EJSIT
European Journal of Science, Innovation and Technology
ISSN: 2786-4936
www.ejsit-journal.com
Volume 3 | Number 2 | 2023

Toxicology Study of Dispersant Corexit 9527 to 14-day Tilapia Guineensis Fingerlings

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Abstract

The sensitivity of 14-day old fresh water tilapia guineensis fingerlings to dispersant corexit 9527 was studied in static renewal bioassay glass tank, using tap water to access the lethal concentrations of the chemical on the test organisms. The test organisms were collected from a fish pond in Warri, Delta State of Nigeria. In the most predictive applications, the LC50 is normally used as an estimate of the incipient lethal level, also known as the lethal threshold concentration, asymptotic LC50 or tolerant limit. In fish tests, the incipient lethal level often occurs within 4 days, and this is one rationale behind the 96-hr 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. This research showed that the median lethal concentration, LC50 for the 14-day old tilapia guineensis fingerlings were 54.82mg/l, 24.21 mg/l and 16.47mg/l for 2, 3 and 4 days respectively; and the 14-day old tilapia guineensis showed high sensitivity to the different lethal concentrations of corexit 9527 at 96-hr duration.

Key words: sensitivity, probit, lethal, asymptotic, threshold, tolerant, tilapia, static, bioassay, corexit 9527

Introduction

Application of chemical dispersants can be an effective means of accelerating the dispersion of oil from the sea surface when used adequately. This aids in the biodegradation of oil in the water surface (Swannel & Daniel, 1999). Chemical dispersant was recommended as a first response option to oil spills by the National Research Council in 1989 (NOAA, 2018). However, there is a divergence of opinion among the European Union (EU) member states on a recent inventory of national practices and policies relating to the use of dispersants as an oil spill response tool, which was undertaken by the European Maritime Safety Agency (EMSA) (EMSA, 2005). In most states, the use of dispersants is secondary to mechanical containment and recovery; and in several states, the use of dispersants is either not allowed or it is restricted. Oil spill dispersants are controversial because unlike traditional clean up techniques, where booms and skimmers are used in attempts to remove oil altogether from water's surface, dispersants do not reduce the total amount of oil entering the sea. Dispersants can dissolve a slick before it reaches the shoreline, where the oil smears marine mammals and turns beaches and coastal wetlands black (Wise & Wise, 2011). The chemical agents used as dispersants work by reducing the tension between oil and water, thereby enhancing the natural process of dispersion that takes place when waves mix large numbers of small oil droplets into the water beneath a spill (Mitchell & Holdway, 2000).

Toxic chemicals cause a wide range of direct and indirect adverse effect on biological systems, ranging from cell to ecosystem. The severity of the effects depends on the types and properties of the chemical and dosage or duration of exposure to ambient concentration. Parallel efforts by the international scientific communities have also emerged, enabling a greater understanding of the potential toxicological effects of dispersants to aquatic organisms (Hemmer et al., 2011). Most acute toxicity data in aquatic toxicology are generated from laboratory tests that are designed to maintain constant concentrations of the test material for the duration of the exposure period. These test conditions are necessary to establish an equilibrium between the exposure media and test organisms, thus ensuring that tissue body

burdens are sufficient to elicit a quantifiable toxicological response (Hansen, et al 2014). Bioassays are used to measure the magnitude of existing biological damage to benthic organisms, fish, earthworms and other organisms using mortality, impaired physiology, biological abnormality and behavioral aberration as end point indicators (Barron et al 2013).

Dispersants are a mixture of chemicals generally containing surfactants and solvents that reduce the surface tension between oil and water, and promote the formation of oil microdroplets that are more readily biodegraded in the water column (NRC, 2005). EC 9527A Corexit 9527 described as surfactant ester is composed of 2-butoxy ethanol and oxyalkylated organic ester. It appears as clear to slightly hazy amber liquid glycol. It has the same odor as ether which is used in disaggregation and dispersion of water.

Aquatic systems reflect perturbation in the environment, so fish and invertebrates can often be used to indicate the health of an aquatic system because chemicals can accumulate in invertebrates from the water and sediment and in fish from water and build up the food chain (Ezemonye & Olomukoro, 2000). 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. Once broken up, the slick poses less of a physical risk to seabirds or marine mammals but may transfer oil into the water column and beaches. Concentration of the dispersants alone may be sufficient to cause toxic effects. This resists biodegradation and they are metabolized incompletely with the result that some of these compounds accumulate in the environment. Dissolved aromatic components of these chemicals disrupt the chemoreception of various organisms which can lead to elimination of many species from the polluted areas since the feeding and mating response largely depends on the chemoreception (Mitchell & Holdway, 2000).

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 environments. The regulatory body in Nigeria, the Department of Petroleum Resources (DPR) in its guidelines and standards (2002) states that “operators shall be required 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”. This is to ensure the sustainability of the environment since physical and chemical test do not reveal completely the toxic nature of these chemicals.

This research tends to assess the acute toxicity of chemical dispersant, corexit 9527, on 14-day old tilapia guineensis in fresh water environment to determine the effect on survival and sensitivity of the fish by renewal static bioassay (ASTM, 1999).

Materials and Methods

Acclimatization of Test Organisms

The fish, tilapia guineensis used in this work were held in the laboratory tap water for one week prior to use in experiments and maintained under laboratory conditions similar to previous experiments (Ohonba & Oronsaye, 1999). In the laboratory, the fish were transferred into 150L plastic containers in which were vigorously aerated tap water which served as the holding tank. The fish were fed daily with fish meal in the morning and there was daily change of water which ensured that the water stayed clear to avoid pollution. During the holding period, the weak, diseased and paralyzed specimen were spotted and removed. Prior to the experiment, the test organisms were not fed but water was changed.

Toxicity Test

Toxicity bioassays began by carrying out a range finding test after which five (5) definitive tests were carried out and the tilapia guineensis were transferred from the

acclimatization tanks with a small hand net into five (5) bioassay experimental glass tanks containing 5 liters of the media samples. 5 liters' experimental media were made up by mixing (v: v) of appropriate amounts of chemical dispersant and water to obtain desired concentration. Five concentrations: 80mg/L, 40mg/L, 20mg/L, 10mg/L, and 5mg/L were selected for the experiment. Dilution water served as control. All treatments were replicated three times using ten test organisms for each treatment, which gives a total of one hundred and fifty test organisms. Test solutions were vigorously aerated and lighting was daylight supplemented by fluorescent lighting. Fresh test solutions were prepared and replaced daily throughout the experiment (Bejarano & Farr, 2013). The fish were not fed throughout the experimental period. Symptoms of toxicity and behavioral changes such as weakness, increased irritability, inactivity and mortality counts were monitored on an hourly basis for 96-hour duration. Dead fish were removed and recorded during the period of the experiment. A fish was considered dead when it ceases respiratory movement (George-Ares & Clark, 2000).

Results and Discussion

The LC50 and 95 % Confidence Limit

The LC50 and 95 % Confidence Limit for Corexit 9527 with (Tilapia Guineensis) for 24 hours, 48 hours, 72 hours and 96 hours are shown in Table 1, Table 2, Table 3 and Table 4 respectively. The mortality was very low in all the concentration within 24 hours of exposure. The lowest concentration, 5 mg/l recorded 10% mortality while the highest concentration 80 mg/l recorded 37% mortality. The effect of the dispersant was low; some organisms were seen at the bottom while others were seen at the surface. The organisms affected displayed frenzied swimming, moved to the surface, followed by loss of balance when the test tanks were approached; the fish became excited, irritated and hyperactive. Fish finally slowed down before death. Dead fish displayed open mouths.

At 24 hours' exposure, there was high survivorship and an LC50 value could not be calculated simply because survivorship was >50%.

At 48 hours' exposure, the lowest concentration recorded 10 % mortality while the highest concentration recorded 63 %. The organisms were getting weaker; some had blood on their gills. The survivorship was <50%, so LC50 was calculated and found to be 54.82 mg/l with confidence limit of 30.83 mg/l - 309.92 mg/l.

At 72 hours' exposure, the lowest concentration recorded 97 % mortality. The organisms were getting weaker, some had blood on their gills, the movement of some organisms was very slow and some organisms swam upside down and of course dead ones were seen. LC50 was calculated and found to be 24.21 mg/l with confidence limit of 14.63 mg/l - 43.60 mg/l.

At 96 hours' exposure, the lowest concentration recorded 30 % mortality while the highest concentration recorded 100 % mortality. At this stage, the organisms have had the full dose of the dispersant; all the test organisms were dead at the highest concentration. LC50 was calculated and found to be 16.47 mg/l with confidence limit of 10.40 mg/l - 25.10 mg/l.

Relative Acute Toxicity

The summary of relative acute toxicity of dispersant Corexit 9527 on tilapia guineensis is shown in Table 5. The median lethal concentration LC50 was determined using a computerized probit analysis according to the methods of Finney. Mortalities recorded in the three test containers, tank A, B, and C for each concentration were determined separately and the average noted. The percentage mortality in the three test containers, for each concentration at different time intervals were calculated separately and the average determined. Hence percentage mortality is given by:

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

The percentage mortality at 96-hour exposure is shown in Table 6.

Table 1: The LC50 and 95% Confidence Limit for Corexit 9527 with *Tilapia Guineensis* for 24 hours

Test Tanks	24hour Lc50	Min 95% Cl	Max 95% Cl	Probit Line Equation	Slope
1	ND	ND	ND	ND	ND
2	ND	ND	ND	ND	ND
3	ND	ND	ND	ND	ND
Mean	ND	ND	ND		ND
SD	ND	ND	ND		ND

Table 2: The LC50 and 95% Confidence Limit for Corexit 9527 with *Tilapia Guineensis* for 48 hours

Test Tanks	48hour Lc50	Min 95% Cl	Max 95% Cl	Probit Line Equation	Slope
1	57.96	32.66	290.67	Y = 2.062 + 1.666 LOG X	3.92
2	57.61	30.29	496.14	Y = 2.476 + 1.434 LOG X	4.91
3	48.87	29.53	142.94	Y = 1.806 + 1.891 LOG X	3.34
Mean	54.81	30.83	309.92		4.06
SD	5.15	1.63	177.38		0.79

Table 3: The LC50 and 95% Confidence Limit for Corexit 9527 with *Tilapia Guineensis* for 72 hours

Test Tanks	72 Hour Lc50	Min 95 % Cl	Max 95% Cl	Probit Line Equation	Slope
1	25.75	15.90	45.88	Y = 2.126 + 2.037 LOG X	3.06
2	20.52	12.22	34.77	Y= 2.358 + 2.014 LOG X	3.10
3	26.35	15.77	50.14	Y= 2.328 + 1.881 LOG X	3.36
Mean	24.21	14.63	43.60		3.17
SD	3.21	2.09	7.94		0.16

Table 4: The LC50 and 95% Confidence Limit for Corexit 9527 with *Tilapia Guineensis* for 96 hours

Test Tanks	96 Hour Lc50	Min 95 % Cl	Max 95 % Cl	Probit Line Equation	Slope
1	16.41	10.46	24.79	Y= 1.899 + 2.552 LOG X	2.44
2	15.35	9.76	23.01	Y= 1.920 + 2.597 LOG X	2.41
3	17.64	10.97	27.49	Y= 2.084 + 2.340 LOG X	2.65
Mean	16.47	10.40	25.10		2.50
SD	1.15	0.61	2.26		0.13

Note: X = dose concentration, mg/L; Y = probit; CL = confidence limit; ND = not detected; SD = Standard deviation

Table 5: 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			
Corexit 9527	14		Test Tank A	Test Tank B	Test Tank C	Average
		24	ND	ND	ND	ND
		48	57.97(32.66-290.67)	57.61(30.29-496.14)	48.87(29.53-142.92)	54.82(30.83-309.92)
		72	25.75(15.90-45.88)	20.52(12.22-34.77)	26.35(15.77-50.14)	24.21(14.63-43.60)
		96	16.41(10.46-24.79)	15.35(9.76-23.01)	17.64(10.97-27.49)	16.47(10.40-25.10)

Note: ND = Not detected

Table 6: The Percentage Mortality at 96hr Exposure

96 hours												
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
5	0.699	10	2	2	2	2	0.00	20	20	20	20	0.00
10	1	10	3	3	3	3	0.00	30	30	30	30	0.00
20	1.3	10	5	6	5	5	0.58	50	60	50	53	5.77
40	1.6	10	10	10	9	10	0.58	100	100	90	97	5.77
80	1.9	10	10	10	10	10	0.00	100	100	100	100	0.00

Conclusion

The study showed that 14-day old tilapia guineensis are very sensitive to dispersant Corexit 9527. The results of previous studies and the results from this work suggest that 14-day old may be a more appropriate life stage for assessing acute toxicity based on the regulatory approach of deriving risk from the most sensitive life stage of species. Their small size makes them convenient test organisms. Their widespread distribution, ease of collection from the field, ease of handling in the laboratory makes it possible to use large numbers of replicate, giving greater statistical validity to the results. They are more cost-effective to use in toxicological studies. The younger the organisms are, the less able they are to avoid toxicants due to immaturity of their tissues and cells.

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