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► To cite this version:

Thomas Milinkovitch, Thomas Milinkovitch, Rami Kanan, H el ene Thomas-Guyon, St ephane Le Floch. Effects of dispersed oil exposure on the bioaccumulation of polycyclic aromatic hydrocarbons and the mortality of juvenile *Liza ramada*. *Science of the Total Environment*, 2011, 409 (9), pp.1643-1650. 10.1016/j.scitotenv.2011.01.009 . hal-00647546

HAL Id: hal-00647546

<https://hal.science/hal-00647546v1>

Submitted on 2 Dec 2011

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

3

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23 **Abstract**

24

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

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

45

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

48

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.

73 In an attempt to do so, past studies have evaluated the acute toxicity of single dispersants
74 (Perkins et al., 1973; Thompson and Wu, 1981; Law, 1995; Adams et al., 1999; George-Ares
75 and Clark, 2000). More recent studies have taken into consideration the toxicity of the
76 petroleum-dispersant interaction (Epstein et al., 2000; Long and Holdway, 2002; Lin et al.,
77 2009) using chemical enhanced water accommodated fractions (CEWAF, Singer et al., 2000)
78 as contamination solutions. These exposure solutions do not take into account most of the
79 particulate oil formed during the dispersion of an oil slick.

80 However, the dispersion of oil provokes the presence of oil droplets, which have been
81 suggested to be a determinant of toxicity (Ramachandran et al., 2004a and Brannon et al.,
82 2006), and does so even more in nearshore areas, where natural dispersion (e.g. waves) can
83 send the whole oil slick from the surface into the water column (as described during the Braer
84 oil spill by Lunel et al., 1995). Thus, in order to simulate actual exposure to dispersed oil in
85 nearshore areas, an experimental approach was designed. This approach, similar to
86 Milinkovitch et al. (2011), considers the presence of oil droplets in the water column.

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

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

107

108

109 **2. Materials and methods**

110

111 **2.1 Materials and experimental organisms**

112

113 **2.1.1. Experimental system**

114

115 The experimental system (**Figure 1**) used in this study was adapted from Blackman et al.
116 (1978), and is composed of 12 experimental tanks (units) covered by a lid. Each tank is a 22 L
117 cylinder fitted with a removable central column (77 mm in diameter), that houses a stainless
118 steel shaft and a three-bladed propeller. The central cylinder has two sets of two apertures,
119 located at the top and the bottom. The apertures are covered with a metallic mesh screen to
120 exclude test animals from the propeller housing. The propeller (30 × 25 mm) is rotated (1000
121 rounds per minute) to produce a small vortex within the central cylinder, drawing the
122 exposure solutions in through the upper apertures and expelling them through the lower ones.

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

127

128 **2.1.2 Chemicals**

129

130 A Brut Arabian Light (BAL) oil was selected for this study and the composition of the oil was
131 evaluated by CEDRE (CEntre de Documentation de Recherche et d'Expérimentations sur les
132 pollutions accidentelles des eaux). The oil was found to contain 54% saturated hydrocarbons,
133 36% aromatic hydrocarbons, and 10% polar compounds. Concentrations of 21 PAHs
134 (including the 16 priority PAHs listed by US-EPA) in the Brut Arabian Light oil are presented
135 in Table 2. Before performing the exposure studies, the oil was weathered by bubbling air
136 through the petroleum in 3 m³ tanks, for 8 days in open air, at a temperature of 12 to 16 °C.
137 This aeration protocol results in a 7 % petroleum weight loss. This, corresponds to the
138 petroleum weight loss occurring in 12 h on a 1 mm oil slick released at sea (personal
139 communication, S. Le Floch). Using this weathered oil, our study simulates a 12 h period of
140 petroleum ageing, i.e. the time it might take for responders to apply dispersant. The
141 composition of the weathered test oil was 54% saturated hydrocarbons, 34% aromatic
142 hydrocarbons and 12% polar compounds and its API (American Petroleum Institute) gravity
143 was 33. Concentrations of 21 PAH (including the 16 US-EPA PAH) are presented in Table 2.
144 Two formulations of dispersants (1 and 2), manufactured by Total Fluides and Innospech,
145 were selected. Both were evaluated by CEDRE and were deemed effective enough
146 (determined using the method, NF.T.90-345) for use in marine environments, non-toxic at
147 concentrations recommended by the manufacturer (determined using a standard toxicity test:

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

154

155 **2.1.3 Experimental Organisms**

156

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

163

164 **2.2 Exposure Methods**

165

166 **2.2.1 Preparation of exposure media**

167

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

173 Practically, 95 g of BAL (Brut Arabian Light) oil was weighed and gently spread out over 20
174 L seawater to simulate an oil slick. Then, the solution was stirred using a magnetic agitator
175 (RCT basic IKA) using the low energy method (no vortexing) for a 24 h period. Only the
176 liquid phase of the WSF of oil was used in the subsequent exposure studies. Chemically
177 dispersed oil (CD1 and CD2) stock solutions, using dispersant 1 and 2, were prepared using
178 20 L of seawater, 95 g BAL oil slick, and 5 g of dispersants 1 and 2 (following the
179 manufacturers' recommended application petroleum:dispersant ratio of 20:1). Dispersant (D1
180 and D2) stock solutions were prepared using 20 L of seawater and 5 g of dispersant. A
181 mechanically dispersed oil stock solution (MD) was prepared using 20 L of seawater and 95 g
182 BAL oil slick. CD1, CD2, D1, D2 and MD stock solutions were each stirred for 24 h using a
183 propeller mixer (RW 16 Basic IKA), fitted with the same propeller as used in the
184 experimental system. The propeller mixer speed was set higher than during exposure (1400
185 rounds per minute instead of 1000 rounds per minute) in order to avoid the formation of an oil
186 slick for the MD stock solution.

187

188 **2.2.2. Exposure conditions**

189

190 Following the 24 h period of exposure media preparation, each stock solution was diluted in
191 seawater, which was previously placed in the experimental system tanks (described in **2.1.1.**).
192 On the basis of one dilution per tank, 6 dilutions of each stock solution were made: 0%, 2.4%,
193 12%, 18%, 24%, and 40%. The final volume of each dilution was 16 L. Two exposure
194 conditions were tested simultaneously: CD1 and D1, followed by CD2 and D2, and then WSF
195 of oil and MD, chronologically. Each group of 10 organisms was exposed to one stock
196 solution dilution (16 L) for 24 h in one experimental tank. At the end of the 24 h exposure
197 period, the animals in each tank were gently transferred to a 22 L glass tank with clean

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

204

205 **2.3. Chemical analysis**

206

207 **2.3.1. Total petroleum hydrocarbon (TPH) seawater concentrations**

208

209 The TPH concentration, which is the sum of dissolved hydrocarbon concentrations plus the
210 amount of oil droplets, was measured for all exposure media in each tank, at T = 0 h, and at
211 the end of fish exposure (T = 24 h), using the mean of three replicated measurements for each
212 time point. Each sample was removed using a Teflon straw, linked to a pipette filler (VWR),
213 and stored in a 60 mL tinted glass bottle (VWR). The seawater samples were extracted with
214 10 mL of Pestipur-quality dichloromethane (Carlo Erba Reactifs, SDS). After separation of
215 the organic and aqueous phases, the seawater was extracted two additional times with the
216 same volume of dichloromethane (2×10 mL). The combined extracts were dried on
217 anhydrous sulphate and then analysed using a UV spectrophotometer (UV-Vis
218 spectrophotometer, Unicam) at 390 nm, as described by Fusey and Oudot (1976). The
219 detection limit of this method is dependent on the precision of the spectrophotometer, and
220 results are not reliable for concentrations under 1 mg/L

221

222 **2.3.2. Polycyclic aromatic hydrocarbon (PAH) seawater concentrations**

223

224 PAH concentrations were assessed in each tank, at T = 0 h and following fish exposure (T =
225 24 h), using the mean of two replicated measurements for each time point. After sampling, a
226 24 h settling phase was used to separate oil droplets and particulate matter from the seawater.
227 Then, 150 μ L of a solution of 5 perdeuterated internal standards (Naphthalene d_8 , Biphenyl
228 d_{10} , Phenanthrene d_{10} , Chrysene d_{12} , and Benzo[a]pyrene d_{12} at concentrations of 210, 110,
229 210, 40 and 40 μ g/mL, respectively in acetonitrile Sigma-Aldrich, France) were diluted in 10
230 mL of absolute methanol (Sigma-Aldrich, France), and this volume of methanol was added to
231 the liquid phase of the samples. PAH were extracted from the seawater using the stir bar
232 sorptive extraction technique (SBSE, stir bar coated with PDMS, Gerstel), and analysed by
233 thermal desorption coupled to a capillary gas chromatography–mass spectrometer (GC–MS).
234 An HP7890 series II (Hewlett Packard, Palo Alto, CA, USA) GC was used, coupled with an
235 HP5979 mass selective detector (MSD, Electronic Impact: 70eV, voltage: 2 000 V). Twenty-
236 one PAH (alkylated and parents), including the 16 PAH listed by the US-EPA and 5
237 additional PAH (benzo[*b*]thiophene, biphenyl, dibenzothiophene, benzo[*e*]pyrene, perylene),
238 were quantified according to published procedures (Roy et al., 2005). Based on the detection
239 limits of this method, accurate results at concentrations of 1ng/L were possible.

240

241 **2.3.3 Polycyclic aromatic hydrocarbon (PAH) concentrations in fish muscles and** 242 **bioaccumulation factor (BAF)**

243

244 The concentrations of 21 PAH (alkylated and parents) in the fish muscles were assessed. The
245 21 PAH represent the 16 US-EPA PAH and 5 supplementary PAH (benzo[*b*]thiophene,
246 biphenyl, dibenzothiophene, benzo[*e*]pyrene, perylene). PAH concentrations in the fish
247 muscles were determined by GC–MS, using a procedure modified from Baumard et al.

248 (1997). Fish samples were pooled according to exposure treatment (one pool per tank).
249 Although most pools contained some fish which survived the exposure experiments, pools
250 were not analysed for exposures to 40 % stock solutions, because there were no surviving fish
251 following CD1 and CD2 exposure.. The mean weight of the fish in the pools was 6.1 ± 0.7 g.
252 Prior to extraction, 150 μ L of a solution of 5 perdeuterated internal standards (Naphthalene d_8 ,
253 Biphenyl d_{10} , Phenanthrene d_{10} , Chrysene d_{12} , and Benzo[a]pyrene d_{12} at concentrations of
254 210, 110, 210, 40 and 40 μ g/mL, respectively, in acetonitrile Sigma-Aldrich, France) and
255 50 mL of an ethanolic solution of potassium hydroxide (2 mol L^{-1} , Fisher Chemicals) were
256 added to fish muscles in 250 mL flasks and placed for 3 h in a drying cupboard at 60 °C.
257 After alkaline digestion, 20 mL of demineralised water was added and samples were extracted
258 with 2×20 mL of pentane (Carlo Erba Reactifs, SDS). The resulting extract was then
259 concentrated using a Turbo Vap 500 concentrator (Zyman, Hopkinton, MA, USA, at 880
260 mbar and 50 °C) to 1 mL, purified on a silica column (5 g of silica, hydrocarbons were eluted
261 with 50 mL of pentane/dichloromethane 80/20) and concentrated to 200 μ L for analysis.
262 Aromatic compounds were analysed by GC–MS, with an approximate quantification limit of
263 $5 \mu\text{g.kg}^{-1}$ of dry weight. PAH levels were quantified relative to the 5 perdeuterated internal
264 standards introduced at the beginning of the sample preparation procedure (one per
265 aromaticity class).
266 Moreover, as described in Baussant et al. (2009), a bioaccumulation factor (BAF) was
267 calculated using the ratio of the total PAH concentration in fish muscles divided by the total
268 PAH concentration in seawater (**2.3.2.**).

269
270
271
272

273 **2.4. Statistical analysis**

274

275 All correlations were tested using Spearman's correlation test (XL Stat 5.2) and the statistical
276 significance of the results was ascertained at $\alpha = 0.05$. Differences between exposure
277 conditions (CD1, CD2, MD, D1, D2, WSF) concerning seawater PAH concentrations, muscle
278 PAH concentrations, and bioaccumulation factors were evaluated using the Quade test. For
279 the Quade test procedure, exposure conditions were defined as treatment and % of stock
280 solutions defined as blocks. Values obtained for each exposure conditions at several % of
281 stock solutions were considered as repeated measurements. The Quade test was carried out
282 using R statistical software and the statistical significance of the results was ascertained at $\alpha =$
283 0.05.

284

285

286 **3. Results**

287

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

290

291 **3.1. Total petroleum hydrocarbon (TPH) seawater concentrations and fish mortality** 292 **(Tables 3a and 3b)**

293

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

298 0.137). Because only soluble compounds are present in WSF of oil exposure media, the low
299 TPH concentrations contained in this exposure media cannot be detected using
300 spectrophotometry.

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

305 For CD1 exposure, no mortality was observed following exposure to 0% and 2.4 % of the
306 stock solution. Following exposure to 12, 18 and 24 % of the stock solution, 30 % mortality
307 was observed. Thus, no mortality increase was observed following exposure between 12 and
308 24 % of the CD1 stock solution, whereas our results show increased TPH concentrations.
309 Following exposure to 40 % of the stock solution, 100 % mortality was observed.

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

314

315 **3.2. Polycyclic aromatic hydrocarbon (PAH) seawater concentration**

316

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

319 A correlation between the sum of the concentrations of the 21 PAH (**Figure 2**) and the
320 percent dilution of the stock solution was found for CD1, CD2 and WSF of oil exposures ($P <$
321 0.05), but not for MD exposure ($P = 0.100$). The sum of the concentrations of the 21 PAH in
322 seawater revealed significant differences between exposure conditions. First, PAH

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

328

329 **3.3. Polycyclic aromatic hydrocarbon (PAH) concentrations in fish muscles and** 330 **bioaccumulation factor (BAF)**

331

332 Polycyclic aromatic hydrocarbon (PAH) concentrations in fish muscles (**Table 4**) were
333 measured to be zero following exposure to 0% of the stock solutions for all exposure media.

334 Correlations were found between PAH concentrations in fish muscles and the percent stock
335 solution dilution used for MD, WSF, CD1 and CD2 ($P < 0.05$). PAH concentrations were
336 significantly higher in chemically dispersed oil (CD1 and CD2) than in either mechanically
337 dispersed oil (MD) or WSF of oil. Even though the PAH concentrations appeared to be much
338 higher following mechanically dispersed oil exposure than WSF of oil exposure, statistical
339 analysis did not reveal any significant difference (P -value = 0.115).

340 No correlation was observed between BAF and the percent stock solution dilution, for all
341 exposure media (**Table 5**). BAF was found to be significantly higher following exposure to
342 chemically dispersed crude oil (CD1 and CD2) than WSF of oil exposure. Although BAF
343 levels appeared to be higher following MD exposure than following WSF of oil exposure, no
344 significant difference was found ($P = 0.064$). No significant difference was found between
345 MD and CD1 exposure ($P = 0.265$). The same is true of MD and CD2 exposure ($P = 0.701$).

346

347

348 **4. Discussion**

349

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

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

365

366 **4.1. Mortality, total petroleum hydrocarbon (TPH), and polycyclic aromatic**
367 **hydrocarbon (PAH) concentrations in seawater**

368

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

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

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

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

397 Regarding to fish mortality, chemically dispersed oil would be more toxic than an untreated
398 and undispersed oil slick (WSF). Indeed, no mortality was found for WSF of oil exposure
399 whereas 100 % fish mortality was observed following exposure to 40% dilutions of CD1 and
400 CD2 exposure. Moreover, mortality due to chemical dispersion of oil (CD1 and CD2) were
401 observed at lower concentrations: at 12 % of the CD1 stock solution and at 24 % of the CD2
402 stock solution. Note that mortality did not increase between 12 % and 24 % of the CD2 stock
403 solution, whereas actual TPH concentrations increased. This phenomenon is likely due to
404 contamination resistance variability between fish. For example, it is possible that some fish
405 exposed to 24 % of the CD2 stock solution were more resistant to hydrocarbon contamination
406 than other fish exposed to 12 % of the CD2 stock solution. This would result in the same
407 mortality percentage for both groups of fish, even if the exposure concentrations were
408 different. In total, these results are in agreement with many studies (Long and Holdway, 2002;
409 Pollino and Holdway, 2002; Lin et al., 2009) and suggest, as it was previously proposed by
410 Cohen and Nugegoda (2000), that when meteorological conditions permit it, recovery and
411 containment of the oil slick should be conducted since it is a less toxic response technique
412 than the application of chemical dispersants. Similarly, chemically dispersed oil seems to be
413 more toxic than mechanically dispersed oil, because no mortality was observed after fish were
414 exposed to this condition. This finding suggests that, when the oil slick is under natural
415 mixing processes, the application of chemical dispersant increases the toxicity of the
416 petroleum, probably by increasing the amount of total petroleum hydrocarbons in the water
417 column (as described above). Moreover, our study results suggest that the toxicity of
418 dispersants (D1 and D2) seems to be too low to be the major determinant of CD1 and CD2
419 exposure toxicity. This result is in accordance with Otitoluju (2005), who showed that the
420 toxicity of dispersant application is not due to dispersant toxicity alone, but rather to the
421 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

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

452

453 **4.2. Bioaccumulation factor (BAF), PAH concentrations in fish muscles**

454

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

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

472 Taken together, these results show that dispersant application increases PAH partitioning
473 from oil to water, and moreover, increases PAH bioaccumulation (at the seawater–organism
474 interface). As a result, PAH concentrations were found to be higher in the muscles of fish
475 exposed to chemically dispersed oil than in the muscles of fish exposed to the water soluble
476 fraction of oil. These results are in agreement with many studies that have revealed an
477 increase in PAH uptake due to dispersant application (Wolfe et al., 2001; Mielbrecht et al.,
478 2005).

479 Similarly, PAH concentration measurements in seawater were found to be higher following
480 MD exposure than WSF of oil exposure. Moreover, these results show that PAH
481 bioaccumulation seems to be higher following MD exposure than WSF of oil exposure ($P =$
482 0.064). Together, these results suggest that mechanical dispersion seems to increase PAH
483 partitioning from oil to water, and, in addition, increase PAH bioaccumulation (at the
484 seawater–organism interface). As a result, PAH concentrations seem to be higher in the
485 muscles of fish exposed to mechanically dispersed oil (MD) than in the muscles of fish
486 exposed to the water soluble fraction of oil (P value = 0.115).

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

495

496

497 **5. Conclusion**

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

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

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

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

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530

531 **Acknowledgements**

532

533 This study was supported by a PhD grant from the Conseil Général of the Charente-Maritime.
534 The Agence Nationale de la Recherche and especially Michel Girin and Gilbert Le Lann are
535 acknowledged for financial support from the project 'DISCOBIOL', managed by F. X.
536 Merlin. The authors also acknowledge Total Fluides and Innospech for providing chemicals.
537 Special thanks go to Gwenaël Quaintenne and Benoit Simon-Bouhet for their help and
538 assistance with statistical analysis and to Maxime Richard and Marie Czamanski for their
539 helpful assistance providing the fish.

540

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

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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 ($\mu\text{g/g}$ of petroleum)	Concentration in weathered BAL ($\mu\text{g/g}$ 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(<i>1,2,3-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_{0h}-T_{24h}) 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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% of stock solution	WSF		D1		D2	
	[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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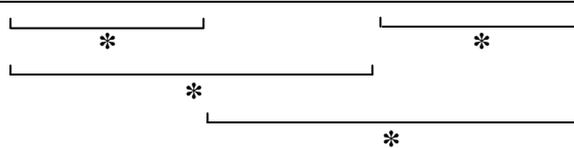
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717 **Table 4:** Concentration of the sum of 21 PAHs (alkylated and parents) in fish muscles ($\mu\text{g/g}$)
 718 for CD1 (Chemically Dispersed oil using dispersant 1), CD2 (Chemically Dispersed oil using
 719 dispersant 2), MD (Mechanically Dispersed oil) and WSF (Water Soluble Fraction of oil)
 720 exposures. Respecting Quade test procedures, values obtained for each exposure conditions at
 721 several % of stock solution are considered as repeated measure. *indicates significant
 722 differences ($P < 0.05$) of concentrations ([sum of 21 PAHs]) between exposure conditions.
 723 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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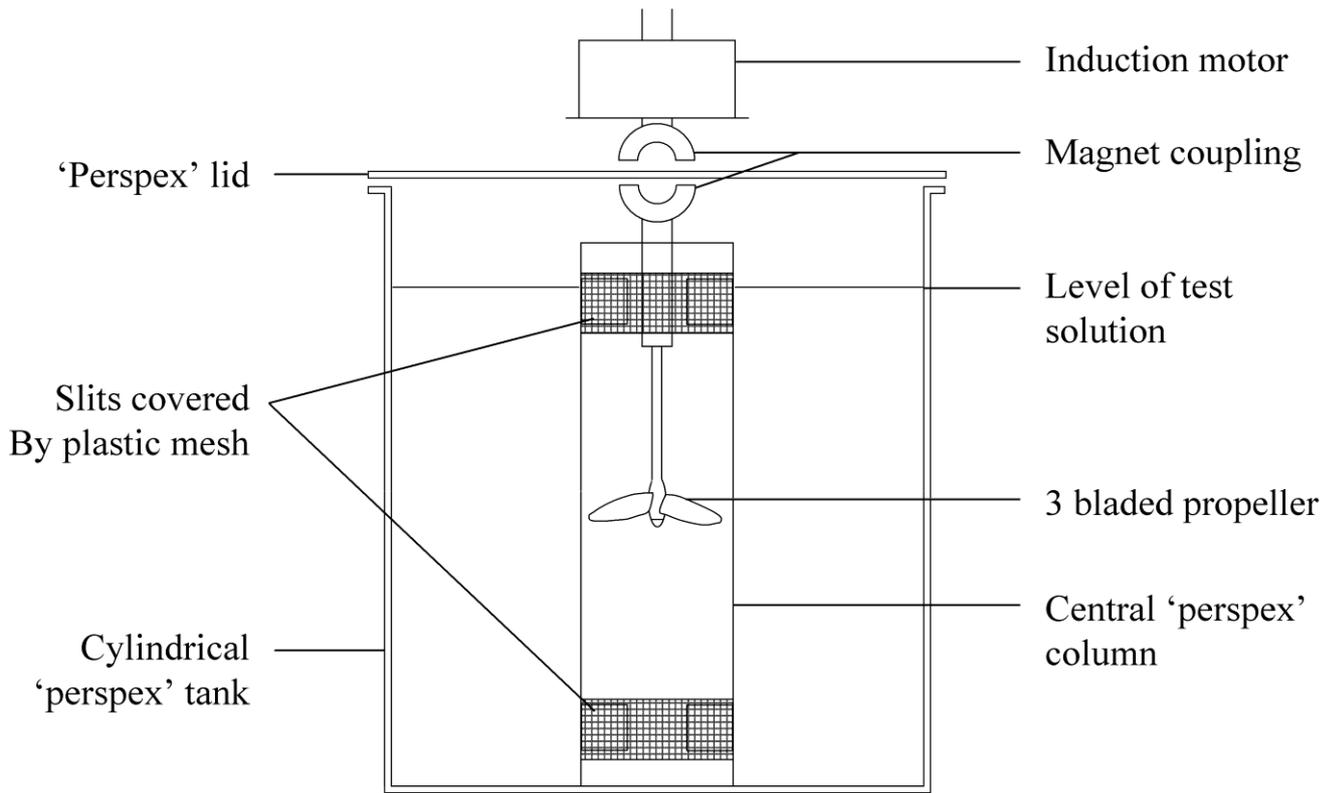
736 **Table 5:** Bioaccumulation factor (BAF = [total PAH] in fish muscle / [total PAH] in sea
737 water) for CD1 (Chemically Dispersed oil using dispersant 1), CD2 (Chemically Dispersed
738 oil using dispersant 2), MD (Mechanically Dispersed oil) and WSF (Water Soluble Fraction
739 of oil) exposures. 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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745 **Figure 1:** One tank of the experimental system (composed of twelve tanks) devised to

746 maintain a mixture of oil-dispersant droplets throughout the water column

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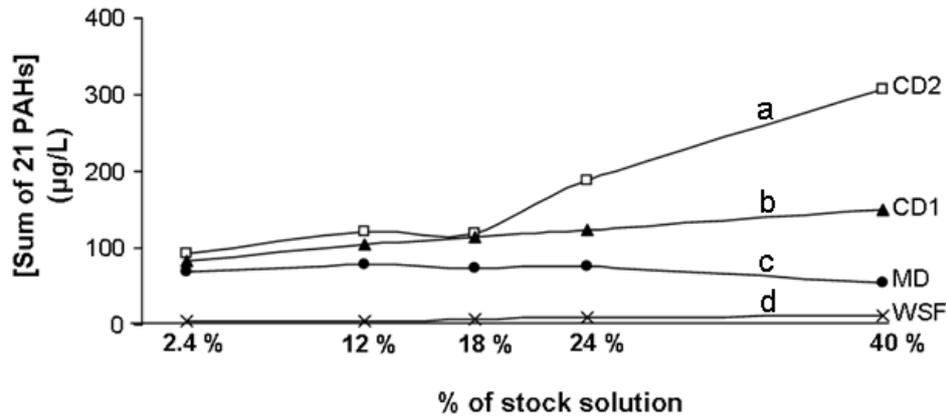
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755 **Figure 2:** Concentration of the sum of 21 PAHs (alkylated and parents) in sea water during
 756 CD1 (Chemically Dispersed oil using dispersant 1), CD2 (Chemically Dispersed oil using
 757 dispersant 2), MD (Mechanically Dispersed oil) and WSF (Water Soluble Fraction of oil).

758 The 21 PAHs represent the 16 US-EPA PAHs and five supplementary PAHs

759 (benzo[*b*]thiophene, biphenyl, dibenzothiophene, benzo[*e*]pyrene, perylene).

760 Values represent means of two measurements (at T = 0 h and at T = 24 h). To determine

761 whether the curves differed significantly, Quade test was conducted. Different letters above

762 curves indicates that curves differed significantly ($P < 0.05$)

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