Assessing the Acute Toxicity of Herders to *Skeletonema sp* and *Tisbe battagliai* in Accordance with the UK Procedures for the Testing and Approval of Oil Spill Treatment Products

Tyler-Rose Woolley[1], Fern Lyne-Temple[1], Daniel Hill[1], Rob Holland[2]

[1] Scymaris Ltd., Brixham Laboratory, Freshwater Quarry, Brixham Devon TQ5 8BA UK, [2] Oil Spill Response Limited, United Kingdom

Introduction

A herding agent (or herder) is a chemical product containing a surface-active ingredient designed to reduce the surface tension of water and change how an insoluble liquid like crude oil spreads, causing oil slicks to rapidly thicken. Thicker oil slicks are more amenable to in-situ burning and are easier to recover by skimming. The use of a herder in a spill would always be guided by applied "Net Environmental Benefit Analysis" principles to ensure that no further harm is caused by the response technique (Ref 1). Herding agents can also be used to aid in the protection of sensitive resources e.g. saltmarshes. In this application, the herder is used to deflect oil away from these areas and reduce the impact risk. Herders are commercially ready for use; however, they require appropriate regulatory approvals before they can be considered for use in event of an oil spill.

Aims and Objectives

Based on assessments of products currently on the UK approved products list, the pass/fail criteria for the testing and approval of Oil Spill Treatment Products sets a threshold EC50 limit of >10 mg/L.

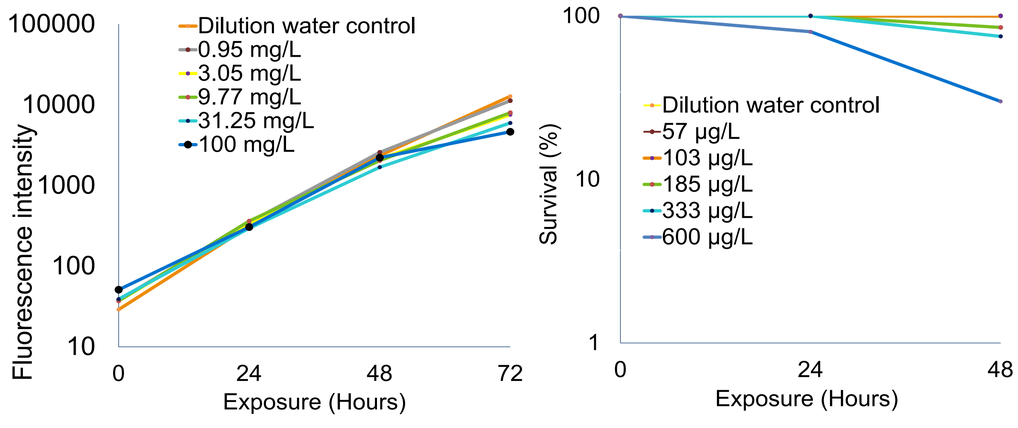
The acute toxicity of a herding agent to the marine alga *Skeletonema sp* and marine copepod *Tisbe battagliai* was determined in accordance with the UK procedures for the testing and approval of Oil Spill Treatment Products (CEFAS, 2020) (Ref 2) to allow submission of the product to the MMO (Marine Management Organisation).

Figure 1 Effect on fluorescence intensity of the marine diatom *Skeletonema sp.* over 0 - 72 hours

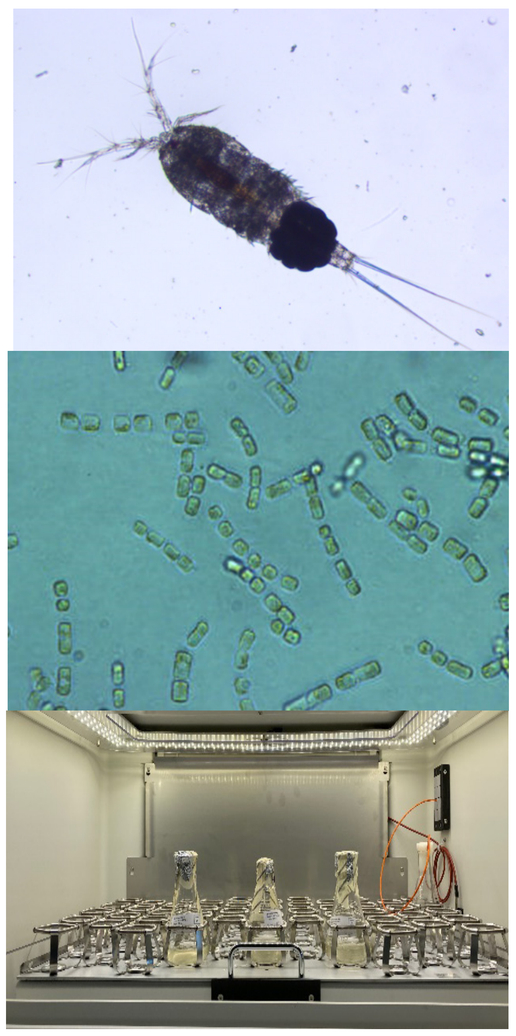
Nominal Loading rate (mg/L)	Replicate	Fluorescence intensity				
		0 hours	24 hours	48 hours	72 hours	72 hour % inhibition compared to the control
Dilution water control	A	27	329	2984	17621	-
	B	30	310	2319	9594	
	C	29	285	2006	13092	
	D	32	350	2515	8719	
	E	25	283	1888	12836	
	F	32	312	2361	14610	
	Mean	**29**	**312**	**2346**	**12745**	
0.95	A	40	349	2417	12226	12
	B	41	371	2634	10681	
	C	33	331	2666	10798	
	Mean	**38**	**350**	**2572**	**11235**	
3.05*	A	34	338	2388	8110	41
	B	34	337	2051	6964	
	C	42	329	1993	7498	
	Mean	**37**	**335**	**2144**	**7524**	
9.77*	A	38	341	1881	8404	37
	B	37	387	2372	8359	
	C	36	359	1848	7171	
	Mean	**37**	**362**	**2034**	**7978**	
31.25*	A	39	307	1676	5481	53
	B	35	281	1391	4845	
	C	44	297	1987	7540	
	Mean	**39**	**295**	**1685**	**5955**	
100*	A	63	304	2314	5984	64
	B	46	312	2364	2342	
	C	44	298	1959	5556	
	Mean	**51**	**305**	**2212**	**4627**	

Fluorescence intensity values are quoted to the nearest integer. * Significant difference ($p < 0.05$) from the dilution water control

Figure 2 Effect on growth of *Skeletonema sp* (left) and survival of *Tisbe battagliai* (right)



Methods



The *Skeletonema* test was run at a nominal temperature of 20±2°C for a duration of 72 hours. The test vessels were glass conical flasks closed with foam bungs. Containing 100 mL of test solution. Cell fluorescence was measured as a surrogate for algal biomass. Readings were taken at 24, 48 and 72 hours using a Tecan Infinite 200 pro Nano+ Microplate reader and the fluorescence intensity and growth rate were calculated for each loading rate. The loading rates employed were: Dilution water control (MM5) and nominal WAF (Water Accommodated Fraction) loading rates of 0.95, 3.05, 9.77, 31.25 and 100 mg/L.

The *Tisbe battagliai* test was run at a nominal temperature of 20±2°C for a duration of 48 hours. The test vessels were tissue culture wells with a working volume of 5mL within a 12-well plate, covered with loose fitting lids. *Tisbe* were assessed for mortality and symptoms of toxicity at 24 and 48 hours. The loading rates employed were: Dilution water control (seawater) and nominal WAF loading rates of 57, 103, 185, 333 and 600 mg/L.

Results and conclusions

The determination of acute toxicity to *Skeletonema sp* generated a fluorescence intensity (measured as a surrogate for algal biomass) EC50 of 25.2 mg/L and a growth rate EC50 of >100 mg/L (Ref 3). The determination of acute toxicity to *Tisbe battagliai* generated an LC50 of 447 mg/L (Ref 4).

Based on assessments of products currently on the UK approved products list, the pass/fail criteria for the testing and approval of Oil Spill Treatment Products sets a threshold EC50 limit of >10 mg/L. As the product exceeded this limit in both tests discussed, the criteria were passed, and the product would be suitable for addition to the UK approved products list.

Figure 3 Effect on survival of the marine copepod *Tisbe battagliai.* over 0 - 48 hours

Time (hour)	Nominal Loading rate (mg/L)	Total number tested	Total number of mortalities	Percentage mortality
24	Dilution water control	60	0	0
	57	20	0	0
	103	20	0	0
	185	20	0	0
	333	20	0	0
	600*	20	4	20
48	Dilution water control	60	0	0
	57	20	0	0
	103	20	0	0
	185*	20	3	15
	333*	20	5	25
	600*	20	14	70

* Significant difference ($p < 0.05$) from the dilution water control

References (1) Response strategy development using net environmental benefit analysis (NEBA) https://www.ipieca.org/resources/response-strategy-development-using-net-environmental-benefit-analysis-neba (2) Walton. H; Milliken. P & Kirby. M. 2020. Procedures for the testing and approval of Oil spill treatment products. Centre for Environment Fisheries & Aquaculture Science (CEFAS). (3) Scymaris (2022) ThickSlick 6535: Determination of acute toxicity to the marine alga Skeletonema sp. (non-GLP) Study number: 1120.00201. (4) Scymaris (2022) ThickSlick 6535: Determination of acute toxicity to Tisbe battagliai (Non-GLP). Study number: 1120.02701.