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United Kingdom Toxicity Test with OSE II Summary

The United Kingdom Marine Management Organization, who's Marine Pollution Response Team, established the requirements to gain approval in the UK. This group required an efficacy test similar to the US EPA's efficacy test requirements to gain approval to the US NCP List. This group in the UK also required an Agitation Test, a Sea Test, and a Rocky Shore Test. The Agitation and Sea Test were to determine if OSE II was non toxic enough to be used on off shore, OSE II was tested with the species the brown shrimp (Crangon crangon), where OSE II proved it was non-toxic, and OSE II was approved for open waters in the UK.

A second test was performed on rocky shores to see if OSE II was nontoxic/safe enough to be used on UK shorelines. OSE II was utilized with Patella vulgata, and was shown to be nontoxic and safe therefore OSE II can be used on shorelines in the UK as well.

"3.2 There are 2 toxicity tests. The first test, the sea test, is carried out using the brown shrimp (Crangon crangon). This test compares the relative toxicity of an oil-product mix to that of the oil alone.

The second test is called the rocky shore test and is carried out using the common limpet (Patella vulgata). This test compares the toxicity of the product alone to that of the standard test oil."

OSE II is approved for use in the UK for open water and shorelines, once again proving to how safe and non-toxic OSE II is to marine species.

Steven Pedigo

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Approval for the use of oil spill treatment products in the sea under the provisions of the Marine and Coastal Access Act 2009

Approval reference	2 number	ODA 241/2015		4	
Name and address of approval holder	P.O. BOX	ater International Cor	rporation (OSEI Corp	oration)	
Postcode					
authority") in exerci Order 2011 approve	ise of the po es the use o	onment, Food and Ru ower conferred by Se f Oil Spill Eater II as a vaters adjacent to Sco	ection 15 of the Marir bioremediation proc	ne Licensing (Ex duct within Unit	empted Activities)
This approval shall r following condition		rce for a period of 5 y	years from the date g	given below sub	oject to the
with any subsequent icensing authority. 2. Only the product used on supplies of a supplies of the approval hold from that given in the icensing authority. The product must be obtained before the	t instruction label provide the product der shall no ne applicati if any change withdrawn product is ne name an	d address details mu	holder and accepted ited Kingdom. sition of the product, but the prior notifical added without the agress the agreement of the prior item.	al holder and ap by the licensin or the source of tion to and the eement of the light of the licensing of	proved by the g authority shall be of its raw material agreement of the icensing authority authority must be
Signature AM	14			Date	e 23/01/2015
[27.8.25	atherine M	orton	Marine	e Pollution Responder	

Approval for the use of oil spill treatment products (Revised 7 April 2011) Page 1 of 1

Toxicity Test Analysis v0.3

A204 Agitation test test

(96hrs exposure and 0 recovery)

Appendix (iv) 12/08/2014

Reference: Fresh Kuwait Crude 04/08/11: 5.4ml per tank

Tank no.	no. dead	no. alive	no. in tank	%Mortality
5	4	16	20	20.0
7	7	13	20	35.0
10	4	16	20	20.0
Total	15	45	60	25.00

Chi-squared 1.600 d.f. 2 p-value for chi-squared test 0.449

Testing at 5% significance level, Reference tanks are HOMOGENEOUS

! 3 tanks used

Test Treatment: Oil Spill Eater (557) : 5.4ml per tank Bioremediation , 50 parts water to 1 part OSE

Tank no.	no. dead	no. alive	no. in tank	%Mortality
1	3	17	20	15.0
4	2	18	20	10.0
8	6	14	20	30.0
9	5	15	20	25.0
Total	16	64	80	20.00

Chi-squared 3.125
d.f. 3
o-value for chi-squared test 0.373

Testing at 5% significance level,
Treatment tanks are HOMOGENEOUS
! 4 tanks used

COMPARISON OF MORTALITY RATES

Reference %mortality Treatment %mortality

D, Treatment %mortality - Reference %mortality -5.00

Standard error of D 7.16

95% Confidence interval for D -19.0

H0: treatment mort. = reference mort. , H1: treatment mort. > reference mort.

25.00

20.00

9.0

Test statistic -0.70 p-value = 0.758

Treatment mortality < reference mortality

Tanks where motors stopped have been removed from analysis

Notes: Tanks where motors stopped have been removed from analysis

Cefas CONFIDENTIAL Toxicity Test Analysis v0.3 C718 Sea to

Sea test test

(100min exposure and 24 hours recovery)

Appendix (iv)

29/10/14

Reference: Fresh Kuwait Crude: 18ml per 18L SW

Tank no.	no. dead	no. alive	no. in tank	%Mortality
2	2	18	20	10.0
5	6	14	20	30.0
7	5	15	20	25.0
9	0	20	20	0.0
13	5	15	20	25.0
Total	18	82	100	18.00

Chi-squared	8.537
d.f.	4
p-value for chi-squared test	0.074

Testing at 5% significance level,

Reference tanks are HOMOGENEOUS

Test Treatment: Oil Spill Eater (557) : 18ml per 18ml oil Type 2 , 10% in sea water

Tank no.	no. dead	no. alive	no. in tank	%Mortality
4	4	16	20	20.0
6	1	19	20	5.0
10	0	20	20	0.0
12	2	18	20	10.0
14	0	20	20	0.0
Total	7	93	100	7.00

Chi-squared	8.602
d.f.	4
p-value for chi-squared test	0.072
Testing at 5% significance level,	
Treatment tanks are HOMOGENEO	ous

COMPARISON OF MORTALITY RATES

Reference %mortality

18.00

Treatment %mortality

7.00

to

D, Treatment %mortality - Reference %mortality

-11.00

Standard error of D

95% Confidence interval for D

4.61

-20.0

 $\label{eq:H0:treatment} \mbox{H0: treatment mort.} \ \ -\ \mbox{reference mort.} \ \ \ +\ \mbox{H1: treatment mort.} \ \ -\ \mbox{reference mort.}$

Test statistic

-2.39

p-value = 0.991

-2.0

Treatment mortality < reference mortality

Notes:

Pass

Cefas CONFIDENTIAL Toxicity Test Analysis v0.3

B135

Rocky shore test test (6 hours exposure and 72 hours recovery)

Appendix (iv)

17/11/14

Reference: Fresh Kuwait Crude 04/08/11: 16ml per plate

Tank no.	no. dead	no. alive	no. in tank	%Mortality
1	8	12	20	40.0
4	16	4	20	80.0
5	17	3	20	85.0
9	18	2	20	90.0
12	18	2	20	90.0
Total	77	23	100	77.00

Chi-squared	20.102
d.f.	4
p-value for chi-squared test	0.000

Testing at 5% significance level,

Reference tanks are NOT HOMOGENEOUS

Test Treatment: Oil spill eater (557) : 16ml per plate Bioremediation , 10% in seawater

Tank no.	no. dead	no. alive	no. in tank	%Mortality
6	5	15	20	25.0
8	4	16	20	20.0
11	3	17	20	15.0
13	1	19	20	5.0
15	1	19	20	5.0
Total	14	86	100	14.00

Chi-squared	5.316
d.f.	4
p-value for chi-squared test	0.256

Testing at 5% significance level,
Treatment tanks are HOMOGENEOUS

COMPARISON OF MORTALITY RATES

Reference %mortality 77.00 Treatment %mortality 14.00

D, Treatment %mortality - Reference %mortality -63.00

Standard error of D 5.45

95% Confidence interval for D -73.7 to -52.3

H0: treatment mort. = reference mort. , H1: treatment mort. > reference mort.

Test statistic -11.55 p-value = 1.000

Treatment mortality < reference mortality

TEST INVALID: Reference tanks are not homogeneous

Reference tanks are not homogeneous, however the test treatment tanks are all lower mortality than the reference tanks. This will not be repeated, as it is a clear pass.

Cefas CONFIDENTIAL

Notes: