



Effects of dispersed oil exposure on the bioaccumulation of polycyclic aromatic hydrocarbons and the mortality of juvenile *Liza ramada*

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Abstract

Dispersing an oil slick is considered to be an effective response to offshore oil spills. However, in nearshore areas, dispersant application is a controversial countermeasure: environmental benefits are counteracted by the toxicity of dispersant use. In our study, the actual toxicity of the dispersant response technique in the nearshore area was evaluated through an experimental approach using juvenile *Liza ramada*. Fish were contaminated via the water column (i) by chemically dispersed oil, simulating dispersant application, (ii) by dispersant, as an internal control of chemical dispersion, (iii) by mechanically dispersed oil, simulating only the effect of natural mixing processes, without dispersant application, and (iv) by the water soluble fraction of oil, simulating the toxicity of an oil slick before recovery. Bioconcentrations of polycyclic aromatic hydrocarbons (PAH) and mortality were evaluated, and related to both total petroleum hydrocarbon (TPH) and polycyclic aromatic hydrocarbon (PAH) concentrations in seawater.

Fish exposed to chemically dispersed oil showed both a higher bioconcentration of PAH and a higher mortality than fish exposed to either the water soluble fraction of oil or the mechanically dispersed oil. These results suggest that (i) dispersion is a more toxic response technique than containment and recovery of the oil slick; (ii) in turbulent mixing areas, dispersant application increases the environmental risk for aquatic organisms living in the water column. Even if the experimental aspects of this study compel us to be cautious with our conclusions, responders could consider these results to establish a framework for dispersant use in nearshore areas.

Keywords: dispersant, chemically dispersed oil, toxicity, polycyclic aromatic hydrocarbons, bioaccumulation, oil droplets, nearshore areas, *Liza ramada*.

49 **1. Introduction**

50 In the last decades, increasing demand for petrochemicals has led to an increase in oil
51 pollution in the sea. Many sources oil pollution, such as industrial wastewater, tanker
52 accidents, and oil leaks from drilling operations (recently Deepwater Horizon) still
53 contaminate the marine ecosystem. Even if oil spills do not represent the major source of
54 pollution (one third of the petroleum hydrocarbons that enter the aquatic environment each
55 year, UNEP/IOC/IAEA, 1992), the consequences of oil spills on local flora and fauna are
56 disastrous (Dauvin, 1998; Claireaux et al., 2004; Cadiou et al., 2004). Secondary to
57 mechanical containment and recovery of the oil slick, chemical dispersants can be used to
58 reduce the environmental and economic impact of an oil spill. Chemical dispersants are
59 composed of surface active agents (surfactants), which contain anionic and nonionic
60 molecules that confer hydrophilic and hydrophobic properties, enabling lower interfacial
61 tension between oil and water. This chemical process facilitates the formation of small, mixed
62 oil-surfactant micelles, dispersed into the water column (Canevari, 1978). Thus, the
63 application of chemical dispersants shows many advantages, by accelerating dilution of the
64 oil slick (Lessard and Demarco, 2000) and consequently accelerating biodegradation of oil
65 compounds (Thiem et al., 1994; Churchill, 1995). However, in nearshore areas, the
66 advantages of dispersant use are counteracted by its toxicity: the low dilution potential of the
67 oil slick in shallow water may expose ecologically sensitive ecosystems to relevant
68 concentrations of petroleum. Therefore, in nearshore areas, the long-term net environmental
69 benefits of dispersant application are counteracted by acute toxicity. A net environmental
70 benefits analysis (NEBA, as conducted by Baca et al., 2005, on a mangrove ecosystem),
71 which considers both the advantages and toxicity of dispersant use, is required in order to
72 establish a comprehensive framework for dispersant use policies for nearshore areas.

In an attempt to do so, past studies have evaluated the acute toxicity of single dispersants (Perkins et al., 1973; Thompson and Wu, 1981; Law, 1995; Adams et al., 1999; George-Ares and Clark, 2000). More recent studies have taken into consideration the toxicity of the petroleum-dispersant interaction (Epstein et al., 2000; Long and Holdway, 2002; Lin et al., 2009) using chemical enhanced water accommodated fractions (CEWAF, Singer et al., 2000) as contamination solutions. These exposure solutions do not take into account most of the particulate oil formed during the dispersion of an oil slick.

However, the dispersion of oil provokes the presence of oil droplets, which have been suggested to be a determinant of toxicity (Ramachandran et al., 2004a and Brannon et al., 2006), and does so even more in nearshore areas, where natural dispersion (e.g. waves) can send the whole oil slick from the surface into the water column (as described during the Braer oil spill by Lunel et al., 1995). Thus, in order to simulate actual exposure to dispersed oil in nearshore areas, an experimental approach was designed. This approach, similar to Milinkovitch et al. (2011), considers the presence of oil droplets in the water column.

Experiments were conducted on juvenile, thin-lipped grey mullets (*Liza ramada*), because this teleost fish species is present in the Atlantic nearshore area during its early life stage (Gautier and Hussenot, 2005), and is consequently considered to be a target organism of anthropogenic contaminants (Bruslé, 1981). Moreover, this species is a key species of ecosystems since it plays a significant role in the global energy budgets of coastal environments, by transporting particulate organic matter from the salt marsh to the marine coastal waters (Lafaille et al., 1998). Four exposure conditions were tested on these organisms: (i) chemically dispersed oil solutions, simulating the application of dispersant when turbulent mixing processes (necessary for this response technique) are presents; (ii) single dispersant solutions as internal controls of chemically dispersed oil solutions; (iii) a mechanically dispersed oil solution, simulating the natural dispersion of an oil slick due to

mixing processes, but without dispersant application; (iv) a water soluble fraction of oil solution, simulating an undispersed oil slick before recovery, when the absence of turbulent mixing processes permit this response technique. Mortality was observed upon increasing exposure concentrations, yielding information on acute toxicity following dispersant application. Moreover, the concentration of 21 polycyclic aromatic hydrocarbons (PAH) was measured in both the sea water and fish muscles, since (i) PAH are considered to be a primary determinant of petroleum toxicity for aquatic organisms (Anderson et al., 1974) and (ii) their bioaccumulation in organisms is enhanced by dispersant application (Wolfe et al., 2001; Ramachandran et al., 2004b; Mielbrecht et al., 2005).

2. Materials and methods

2.1 Materials and experimental organisms

2.1.1. Experimental system

The experimental system (**Figure 1**) used in this study was adapted from Blackman et al. (1978), and is composed of 12 experimental tanks (units) covered by a lid. Each tank is a 22 L cylinder fitted with a removable central column (77 mm in diameter), that houses a stainless steel shaft and a three-bladed propeller. The central cylinder has two sets of two apertures, located 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 (30 × 25 mm) is rotated (1000 rounds per minute) to produce a small vortex within the central cylinder, drawing the exposure solutions in through the upper apertures and expelling them through the lower ones.

Even if residual oil is observed in the experimental system following exposure, the system is devised to maintain a mixture of oil-dispersant droplets throughout the water column. The system is a static water system, stored in a temperature controlled room (19 °C). Physicochemical parameters were measured during exposure (**Table 1**).

2.1.2 Chemicals

A Brut Arabian Light (BAL) oil was selected for this study and the composition of the oil was evaluated by CEDRE (CEntre de Documentation de Recherche et d'Expérimentations sur les pollutions accidentelles des eaux). The oil was found to contain 54% saturated hydrocarbons, 36% aromatic hydrocarbons, and 10% polar compounds. Concentrations of 21 PAHs (including the 16 priority PAHs listed by US-EPA) in the Brut Arabian Light oil are presented in Table 2. Before performing the exposure studies, the oil was weathered by bubbling air through the petroleum in 3 m³ tanks, for 8 days in open air, at a temperature of 12 to 16 °C. This aeration protocol results in a 7 % petroleum weight loss. This, corresponds to the petroleum weight loss occurring in 12 h on a 1 mm oil slick released at sea (personal communication, S. Le Floch). Using this weathered oil, our study simulates a 12 h period of petroleum ageing, i.e. the time it might take for responders to apply dispersant. The composition of the weathered test oil was 54% saturated hydrocarbons, 34% aromatic hydrocarbons and 12% polar compounds and its API (American Petroleum Institute) gravity was 33. Concentrations of 21 PAH (including the 16 US-EPA PAH) are presented in Table 2. Two formulations of dispersants (1 and 2), manufactured by Total Fluides and Innospech, were selected. Both were evaluated by CEDRE and were deemed effective enough (determined using the method, NF.T.90-345) for use in marine environments, non-toxic at concentrations recommended by the manufacturer (determined using a standard toxicity test:

method NF.T.90-349), and biodegradable. Dispersants 1 and 2 are composed of surfactants (surface active agents) and solvents. Because they are “third generation” dispersants, these surfactants are blends of anionic and non-ionic types (Fiocco and Lewis, 1999). The manufacturers state that the chemical compounds in their surfactants which represent a health risk are non-ionic surfactants (24 %) and anionic surfactants (between 12 and 24%) for dispersant 1; and saturated hydrocarbons with a flash point higher than 60 °C for dispersant 2.

2.1.3 Experimental Organisms

Juvenile, thin-lipped grey mullets (*Liza ramada*) were caught in Daoulas Bay (France) and acclimatised for 1 month in 300 L flow-through tanks (35 ± 0.5 ‰, 19 ± 0.2 °C, with 12 h light:12 h dark photoperiods) prior to the bioassays. During acclimatisation, no mortality was observed and mullets were fed daily with fish food (Neosupra AL2 from Le Gouessant aquaculture). The fish were not fed 48 h prior to the bioassays, and throughout the exposure period. For each exposure condition, ten fish were weighed prior to the exposure (**Table 1**).

2.2 Exposure Methods

2.2.1 Preparation of exposure media

Stock solutions were prepared in 22 L glass beakers. All stock solutions were stirred for 24 h as described below. A Water Soluble Fraction of oil (WSF) stock solution was prepared as the Water Accommodated Fraction (WAF) recommended by CROSSERF, with the exception that the WAF preparation used in this study did not have a lid on the glass beaker in order to simulate the evaporation of light compounds which occur during oil slick confinement.

Practically, 95 g of BAL (Brut Arabian Light) oil was weighed and gently spread out over 20 L seawater to simulate an oil slick. Then, the solution was stirred using a magnetic agitator (RCT basic IKA) using the low energy method (no vortexing) for a 24 h period. Only the liquid phase of the WSF of oil was used in the subsequent exposure studies. Chemically dispersed oil (CD1 and CD2) stock solutions, using dispersant 1 and 2, were prepared using 20 L of seawater, 95 g BAL oil slick, and 5 g of dispersants 1 and 2 (following the manufacturers' recommended application petroleum:dispersant ratio of 20:1). Dispersant (D1 and D2) stock solutions were prepared using 20 L of seawater and 5 g of dispersant. A mechanically dispersed oil stock solution (MD) was prepared using 20 L of seawater and 95 g BAL oil slick. CD1, CD2, D1, D2 and MD stock solutions were each stirred for 24 h using a propeller mixer (RW 16 Basic IKA), fitted with the same propeller as used in the experimental system. The propeller mixer speed was set higher than during exposure (1400 rounds per minute instead of 1000 rounds per minute) in order to avoid the formation of an oil slick for the MD stock solution.

2.2.2. Exposure conditions

Following the 24 h period of exposure media preparation, each stock solution was diluted in seawater, which was previously placed in the experimental system tanks (described in **2.1.1.**). On the basis of one dilution per tank, 6 dilutions of each stock solution were made: 0%, 2.4%, 12%, 18%, 24%, and 40%. The final volume of each dilution was 16 L. Two exposure conditions were tested simultaneously: CD1 and D1, followed by CD2 and D2, and then WSF of oil and MD, chronologically. Each group of 10 organisms was exposed to one stock solution dilution (16 L) for 24 h in one experimental tank. At the end of the 24 h exposure period, the animals in each tank were gently transferred to a 22 L glass tank with clean

seawater flow-through for 24 h, as recommended by Blackman et al. (1978). After 24 h, each tank was inspected and dead animals were counted. Animals were considered to be dead when no gill movement was visible and no response to a caudal pinch was observed. Surviving fish were euthanised using Eugenol (4-allyl-2-methoxyphenol). The whole axial muscle of each fish was removed and stored at -20°C for later assessment of polycyclic aromatic hydrocarbon (PAH) concentrations.

2.3. Chemical analysis

2.3.1. Total petroleum hydrocarbon (TPH) seawater concentrations

The TPH concentration, which is the sum of dissolved hydrocarbon concentrations plus the amount of oil droplets, was measured for all exposure media in each tank, at T = 0 h, and at the end of fish exposure (T = 24 h), using the mean of three replicated measurements for each time point. Each sample was removed using a Teflon straw, linked to a pipette filler (VWR), and stored in a 60 mL tinted glass bottle (VWR). The seawater samples were extracted with 10 mL of Pestipur-quality dichloromethane (Carlo Erba Reactifs, SDS). After separation of the organic and aqueous phases, the seawater was extracted two additional times with the same volume of dichloromethane (2×10 mL). The combined extracts were dried on anhydrous sulphate and then analysed using a UV spectrophotometer (UV-Vis spectrophotometer, Unicam) at 390 nm, as described by Fusey and Oudot (1976). The detection limit of this method is dependent on the precision of the spectrophotometer, and results are not reliable for concentrations under 1 mg/L

2.3.2. Polycyclic aromatic hydrocarbon (PAH) seawater concentrations

PAH concentrations were assessed in each tank, at T = 0 h and following fish exposure (T = 24 h), using the mean of two replicated measurements for each time point. After sampling, a 24 h settling phase was used to separate oil droplets and particulate matter from the seawater. Then, 150 µL of a solution of 5 perdeuterated internal standards (Naphthalene d₈, Biphenyl d₁₀, Phenanthrene d₁₀, Chrysene d₁₂, and Benzo[a]pyrene d₁₂ at concentrations of 210, 110, 210, 40 and 40 µg/mL, respectively in acetonitrile Sigma-Aldrich, France) were diluted in 10 mL of absolute methanol (Sigma-Aldrich, France), and this volume of methanol was added to the liquid phase of the samples. PAH were extracted from the seawater using the stir bar sorptive extraction technique (SBSE, stir bar coated with PDMS, Gerstel), and analysed by thermal desorption coupled to a capillary gas chromatography–mass spectrometer (GC–MS). An HP7890 series II (Hewlett Packard, Palo Alto, CA, USA) GC was used, coupled with an HP5979 mass selective detector (MSD, Electronic Impact: 70eV, voltage: 2 000 V). Twenty-one PAH (alkylated and parents), including the 16 PAH listed by the US-EPA and 5 additional PAH (benzo[*b*]thiophene, biphenyl, dibenzothiophene, benzo[*e*]pyrene, perylene), were quantified according to published procedures (Roy et al., 2005). Based on the detection limits of this method, accurate results at concentrations of 1 ng/L were possible.

2.3.3 Polycyclic aromatic hydrocarbon (PAH) concentrations in fish muscles and bioaccumulation factor (BAF)

The concentrations of 21 PAH (alkylated and parents) in the fish muscles were assessed. The 21 PAH represent the 16 US-EPA PAH and 5 supplementary PAH (benzo[*b*]thiophene, biphenyl, dibenzothiophene, benzo[*e*]pyrene, perylene). PAH concentrations in the fish muscles were determined by GC–MS, using a procedure modified from Baumard et al.

(1997). Fish samples were pooled according to exposure treatment (one pool per tank). Although most pools contained some fish which survived the exposure experiments, pools were not analysed for exposures to 40 % stock solutions, because there were no surviving fish following CD1 and CD2 exposure.. The mean weight of the fish in the pools was 6.1 ± 0.7 g. Prior to extraction, 150 μ L of a solution of 5 perdeuterated internal standards (Naphthalene d_8 , Biphenyl d_{10} , Phenanthrene d_{10} , Chrysene d_{12} , and Benzo[a]pyrene d_{12} at concentrations of 210, 110, 210, 40 and 40 μ g/mL, respectively, in acetonitrile Sigma-Aldrich, France) and 50 mL of an ethanolic solution of potassium hydroxide (2 mol L^{-1} , Fisher Chemicals) were added to fish muscles in 250 mL flasks and placed for 3 h in a drying cupboard at 60 °C. After alkaline digestion, 20 mL of demineralised water was added and samples were extracted with 2×20 mL of pentane (Carlo Erba Reactifs, SDS). The resulting extract was then concentrated using a Turbo Vap 500 concentrator (Zyman, Hopkinton, MA, USA, at 880 mbar and 50 °C) to 1 mL, purified on a silica column (5 g of silica, hydrocarbons were eluted with 50 mL of pentane/dichloromethane 80/20) and concentrated to 200 μ L for analysis. Aromatic compounds were analysed by GC–MS, with an approximate quantification limit of 5 μ g.kg⁻¹ of dry weight. PAH levels were quantified relative to the 5 perdeuterated internal standards introduced at the beginning of the sample preparation procedure (one per aromaticity class). Moreover, as described in Baussant et al. (2009), a bioaccumulation factor (BAF) was calculated using the ratio of the total PAH concentration in fish muscles divided by the total PAH concentration in seawater (**2.3.2.**).

2.4. Statistical analysis

All correlations were tested using Spearman's correlation test (XL Stat 5.2) and the statistical significance of the results was ascertained at $\alpha = 0.05$. Differences between exposure conditions (CD1, CD2, MD, D1, D2, WSF) concerning seawater PAH concentrations, muscle PAH concentrations, and bioaccumulation factors were evaluated using the Quade test. For the Quade test procedure, exposure conditions were defined as treatment and % of stock solutions defined as blocks. Values obtained for each exposure conditions at several % of stock solutions were considered as repeated measurements. The Quade test was carried out using R statistical software and the statistical significance of the results was ascertained at $\alpha = 0.05$.

3. Results

Physicochemical parameters were stable and no difference was observed between exposure conditions (**Table 1**).

3.1. Total petroleum hydrocarbon (TPH) seawater concentrations and fish mortality (Tables 3a and 3b)

No mortality was observed and the TPH concentration was zero for 0% stock solutions of all exposure media. Spearman's test revealed a correlation between TPH concentration (the mean of measurements at $T = 0$ h and at $T = 24$ h) and the percent dilution of stock solutions for CD1 and CD2 exposure ($P < 0.05$), but no correlation was found for MD exposure ($P =$

0.137). Because only soluble compounds are present in WSF of oil exposure media, the low TPH concentrations contained in this exposure media cannot be detected using spectrophotometry.

No mortality was observed following WSF of oil and MD exposure. For D1 exposure, 10 % mortality and 30 % mortality was observed for 18 and 40 % stock solution exposures, respectively. Approximately the same pattern was observed for D2 exposure: 10% mortality and 20 % mortality was observed for 12 and 40 % stock solution exposures, respectively.

For CD1 exposure, no mortality was observed following exposure to 0% and 2.4 % of the stock solution. Following exposure to 12, 18 and 24 % of the stock solution, 30 % mortality was observed. Thus, no mortality increase was observed following exposure between 12 and 24 % of the CD1 stock solution, whereas our results show increased TPH concentrations. Following exposure to 40 % of the stock solution, 100 % mortality was observed.

For CD2 exposure, 0 % mortality was observed following exposure between 0 and 18 % of the stock solution. 10% mortality was observed following exposure to 24 % of the stock solution and 100 % mortality was observed following exposure to 40 % of the CD2 stock solution.

3.2. Polycyclic aromatic hydrocarbon (PAH) seawater concentration

No polycyclic aromatic hydrocarbons (PAH) were detected for 0% of the stock solutions for all exposure media.

A correlation between the sum of the concentrations of the 21 PAH (**Figure 2**) and the percent dilution of the stock solution was found for CD1, CD2 and WSF of oil exposures ($P < 0.05$), but not for MD exposure ($P = 0.100$). The sum of the concentrations of the 21 PAH in seawater revealed significant differences between exposure conditions. First, PAH

concentrations were significantly higher following CD2 exposure than CD1 exposure. Moreover, our results reveal that exposure to chemically dispersed oil solutions (CD1 and CD2) is associated with higher concentrations of PAH than mechanically dispersed oil media. Finally, PAH concentrations following WSF of oil exposure were significantly lower than PAH concentrations measured following other exposure conditions (MD, CD1, CD2).

3.3. Polycyclic aromatic hydrocarbon (PAH) concentrations in fish muscles and bioaccumulation factor (BAF)

Polycyclic aromatic hydrocarbon (PAH) concentrations in fish muscles (**Table 4**) were measured to be zero following exposure to 0% of the stock solutions for all exposure media. Correlations were found between PAH concentrations in fish muscles and the percent stock solution dilution used for MD, WSF, CD1 and CD2 ($P < 0.05$). PAH concentrations were significantly higher in chemically dispersed oil (CD1 and CD2) than in either mechanically dispersed oil (MD) or WSF of oil. Even though the PAH concentrations appeared to be much higher following mechanically dispersed oil exposure than WSF of oil exposure, statistical analysis did not reveal any significant difference ($P\text{-value} = 0.115$). No correlation was observed between BAF and the percent stock solution dilution, for all exposure media (**Table 5**). BAF was found to be significantly higher following exposure to chemically dispersed crude oil (CD1 and CD2) than WSF of oil exposure. Although BAF levels appeared to be higher following MD exposure than following WSF of oil exposure, no significant difference was found ($P = 0.064$). No significant difference was found between MD and CD1 exposure ($P = 0.265$). The same is true of MD and CD2 exposure ($P = 0.701$).

4. Discussion

The aim of this study was to evaluate the toxicity due to dispersant application in nearshore areas as an oil spill response technique. Using an experimental approach, this study took into account the turbulent mixing processes inherent in nearshore waters, and also accounted for the presence of oil droplets resulting from oil slick dispersion. Four exposure conditions were tested: (i) chemically dispersed oil solutions, simulating the application of dispersant when turbulent mixing processes permit this response technique; (ii) dispersant alone (D1 and D2) in seawater, as internal controls of chemically dispersed oil solutions; (iii) a mechanically dispersed oil solution, simulating the natural dispersion of an oil slick due to mixing processes but without dispersant application; (iv) a water soluble fraction of oil, simulating an undispersed and untreated oil slick before recovery, when calm weather conditions permit this response technique.

For 0% dilutions of the stock solution of all treatments, mortality did not occur and contaminants were not detected in seawater or fish tissues. These results, coupled with the stability of physicochemical parameters (T °C, pH, dissolved oxygen, salinity) validate the experimental procedure used in this study.

4.1. Mortality, total petroleum hydrocarbon (TPH), and polycyclic aromatic hydrocarbon (PAH) concentrations in seawater

The TPH concentration decrease observed in this study between T = 0 h and T = 24 h, is in agreement with field operation measurements, although the drastic decrease observed in literature (Cormack, 1977; Lessard and Demarco, 2000) was slighter less in our experiment. Although the experimental system used for this study attempts to simulate natural dispersion

at nearshore areas, we admit that the evolution of TPH concentration depends on the turbulent mixing energy of the experimental system.

Correlations between the percentage stock solution used for exposure and total petroleum hydrocarbon concentrations (mean of measurements at $T = 0\text{h}$ and at $T = 24\text{h}$) were observed following CD1 and CD2 exposure, whereas no correlation was found following MD exposure. Indeed, following CD1 and CD2 exposure, TPH concentrations increase linearly as a function of the percentage stock solution used for exposure, whereas TPH concentrations for MD exposure did not increase between 12% of the stock solution and 40% of the stock solution. Extrapolated to oil spill response techniques, this result shows that, for a given sea energy (in this study, the experimental system energy), a threshold water column concentration cannot be exceeded if the oil slick is dispersed mechanically, whereas this threshold can be exceeded if the oil slick is dispersed chemically.

In parallel, an oil slick was observed following exposure to 12, 18, 24, and 40% MD stock solution dilutions, whereas no oil slick was observed following CD1 and CD2 exposure. Thus we can hypothesise that, following MD exposure, increasing the petroleum quantity led to an increase in oil slick thickness instead of an increase in water column TPH concentration. The formation of an oil slick during mechanical dispersion, but not during chemical dispersion is in agreement with the mode of action of chemical dispersants and with observations made at oil spill sites. Lewis and Daling (2001) gave a complete explanation of this phenomenon: when turbulent mixing energy permits natural dispersion of the oil slick, oil droplets are large (from 0.4 to several mm in diameter) and rise quickly back to the sea surface, where they coalesce and reform the oil slick. In contrast, chemical dispersant application mediates the formation of smaller oil droplets (10 to 50 μm), which have a low rise velocity and are disseminated, for instance by water currents, before they can reform an oil slick.

Regarding to fish mortality, chemically dispersed oil would be more toxic than an untreated and undispersed oil slick (WSF). Indeed, no mortality was found for WSF of oil exposure whereas 100 % fish mortality was observed following exposure to 40% dilutions of CD1 and CD2 exposure. Moreover, mortality due to chemical dispersion of oil (CD1 and CD2) were observed at lower concentrations: at 12 % of the CD1 stock solution and at 24 % of the CD2 stock solution. Note that mortality did not increase between 12 % and 24 % of the CD2 stock solution, whereas actual TPH concentrations increased. This phenomenon is likely due to contamination resistance variability between fish. For example, it is possible that some fish exposed to 24 % of the CD2 stock solution were more resistant to hydrocarbon contamination than other fish exposed to 12 % of the CD2 stock solution. This would result in the same mortality percentage for both groups of fish, even if the exposure concentrations were different. In total, these results are in agreement with many studies (Long and Holdway, 2002; Pollino and Holdway, 2002; Lin et al., 2009) and suggest, as it was previously proposed by Cohen and Nugegoda (2000), that when meteorological conditions permit it, recovery and containment of the oil slick should be conducted since it is a less toxic response technique than the application of chemical dispersants. Similarly, chemically dispersed oil seems to be more toxic than mechanically dispersed oil, because no mortality was observed after fish were exposed to this condition. This finding suggests that, when the oil slick is under natural mixing processes, the application of chemical dispersant increases the toxicity of the petroleum, probably by increasing the amount of total petroleum hydrocarbons in the water column (as described above). Moreover, our study results suggest that the toxicity of dispersants (D1 and D2) seems to be too low to be the major determinant of CD1 and CD2 exposure toxicity. This result is in accordance with Otitoluju (2005), who showed that the toxicity of dispersant application is not due to dispersant toxicity alone, but rather to the synergistic toxicity of dispersant and petroleum.

422 With respect to seawater concentrations of the 21 PAH compounds tested, our results show
423 that PAH concentrations were significantly higher following CD1 and CD2 exposure than
424 WSF of oil exposure. This result is in line with the mode of action of chemical dispersants,
425 which increase the total surface area for the partitioning of PAH from oil to water by
426 increasing the number of droplets and decreasing their size. Mechanical dispersion also
427 increases the number of droplets in the water column, and, logically, based on our results, this
428 phenomenon led to an increase in PAH concentrations (comparing WSF of oil exposure and
429 MD exposure).

430 Our study results demonstrate that PAH concentrations are higher in chemically dispersed oil
431 than in mechanically dispersed oil. This result could also be due to an increase in surface area
432 available for PAH partitioning, since part of the petroleum is taking part in an oil slick
433 (instead of droplets) in mechanically dispersed oil.

434 Moreover, our results indicate that, through comparison of both chemical dispersants (CD1
435 and CD2), dispersant 2 induces higher PAH concentrations in seawater than dispersant 1.
436 However, TPH concentrations in the water column do not seem to be different between both
437 dispersed oil solutions. It is therefore possible to hypothesise that dispersant 1 retards the
438 PAH partitioning from oil droplets to water.

439 In our study, the sum of 21 PAH concentrations in seawater were never higher than 308 µg/L
440 (following exposure to 40 % of the CD2 stock solution). In a study by Maria et al. (2002),
441 conducted on *Anguilla anguilla*, the exposure period was 9 times longer (216 h) than in our
442 study, and the concentration of benzo[*a*]pyrene -considered to be the most toxic of the 21
443 PAH (Eisler, 1987)- was measured to be 2 times higher (680 µg/L) than the sum of 21 PAH
444 concentrations measured in our study. Nevertheless, in this last study, mortality was not
445 observed. Comparison of these two studies suggests that, in our study, PAH concentration
446 was not sufficient to induce mortality. Thus, PAH were not the only determinant of acute

toxicity. In fact, acute toxicity could be due to other petroleum compounds (such as saturated hydrocarbons), or the presence of oil droplets (Brannon et al., 2006). Therefore, as stated in the introduction of this article, the presence of total petroleum hydrocarbons and/or droplets in the water column seems to be very important for evaluation of the toxicity of chemically dispersed oil.

4.2. Bioaccumulation factor (BAF), PAH concentrations in fish muscles

PAH compounds are considered to be mutagenic and carcinogenic (Ohe et al., 2004) and, consequently, their bioaccumulation is relevant and interesting because it provides information about long-term toxicity (Ramachandran et al., 2004a). In the present study, PAH bioconcentrations were measured following a 24 h period in clean seawater. There is evidence that biological detoxification processes (such as the induction of EROD activity described in Camus et al., 1998) can be induced during this period. However, because the detoxification period was the same for all fish, comparison of results between exposure conditions is reliable.

In a comparison of chemically dispersed oil and the water soluble fraction of oil, our results show that dispersion of the oil slick led to an increase in PAH concentrations in seawater (previously discussed in section 4.1. above). Moreover, our results show that PAH bioaccumulation (via BAF calculation) is higher following CD1 and CD2 exposure conditions than WSF of oil exposure conditions. This phenomenon could be due to other contaminants (such as saturated hydrocarbons or the chemical dispersant), which can induced functional morphology alterations in the gills (as described in Rosety-Rodriguez et al., 2002). This alteration would have induced a decrease of the selective permeability of gills and by the way an increase of PAH bioaccumulation.

Taken together, these results show that dispersant application increases PAH partitioning from oil to water, and moreover, increases PAH bioaccumulation (at the seawater–organism interface). As a result, PAH concentrations were found to be higher in the muscles of fish exposed to chemically dispersed oil than in the muscles of fish exposed to the water soluble fraction of oil. These results are in agreement with many studies that have revealed an increase in PAH uptake due to dispersant application (Wolfe et al., 2001; Mielbrecht et al., 2005).

Similarly, PAH concentration measurements in seawater were found to be higher following MD exposure than WSF of oil exposure. Moreover, these results show that PAH bioaccumulation seems to be higher following MD exposure than WSF of oil exposure ($P = 0.064$). Together, these results suggest that mechanical dispersion seems to increase PAH partitioning from oil to water, and, in addition, increase PAH bioaccumulation (at the seawater–organism interface). As a result, PAH concentrations seem to be higher in the muscles of fish exposed to mechanically dispersed oil (MD) than in the muscles of fish exposed to the water soluble fraction of oil (P value = 0.115).

By comparing chemically and mechanically dispersed crude oil, we have shown that PAH concentrations in seawater were higher following chemically dispersed oil exposure (either CD1 or CD2) than following MD exposure. PAH bioaccumulation (BAF) was not different following these two exposures whereas PAH concentrations were significantly higher in the muscles of fish exposed to chemically dispersed oil than in the muscles of fish exposed to mechanically dispersed oil. These results suggest that, following chemical dispersant application, PAH partitioning from oil to water is the main factor inducing the observed increase in PAH bioconcentrations.

5. Conclusion

By comparing chemically dispersed oil exposure and water soluble fraction of oil exposure, our results demonstrate that chemical dispersion is more toxic than an untreated and undispersed oil slick, both in terms of acute toxicity (i.e. mortality observations) and chronic toxicity (increased PAH bioconcentration in fish muscles). The higher toxicity found for chemically dispersed oil solutions is probably due to the presence of oil droplets and the resulting increase in the concentration of PAH in the water column. Based on these results, responders should consider the increased toxicity due to chemical dispersion before using this response technique. For instance, when an oil spill site is ecologically sensitive, oil dispersion, by applying dispersants and inducing mixing processes (e.g. using a boat propeller as recommended in Merlin et al., 2005), would not be appropriate. In this case, oil slick containment and recovery should be considered if technical facilities and meteorological conditions permit.

Our comparison of chemically and mechanically dispersed oil exposure effects has yielded information on dispersant application toxicity when turbulent mixing processes are present in nearshore areas. Under these conditions, oil slick containment and recovery is impossible, because of natural dispersion of the slick. Our results show that dispersant application increases both fish mortality and PAH bioconcentrations (by increasing the amount of dissolved PAH in the water column). These results suggest that, when the oil slick is under natural mixing processes, such as waves, the application of dispersant increases the environmental risk for aquatic organisms living in the water column.

However, these results were obtained at an experimental given mixing energy and concentrations used were significantly higher than those normally encountered during oil spills (Cormack, 1977; Lunel et al., 1995). These limitations of our study compel us to be

cautious in our conclusion. Nevertheless, responders must take into account these results and also need more information on potentially sublethal effects, to better evaluate the long-term toxicity of dispersant application. Because of this, our study is part of an on-going project (DISCOBIOL project: DISpersant and response techniques for COastal areas; BIOLogical assessment and contributions to the regulation). Considering both the toxicity of dispersant application and its advantages, this project aims to obtain information about the ecological effects of dispersant application in nearshore areas.

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657 larval topsmelt (*Atherinops affinis*). *Aquatic Toxicol* 2001; 52: 49-60.

668 **Table 1:** Fish weight (Values represent mean \pm standard error mean; n = 10 per treatment)
669 and physicochemicals parameters measured during organism exposure (Values represent
670 mean \pm standard error mean of 6 tanks measurement at T = 0 h and at T = 24 h)

Parameters	MD	CD1	CD2	WSF	D1	D2
Temperature (°C)	19.1 \pm 0.1	18.8 \pm 0.1	18.6 \pm 0.2	19.1 \pm 0.1	18.9 \pm 0.2	18.6 \pm 0.2
pH	7.96 \pm 0.03	7.92 \pm 0.04	7.90 \pm 0.03	8.00 \pm 0.01	7.98 \pm 0.02	7.93 \pm 0.02
Dissolved oxygen (% AS)	95.2 \pm 0.8	92.1 \pm 0.6	94.6 \pm 1.1	95.2 \pm 0.8	93.8 \pm 0.6	96.8 \pm 0.6
Salinity (‰)	35.1 \pm 0.1	35.2 \pm 0.1	35.2 \pm 0.1	35.1 \pm 0.1	35.1 \pm 0.1	35.2 \pm 0.1
Fish weight (g)	1.74 \pm 0.11	1.60 \pm 0.12	1.65 \pm 0.09	1.71 \pm 0.12	1.78 \pm 0.11	1.87 \pm 0.14

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674 **Table 2:** Concentration of 21 PAHs (alkylated and parents) in the Brut Arabian Light (BAL)
675 and in the weathered Brut Arabian Light. The 21 PAHs represent the 16 US-EPA PAHs and
676 five supplementary PAHs (benzo[*b*]thiophene, biphenyl, dibenzothiophene, benzo[*e*]pyrene,
677 perylene). n.d. = not detected.

	Molecular weight (g/mol)	Concentration in BAL (μ g/g of petroleum)	Concentration in weathered BAL (μ g/g of petroleum)
Naphtalene	128.2	222	211
C1-Naphtalene	143.2	955	854
C2-Naphtalene	158.2	2 099	1 819
C3-Naphtalene	173.2	2 084	1 796

C4-Naphtalene	188.2	1480	1317
Benzo[<i>b</i>]thiophene	134.2	5	5
C1-benzo[<i>b</i>]thiophene	149.2	63	22
C2-benzo[<i>b</i>]thiophene	164.2	298	292
C3-benzo[<i>b</i>]thiophene	179.2	681	1 030
C4-benzo[<i>b</i>]thiophene	209.2	606	537
Acenaphtylene	152.2	30	25
Biphenyl	154.2	15	14
Acenaphtene	154.2	4	3
Fluorene	166.2	45	39
C1-Fluorenes	181.2	132	116
C2-Fluorenes	196.2	269	230
C3-Fluorenes	211.2	304	261
Phenanthrene	178.2	112	95
Anthracene	178.2	112	95
C1-phenanthrenes/anthracenes	193.2	396	335
C2-phenanthrenes/anthracenes	208.2	603	498
C3-phenanthrenes/anthracenes	223.2	493	416
C4-phenanthrenes/anthracenes	238.2	318	273
Dibenzothiophene	184.3	373	330
C1-dibenzothiophenes	199.3	1115	987
C2-dibenzothiophenes	214.3	2021	1759
C3-dibenzothiophenes	229.3	1764	1546
C4-dibenzothiophenes	244.3	1040	936
Fluoranthene	202.3	7	6

Pyrene	202.3	11	9
C1-fluoranthenes/pyrenes	217.3	62	51
C2-fluoranthenes/pyrenes	232.3	137	119
C3-fluoranthenes/pyrenes	247.3	222	191
Benzo[<i>a</i>]anthracene	228.3	19	16
Chrysene	228.3	18	15
C1-chrysenes	243.3	37	29
C2-chrysenes	258.3	57	45
C3-chrysenes	273.3	84	88
Benzo[<i>b+k</i>]fluoranthene	252.3	3	3
Benzo[<i>e</i>]pyrene	252.3	2	2
Benzo[<i>a</i>]pyrene	252.3	11	9
Perylene	252.3	3	7
Benzo(<i>g,h,i</i>)perylene	276.3	2	2
Indeno(1,2,3- <i>cd</i>)pyrene	276.3	n.d.	n.d.
Dibenz(<i>a,h</i>)anthracene	278.4	n.d.	1

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686 **Table 3a:** Mortality and Total petroleum hydrocarbon (TPH) concentration (values represent
687 the concentration at T_{0 h}-T_{24 h}) measured for each % of stock solution, for MD (mechanically
688 dispersed oil), CD1 (chemically dispersed oil using dispersant 1), CD2 (chemically dispersed
689 oil using dispersant 2). n.d. = not detected.

	MD		CD1		CD2	
% of stock solution	[TPH] (mg/L)	Mortality (%)	[TPH] (mg/L)	Mortality (%)	[TPH] (mg/L)	Mortality (%)
0	n. d.	0	n. d.	0	n. d.	0
2.4	67-15	0	55-35	0	111-64	0
12	281-228	0	491-398	30	616-548	0
18	173-158	0	873-605	30	777-625	0
24	383-340	0	1223-1096	30	1203-1055	10
40	374-122	0	1606-1457	100	1641-1438	100

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701 **Table 3b:** Mortality and contaminants concentration (TPH for WSF exposure and Dispersant
702 nominal concentration for D1 and D2 exposure). n.d. = not detected.

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	WSF		D1		D2	
% of stock solution	[TPH] (mg/L)	Mortality (%)	[Disp] (mg/L)	Mortality (%)	[Disp] (mg/L)	Mortality (%)
0	n. d.	0	0	0	0	0
2.4	n. d.	0	6	0	6	0
12	n. d.	0	30	0	30	10
18	n. d.	0	45	10	45	0
24	n. d.	0	60	0	60	0
40	n. d.	0	100	30	100	20

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Table 4: Concentration of the sum of 21 PAHs (alkylated and parents) in fish muscles (µg/g) for CD1 (Chemically Dispersed oil using dispersant 1), CD2 (Chemically Dispersed oil using dispersant 2), MD (Mechanically Dispersed oil) and WSF (Water Soluble Fraction of oil) exposures. Respecting Quade test procedures, values obtained for each exposure conditions at several % of stock solution are considered as repeated measure. *indicates significant differences ($P < 0.05$) of concentrations ([sum of 21 PAHs]) between exposure conditions. n.d. = not detected.

% of stock solution	MD	CD1	CD2	WSF
0	n. d.	n. d.	n. d.	n. d.
2.4	2.27	6.30	5.70	0.08
12	3.34	10.23	8.13	0.09
18	4.24	11.43	17.00	0.14
24	9.80	12.10	11.25	0.12


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736 **Table 5:** Bioaccumulation factor (BAF = [total PAH] in fish muscle / [total PAH] in sea
 737 water) for CD1 (Chemically Dispersed oil using dispersant 1), CD2 (Chemically Dispersed
 738 oil using dispersant 2), MD (Mechanically Dispersed oil) and WSF (Water Soluble Fraction
 739 of oil) exposures. Respecting Quade test procedure, values obtained for each exposure
 740 conditions at several % of stock solution are considered as repeated measure. *indicates
 741 significant differences (P<0.05) of BAF between exposure conditions. n.c. = not calculated.

% of stock solution	MD	CD1	CD2	WSF
0	n. c.	n. c.	n. c.	n. c.
2.4	33.1	75.1	61.4	16.1
12	42.5	98.4	66.7	16.5
18	58.2	99.3	141.4	20.6
24	126.9	98.1	59.2	12.1

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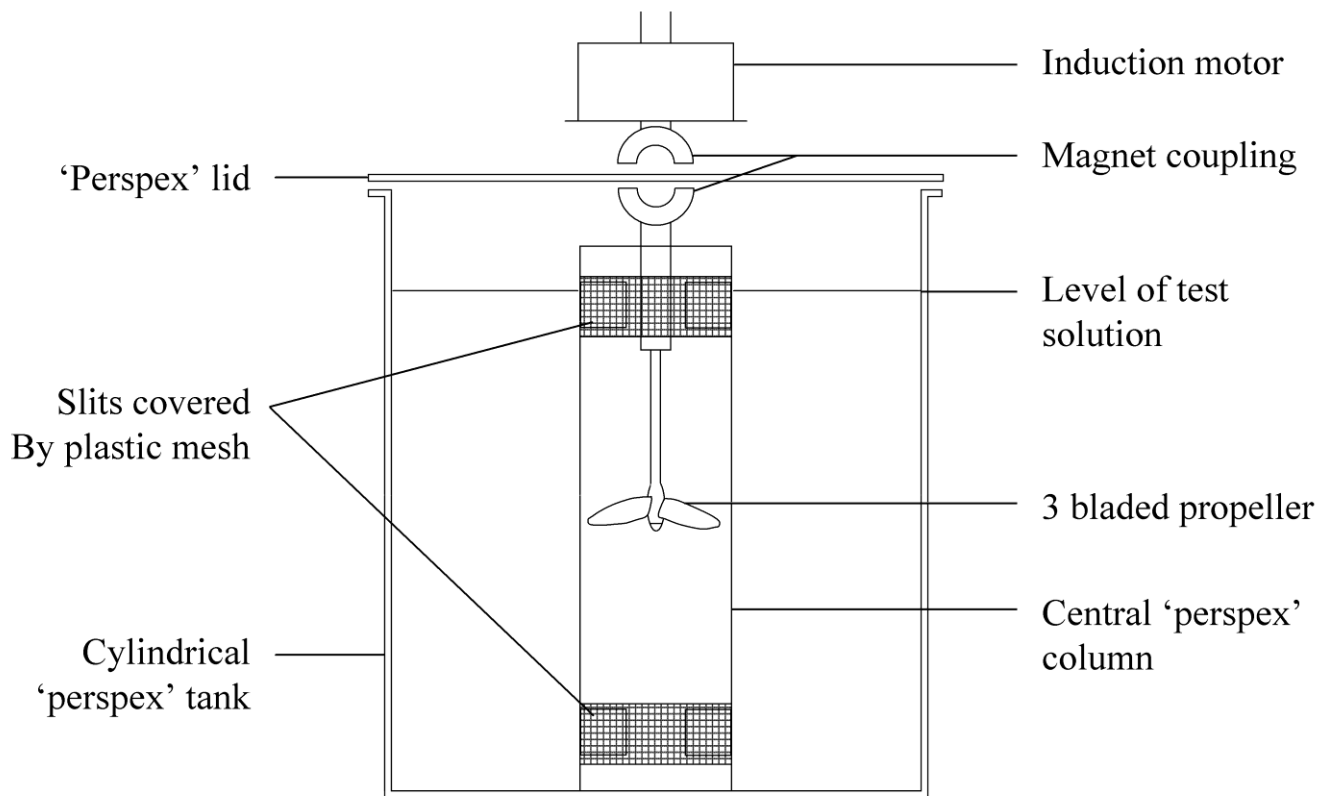


Figure 1: One tank of the experimental system (composed of twelve tanks) devised to maintain a mixture of oil-dispersant droplets throughout the water column

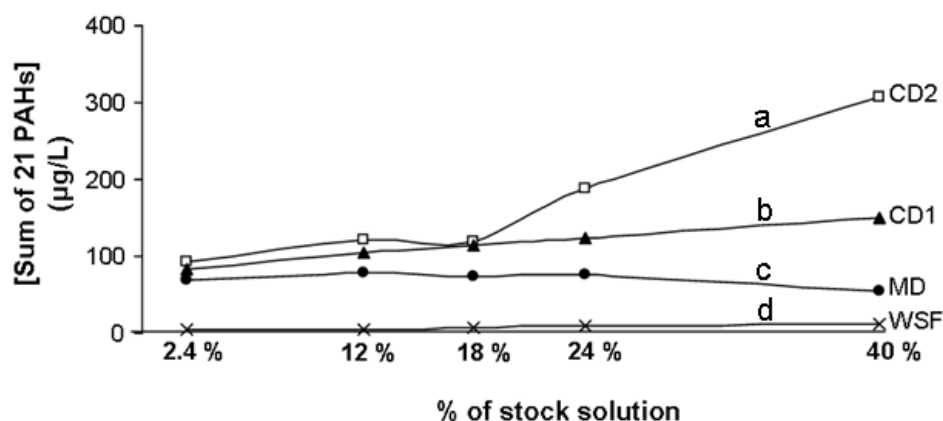


Figure 2: Concentration of the sum of 21 PAHs (alkylated and parents) in sea water during CD1 (Chemically Dispersed oil using dispersant 1), CD2 (Chemically Dispersed oil using dispersant 2), MD (Mechanically Dispersed oil) and WSF (Water Soluble Fraction of oil). The 21 PAHs represent the 16 US-EPA PAHs and five supplementary PAHs (benzo[*b*]thiophene, biphenyl, dibenzothiophene, benzo[*e*]pyrene, perylene). Values represent means of two measurements (at T = 0 h and at T = 24 h). To determine whether the curves differed significantly, Quade test was conducted. Different letters above curves indicates that curves differed significantly ($P < 0.05$)