Journal of Petroleum & Environmental Biotechnology

Dussauze et al., J Pet Environ Biotechnol 2016, 7:3
DOI: 10.4172/2157-7463.1000282

Short Communication Open Access

An Experimental System to Assess Potential Biological Impact of Operational Response During an Oil Spill

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Abstract

In order to evaluate the potential toxicity of dispersant application in an oil slick in near-shore areas, this study presents an experimental system designed to perform toxicity tests on fish. Three possible oil spill scenario issues during an oil spill were tested on juvenile sea bass (*Dicentrarchus labrax*); the Water Soluble Fraction of oil; the Mechanical Dispersion of oil; the Chemical Dispersion of oil. Preliminary toxicity assays suggest that the experimental system is appropriate to assess the three experimental conditions during a period of 24 hours. This experimental system allowed obtaining "quick" and relevant results of acute toxicity in an emergency context such an oil spill.

Keywords: Dispersed oil; Acute toxicity test; Water soluble fraction of oil; Oil droplets; *Dicentrarchus labrax*

Highlights

- A communication for the validation of an experimental system.
- A system designed to perform toxicity tests on small water organisms.
- A system devised to simulate the behaviour and the toxicity of the petroleum following dispersant use.

Introduction

The application of chemical dispersants is a commonly used technical response in case of oil spill at sea [1]. Dispersant shift the oil slick from the surface to the water column. In offshore areas, dispersants are often used since their application shows many environmental advantages: they increase the natural dilution of oil and consequently the biodegradation; they also decrease the amount of oil slick grounded on the shore [2]. However, in near-shore areas, dispersant application is a controversial countermeasure: the low dilution potential of the oil slick (in a limited water column depth) is able to enhance the toxicity and consequently reduces the environmental advantages of dispersant use. For this reason, in an emergency context such an oil spill, it is necessary to have as soon as possible toxicity data to evaluate the potential biological impact. Many studies have evaluated the acute toxicity of dispersant alone [3-6] or dispersant enhanced water accommodated fractions [7-10]. These methods presented a major disadvantage. Indeed, they do not take into account the presence of oil droplets in the water column, especially in near-shore areas where the mechanical agitation, e.g. wave action, promotes their formation. Moreover oil droplets are suggested as a determinant of toxicity [11]. On this basis, an experimental system adapted from Blackman et al. [12] was proposed to measure the total petroleum hydrocarbons transferred in the water column and the toxicity following dispersant application. The present methodological paper is a prolongation of a preliminary work of Milinkovitch et al. [13] and discusses the validation of this experimental system to recreate the three possible oil exposure issues for water column organisms during an oil spill: (I) the Water Soluble Fraction of oil; (II) the Mechanical Dispersion of oil; (III) the Chemical Dispersion of oil (Figure 1).

Materials and Methods

Fish

Experimentations were done on juvenile European seabass, *Dicentrarchus labrax*. Seabass is a commercially important demersal species present in near-shore areas. Sea bass (4.6 ± 0.2 g) were provided by an aquaculture facility (E.M.G., Gravelines, France) and acclimated for 1 month in a 300-L flow-through tank (35‰, 19.0 ± 0.1°C, with 12 hours light:12 hours dark photoperiod) prior to bioassays. During acclimation, they were fed daily with fish food (Neosupra AL2 from Le Gouessant Aquaculture). The diet composition was 58% proteins, 13% lipids, 0.5% cellulose, 10% ash, 10% moisture.

Chemicals

The petroleum used in the study was a crude Arabian light (CAL). The CAL is oil used in other eco-toxicological studies [14-17]. This oil is composed of 54% saturated hydrocarbons, 10% polar compounds and 36% aromatic hydrocarbons. To recreate the most realistic conditions of an oil slick drifting at sea for a few days, the oil was evaporated in a 1 m^3 tank for 24 hours. This weathering caused initial evaporation of the lighter compounds inducing change in the oil composition. The weathered CAL contained 54% saturated hydrocarbons, 12% polar compounds and 34% aromatic hydrocarbons. The total evaporation of oil was approximately 7%. Details of CAL are presented in supplementary data. The viscosity of the oil was under 5000 cst, allowing the application of dispersants [18]. Two formulations of third

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Receive April 29, 2016; **Accepted** June 08, 2016; **Published** June 15, 2016

Citation: Dussauze M, Pichavant-Rafini K, Theron M, Floch SL, Milinkovitch T, et al. (2016) An Experimental System to Assess Potential Biological Impact of Operational Response During an Oil Spill. J Pet Environ Biotechnol 7: 282. doi:10.4172/2157-7463.1000282

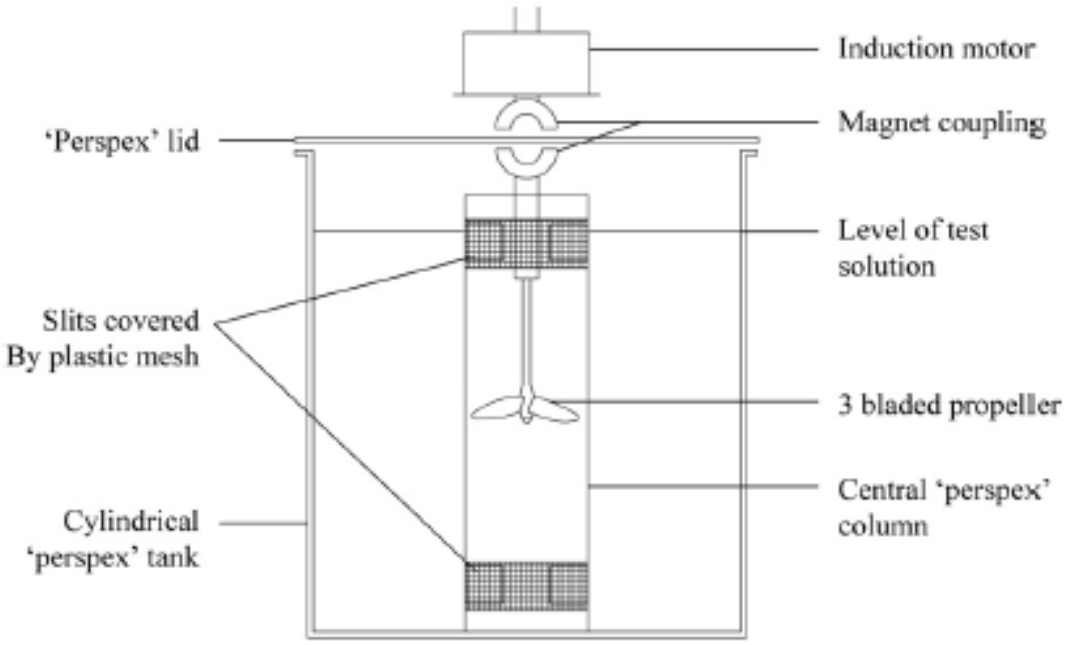

Figure 1: Experimental system provided by Blackman *et al* 1977.

generation dispersant (1 and 2), manufactured by Total Fluides and Innospec (Gamlen) were selected.

Experimental system

The experimental system was adapted from Blackman et al. [12]. It is composed of twelve experimental tanks (units). Each tank is a 20 L cylinder fitted with a removable central column 77 mm in diameter that houses a stainless steel shaft and 3 bladed propellers. The central cylinder has two sets of two apertures situated at the top and the bottom. The apertures are covered with a metallic mesh screen to exclude test animals from the propeller housing. The propeller rotates (at 1000 rpm) to produce a small vortex within the central cylinder, thus drawing the exposure solutions in through the upper apertures and expelling them through the lower ones. This homogenization allowed maintaining oil droplets throughout the water column. The system is a static water system, e.g. without water supply, maintained in a temperature controlled room (19°C) and equipped of an aeration supply. The experimental device complies with the French AFNOR standard [19] to determine the acute toxicity of a substance.

Exposure condition

All the exposure conditions were prepared in 22 L glass exposure tanks. The four basic exposure conditions to be used were prepared separately; they included Water Soluble Fraction (WSF), Mechanically Dispersed oil (MD) and Chemically Dispersed oil using the two dispersant formulations (CD1 and CD2). The WSF was prepared with 95 g of weathered CAL in 20 L of seawater following the lower energy method of Singer et al. [20]. Only the liquid phase was used as the exposure environment. Mechanically dispersed oil (MD) was prepared using 20 L of sea water and 95 g of weathered CAL; the mixture was agitated using a propeller mixer (RW 16 Basic IKA) fitted with the same 3 bladed-propeller and using the same rotor speed (1000 rpm) as used in the experimental system (described in 2.3). Chemically dispersed oil solutions, using dispersants 1 and 2 (CD1 and CD2), were prepared using 20 L of sea water, 95 g of weathered CAL, and 5 g of dispersants 1 or 2 respectively (following the manufacturer's recommended application ratio of 20:1), and with the same mixing procedure as for the mechanically dispersed oil solution. Once the exposure media had been prepared, they were diluted in sea water at six concentrations (0%, 2.4%, 12%, 18%, 24% and 40%) and distributed in the experimental system.

Experimental design

Groups of 10 fish were exposed to one dilution of each exposure condition medium for 24 hours in an experimental tank. Physicochemical parameters (pH, dissolved oxygen, water temperature, salinity) were monitored. Two exposure conditions were tested simultaneously: chronologically CD1 and WSF, then CD2 and MD. At the end of the 24 hours exposure period, the fish in each tank were gently transferred to clean sea water for a 24 hour period, as recommended by Blackman et al. [12]. For this purpose, 22 L glass flow-through tanks were used. After 24 hours, each tank was inspected and dead fish were counted. Fish were considered dead when no gill movement and no response to a caudal pinch were observed.

Analytical methods

Measurements of total petroleum hydrocarbon (TPH) seawater concentrations: The total petroleum hydrocarbon (TPH) concentration in each dilution of each exposure medium was assessed at the beginning (T_0) and at the end of the exposure period (T_1), using the mean of three replicated measurements for each time point. TPH concentrations were quantified by spectrophotometry (UV-Vis spectrophotometer, Unicam at 390 nm) of dichloromethane extracted samples, as described by Fusey and Oudot [21].

Measurement of the droplet size of dispersed oil: The oil droplet size distribution (diameter in microns) of CD1, CD2 and MD conditions were analyzed 6 hours after the beginning of fish exposure at a nominal concentration of 1250 mg/L. The measurements were performed by laser granulometry (Malvern Mastersizer 2000) based on the principle of Fraunhofer according to the intensity of diffracted

	MD		CD1		CD2	
% of stock solution	**[TPH] (mg/L)**	**Fish mortality (%)**	**[TPH] (mg/L)**	**Fish mortality (%)**	**[TPH] (mg/L)**	**Fish mortality (%)**
0	nd.	0	nd.	0	nd.	0
2.4	45 (65-25)	0	107 (118-96)	0	102 (130-74)	0
12	214 (235-196)	0	554 (659-449)	0	585 (647-523)	0
18	213 (293-133)	0	971 (1037-905)	50	744 (881-607)	0
24	306 (405-207)	0	1116 (1269-963)	100	964 (1050-878)	30
40	373 (443-302)	0	1547 (1542-1553)	100	1879 (1948-1810)	100
LC50 (mg/L)	n.c.		873 (782-976)		1227 (1091-1379)	
The results are expressed as mean concentrations over 24 hours (concentration at T0 - concentration at T1). Respecting Quade test procedures, values obtained for each exposure condition at several % of stock solution are considered as repeated measure and *indicates significant differences (P < 0.05) of TPH concentrations between exposure conditions. LC_{50} values are expressed as values (lower 95% CI-upper 95% CI). n.d. = not detected. n.c. = not calculable.						

Table 1: Fish mortality (%). % of stock solution and total petroleum hydrocarbon concentration (mg/L) in sea water for mechanical dispersion (MD) and chemical dispersion (CD1 and CD2) during the 24 hour exposures.

	d (0.1)	d (0.5)	d (0.9)
CD1	2.0 ± 0.0 a	5.2 ± 0.0 a	12.5 ± 0.0 a
CD2	1.8 ± 0.0 a	4.1 ± 0.0 a	11.8 ± 0.2 a
MD	107.6 ± 0.9 b	227.9 ± 3.3 b	437.3 ± 11.4 b
In this table, d (0.5), d (0.1) and d (0.9) correspond respectively to the median and the two deciles of a Normal distribution. (n = 7). Differences in letters indicate statistical differences between groups (p < 0.05).			

Table 2: Size (µm) of oil droplets in CD1, CD2 and MD obtained with laser granulometry.

radiation, whereby the diffraction angle dependent on the particle size. A water sample flow rate of 1200 mL/min and an obscuration of 10% were the conditions used during the measurements.

Statistical analysis

The LC_{50} values (the TPH concentration of the exposure media that caused the death of 50% of a group of test animals) were calculated using the trimmed Spearman-Karber method and a US EPA probit program, and expressed as values (lower 95% confidence interval - upper 95% confidence interval). The difference between MD, CD1 and CD2, concerning TPH concentration, was evaluated following the Quade test procedure: exposure media (MD, CD1 and CD2) were considered as treatment and the % of stock solutions (0%, 2.4%, 12%, 18%, 24%, 40%) were considered as blocks. Thus, the values obtained for each exposure environment at several dilutions were considered as repeated measurements. The statistical analysis was carried out using Systat 12 software and the significance of the results was ascertained at α=0.05.

Results and Discussion

The goal of this study was to simulate a possible scenario of dispersion of a drafting oil spill. For fish exposed to 0% of stock solution, no mortality was found. Moreover all physicochemical parameters, temperature (19.1 ± 0.2°C), pH (8.04 ± 0.03), dissolved oxygen (97.5 ± 0.9% of O_2 saturation) and salinity (35.2 ± 0.0 PSU), remained constant throughout the experimental periodfor all exposure conditions. The possibility to maintain viable juvenile of *Dicentrarchus labrax* suggests that the experimental system makes possible to use early life stage in oil and dispersant toxicity assessment.

When measurements of total petroleum hydrocarbon concentration for mechanical (MD) and chemical (CD1 and CD2) dispersion are compared, it appears that the dispersant application increases significantly the concentration of TPH (mean over 24 hours) in the water column (Table 1). This result is in accordance with previous observations obtained in field operations and *in situ* experimentation [22,23]. Taken together and very logically, these results show that the transfer of petroleum from the surface to the water column is increased when dispersant is applied.

Analysis of oil droplet size distribution showed a significant difference between the two chemically dispersed conditions and the mechanically dispersed condition (Table 2). This result can explain a part of the higher TPH concentration of CD1 and CD2 conditions compared to those observed in MD condition. Indeed, application of surfactant increases the bioavailability of oil [24]. The two commercial formulations of dispersant used in this study had different surfactant concentrations. This difference could conduct to difference in oil bioavailability for the two chemical dispersion conditions. Consequently, oil bioavailability could be one of the reasons of differences observed between MD, CD1 and CD2 and CD2 conditions in their fish mortalities.

Without dispersant application a part of the oil slick could solubilised in the water column. The water soluble fraction (WSF) of oil is commonly used in dispersed crude oil toxicity tests. Thus, we exposed fish to this treatment in order to compare our results with those obtained in the literature. Preliminary results of this study suggest that chemically dispersed oil was more toxic than WSF of oil since no mortality was found for WSF whatever the percentage of stock solution tested (0, 2.4, 12, 18, 24 and 40% of stock solution in seawater for WSF). In our results, no TPH were detected for each % of stock solution tested. These results are in accordance with other studies using different experimental approaches [8,10,25- 27]. It can therefore, be concluded that our experimental system is suitable for assessing the toxicity of dispersant application.

A rapid decrease in TPH concentration is commonly observed in offshore field operations [2]. At the opposite, since near-shore areas have a lower dilution potential and important natural mixing processes (e.g. wave action), natural dispersion of the oil slick can be maintained and the oil slick can even be displaced from the surface to the water column (as described by Lunel [28] during the *Braer* oil spill). For example, during Sea Empress and Braer oil spills, high concentration of oil was observed over more than one week (respectively [29] and [28]. In this study, the experimental system was under static condition (e.g. without dilution due to water supply), consequently the evolution

of TPH concentration depends on the turbulent mixing energy. This allows reproducing different scenarios making possible to simulate a natural dispersion in near-shore areas.

The device presented here makes possible to measure the evolution of the total petroleum hydrocarbon concentration during this period and our results show no important reduction of TPH concentration over the 24 hours of contamination (Table 1). Thus, the experimental approach succeeds in simulating dispersant application in near-shore areas.

Conclusion

Bioassays must be considered with caution due to the complexity of work with living material [30]. Indeed, numerous settings could influence the results of the experimentation if they cannot be controlled. However, during an emergency context such an oil spill, bioassay could be relevant to evaluate the potential biological impact of operational response.

Our concern was to find an efficient and reliable method for rapid toxicity assessment of a dispersed oil spill even in near-shore areas. This study presents an experimental system designed to perform reproducible toxicity tests on small marine organisms and to simulate the increasing transfer of petroleum from the surface to the water column when dispersant is applied (comparing MD and CD1, CD2). Moreover comparisons of preliminary toxicity assays performed on juvenile fish with published literature suggest that the experimental system is suitable for assessing the toxicity of dispersant application. Nevertheless, in this system attention must be paid to the fact that a part of the evolution of TPH concentrations and size of oil droplets are dependent on the given turbulent mixing energy. This mixing energy can be changed to respond to different oil spill scenario.

Thus, the present experimental approach seems of interest in order to establish a comprehensive framework in an emergency context and especially to dispersant use during an oil spill. However, the natural environment is complex and full of interaction between biotic and abiotic parameters. These laboratory results can differ with *in situ* observations. Consequently, for a better comprehension of these interactions, these acute toxicity studies can be coupled with studies focused on the sub-lethal effects of oil-dispersant mixtures.

Acknowledgements

This study was supported by a PhD grant from the Conseil Général of the Charente-Maritime. The Agence Nationale de la Recherche is acknowledged for financial support for the project 'DISCOBIOL'. The authors also acknowledge Total Fluides and Innospech for providing chemicals.

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Supplementary data

Supplementary data 1: Concentration of PAHs (alkylated and parents) in the weathered Crude arabian light. The 21 PAHs represent the 16 US-EPA PAHs and five supplementary PAHs (benzo[*b*]thiophene, biphenyl, dibenzothiophene, benzo[*e*]pyrene, and perylene).

PAH	Molecular weight (g/mol)	Concentration in weathered CAL (µg/g of petroleum)
Benzo[*b*]thiophene	134	5
C_1-benzo[*b*]thiophene	148	23
C_2-benzo[*b*]thiophene	162	292
C_3-benzo[*b*]thiophene	176	1 031
C_4-benzo[*b*]thiophene	190	537
Naphtalene	128	211
C_1-Naphtalene	142	854
C_2-Naphtalene	156	1 820
C_3-Naphtalene	170	1 796.
C_4-Naphtalene	184	1 317
Biphenyl	154	14
Acenaphtylene	152	25
Acenaphtene	154	3
Fluorene	166	39
C_1-Fluorene	180	116
C_2-Fluorene	194	230
C_3-Fluorene	208	261
Phenanthrene	178	95
Anthracene	178	95
C_1-phenanthrene/anthracene	192	335
C_2-phenanthrene/anthracene	206	498
C_3-phenanthrene/anthracene	220	416
C_4-phenanthrene/anthracene	234	273
Dibenzothiophene	184	330
C_1-dibenzothiophenes	198	987
C_2-dibenzothiophene	212	1 759
C_3-dibenzothiophene	226	1 546
C_4-dibenzothiophene	240	936
Fluoranthene	202	6
Pyrene	202	9
C_1-fluoranthene/pyrene	216	51
C_2-fluoranthene/pyrene	230	119
C_3-fluoranthene/pyrene	244	191
Benzo[*a*]anthracene	228	16
Chrysene	228	15
C_1-chrysene	242	29
C_2-chrysene	256	45
C_3-chrysene	270	88
Benzo[*b*+*k*]fluoranthene	252	3
Benzo[*e*]pyrene	252	2

Benzo[*a*]pyrene	252	9
Perylene	252	7
Indeno[1,2,3-*cd*]pyrene	276	0
Dibenz[*ah*]anthracene	278	1
Benzo[g*hi*]perylene	276	2

Supplementary data 2: Concentration of C11-C33 petroleum hydrocarbons in the weathered Crude arabian light.

Hydrocarbon	**Molecular weight (g/mol)**	**Concentration in weathered CAL (µg/g of petroleum)**
n-C11	57	7707
n-C12	57	7594
n-C13	57	7525
n-C14	57	7244
n-C15	57	6448
n-C16	57	5753
n-C17	57	5088
pristane	57	1289
n-C18	57	4491
phytane	57	2048
n-C19	57	3819
n-C20	57	3440
n-C21	57	2908
n-C22	57	2467
n-C23	57	2614
n-C24	57	1976
n-C25	57	1671
n-C26	57	1634
n-C27	57	1640
n-C28	57	1726
n-C29	57	1869
n-C30	57	2109
n-C31	57	1973
n-C32	57	1817
n-C33	57	1487