Procedures for the approval of oil spill treatment products

M. F. Kirby, P. Matthiessen and R. J. Rycroft

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INTRODUCTION

The Food and Environment Protection Act, 1985 (Great Britain - Parliament, 1985) requires a licence 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. Such exemptions are currently contained in the Deposits in the Sea (Exemptions) Order, 1985 and the Deposits in the Sea (Exemptions) Order (Northern Ireland), 1995. The 1985 and 1995 Orders provide, amongst other things, that 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 Ministry of Agriculture, Fisheries and Food (MAFF), the Scottish Office - Agriculture, Environment and Fisheries Department (SOAEFD) and the Department of the Environment for Northern Ireland (DOE(NI)) are the Licensing Authorities under the Act and the Act requires that they must approve all oil treatment products for use in waters over which they have jurisdiction. This report explains the approval process and gives details of the toxicity test procedures.

The Final Report of the Government Review on the Testing, Approval and Use of Oil Dispersants (MAFF, 1996) made a number of recommendations affecting approval of oil dispersants. In addition toxicity testing protocols have recently been developed to assess new types of oil treatment products such as sorbents and bioremediation agents. This document reflects these changes. It supersedes Fisheries Research Technical Report No. 39 - New procedures for the toxicity testing of oil slick dispersants (Blackman *et al*., 1977). Until April 1996 all toxicity assessments were carried out by the MAFF, Directorate of Fisheries Research (DFR). However, from that date appropriate toxicity testing can be carried out by suitably accredited or recognised laboratories, as an alternative to DFR, and results submitted to MAFF for consideration. This document is designed as an aid to manufacturers and potential testing laboratories.

Under the scheme there is a need to assess and approve all types of oil treatment products. At present four different groups are recognised; dispersants, sorbents, bioremediation agents and miscellaneous.

APPROVAL REQUIREMENTS

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 Ministry of Agriculture, Fisheries and Food, Marine Protection (Policy) Branch, Environmental Protection Division, London (see Appendix 1).

1. PRE-TOXICITY TESTING REQUIREMENTS

All chemical dispersants need to undergo efficacy testing procedures before toxicity assessment. The efficacy tests were developed by AEA Technology and advice on test procedures can be obtained from them (see Appendix 1). The current test specification for dispersants is LR 448 (Morris and Martinelli, 1983). Efficacy testing is not currently required for sorbent or miscellaneous (e.g. gelling agents, demulsifiers, herders, surface cleaners etc.) products although MAFF will request appropriate information on a case by case basis and may review this situation at a later date. Bioremediation products will also require an efficacy assessment before they are toxicity tested. Further details can be obtained from AEA Technology (see Appendix 1).

In addition to efficacy and toxicity testing, bio-remediation agents require more extensive assessment than other products due to the greater concern expressed with regard to the addition of biological agents or microbial activity enhancing agents to the natural environment. There are two recognised types of bioremediation agent.

Bacterial based - These involve the addition to the oil of pre-cultured bacterial assemblages either as a pre-prepared broth or a dry powder.

Nutrient based - These comprise of nutrient formulations designed to stimulate indigenous bacterial growth.

Some bioremediation products may comprise of both bacterial and nutrient elements.

Prior to efficacy or toxicity assessment, bacterial based bioremediation products must be subject to a basic microbiological hazard assessment by the MAFF Fish Diseases Laboratory (FDL) Weymouth to ensure that the microbial strains present do not constitute a pathogenic risk to humans, fish or shellfish. In order for this to be done the applicant needs to provide:

(i) a full bacterial species analysis;

(ii) relative species ratios (i.e. concentrations of each species in the final product);

(iii) intended dose ratios to the marine environment;

(iv) safety procedures and precautions required during the preparation and application of the formulation; and

(v) a quality control assurance from the manufacturer that the composition of the product is consistent over time in microbiological terms (i.e. a guarantee that the bacterial composition is consistent between different batches and that potential pathogens cannot adventitiously enter the system).

Once a product is deemed to be of acceptable microbiological hazard to the marine environment and to users it then has to be subject to efficacy testing as mentioned above.

The hazard assessment is only required for bacterial based products. Nutrient based products do not require this preliminary assessment.

2. TOXICITY TESTING

Once a product has met the required efficacy criteria, it has to undergo a toxicity assessment. All products (except sorbents and some miscellaneous products) have to pass two tests in recognition of the different environments in which they may be used; the Sea Test and the Rocky Shore Test (formerly referred to as the Beach Test). Dispersants must pass both tests before an approval can be granted; sorbents must pass the Sea Test but only need to pass the Rocky Shore Test if they are to be used in this environment. Bioremediation agents must also pass two additional agitation toxicity tests.

2.1 Reference oil

The oil used in all toxicity tests is Kuwait crude (see Appendix 1). This medium crude oil has been used extensively in toxicity studies on the effects of dispersants (Portmann and Connor, 1968; Dicks, 1973; Wilson, 1974) for many years at the MAFF Fisheries Laboratory, so a good reference base of results exists. On reception, a fresh batch of test oil must be decanted into smaller air-tight storage containers that are filled to the brim to avoid losses of volatile fractions during storage. They must be stored in a cool area and a new container used for each test. New oil batches must be chemically analysed by gas chromatography (GC-FID) or gas chromatography-mass spectrometry (GC-MS) and the chromatogram examined to confirm that no loss of volatiles has occurred. Such a loss of volatile components (weathering) can result in a dramatic reduction in oil toxicity. Figure 1 shows chromatograms of whole (Figure 1(a)) and weathered (Figure 1(b)) Kuwait crude oil. The chromatograms in Figure 1 were produced with the following gas chromatograph set up: Hewlett Packard 5890a GC coupled to a Hewlett Packard 7673 a auto-injector, on-column injector and

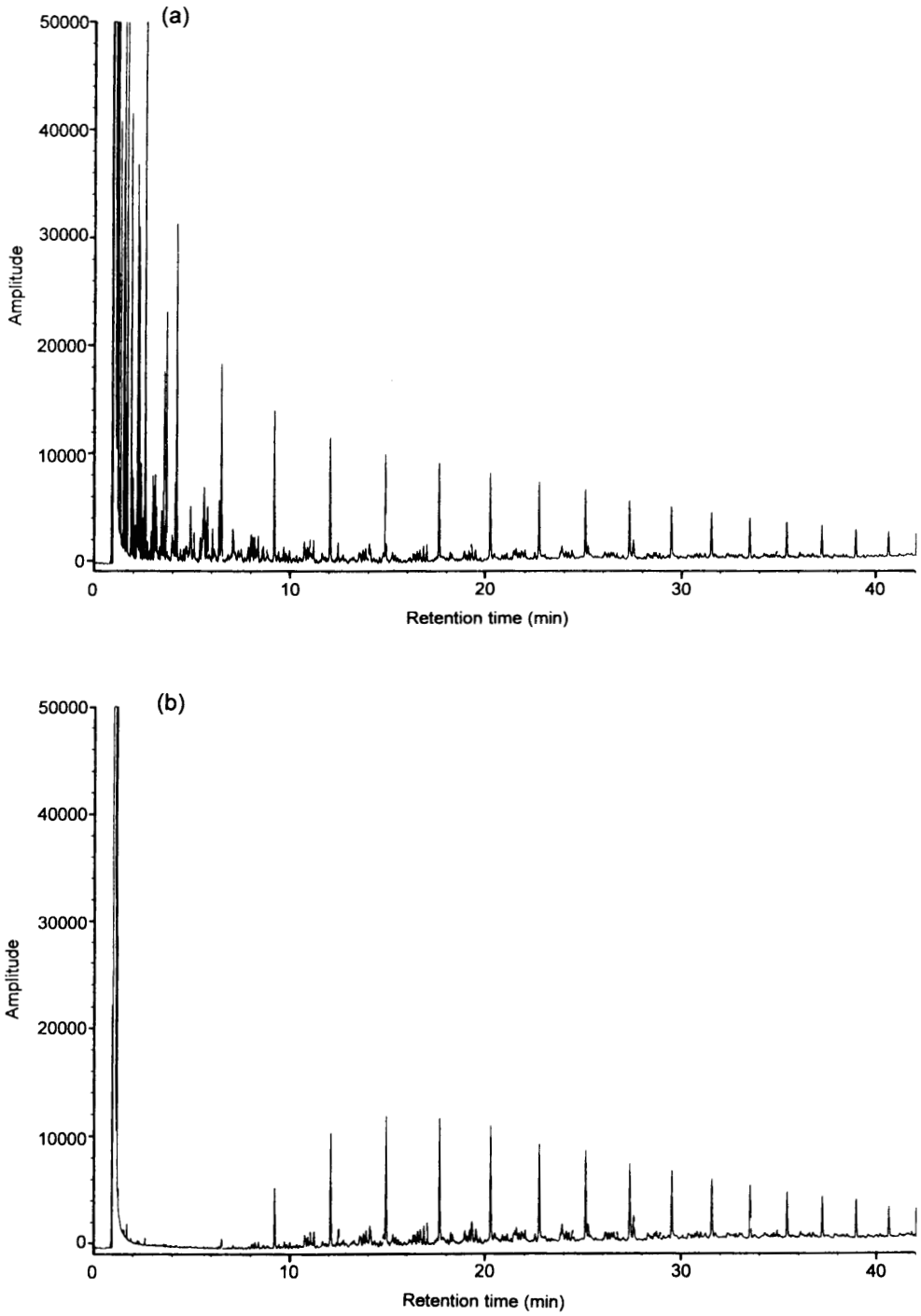

Figure 1. Gas chromatographs showing spectra of whole (a) and weathered (b) Kuwait crude oil

hydrogen gas carrier. The column was a fused silica capillary column, 25 m by 0.3 mm internal diameter, coated with SE-54 type phase. Sample volumes of 1 µl were injected at 60°C and held for one minute. Oven programme set at 60-320°C, hold for seven minutes and a run time of 60 minutes. Detection was by flame-ionisation or mass-spectrometer (MS). Any equivalent GC set-up will suffice.

2.2 Test water

The maintenance of test animals in stock tanks and all toxicity tests must use natural seawater taken from a site known to be relatively free of industrial, agricultural and sewage pollution. It should be filtered to 10 µm and brought to the test temperature of 15°C (± 1°C) prior to use. The salinity must be in the range of 28 to 35‰.

2.3 Sea Test

This test is compulsory for all oil treatment products.

2.3.1 Rationale

This test is based on the premise that if oil treatment products are correctly applied to an oil slick at sea, marine organisms will be exposed to a mixture of oil and product, rather than to a suspension or solution of product alone. The test therefore compares the toxicity of oil dispersed under standard conditions of mechanical agitation, with that of the same amount of oil treated with the product in question under the same conditions of mechanical agitation.

Research has shown that concentrations of oil (especially dispersed oil) under slicks reduce rapidly in the first few hours (Cormack, 1977). It was, however, technically difficult and unrealistic in a routine and reproducible laboratory test to reproduce this phenomenon. Therefore, it was decided to base the test on the exposure of a marine organism to a fixed concentration for a fixed period.

2.3.2 Test species

The test species is the brown shrimp (*Crangon crangon* L.). They should be between 50-70 mm total length and caught from an area known to be relatively free of contamination. They must be acclimated in well aerated, gently flowing seawater for 4-5 days prior to use in a test. They are not fed during this acclimation period.

Developmental work showed that exposure of shrimp to 1000 ppm of Kuwait crude oil produced a measurable mortality during the Sea Test procedure and this concentration was therefore adopted. The concentration of oil used in the test is very high compared to those that might be observed in the field, and thus includes a safety factor for species more sensitive than shrimps to the acute toxic effects of oil.

2.3.3 Apparatus

The test system has been devised to maintain oils and dispersed oils (for chemical dispersants) as an homogenous dispersion of small droplets throughout the test tank.

The apparatus consists of a set of replicate cylindrical Perspex tanks fitted with a removable central cylinder which has two sets of three apertures at the top and bottom, such that when 18 litres of water are placed in the tank, the water level is about 2 mm from the top of the upper apertures. A propeller mounted inside the cylinder draws water in through the upper apertures and expels it through the lower set without drawing air into the water column. A compressed air motor above each tank is fitted with its own individual pressure and flow regulators and drives the propeller via a magnetic coupling. The apparatus is represented in Figure 2 and technically described below. A complete test apparatus is shown in Figure 3.

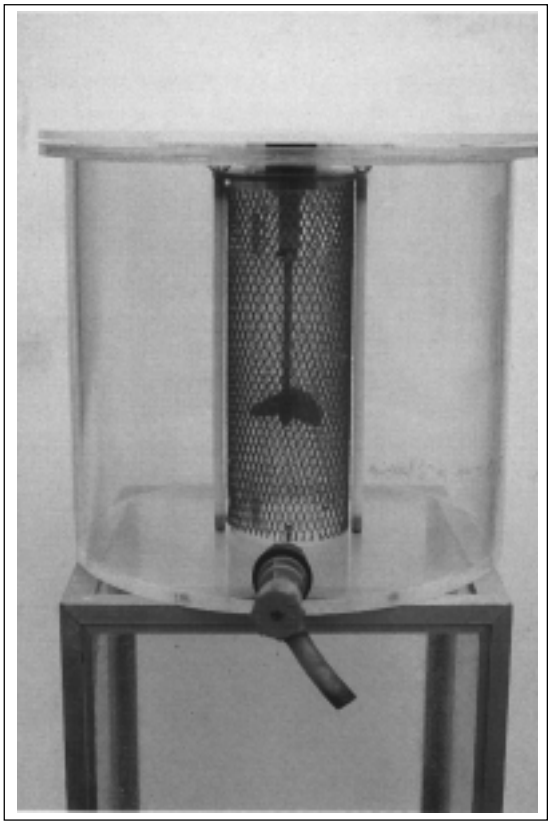

Figure 2. Sea Test agitation tank

Figure 3. Sea Test apparatus set up showing agitation and recovery tanks

2.3.3.1

A cylindrical tank, with an internal diameter of 314 mm and height of 300 mm and a 25 mm wide flange around the top, made from 6 mm Perspex. A circular register of 80 mm diameter and 12 mm height is glued centrally to the inside of the base and a 24.5 mm hole is drilled low in the side of the tank and tapped ¾" BSP for the fitment of a drain valve. Two 8 mm diameter nylon rods are fitted either side of the central register and have a 20 mm length of 4 mm stainless steel studding tapped into their upper ends.

2.3.3.2

A Perspex cylinder, with an internal diameter of 80 mm, height 265 mm and wall thickness of 5 mm. This fits over the central register in the tank. Three sets of slots 55 mm wide by 30 mm high are symmetrically arranged 12 mm above the base of the cylinder and 22 mm from the top. The upper end of the cylinder is machined internally to accept part 2.3.3.3. The apertures are screened by a disposable sleeve of plastic mesh, which excludes the test animals from the central column.

2.3.3.3

A moulded plastic propeller (Graupner 60 mm diameter, 2 bladed racing propeller No. 455/11 right handed rotation or equivalent) is mounted on the bottom of a stainless steel shaft which runs in a cylindrical nylon bearing fitted to the centre of a circular Perspex carrier that attaches to the top of the cylinder (part 2.3.3.2). The top end of the shaft is attached to a magnet which is shrouded by a circular upstand on the Perspex carrier.

2.3.3.4

A circular collar of uPVC fits over the top of part 2.3.3.3 and attaches both it and the internal cylinder 2.3.3.2 to the tank, and is held in place on the nylon tie-rods with stainless steel wing nuts.

2.3.3.5

A circular Perspex lid with a pair of 3 mm thick Perspex strips glued to its lower surface to leave an air gap between lid and the tank.

2.3.3.6

A second magnet is mounted beneath a compressed air motor and drives the propeller assembly indirectly through the tank lid. The speed of each individual air motor is controlled by its own air pressure and flow regulator.

2.3.3.7

A shallow Perspex 'recovery' tank 515 x 214 x 127 mm, fitted with an overflow outlet at the 10 litre level and a Perspex lid.

2.3.4 Test procedure

The test tanks are filled to approximately 2 mm from the top of the column apertures (approx. 18 litres) with filtered seawater at 15°C (± 1°C) and aerated for at least one hour. The air motors are then started and speed of rotation adjusted to 800 rpm (±10) using an optical tachometer. The water level may need to be adjusted to give a vortex of about 17-25 mm and to ensure that air is not dragged into the water column.

The motors are then stopped and twenty healthy shrimp (*Crangon crangon* L.) are added randomly to each tank. The lids are replaced and the shrimps left to acclimate for two hours, after which time the tank temperatures are noted. After acclimation the lids and aerators are removed and 18 ml of reference oil (1000 $\mu l\ l^{-1}$ or ppm) is added evenly to the surface water of each tank. If the oil spreads rapidly over the surface, possibly due to the action of surfactant residues in the tank after imperfect cleaning, then that tank must be abandoned.

The test product is now added. Use the method below appropriate to the test product.

Type 1 dispersant - Applied neat at an oil to dispersant ratio of 1:1. Gently pour 18 ml of neat dispersant from a measuring cylinder onto the patches of floating oil.

Type 2 dispersant - Applied as a 10% dilution in seawater. Prepare 100 ml of dilute dispersant and add 18 ml to each tank as above.

Type 3 dispersant - Applied at a dispersant to oil ratio of 1:10 but added neat Pour 18 ml of oil onto the water inside a floating PVC tubing 'containment ring'. Then add 1.8 ml of dispersant dropwise onto the oil. The rings are removed 1 minute after the start of the test.

Type 2/3 dispersant - Test as a type 2.

Sorbent - Spread evenly onto the oil at the maximum product to oil dose ratio recommended by the manufacturer.

Bioremediation agent - Follow carefully all manufacturer's preparation instructions. Always prepare in a fume cupboard if bacterial based (NB. Always follow a code of safe working practice and procedure - see Appendix 2). Spread or pour evenly onto the patches of oil at the maximum product to oil ratio recommended by the manufacturer.

Miscellaneous - Applied as per manufacturer's instructions at the maximum recommended product to oil ratio.

Start the motors one minute after addition of the product, record motor speeds and adjust to 800 rpm ($\pm$10).

Ten minutes after the test start and also just before its end, note the temperature of the test mixtures.

Dissolved oxygen and pH should not be measured as the oil may foul the probe membranes.

One hundred minutes after the test start, stop the air motors and run the oily water to waste via an oil trap. All the shrimp (alive, dead or moribund) should be carefully transferred into gently running, aerated seawater in the recovery tanks. Temperatures and mortalities are recorded immediately and then again after a 24 hour recovery period.

Routine testing is carried out with five tanks containing 1000 ppm oil treated with product and five 'control' tanks containing 1000 ppm of oil alone. The oil controls are used on each occasion to compensate for seasonal and other variations in the sensitivity of the test organism.

Immediately after use the tanks should be dismantled and all components sprayed with a water rinsable degreaser (e.g. Jizer®). They should be given a hot detergent wash followed by hot and cold rinses and a thorough drying. It is imperative that all traces of oil, dispersant and cleaning detergent are removed before a new test.

2.4 Rocky Shore Test

This test was formerly known as the Beach Test.

This test is compulsory for all dispersants, bioremediation agents and 'non-recoverable' miscellaneous products. It will also be required for 'recoverable' sorbents or miscellaneous products if they are to be used in a rocky shore environment or stand any chance of reaching such an environment after usage.

A Rocky Shore Test should only be carried out if the candidate product has already passed the compulsory Sea Test.

2.4.1 Rationale

The intertidal zone is of great value both in amenity and ecological terms. Toxic effects of beached oil treatment (dispersant spraying etc.) are likely to have only limited impact on commercial fisheries e.g. cockles etc., on sandy beaches and will be relatively benign on dynamic pebble beaches where there is good drainage and a relatively impoverished species community. Therefore, for these environments (i.e. sandy/pebble amenity beaches) it is assumed that a product passing the Sea Test will be of an acceptably low risk. However, the death of grazing organisms (e.g. winkles and limpets) that inhabit rocky shores can lead to a much more significant deleterious ecological change due to extensive uncontrolled growth of seaweed. Consequently a toxicity test was developed using a typical intertidal grazing organism, the common limpet (*Patella vulgata* L.).

When products are used to clean oil from beaches, animals are exposed to very different conditions to that experienced at sea. Both oiled and unoiled animals may be exposed to neat product and left exposed until they are washed by the next incoming tide or the use of water hoses. The Rocky Shore toxicity test for all dispersants and bioremediation agents has therefore been based on these exposure conditions.

Preliminary tests in the laboratory showed that the mortality of limpets exposed to oil is high and the detection of a toxic effect due to the product over and above that of the oil would be difficult and less accurate than determination of the effect of the product alone. Additionally, a product is likely to be applied over wide areas of shore and the evaluation of a particular product should also take into account the effect of the product on those parts which are un-oiled as well as those which are oiled. Therefore, the test finally adopted was designed to assess the effect of application of the product on un-oiled limpets. The amounts of product applied to the test organism were based on the density of application likely to be encountered in practice. Similarly, the test sought to simulate the initial exposure to a product for an average period of 6 h followed by successive tidal rinsing. In order to compensate for seasonal variations in the susceptibility of the test species, the effects of a standard oil alone were also assessed.

Granular sorbents and some miscellaneous products have been shown in preliminary tests to also express a different type of deleterious effect than that of direct toxicity, that of physical adhesion interference. This phenomenon also appears to be much more acute when the product is sorbed with oil, therefore the Rocky Shore Test for these products is slightly modified to take this into account.

2.4.2 Test species

The test species is the common limpet, *Patella vulgata* L. of 30-40 mm shell width. The limpets should be collected from a relatively uncontaminated beach. In order to avoid damage, the limpets should be taken from a beach with chalk boulders from which they can be carefully prised with an oyster knife without breaking or chipping their shells. They should be kept as cool as possible and returned to the laboratory immediately. They are placed, shell uppermost, in polyethylene stock tanks lined with polythene sheeting to facilitate subsequent removal. The animals are maintained in well aerated, gently flowing seawater and subjected to intermittent immersion (about 18 h immersed and 6 h dry) to simulate tidal action. The limpets should be acclimated to laboratory conditions (i.e. 15°C $\pm$ 1°C air and water temperature) for at least 96 h before use. They are not fed during their stay in the laboratory.

2.4.3 Apparatus

2.4.3.1
A test plate (350 x 125 mm) made from 6 mm Perspex with sharply bevelled edges to deter limpets from moving from one side of the plate to the other. Each plate has two stainless steel hooks by which it can be suspended vertically. Before use each plate surface should be gently roughened with fine abrasive paper to aid limpet adhesion.

2.4.3.2
A lidded polyethylene tank (770 x 490 x 210 mm) with two 12 mm wooden dowels running the length of the tank approximately 40 mm below the top.

2.4.3.3
A hand-operated pump sprayer which delivers approximately 0.8 ml ($\pm$0.1) per pump stroke.

2.4.3.4
A rectangular Perspex recovery tank (430 x 150 x 230 mm) with an overflow outlet at the 10 litre level and a ½" BSP threaded hole to accept a drain valve low down in the end of the tank.

2.4.3.5
A pair of 6 mm Perspex lids to fit the recovery tank (one lid 378 x 150 mm, the other 52 x 150 mm).

2.4.4 Test procedure

2.4.4.1 Dispersant and bioremediation agents

At least 24 h before the start of a test 24 limpets should be placed on the non-bevelled side of each of a sufficient number of test plates (see 2.4.3.1) to allow 7 plates for each product being tested and 7 for the reference oil. Once the limpets have settled they should be suspended in well aerated seawater with the same tidal regime as the stock tanks.

On the morning of the test reduce the number of limpets on each plate to 20, preferentially keeping those that are well settled and rejecting those that are unsettled or have excessive curvature to the base of their shells.

Routine testing is carried out with five plates with product alone (or oil + product for sorbents) and five 'control' plates with oil alone. The oil controls are used on each occasion to compensate for seasonal and other variations in the sensitivity of the test organism.

Place 5 plates onto the dowels in the spraying bins (see 2.4.3.2) leaving enough space between the plates to prevent the limpets from transferring from plate to plate. Use separate bins for spraying oil and spraying or applying the test product.

After an hour place the first bin into a fume cupboard and spray each limpet with 0.8 ml of reference oil from a height of about 10 cm (Figure 4). This gives an application rate of 0.4 l m^{-2} which is similar to the recommended application rate for dispersant when cleaning rocky shores. Lift the bin out of the fume cupboard and pour 1 l of seawater into the bottom of the bin, without wetting the plates and cover with the lid, to maintain humidity.

Treat the other set(s) of test plates in a similar manner with the test product. For each type of product apply as below:

Type 1 dispersant - Spray neat 0.8 ml per limpet.

Type 2 dispersant - Make a 10% dilution in seawater and spray 0.8 ml of dilute dispersant per limpet.

Type 3 dispersant - Adjust pump sprayer to spray only 0.08 ml of neat dispersant onto each limpet. If this is impossible then it must be ensured that the 1.6 ml required for an entire plate is evenly distributed.

Type 2/3 dispersant - Treat as a Type 2.

Sorbent - Due to possible physical interference of granular sorbents they are treated slightly differently (see below).

Bioremediation Agent - Follow carefully all manufacturer's preparation instructions. Always prepare in a fume cupboard if bacterial based. Spray (if liquid) or sprinkle (if granular) enough product onto each limpet equivalent to the maximum product to oil ratio recommended by the manufacturer (i.e. maximum amount needed to treat 0.8 ml of oil).

Miscellaneous - Apply to each limpet at the maximum product to oil ratio recommended by the manufacturer. (Note: If the product is granular or powdery in nature then they should be treated in the same way as a sorbent - see below).

Six hours after treatment the plates should be removed from the spraying tanks and rinsed under gently running seawater for approximately 15 seconds. Each plate is then suspended in gently flowing, well aerated seawater in one of the recovery tanks (Figure 5) (see 2.4.3.4).

The oily water from the spraying bins should be run to waste via the oil trap. They should then be sprayed with Jizer® or equivalent and given a hot detergent wash followed by several hot rinses.

One hour after initial suspension the number of live limpets on each plate should be counted and any which have become detached should be recorded as dead. Water temperatures in the recovery tanks should also be noted.

Figure 4. Rocky Shore Test exposure tank showing the spraying of limpets with crude oil

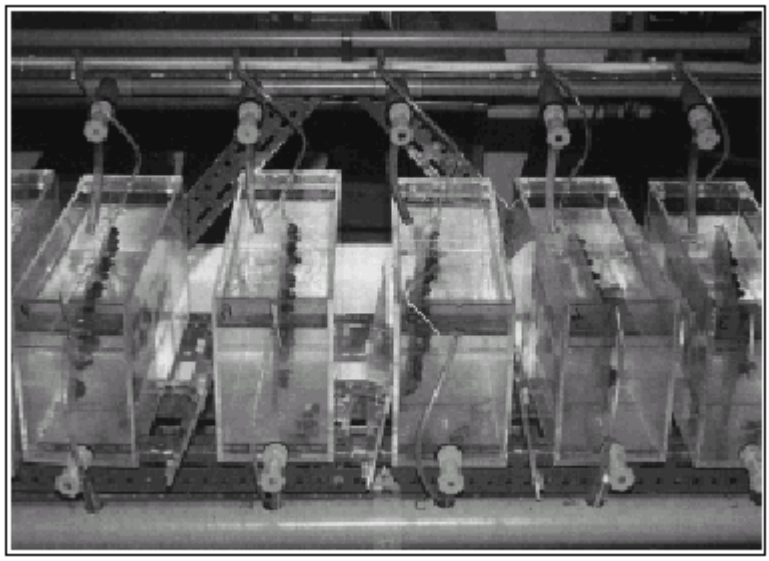

Figure 5. Rocky Shore Test recovery tanks

At both 24 h and 48 h post spraying water temperature is noted and the recovery tanks drained. One hour later any further limpet mortalities should be noted. Any limpets which have detached from the plates singly, whether apparently live or dead, should be counted as dead. Limpets which have fallen in pairs or groups should be examined and any which are alive should be resettled on the bottom of the tank. Six hours after draining the recovery tank drain valves should be closed and the water flow restarted. The aeration must be checked as the tanks begin to fill again.

Seventy two hours after the start of the test each recovery tank water temperature should be recorded and then drained. The live and dead limpets should be counted as before. Any limpets which detach easily from their plates should be recorded as dead.

2.4.4.2 Sorbents and granular/ powdery products

As mentioned in the rationale, the Rocky Shore Test for sorbents and granular/powdery miscellaneous products is slightly modified to take into account their potential for physical adhesion interference. The test is exactly the same in all respects except that the product treatment is made onto oiled limpets. The limpets in the test product tank are sprayed with oil in exactly the same manner as the reference tank (i.e. 0.8 ml per limpet). The product is then applied to each limpet in accordance with the manufacturer's maximum product to oil dose ratio. The treated plates are then left for six hours (as per normal protocol) and rinsed with gently flowing seawater for approximately 15 seconds. While rinsing the plates, an attempt should be made to remove as much of the oil/sorbent complex as possible without disturbing the limpets. This attempts to mimic genuine environmental conditions. The plates are then placed in the recovery tanks and mortalities etc. are monitored according to the normal protocol.

Once the test is complete all limpets (alive and dead) should be bagged up and autoclaved before disposal.

Immediately after use the tanks should be dismantled and all components sprayed with a water rinsable degreaser (e.g. Jizer®). They should be given a hot detergent wash followed by hot and cold rinses and a thorough drying. It is imperative that all traces of oil, dispersant and cleaning detergent are removed before a new test.

2.5 Agitation toxicity tests for bioremediation agents

These two tests are compulsory for the approval of bioremediation agents and will only be completed once the product has been assessed as microbiologically safe by FDL Weymouth, efficacious, and has passed both the Sea Test and the Rocky Shore Test in that order. Two agitation tests are required, one using a crustacean as the test species and the other a fish.

2.5.1 Rationale

Due to the unique environmental impacts which might be associated with the addition of biological agents or bacterial growth enhancing agents to the marine environment, bioremediation agents must be subject to longer term toxicity tests than those for other types of oil treatment product. Their potential biological hazard to fish species also requires that a fish toxicity test as well as a crustacean test is carried out.

All other oil treatment products are tested for their safety with respect to direct toxicity (or adhesion interference with granular/powdery sorbents) but it is perceived that bioremediation agents could exhibit deleterious effects by two other modes: (i) unforeseen pathogenic effects caused by artificially elevated bacterial concentrations, and (ii) toxic effects caused by the crude oil breakdown intermediates of biodegradation. In both cases these effects may take longer to manifest themselves than the duration of the standard Sea Test and therefore the Agitation Tests are run for 96 h.

2.5.2 Test species

The crustacean Agitation Test uses the brown shrimp *Crangon crangon* L. as the test species. These are collected and acclimated as per the Sea Test (see section 2.3.2).

The fish Agitation Test is conducted using the Armed Bullhead, *Agonus cataphractus*. The fish should be between 50-100 mm length and should be caught from an area known to be relatively free of pollution. The fish should be acclimated to the test temperature (15 $\pm$ 1°C) in well aerated, gently flowing seawater for 4-5 days prior to use in a test. They are not fed during this acclimation period.

2.5.3 Apparatus

The apparatus is exactly as that used in the Sea Test (see section 2.3.3) with the exceptions that no recovery tank is required and a 'shrimp mat' consisting of a ring of plastic mesh (see Figure 6) is used to avoid exhaustion related deaths in the crustacean test.

2.5.4 Test procedure

The tanks are filled with seawater and the air motors checked and set to 800 rpm as for the Sea Test (section 2.3).

The motors are stopped and to each tank twenty healthy shrimp (for the crustacean test) or ten healthy fish (for the fish test) are added and left to acclimate to test conditions (temp. 15°C $\pm$ 1) for a further two hours.

After acclimation is complete the standard oil is added to each tank. The concentration used is 300 ppm (i.e. 5.4 ml to each tank) for both the shrimp and fish test. This is much lower than that used in the Sea Test but is at a level that will elicit mortalities after 96 h exposure. The test product is prepared carefully in accordance with the manufacturer's instructions. If the candidate product is bacterially based follow a code of safe working practice and procedure (Appendix 2). The product is spread or poured evenly onto the patches of oil

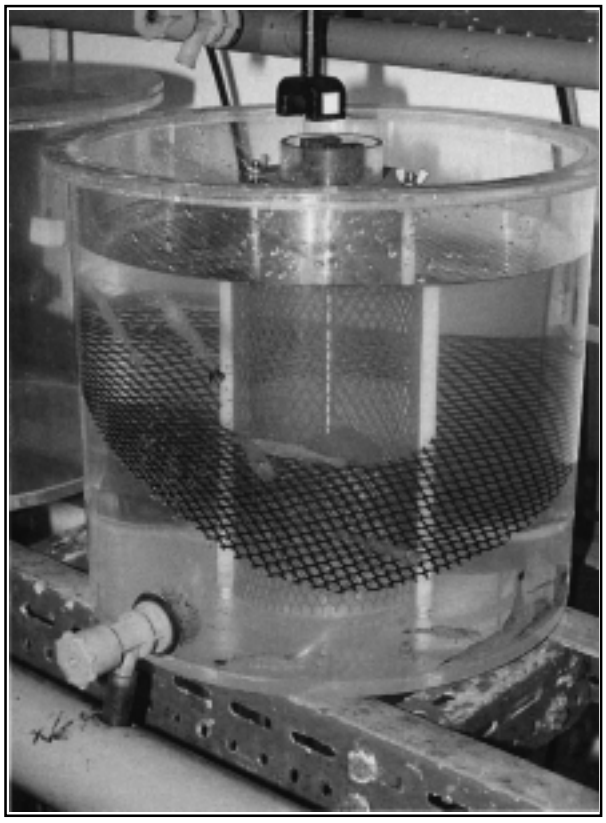

Figure 6. Agitation tank showing position of shrimp mat for use with longer term agitation tests

at the maximum product to oil ratio as recommended by the manufacturer. After one minute the agitation motors are started and set to 800 rpm (± 10). Test tank temperatures, motor speeds and organism mortality are recorded (all dead organisms are removed) in the morning and afternoon of each day (speeds adjusted if required) and the test is ended after 96 h exposure. Each test comprises of five 'control' (oil alone) and five test (oil plus products) tanks.

3. TEST VALIDITY

For any of the described tests to be considered valid the following criteria must be met:

(i) Significant mortality (>20% of total) of test organisms must not occur during any storage or acclimation periods prior to the test.

(ii) The test temperature does not deviate outside of the accepted limits (i.e. 15°C ± 1).

(iii) The motor speeds (for Sea and Agitation Tests) do not deviate outside of the accepted limit i.e. 800 rpm ± 20 rpm for extended periods of time.

(iv) The mean control mortalities (i.e. the tanks dosed with reference Kuwait crude only) are not <10% or >80% at the end of the test.

(v) Any set of 5 control or 5 test tank mortality replicates must be found to be homogenous (see below).

If any of the above criteria are not met the test must be repeated.

4. PASS/FAIL CRITERIA

All the above tests are assessed on the basis of comparing the mortalities occurring in five replicate 'controls' against those in five replicate 'treatments'. Each set of five replicates must be subject to statistical analysis to ensure that the set is homogenous. If this is not the case for treatment or control, the test is invalid. Once it has been confirmed that the replicate groups are homogenous the two sets are compared statistically (Student's t-test, F variance ratio) for differences in their mean. If the oil toxicity is significantly ($p<0.05$) greater in the treatment than in the controls then the product has failed the test.

5. REFERENCES

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The reference to proprietary products in this report should not be construed as an official endorsement of these products, nor is any criticism implied of similar products which have not been mentioned.

APPENDIX 1. Contact addresses for further information

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

Marine Protection (Policy) Branch
Environmental Protection Division
Ministry of Agriculture, Fisheries and Food
Nobel House
17 Smith Square
London
SW1P 3JR
Tel: 0171 238 5880

For further information with regard to toxicity test procedures or microbiological hazard assessments contact:

Mr M Kirby
Ministry of Agriculture, Fisheries and Food
Directorate of Fisheries Research
Fisheries Laboratory
Burnham-on-Crouch
Essex
CM0 8HA
Tel: 01621 787200

For further information with regard to efficacy testing procedures contact:

AEA Technology
National Environmental Technology Centre
Culham
Abingdon
Oxfordshire
OX14 3DB
Tel: 01235 4633040

For information with regard to gaining samples of standard Kuwait crude oil contact:

Mr Bob Barnes
Q8 Petroleum (GB) Ltd
Burgon House
The Causeway
Staines
Middlesex
TW18 3PA

APPENDIX 2. Safe working practice and procedure

Handling of bacterial bioremediation products

1. Task

Increasing numbers of applications for the toxicity testing of bioremediation products for the treatment of oil spills under the Food and Environment Protection Act, Part II, 1985 require that a standard safe working practice is formulated and monitored.

2. 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) can be accepted as of sufficiently low risk.

The definitions of hazard groups 1 and 2 are as follows:

H.G.1.: An organism that is most unlikely to cause human disease.

H.G.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.

3. Safety Requirements and Procedures

All procedures should be carried out in accordance with good laboratory practice with due consideration to health and safety issues.

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

(ii) 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.

(iii) Access to laboratories where tests are taking place should be limited to authorised personnel only.

(iv) The laboratory must contain a wash-basin which should be located near the laboratory exit. Taps must be of a type that can be operated without being touched by hand.

(v) An autoclave for the sterilisation of waste materials and solid glassware must be readily available.

(vi) The laboratory door must be closed whilst work is in progress.

(vii) Laboratory coats (preferably side or back fastening) must be worn in the laboratory and removed when leaving the tank room area.

(viii) Eating, chewing, drinking, storing of food and application of cosmetics must not take place in the laboratory.

(ix) Mouth pipetting must never be attempted.

(x) 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.

(xi) 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.

(xii) Effective disinfectants must be available for routine disinfection and immediate use in the event of a spillage.

(xiii) All bench tops must be disinfected after use.

(xiv) Used laboratory glassware and other materials awaiting sterilisation must be stored in a safe manner. Pipettes, if placed in disinfectant, must be totally immersed.

(xv) Material for autoclaving must be transported to the autoclave in robust containers without spillage.

(xvi) 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.

(xvii) All accidents and incidents must be immediately recorded in the accident record book and the line manager and safety officer informed as soon as possible.

(xviii) No person should be working on their own in the laboratory when directly handling these materials.

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Procedures for the approval of oil spill treatment products

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