaldehyde

Concentration (ppm)	Manifestation Time (Hrs)	Over-turning Time (Hrs)	Survival Time (Hrs)
0.00	1.33	2.56	3.00
30.0	1.23	2.47	2.45
50.0	1.20	2.40	2.35
70.0	1.19	2.36	2.25
100.0	1.16	2.20	2.10

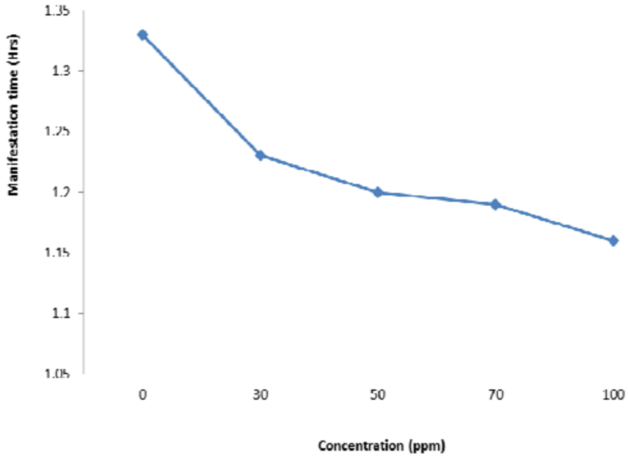

Fig 1: Relationship between concentration and manifestation time of *Clarias gariepinus* exposed to formaldehyde

3.2 Overturning time

Overturning time, (the time between the introduction of the toxicant and the loss of equilibrium or righting balance) showed a similar trend with manifestation time, decreasing with increase in toxicant concentration (Table 1 and Fig. 2). In all concentrations, overturning was preceded by uncoordinated, fast swimming bursts, swimming head up and tail down and swimming upside down.

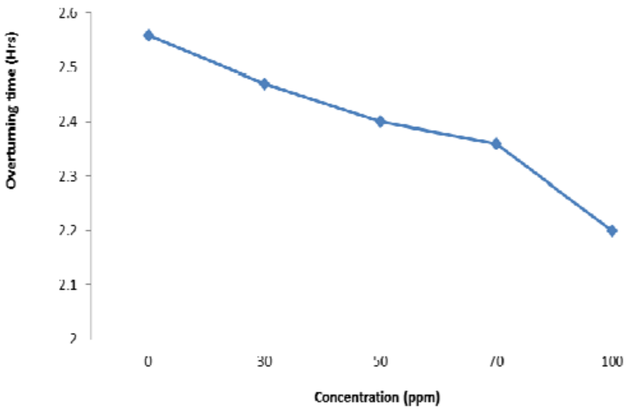

Fig 2: Concentration – Overturning time relationship of *Clarias gariepinus* exposed to concentrations formaldehyde

3.3 Survival time

Survival time, the time interval between the initial exposure of *Clarias gariepinus* to toxicant and the time the first mortality occurs. Survival time of *C. gariepinus* exposed to concentrations of formaldehyde decreased as exposure concentration increased (Table 1). Drops in survival times were steeper from 30.0 to 70.0 ppm but ended more gradually towards 100.0 ppm (Fig. 3).

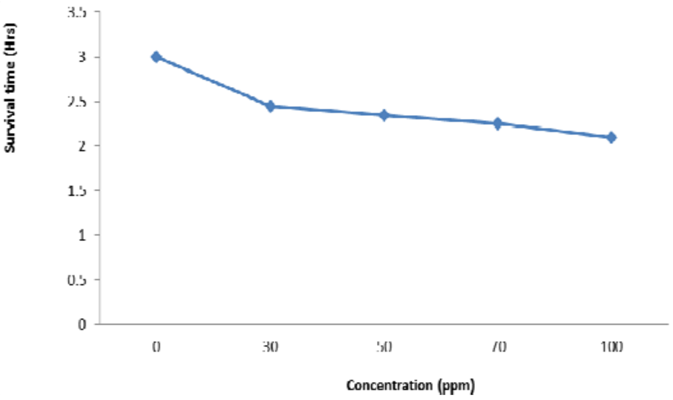

Fig 3: Survival times of *Clarias gariepinus* exposed to concentrations formaldehyde

3.4 Probit Analysis

Table 2 shows the results of probit analysis performed on mortality data. The trend in mortality data of fingerlings indicate that fingerling's mortality rate increased with increasing concentration of formaldehyde. Probit transformation of mortality rate-log concentration relationship determined to be linear by regression analysis. A log concentration probit regression analysis was significant at $p<0.05$ yield a co-efficient of determination, r^2 of 0.637, and the LC_{50} with 95% confidence limit was also determined to be 1.80 (Figure 4).

Table 2: Probit Transformation / analysis of mortality data of *C. gariepinus* exposed to concentration of formaldehyde

Conc (ppm)	Log. conc (x)	n	R	p	M_R	Y	R_P	*P*
0.00	0.00	25	0	0	0	0	0.00	0.00
30.0	1.4771	25	5	0.20	20	6.27	-1.269	0.251
50.0	1.6989	25	10	0.40	40	7.82	2.179	0.313
70.0	1.8450	25	15	0.60	60	17.10	-2.091	0.684
100.0	2.00	25	25	1.00	100	24.4	0.572	0.977

Where;
n = Number of fish fingerling tested at each concentration
r = Number of fish fingerling responding
p = Response rate, r/n
M_R = Mortality rate
Y = Expected probit from visual regression line
R_P = Residual probit
P = Probability

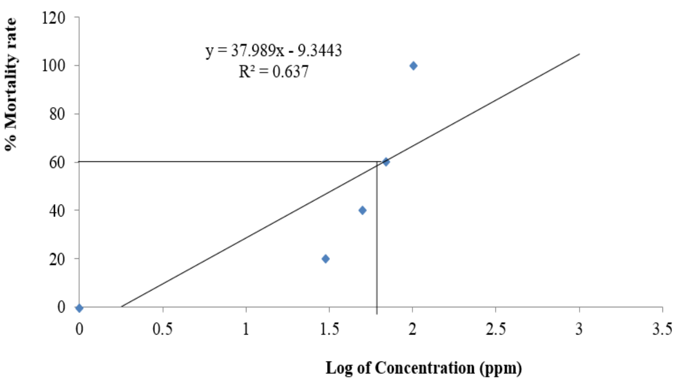

Fig 4: Probit graph of *Clarias gariepinus* fingerlings exposed to concentrations of formaldehyde

Table 3 shows the regression analysis of Log. Concentration – probit relationship of *Clarias gariepinus* fingerlings exposed to a concentration of formaldehyde, Table 4. shows the Chi-square Tests of *C. gariepinus* fingerlings exposed to concentration of formaldehyde and Table 5. shows the Covariance's and correlation of *C. gariepinus* fingerlings exposed to concentration of formaldehyde. The LC_{50} (medium lethal concentration) the concentration of the toxicant that kills 50% of exposed organisms) was determined by the method of Finney, (1971) [9] and the 95% confidence limit computed. The LC_{50} and 95% confidence limits for *C. gariepinus* exposed to concentration of formaldehyde was determined as 1.80 ± 0.25 ppm and LC_{50} interval of 0.706 – 2.094 ppm as shown in Table 6.

Table 3: The regression analysis of Log. Concentration – probit relationship of *Clarias gariepinus* fingerlings exposed to concentration of formaldehyde

Conc. (Log Unit)	Response rate, p	Equation	Co-efficient of determination, r^2	Significant level, α
0.00	0	9.344 + 37.989x	0.637	0.05 (S)
1.4771	0.20			
1.6989	0.40			
1.8450	0.60			
2.00	1.00			

Table 4: Chi-square Tests of *Clarias gariepinus* fingerlings exposed to concentration of formaldehyde

	Chi square	df[a]	Sig.
Probit Pearson Goodness-of- Fit Test	2.620	1	0.106[b]

Table 5: Covariance's and correlation of *Clarias gariepinus* fingerlings exposed to concentration of formaldehyde

Probit	Concentration	Natural Response
Concentration	16.63	0.891
Natural Response	0.588	0.026

Table 6: LC_{50} with 95% confidence limits of *Clarias gariepinus* fingerlings exposed to concentrations of formaldehyde

LC_{50} with ± 95%CL	Confidence limits	
	Lower	Upper
1.80 ± 0.25	0.706	2.094

4. Discussion

The exposure of formaldehyde to fishes showed restlessness, rapid body movement, and difficulty in respiration displayed by fishes moving to the surface to gulp air, intense opercula movement, accumulation of mucus on the body, loss of equilibrium by swimming sideways, finally fishes collapsed and died. The mortality recorded increased with a rise in concentration. The first death was noticed two minutes after the introduction of toxicant in the bowl with the lowest concentration in formaldehyde (30.0mg/L). Similar observation was also made by Ayuba *et al.*, (2013) [3] after introduction of Acute Toxicity of Formalin on Juveniles of *Clarias gariepinus*. Olaifa *et al.*, (2004) reported the first death of *C. gariepinus* in three hours after exposure to lethal and sub-lethal concentrations of copper, while, Guedenon *et al.*, (2012) [12] reported the first death after thirty hours while treating *C. gariepinus* with 120 mg/L of cadmium sulphate. Datta and Kaviraj, (2002) [5], Fafioye *et al.*, (2004) [8] and Okomoda *et al.*, (2010) [15] also recorded the first death in 36 hours after the exposure to acute toxicity treatments of *C.*

gariepinus with Synthetic Pyrethroid Deltamethrin, *Parkia biglobosa* and *Raphia vinifera* extracts and Formalin respectively.

The duration of resistance of *C. gariepinus* in this study appeared to be the lowest compared to previous studies. Although *C. gariepinus* has proved to be very resistant to various toxicants, it has shown very little resistance to formaldehyde. The LC_{50} value from the toxicity test revealed that *C. gariepinus* fingerlings were extremely sensitive to the formaldehyde. The regression equation obtained by Kori-Siakpere (2008) [14] when *C. gariepinus* fingerlings were exposed to potassium permanganate was $y = 1.38 + 7.84x$, $R^2 = 0.84$, while the present study was $y = 9.344 + 37.989x$, $R^2 = 0.637$. This disparity may be due to the difference in toxicant concentration and weight of the fish though both equations indicated that the mortality rate of *C. gariepinus* fingerlings and formaldehyde concentrations were positively correlated and increased mortality of the fish with increase in the concentration of toxicants. The 96hour LC_{50} had earlier been reported for *C. gariepinus* by Onusiruka and Ufodike, (1994) [19], to be 25.71, 26.92 and 8.3 mg/l for the floral parts of Akee Apple (*Blighia sapida)*, the bark of sausage plant (*Kigelia africana)* and the block of *Blighia sapida* respectively, while Oronsaye and Ogbebo, [20] (1995) reported that LC_{50} (96hour) for copper sulphate to be 0.4 mg/l for *C. gariepinus.* Also, Ayuba and Ofojekwu, (2002) [2] reported the 96hours LC_{50} of *C. gariepinus* fingerlings exposed to *Datura innoxia* root extracts to be 204.17 mg/L with lower and upper confidence limits being 125.89 and 384.59 mg/L respectively. The lower value in the 96 hour LC_{50} of the present study may be due to difference in toxicant concentration, acclamation period and the age of the fish.

Behavioral changes were observed such as jumping out due to skin irritation, restlessness, respiratory distress, loss of balance, gulping for air due to respiratory rate impairment, darkening of the body, sudden and quick movement, rolling movement, back stroke, excessive accumulation of mucus, all these ending in death. The reaction to the toxicant was more noticeable in the media containing the highest three concentrations of formaldehyde. These observations are similar to reports by Hirt and Domitrovic, (1999) [13], Oti, (2002) [18], Oshode *et al.,* (2008) [17], Ezike and Ufodike, (2008) [7] and Guedenon *et al*., (2011). The accumulation of mucus on the body could be connected to the intensification of mucus secretion of mucous cells activated by the toxicants (Omitoyin, 2007) [16]. The ensuing death might be due to increased heart failure, hypertension, gastric hemorrhage, convulsion, paralysis, heart failure and suffocation (Valee and Ulmer, 1972) [23].

5. Conclusion

The exposure of *Clarias gariepinus* to formaldehyde highlight the high toxicity of this chemical to fish. This information can be exploited by the aquaculturists to formulate the safety levels of formaldehyde into the water bodies. It also demonstrated the necessity to regulate the discharge of formaldehyde from domestic and industrial sources into aquatic systems. There is a need for further research on this area so as to establish standards for other tropical fishes which are meant for human consumption. Also, the toxicant has to be taken into more consideration as an environmental contaminant

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