



Discobiol project –phase 1B Description:

Impact of the dispersed oil on organisms living in the water column (sub-lethal effects) in sea water with and without suspended particles material

DESCRIPTION

FXM

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TITLE

**Discobiol project –phase 1B - description
Impact of the dispersed oil on organisms living in the
water column (sub-lethal effects) in sea water with and
without suspended particles material**

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OUR REFERENCE

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OBJECTIVE

The study aims at assessing the impact (sub-lethal effects) of dispersed oil on organisms living in the water column, in presence of and without suspended particulate matter.

The work will consist in toxicity tests carried out on fish (pelagic and benthic) and bivalves with oil dispersed and not dispersed in sea water with and without suspended materials.

The work described is DISCOBIOL phase 1 B program

Starting year: 2009

Completion year: 2009

Total planned expenditure: 1 year

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1 BACKGROUND

Dispersants are known to be an appropriate response option offshore where dilution has practically no limit and allows dispersed oil concentrations to decrease rapidly under harmful level for the environment. In coastal areas, where dilution can be restricted due to limited depth and to the vicinity of the coastline, dispersant use is often limited: as an example of such limitations, along the French coast, geographical limits have been defined taking into account minimum depth and distance to the shoreline and to sensitive items (such as aquaculture, ecological reserve...) for different pollution sizes (10, 100 and 1000 tons of oil to be dispersed).

However, these general limits do not take into account special areas like estuaries and closed bays. In these situations due to the vicinity of the different resources, it is necessary to analyse and balance the advantages and the potential impact, to the different sensitive resources, of dispersing the oil. In other words, it is necessary to consider simultaneously the different sensitive resources by completing a Net Environmental Benefit Analysis.

Such an analysis requires knowing the dispersed oil toxicity level towards the different habitats.

Much work has already been done on this topic; however, results are rarely comparable as the methodologies used to get these data are different (e.g. oil type, time exposure...).

The question of using dispersant in very coastal areas, particularly estuaries, remains an unsolved question for responders and this is especially problematic as estuaries are often locations where the pollution risk is high due to the concentration of human activities (harbours, industries and ship traffic).

An extensive work program named DISCOBIOL has been undertaken. It aims at bringing to responders a clear and practical information on which they can decide whether the chemical dispersion is an advisable option in case of an incident in an estuary or in the close vicinity of the coast, in shallow waters.

This work program aims at acquiring comparable and robust information on the impact of dispersed oils towards the different habitats and resources of estuaries and/or close bays. This information will give the possibility for responders to carry out reliable NEBA on dispersant use according to their own scenarios.

The DISCOBIOL program consists in an experimental study program in order to compare the toxicity and impact of dispersed oil to different eco-compartment of an estuary in temperate climate (organisms in the water column –pelagic and benthic fish, bivalves and crustaceans- mudflats and salt marshes): tests involved in this program are carried out using the same oil to get comparable data between the sensibility of the different resources.

The different resources considered in this program, are: organisms in the water column (adult or juvenile), mudflats and salt marshes.

As an additional task to this program different oils will be compared in terms of their toxicity using a simple toxicity test method, and in terms of chemical composition.

This scientific program is carried out by Cedre with different French institutes or universities: Agence Francaise de Securite Sanitaire de Aliments, Universite de Bretagne

Occidentale, Universite de La Rochelle. It is also technically supported by two dispersant suppliers, Gamlen-Innospec and Total Fluides Speciaux.

This program involves also a technical cooperation with COGER-DFO Canada (which plans to carry out the experimental work on saltmarshes), and receives supports from Exxon, OSR Limited and CRRC (US). Contribution from ITOPF is expected.

At last but not the least, the technical information gained from this work program will be shared and discussed at an international level with other scientists, and when possible, this program will be linked to other similar studies in order to avoid duplication work and to enhance synergies (e.g; contacts have been taken with Sintef and Aquaplan Niva which are setting a similar program for Arctic waters).

This large program is planned to be completed within on 3 years as followed:

2008-2009	experimental work on the organisms living in the water column	DISCOBIOL 1A acute toxicity DISCOBIOL 1B sub lethal effects
2009-2010	experimental work on mudflats	DISCOBIOL 2
2010-2011	experimental work on salt marshes	DISCOBIOL 3

Annex 1 provides a general description of the project.

However, considering the available funding, the original program of Discobiol did not consider some other important issues related to the use of dispersants in coastal and estuarine habitats such as the effect of the suspended particulate material –SPM- (which can interact with the dispersed oil) on the impact of dispersed oil, the sensitivity of larvae stages to dispersed oil, the possibility for dispersed oil to taint the commercial living resources.....

This revised work program is considering the effect of SPM.

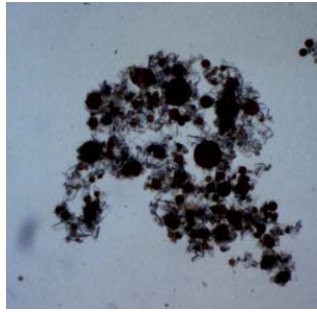
2 OBJECTIVE OF THE STUDY

The proposed study aims at assessing the impact of dispersed oil on organisms living in the water column, fish and bivalves, in presence and without suspended particulate material.

3 RATIONAL CONCERNING THE SPM IN THE STUDY

Estuarine waters are often highly loaded with fine mineral particles. This is due to the presence of the “mud plug” (which results from the contact between the fresh water coming from the river with the sea water), and also due to the muddy sediment brought to suspension in shallow waters by the natural agitation.

This suspended particulate material (SPM) can interact with the oil, especially when the oil is itself in a divided form, as an oil dispersion, to form aggregates. The behaviour of these aggregates can differ from the one of the initial oil droplets (as an example the formation of aggregates reduces the possibility of oil droplets to coalesce at the water surface).



Oil fine mineral aggregates (picture from DFO Canada)

These changes have already been studied in the past years from physical-chemical (conditions of formation according to the type of particles, the type and state of oil...), and biodegradation viewpoints. However, few works (if any) have considered the influence on the toxicity and impact of the oil-mineral aggregate formation, which could have an effect on the bioavailability of the oil.

4 PHASE 1B WORK DESCRIPTION

The work will consist in toxicity tests carried out on fish (pelagic and benthic) and bivalves with oil dispersed and not dispersed in sea water, with and without suspended materials. The work described is a modification to the original DISCOBIOL program phase 1 B (sub lethal effect on organisms living in the water column), by adding the mineral particles issues.

The experimental program will be conducted in close cooperation with the biologists from the Universities of Brest and La Rochelle both partners of the DISCOBIOL project.

4.1 Activity 1: Short bibliographical study

A short bibliographical research will be undertaken in order to know the typical amounts and the types of mineral particles currently encountered in estuaries in temperate climates. From this information, the experimental testing conditions will be defined (type and concentration of clay particles).

This issue will be discussed with the partners of the project and particularly with COOGER-DFO which has expertise on this topic.

Additionally, a short bibliographical survey will be carried out to know what are the typical oil concentrations which have been measured at sea following a chemical dispersion operation.

4.2 Activity 2: Tests on organisms

4.2.1 Choice of the organisms

The tests will be conducted on the following species:

Fish:

Turbot
Sea Bass
Grey mullet

Bivalves:

Mussel
Oyster

These species have been already chosen in the original program of DISCOBIOL as representative of the varying nature of the organisms to be found in the water column: a pelagic fish, a benthic fish, another benthic fish which feed on the sea floor, and two bivalves which are filter organisms. In addition, the sea bass, the turbot, the mussel and the oyster are well known experimental models and represent commercial value.

4.2.2 Test conditions

The experimental program will be conducted in Cedre's testing facilities, described in the annex 1.

6 testing conditions will be considered:

- C = control (no oil).
- M = oil mechanically dispersed.
- CD1 = oil chemically dispersed (with dispersant 1).
- CD2 = oil chemically dispersed (with dispersant 2).
- SPM = water added with SPM.
- CD1+SPM = oil chemically dispersed with dispersant 1- in water added with SPM.



General view of Cedre testing facility

The oil introduced in the equipment will be adjusted in order to reach a realistic oil concentration (typical concentration to be found in real situation at sea). This concentration will be far less than the lethal concentration at 24h found in previous tests (between 300 and 1200 ppm):

- Mechanically dispersed oil: the SPM will be introduced into the tank first; then, the oil will be poured into the tank as an artificial dispersion resulting from the mixing of a turbine (Ultra Turax).
- Chemical dispersion: the SPM will be introduced into the tank first then the dispersed oil will be poured into the tank after a regular mixing in a beaker.

The tank will be equipped with a recycling pump in order to keep the oil and/or the clay in suspension during the test (see annex 2 - testing equipment).

The exposure time will be short (2 days which represent 4 tidal cycles) in order to keep in realistic conditions followed by 14 days of restoration in clear water.

The tests will be run with a mixture of mineral particles (clay) whose cation exchange capacity will be taken into consideration. The amount of mineral particles introduced into the test tanks will be in agreement with the typical concentrations found in real world as previously determined in the short bibliographical study.

Tests will be conducted successively on pelagic fish, seabass (*Dicentrarchus labrax*), benthic fish, turbot (*Scopholamus maximus*), grey mullet, mussel and oyster. These organisms will be adult or juvenile and will come from marine farming, except the grey mullet whose origin will remain wild; they will be sized in order to work with homogeneous batches of animals. These species have been already chosen in the original program of DISCOBIOL as representative of the varying nature of the organisms to be found in the water column: pelagic and benthic fish species and two bivalves. The grey mullet has the advantage to feed on the sea floor and will be used in the next part of the Discobiol project planned on mudflat; moreover, seabass, turbot, mussel and oyster are well known experimental models and represent commercial value species.

The test conditions will be discussed with the partners of the project whose technical advice will be required.

4.2.3 Control

4.2.3.1 *Quality of the water*

The quality of the water will be daily monitored for oxygen and nitrogen content.

4.2.3.2 *Biological parameters*

During the experiment the mortality, if any, will be recorded daily in the tanks. However, as the testing oil concentration will be far less than lethal concentration 24h. assessed in previous tests, (few tens ppm to be compared to several hundreds ppm), it is expected not to reach lethality in these tests.

The biological impact of oil will be assessed through 3 different types of parameters:

Early response stress indicator

General criteria:

Biochemical criteria showing an effect on the immune system,

Physiological criteria showing an effect of the general capability of the organisms,

Bio-accumulation in tissues of molecules coming from oil

On fish:

Early response stress indicator: *hepatic metabolites*

Biochemical criteria: *superoxyde, dismutase, catalase*

Physiological criteria: *challenge test, growth rate assessment,*

Bio-accumulation: *analysis of oil in liver or flesh (GC-MS after esterification)*

On bivalve:

Early response stress indicator: *cortisol*

Biochemical criteria: *phenol-oxydase*

Physiological criteria: *lysosomal stability, growth rate assessment,*

Bio-accumulation: *analysis of oil in liver or flesh (GC-MS after esterification)*

The organisms will be sampled at the end of the exposure and at the end of the restoration time (14 days after exposure).

The growth rate assessment will last, after the restoration time, for 21 more days. For these 21 days, the organisms will be put in another tank in order to let the testing tank free for the next test.

4.2.3.3 *Oil concentration in the water*

As dispersion is not perfectly stable (possible coalescence, oil sticking on solid surface of equipment) the oil concentration in the water column will be monitored daily during the exposure period in order to know the real exposure level of the organisms.

The global amount of dispersed oil will be assessed; the level of oil soluble compounds will be analysed by GC-MS.

4.2.4 Experimental plan

Each species will be tested independently except the bivalves which will be tested together. The following picture summarizes the experimental plan of the program.

General experimental plan

Sea bass



Bivalve (mussel + oyster)



Turbo



Grey mullet



4.3 Schedule

The technical preparation of this test program, especially definition and preparation of the testing equipment, is already ongoing and will last up to the end of 2008.

The Activity 1, the short bibliographical study, will be conducted during the preparation of the testing equipment during the end of 2008.

The Activity 2, the test program itself, will begin in January 2009.

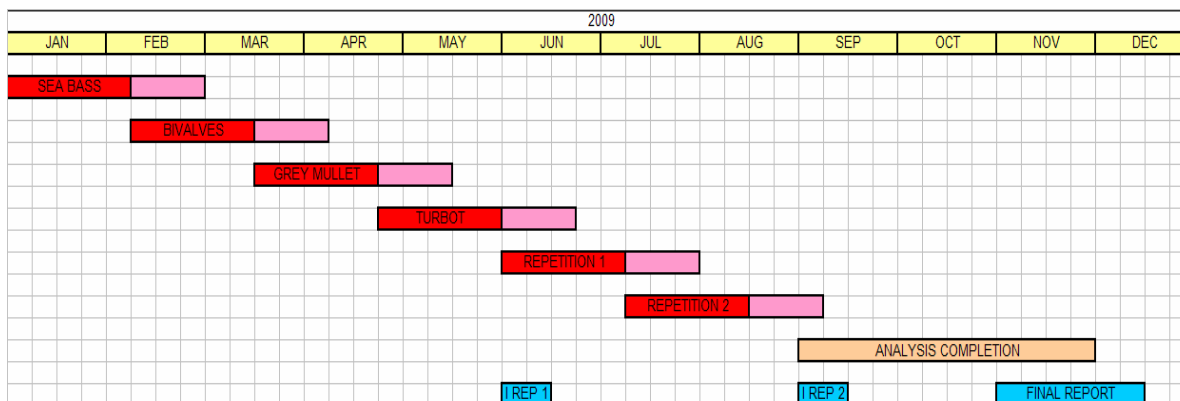
Considering the planned protocol (acclimatization 14 days + exposure 2 days + restoration 14 days), the duration of a test will be 5 weeks (not included: the growth rate assessment carried out in an additional tank).

It is planned to complete the testing program in between January and June, keeping the summer time to repeat the tests which would have not been satisfactory; (past experience proved that experimental work could faced unexpected difficulties).

However, some analyses of the samples collected during the tests will last after the tests program, between September and November.

This program will end with the final report in December.

The following bar chart presents the planned schedule for the Activity 2 (completion of the tests).



4.4 Reporting

3 reports are planned to be issued:

- an intermediary report by June to report on the progress of the testing program,
- an intermediary report by September to summarize the testing program which has been completed,
- and the final report by the 15th of December.

Annex 1:

GENERAL DESCRIPTION OF DISCOBIOL PROJECT



DISCOBIOL JSP.

Joint Study Project on the use of chemical dispersion in coastal areas

CONTEXT

Dispersants are known to be an appropriate response option offshore where dilution conditions are infinite and allow dispersed oil concentrations to decrease rapidly under environmental harmful levels. In coastal areas where dilution can be restricted due to limited depth and the vicinity of the coastline, dispersant use is often limited: as an example of such limitations, along the French coast geographical limits have been defined taking into account minimum depth and distance to the shoreline and to sensitive areas (such as aquaculture, ecological reserve...) for different pollution sizes of 10, 100 and 1000 tonnes of oil to be dispersed.

However these general limits do not take into account special areas especially estuaries and closed bays. In these situations due to the vicinity of the different resources, it is necessary to analyse and weigh up the advantages and the potential risk for the different sensitive resources of dispersing the oil. In other words it is necessary to consider simultaneously the different sensitive resources by completing a Net Environmental Benefit Analysis.

Such an analysis requires knowing the dispersed oil toxicity level for the different habitats.

A lot of work has already been done on this topic, however, results are not often comparable as the methodologies used to get these data are different (e.g. oil type, exposure time...).

The question of using dispersant in very coastal areas particularly estuaries remains an unsolved question for responders and this is especially problematic as estuaries and coastal areas are often locations where the pollution risk is high due to the concentration of human activities (harbours, industries and ship traffic).

OBJECTIVE

The work program aims to provide responders with a clear and practical information with which they can decide if chemical dispersion is an advisable option in case of an incident in an estuary or in the close vicinity of the coast.

The work program aims to acquire comparable and robust information on the impact of dispersed oils on the different habitats and resources of estuaries and/or close bays. This information will give the possibility for responders to carry out reliable NEBA on dispersant use according to their own scenarios.

PRINCIPLE

The program consists in:

1) Experimental program :



- 1-1) **Comparable assessments of the toxicity and impact of dispersed oil towards different eco-compartments** of an estuary in a temperate climate (organisms in the water column –pelagic and benthic fish, bivalves and crustaceans- mudflats and salt marsh); these tests will be carried out using the same oil to obtain comparable data for the sensitivity of the different resources.
- i. **Organisms in the water column** (Year 1 – in progress):
 - a) short term acute toxicity of the oil towards the different species
 - b) sub-lethal effect of short term exposure to dispersed oil.
 - ii. **Mudflat** (Year 2): assessed in mesocosms reproducing mudflat conditions. -
 - iii. - **Salt marshes** (Year 3): assessed through a field trial.



- 1-2) **Classifying the relative toxicity of different oils**, and analyse them in order to link, when possible, the toxicity to the chemical composition. The information from all these toxicity tests carried out on the different compartments of a coastal environment and the ranking of the toxicity of the oils will be used as a basis for implementing recommendations on the use of the chemical dispersion in coastal areas.

2- Exchange of information between professionals concerned by the impact of dispersed oil to:

- Discuss the content, methodology and results of the technical work,
- Bring and share additional information coming from other scientific programs conducted elsewhere. In this respect the original scope of work may be enlarged to other sub-topics (other climates, fine mineral effect on toxicity, tainting...).
- Harmonize the methodologies/protocols used by research teams in order to studies which generate comparable data (e.g. definition of reference crude oils for toxicity assessments or standard testing conditions...).





In addition to the scientific participants discussions will include scientific and operational persons in order to bring in the practical experience and real pieces of information from case stories in order to enrich the whole study.

This discussion group will be a platform to define relevant recommendations on the use of chemical dispersion in very coastal areas.

A possible objective to further this study, would be to write official and widely accepted guidelines on dispersant use in the frame of IMO (revision of the current IMO-PNUE guide line on dispersant).

PARTICIPANTS - PARTNERS

Participants / contributors: *Cedre* (leader), universities of Brest and La Rochelle, AFSSA, Total special fluids, Innospec-Gamlen, and....

 <p>OSRL (UK) Oil Spill Response and East Asia Response Limited</p>	<p>CRRRC (US)</p>  <p>Coastal Response Research Center</p>	<p>Exxon (US)</p> 
<p>Department Fisheries Oceans (Canada) Fisheries and Oceans Pêches et Océans Canada</p> 		<p>ITOPF (UK)</p> 