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Effect of Chemical Corexit 9527 on 28 Day old *TILAPIA GUINEENSIS FINGERLINGS*

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ABSTRACT

The primary focus of this study is to assess the toxicity of the chemical corexit 9527 to 28 day old fresh water tilapia guineensis fingerlings. The toxicity of the chemical corexit 9527 to 28 day old fresh water tilapia guineensis fingerlings was studied in the static renewal bioassay glass tank, using tap water. The 28 day old tilapia guineensis fingerlings collected from Ikpoba Hill River, Edo State was used to access lethal concentrations of dispersant corexit 9527. Differential sensitivity of test organisms to corexit 9527 was observed. This was achieved by predictive application. The LC50 is actually used as an estimate of the incipient lethal level which is also called the lethal threshold concentration, asymptotic LC50 or tolerant limit. The incipient lethal level is the concentration at which half the population will die in an indefinite period of time. It is estimated by the LC50 at that time for which no further toxicity has been observed. This steady state LC50 is the incipient lethal level. In fish tests, this often occurs within 4 days, and this is one rationale behind the 96-hour length for a standard acute fish test.The median lethal concentration LC50 of dispersant corexit 9527 on tilapia guineensis was determined using a computerized probit analysis according to the methods of finney. The median lethal concentration, LC50 for the 28 day old tilapia guineensis were 409.71mg/l and 367.20mg/l for 3 and 4 days respectively. The 28 day old tilapia guineensis showed a high level tolerance to lower concentrations of corexit 9527 and high level toxicity at the highest concentration which recorded 100% mortality even at 24-hour exposure period.

Keywords: Bioassay, Chemical, Fingerling, Lethal, Tolerant, Toxicity.

1. INTRODUCTION

After oil spills, mechanical containment and recovery is normally used during clean-up exercise but there are situations during oil spills that the use of chemical dispersants was preferred. This is because the mechanical clean-up methods could only cover limited areas [1]. Chemical dispersant was commonly recommended as a first response option to oil spills since 1969 and have been used severally [2].Dispersants are a mixture of chemicals that contain surfactants which reduce the surface tension between oil and water and improve the formation of oil micro droplets that are easily biodegraded in the water column [3].Oil spill dispersants are controversial because unlike traditional clean up techniques, where booms and skimmers are used to remove oil altogether from water's surface, dispersants do not reduce the total amount of oil entering the sea; they accelerate the dispersion of oil from the sea surface into the water column. This helps to improve the dilution and biodegradation of the oil [4]. The chemical agents used as dispersants work by reducing the tension between oil and water, thereby improving the natural process of dispersion that takes place when waves mix large numbers of small oil droplets into the water beneath a spill [5]. It was recorded that during the Deepwater Horizon oil spill, a large volume of dispersants were used as a response tool to enhance the natural biodegradation of oil and to minimize oil impacts on aquatic habitats[6]. This act raised serious concerns about the potential toxicity of these chemicals to aquatic organisms [7].Toxicity of dispersants was characterized to a diverse number of aquatic species under controlled laboratory exposures using standard toxicity testing methods [8].

Toxic chemicals cause a wide range of direct and indirect negative effects on biological systems, ranging from cell to ecosystem. The severity of the effects depends on the type or properties of the chemical dispersant. It also depends on the dosage or duration

of exposure to ambient concentration. Bioassays are used to measure the magnitude of biological damage to benthic organisms, fish, earthworms and other organisms using mortality, impaired physiology, biological abnormality and unusual behavior as end point indicators [9].

EC 9527A Corexit 9527 is a mixture of surfactant ester and mixed oxygenated solvents mainly ethylene glycol monobutyl ether (EGMBE) at 38% v/v. It appears as clear to slightly hazy amber liquid glycol and has the same odour as ether [3]. Corexit 9527 is a third generation dispersant that is generally applied to oil spills in dilution with seawater [10]. Aquatic systems reflect perturbation in the environment, so fish and invertebrates can often be used as indicators to assess the health of an aquatic system because chemicals can accumulate in invertebrates and fish from the water and sediment to build up the food chain [11].

Chemical dispersants are used to break up the slick into a large number of small droplets as a way of dealing with oil spills passing over reefs or coming ashore. They are generally applied from boats or small aircraft, and they can be used on light, medium and heavy oil spills [12].The broken slick constitutes less of a physical risk to marine organisms but may transfer oil into shallow water reefs, water column and beaches; which likely has detrimental effects on marine organisms. Concentration of the dispersants may be sufficient to cause toxic effects which resist biodegradation and result to accumulation of these compounds in the environment. Dissolved aromatic components of dispersants destroy the chemoreception of various organisms which can lead to elimination of many species from the polluted areas since the feeding and mating response are dependent on chemoreception [13]. Pollution problems caused by organic chemicals to marine environment can be destructive to fish, birds, earthworms, amphipods and other marine organisms. When these organic chemicals are driven ashore, they can cause serious economic damage as well as rendering the water unfit for various uses [9].

Based on these facts, there is growing need to protect and preserve the environment from human activities that could lead to the disposal or use of these chemicals on both aquatic and terrestrial environment. The regulatory body in Nigeria, the Nigerian Midstream and Downstream Petroleum Regulatory Agency (NMDPRA) requires operators to undertake toxicity test of all low based oil, oil based mud systems, drilling fluid, chemical dispersants and any other chemical on the standard aquatic organism under Nigeria environment condition prior to any operation [14]. This is to ensure the sustainability of the environment since physical and chemical test do not reveal completely the toxic nature of these chemicals [3].The acute toxicity test remains a better screening technique and a comparative tool [2].
This research tends to help address concerns regarding acute toxicity of chemical dispersant, corexit 9527 on 28 day old *tilapia guineensis* in fresh water environment by renewal static bioassay to enhance operational decisions during an oil spill.

2. MATERIALS AND METHODS

The 28 day old fingerlings of*tilapia guineensis* used in this work were collected from a concrete pond inside the University of Benin, Nigeria. The fingerlings were collected with dip nets and transferred rapidly into plastic bags which were aerated before transferring to the laboratory. In the laboratory, the fingerlings were transferred into large plastic containers; which served as the holding tank with about two-third of the total volume filled with the tap water. The plastic containers were vigorously aerated with aerators equipped with air stones.The fingerlings were acclimatized to tap water from the laboratory and maintained under laboratory conditions for one week before the experiments [15].The fish were fed daily with fish meal in the morning during the period of acclimatization to prevent starvation. The water in the holding tank was changed daily in the morning to avoid contamination and pollution. During the holding period, the weak, diseased and paralyzed specimen were spotted and removed. The organisms were considered acclimatized when there was no mortality within a seven-day period.

The feeding stopped a day prior to the day of the experiment but water was changed. Previous experiments showed that 5 litres of water can support ten fish for more than five days without stress. Toxicity bioassays began by producing 5 litres of experimental media; which were produced by mixing (V: V) appropriate amounts of chemical and dilution water to obtain required concentrations. The test concentrations were obtained from a range finding test, which is a preliminary test conducted before the actual test to enable the achievement of appropriate dilutions. A broader concentration range was used for the range finding test to reduce the number of concentration to be tested and approximate the range that will produce a desired response. The test was run in 24 hours using different chemical concentrations with ten (10) fish per concentration. The result enabled a definitive acute toxicity test. Appropriate geometric dilution series was used in which each successive concentration was about 10 % of the previous one. 5 litres experimental media were made by mixing (V: V) of appropriate amounts of chemical dispersant and dilution water to obtain desired concentrations. The test solutions were prepared from the lowest concentration to the highest concentration to prevent contamination.

Bioassays were carried out by transferring the test organisms from the acclimatization tanks with a small hand net (to prevent water from the transfer container diluting the test solutions) into labeled five bioassay experimental glass tanks containing 5 liters

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of the media samples. The fish were randomly distributed, one at a time, until each tank has 10 fish. The hand net must not touch the test solution to avoid contamination of the fish in the holding tank. Hence, if contamination occurs, another hand net must be used. The tanks were washed with detergents and rinsed thoroughly with water. The tank preparation was done according to the method of United States Environmental Protection Agency [USEPA].All treatments were replicated three times of the test materials and two controls; dilution water with no toxicant served as control. Appropriate concentrations of the test materials based on the range finding toxicity trial were selected: 700mg/l, 350mg/l, 175mg/l, 87.5mg/l and 43.75mg/l. Test solutions were vigorously aerated and lighting was daylight supplemented by fluorescent lighting. The fish were not fed throughout the experimental period. Fresh test solutions were prepared and renewed daily throughout the experiment. The time when the first fish was transferred into the test solution was noted, which marks the time when the test started. The fish were monitored at 24 hours, 48 hours, 72 hours and 96 hours after commencing the test and observing mortality of the fish firstly in the control solution and then in the replicates for each test sample.

Symptoms of chemical toxicity and behavioural changes such as disorientation, weakness, increased irritability, inactivity and mortality counts were monitored on an hourly basis for 96-hour duration. Dead fish were removed using a disposable plastic spoon that can be used for one test sample and then discarded to prevent cross contamination. The dead fish were recorded during the period of the experiment [16]. The fact that there was no mortality in the control experiments suggests that the cleaning agent and dilution water had no effect on the mortality rate of the fingerlings. As a rule of thumb, a toxicity test is valid if control mortality is not more than 10 % [17]

3. RESULTS AND DISCUSSION

The summary of relative acute toxicity of dispersant Corexit 9527 on 28 day old*tilapia guineensis* is shown in table 1. The median lethal concentration LC50 was determined using a computerized probate analysis according to the methods of Finney. Mortalities recorded in the three test containers, tank A, tank B, and tank C for each concentration were determined separately and the average was calculated.

The percentage mortality in the three test containers, tank A, tank B and tank C for each concentration at different time intervals were calculated separately and the average was determined. Hence percentage mortality is given by

$$\%\text{mortality} = \frac{\text{No of Organisms dead}}{\text{Total No of organism tested}} \times 100\% \quad (1)$$

The percentage mortality at different time intervals of exposure are shown in Table 2, table 3, table 4 and table 5.

Table 1.1: Summary of Relative Acute Toxicity of dispersant Corexit 9527 on *Tilapia Guineensis*

Treatment	**Age of Organism (Days)**	**Time (Hours)**	**Freshwater Fish LC50 (95% C.L) mg/l**			
			Test Tank A	**Test Tank B**	**Test Tank C**	**Average**
Corexit 9527	28	24	ND	ND	ND	ND
		48	ND	ND	ND	ND
		72	ND	384.15(284.90-521.93)	435.27(325.56-590.52)	409.71(305.23-556.23)
		96	361.02(266.86-489.69)	384.15(284.90-521.93)	356.43(249.90-512.69)	367.20(267.04-508.10)

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Table 1.2: The Percentage Mortality at 24 hour Exposure

		No dead						%Mortality				
Conc. mg/l	**Log of Dose**	**No Tested**	**X1**	**X2**	**X3**	**Mean**	**SD**	**X1**	**X2**	**X3**	**Mean**	**SD**
0	0	10	0	0	0	0	0.00	0	0	0	0	0.00
43,75	1.64	10	0	0	0	0	0.00	0	0	0	0	0.00
87.6	1.94	10	0	0	0	0	0.00	0	0	0	0	0.00
175	2.24	10	0	0	0	0	0.00	0	0	0	0	0.00
350	2.54	10	0	0	0	0	0.00	0	0	0	0	0.00
700	2.85	10	10	10	10	10	0.00	100	100	100	100	0.00

Table 1.3: The Percentage Mortality at 48 hour Exposure

		No dead						%Mortality				
Conc. mg/l	**Log of Dose**	**No Tested**	**X1**	**X2**	**X3**	**Mean**	**SD**	**X1**	**X2**	**X3**	**Mean**	**SD**
0	0	10	0	0	0	0	0.00	0	0	0	0	0.00
43,75	1.64	10	0	0	0	0	0.00	0	0	0	0	0.00
87.6	1.94	10	0	0	0	0	0.00	0	0	0	0	0.00
175	2.24	10	1	1	2	1	0.58	10	10	20	13	5.77
350	2.54	10	0	3	2	2.5	1.53	0	30	20	25	15.28
700	2.85	10	10	10	10	10	0.00	100	100	100	100	0.00

Table 1.4: The Percentage Mortality at 72 hour Exposure

		No dead						%Mortality				
Conc. mg/l	**Log of Dose**	**No Tested**	**X1**	**X2**	**X3**	**Mean**	**SD**	**X1**	**X2**	**X3**	**Mean**	**SD**
0	0	10	0	0	0	0	0.00	0	0	0	0	0.00

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43,75	1.64	10	0	0	0	0	0.00	0	0	0	0	0.00
87.6	1.94	10	0	0	0	0	0.00	0	0	0	0	0.00
175	2.24	10	0	1	1	1	0.58	0	10	10	10	5.77
350	2.54	10	3	4	2	3	1.00	30	40	20	30	10.00
700	2.85	10	10	10	10	10	0.00	100	100	100	100	0.00

Table 1.5: The Percentage Mortality at 96hour Exposure

			No dead					**%Mortality**				
Conc. mg/l	**Log of Dose**	**No Tested**	**X1**	**X2**	**X3**	**Mean**	**SD**	**X1**	**X2**	**X3**	**Mean**	**SD**
0	0	10	0	0	0	0	0.00	0	0	0	0	0.00
43,75	1.64	10	0	0	0	0	0.00	0	0	0	0	0.00
87.6	1.94	10	0	0	0	0	0.00	0	0	0	0	0.00
175	2.24	10	1	1	2	1	0.58	10	10	20	13	5.77
350	2.54	10	5	4	4	4	0.58	50	40	40	43	5.77
700	2.85	10	10	10	10	10	0.00	100	100	100	100	0.00

NOTE:

X = dose concentration, mg/L

CL = confidence limit

ND = not detected

SD = Standard deviation

There was a dose dependent response and toxicity increased with exposure time. The mortality in 28 day old *tilapia guineensis*, was zero within 24hours of exposure in all the concentrations except the highest concentration (700mg/l) which recorded 100 % mortality. The survivorship of organisms in the other concentrations was >50%.

At 48 hours exposure, the fish continued to tolerate the solutions at lower concentrations. Hence, no mortality was recorded in the 43.75 mg/l, 87.5 mg/l and 175mg/l concentrations. 350mg/l concentration recorded 25 % mortality and 700mg/l concentration recorded 100 % mortality.

At 72 hours exposure, 175mg/l concentration recorded 10 % mortality, 350mg/l concentration recorded 30% mortality and 700mg/l concentration recorded 100 % mortality. The organisms were getting the effect of the toxicant gradually which resulted in the death of the organisms. The LC50 value was calculated.

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At 96 hours exposure, 175mg/l concentration recorded 13% mortality while 350mg/l concentration recorded 43% mortality and 700 mg/l concentration recorded 100 % mortality. LC50 was also calculated.

Throughout the test period, the control and lowest test concentrations recorded 100% survivorship; but all the organisms died in the highest test concentration(700mg/l) after 24 hours. Survivorship decreased at increased concentration of the toxicant and at each time interval the test was monitored.

4. CONCLUSION

The research has attempted to establish an organism/concentration response to dispersant Corexit 9527 on 28 day old *tilapia guineensis*. The toxicity of the chemical should not be underestimated even though 28 day old *tilapia guineensis*showed resistance to dispersant Corexit 9527 at lower concentrations, within 24hours of exposure. It is lethal to the test organism which by extension to other aquatic organisms. Therefore, it is established that chemical corexit 9527, though a surfactant, should not be considered as a response option to oil spills.

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