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Preliminary Investigation of Comparative Toxic Effects of Locally Formulated and Commercial Demulsifiers on C-Callichytes Fingerlings

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Authors' contributions

This work was carried out in collaboration between all authors. Author RUD designed and managed the analysis of the study. Author CJU provided some of the journals and books used for the work. Author OA supervised the work and corrected the initial manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Demulsifier is one of the crude oil production chemicals that constitute the contaminants in the resulting produced water that is usually discharged into the marine environment. Most commercial demulsifiers list the toxic effects of individual components of their formulations which on application, may require the addition of one or more other chemicals. The synergic effects of these components in the final products are not usually accounted for. Different concentrations (10, 20, 30, 40, 50, 60 and 70 mg/l) of a typical commercial and locally formulated demulsifiers were application-ready prepared. Each concentration was introduced into an aquarium containing 20 *Callichthyes callichthyes* fingerlings with an average weight of 9.73 g and observed for 60 hours. The values for 36-hour LC_{50} of the formulated demulsifier was found to be higher than that of the commercial one

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by 15.75 mg/l, using Miller & Tainter method and 18.70 mg/l in the arithmetic of Karber method. These preliminary results show that there is a difference between the toxicity of the individual components of the demulsifiers and their final formulations or when mixed with other chemicals at application stages.

Keywords: Demulsifiers; crude oil; production chemicals; contaminants; marine environment; toxicity.

1. INTRODUCTION

Despite the organic and inorganic materials resulting from the geological location and formation of the oil fields and the type of hydrocarbon produced, production chemical compounds have been identified as one of the major sources of contaminants in produced water [1]. Ray and Engelhardt had advised that produced water discharged from petroleum production platforms in the offshore environment should not have adverse effects on the receiving water bodies or the surrounding aquatic life [2]. Although local and international regulatory agencies have put strict limits on levels of contaminants that can be discharged to the sea [3], the cost of treating produced water can be reduced with a careful selection of production chemicals employed.

Demulsifiers used for emulsion treatments are examples of some of these chemicals. They remain with the produced water and are discharged with it to the water bodies giving rise to some adverse effects on the biota [4,5]. Khan et al. [6] for example, reported the influence of crude oil effluent on mixed infections of Trichodina Cortinarius and T. saintjohnsi (Ciliophora) parasites on certain fish (Myoxocephalus octodecemspinosus and M. Scorpius) hosts. One of the concerns in the use of chemical demulsifiers, therefore, is the issue of increase in contaminants in the effluent water which will ultimately impact on the aquatic environment. As a result, it is necessary to determine the acute toxicity of formulated demulsifiers before they are approved for trial. Acute toxicity is a discernible adverse effect (lethal and sublethal) induced on the test organisms within a short period of exposure to a test material, usually ≤4 days for fish [7]. It is usually expressed as medial lethal concentration or dose, LC_{50} (or LD_{50}) which is the concentration of the test substance estimated to be lethal to 50% of the test population.

In the Material Safety Data Sheet (MSDS) of most commercial demulsifiers, the toxic impacts of only the individual components were provided with regards to the targeted environment. These do not account for the positive or negative synergy that may result in the final formulations or when these formulations will require further mixing with certain solvents before application. In this work, the toxic aqua effect of a typical commercial demulsifier was determined alongside another (PXPNG442) locally formulated in an earlier work [8].

2. MATERIALS AND METHODS

Fingerlings, Callichthyes callichthyes (C. callichthyes) species were purchased from the African Regional Aquaculture Centre (ARAC) at Aluu near The University of Port Harcourt and acclimated for 48 hours in the holding water and laboratory conditions. Three sets of ten fingerlings were weighed to estimate the average weight (9.73 g) of each. The commercial demulsifier was prepared based on a prescribed method for field application (Appendix B). Different concentrations (10, 20, 30, 40, 50, 60 and 70 mg/l) were measured into seven rectangular plastic aquaria (20 x 30 cm) containing 5000 ml of groundwater and thoroughly stirred. This was repeated in another set of seven with the same concentrations of the formulated demulsifier while the 15th aquarium was left free of the chemicals to serve as a control. A total of 300 fingerlings employed for the test were distributed (20 each) into each aquarium and labelled accordingly. They were observed for 70 hours and mortalities at six hours' intervals were recorded. The data obtained were converted to % mortalities and used to calculate medial lethal concentration (LC_{50}) using Tainter and miller graphical method and Arithmetic method of Karber [9,10].

3. RESULTS AND DISCUSSION

As shown in Tables 1 and 2, zero death was recorded for both demulsifiers at 10 mg/l concentration. Initial death records were observed for the commercial and formulated demulsifiers at 20 and 40 mg/l respectively. The commercial demulsifier showed the first 100% mortality at the 60th hour of 40 mg/l concentration

while that of the formulated demulsifier was observed at the 48th hour of the 60 mg/l concentration. The medial lethal concentration at the 36th hour (36-hour LC_{50}) reproduced in Table 3 was determined, using two methods:

3.1 Miller and Tainter Graphical Method

The % mortalities were transformed into probit values using appendix A which is then plotted against log concentration. The 0 and 100% mortalities were corrected with the formula below before taking their corresponding probit values.

$$\text{Corrected 0\% mortality} = 100(0.25/n) \quad (3.1)$$

$$\text{Corrected 100\%} = 100(n-0.25/n) \quad (3.2)$$

Where n is the number of population in a group [9].

From the graph (Figs. 1 and 2), the concentration corresponding to probit 5 (which is 50%) is taken as the 36-hour LC_{50}. The value for Log LC_{50} for commercial demulsifier is 1.5, and that of formulated demulsifier is 1.7

Therefore, 36-hour LC_{50} for the commercial and formulated demulsifiers are 31.6 and 50.1 mg/l respectively.

3.2 Arithmetic Method of Karber

The formula for the arithmetic method of Karber is

$$LD_{50} = LD_{100} - \Sigma(a \times b)\, n \quad [11] \quad (3.3)$$

Where,

n = total number of animal in a group.
A = the difference between two successive doses of administered extract/substance.
B = the average number of dead animals in two successive doses.
LD_{100} = Lethal dose causing the 100% death of all test animals.

Table 1. Concentration-time effect of commercial and formulated demulsifiers on *C. callichthyes* fingerlings

	Number of surviving fingerlings							
Concentration (mg/l)	**00**	**10**	**20**	**30**	**40**	**50**	**60**	**70**
Commercial demulsifier (CD)								
6 hours	20	20	20	20	19	16	11	00
12 hours	20	20	20	20	17	14	05	00
18 hours	20	20	20	18	15	11	02	00
24 hours	20	20	19	18	12	07	00	00
30 hours	20	20	18	16	09	05	00	00
36 hours	20	20	17	15	07	04	00	00
42 hours	20	20	17	13	06	01	00	00
48 hours	20	20	16	10	04	00	00	00
54 hours	20	20	16	70	01	00	00	00
60 hours	20	20	15	05	00	00	00	00
Formulated demulsifier (FD)								
6 hours	20	20	20	20	20	20	17	11
12 hours	20	20	20	20	20	20	15	07
18 hours	20	20	20	20	20	19	12	04
24 hours	20	20	20	20	20	17	10	00
30 hours	20	20	20	20	19	15	08	00
36 hours	20	20	20	20	17	12	05	00
42 hours	20	20	20	20	17	09	02	00
48 hours	20	20	20	20	14	05	00	00
54 hours	20	20	20	20	11	04	00	00
60 hours	20	20	20	20	09	01	00	00

Table 2. Mortality (%) of test population in commercial and formulated demulsifiers

	Mortality (%)							
Concentration (mg/l)	**00**	**10**	**20**	**30**	**40**	**50**	**60**	**70**
Commercial demulsifier (CD)								
6 hours	0	0	0	0	5	20	45	100
12 hours	0	0	0	0	15	30	75	100
18 hours	0	0	0	10	25	45	90	100
24 hours	0	0	5	10	40	65	100	100
30 hours	0	0	10	20	55	75	100	100
36 hours	0	0	15	25	65	80	100	100
42 hours	0	0	15	35	70	95	100	100
48 hours	0	0	20	50	80	100	100	100
54 hours	0	0	20	65	95	100	100	100
60 hours	0	0	25	75	100	100	100	100
Formulated demulsifier (FD)								
6 hours	0	0	0	0	0	0	15	45
12 hours	0	0	0	0	0	0	25	65
18 hours	0	0	0	0	0	5	40	80
24 hours	0	0	0	0	0	15	50	100
30 hours	0	0	0	0	5	25	60	100
36 hours	0	0	0	0	15	40	75	100
42 hours	0	0	0	0	15	55	90	100
48 hours	0	0	0	0	30	75	100	100

Table 3. 36-hour medial lethal concentration determination based on Miller and Tainter method

Group	Conc. (mg/l)	Log Conc.	% Mortality	Corrected %	Probits
Commercial demulsifier					
1	10	1.0	0	1.25	2.74
2	20	1.30	15	15	3.96
3	30	1.48	25	25	4.33
4	40	1.60	65	65	4.75
5	50	1.70	80	80	5.84
6	60	1.78	100	98.75	7.26
Formulated demulsifier					
3	30	1.48	0	1.25	2.74
4	40	1.60	15	15	3.96
5	50	1.70	40	40	4.75
6	60	1.78	75	75	5.67
7	70	1.85	100	98.75	7.26

Table 4 was reproduced from Table 1 to get the values for a and b.

For commercial demulsifier,

36-hoiur LC_{50} = 60 - (470÷20) = 36.5 mg/l

For formulated demulsifier,

36-hour LC_{50} = 70 - (355÷20) = 52.25 mg/l

For both Miller and Tainter graphical method and arithmetic method of Karber, the LC_{50} of the commercial demulsifier is higher than that of the formulated with a difference of 15.75 and 18.50 mg/l respectively.

Table 4. 36-hour LC_{50} determination based on arithmetic method of Karber

Conc. (mg/l)	Conc. difference	Death	Mean death	Mean death x Conc. difference
Commercial demulsifier				
0	-	0	-	0
10	10	0	-	0
20	10	3	1.5	15
30	10	5	4.0	40
40	10	13	9.0	90
50	10	16	14.5	145
60	10	20	18.0	180
				470.0
Formulated demulsifier				
0	-	0	0	0
10	10	0	0	0
20	10	0	0	0
30	10	0	0	0
40	10	3	1.5	15.0
50	10	8	6.0	60.0
60	10	15	11.5	115.0
70	10	20	17.5	175.0
				355.0

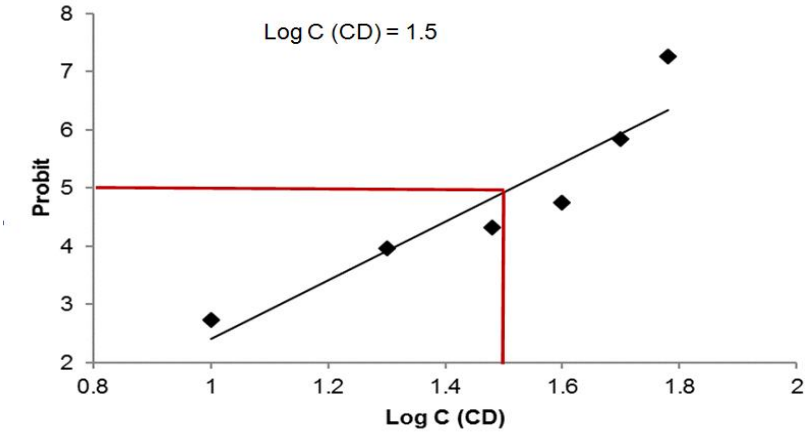

Fig. 1. 36-hour LC50 for commercial demulsifier

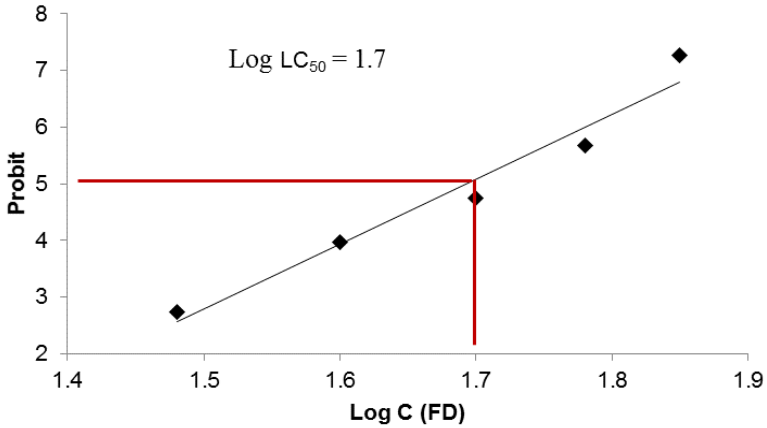

Fig. 2. 36-hour LC50 for formulated demulsifier

4. CONCLUSION

This work has shown that two or more relatively safe components of demulsifier may synergistically give rise to toxic effects when compounded or when the demulsifier is mixed with another chemical (s). It provides the need for the re-assessment of the environmental safety of demulsifiers and other crude oil production chemicals based on local applications irrespective of what is contained in the MSDS of the manufacturers. Except for the methanol component, the ecological toxicity test of the commercial demulsifier used in this work was not provided as shown in a section of the MSDS (appendix B), considering that produced water and the production chemicals contained therein, eventually end up in the water bodies. The results also reveal that the locally formulated demulsifier is less toxic compared to the commercial one with an LC_{50} difference of 16-19 mg/l. We, therefore, recommend that lethality of demulsifier formulations on aquatic biota should be determined on the final products, rather than its individual components.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Appendix A: Transformation of % Mortalities to Probits

%	0	1	2	3	4	5	6	7	8	9
0	-	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.72	4.69
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	52.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33

APPENDIX B: A Section of MSDS of the Commercial Demulsifier

Safety Data Sheet in accordance with Regulation (EU) No.453/2010

Substance key: 000000025500	Revision Date: 15.06.2015
Version : 4 - 0 / EU	Date of printing : 29.01.2016

6,65 mg/l, Rat)
Method : OECD Test Guideline 414
Source : literature
Route of application: oral (gavage)
NOAEL: no NOAEL defined (Exposure time : one time day 10 of gestation, Frequency of treatment: single treatment, Dose: 1027 - 2054 - 4108 mg/kg, Rat)
NOAEL (maternal): 2.054 mg/kg (Exposure time : one time day 10 of gestation, Frequency of treatment: single treatment, Dose: 1027 - 2054 - 4108 mg/kg, Rat)
Method : OECD Test Guideline 414
Source : literature

Toxicity to reproduction/fertility : Two generation study
NOAEL parent: 1,3 mg/l (Exposure time : F0<=108d, F1<=153d, F2<=56d, Frequency of treatment: ca. 20 h/day, Dose: 0,013 - 0,13 - 1,3 mg/l, Rat, male and female)
NOAEL F1: 0,13 mg/l (Exposure time : F0<=108d, F1<=153d, F2<=56d, Frequency of treatment: ca. 20 h/day, Dose: 0,013 - 0,13 - 1,3 mg/l, Rat, male and female)
NOAEL F2: 0,13 mg/l (Exposure time : F0<=108d, F1<=153d, F2<=56d, Frequency of treatment: ca. 20 h/day, Dose: 0,013 - 0,13 - 1,3 mg/l, Rat, male and female)
Method : OECD Test Guideline 416
Source : literature

Assessment of toxicity to reproduction : No reproductive toxicity to be expected.

Assessment of teratogenicity : No teratogenic effects to be expected.

Specific target organ toxicity (STOT) - single exposure :	Assessment :	Causes damage to organs.
Specific target organ toxicity (STOT) - repeated exposure :	Assessment :	The substance or mixture is not classified as specific target organ toxicant, repeated exposure.

Aspiration hazard :
No aspiration toxicity classification

SECTION 12: Ecological information

12.1. Toxicity

Information related to the product itself:

Fish toxicity :	not tested.
Daphnia toxicity :	not tested.
Algae toxicity :	not tested.
Bacteria toxicity :	not tested.

Safety Data Sheet in accordance with Regulation (EU) No.453/2010

Substance key: 000000025500 Revision Date: 15.06.2015
Version : 4 - 0 / EU Date of printing : 29.01.2016

Information related to the component: Methanol

Fish toxicity :	LC50 15.400 mg/l (96 h, Lepomis macrochirus (Bluegill sunfish)) Method : EPA Source : literature
Fish toxicity (chronic) :	NOEC 446,7 mg/l (28 d, Pimephales promelas (fathead minnow)) Method : Other Source : literature
Daphnia toxicity :	EC50 18.260 mg/l (96 h, Daphnia magna (Water flea)) Method : OECD Test Guideline 202 Source : literature The details of the toxic effect relate to the nominal concentration.
Daphnia toxicity (chronic) :	NOEC 208 mg/l (21 d, Daphnia magna (Water flea)) Method : calculated Source : literature
Algae toxicity :	EC50 (Growth rate) ca. 22.000 mg/l (96 h, Pseudokirchneriella subcapitata (microalgae)) Method : OECD Test Guideline 201 Source : literature
Bacteria toxicity :	EC50 > 1.000 mg/l (3 h, activated sludge) Method : OECD Test Guideline 209 Source : literature
Toxicity to soil-dwelling organisms :	LC50 > (48 h, Eisenia fetida (earthworms)) Method : OECD Test Guideline 207 Source : literature
Toxicity to terrestrial plants :	IC50 ca. 41.000 mg/l (3 d, Lactuca sativa (lettuce)) Method : Other Source : literature
Toxicity to other environmentally relevant organisms :	Not applicable
Sediment toxicity :	Not applicable

12.2. Persistence and degradability

Information related to the product itself:

Biodegradability :	Biodegradable

Information related to the component: Methanol

Photodegradation :	17,2 d 50 % Method : other (measured) Source : literature

Peer-review history:
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