



World Class Science for the Marine and Freshwater Environment

# Procedures for the testing and approval of oil spill treatment products

# **Final**

Author(s): Helen E Walton, Paula Milliken & Mark F Kirby

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Compiled by:	Helen E Walton, Paula Milliken & Mark F Kirby
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## 1 Introduction

The Marine and Coastal Access Act, 2009 requires a license to be issued for the deposit of any substance or article in the sea. It also enables provision to be made by statutory instrument for exemption to this general requirement and as such a licence is not required for the deposit of a substance for the purpose of treating oil on the surface of the sea, subject to certain conditions. The decision of whether or not to use an oil spill treatment product (e.g. chemical dispersants) is made by the following licence authorities under the Act; Marine Management Organisation (MMO) (England), Natural Resources Wales, Marine Scotland and Department of Agriculture, Environment and Rural Affairs Northern Ireland (DAERA(NI)) in waters over which they have jurisdiction. However, before oil spill treatment products can be considered for use in UK waters, they must be on the UK approved products list. Only then can they be marketed and, ultimately, stockpiled for mobilisation in the event of an oil spill. The approved products list and approval process is managed by the MMO on behalf of all UK licencing authorities. This report explains the approval process with regards to toxicity tests and procedures.

## 1.1 Background

The primary purpose of using oil spill treatment products is to prevent an oil slick from reaching the coastline, impacting sensitive biota and to promote natural degradation. However, while the major toxicological issue is the oil, spill treatment products (e.g. dispersants) have been shown to have an inherent toxicity of their own and have the potential to affect the toxicity of the oil they are applied to. Since chemical products where first used, developments in the production of dispersants has resulted in the far less toxic second and third generation oil dispersants (European Maritime Safety Agency, 2009) which generally comprise of a surfactant and a solvent. The solvent facilitates the transport of the surfactant through the oil layer to the oil-water interface where the surfactant reduces the interfacial tension between the oil and water, encouraging the oil to disperse into smaller droplets. Whilst these smaller droplets are more bioavailable to marine organisms (Rico-martínez et al., 2013), the chemical dispersion results in faster dilution and biodegradation of the oil. Despite the evolution of oil dispersants, there is still a need to ensure the most environmentally acceptable products (e.g. lowest toxicity) are approved for use.

Previous experimental protocols for assessing whether products were suitable for inclusion in the UK approved list were established in the 1970s (Blackman et al., 1978) and have not been significantly updated since 1996 (Kirby et al., 1996). To address health and safety, cost and scientific robustness issues, the UK approach for oil spill treatment product testing and approval has been reviewed and updated. To provide more robust scientific advice for the risk assessments that enable effective decision making on the use of products in the event of a spill there has been a focus on methods that already have internationally accepted protocols. This standardisation of dispersant testing will promote more effective cross-institute comparisons of toxicity data and will enable further harmonisation of approaches in the future. Dispersants are not the only oil spill treatment products on the market; demulsifiers, surface cleaners, bioremediation products, sorbents and degreasers are also options and appropriate under specific scenarios. The choice of product depends on factors such as oil type, sea conditions and the surface that the oil has come into contact with (be it water, rocky shore or other) and therefore the decision on an appropriate oil spill treatment for use needs to take these factors into consideration in an overall risk assessment.



# 2 Pre-toxicity testing requirements

Approval to market a new or rebranded oil spill treatment product for use in UK waters must be made through an application to the MMO. The toxicity assessment to which this report refers, is the final stage in the approval process. The first step for any applicant and for any product type is the completion of the appropriate application form. This can be obtained along with any advice on the scheme (costs etc) from the MMO (Appendix 1). Prior to toxicity testing, oil dispersants must meet criteria regarding their performance and efficacy as determined by one of two accepted methods; i) that set out in annex 1 of the WLS Report LR448 'Specification for Oil Spill Dispersants' (LR448 Protocol, 1983), or ii) the Cefas 'Protocol for efficacy testing' based on the published 'Baffled Flask test for dispersant effectiveness' (Suhring et al., 2017). For other types of oil spill treatment product, where a standard method for efficacy does not exist, scientific evidence of their effectiveness for efficacy will be requested by the approval authority. For example, bioremediation product efficacy can be based on the OECD 306 marine biodegradation test (OECD 306, 1992) with modifications as necessary for poorly soluble substances. This approach can allow oil degradation, under standard conditions with and without the presence of the test product, to be compared and thus develop evidence that product enhances oil degradation.

# 3 Toxicity testing

#### 3.1 Rationale

The following methods act as a screening process to filter out the most toxic products and generate data that will feed into risk assessments to decide if a dispersant should be used in the event of an oil spill. The methods have been chosen to adhere to standardised protocols to ensure robust, repeatable, interpretable and comparable data and cost-effective tests.

#### 3.1.1 Test species

#### 3.1.1.1 Algal species

*Skeletonema* sp. (CCAP 1077/1C, NIVA BAC 1). *Skeletonema sp.* is an important and widely distributed phytoplankton species (phylum *Bacillariophyta*) in estuarine and coastal areas worldwide.

#### 3.1.1.2 Crustacea species

*Tisbe battagliai*: copepodids  $6 \pm 2$  days old. *T. battagliai* is a sibling species of the *holothuriae* group, has been found in shallow waters in coastal regions of Europe and the Atlantic coast of the USA.

#### 3.1.2 Apparatus

Ordinary laboratory apparatus as outlined in ISO 10253 and ISO 14669 guidelines, and in particular:

- Apparatus for measuring dissolved oxygen, salinity, temperature and pH;
- Low-power stereo microscope;
- Optical microscope;
- Haemocytometer;
- Temperature-controlled cabinet, incubator or room;
- Apparatus for measuring algal cell density Plate reader capable of measuring fluorescence;



- Apparatus for membrane filtration;
- Erlenmeyer flasks;
- 96 well-plates;
- Tissue culture 12 well-plates; and
- Ultrasonic bath or other apparatus for the preparation of stock solutions of poorly soluble substances.

#### 3.2 Procedures

#### 3.2.1 Dispersants

#### 3.2.1.1 Algae

The algae test should be run according to the guideline ISO 10253:2016 Water quality – Marine algal growth inhibition test with *Skeletonema* sp. and *Phaeodactylum tricornutum* with specifics as outlined below. The test species should be *Skeletonema* sp. strain CCAP 1077/1C or NIVA BAC 1.

#### Procedure in brief:

Growth medium should be prepared using natural sea water obtained from a non-polluted source and filtered through a 0.45 µm membrane filter in order to remove particulate material and algae. Then, 15 mL of nutrient stock solution 1, 0.5 mL of nutrient stock solution 2 and 1 mL of nutrient stock solution 3 (If the dispersant is considered to be suitably soluble, test solutions should be prepared on the day of testing by first preparing a stock solution with growth medium and dispersant. The pH of the stock should be  $8.0 \pm 0.2$ , if it is outside these parameters it can be adjusted using either hydrochloric acid or sodium hydroxide solution. The required test concentrations should be prepared from the stock solutions ensuring all solutions are thoroughly mixed. A concentration range should be selected based on the results of a preliminary test but with a smaller gap between test concentrations i.e. algae should be exposed to concentrations of the dispersant in a geometric series with a factor not exceeding 3.2 between concentrations. Enough of each test solution to make at least three replicates (each containing 100 mL) should be prepared. Controls should contain growth medium without dispersant. A minimum of six control vessels should be prepared (each containing 100 mL). The pH, salinity and temperature should be measured and recorded before inoculating with sufficient preculture to obtain no more than 10,000 cells per mL. The salinity should be 30 ± 5 ppt and temperature 20°C ± 2°C at the beginning of the test, but the pH should not be adjusted at this stage. 100 mL of the test solution should be added to each Erlenmeyer flask. Before inoculating the flasks, the same volume of inoculum that will be added should be removed so the final volume in each flask is 100 mL.

) should be added to the filtered natural sea water for every 1 L of growth medium required. Growth medium should be adjusted to pH of  $8\pm0.2$  with dilute hydrochloric acid or sodium hydroxide solution as required.

Table 1. Nutrient stock solutions from ISO 10253:2016 Water quality – Marine algal growth inhibition test with *Skeletonema* sp. and *Phaeodactylum tricornutum* guideline.

Nutrient	Concentration in stock	Final concentration in test
Stock solution 1		
FeCl <sub>3</sub> 6H <sub>2</sub> O	48 mg/L	149 μg/L (Fe)
MnCl <sub>2</sub> 4H <sub>2</sub> O	144 mg/L	605 μg/L (Mn)
ZnSO <sub>4</sub> 7H <sub>2</sub> O	45 mg/L	150 μg/L (Zn)
CuSO <sub>4</sub> 5H <sub>2</sub> O	0.157 mg/L	0.6 μg/L (Cu)
CoCl <sub>2</sub> 6H <sub>2</sub> O	0.404 mg/L	1.5 μg/L (Co)



H <sub>3</sub> BO <sub>3</sub>	1140 mg/L	3.0 mg/L (B)		
Na₂EDTA	1000 mg/L	15.0 mg/L		
Stock solution 2				
Thiamin hydrochloride	50 mg/L	25 μg/L		
Biotin	0.01 mg/L	0.005 μg/L		
Vitamin B <sub>12</sub> (cyanocobalamin)	0.10 mg/L	0.05 μg/L		
Stock solution 3				
K <sub>3</sub> PO <sub>4</sub>	3.0 g/L	3.0 mg/L; 0.438 mg/L P		
NaNO <sub>3</sub>	50.0 g/L	50.0 mg/L; 8.24 mg/L N		
Na <sub>2</sub> SiO <sub>3</sub> 5H <sub>2</sub> O	14.9 g/L	14.9 mg/L; 1.97 mg/L Si		

A pre-culture should be prepared two to four days before the beginning of the test to ensure cells are in exponential growth phase. The cell density of the existing algal cell culture should be calculated by performing multiple cell counts using an optical microscope and a haemocytometer and then approximately  $2 \times 10^3$  to  $2 \times 10^4$  cells per millilitre should be added to the pre-culture. The pre-culture should then be incubated under the same conditions as the test.

If the dispersant is considered to be suitably soluble, test solutions should be prepared on the day of testing by first preparing a stock solution with growth medium and dispersant. The pH of the stock should be  $8.0 \pm 0.2$ , if it is outside these parameters it can be adjusted using either hydrochloric acid or sodium hydroxide solution. The required test concentrations should be prepared from the stock solutions ensuring all solutions are thoroughly mixed. A concentration range should be selected based on the results of a preliminary test but with a smaller gap between test concentrations i.e. algae should be exposed to concentrations of the dispersant in a geometric series with a factor not exceeding 3.2 between concentrations. Enough of each test solution to make at least three replicates (each containing 100 mL) should be prepared. Controls should contain growth medium without dispersant. A minimum of six control vessels should be prepared (each containing 100 mL). The pH, salinity and temperature should be measured and recorded before inoculating with sufficient pre-culture to obtain no more than 10,000 cells per mL. The salinity should be  $30 \pm 5 \text{ ppt}$  and temperature  $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$  at the beginning of the test, but the pH should not be adjusted at this stage. 100 mL of the test solution should be added to each Erlenmeyer flask. Before inoculating the flasks, the same volume of inoculum that will be added should be removed so the final volume in each flask is 100 mL.

The test vessels should be covered to avoid airborne contamination and to reduce water evaporation, but they should not be airtight in order to allow  $CO_2$  to enter the vessels, the use of foam bungs for this purpose is advised. The test vessels should be incubated at  $20^{\circ}C \pm 2^{\circ}C$  and under continuous white light intensity of 60 to 120  $\mu$ mol/m²s, when measured in the photosynthetically effective wavelength range of 400 nm to 700 nm using an appropriate receptor. Algal cells should be kept in suspension by shaking at approximately 100 rpm.

Care should be taken to ensure even distribution of algal cells before calculating the cell density and whilst inoculating the flasks. The cell density should be measured in each test vessel (including the controls) after inoculation (0 hours) and at 24; 48 and 72 hours ( $\pm$  2 hours) by measuring fluorescence intensity on a plate reader. The cell density is measured at each timepoint from a small volume ( $\leq$ 5 mL) that is removed from each test vessel and not replaced. From each aliquot removed, 10 replicates of 200  $\mu$ L for each test vessel should be added to a 96 well-plate. The fluorescence intensity should be measured with an excitation filter at 440 and an emission filter at 670. The outermost wells of the 96-well plate are subject to refraction effects so should not be used for measuring cell density. These wells should have filtered seawater or growth medium added to them. To account for background fluorescence blank plates containing test solutions (or growth medium for the controls) without algal cells should be prepared at test initiation and read along with the test plates. The blank plates can be



stored in the incubator along with the test vessels and re-used at each time point. At test termination the pH should be measured at each concentration (including the controls) to ensure the pH did not increase by more than 1.0 from the test initiation.

Once the fluorescence intensity has been measured at each time point, these results should be tabulated and corrected for blank fluorescence. Then, the percentage inhibition should be calculated using the following formula:

$$\mu = \frac{\ln N_L - \ln N_0}{t_L - t_0}$$

#### where

t<sub>0</sub> is the time of the test start

 $t_{\text{L}}$  is the time of the test termination of the time of the last measurement within the exponential growth period

N<sub>0</sub> is the nominal initial cell density (fluorescence)

 $N_L$  is the measured cell density (fluorescence) at time  $t_L$ 

#### 3.2.1.1.1 Test validity

The test will be considered valid if the following conditions are met:

- The control cell density shall have increased by a factor of more than 16 in 72 h;
- The variation coefficient of the control specific growth rates should not exceed 7 %; and
- The control pH shall not have increased by more than 1.0 during the test.

#### 3.2.1.2 Crustacean

The crustacean test should be run according to the guideline ISO 14669:1999 Water quality — Determination of acute lethal toxicity to marine copepods (*Copepoda, Crustacea*) with specifics as outlined below. The test species should be *Tisbe battagliai*: copepodids 6 ± 2 days old taken from an in-house culture or reliable supplier.

#### Procedure in brief:

Dilution water can either be natural or artificial sea water. Both should be filtered through 0.45  $\mu$ m membrane filter and should be from the same source as water that has been found to support the culture organisms through at least two generations. The salinity should be between 29 – 36 ppt, the dissolved oxygen concentration should be above 80 % of the air saturation value and a pH of 8.0  $\pm$  0.3.

If the dispersant is considered to be suitably soluble, a stock solution should be prepared using dilution water on the day of testing. The pH of the stock solution should be  $8.0 \pm 0.3$ , if it is outside these parameters it can be adjusted using either hydrochloric acid or sodium hydroxide solution. The stock solution should be used to prepare the test concentrations with the same dilution water. A concentration range should be selected based on the results of a preliminary test but employing a smaller factor (typically 1.8 or 2) between concentrations. Ensure all solutions are thoroughly mixed. Water quality parameters should be measured and recorded from each concentration before addition of *Tisbe battagliai*. Salinity should be between 29-36 ppt, dissolved oxygen concentration should be above 80 % of the air saturation value, the temperature should be  $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ , but the pH should not be adjusted at this stage.



Disposable rigid plastic tissue-culture 12 well-plates that have been soaked in deionised water then rinsed with dilution water are recommended as test vessels. For each concentration, 5 mL of solution should be added to four replicate wells and an additional eight replicates of dilution water only should be used for controls. Excess solutions should be kept under the same conditions as the test vessels to measure the water quality parameters at test termination.

Five *Tisbe battagliai* copepodids  $6 \pm 2$  days old should be added to each well, taking care not to contaminate the culture pool with the test item and minimizing the quantity of water transferred. A dissection microscope should be used when adding copepodids to ensure the correct number are added to each well. The plates should then be covered with loose-fitting lids or covers. Stored the test vessels at  $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$  under a 16-hour: 8-hour light: dark cycle.

Observations should take place after 24 and 48 hours using a low-power microscope. The number of mortalities should be recorded. Mortalities are defined as those individuals showing no swimming or appendage movements within an observation period of 10 seconds. At test termination (48 hours), pH; temperature; salinity and dissolved oxygen should be measured from the excess solutions or from pooling the replicates at each concentration.

#### 3.2.1.2.1 Test validity

The test will be considered valid if the following conditions are satisfied:

- the dissolved oxygen concentration at the end of the test is greater than or equal to 4 mg/l; and
- the percentage mortality of the controls is less than or equal to 10 %.

#### 3.2.1.3 Reference test

A reference test will be run with 3,5-dichlorophenol on the same batch/culture of test organism as the dispersant test. This is done to confirm the organisms are in an appropriate health condition with normal levels of sensitivity.

For the reference test, prepare a 1000 mg/L stock solution and sonicate for approximately 5 minutes to ensure 3,5-dichlorophenol is in solution. From the stock solution prepare a 100 mg/L working solution. Use the working solution to prepare the test concentrations (e.g. 0.22; 0.46; 1.0; 2.2; 4.6; 10 mg/L). After stock preparation, the procedure for *Skeletonema* sp. or *Tisbe battagliai* should be followed.

For *Skeletonema* sp. the expected  $EC_{50}$  is 0.7 to 2.5 mg/L over 72 hours. For *Tisbe battagliai*, the reference chemical should give an  $EC_{50}$  of 1.1 to 3.5 mg/L over 48 hours. However, in-house control charts may be used where there is sufficient data from previous reference tests to inform on the normal response of in-house cultures. If the reference test  $EC_{50}$ s are exceeded or the  $EC_{50}$  is outside of two standard deviations of the control chart mean on two or more occasions, or outside of three standard deviations of the control chart mean on one occasion, then the test organisms cannot be assumed to be behaving normally and the tests must be repeated.

## 3.2.2 Sorbents, granular/powdery and insoluble products

When dealing with sorbents, granular/powdery substances or insoluble products Water Accommodated Fractions (WAFs) or Water Soluable Fractions (WSFs) should be used according to the guideline ISO 14442:2006 Water quality — Guidelines for algal growth inhibition tests with poorly



soluble materials, volatile compounds, metals and waste water. A WAF is an aqueous medium containing only that fraction of a substance which remains in the aqueous phase after the preparation procedure has been terminated. When filtered through suitable filters, water-soluble fractions (WSFs) are obtained.

#### Procedure in brief:

Measure the appropriate amount of test media followed by test substance into a cylindrical glass vessel of sufficient volume to contain the volume of liquid required for each test concentration with minimal headspace. The vessel should also be fitted with a drain port near the base, for example a glass aspirator is suitable. The vessel should then be sealed to prevent the loss of volatiles and placed in the dark to prevent photodegradation, before being stirred continuously (with suitable apparatus such as a magnetic stirrer platform with stirrer bar) for 20 to 24 hours. The stirring vortex should be maintained at one third the distance from the top to the bottom of the vessel unless an emulsion is formed in which case stirring speed and vortex depth should be reduced. Following the stirring period, vessels should be left undisturbed for 1 to 4 hours in the dark before being drawn off into test vessels avoiding the introduction of any insoluble material.

Where it is likely that small particles may end up in the test vessels as is the case with granular or powdery substances, WSF should be prepared to avoid interference with the *Tisbe battagliai* or the measurement of cell density. For WSFs the same preparation method is followed as for WAFs, however before drawing off the solution to test vessels, it should be filtered through a membrane filter or centrifuged to remove particles.

Where possible a WAF or WSF should be prepared for each test concentration separately and should not be diluted, however if lower concentrations do not allow for accurate weighing of the test item then serial dilution may be required. Following production of the WAFs or WSFs the same procedure for *Skeletonema* sp. or *Tisbe battagliai* as outlined in section 3.2.1 should be followed.

#### 3.3 PASS/FAIL criteria

EC<sub>50</sub>s should be generated for the *Tisbe battagliai* mortality and *Skeletonema* sp. growth inhibition using an appropriate statistical analysis. The most sensitive of the tests (i.e. the lowest EC<sub>50</sub>) will be used in the assessment to determine a pass or a fail of the product. Based on assessments of products currently on the UK approved products list, a threshold limit of 10 mg/L has been set. If the product has an EC<sub>50</sub> below the 10 mg/L threshold but within 20 % it may be retested, however if the repeat test fails to meet or exceed 10 mg/L a second time the product will fail the criteria. If a product exceeds the 10 mg/L threshold on either the first test or retest, then it will pass the criteria and can be added to the approved products list.

## 4 Application for addition to the approved products list

Toxicity testing can be undertaken either through an MMO commissioned laboratory or an alternative suitable facility. If the product has been tested in a laboratory not commissioned by MMO, the test reports should be included with the application form sent to the MMO (original test raw data may also be requested as part of the assessment). The MMO will charge a fee to assess that the test data conforms to standard protocols and quality. If a laboratory commissioned by the MMO is used, a sample of the product should be sent separately from the application form, to the test laboratory and



the MMO will charge a testing fee. Full details such as the size of sample required, and fee amounts are given on the application form or can be requested from the MMO.

The proposed product label must also be approved by the MMO and must contain:

- product name;
- name, address and daytime or out of office hours telephone number of manufacturer, importer or re-brander;
- list of ingredients;
- oil spill treatment product type dispersant type, sorbent, bioremediation agent or other;
- a warning against mixing the product with any other products;
- date of manufacture, batch number and expiry date (subject to extension);
- recommended storage instructions;
- risk symbol and description;
- instructions on use including a statement that the product should not be used in sea depths of fewer than 20 metres or within 1 nautical mile of such depths; and
- basic safety instructions or caution and any suitable chemical hazard signs.

The label should also meet the requirements of the Classification, Labelling, and Packaging (CLP) Regulations 2009

Once the application form has been received the MMO will:

- consider the application;
- examine the proposed labelling; and
- arrange for toxicity and efficacy testing to be conducted if required.

The product may be marketed for use in English and Welsh waters as soon as MMO issues the formal approval notice. However, the product should not be marketed for use in Scottish or Northern Irish waters until the applicant has also received confirmation of the approval from Marine Scotland or DAERA. Details of the product and the name and address of the approval holder will be registered, and this information will be made publicly available on the approved oil spill treatment products list.

Approval must be renewed after 5 years, or sooner if specified, and the MMO must be notified of any change in the company's name, address or the product's composition. Renewing or rebranding an existing approved product will normally be considered on the basis that the product's composition is not significantly changed.

# 5 Approval for use of approved products

The MMO acts on behalf of the UK for testing and adding products to the approved oil spill treatment products list intended for use in all UK waters. Products that make it onto the approved products list can be marketed for use in UK waters and can be considered as a response option for UK based spills subject to further risk assessments, where the type of spill, location and weather conditions are also taken into account. Before a product can be used to treat an oil spill the user must have approval from the licencing authority who has jurisdiction over the waters it is to be used in and the product must only be used in line with conditions of its approval. Approved oil spill treatment products can only be



used without consulting with the licencing authority where there is a genuine risk to human life or to the safety of an installation or vessel. However, the relevant licencing authorities should be notified as soon as possible after the incident. Further information on the approval to use products from the approved products list can be obtained from the MMO (Appendix 1).



## 6 References

Blackman, R.A.A., Franklin, F.L., Norton, M.G., Wilson, K.W., 1978. New Procedures for the Toxicity Testing of Oil Slick Dispersants in the United Kingdom. Mar. Pollut. Bull. 9, 234–238.

European Maritime Safety Agency, 2009. Manual on the Applicability of Oil Spill Dispersants - Version 2.

ISO 10253: 2016 Water Quality – Marine algal growth inhibition test with *Skeletonema sp.* and *Phaeodactylum tricornotum* 

ISO 14442: 2006 Water Quality – Guidelines for algal growth inhibition with poorly soluble materials, volatile compounds, metals and waste water

ISO 14669: 1999 Water Quality - Determination of acute lethal toxicity to marine copepods (Copepoda, Crustacea)

Kirby, M.F., Matthiessen, P., Rycroft, R.J., 1996. Procedures for the approval of oil spill treatment products, Fisheries Research Technical Report.

LR448 Protocol, 1983. Specification for Oil Spill Dispersants. Appendix A to WSL. Report LR 448 (OP) revised February 2007. Available at:

https://www.gov.uk/government/uploads/system/uploads/attachment\_data/file/308092/approval\_lr448.pdf, Accessed date: 13<sup>th</sup> March 2020

OECD Guideline for Testing of Chemicals 306, 1992. Biodegradability in Seawater Closed Bottle Method

Rico-martínez, R., Snell, T.W., Shearer, T.L., 2013. Synergistic toxicity of Macondo crude oil and dispersant Corexit 9500A Ò to the Brachionus plicatilis species complex (Rotifera). Environ. Pollut. 173, 5–10. https://doi.org/10.1016/j.envpol.2012.09.024

Suhring, R., Smith, A., Emerson, H., 2017. Qualification of Oil-spill Treatment Products. Protocol for efficacy testing.



# **Appendix 1: Contact addresses for further information**

For further information regarding running of the approval scheme (i.e. policy, costs, etc) contact:

Marine Management Organisation (MMO)
Lancaster House
Hampshire Court
Newcastle upon Tyne
NE4 7YH
United Kingdom
Marine Conservation and Enforcement Team: 0300 123 1032
dispersants@marinemanagement.org.uk

To report a marine pollution incident to the MMO call:

- 0300 200 2024 within office hours
- 07770 977 825 outside office hours
- 0345 051 8486 or 0845 051 8486 at all times if other numbers out of order

For further information regarding toxicity and efficacy test procedures contact:

Centre for Environment Fisheries and Aquaculture Science (Cefas) Lowestoft Pakefield Road Lowestoft Suffolk NR33 OHT Tel: +44 (0) 1502 562244

ecotox@cefas.co.uk



# **Appendix 2: Safety requirements and procedures**

## Handling of bacterial bioremediation products

#### 1. Hazard

These products may contain a variety of bacterial species that have the potential to cause infection. Therefore, precautions are required to ensure that the risk of any infection is minimised. All bioremediation products must have their species compositions identified and categorised into hazard groups by the National Collection of Industrial and Marine Bacteria (NCIMB) or a similar organisation before a sample will be accepted. Only products that contain bacteria, categorised in hazard groups 1 and 2 (Advisory Committee on Dangerous Pathogens categorisation), as shown below, can be accepted as sufficiently low risk:

Hazard group 1: An organism that is most unlikely to cause human disease

Hazard group 2: An organism that may cause human disease and which might be a hazard to laboratory workers but is unlikely to spread to the community. Laboratory exposure rarely produces infection and effective prophylaxis, or effective treatment is usually available.

#### 2. Safety requirements and procedures

Any product sent to the laboratory must be properly labelled and include any necessary hazard warnings and a safety data sheet. On receipt, the product must be placed and stored in a fume cupboard until use. The user must ensure the container is properly labelled and details of arrival, use and disposal are kept in a record book.

- Benches/worksurfaces used in making up dilutions etc. should be easy to clean, impervious
  to water and resistant to acids, alkalis, solvents and disinfectants. Use of a clean disposable
  surface such as benchcote is suggested.
- Access to laboratories where tests are taking place should be limited to authorised personnel only.
- The laboratory must contain a washbasin which should be located near the laboratory exit.
- Taps must be of a type that can be operated without being touched by hand.
- An autoclave for the sterilisation of waste materials and solid glassware must be readily available.
- The laboratory door must be closed whilst work is in progress.
- Laboratory coats (preferably side or back fastening) must be worn in the laboratory and removed when leaving the tank room area.
- Eating, chewing, drinking, storing of food and application of cosmetics must not take place in the laboratory.
- Disposable gloves must be worn at all times. Hands must be disinfected or washed immediately when contamination is suspected, after handling infective materials and also before leaving the laboratory.



- In general, work may be conducted on the open bench, but care must be taken to minimise the production of aerosols. All vigorous shaking or mixing must take place in a fume cupboard.
- Effective disinfectants must be available for routine disinfection and immediate use in the event of a spillage.
- All bench tops must be disinfected after use.
- Used laboratory glassware and other materials awaiting sterilisation must be stored in a safe manner. Pipettes, if placed in disinfectant, must be totally immersed.
- Material for autoclaving must be transported to the autoclave in robust containers without spillage.
- All waste materials must be made safe before disposal including test organisms by autoclaving or disinfection as appropriate.
- Floors and floor drains must be disinfected after test solutions have been poured to waste.
- All accidents and incidents must be immediately recorded in the accident record book and the line/lab manager and health and safety officer informed as soon as possible.
- No person should be working on their own in the laboratory when directly handling these materials.





## About us

We are the Government's marine and freshwater science experts. We help keep our seas, oceans and rivers healthy and productive and our seafood safe and sustainable by providing data and advice to the UK Government and our overseas partners.

We are passionate about what we do because our work helps tackle the serious global problems of climate change, marine litter, over-fishing and pollution in support of the UK's commitments to a better future (for example the UN Sustainable Development Goals and Defra's 25 year Environment Plan).

We work in partnership with our colleagues in Defra and across UK government, and with international governments, business, maritime and fishing industry, non-governmental organisations, research institutes, universities, civil society and schools to collate and share knowledge.

Together we can understand and value our seas to secure a sustainable blue future for us all, and help create a greater place for living.

#### **Head office**

Pakefield Road Lowestoft Suffolk **NR33 0HT** 

Tel: +44 (0) 1502 56 2244 Fax: +44 (0) 1502 51 3865

## Weymouth office

Barrack Road The Nothe Weymouth DT4 8UB

Tel: +44 (0) 1305 206600 Fax: +44 (0) 1305 206601

Innovative, world-class science is central to our mission. Our scientists use a breadth of surveying, mapping and sampling technologies to collect and analyse data that are reliable and valuable. We use our state-of-the-art Research Vessel Cefas Endeavour, autonomous marine vehicles, remotely piloted aircraft and utilise satellites to monitor and assess the health of our waters.

In our laboratories in Lowestoft and Weymouth we:

- · safeguard human and animal health
- · enable food security
- · support marine economies.

This is supported by monitoring risks and disease in water and seafood; using our data in advanced computer models to advise on how best to manage fish stocks and seafood farming; to reduce the environmental impact of man-made developments; and to respond to serious emergencies such as fish disease outbreaks, and to respond to oil or chemical spills, and radioactivity leaks.

Overseas our scientists currently Commonwealth countries, United Kingdom Overseas Territories, South East Asia and the Middle East.

Our customer base and partnerships are broad, spanning Government, public and private sectors, academia, non-governmental organisations (NGOs), at home and internationally.









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