Tool for the Impact Assessment of Chemicals on Marine Organisms

Matthieu Dussauze [1,2], Stéphane Le Floch [1], François-Xavier Merlin [1], Michael Theron [2] and Karine Pichavant-Rafini [2]

[1] *Cedre*, Centre de Documentation, de Recherche et d'Expérimentations sur les pollutions accidentelles des eaux, 715, rue Alain Colas, CS 41836, F-29218 Brest Cedex 2, France.

[2] ORPHY (EA-4324), Université de Bretagne Occidentale, UFR Sciences et Techniques, 6 Avenue Le Gorgeu, Brest, 29285-Cedex 3, France

1. Introduction

Marine pollutions are a major concern for environmental protection; they not only directly impact the marine ecosystems but also the human populations relying on marine resources (Worm *et al.*, 2006; Garcia and Rosenberg 2010). Marine environments are particularly susceptible to pollution by petroleum hydrocarbons, and as far back as in the 1970[s] it was estimated that 6 to 7 million tons of hydrocarbons were introduced in marine ecosystems every year (Clark and MacLeod, 1977).

Chemical dispersion of oil is the main technique available to cope with offshore oil spill incidents resulting from the oil industrial activities. Chemical dispersion is considered as an appropriate response option offshore, where dilution conditions allow dispersed oil concentrations to decrease rapidly under environmental harmful levels. Nevertheless, dispersant use is a controversial issue and can be viewed as adding a new contaminant in the environment.

The dispersion process aims at keeping at sea the oil as a plume of dispersed oil, in order to avoid the shoreline contamination, to limit the risk of contamination to the surface occupying organisms (e.g. seabirds, marine mammals) and to enhance the oil biodegradation

(Tiehm, 1994; Churchill *et al*., 1995). The chemical dispersion is fully legitimated since the Net Environmental Benefits Analysis (NEBA) allow considering that dispersed oil is less harmful for the environment than an oil slick at the sea surface or on a sensitive shore line (Lunel *et al*., 1997). So far, this technique had mainly been used on many incidents of limited size; for example in the case of the Sea Empress incident, in 1995, 440 t of dispersant have been released at sea (Chapman *et al*., 2007), but the recent release of oil from the Macondo well into the Gulf of Mexico, changed the situation. This evolution increases the need for standardized and reproducible eco-toxicological tests allowing assessing the effects of chemically dispersed oil.

This study aims at evaluating the efficiency of a new tool: the ecotoxicological bench for the analysis of chemically dispersed oil effects on marine organisms. The eco-toxicological bench has been designed by the Cedre for the assessment of chemical substances on aquatic organisms. It is used here to establish the LC_{50} of one oil dispersed with two different commercial formulations of dispersants on juvenile sea bass (*Dicentrarchus labrax*).

2. Material and methods

2-1 Animals

Juveniles seabass *Dicentrarchus labrax* (mass: 0.6 ± 0.18g) were purchased from a local hatchery Aquastream (Ploemeur, France). They were acclimatised three weeks in a 300 litres tank were sterilized and filtered seawater was continuously renewed.. pH (7.93 ± 0.10), oxygen saturation (upper than 90%), and temperature (15.8 ± 0.1°C) were measured daily. Seabass 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%, moisture 10%)..

2-2 Chemicals

The petroleum oil used in the study was a weathered Brut Arabian Light (BAL) previously used in other studies (Danion *et al*., 2011; Milinkovitch *et al*., 2011). The BAL is composed of 54% saturated hydrocarbons, 10% polar compounds and 36% aromatic hydrocarbons. 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 BAL contains 54% of saturated hydrocarbons, 34% of aromatic hydrocarbons and 12% of polar compounds.

Two commercial formulations of 3rd generation dispersants were used in this work. They are both oil-based dispersants composed of surfactants (anionic and non-ionic type surface active agents) and solvents. The oil dispersed by dispersant A was called mixture A and the oil dispersed by dispersant B was called mixture B.

2-3 Experimental system

- Ecotoxicological Bench

The ecotoxicological bench (Figure 1) is composed of 12 exposure tanks and 12 depuration tanks. The ecotoxicological bench is located in thermostated room (1 to 30°C), and connected to an air extractor. Each exposure tanks is connected to water and gas supplies (Figure 2), that allow to perform static, semi-static or flow-through tests.

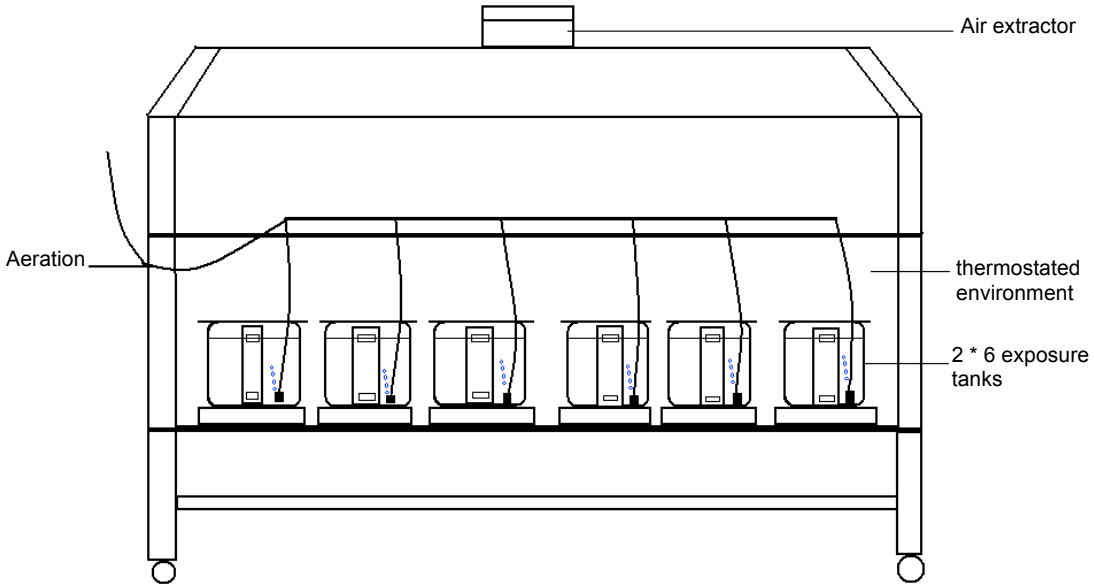

Figure 1: Side view of the ecotoxicological bench, showing the exposure tanks and the gaz inlet and outlet.

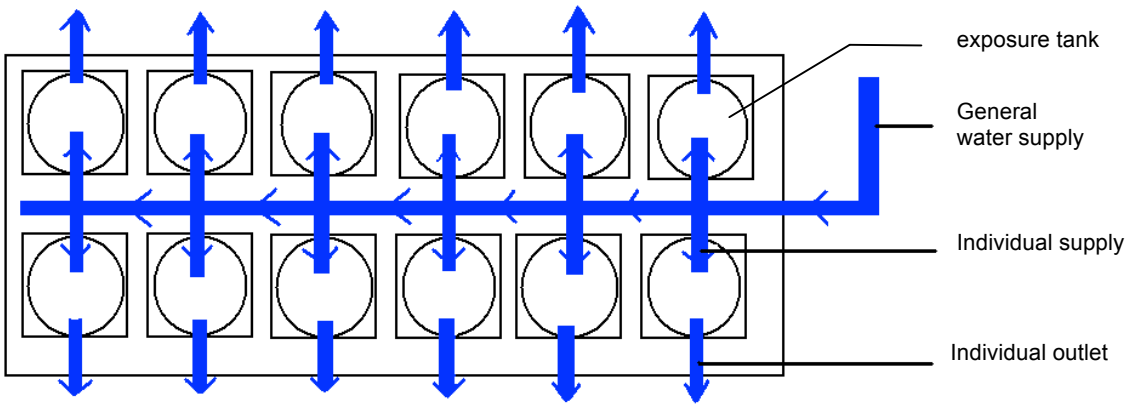

Figure 2: Upper view of the ecotoxicology bench, showing the water supply and outlet.

- **Exposure tank**

The glass tanks have a volume of 16 liters. Each tank is equipped with a magnetic stirrer for homogenization of the chemicals to be tested in the water column. A vertical PVC tube with screened openings on the top and the bottom is placed in the center of each tank. A magnetic rod positioned in the center of the tube generates the water flow in the tank (Figure 3),

drawing the exposure solutions in through the upper apertures and transferring them into the bottom of the tank. All exposure tanks were equipped of an aeration system. A tap on the bottom of tanks allows water sampling.

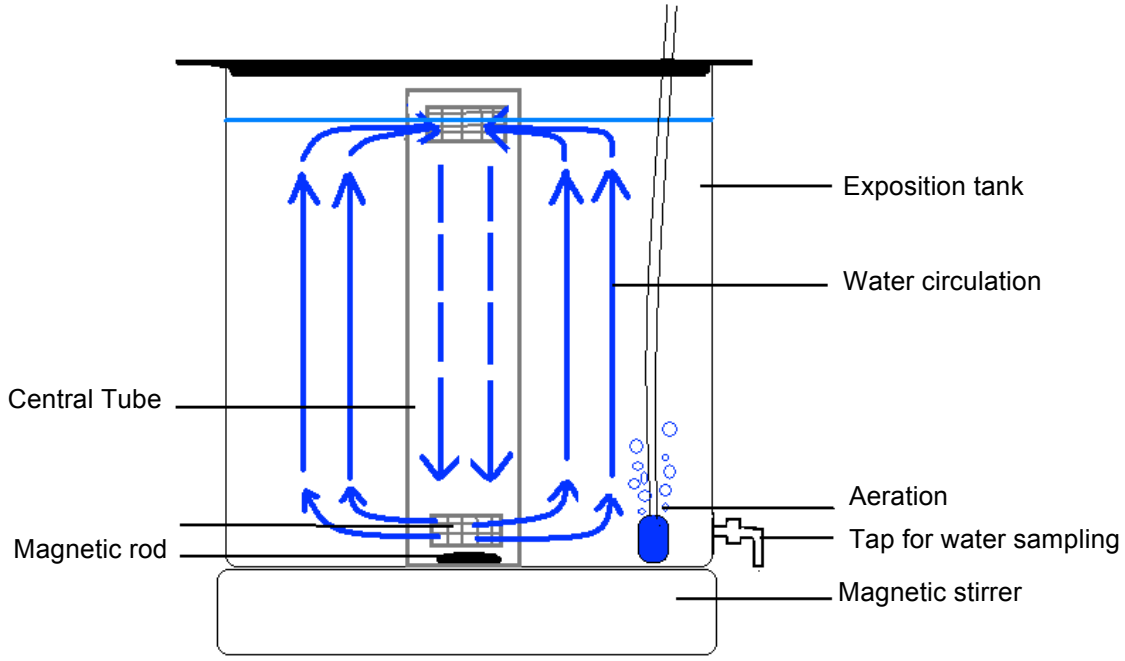

Figure 3: Exposure tank and water circulation

2-4 Experimental conditions

The measurements were carried out on a period of 24 hours. Tests were conducted in static condition. The weight ratio of oil / dispersant was 95 / 5. The fish were placed in tanks 6 hours after the release of oil/dispersant mixture. This delay allows the stabilization of hydrocarbon concentrations in the tanks. 16 fish were tested per tanks. Each concentration was tested in duplicate (n=2x16 fishes per condition). The total weight of the fishes did not exceed one gram per liter of water. The two experiments were performed at 16 ° C. Fish were considered dead when no gill movement and no response to a caudal pinch were observed.

- Total petroleum hydrocarbon (TPH) seawater concentrations

The total petroleum hydrocarbons concentration (THC), corresponding to the combination of dissolved hydrocarbons and oil droplets, was measured at the beginning and at the end of the fish exposure (two measures in each tank) . Water samples of 30 ml were extracted third times with 10 mL of Pestipur quality dichloromethane (Carlo Erba Reactifs, 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 (UVeVis spectrophometer, Unicam, France). THC concentrations in water samples contaminated with mixtures A and B were calculated from two different calibrations curves including 5% of the corresponding dispersant.

2-5 Statistical analysis

At initial time, and for each nominal oil concentration, statistical differences between measured concentrations from mixtures A and B were assessed with Mann and Whitney tests using Statistica (Version 10.0, Statsoft). LC_{50} were calculated using the trimmed Spearman-Karber method with a probit program of the USEPA, and expressed as mg/L and given with their confidence interval.

3. Results and discussion

Throughout the experiment, environmental measured parameters remained constant: oxygen saturation was higher than 90%, temperature was 16°C and pH 8.2. 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.

Table 1: Measurement of Total Petroleum Hydrocarbon (THC) concentration (mg/L) in mixture A and B at the beginning and the end of exposure.

	Mixture A		Mixture B	
	Initial time	Final time	Initial time	Final time
[THC] nominal (mg/l)	[THC] (mg/l)	[THC] (mg/l)	[THC] (mg/l)	[THC] (mg/l)
0	0	0	0	0
188	162 ± 16	101 ± 7	152 ± 7	68 ± 10
313	287± 19	217 ± 12	285 ± 3	191 ± 6
500	-	-	453 ±27	254 ± 36
625	667 ± 52	613 ± 17	570 ± 15	474 ± 13
938	774 ± 27	712 ± 20	899 ± 12	824 ± 38
1250	1135 ± 27	1019 ± 28	-	-

No statistical difference could be observed between measured concentrations from mixtures A and B at initial time for each nominal oil concentration (Mann and Whitney tests).

As shown in table 1, oil measured concentrations at time 0 are closed to nominal concentrations. Moreover, for each nominal concentration no significant difference was observed between mixture A and mixture B measured oil concentration. These results show that our experimental setup warrants duplicable oil-concentrations closed to those expected. Obviously, a decrease in oil was observed throughout the exposure time for the two mixtures. Therefore, LC_{50} was determined using measured concentrations at time 24 hours as recommended by the OECD test guideline n°203.

Table 2: Mortality (%) of fish and Total Petroleum Hydrocarbon (THC) concentration (mg/L) for mixture A and B at the end of the 24 hours exposures (± standard error mean).

Mixture A		Mixture B	
[THC] (mg/l)	**Mortalities (%)**	**[THC] (mg/l)**	**Mortalities (%)**
0	0	0	0
101 ± 7	0	68 ± 10	9
217 ± 12	9	191 ± 6	59
613 ± 17	88	254 ± 36	94
712 ± 20	97	474 ± 13	100
1019 ± 28	100	824 ± 38	100

Table 3: LC_{50} values at 24 hours are expressed in mg/l of THC considering the confidence interval (lower 95% -upper 95%).

	Mixture A	**Mixture B**
Spearman-karber estimates : LC_{50} at 24h :	**468 mg/l**	**247 mg/l**
95% lower confidence :	**423 mg/l**	**220 mg/l**
95% upper confidence :	**528 mg/l**	**277 mg/l**

Observed mortalities after 24 hours exposure of juvenile‘s sea bass to the tested concentrations of the two mixtures are presented on table 2. For the same nominal concentration, mortality was higher in presence of mixture A compared to mixture B leading to significant difference in determined LC_{50} (table 3). As used oil was the same for the two mixtures, these results suggest that the dispersant could play an important role in the toxicity of the mixture. Therefore, the differences observed in terms of toxicity between the two mixtures tested could be linked to the action mechanism of dispersant. Indeed, when dispersants were used, oil droplets were formed. Their size distribution depends not only on composition and viscosity of oil, mixing energy but also on dispersant efficiency. Previous study has shown that depending on their size these oil droplets could attach to the gill surface of fish and thereby cause enhanced uptake of polyaromatics hydrocarbons (Ramachandran *et al*., 2004). Moreover, oil droplets could be ingested directly or indirectly through

contaminated food items by fish larvae. Therefore, it could be hypothesized that oil droplet size could be an important parameter implicated in dispersed oil toxicity. Further investigations aimed at measuring droplet size could bring important information.

4. Conclusion

In these experiments, dispersed oil toxicity has been successfully tested. Interestingly, difference in LC_{50} has been observed between the two chemical dispersed-oil tested. Moreover, these experiments allowed us to test a new and innovative experimental tool the "ecotoxicological bench". It permits to obtain duplicable and expected oil measured concentrations. Moreover, environmental parameters such as temperature, oxygen concentration, pH, salinity, and photoperiod are controlled and therefore could be adapted to each used animal species. In conclusion, this "ecotoxicological bench" is an interesting experimental tool providing standard and parameterized data on impact of chemicals on aquatic organisms.

5. Bibliography

Chapman H., Purnell K., Law R.J., Kirby M.F., 2007, The use of chemical dispersants to combat oil spills at sea: A review of practice and research needs in Europe. Marine Pollution Bulletin, 54, 827-838.

Churchill, P.F., Dudley, R.J., Churchill, S.A., 1995. Surfactant-enhanced bioremediation. Waste Management. 15, 371-377.

Clark R.C., MacLeod W.D. 1977. Inputs, transport mechanisms and observed concentrations of petroleum on arctic and subarctic marine environments and organisms. *In* Nature and Fate of Petroleum, Malins D.C. ed, Academic Press N.Y., 91-224.

Danion, M., Le Floch, S., Kanan, R., Lamour, F., Quentel, D. 2011. Effects of an in vivo chronic exposure to hydrocarbons on sanitary status and immune system in seabass (*Dicentrarchus labrax*.L). Aquatic Toxicology. 105, 300-311

Garcia S.M., Rosenberg A.A. 2010, Food security and marine capture fisheries: characteristics, trends, drivers and future perspectives. Philosophical Transactions of the Royal Society of London Series B Biological Sciences. 365(1554): 28 69-80.

Lunel T, Rusin J, Bailey N, Halliwell C, Davies L, 1997. The net environmental benefit of a successful dispersant operation at the *Sea Empress* incident. In Proceedings of the 1997 International oil Spill Conference API Washington, DC, 184-194.

Milinkovitch, T., Kanan, R., Thomas-Guyon, H., Le Floch, S., 2011. Effects of dispersed oil exposure on the bioaccumulation of polycyclic aromatic hydrocarbons and the mortality of juvenile *Liza ramada*. Science of the Total Environment. 409, 1643-1650

Ramachandran SD, Hodson PV, Khan CW, Lee K, 2004. Oil dispersant increases PAH uptake by fish exposed to crude oil. Ecotoxicol. Environmental Safety. 59, 300-308.

Tiehm, A., 1994. Degradation of polycyclic aromatic hydrocarbons in the presence of synthetic surfactants. Applied and Environmental Microbiology. 60, 258-263.

Worm B., Barbier E.B., Beaumont N., Duffy J.E., Folke C., Halpern B.S., Jackson J.B., Lotze H.K., Micheli F., Palumbi S.R., Sala E., Selkoe K.A., Stachowicz J.J., Watson R., 2006, Impacts of biodiversity loss on ocean ecosystem services. Science, 314 (5800): 787-90.