

## Toxicity of Oil-Dispersant Mixtures on Juveniles Sea Bass (*Dicentrarchus labrax*)

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### Abstract

In case of oil spill, the use of chemical dispersant has been recognized as an appropriate technical response (Chapman *et al.*, 2007). Indeed, surface active agents contained in dispersants fragment the oil slick into fine droplets facilitating its dilution and subsequently biodegradation (Tiehm, 1994). However, despite their efficiency, use of dispersants in coastal areas is controverted mainly due to the lack of information about toxicity of the mixture (oil plus chemical dispersant). Therefore, the aim of this study was to investigate the toxicity of a reference oil (weathered Arabian Light Crude) dispersed mechanically and chemically with 2 different commercial formulations (Finasol OSR 52 and COREXIT 9500). Experimentations were carried out on juvenile sea bass (*Dicentrarchus labrax*). Tests were conducted under static conditions at a temperature of  $16 \pm 1$  ° C with a 12/12 photoperiod during 96 hours. Mortality of fish was assessed twice a day during 96 of exposure. Moreover, droplet size of dispersed oil and total petroleum concentrations was measured during the study. Results showed that during the first 24 hours of exposure mortalities induced by mechanical dispersion of oil were lower than those induced by the two chemical dispersion of oil (CL50 at 24 h: Mechanical dispersion: not calculable; oil dispersed by Finasol OSR 52:  $385 \text{ mg.L}^{-1}$ ; oil dispersed by Corexit 9500 :  $396 \text{ mg.L}^{-1}$ ). However, from 48 hours until the end of exposure, LC<sub>50</sub> and LC<sub>20</sub> were similar for the mechanical and the two chemical dispersions of oil.

### 1 Introduction

Since the second part of the twentieth century, the increase of world demography and economic developments led to an increase in energy requirements. Among all energies, oil remains the most coveted and consumed in the world. With four billion tons consumed in 2010, oil represents 33.6% of the total worldwide energy use. This oil demand involves risks of pollution during extraction and transport. During the last decades many maritime accidents occurred resulting in major releases of oil in the environment (*Torrey Canyon*, 1967; *Amoco Cadiz*, 1978; *Exxon Valdez*, 1989; *Deepwater Horizon*, 2010). This Oil spill can lead to important ecologic impacts and disturb ecosystems on a long term basis (Bocard *et al.*, 2006). To limit the ecologic and economic impact of an oil spill, three mains technical response can be used: containment followed by recovery, *in situ* burning and chemical dispersion of the oil slick.

Since the 1970s, use of chemical dispersants in offshore environments has been recognized as an appropriate technical response (Chapman *et al.*, 2007). Indeed, surface active agents contained in dispersants, fragment the oil slick into fine droplets facilitating its dilution and subsequently biodegradation (Tiehm, 1994). Despite these advantages, the use of dispersants in coastal areas is still a controversial subject and only few information on toxicity of the mixture oil plus dispersant are available. Amongst other things, it has been shown that chemical dispersion of an oil slick leads to a significant increase of oil concentration and oil

bioavailability in lower part of the water column (Wolfe et al., 1998) increasing the short term toxicity (Lunel et al., 1997; Chapman et al., 2007; Milinkovitch et al., 2011).

Thus, the aim of this study was to evaluate the acute toxicity of a reference oil dispersed mechanically and chemically with two commercial formulation of dispersants. Acute toxicity was evaluated through the determination of lethal concentration (LC<sub>20</sub>, LC<sub>50</sub> and LC<sub>80</sub>), considered as integrative parameters of toxicity. Experimentations were performed on juvenile sea bass (*Dicentrarchus labrax*) following OECD guidelines (OECD, 1992) and the AFNOR French standard (NF T 90-307 AFNOR, 1985) characterizing the acute toxicity tests on fish.

## **2 Materials and Methods**

### **2.1 Animals**

Juvenile seabass *Dicentrarchus labrax* ( $0.99 \pm 0.17$  g) were purchased from a local hatchery Aquastream (Ploemeur, France). They were acclimatized three weeks in a 300 liters tank supplied with a continuous flow of sterilized, filtered and aerated seawater. pH ( $7.93 \pm 0.10$ ), oxygen saturation (upper than 90%), and temperature ( $15.8 \pm 0.1$  °C) were measured daily. Fish were fed daily with dried pellets (Neo Start Coul 2 from Le Gouessant aquaculture, total proteins 52%, lipids 17%, cellulose 1.1%, ash 7.35%, and moisture 10%).

### **2.2 Chemicals**

#### **2.2.1 Oil**

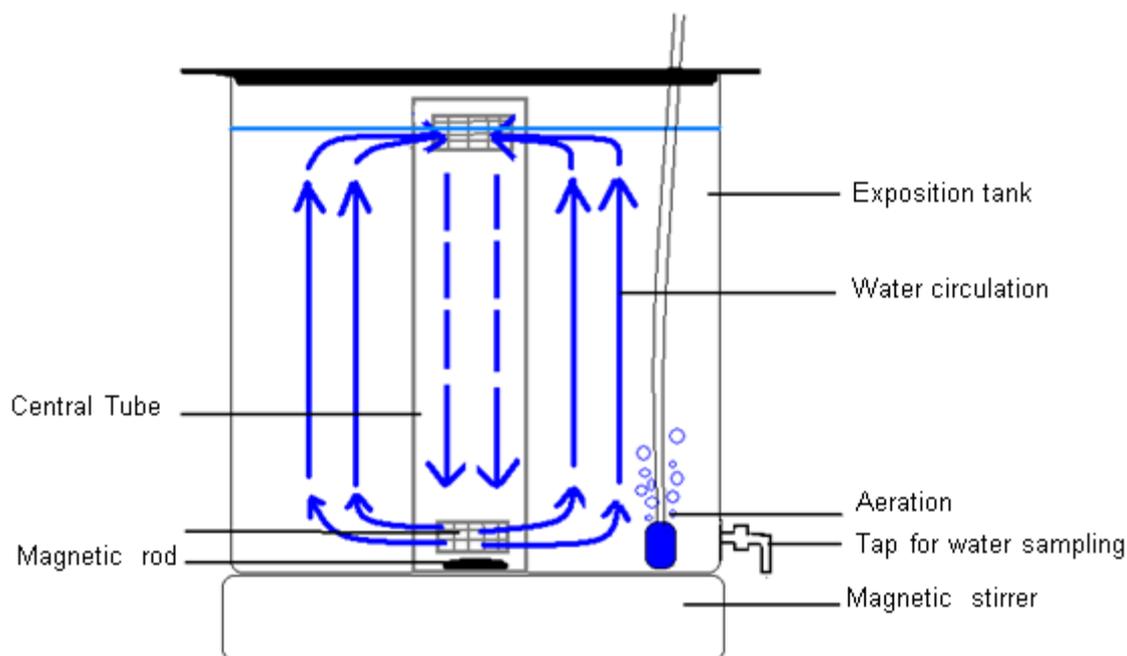
The oil used in the study was an Arabian Light Crude (ALC) composed of 54% saturated hydrocarbons, 10% polar compounds and 36% aromatic hydrocarbons and previously used in other studies (Danion et al., 2011; Milinkovitch et al., 2011). Before use in our experiments, ALC was artificially weathered. The weathering process consisted in air bubbling through the oil at low temperature (12 to 16 °C) until a loss of 7% of petroleum weight. Such a treatment simulates ageing of a slick released at sea. The weathered ALC contains 54% of saturated hydrocarbons, 34% of aromatic hydrocarbons and 12% of polar compounds.

#### **2.2.2 Dispersants**

Two commercial formulations of 3<sup>rd</sup> generation dispersants were used in this study: Finasol OSR 52 from Total Fluides and Corexit 9500 from Nalco Holding Company. They are both oil-based dispersants composed of surfactants (anionic and non-ionic type surface active agents) diluted in water-soluble solvents.

### **2.3 Experimental System**

Twelve glass tanks (16 liters) were equipped with a magnetic stirrer allowing homogenization of the chemicals to be tested. A vertical high density polyethylene tube with screened openings on the top and the bottom was placed in the center of each tank. A magnetic rod positioned in the center of the tube generated a water flow in the tank (Figure 1), drawing the exposure solutions through the upper apertures to the bottom of the tank. All exposure tanks were equipped with an aeration system. A tap on the bottom of tanks allows water sampling.



**Figure 1: Diagram of the exposure tank**

## 2.4 Experimental Conditions

Three experimental conditions were performed: weathered ALC dispersed by Finasol OSR52 (mixture F), weathered ALC dispersed by Corexit 9500 (mixture C), and weathered ALC dispersed mechanically. For the two chemical dispersions, the dispersant to oil ratio (DOR) was 1:20

Before addition of chemicals, tanks were filled with 16 liters of seawater and each magnetic stirrer was set at 800 rpm. Seawater (35PSU) provided from Oceanopolis aquarium. Before use, seawater was filtered and UV sterilized. The oil - dispersant mixture was poured in the tanks. Six hours after (allowing the stabilization of hydrocarbon concentrations) 16 two-days-starved fish, were randomly assigned in one of the tanks. Total concentration of fish in each tank did not exceed 1 g fish /liter of water (Lines OECD Test No. 203, 1992).

Then chemicals were added and fish mortalities were evaluated every 24 hours under static conditions at a temperature of  $16 \pm 1$  °C with a 12/12 photoperiod during 96 hours. Dead fish (no gill movement and no response to a caudal pinch) were removed from the tanks at the beginning and at the end of the day to reduce stress which might be induced by samplings. The range of hydrocarbons nominal concentrations of oil and dispersant used in this study were shown in Table 1. One tank was used per concentration. Measurements of hydrocarbons concentrations were also performed every 24 hours (T0, T24, T48, T72 and T96) on water samples (25 mL duplicates).

**Table 1: Amounts (g) of oil and dispersant required to achieve ranges of hydrocarbons nominal concentrations ( $\text{mg.L}^{-1}$ ).**

		Hydrocarbon nominal concentrations ( $\text{mg.L}^{-1}$ )						
		0	188	313	500	625	938	1250
<b>Mixture F and C</b>	<b>oil (g)</b>	0	3	5	8	10	15	-
	<b>Dispersant (g)</b>	0	0,15	0,25	0,4	0,5	0,75	-
<b>Mechanical dispersion</b>	<b>oil (g)</b>	0	-	5	8	10	15	20

## 2.5 Measurement of Total Petroleum Hydrocarbon (TPH) Seawater Concentrations

The total petroleum hydrocarbons concentration (TPH), corresponding to the combination of dissolved hydrocarbons and oil droplets, was measured each day in duplicate in each tank. Water samples were extracted three times with 10 mL of Pestipur quality dichloromethane (Carlo ErbaReactifs, SDS). After separation of the aqueous and the organic phases, combined organic phases were dried on anhydrous sulphate. The absorbance of the organic phase was measured and quantified at a wavelength of 390 nm (UveVispectrophometer, Unicam, France). TPH concentrations in water samples were calculated from three different calibrations curves including or not 5% of the corresponding dispersant.

## 2.6 Measurements of Droplet Size of Dispersed Oil

Droplet sizes (diameter in  $\mu\text{m}$ ) were analyzed at T0 at a nominal concentration of 1250 mg/L. Measurements were performed by laser granulometry (Malvern Mastersizer 2000) based on the principle of Fraunhofer where particle size depends of the intensity of diffracted radiation and the diffraction angle. It is completed in our case (particles diameter <50 microns) with the Mie theory taking into account the refractive indices of the sample and the carrier medium (ISO 13320-1, 1999). A flow rate of 1200 mL/min and an obscuration of 10  $\pm$  0.01% were the conditions used during measurements.

## 2.7 Statistics

Dose response curves and lethal concentrations ( $\text{LC}_{50}$ ,  $\text{LC}_{20}$ ) were calculated using a logistic dose-response curve model from the TableCurve2D software (SYSTAT Software Inc.), and expressed as  $\text{mg.L}^{-1}$ . They are given with their confidence interval (lower 95% confidence interval-upper 95% confidence interval).

## 3 Results

Throughout the experiment, environmental measured parameters remained constant: oxygen saturation was higher than 90%; temperature was 16°C and pH 7.9. Moreover, no mortality was observed in control tanks. Taken together, these results indicated that all experimental requirements were met to perform toxicity studies of chemical dispersed oil on juvenile sea bass.

### 3.1 Total Petroleum Hydrocarbon (TPH) Seawater Concentrations

TPH seawater concentrations during the 96 hours of exposure were showed in Table 2.

**Table 2: Total Petroleum Hydrocarbon (TPH  $\text{mg.L}^{-1}$ ) concentrations in the three experimental conditions during 96 hours.**

	[TPH] ( $\text{mg.L}^{-1}$ )					
	Nominals	T0	T24	T48	T72	T96
	0	0	0	0	0	0
	188	155 $\pm$ 5	93 $\pm$ 1	33 $\pm$ 1	31 $\pm$ 0	28 $\pm$ 0
Mixture F	313	262 $\pm$ 1	228 $\pm$ 1	80 $\pm$ 1	51 $\pm$ 1	41 $\pm$ 1
	500	390 $\pm$ 15	301 $\pm$ 2	179 $\pm$ 0	-	-
	625	553 $\pm$ 7	297 $\pm$ 3	146 $\pm$ 2	-	-

	<b>938</b>	<b>821 ± 76</b>	<b>772 ± 9</b>	<b>-</b>	<b>-</b>	<b>-</b>
	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
	<b>188</b>	<b>173 ± 9</b>	<b>94 ± 2</b>	<b>63 ± 0</b>	<b>46 ± 1</b>	<b>34 ± 0</b>
	<b>313</b>	<b>261 ± 1</b>	<b>116 ± 12</b>	<b>61 ± 0</b>	<b>62 ± 0</b>	<b>51 ± 2</b>
<b>Mixture C</b>	<b>500</b>	<b>441 ± 6</b>	<b>183 ± 4</b>	<b>181 ± 8</b>	<b>110 ± 2</b>	<b>-</b>
	<b>625</b>	<b>580 ± 4</b>	<b>466 ± 6</b>	<b>261 ± 6</b>	<b>-</b>	<b>-</b>
	<b>938</b>	<b>866 ± 4</b>	<b>657 ± 9</b>	<b>-</b>	<b>-</b>	<b>-</b>
	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
	<b>313</b>	<b>47 ± 3</b>	<b>112 ± 4</b>	<b>76 ± 4</b>	<b>39 ± 3</b>	<b>39 ± 3</b>
<b>Mechanical dispersion</b>	<b>500</b>	<b>57 ± 15</b>	<b>162 ± 4</b>	<b>113 ± 11</b>	<b>60 ± 4</b>	<b>111 ± 10</b>
	<b>625</b>	<b>125 ± 2</b>	<b>209 ± 1</b>	<b>104 ± 9</b>	<b>194 ± 10</b>	<b>188 ± 1</b>
	<b>938</b>	<b>276 ± 41</b>	<b>573 ± 15</b>	<b>309 ± 63</b>	<b>159 ± 1</b>	<b>251 ± 34</b>
	<b>1250</b>	<b>178 ± 25</b>	<b>476 ± 16</b>	<b>281 ± 72</b>	<b>285 ± 90</b>	<b>144 ± 3</b>

(-) stop of experimentation due to 100% of mortality in the tank. Means ± SEM, n= 2

For the two mixtures, measured TPH concentrations at T0 were closed from nominal concentrations. Then they decreased throughout the experiment. At the opposite, the TPH concentrations of mechanical dispersion presented positive and negative variation during the 96 hours of exposure. Furthermore, TPH concentrations measured in some tank were higher than TPH concentrations of tanks with a higher nominal concentration.

### 3.2 Measurements of Hydrocarbons Droplet Size

Droplet sizes obtained with laser granulometry are reported in Table 3. In this table, d (0.5), d (0.1) and d (0.9) correspond to the median and the two deciles of a Normal distribution.

**Table 3: Measurements of hydrocarbons droplet size (µm) for the two mixture (n = 15).**

	<b>Mixture F</b>	<b>Mixture C</b>	<b>Mechanical dispersion</b>
<b>d(0,1)</b>	<b>4,0 ± 0,0</b>	<b>1,7 ± 0,0</b>	<b>N.D</b>
<b>d(0,5)</b>	<b>25,0 ± 0,2</b>	<b>17,9 ± 0,0</b>	<b>N.D</b>
<b>d(0,9)</b>	<b>87,8 ± 1,1</b>	<b>37,2 ± 0,2</b>	<b>N.D</b>

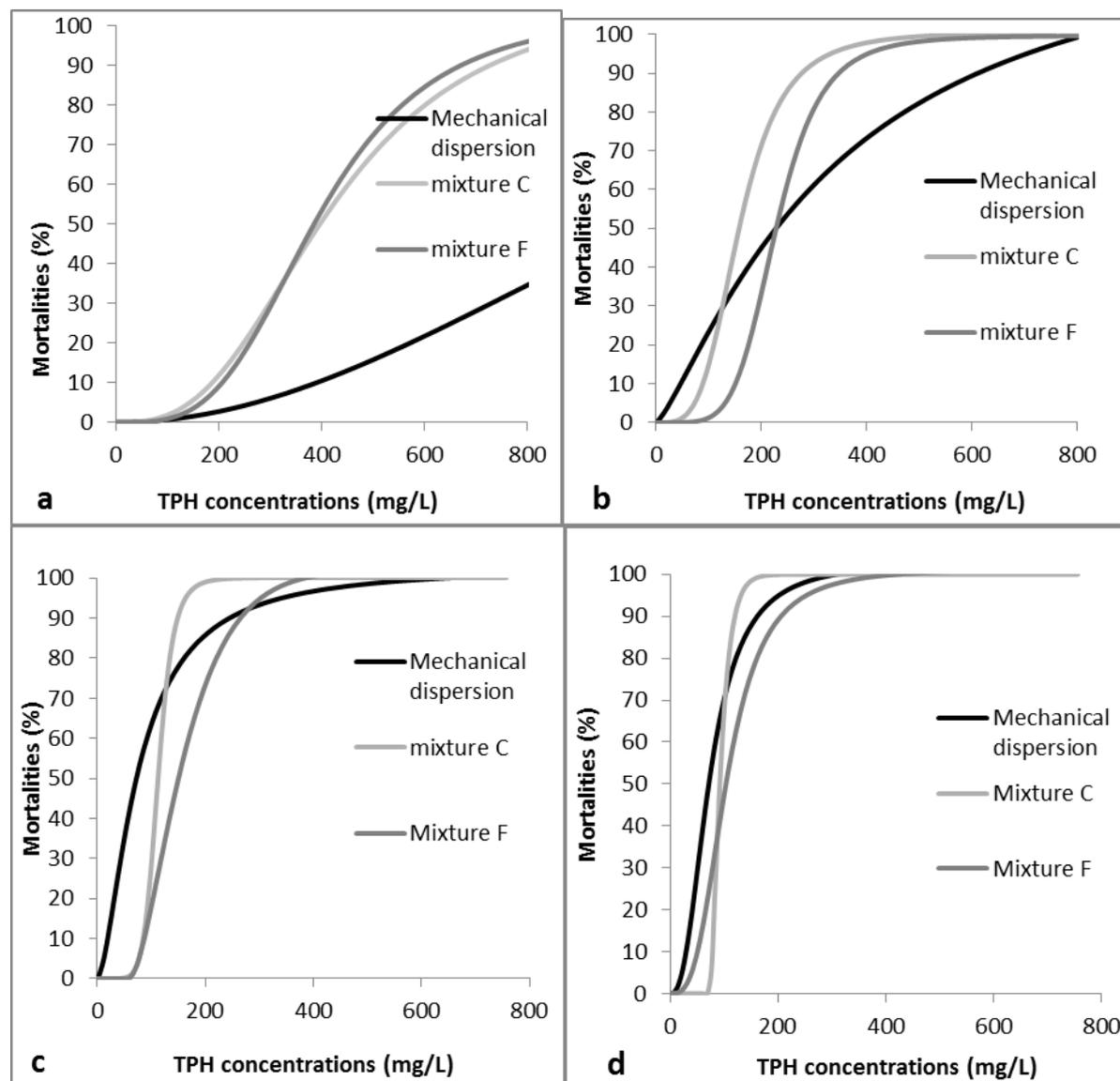
ND: not determined Median ± SEM, n= 15

No statistical differences were observed between the median of droplet distribution for the two mixtures. For the mechanical dispersion of the oil, it was impossible to reach a minimum obscuration of 10% and consequently it was not possible to determine droplet size in this condition.

### 3.3 Analysis of Fish Mortality

#### 3.3.1 Mortality Curves

Mortality curves at different time were presented in Figure 2 for the three conditions. Figure 2 a, b, c and d presented respectively results after 24, 48, 72 and 96 hours of exposure.

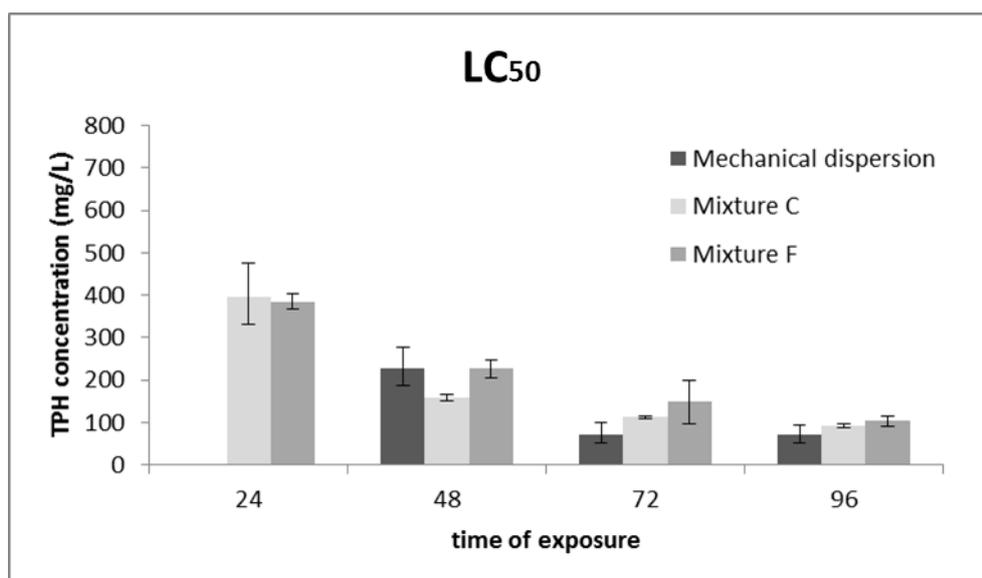


**Figure 2: Mortality curves after 24, 48, 72 and 96 hours of exposure**

After 24 hours of exposure, the curves of the two mixtures seemed similar and higher than the mechanical dispersion curve. For the 3 last times of observation, curves of the three conditions showed a similar pattern.

#### 3.3.2 Lethal Concentration (LC<sub>50</sub>, LC<sub>20</sub>)

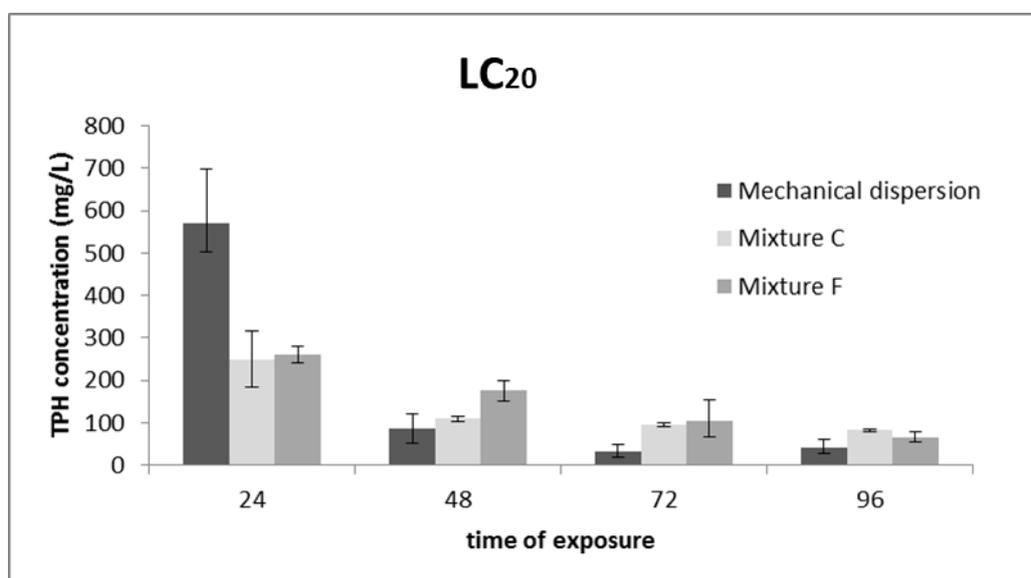
LC<sub>50</sub> and LC<sub>20</sub> values at 24, 48, 72 and 96 hours presented respectively in Figures 3 and 4 are expressed in mg/l of TPH considering the confidence interval (lower 95%-upper 95%).



**Figure 3: LC<sub>50</sub> values at 24, 48, 72 and 96 hours (mg.L<sup>-1</sup> of TPH) for the three experimental conditions.**

After 24 h of exposure, the low mortalities observed for the mechanical dispersion condition doesn't allowed the calculation of LC<sub>50</sub>. During the first 24 hours of exposure, mortalities for the two chemical dispersion of oil are higher than mortalities observed for mechanical dispersion of oil.

From 48 hours until the end of exposure, no differences of cumulated mortalities were observed between the 3 conditions. For the three conditions, there is a progressive decrease of LC<sub>50</sub> values during time.



**Figure 4: LC<sub>20</sub> values at 24, 48, 72 and 96 hours (mg.L<sup>-1</sup> of TPH) for the three experimental conditions.**

During the first 24 hours of exposure, LC<sub>20</sub> for the mechanical dispersion were higher than for the two chemical dispersion of oil. From 48 hours until the end of exposure, LC<sub>20</sub> observed between the 3 conditions were closer. For the three conditions, LC<sub>20</sub> at 72 and at 96 hours of exposure were quite similar.

## 4 Discussion

### 4.1 Comportment of Total Petroleum Hydrocarbon (TPH) in Tanks

In agreement with the work of Milinkovitch et al. (2011), TPH concentrations decreased with time in the three conditions. Oil has a hydrophobic comportment, making difficult to keep stable concentrations of TPH during few days. The dispersant added to the oil requirements will limit the oil adhesion by stabilizing the oil droplets. On the contrary, for mechanical dispersion, the particles of oil will be able to more easily aggregate (Li et al., 2009). The presence of grids, the micro-porosity of materials may increase the adhesion of the particles of oil on the experimental device and have an important role in the decrease of TPH concentration in the experimental system.

In the experimental conditions applied in this study, measurements of droplet sizes of different mixtures are in agreement with efficiency measures previously conducted by *Cedre* on those two dispersants, in accordance with standard NF T 90-345 (AFNOR, 1990). The efficiency of a dispersant is measured by determining the proportion (or percentage) of oil which was dispersed in a water sample, an efficiency of 100% is equivalent to a total dispersion (REMPEC, 1998). Previous studies show that Corexit 9500 and Finasol OSR 52 efficiencies are not significantly different (75%, 77%). This is consistent with measurement of the median of droplet distribution of mixture F and mixture C.

Some studies performed on the chemical dispersion of oil have shown that the use of dispersant decrease the size of oil droplets (Li and Garrett, 1998) and increase the bioavailability (Ramachandran et al., 2004). So even it was impossible in this study to determine droplet size for mechanical dispersion, we can assume that the droplet size in the case of the mechanical dispersion were higher than droplets from the two mixtures.

### 4.2 Analysis of Fish Mortality

Results obtained during this acute toxicity experiment showed that chemical dispersion increased the oil toxicity during the first 24 hours. Those results were in accordance with a study performed, on sheephead minnow *Cyprinodon variegatus* (Adams et al., 1999), and with the study of Ramachandran et al. (2004) which determined that oil toxicity was increased by chemical dispersion especially on sensitive life stages such as eggs and larvae.

In this study, LC<sub>50</sub> at 24 hours for the mechanical dispersion of oil were too high to be measured. LC<sub>50</sub> and LC<sub>20</sub> at 24 hours for the mechanical dispersion were higher, i.e. less toxic, than for the two mixtures. However, after the first 24hours of exposure, no difference of LC<sub>50</sub>, LC<sub>20</sub>, and mortality curves were observed between the three experimental conditions. In the context of this experimentation, it seems that the use of chemical dispersants doesn't increase mortalities over a 96 hours period and therefore the impact of mixture F and C on juvenile sea bass are not higher than for mechanical dispersion of oil. So, in this study, mortalities do not seem to be caused by dispersants but rather by oil. However it would be interesting to compare the toxicity of dispersants alone to the toxicity of the two mixtures used here, to confirm their low impact at the concentrations used in oil-dispersant mixtures.

## 5 Conclusion

The results obtained from the search of lethal concentrations showed that chemical dispersion increases the oil toxicity, but only in the first 24 hours. No difference of fish mortalities can be observed over a 96 hours period between chemical and mechanical dispersion of oil. Physico-chemical parameters such as droplet size of oil don't allow to explain the differences in acute toxicity between the three oil dispersions during the first 24 hours of exposure.

Additional information may also be provided on the toxicity induced by dispersants alone.

## 6 References

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