DISCOBIOL Program: Investigation of Dispersant Use in Coastal and Estuarine

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ABSTRACT

Dispersants are known to be an appropriate solution for offshore spill response when dilution conditions are high and dispersed oil concentrations will decrease rapidly below levels that could potentially harm the environment. In coastal areas, however, where dilution can be restricted due to limited depth and proximity to various coastal resources, dispersant use requires further consideration. In certain cases, the use of dispersants could be beneficial to these regions while in others their use may be more problematic. In response to these situations, it is necessary to analyze and assess the advantages and potential risks of dispersing oil in these sensitive regions.

The Discobiol work program aims to acquire comparable and robust information on the impact of mechanically and chemically dispersed oil on different habitats and resources, most notably estuaries and/or closed bays. Information regarding lethal and sub-lethal effects will be analyzed for several organisms in the water column, mudflats, and salt marsh communities. The information gathered through this work program will be used to make recommendations for the use of dispersants in such areas.

In this program, the determination of an interspecific sensitivity scale with organisms from different trophic levels exposed to the soluble fraction of different oils was included.

For this purpose, tests were conducted on *Vibrio fischeri* using Delta Tox equipment and on brine shrimps *Artemia sp* using more conventional LC_{50} tests. Each species was exposed to a Water Accommodated Fraction (WAF) and to a Water Soluble Fraction (WSF) of 4 different oils.

INTRODUCTION

Aquatic ecosystems have some of the world's richest biodiversity. Pollution in this environment is therefore as much a concern for flora and fauna as it is for humans using the wealth of these resources (Galloway and Depledge, 2001). The shoreline is one of the most contaminated areas. It is therefore most affected by anthropogenic discharges, whether urban, agricultural or industrial. Due to increased shipping, the marine environment has a higher pollution risk, whether owing to spills or operational discharge. In the face of increasingly severe environmental risks, the European Community has made environmental protection and resource management one of its main priorities.

Although the risk of oil pollution has considerably dropped since the tightening of safety regulations and maritime surveillance, it is still far from negligible. When a spill occurs offshore, response means are deployed to contain and recover the pollutant. In the case of an oil spill, the use of dispersants may be required. Dispersants transfer the oil slick from the surface into the water column. This diffusion of the oil will affect all the flora and fauna exposed at effects-based levels in the spill area. The use of dispersant in the event of an oil spill will therefore increase exposure to hydrocarbons by certain resources while reducing exposure to others (Ramachandran *et al*, 2003). Its use in coastal areas, especially in fisheries and aquaculture areas, should be carefully considered through an environmental cost-benefit analysis (Koyama and Kakuno, 2004). For these reasons, this response strategy is only used in very specific conditions which take into account the weather conditions, the water depth in the affected area, the biological resource types exposed to the dispersed or undispersed oil, the distance from the coast and the type of oil.

In this respect, Cedre is currently carrying out a research programme on this topic: the Discobiol project.

The Discobiol program involves comparable assessments of the toxicity and impact of dispersed oil towards the three main eco-compartments of the coastal or estuarine environment of a temperate climate (organisms in the water column, mudflat habitats and salt marshes). No research was carried out on organisms that interact at the sea water interface.

- Phase 1: Organisms in the water column, involves short-term acute toxicity assessment of the oil towards different species (pelagic fish (sea bass), benthic fish (turbot and additionally grey mullet), bivalves (oysters and mussels) and crustaceans (shrimp)) [phase 1A], followed by a sub-lethal effects assessment on the same species except shrimp [phase 1B].
- Phase 2: Mudflat habitats will involve mesocosm experiments.
- Phase 3: Salt marshes are to be assessed through a field trial.

These experiments were conducted with rather short exposure times of 24 to 48 hours (i.e. 2 to 4 tidal movements) in order to reflect realistic conditions of coastal pollution in which the dilution process is expected to reduce the dispersed oil concentration. The tests were carried out on the entire dispersed oil (and not only on the water accommodated fraction) in order to best reflect the impact of a real spill, including the chemical toxicity of the oil-dissolved compounds and the damage resulting from contact of the animals with the suspended oil droplets.

In order to obtain comparable data for the sensitivity of the different resources, all these tests were carried out using the same oil. This oil was an Arabian Light Crude. Oil was pre-evaporated to simulate realistic situations (i.e. oil that would have spent a few hours at sea before reaching the shore or being dispersed). This evaporation was performed under atmospheric conditions and natural UV-sunlight. The resulting chemical composition of the oil was 54% saturated hydrocarbons, 34% aromatic hydrocarbons and 12% polar compounds. The dispersants used were third generation dispersants and their efficiencies were 62% for dispersant 1 and 45% for dispersant 2 (these measurements were obtained using the French IFP test method). They were deemed effective enough to be used in the marine environment (preliminarily determined using the method NF.T.90-345, November 1990), non-toxic at the

concentration recommended by the manufacturer (preliminarily determined assessing standard toxicity test: method NF.T.90-349, April 1997) and biodegradable.

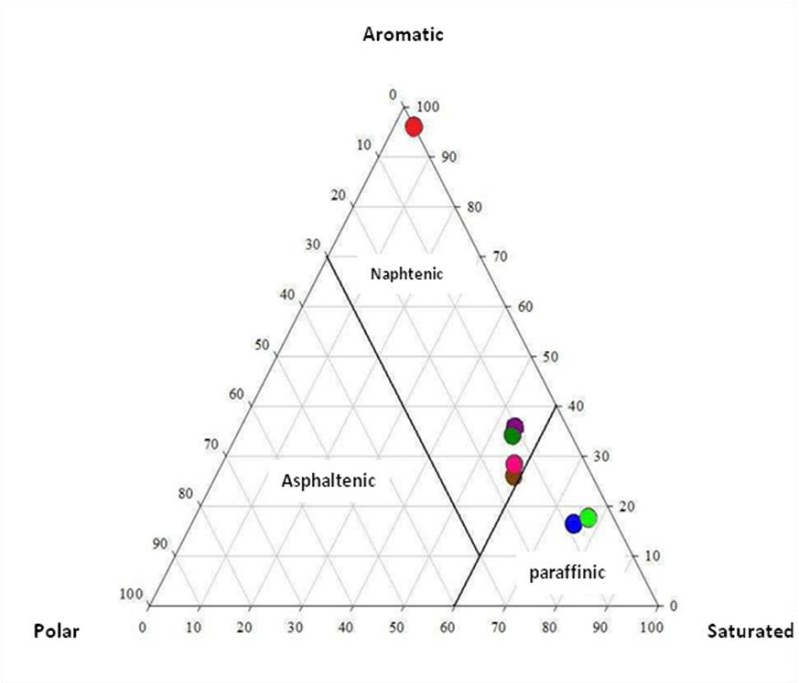
However, an additional study was carried out in order to classify this oil in terms of toxicity in relation to other commonly encountered oils. To do so, an interspecies sensitivity scale was established for different oils with characteristic compositions (heavy, light, naphthenic and paraffinic). This interspecies sensitivity scale was based on three ecotoxicity trials conducted using recognised simple, rapid protocols in order to respond to the inherent operational urgency of a spill situation:

1- Delta Tox measurement in *Vibrio fischeri*, a bioluminescent marine bacterium. Field instrument
2- Lethal concentration LC_{50} investigation in *Artemia sp*, a halophilic crustacean. Laboratory trials
3- *Artemia sp* cyst hatching success. Laboratory trials

MATERIALS AND METHODS

Oils

In order to obtain a wide range of results, different oils were tested. The choice of different oil was determined according to their paraffinic compound and saturated compound contents (Figure 1).



Light Cycle Oil (LCO)
Weathered Arabian Light Crude
Djeno Oil Blend (Congo)
Light Nigerian Oil
Arabian Light Crude (ALC)
Kole Oil Blend (Cameroon)
Forties Oil (North Sea)

Figure 1: Distribution of tested oil compound

These 7 oils can be separated into 2 groups: light and heavy oils.

The light oils (LCO, Nigerian and Forties) are characterised by the lowest polar compound contents and the higher solubility's (between 0.58 and 0.77 ppm). The heavy oils have a solubility of less than 0.3 ppm.

In order to conduct the Delta Tox trials, Water Accommodated Fractions (WAF) were prepared for all the oils following the method described by Barron *et al*., 1999 as well as by Hokstad *et al*, 2000 (Figure 2).

Figure 2: Preparation of an oil WAF

For the two trials conducted in the laboratory on *Artemia*, the aim was more to validate simple, robust laboratory tests. They were therefore performed only on the WAF and Water Soluble Fraction of ALC and artificially weathered ALC. Concentrations of 21 Polycyclic Aromatic Hydrocarbons (including the 16 US-EPA PAH) are presented in the table 1.

Table 1: Concentration of 21 PAH (alkylated and parents) in the Arabian Light Crude (ALC) and in the weathered Arabian Light Crude. The 21 PAH represent the 16 US-EPA PAH and five supplementary PAH (benzo[b]thiophene, biphenyl, dibenzothiophene, benzo[e]pyrene, and perylene). n.d.= not detected.

Molecular	weight (g/mol)	Concentration in ALC (µg/g of petroleum)	Concentration in weathered ALC (µg/g of petroleum)	WAF (100mg of ALC in 1 L of sea water) (ng/L)
Naphtalene	128.2	222	211	4117
C1-Naphtalene	143.2	955	854	34021

C2-Naphtalene	158.2	2099	1819	73409
C3-Naphtalene	173.2	2084	1796	80199
C4-Naphtalene	188.2	1480	1317	24884
Benzo[b]thiophene	134.2	5	5	179
C1-benzo[b]thiophene	149.2	63	22	813
C2-benzo[b]thiophene	164.2	298	292	7425
C3-benzo[b]thiophene	179.2	681	1030	15671
C4-benzo[b]thiophene	209.2	606	537	n.d.
Acenaphtylene	152.2	30	25	348
Biphenyl	154.2	15	14	320
Acenaphtene	154.2	4	3	82
Fluorene	166.2	45	39	1838
C1-Fluorenes	181.2	132	116	2219
C2-Fluorenes	196.2	269	230	1272
C3-Fluorenes	211.2	304	261	1331
Phenanthrene	178.2	112	95	930
Anthracene	178.2	112	95	5
C1-phenanthrenes/anthracenes	193.2	396	335	968
C2-phenanthrenes/anthracenes	208.2	603	498	372
C3-phenanthrenes/anthracenes	223.2	493	416	111
C4-phenanthrenes/anthracenes	238.2	318	273	n.d.
Dibenzothiophene	184.3	373	330	2969
C1-dibenzothiophenes	199.3	1115	987	2910
C2-dibenzothiophenes	214.3	2021	1759	1271
C3-dibenzothiophenes	229.3	1764	1546	312
C4-dibenzothiophenes	244.3	1040	936	n.d.
Fluoranthene	202.3	7	6	4
Pyrene	202.3	11	9	14
C1-fluoranthenes/pyrenes	217.3	62	51	15
C2-fluoranthenes/pyrenes	232.3	137	119	17
C3-fluoranthenes/pyrenes	247.3	222	191	6
Benzo[a]anthracene	228.3	19	16	n.d.
Chrysene	228.3	18	15	11
C1-chrysenes	243.3	37	29	5
C2-chrysenes	258.3	57	45	n.d.
C3-chrysenes	273.3	84	88	n.d.
Benzo[b+k]fluoranthene	252.3	3	3	7
Benzo[e]pyrene	252.3	2	2	n.d.
Benzo[a]pyrene	252.3	11	9	n.d.
Perylene	252.3	3	7	n.d.
Benzo(g,h,i)perylene	276.3	2	2	n.d.
Indeno(1,2,3-cd)pyrene	276.3	n.d.	n.d.	n.d.
Dibenz(a,h)anthracene	278.4	n.d.	n.d.	n.d.

Delta Tox

Delta Tox (Figure 3) is the field version of Microtox and uses reference bioluminescent bacteria (*Vibrio fischeri*) (Girotti *et al*, 2008; M.D. Hernando *et al*, 2006) to assess each sample's toxicity. This bioluminescence is a metabolism indicator, therefore demonstrating the bacteria's health condition (Adoki and Odokuma, 2007).

The photons produced by the bacteria were amplified and measured with a Photon Multiplier Tube (PMT); the difference between the number of photons produced before and after contamination was used to estimate the toxicity of the sample tested.

Figure 3: Delta Tox field instrument

Tests on *Artemia sp*

a) LC50 tests

Cysts were exposed to natural light and a temperature of between 25°C and 30°C. After 24 hours of contact, the hatched larvae were used for the tests.

The *Artemia* larvae were exposed to WAF and WSF in multiwell plates (Figure 4). Only one type of contaminant was tested in each dish in order to produce 4 dilutions in triplicate. The dilutions were performed directly in each well using a micropipette.

The nauplii were placed in suspension in 2 mL of diluted solution. 10 larvae were tested per well. The larvae were exposed to the WAFs and WSFs of ALC and artificially weathered ALC. In order to prevent too high a level of photodegradation of the PAHs, exposure was performed in the dark.

Figure 4: Multiwell plate used for toxicity testing on *Artemia* larvae and cysts.

b) Hatching tests

In order to obtain better characterisation of the reference oil in the Discobiol project (Arabian Light Crude) a study on the hatching success of *Artemia* cysts was conducted.

Artemia cysts were exposed in the same type of multiwell plates as in the toxicity experiment on *Artemia* nauplii. Only one type of contaminant was tested in each dish in order to produce 4 dilutions in triplicate. The dilutions were performed directly in each well using a micropipette.

The cysts were placed in suspension in 2 mL of diluted solution. 30 cysts were tested per well. The cysts were exposed to the WAFs and WSFs of ALC and artificially weathered ALC.

In order to prevent too high a level of photodegradation of the PAHs, exposure was performed in the dark. After 24 hours of contact of cysts with the mixture, the hatched larvae were counted.

RESULTS AND DISCUSSION

Delta Tox

The graph in Figure 5 shows the LC_{50} obtained with the different oils tested.

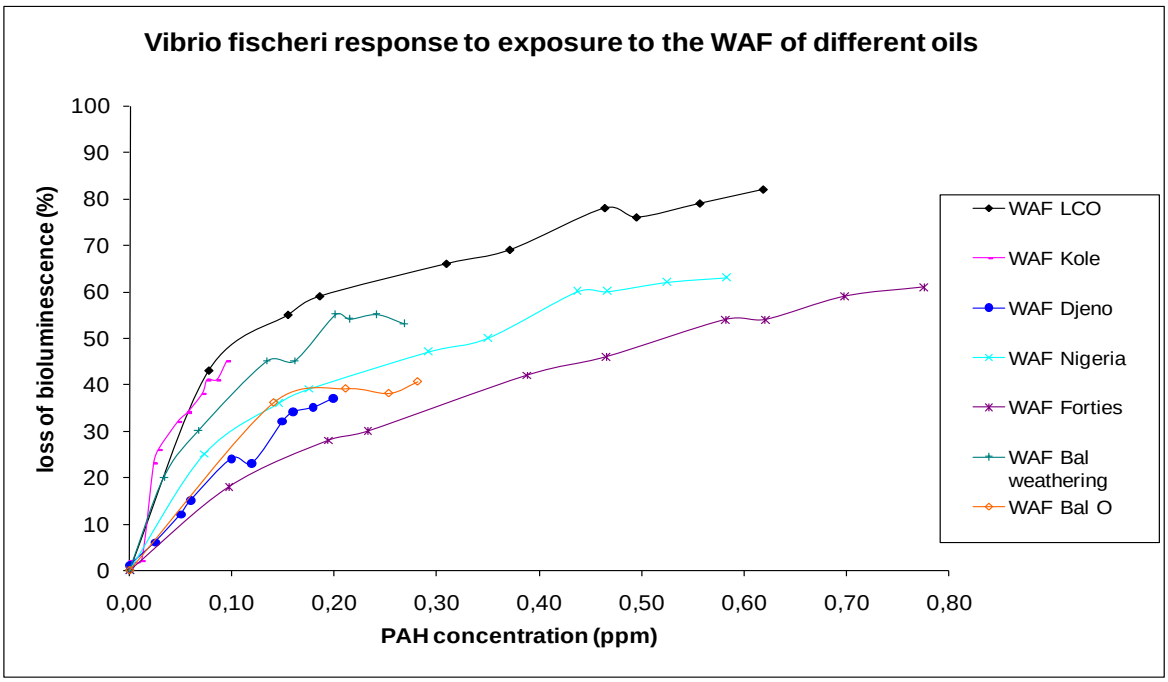


Figure 5: Graph showing the *Vibrio fischeri* responses to the WAFs of different oils. PAH concentration is the sum of the PAH listed in the table 1.

The lightest oils (LCO, Forties, Nigerian) produced the most concentrated soluble fraction and their pure WAF was therefore also the most toxic. A LC50 was obtained for each of these oils. These were the most potentially noxious oils.

A LC_{50} could not be obtained for the heaviest oils (Kole, Djeno). Nevertheless, these results showed very strong inhibition (up to over 30%) of the bioluminescence of the bacteria.

Study of different lethal concentrations of *Artemia* nauplii with ALC

The mortality of control samples was always lower than 10%, demonstrating the quality of the test. No LC_{50} could be obtained with the WAFs or WSFs of the different ALCs and the LC_{10} was not obtained for any of the samples.

This experiment appears to indicate that the *Artemia* model is not relevant in this study. However, another life phase of this biological model could provide more accurate information on the toxicity of soluble oil fractions, hence the hatching tests.

Futhermore, while this experimental protocol is perfectly suited for a study as part of a research programme, it lacks the reactivity required for to be used in an emergency situation.

Hatching success of *Artemia sp* with pollutant

Table 2 below shows the decrease in the hatching success of *Atemia* cysts in relation to the control in percent according to the percentage of contaminant (dilution from the initial WAF or WSF). The negative values represent an increase in hatchings in relation to the control sample.

Table 2: Decrease in the hatching success of *Atemia* cysts in relation to the control in % according to the percentage of contaminant.

	% of contaminant			
petroleum	**25**	**50**	**75**	**100**
WSF ALC	34.3	30.7	30.8	39.9
WAF ALC	10.14	22.1	10	34
WAF weathered ALC	-4.9	18.7	-7.8	14.7

No hatching inhibition rate of over 50% was obtained and the responses remained rather variable. Nevertheless, a clear trend of hatching inhibition emerged.

As with the *Artemia* mortality tests, this bioassay is not suitable for an operational context due to its long implementation time.

Nearly 10,000 cysts were required to conduct the hatching tests on *Artemia*.

CONCLUSION

The aim of this supplementary DISCOBIOL project study was to obtain an interspecies sensitivity scale for different oils.

As bioassays, by definition, involve *in vitro* observation, they must be handled with care as a large number of parameters can influence the results. However in an operational response context, bioassays can be considered as reliable diagnosis instruments.

The aim of these additional experiments was to identify a rapid and efficient method of obtaining a toxicity assessment of an oil spill at sea. This toxicity was studied through different bioassays on the soluble fractions of different oils. Two trophic levels were involved: *Vibrio fischeri* and *Artemia sp*.

The inhibition of the bioluminescence of *Vibrio fischeri* was measured using Delta Tox, a field instrument whose simple and rapid implementation makes it an ideal tool for use in spill situations. The fact that 4 LC_{50} and 7 LC_{10} were obtained for 7 characteristic crude oils using Delta Tox demonstrates its utility in this context. The comparison of these results with those obtained from other bioassays would refine this toxicity assessment. A comparative study at different trophic levels would provide more fully representative results on the toxicity of the oil tested.

Mortality and hatching counts were performed on *Artemia sp.* The low cost of these measurements makes this procedure a good tool to work with. However, the duration of these tests (24 or even 48 hours minimum) is a limiting factor in emergency contexts.

The inability to obtain LC_{50} or even LC_{10} values during *Artemia sp* mortality tests undermines the relevance of the use of this bioassay in this context. The sensitivity of these crustaceans was not high enough to efficiently respond to the constraints imposed. Likewise, the results showing clear inhibition of hatching are not robust enough to provide a diagnosis.

The use of a single species is not sufficient to assess the toxicity of an oil slick. It would be of interest to identify another model at a higher trophic level, such as corals or fish larvae, whose sensitivity to dissolved hydrocarbons would help to refine the initial diagnosis established using *Vibrio fischeri*.

A website is dedicated to the project "Discobiol": more information can be found at http://www.cedre.fr/project/discobiol/

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