DISCOBIOL Project

1) GENERAL INTRODUCTION

For countries where dispersant use is considered a response option, most national policies impose depth/ distance restrictions to ensure there is sufficient dilution of the dispersed oil in order to minimise potential impacts on sensitive coastal resources. Nevertheless, the benefits associated with dispersant use are still applicable to near shore areas. In making a decision on dispersant use in these areas, the potential benefits need to be weighed against the potential risks and impacts. In an effort to answer the questions of whether dispersant use is advisable in coastal and estuarine areas, Cedre have been leading a three year research project. The project has a budget of just over €1 million covered by the French National Agency for Research with complement from the French Navy, Total SA, with a number of additional contributing external partners including Total, ExxonMobil, and OSR.

The overall aim of the project is to generate robust, comparable technical information on the effects of mechanically and chemically dispersed oil toxicity on habitats and resources found in estuaries and other inshore areas. Information regarding the lethal and sub-lethal effects will be analysed for several organisms found in the water column, in mudflats and in salt marshes. It is hoped that the information gathered by this project will improve operational guides and so enable responders to carry out a reliable NEBA when making decisions on dispersant use in nearshore waters.

2) WORK PROGRAMME

The experiments have been designed to simulate, as closely as possible, spill conditions with the objective of generating comparable datasets. Therefore, only one oil (Arabian light crude oil weathered to simulate 4-8hrs at sea) has been used for all of the experiments. All of the oily water is used in the tests (not just the soluble compounds found in the water accommodated fraction), and the tests are run over a reasonably short exposure 24-48 hours which replicates 2-4 tidal cycles in order to cope with a realistic situation.

The study is divided into the following phases:

Phase 1: compared toxicity of the chemically and mechanically dispersed oil towards organism living in the water column (or directly in contact with).

This phase is divided into 3 sub-phases:

1A: acute toxicity of dispersed oil

1B: sub-lethal effect of dispersed oil

1C: level of toxicity of different oils

Phase 2: effects of dispersed oil in mudflat habitat

This phase is divided into 2 sub-phases:

2A: effect of dispersed oil in mudflat

2B: long term effects on fish first exposed to dispersed oil, and then put in situ in marsh environment.

Phase 3:

Discussion of results with other parties and implementation of recommendations on the use of dispersants

Progress of the study:

See table below:

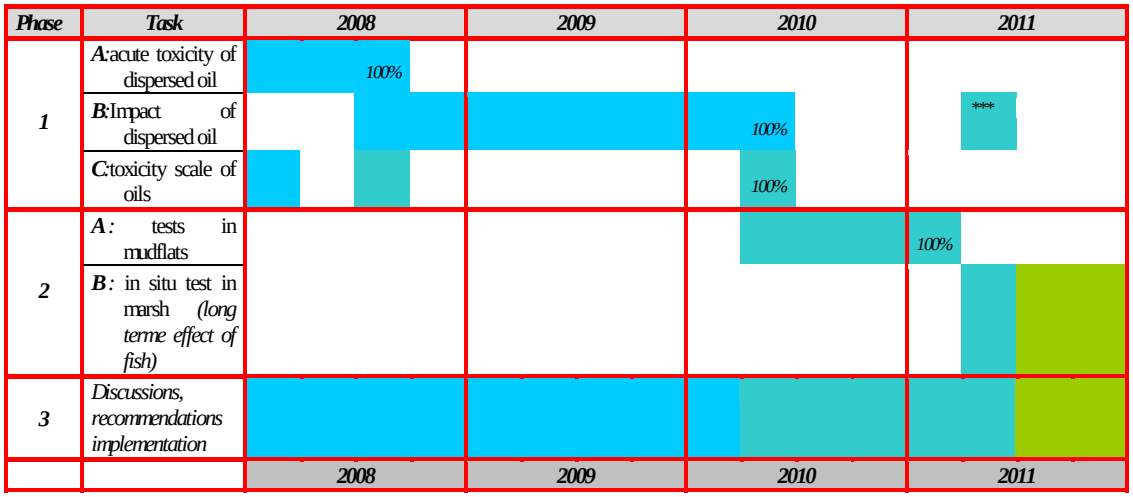


table 1 : study progress timetable

3) EXPERIMENTAL WORK DESCRIPTION AND RESULTS

Phase 1: compared toxicity of the chemically and mechanically dispersed oil towards organism living in the water column (or directly in contact with).

3-1) Phase 1A: short term toxicity assessment:

The work is conducted on shrimp, pelagic fish (sea bass) and benthic fish (turbot and grey mullet), bivalves (mussels and oysters) and crustaceans (shrimp). Adult animals are exposed to various conditions for 24 hours followed by 24 hours in clean sea water. Tests involve exposure to mechanically dispersed oil, chemically dispersed oil using two different dispersants, the dispersants themselves and a control without oil.

The mortality (acute toxicity) is observed at the end of the tests.

The conclusions of phase 1A were:

- *The toxicity with dispersant was higher than the one without dispersant*
- *No mortality was observed without dispersant*
- *Dispersed oil seemed to alter respiration process (on fish)*

- *On bivalve, the tests were no conclusive; as the bivalve closed their shell (chemo-detection) it was not possible to reach a lethal concentration (LC_{50}).*
- *On fish, the toxicity level (LC_{50} 24h) was extremely high (x100ppm to few 1000ppm) and much higher than the oil concentration actually observed in real incidents (ppm to x10 ppm)*
- *According to these results, the chemical dispersion should not promote mortality in real situations (at least on adults).*

3-2) Phase 1B: sub-lethal effects on pelagic and benthic fish as well as bivalves promoted by realistic dispersed oil exposures:

According to observations made in phase 1A, it seemed necessary to look for possible sub-lethal effects resulting from the exposure to chemically or not dispersed oil. Additionally, the exposure time was extended to 48 h.

The principle of these tests was to expose the animals to the same amount of oil with and without dispersant in the same conditions especially the mixing energy; therefore the oil concentrations in the water column varied from 20 to 70 ppm.

Since the project focuses on inshore areas, this phase of the project includes additional testing to investigate the effect the presence of suspended mineral particles and the subsequent formation of aggregates can have on the toxicity and the bioavailability of the oil.

Tests were conducted on sea bass, grey mullet, turbot, mussel and oyster.

The impact of the oil was assessed after the exposure and after 2 weeks recovery through a large number of bio-indicators, physiology, stress indicator, immunology, as well as oil bioaccumulation.

The main conclusions of phase 1B were:

- *As expected from phase 1A, no mortality was observed for 48h exposure at 30 to 70 ppm either for chemically or mechanically dispersed oil.*
- *Compared to the control (no oil) sub-lethal effects were observed just after the exposure for both chemically and mechanically dispersed oil.*
- *The presence of mineral particle tends to reduce the effects observed after exposure on the chemically dispersed oil to the level of mechanically dispersed oil for pelagic fish; for benthic fish, the presence of mineral particles leads to respiratory problems (particles with and without dispersed oil).*
- *For fish, most of the effects observed after exposure were no longer observed after 2 weeks recovery; and when still observed, they were highly reduced. (See table 2)*
- *For fish, when comparing chemically dispersed with mechanically dispersed, they no difference after 2 weeks recovery (see table 3).*
- *For bivalves, effects are still observed after the depuration time; data need to be analysed more carefully.*

See in Annex the text of the presentation given at AMOP 2010 seminar.

Sublethal effects of dispersed oil

Effect on fish of the presence of oil (DM, DC1&2, PS+DC) compared with the control (T) at the end of the exposure time (T1) and at the end of the depuration time (T2)

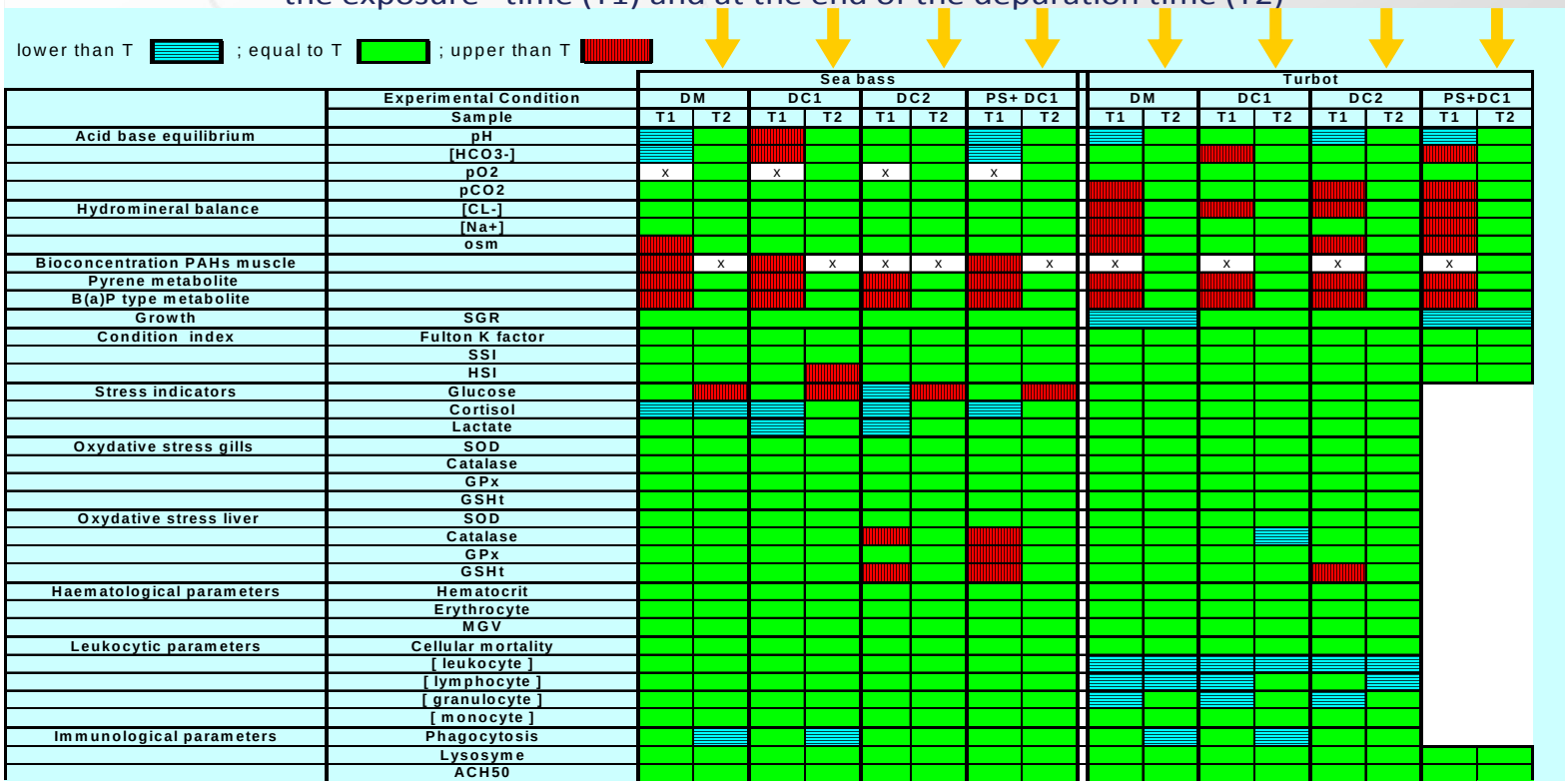


Table 2 : effect on fish of the presence of oil (mechanical dispersion [DM], chemical dispersion with dispersant 1 & 2 [DC1], [DC2], and chemical dispersion with mineral particles [PS+CD1], compared with the control [T] at the end of the exposure (T1) and at the end of the depuration time , two week (T2)

Sublethal effects of dispersed oil

Effect on fish of chemical dispersion (DC) compared with mechanical dispersion (DM) at the end of the exposure time (T1) and at the end of the depuration time (T2)

lower than DM ; equal to DM ; upper than DM

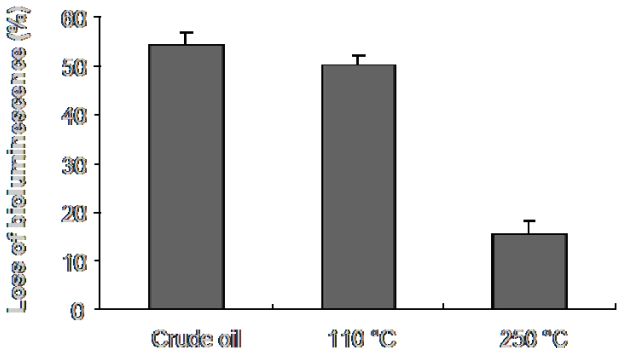
	Experimental condition	Sea bass				Turbot			
		DC1		DC2		DC1		DC2	
	Sample	T1	T2	T1	T2	T1	T2	T1	T2
Acid base equilibrium	pH								
	[HCO3-]								
	pO2	x		x					
	pCO2								
Hydromineral balance	[CL-]								
	[Na+]								
	osm								
Bioconcentration PAHs muscle			x	x	x	x		x	
Pyrene metabolite									
B(a)P type metabolite									
Growth	SGR								
Condition index	Fulton K factor								
	SSI								
	HSI								
Stress indicators	Glucose								
	Cortisol								
	Lactate								
Oxydative stress gills	SOD								
	Catalase								
	GPx								
	GSHt								
Oxydative stress liver	SOD								
	Catalase								
	GPx								
	GSHt	x		x		x		x	
Haematological parameters	Hematocrit	x	x	x	x	x	x	x	x
	Erythrocyte	x	x	x	x	x	x	x	x
	MGV	x	x	x	x	x	x	x	x
Leukocytic parameters	Cellular mortality								
	[leukocyte]								
	[lymphocyte]								
	[granulocyte]								
	[monocyte]								
Immunological parameters	Phagocytosis								
	Lysosyme								
	ACH50								

Table 3 : effect on fish of the chemical dispersion with dispersant 1 & 2 [DC1], [DC2], compared with the mechanical dispersion [DM], at the end of the exposure (T1) and at the end of the depuration time , two week (T2)

3-3) Phase 1C: level of toxicity of different oils.

Although only on a single oil Arabian light crude is being used in the toxicity experiments, other complimentary work will rank a number of different oils according to their relative toxicity.

This task was carried out using a simple and cheap toxicity assessment method the DELTA tox *(similar to micro-tox).*
The results did not show large differences between oils. However, these tests confirmed the importance of the weathering stage of the oil: the more weathered the oil, the less toxic it is (see picture 1)



Picture 1 : Oil toxicity according to its weathering stage

This importance of the weathering may explain why other studies (e.g. North American) conducted on the same issue which are often with fresh crude, use to give higher toxicity results. However, in a real incident, it should be kept in mind that the oil will have spent few hours weathering at sea before dispersant treatment could be achieved.

3-4) Phase 2A: looking for sub-lethal effects promoted by dispersed oil on mudflat habitats

There are two aspects to this phase, firstly an investigation of the interactions between mudflats and pollutants and secondly a study of interactions between polluted mudflats and organisms (grey mullet, mussels and oysters).
This task has been completed in mesocosm experiments.
The interactions between the dispersed and non dispersed hydrocarbons with the mud and with phytobenthos (algae film) were assessed by looking to the oil penetration into the first centimetres of the mud and to the variations of chlorophyll *a* biomass.
A first set of experiments consisted in letting exposing the mudflat (mesocosm) and its biofilm to oil and dispersed oil at 100 ppm for 48 h with tidal movement (9 hours emersion and 3 hours immersion).

The main conclusions were:

- *at 66 ppm the dispersed oil has an effect on the photosynthetic activity (30 to 40% reduction), the non dispersed oil, not, however, there was no difference on the chlorophyll A biomass between chemically and mechanical dispersed.*
- *The mud contamination remained low (150 ppm over the background level) and limited to the first centimetre*

- *To achieve a clear contamination of the mud (400 ppm over the background level) it was necessary to increase 4 times the oil concentration during the exposure phase.*
-

A second set of experiments consisted in growing fish and oyster on mud previously contaminated as defined in the first set of experiments.

The first conclusions were:

- *With fish effects can be observed after 5 days of exposure to oil while for oyster, such effects are observed after 7 to 10 days..*

Full analysis has still been achieved.

3-5) Phase 2A: looking for long term effect promoted by dispersed oil on fish in natural habitat (saltmarsh)

Note: initially it was plan to carry out an experiment with dispersed oil release in salt marsh in Canada; due to the fact that the oil release permit was not gained this plan has been moved for another experiment (complementary of previous Discobiol ones), looking for long term effect of dispersed oil on fish.

The last task of Discobiol program consists in looking for long term effect (4-5 months) oil on fish (Sea Bass) from an exposure to dispersed oil.
Fish are exposed to chemically or dispersed oil as they were in the phase 1B, then put in a natural environment, (salt marsh close to La Rochelle).
The performances of the fish are measured before, after oil exposure, and further from month to month.

This experiment is in progress up to September.

3-6)Complementary works

In addition to the work identified in Phases 1A and 1B, other complimentary projects has been carried out by the project partners.

a) effect of Discobiol oil on the sea bass juvenile, by the university of Cote d'Opale, in this task the some exposure have been extended to 96 hours

Conclusions

- *Effect more important after 96 hours exposure*
- *Decrease of growth, condition indice and RNA/DNA ratio = risk for fish well-being and survival*
- *EROD induction right from 48 hours exposure and GST induction after 96 hours exposure*
- *Induction still monitored after the decontamination period*
- *No catalase induction : indirect effects*
- *Dispersant application induce an increase of hydrocarbons concentrations*
- *But no difference on growth between mechanical and chemical dispersion*
- *Surprisingly dispersant use led to a lower induction of EROD*

b) effect of dispersed oil exposure on key physiological system; innate immune system, cardiovascular system, metabolism

Conclusions

- *observed hepatic impairment (SOD-super Oxyde Dismutase activity) with oil mechanically dispersed and the oil water soluble fraction but not with the other testing conditions (dispersant alone, mixture oil and dispersant and control)*
- *Effect on cardiac performance for oil mechanically and chemically dispersed as well as the oil water soluble frat ions)*

c) toxicity of Discobiol oil on cod and herring embryos by DFO/COOGER Canada

Conclusions

- *CEWAF subjects fish to higher concentration of PAHs than WAF*
- *CEWAF is 100X more toxic than WAF to Atlantic herring embryos*
- *Herring embryos are most sensitive to CEWAF at day 2 post-fertilization*

4) PART TWO - EXCHANGE OF INFORMATION

This part of the project focuses on the exchange of information between scientific professionals and those involved with response operations in order to collate information on the impacts of dispersed oil based on information generated from previous research and that gained during incidents. The project aims to produce guidance/ recommendations on dispersant use in near shore areas which can be used to update and enhance operational guides (such as the IMO manual on dispersant use).
So far, according to what is already known, it appears that chemical dispersion does not generate permanent damages to the tested animals, and is not much detrimental in comparison with the non use of dispersant. This preliminary conclusion will have to be validated in the final synthesis of the study.

However, based on this preliminary observation, it has been proposed, in the national policy implementation carried out during recent seminars and revisions, to ease the definition of the geographical limits for dispersion. (Tiers 1 and 2 10 m depth, and Tiers 3, 20 m depth). These proposition concerned, the country of the West Indian Ocean Commission, the West and Central Africa coastal states (WACAF), and the coastal countries of the Mediterranean sea (through the REMPEC document on dispersant).
The revision of the IMO guidelines which will follow in Marsh 2012, will have benefit of the final conclusion of the study. This revision will be carried out through an international working group which will be an appropriate platform for scientific exchanges with the other scientists working on the same issue.

5) TIMESCALES

Refer to "*table 1: study progress timetable"* in 2) WORK PROGRAM

The administrative closing date of Discobiol is the 31 October.

The discussion of the results with other scientific teams and the implementation of recommendations on the use of dispersants will be pursued through the working group in charge of the revision of the IMO guidelines on dispersant. The final version of the IMO guidelines will be issued from the technical workshop planned to take place at (or just before) the next MEPC meeting (March 2012)

ANNEX

Discobiol presentation at AMOP 2010

Discobiol Program: Investigation of Dispersant Use in Coastal and Estuarine Waters

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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 decrease rapidly below levels that could potentially harm the environment. In coastal areas, however, where dilution can be restricted due to limited depth and vicinity to various coastal resources, dispersant use should be limited. In contrast, for certain cases, the use of dispersants could be beneficial to these regions. 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 close 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 in this work program will be used to make recommendations for the use of dispersants in such areas.

This paper presents the details of the study and the preliminary results of the current phase of this study which is the assessment of lethal and sub-lethal effects of dispersed oil towards organisms in pelagic and benthic communities. These tests involve the comparative assessment of the effects from mechanically and chemically dispersed oil, and, in order to reflect estuarine conditions, suspended particulate matter.

1 Program description

The Discobiol program involves comparable assessments of the toxicity and impact of dispersed oil towards the 3 main eco-compartments of the coastal or estuarine environment of a temperate climate (organisms in the water column, mudflat habitat and salt marsh).

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

These experiments are conducted with rather short durations of 24 to 48 hours, (*i.e.,* 2 to 4 tidal movement), in order to reflect realistic conditions of a coastal pollution in which the dilution process is expected to bring down the dispersed oil concentration. The tests are carried out on the whole dispersed oil (and not only on the water-accommodated fraction) in order to reflect as much as possible 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 are carried out using the same oil. This oil is a Brut Arabian Light. Oil has been 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). Dispersants used were of the third generation and their efficiencies are, for dispersant 1, 62% and for dispersant 2, 45% (these measurements were obtained using the French IFP test method).

2 Phase 1A: Acute Toxicity Assessment Towards Organisms Living in the Water Column

2.1 Description

The objectives of this phase were to get a first set of data on oil toxicity, with and without the addition of chemical dispersant.

These tests were performed in the regular testing equipment used for the French dispersant approval procedure: this equipment is composed of twelve 16-L tanks, each equipped with a central stirrer which provides the agitation needed to keep the oil dispersed (Figure 1).

Tests were conducted on juvenile animals (sea bass, turbot, grey mullet, oyster, mussel and shrimp). The exposure duration was 24 hours, followed by 24 hours of restoration time in clean sea water. Different testing conditions were considered among which were mechanically dispersed oil (DM), chemically dispersed oil with two different dispersants (DC 1 and DC 2), the two dispersants themselves (ds1 and ds2) and a control (T) (without oil).

LC_{50} values (contaminant concentration that causes 50% mortality) are calculated using the trimmed Spearman-Karber method conducted by US-EPA.

For the dispersed oil conditions, the oil was previously dispersed, (mechanically with or without addition of dispersant) before being introduced in the test tanks.

Figure 1 The Testing Equipment: Set of 12 Tanks

2.2 Results

On Sea Bass, the lethal concentration of dispersed oil could be determined only for the chemically dispersed oil, which set between **[687 - 1074]** ppm for oil and dispersant 1 and **[687 - 1435]*** ppm for oil and dispersant 2. For the mechanically dispersed oil, the lethal concentration appeared to be higher than the maximum stable dispersed oil concentration that could be reached. The concentration of lethal dispersant lethal dispersant concentration could be found only for the dispersant 2 the efficiency of which was a bit lower than that of dispersant 1 (Table 1). During the tests on fish, normal oxygen level (upper than 90% of O_2 saturation) in the water were observed, the animals were observed to swim at the water surface as they would do if they would be lacking oxygen. At the end of the experiment, oil was found in the gills of the fish.

For the bivalves, (mussels and oysters), the mortality curves were not relevant to determine the LC_{50}: the mortality started to decrease over a certain oil concentration, as the animal closed its shell to protect itself from the pollutant. It was only possible to identify a concentration for which the animal shows a positive “chemo-detection”.

For the shrimps, oil droplets were found trapped in the gills at the end of the test.

Table 1 Results of the acute toxicity test on fish and shrimp

Species	**CL_{50} on 24 hours exposure**		
Experimental condition	Mechanically dispersed oil	Chemically dispersed oil Dispersant 1	Chemically dispersed oil Dispersant 2
Sea bass (*Dicentrachus labrax*) weight :4,8 ± 1g	Not reached	**[687 - 1074]*** ppm	**[687 - 1435]*** ppm
Turbot (*Scophtalamus maximus*) **weight :4,6 ± 0,2g**	Not reached	**[241 - 414]* ppm**	**[440 - 506]*** ppm
Grey mullet (*Liza aurata*) **weight :1,8 ± 0,1g**	Not reached	**[436 - 1055]* ppm**	**[1177 - 1636]* ppm**
White shrimp (*Paleomonetes varians*)	Not reached	--	700 ppm

***:** LC_{50} was determined with the US-EPA software using the Trimmed Spearman-Karber Method.

2.3 Conclusion of Phase 1A: Acute Toxicity Tests

For all species, with the mechanically dispersed oil, it was not possible to obtain oil concentrations high enough to reach the LC_{50}. For fish, the chemically dispersed oil proved to be more toxic than the mechanically dispersed oil due to the fact that higher dispersed oil concentrations can be achieved with the addition of chemical dispersant. For the bivalves, the animals demonstrated that they were able to protect themselves from a short duration pollution (at least 24 hours), by closing their shell. Concerning preliminary impact assessment, it appears that concentrations lower than the LC_{50} could affect respiratory function of the animals by impacting gills.

However, for the shrimp and the different fish species, the dispersed oil LC_{50} remains far higher (from 300 to 1200 ppm) than the dispersed oil concentrations which are found in real cases of pollution, which range from a few ppm to several tens of ppm. From these results, we can conclude that a chemical dispersion should not lead to direct or acute mortality on juvenile animals that are living in the water column. However, this conclusion does not necessarily apply to other stages such as larval stages.

3 Phase 1B: Sub-lethal Effect of Dispersed Oil on Organisms Living in the Water Column

The next phase of the project was devoted to the research of possible sub-lethal effects of dispersed oil on organisms living in the water column.

As previously, the experimental plan compares the following situations: oil mechanically or chemically dispersed. Taking into consideration the scope of the study, and that estuarine waters are often highly loaded with fine mineral particles, additional testing conditions involving suspended mineral particles (SPM) are studied: oil dispersed with dispersant A in the presence of SPM and a control with SPM (no oil, but SPM). In fact, few studies (if any) have considered the influence of mineral aggregate formation on the toxicity and impact of the oil. Nevertheless, mineral particles could have an effect on the bioavailability of the oil.

The chosen oil exposure conditions were rather severe, 48 hours exposure and the quantity of oil introduced in the system represented a theoretical concentration of 80 ppm, assuming that real dispersed oil concentrations would be around 40 to 60 ppm for the chemically dispersed oil and 20 to 40 ppm for the mechanically dispersed oil. According to the quantity of silt introduced in the tank, the SPM concentration was in the range of 150 to 200 ppm.

In summary, conditions studied are:

T : Control

DM : Mechanical dispersion of 25 g oil BAL 110

DC1 : Chemical dispersion of 25 g oil BAL 110 by 1.2 g of dispersant 1

DC2 : Chemical dispersion of 25 g oil BAL 110 by 1.2 g of dispersant 2

PS : 80 g suspended particulate materials (SPM)

PS + DC 1: Chemical dispersion of 25 g BAL 110 by 1.2 g of dispersant 1 plus 80 g of SPM

Figure 2 The test tanks with the pumping system designed to keep the oil from resurfacing.

The tests were carried out in 300-L tanks equipped with a pumping system to re-circulate continuously in the water column the oil which would come back to the surface (Figure 2). In total, 14 tanks were used (Figure 3).

Figure 3 View of the eco-toxicological testing facilities of Cedre: on the left, the 14 tanks used to expose the animals to the pollutant, on the right the stabilization tanks

Tests were undertaken on fish (sea bass, turbot, and grey mullet) and bivalves (oysters and mussels) to look for effects on the physiology of the animals, their immune system, their behavior and their growth. Study animals were acclimated for 15 days before experimentation. After the acclimatization period, the first group of ten animals were sampled (T0). For each species, 30 animals were released in exposition tank for 48 hours. For each condition, 5 fish and 10 bivalves were sampled at the end of the exposition period (T1) and depuration period (T2). At the end of T1, 15 fishes for each condition have been transferred in clean water for a growth experiment of one month and a hypoxic challenge.

Table 2 summarizes the main testing conditions studied for each of the four main species: sea bass, turbot, oysters and musses.

Table 2 Experimental condition assessed in phase 1B of Discobiol project for each species.

Animals' Condition	T	DM	DC1	DC2	PS	PS+DC 1
Seabass	x	x	x	x	x	x
Turbot	x	x	x	x	x	x
Oysters	x	x	x		x	x
Mussels	x	x	x	x	x	x

The chemical parameters, which were monitored, were the dispersed oil concentration evolution along the exposure, the concentration of dissolved oil compound in the water (PAH and substituted), and the concentration of oil in the tissue of the animals.

The biological impact of oil was assessed through different types of parameters which are presented in table 3. These parameters were selected through a literature survey which has identified some specific and non-specific biomarkers (Aarab, 2004; Bado et al., 2009; Goanvec et al., 2008; van der Oost *et al*, 2003).

Table 3 Biological parameter assessed in phase 1B of Discobiol project.

	On fish	On bivalves
Stress indicators	Cortisol, lactate, glucose	Cortisol, lactate, glucose
Oxydative stress	SOD, catalase, GPx, GSHt	Laccase activity, GPx, phenoloxydase
Acid base equilibrium	pH, $[HCO3^-]$, pO_2, pCO_2	
Hydromineral balance	$[Cl^-]$, $[Na^+]$, osmolality	
Condition index	K factor, HSI, SSI, SGR	
Immunology	Cellular mortality, leukocyte, lymphocyte, granulocyte, monocyte, phagocytose, lysosyme, ACH50	Cellular mortality, hyalinocyte, granulocyte, phagocytose,LMS
Erythrocytar parameters	Hematocrite, erythrocyte, MGV	
PAHs in organism	Bioconcentration in muscle, metabolite of pyren and	Bioconcentration in muscle
Hydrocarbon concentration in water	Particular and dissolved	Particular and dissolved

SOD: Super oxide dismutase
GPx: Glutathione peroxydase activity
GSHt: Total glutathione
HSI: Hepatosomatic index
SSI: Spleenosomatic somatic index
GRS: Specific growth rate
ACH50: Total haemolytic complement activity
LMS: Lysosome membrane stability
MGV: Medium globular volume

3.1 Conclusion of Phase 1B: Sub-lethal Tests

The main objective of this discussion is not to conclude if biomarkers that were used are relevant to show the impact of pollutants. They were used to investigate whether there is or not a significant difference between oil treatments. The full results will be soon described in future specific communications that written by the different scientific teams that performed the different analysis.

However, as a first overview of the whole study, the results can be presented in a very synthetic form through 3 main questions.

1. Does the presence of oil lead to different effects that from the control (T)?
2. Does the chemically dispersed oil (DC1 & DC2) lead to different effects than from the mechanical dispersion (DM)?
3. Does chemical dispersion in the presence of suspended material (PS+DC1) lead to different effects from chemical dispersion in clear sea water (DC1)?

For each of these questions and for each tested animal, tables have been produced to give the response of the different biomarkers with no line when there is no significant difference, with vertical lines which the indicator was significantly lower, and some horizontal lines when it was significantly higher.

Does the presence of oil lead to different effects than the control (T)?

Comparing relative to control condition, it appears clearly that oil has an impact on fish and also on bivalves (Table 4). In addition, concerning fish, biomarker responses seem to be slightly different between pelagic and benthic species. After the exposure period (T1), for pelagic, 7 biomarker responses are significantly different from the control and, for benthic ones, 10 biomarkers are impacted. After the recovery period (T2), this difference is also observed even if fewer biomarkers are significantly different from the control. First analysis, it appears that sea bass are more resistant than turbot at low contamination of the sea water column.

Concerning bivalves, all biomarker measurements are incomplete. But, preliminary results show that all organisms are impacted after the exposure period (T1) and, this effect seems to be reversible.

At this stage, we are not able to further discuss the impacts of these treatments on the physiology of organisms: why after the recovery period some biomarker responses are still significantly different from the control, i.e., the phagocytosis.

Does the chemically dispersed oil (DC1 & DC2) give different effects than the mechanical dispersion (DM)?

Treatments are compared to the mechanical dispersion to determine any significant difference between them (Table 5).

On sea bass, compared to the mechanical dispersion (DM), the chemical dispersions (DC1&2) give an effect at the end of the exposure (T1), particularly on the acid base equilibrium, on the osmolality, and in terms of bio-accumulation of oil in tissues, as well as for the metabolites biliary. After the two weeks of depuration (T2), all these effects have disappeared. No real difference is observed between the two oil dispersions (made with the dispersant 1 and 2).

Surprisingly, few effects that were not observed at T1 (after exposure) are present after depuration (T2), such as the osmolality (on sea bass), and leukocyte and lymphocyte (on turbot). Concerning turbots, same trends could be observed.

Nevertheless, the number of biomarker responses of sea bass is higher than for turbot at T1 and T2, which could indicate that this species is more sensitive to chemically dispersed oil dispersed than turbot. Turbots are more impacted than sea bass independently of the treatment applied to the oil. This difference could be explained by the target organs of the pollutant. At T1, sea bass seem to be more impacted by Reactive Oxygen Species (ROS) in the liver whereas turbot seem to be more impacted in the gills. In addition, Polycyclic Aromatics Hydrocarbons (PAH) metabolites are more present in the bile of sea bass than in turbot which can illustrate a better detoxification rate for the pelagic. These observations should be linked to the way of life of these animals.

Considering the bivalves, results obtained on the variation of hemolymph cellular composition (Reynaud and Deschaux, 2006), show that both oysters and mussels are impacted. If several differences are observed after exposure (T1) and after depuration (T2), some indicators invert their effects between T1 and T2 (laccase and GPx, Table 6). A deeper analysis of biomarker responses suggests that chemical dispersion has an impact on bivalves, particularly on the immune defence

system and on the digestive gland. At this time, mussels are not well enough documented to draw up relevant conclusions.

Does the presence of suspended material (PS+DC1) change the effect of the chemical dispersion (comparison with chemical dispersion in clear sea water) (DC1)?

This condition was chosen to evaluate the impact of particles on the bioavailability of oil and consequently its impact.

The oil concentrations in sea water were systematically lower than for the other treatments. But, at T1, the number of biomarker responses is always higher than for higher oil concentrations. For turbot, if we look at the acid base equilibrium and hydromineral balance, the presence of mineral particles induces a higher response: gas exchanges are directly impacted and these results can be linked to previous observations made on gills (phase 1A). For sea bass, the oxidative stress liver response is also increased by particles.

Nevertheless, at T2, only few significant responses are observed with this treatment: no more impact is recorded. This result could be linked to metabolites biliary measurements, which are a well known biomarkers of oil exposition (van der Oost *et al*, 2003; Vuorinen *et al*, 2006), because with particles these concentrations are lower than with the other treatments.

4 Conclusion

At this stage of the study, it is still too early to draw any final conclusion for the Discobiol Project which aims to compare the impact of mechanical and chemical dispersion of oil in coastal and estuarine areas.

The first phase of the project (phase 1A) was devoted to the evaluation of LC_{50} at 24 hours of exposure to oil with and without dispersant. Results showed that it was not possible to reach the same concentration of oil in the water column with and without dispersants. Consequently, it was not possible to reach the LC_{50} value with mechanical dispersion. With chemical dispersant, it was possible to distinguish species: turbot seems to be less resistant than pelagic fish (sea bass and grey mullet). No LC_{50} were obtained for bivalves due to their capability to detect oil and to close their shell. Nevertheless, LC_{50} found are higher oil concentrations monitored after a real oil spill and concentrations used during phase 1B of this project.

The second phase of the projet (phase 1B) was devoted to checking for sublethal effects, (biomarkers), following the exposure of animals to oil with and without dispersant (48 h exposure at 20 to 70 ppm). Some responses obtained with biomarkers are contradictory. Consequences of the same contamination can stimulate or depress in accordance with the species. It underlines the intra-specific and inter-specific variabilities, which increase the difficulty in interpreting biomarkers on different species. For this main reason, in this paper, we decided to consider only the responses of these biomarkers as discrete criteria (existence or absence of a significant response, positive or negative, in comparison with the control) and not to take into account the level of the responses, or to explain how oil can affect the physiology and/or the immunology system of organisms. It is true that the importance of these biomarkers for determining an oil impact may be different from one to

another (some biomarkers are more relevant than others). In this paper we just wanted to identify eventual global modification in organisms.

Phase 1B of the project had clearly shown the reversible impact of oil intoxication for the majority of the studied biomarkers. At T2, only stress indicators (sea bass) and leukocytic parameters (turbot) were still different than for the control. For bivalves, these results are less clear and more analyses are needed before concluding.

Nevertheless, these preliminary results tend to open the use of chemical dispersion of oil slicks in coastal areas: the mixture of dispersant plus oil seems to be less detrimental than oil alone, especially for turbot. Coastal ecosystem is the result of connection of biotic and abiotic factors. Consequently, it is required to wait until the end of the project to formulate a global conclusion of chemical dispersion of oil slicks in coastal area.

At last but not least, a final experimental study (project phase 2) is planned to determine the impact of chemically dispersed oil in a realistic environment at La Rochelle: on salt marshes ecosystem. This experiment will last several months and will bring additional pieces of information that will contribute to drawing up better rules on the use of dispersants in coastal areas.

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

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Table 4 : Effect on fish and bivalves of the presence of oil (DM, DC1&2, PS+DC) compared with the control (T) at the end of the exposure time (T1) and at the end of the depuration time.

lower than T ; equal to T ; upper than T

		Sea bass								Turbot							
	Experimental Condition	DM		DC1		DC2		PS+ DC1		DM		DC1		DC2		PS+DC1	
	Sample	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
Acid base equilibrium	pH																
	[HCO3-]																
	pO2	x		x		x		x									
	pCO2																
Hydromineral balance	[CL-]																
	[Na+]																
	osm																
Bioconcentration PAHs muscle			x		x	x	x		x	x		x		x		x	
Pyrene metabolite																	
B(a)P type metabolite																	
Growth	SGR																
Condition index	Fulton K factor																
	SSI																
	HSI																
Stress indicators	Glucose																
	Cortisol																
	Lactate																
Oxydative stress gills	SOD																
	Catalase																
	GPx																
	GSHt																
Oxydative stress liver	SOD																
	Catalase																
	GPx																
	GSHt																
Haematological parameters	Hematocrit																
	Erythrocyte																
	MGV																
Leukocytic parameters	Cellular mortality																
	[leukocyte]																
	[lymphocyte]																
	[granulocyte]																
	[monocyte]																
Immunological parameters	Phagocytosis																
	Lysosyme																
	ACH50																

Oysters									Mussels								
Experimental condition	DM		DC1				PS+DC1		Experimental condition	DM		DC1		DC2		PS+DC1	
Sample	T1	T2	T1	T2			T1	T2	Sample	T1	T2	T1	T2	T1	T2	T1	T2
LMS					x	x			LMS								
Phenoloxydase					x	x			Phenoloxydase								
Laccase activity hemocyte					x	x			Hyalinocyte		x		x		x		x
Laccase activity plasma					x	x			Phagocytosis		x		x		x		x
GPx gills					x	x			Cellular mortality		x		x		x		x
GPx digestive glande					x	x			Granulocyte		x		x		x		x

Table 5 : Effect on fish of chemical dispersion (DC) compared with mechanical dispersion (DM) at the end of the exposure time (T1) and at the end of the depuration time.

lower than DM ; equal to DM ; upper than DM

		Sea bass				Turbot			
	Experimental condition	DC1		DC2		DC1		DC2	
	Sample	T1	T2	T1	T2	T1	T2	T1	T2
Acid base equilibrium	pH								
	[HCO3-]								
	pO2	x		x					
	pCO2								
Hydromineral balance	[CL-]								
	[Na+]								
	osm								
Bioconcentration PAHs muscle			x	x	x	x		x	
Pyrene metabolite									
B(a)P type metabolite									
Growth	SGR								
Condition index	Fulton K factor								
	SSI								
	HSI								
Stress indicators	Glucose								
	Cortisol								
	Lactate								
Oxydative stress gills	SOD								
	Catalase								
	GPx								
	GSHt								
Oxydative stress liver	SOD								
	Catalase								
	GPx								
	GSHt	x		x		x		x	
Haematological parameters	Hematocrit	x	x	x	x	x	x	x	x
	Erythrocyte	x	x	x	x	x	x	x	x
	MGV	x	x	x	x	x	x	x	x
Leukocytic parameters	Cellular mortality								
	[leukocyte]								
	[lymphocyte]								
	[granulocyte]								
	[monocyte]								
Immunological parameters	Phagocytosis								
	Lysosyme								
	ACH50								

Table 6 : Effect on bivalve of chemical dispersion (DC) compared with mechanical dispersion (DM) at the end of the exposure time (T1) and at the end of the depuration time.

Oysters		
Experimental condition	DC1	
Sample	T1	T2
LMS		
Phenoloxydase		
Laccase activity hemocyte		
Laccase activity plasma		
GPx gills		
GPx digestive glande		

Mussels				
Experimental condition	DC1		DC2	
Sample	T1	T2	T1	T2
LMS				
Phenoloxydase				
Hyalinocyte		x		x
Phagocytosis		x		x
Cellular mortality		x		x
Granulocyte		x		x