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# **Biological Effects of Dispersants and Dispersed Oil on Surface and Deep Ocean Species**

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## **INTRODUCTION**

### **Effects of Dispersant Use on Biological Systems**

Beginning with the use of industrial-strength detergents, dispersing agents have been employed in spill response for decades. The Corexit series of agents in common use today generally consist of non-ionic and/or anionic surfactants in a solvent base designed to enhance miscibility under varying temperature and salinity conditions; cationic surfactants tend to be too toxic for use. While dispersants generally serve to decrease the interfacial surface tension of oil, thus facilitating its weathering under low-energy conditions, their surface-active nature also causes their interaction with cell surfaces – those of single-celled organisms as well as the gills of vertebrates and invertebrates.

### **Knowledge from Previous Oil Spills**

#### ***Biological Impacts***

Dispersant use is usually considered by spill responders when other means of response, such as containment and removal, are not deemed to be adequate<sup>1</sup>. For instance, during the Deepwater Horizon (DWH) spill dispersants were quickly employed when it became apparent that other means of response were insufficient<sup>2</sup>. However, there are usually consequences for both hydrocarbon bioavailability and toxic impacts, thus environmental tradeoffs must be evaluated. For instance, while undispersed oil generally poses the greatest threat to shorelines and surface-dwelling organisms, most dispersed oil remains in the water column where it mainly threatens pelagic and benthic organisms<sup>1</sup>. This tradeoff was a prime consideration during the DWH spill<sup>3</sup>.

#### ***Bioavailability of Oil Constituents***

Crude oil consists of hundreds of individual hydrocarbons, both aliphatic and aromatic; water solubility is directly related to temperature but inversely related to molecular mass and salinity<sup>4</sup>. Undispersed oil generates a relatively small particulate fraction, as the bulk of the hydrocarbons remain near the water surface, while dispersion results in the generation of a large particulate fraction, which forms a pelagic “cloud.” Adverse effects resulting from spilled oil can be a result of: (1) dissolved materials, (2) physical effects due to contact with oil droplets, (3) enhanced uptake of petroleum hydrocarbons through oil/organism interactions, or (4) a combination of these factors<sup>5</sup>. Both particulate (via ingestion and surface coating) and dissolved hydrocarbons can have adverse effects<sup>1</sup>, but bioavailability is generally defined as the hydrocarbon fraction available for diffusion across cell membranes (i.e. the dissolved fraction). While the intentional dispersion of an oil spill places a larger load of particulate hydrocarbons nearer to pelagic and/or benthic organisms, they are initially contained within surfactant-enclosed micelles and generally

unavailable for membrane diffusion. However, over time, the surfactant will dissolve and dissipate.

The bioavailability question in relation to oil dispersal has been addressed for many years, but much of the research has been hampered by a lack of adequate analytical support<sup>1</sup>. Early on, nominal concentrations were often used to characterize exposures, but even more recently characterizing exposure concentrations has been a formidable challenge in regards to the separation of dissolved versus particulate fractions. Recent investigations involving the use of metabolomics have demonstrated that, while traditional bioassays have shown naturally dispersed oil to be significantly more potent than chemically-dispersed oil, metabolic effects are surprisingly similar<sup>6,7</sup>. Recently, using semi-permeable membrane devices (SPMDs), it was discovered that, while chemical dispersal places more total oil in the water column, dissolved PAH fractions were very similar (Van Scoy, pers. comm., 2011).

### **Testing/Field-Monitoring Procedures**

#### ***Methods Used to Assess Impacts from Dispersants and Dispersed oil***

Many methods have been developed to assess the impacts from dispersants and dispersed oils over the years. Starting in the 1970s and continuing through the 1990s, the main focus was on Corexit 9527, as it was the primary agent stockpiled for use in the United States<sup>1</sup>. In the 1990s, the focus shifted to the newer Corexit 9500, but as available research funding declined following the Exxon Valdez spill, research efforts concurrently declined.

Early methods focused on ecological impacts and involved field studies following major spills<sup>1</sup>. Most employed commonly-used ecological tools and approaches to determine changes in populations and communities, with an emphasis on migrating offshore surface spills and their impacts on sensitive shallow nearshore water areas (coral reefs, mangroves, etc.) and shorelines (subtidal through intertidal zones). The impacts of deep water well blowouts (such as was observed with the DWH spill) on both benthic and pelagic regions have been little studied, and remain relatively unknown today.

#### ***Analytical Chemistry and Toxicity Testing of Dispersants and Dispersed oil***

For many years the standard for analysis of crude oil, dispersants and dispersed oil has been gas chromatography equipped with flame-ionization detection (FID-GC)<sup>1</sup>. In recent years mass spectrometry (GC-MS) has replaced FID due to its increased sensitivity and availability. Rapid field analysis has been routinely performed by deploying a specially-equipped fluorometer—which detects fluorescent PAHs at very low concentrations, but is not generally useful in the detection of dispersants.

Toxicity bioassays have been conducted since at least the 1970s, with early methods involving a variety of organisms, open static or serial-dilution exposure systems, and constant concentrations of either dispersants and/or dispersed oil<sup>1</sup>. The varied solubility and vapor pressures of the different hydrocarbons made control of exposure concentrations nearly impossible, thus flow-through systems were developed<sup>8</sup>; they facilitated better control of both constant and declining exposure concentrations, which can better mimic the actions of dilution in the environment<sup>9</sup>.

Due to the nearly endless number of permutations in variables modeling natural spill conditions, in more recent years a group of researchers attempted to standardize testing conditions to minimize variability between research groups and make results more directly comparable (reducing the “apples and oranges” problem). Thus, CROSERF (Chemical Response to Oil Spills: Ecological Effects Research Forum) was created. Utilizing the methods of Singer *et al.*<sup>9</sup>, CROSERF also sought to standardize a suite of marine test organisms (sensitive early life stages) and the formation of both the water-accommodated fraction of crude oil (WAF, naturally-dispersed oil) and the chemically-enhanced water accommodated fraction of crude oil (CEWAF, via Corexit 9500)<sup>10</sup>.

Early test methods involving crude oil and dispersants also reported nominal exposure concentrations, which today are no longer generally acceptable<sup>1</sup>. Bioassays now routinely employ either FID-GC or GC-MS to confirm exposure concentrations. However, due to the difficulty in separating dissolved from particulate oil, exposures are generally characterized by their total petroleum hydrocarbon content (TPH), which may result in the reporting of excessively high median-effect concentrations. Ideally, analytical methods should separately report dissolved and particulate hydrocarbons, but separation methods (centrifugation or filtering) often disturb particulates to produce an unrealistically high dissolved concentration.

## **STATE OF KNOWLEDGE FROM THE DEEPWATER HORIZON**

Several hundred water and sediment samples were collected during the DWH response from nearshore and deepwater areas. The Operation Science Advisory Team (OSAT) analyzed the bulk of these samples to characterize the risk of oil and dispersants to aquatic receptors and humans<sup>11</sup>, including samples collected through monitoring missions implemented during the response. Such missions included Special Monitoring of Applied Response Technologies (SMART) and Measurement of Concentration and Size Distribution of Surface and Subsurface Small Particles. Samples were also collected as part of the Natural Resource Damage Assessment (NRDA), but these were not available for inclusion in this synthesis.

### **Dispersant Indicators in Water and Tissue Samples**

The U.S. Environmental Protection Agency (USEPA) established analytical methods and screening levels for selected dispersant-related chemicals in water samples (Table 1). Comparisons of measured versus screening level concentrations were widely used during the response to characterize risks to aquatic receptors<sup>11</sup>. Approximately 28% (2,791 samples) of the 10,000 water samples collected for dispersant analysis were from the area with the highest concentration of dispersant application (Figure 1). In this area, propylene glycol, DPnB, and DOSS were detected in a few samples collected during the surface and sub-surface dispersant application periods (22 April-19 July 2010, and 30 April-15 July 2010, respectively), but none exceeded the recommended benchmarks. The large majority of samples collected at depths >200 m with detected dispersant indicators (89%) were from 1,025 to 1,425 m depths consistent with the location of the subsurface plume (1,000-1,500 m)<sup>12-14</sup>.

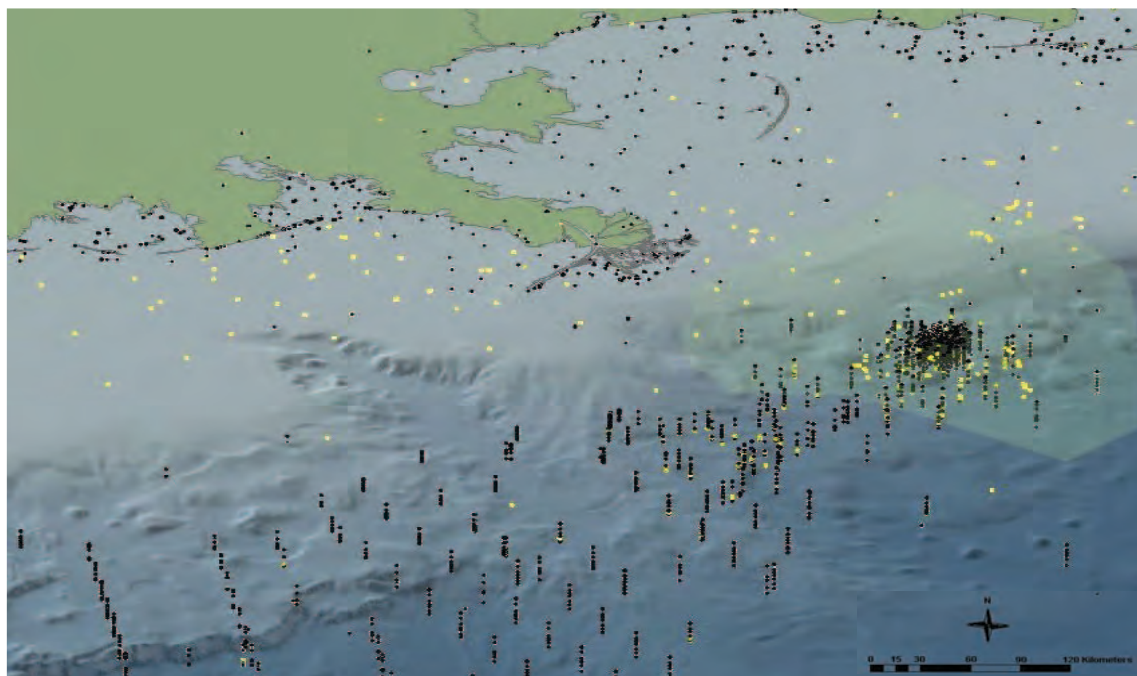
Dispersants in seafood tissues were analyzed by the Food and Drug Administration (FDA). Laboratory tests with Eastern oyster (*Crassostrea virginica*), blue crab (*Callinectes sapidus*), and red snapper (*Lutjanus campechanus*) exposed to Corexit 9500 (100 mg/L)<sup>15</sup> indicated little to no

bioconcentration potential, and depuration from tissues within 24-72 h. DOSS was detected in 4 of 299 tissue samples (concentration range: 0.011-0.1 µg/g) from seafood species collected in State and Federal waters between June and October 2010. Based on the FDA data (low tissue concentrations, low bioconcentration, fast depuration), it is unlikely that DOSS may pose a significant risk to aquatic receptors.

**Table 1.** Analytical methods and screening levels for selected dispersant-related chemicals in water samples established by the EPA in response to the Deepwater Horizon oil spill (<http://www.epa.gov/bpspill/dispersant-methods.html>).

Compound	CAS Number	EPA Method ID	Reporting Limits (µg/L)	EPA Aquatic Life Benchmark (µg/L)
Propylene Glycol	57-55-6	EPA SW 846 Modified 8270	500	500,000
2-Butoxyethanol	111-76-2	EPA R5/6 LC	125	165
Di(Propylene Glycol) Butyl Ether (DPnB)	29911-28-2	EPA R5/6 LC	1	1,000 chronic*
2-Ethylhexanol	104-76-7	EPA SW 846 Method 8260	10	NA
Diocylsulfosuccinate, sodium salt (DOSS)	577-11-7	EPA RAM-DOSS	20	360 acute 40 chronic

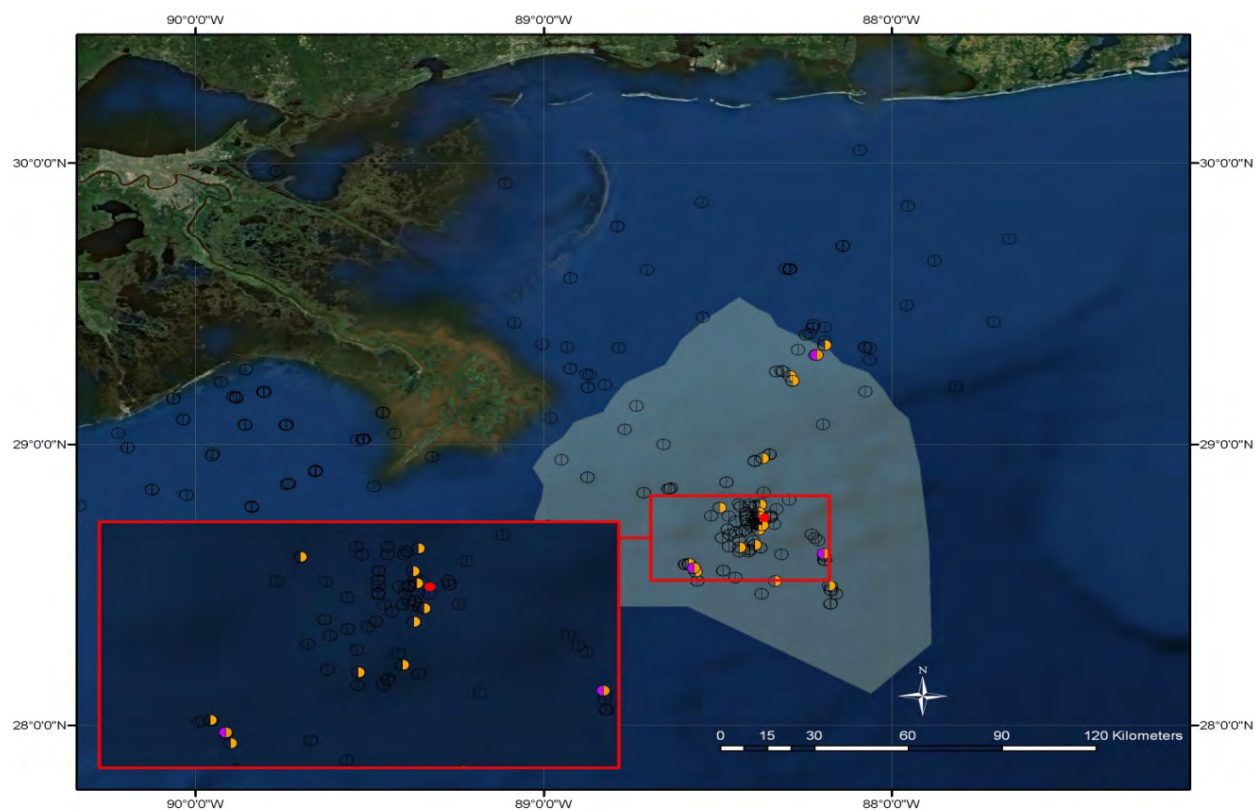
\*Chronic screening level for DPnB agreed upon by BP and EPA during the DWH response



**Figure 1.** Three-dimensional location of water samples collected for dispersant analysis. The yellow-shaded, offshore-polygon depicts the area with the highest concentration of dispersant application. Black symbols: samples with no detected dispersant-related chemicals; yellow symbols: samples with detected dispersant-related chemicals.

## Chemically Dispersed Oil

Response data<sup>11</sup> were used to characterize the potential adverse effects of dispersed oil to aquatic receptors. Several hundred water samples were collected for chemical analysis including PAH quantification<sup>i</sup>. However, not all samples for which PAHs were measured were associated with the use of dispersants. Therefore, samples with detected concentrations of one or more dispersant-related chemicals were used to screen all samples with measured PAHs concentrations. Samples with PAH composition not consistent with the source oil were not included. This screening yielded a total of 605 unique water samples. Although this criterion may have excluded chemically dispersed samples, it is impossible to distinguish natural from chemical dispersion based solely on hydrocarbon composition. Potential acute and chronic adverse effects from exposures to PAH mixtures (under the assumptions of narcosis as the mode of toxicity and PAH additively) were characterized using the Equilibrium Partitioning Benchmark Toxic Unit (TU) approach<sup>16,ii</sup> where values greater than one suggests that the PAH mixture may be unacceptable for the protection of aquatic organisms<sup>16</sup>. Using this approach, 3 and 23 of 605 samples exceeded acute and chronic benchmarks, respectively (Figure 2).



**Figure 2.** Acute (pink) and chronic (yellow) exceedances of PAH mixture benchmarks in samples with detected indicators of dispersants. The grey-shaded offshore-polygon depicts the area with the highest concentration of dispersant application. Red symbol approximate wellhead location.

<sup>i</sup> Analytical methods included among others EPA Method 8260B, EPA Method 8260C, EPA Method 8270C SIM, EPA Method 8270C, EPA Method 8270D, EPA Method 8272

<sup>ii</sup> <http://www.epa.gov/bpspill/water-benchmarks.html>

Some of the samples identified above were collected during the SMART missions (17 May-13 July 2010)<sup>17</sup>. This dataset is important because the greatest risks to aquatic receptors (primarily plankton, planktonic eggs and larval fish) are from the dispersed oil in the upper portion of the water column below the dispersing oil slick. Samples collected at 1 m depth after dispersant application exceeded acute and chronic benchmarks (5 and 13 samples, respectively) consistent with a 1 to 35 fold increase in the CEWAF concentration of PAHs compared to WAF samples. Some samples collected before and after dispersant application with acute and/or chronic exceedances had detected concentrations of linear alkane analytes with low solubility and recalcitrant characteristic (i.e., phytane, pristane) suggesting the presence of non-dissolved particulate oil (oil droplets).

Of a total of ~16,000 unfiltered water samples collected concurrently with particle size analysis measurements<sup>14</sup>, 139 exceeded acute PAH benchmarks, but most (119 samples) had detected concentrations of phytane and pristane indicative of the oil droplets. The remainder 20 samples were collected early during the response (17 May 2011) within 1 km of the wellhead. However, none of these samples had detectable concentrations of dispersant markers (propylene glycol or DPnB). An important contribution of the particle size analysis data was that most of the oil droplets (>80%) suspended in the water column (up to ~160 microns [ $\mu\text{m}$ ]) had a diameter of <70  $\mu\text{m}$ . Although it was not completely resolved whether the formation of these droplets was the result of chemical dispersion, high concentration of small particles were also observed during oil dispersion in wave tank simulations (see<sup>14</sup>). The formation of small particles and their slow rise through the water column (due to neutral buoyancy) are important drivers of exposure to aquatic receptors because particulate oil may have a different mode of toxicity (e.g., physical coating of body surfaces, gill uptake, ingestion; see below) than dissolved oil.

The assessments of the potential effects of dispersants and dispersed oil to benthic fauna proved difficult. Of the 775 sediment samples collected for dispersant analysis, only 8 had detected concentrations of dispersant-related compounds (only propylene glycol was detected)<sup>11</sup>. Only two of these samples were from offshore/deepwater areas. In light of this limited information, effects of dispersants and dispersed oil on benthic fauna represent a data gap from the DWH oil spill.

### **Toxicity Testing**

All the toxicity testing conducted during the response focused solely on assessing acute, short-term effects and did not address chronic and declining exposures to low dispersed oil concentrations, or long-term effects. The large majority of toxicity testing was performed on water samples containing dispersants only<sup>18,19</sup>, or laboratory and field collected samples with chemically dispersed oil<sup>20-23</sup>.

### ***Toxicity from Exposures to Dispersants and Chemically Dispersed Oil***

Dispersant-only tests were performed by the USEPA<sup>18,19</sup> with the eight oil dispersants listed on the USEPA National Contingency Plan (NCP) Product Schedule. These aquatic toxicity tests ranked Corexit 9500A as slightly toxic to mysids, and practically non-toxic to inland silversides<sup>18</sup>. *In vitro* tests conducted to assess the endocrine induction potential of oil dispersants<sup>19</sup>, found cytotoxicity at concentrations between 10 and 1000  $\mu\text{L/L}$ , and no

biologically significant activation of estrogenic or androgenic signaling pathways by any of the dispersants tested<sup>19</sup>. These tests found no indications of estrogenic activity for Corexit 9500A, and revealed generally low dispersant toxicity.

Toxicity tests performed by the USEPA showed that in all cases the dispersants alone were less toxic than the CEWAF, which in most cases had similar toxicity to Louisiana sweet crude oil WAF (Table 2). These tests also showed that oil dispersed with Corexit 9500A was moderately toxic to two standard test species<sup>22</sup>, less toxic than oil dispersed with Dispersit SPC 1000™, and more toxic than oil dispersed with JD 2000<sup>20</sup>. These tests also showed the low sensitivity of the marine rotifer *Brachionus plicatilis* to MC252 oil dispersed with Corexit EC9500A compared to that of the mysid and fish test species (Table 2). Although several hundred water, pore-water, and sediment samples were collected for toxicity testing (see<sup>23</sup>), the response missions guiding most sample collection were not targeted for dispersants and/or chemically dispersed oils (Table 2). Some tests with samples collected during SMART (43 out of 335 tests) showed signs of toxicity, but most of these were inconclusive<sup>21, 23</sup>. Toxicity testing performed during subsurface dispersant application operations showed little toxicity to both *B. plicatilis* (RotoxKit M™) and the marine bacteria *Vibrio fischeri* (Microtox®)<sup>23</sup>.



**Table 2.** Summary of toxicity testing with dispersants performed during Deepwater Horizon oil spill. Only currently available information was included.

Test species	Test conditions	Dispersants tested	Endpoint	Source
Juveniles (3-5 day old) mysid shrimp- <i>Americamysis bahia</i>	Continuous exposure (static, non-renewal) to CEWAF <sup>1, 2</sup>	Corexit® EC9500A	24 hr-LC <sub>50</sub> : 432 µg/L 48 hr-LC <sub>50</sub> : 186 µg/L	20
		Dispersit SPC 1000™	24 hr-LC <sub>50</sub> : 390 µg/L 48 hr-LC <sub>50</sub> : 198 µg/L	
		JD 2000	24 hr-LC <sub>50</sub> : 1,298 µg/L 48 hr-LC <sub>50</sub> : 1,012 µg/L	
Juvenile (9-14 days old) inland silverside fish- <i>Menidia beryllina</i>		Corexit® EC9500A	24 hr-LC <sub>50</sub> : 634 µg/L 48 hr-LC <sub>50</sub> : 571 µg/L	
		Dispersit SPC 1000™	24 hr-LC <sub>50</sub> : 259 µg/L 48 hr-LC <sub>50</sub> : 173 µg/L	
		JD 2000	24 hr-LC <sub>50</sub> : 4,130 µg/L 48 hr-LC <sub>50</sub> : 2,640 µg/L	
Newly hatched marine rotifer- <i>Brachionus plicatilis</i>		Corexit® EC9500A	24 hr-LC <sub>50</sub> : 9,543 µg/L	
		Dispersit SPC 1000™	24 hr-LC <sub>50</sub> : 486 µg/L	
		JD 2000	24 hr-LC <sub>50</sub> : 5,609 µg/L	
Inland silverside fish- <i>M. beryllina</i>	Continuous exposure (static, renewal) to 100%, 50%, 10% water samples collected at 1 and 10 m depths below water surface both before and after dispersant application	Corexit® EC9500A Corexit® EC9527A	No significant mortality above controls	21
Juvenile (3-5 day old) mysid shrimp- <i>A. bahia</i>			Reduced mean algal cell growth. Inconclusive results	
Marine algae- <i>Skeletonema costatum</i>				
24-48 hours old mysid shrimp- <i>A. bahia</i>	Continuous exposure (static, non-renewal) to CEWAF and WAF <sup>1, 3</sup>	Corexit® EC9500A <sup>4</sup>	48 hr-LC <sub>50</sub> -dispersant: 42 µg/L 48 hr-LC <sub>50</sub> -CEWAF: 5.4 mg/L 48 hr-LC <sub>50</sub> -WAF: 2.7 mg/L	18, 22
9-14 day old inland silverside fish- <i>M. beryllina</i>			96 hr-LC <sub>50</sub> -dispersant: 130 µg/L 96 hr-LC <sub>50</sub> -CEWAF: 7.6 mg/L 96 hr-LC <sub>50</sub> -WAF: 3.5 mg/L	

<sup>1</sup> CEWAF = chemically enhanced, water-accommodated fraction; WAF = water accommodated fraction.

<sup>2</sup> CEWAF was prepared using a Dispersant-to-Oil ratio of 1:20 with fresh MC252 oil. CEWAF was analyzed using the Modified EPA Method 8270 with endpoints reported as total petroleum concentrations. <sup>3</sup> CEWAF and WAF were prepared following CROSERF methods<sup>10</sup>. CEWAF was prepared using a Dispersant-to-Oil ratio of 1:10 with fresh Louisiana sweet crude oil (lot # WP 681). CEWAF was analyzed for total petroleum hydrocarbons (TPH) following EPA SW-846, Method 8015B-DRO with endpoints reported as TPH concentrations. <sup>4</sup> Only showing the results for one of the eight oil dispersants. These tests followed a slight modification of the USEPA Test Method 821-R-02-012

While independent research was conducted during the response to address scientific questions regarding the effects of dispersants and chemically dispersed oils on biological receptors, it is still too early to see the results of these studies in the peer-reviewed literature. To date, one study<sup>25</sup> found significant reductions in the production and viability of hydrocarbon-degrading bacteria (*Acinetobacter* and *Marinobacter*) in the presence of Corexit EC9500A at concentrations of 1-10 mg/ml. However, these concentrations were several orders of magnitude above the levels in the field. Preliminary studies have also shown the uptake of dispersed oil droplets (5 µm) in an important zooplankton species in the Gulf of Mexico, demonstrating an exposure pathway to meiobenthos (Lee in <sup>26</sup>). Others (Wetzel in <sup>26</sup>) also examined coral larval mortality and settlement success (*Porites astreoides* and *Montastraea faveolata*) following exposures to spiked and declining concentrations of CEWAF from oil dispersed with Corexit 9500, and found evidences of adverse effects warranting further studies.

Toxicological testing designed to assess the effect of dissolved (e.g., filtered) vs. particulate oil (e.g., whole water) in water, to our knowledge, were not part of the response; therefore, we were unable to analyze these types of data to infer effects to aquatic receptors. Ephemeral data collection of water samples for chemical analysis that takes into account dissolved vs. particulate oil phases are part of Natural Resource Damage Assessment (NRDA) evaluations, and these datasets may become available in coming years. Other data collected as part of the NRDA process may also include samples used to evaluate acute and chronic effect of dispersants and dispersed oil.

### **Potential Effects at a Larger Ecological Scale**

The challenges of characterizing risks from dispersants and chemically dispersed oil to potential receptors are great, particularly in such a vast area impacted by the DWH oil spill. A monumental effort, undertaken in recent years, which gathered an inventory of species (from unicellular organisms to vertebrates) of the entire Gulf of Mexico, documented at least 15,419 species belonging to over 40 phyla<sup>27</sup>. Given such high species richness, it is virtually impossible to assess the effects of dispersants and dispersed oil to most receptors. Furthermore, for most taxa, including deepwater and benthic species, substantial gaps exist in our understanding of their spatial and temporal distributions, their basic biology (rates of growth, reproduction, and recruitment) and ecology (community structure and trophic interactions), and their sensitivity to stressors. Benthic habitats in the Gulf of Mexico (mesophotic and deep water coral reefs, other hard bottoms and soft bottoms) may be the ultimate sink of oil dispersed at the wellhead, as oil particles flocculate with suspended particles or are excreted with fecal pellets and settle out of the water column. In these habitats sessile and small species with limited mobility were likely unable to escape the cloud of chemically dispersed oil, and may have experienced long term, sub-lethal effects. These communities may have also been exposed to less weathered oil than biological communities at the surface. The poorly understood behavior of dispersed oil at depth (effects of high pressure and low temperature), and the lack of understanding on the biology of deepwater species, makes it difficult to assess short- and long-term effects. In addition, potential issues associated with the collection of soft bottom samples for toxicity testing (i.e., disruption of the surface micro layer containing dispersed oil droplets), and the lack of standard deepwater test species further complicate these assessments. Information on the long-term effects of the DWH oil spill is being assessed under subject-specific NRDA technical working groups (TWGs). Funded research projects are also underway to assess the effects of dispersants, dispersant

constituents and naturally/chemically dispersed oil on Gulf of Mexico species (reef biota, deep water species, commercially important species), as well as on offshore habitats, food webs, and ecological interactions.

### ***Effect on Planktonic Food Webs***

After an oil spill, microorganisms are an important part of the degradation process but they also serve as essential members of a healthy ecosystem. Questions still remain as to how oil and dispersants affect microbial communities. Hamdan and Fulmer<sup>28</sup> have shown that, even at prescribed concentrations, the dispersant Corexit EC9500A is toxic to microbes involved in hydrocarbon bioremediation, but the levels of cell death from exposures differed among species. Widger *et al*<sup>29</sup> revealed that microbial population in water and soil samples exposed to oil and dispersant related to the DWH event showed reduced biodiversity, reduction in oxygen producing microorganisms and increased oxygen consumption by hydrocarbon metabolizing bacteria. In addition, selective degradation of hydrocarbons by different bacterial species can either increase or decrease toxic components in oil and the use of dispersant could enhance this toxicity<sup>30</sup>. These results indicate a species-specific tolerance of oil and dispersant and that the presence of hydrocarbons may enhance or reduce dispersant toxicity for some species of bacteria<sup>28</sup>. A better understanding is needed regarding the effect of oil bioremediation on microbial communities.

Although dispersed oil has been shown to negatively impact some organisms<sup>18, 20, 22, 28, 29</sup>, satellite observations of the northeastern region of the Gulf of Mexico in August 2010 revealed increased phytoplankton biomass attributed to the DWH oil spill<sup>31</sup>. It should be noted that this data is based on correlation and not direct evidence due to a scarcity of field observations before and after the spill. The region in which this phytoplankton bloom occurred overlaps with the Gulf's hypoxic zone<sup>32</sup>, leading to concerns about the impact of the oil spill and dispersant use on the Gulf's Dead Zone<sup>33</sup>. Bacterial decomposition of algae reduces oxygen and the presence of dispersed oil increases the abundance of hydrocarbon-degrading microbes which also consume oxygen, which could lead to further hypoxia. Dispersed oil may also be toxic to zooplankton grazers, resulting in increased algal blooms. However, dispersed oil could show toxicity to the algae itself, which may have a mitigating effect on hypoxia. Further research is required to fully understand how dispersed oil affects hypoxic systems.

Preliminary reports suggest that, shortly after the DWH incident, oil and dispersant constituents became entrained in the pelagic food web<sup>34, 35</sup>. Graham<sup>35</sup> showed that dispersed oil in the shallow water column has been incorporated into at least two trophic levels beyond prokaryotic hydrocarbon consumers. Dispersed oil has also been observed in blue crab larvae and researchers are finding potential signs of exposure extending throughout the water column based on the unusual appearance of planktonic organisms pulled up in nets (*unpub. data*)<sup>34, 36, 37</sup>. Contaminants from dispersed oil may result in long-term adverse effects such as carcinogenesis, impaired reproduction, shortened life-spans and decreased population numbers in planktonic organisms<sup>34, 38, 39</sup>. Additionally, exposure to contaminants found in oil and dispersants during early phases of the life cycle can lead to infertility and a host of developmental problems<sup>39-41</sup>. This is important because the area in the Gulf that was exposed to oil and dispersants included a significant portion of offshore larval and spawning grounds<sup>27, 36</sup>. However, exposure data from the DWH do not consistently reflect data from controlled laboratory experiments, which may not

accurately reproduce field conditions or exposure regimes. The ultimate long-term effects will depend on the concentration, location and persistence of dispersed oil and the duration and timing of exposure to organisms. These factors should be further tested in ecologically relevant conditions.

## **Fate of the Oil and Dispersants at Depth**

### ***Subsurface Plumes***

Little is known regarding dispersant behavior and oil droplet microstructure at the high pressures and low temperatures of the deep-sea. But, it has been documented that the treatment of oil with dispersant at the wellhead resulted in the formation of large, subsurface plumes made up of fine droplets of oil suspended in deep waters<sup>42-44</sup> (Figure 3). However, the formation of plumes is complicated due to “the interplay of gas and oil in multiphase flow, preferential solubility of each oil constituent, and potential gas hydrate formation”<sup>42</sup>. The effects of temperature and density gradients on oil droplet phases, changes in buoyancy during transport and transformations to the plume over time are poorly understood. It is uncertain exactly how many plumes existed and their exact fate is unknown, but they have the potential of persisting for months at depth<sup>42, 45</sup>. Research on plume formation and the behavior of dispersants in the deep-sea is needed to model, track and predict the fate of subsurface oil and dispersant.

### ***Dispersed Oil Byproducts at Depth***

Although the ultimate fate of petroleum hydrocarbons in deep water plumes is undetermined, Reddy *et al.*<sup>46</sup> demonstrated that most light-weight, water-soluble hydrocarbons (C<sub>1</sub>-C<sub>3</sub>) were retained in the deep water column, while insoluble fractions were deposited in sea floor sediments or transported to the surface. The retained water-soluble portions persist longer and have a much slower degradation rate than gas and n-alkane fractions<sup>46</sup>. Similarly, Kujawinski *et al.*<sup>47</sup>, quantitatively revealed the sequestration of a highly water-soluble dispersant component (DOSS) at depth undergoing minimal rates of biodegradation (Figure 4). Dispersant applied at the wellhead reduced the amount of oil reaching the surface and likely increased the retention of dissolved petroleum hydrocarbons and dispersant components in the deep-sea. Additionally, MC252 oil contained lighter molecular weight hydrocarbons than typical, which would also result in increased retention of these soluble components in deep waters<sup>34, 46</sup>. The fate of dispersed oil byproducts in deep waters is unknown at this time; research is needed regarding their use in deep waters.

### ***Oil and Dispersant in Deep Sediments***

When interacting with suspended and deposited sediments, oil droplets form oil-sediment aggregates (OSA) and dissolved oil partitions into sediments due to capillary action and surfactant ion adsorption<sup>48</sup>. Model simulations demonstrate that when oil droplets and sediment particles are small (less than 0.1 mm), more OSAs are formed<sup>48</sup>. During DWH, the subsurface injection of dispersants facilitated the formation of small particle size oil droplets<sup>14</sup>, potentially influencing the formation of OSAs. In addition studies have shown that due to the composition of the MC252 oil, conditions in the deep-sea and use of dispersants, more oil and dispersant remained at depth than predicted<sup>34, 46, 48</sup>. This has been supported with analysis of sediment samples from the sea floor which revealed the presence of oil constituents linked to MC252 oil<sup>11, 49</sup>. Sediments collected from within 3 km of the wellhead contained MC252 oil at levels

exceeding aquatic life benchmarks, but these levels returned to reference standards within 10 km of the wellhead<sup>11, 49</sup>. Careful analysis of current data and further studies are required to provide a better understanding of how oil and dispersants interact with deep marine sediments.

### ***Biodegradation***

Due to the depth of the leak and difficulty in obtaining consistent samples, uncertainty and controversy surround the actual amount of microbial biodegradation of dispersed oil from the DWH spill. One study questioned the magnitude of the microbe-directed biodegradation of hydrocarbons in the plumes and concluded that the oil/dispersant plume may have persisted for months without substantial attenuation<sup>42</sup>. Other research has suggested that a variety of hydrocarbon-degrading microbial populations in the deep-sea responded to oil contamination by undergoing rapid dynamic adaptation and that this implies an inherent bioremediation of oil contaminants in the deep-sea<sup>50</sup>. The research of Kessler *et al.*<sup>51</sup> reports that aerobic methanotrophic bacterial communities consumed a significant portion of the total hydrocarbon discharge over several months. Finally, a separate study found that the plume closest to the wellhead with the highest levels of hydrocarbons showed the least evidence of biodegradation<sup>52</sup>. Yet, the authors predict attenuation of the plumes over time due to highly fluctuating cycles of microbial communities influenced by persistent mixing of bacteria species, oxygen and hydrocarbons with background waters. This lack of certainty regarding the extent of biodegradation by microbes in deep-sea plumes is enhanced by the lack of knowledge regarding the effects of dispersant and dispersed oil on deep-sea bacteria. More research is required to understand the impact on oil degrading bacteria when dispersants are applied at depth.

## **DATA GAPS ON THE EFFECTS OF DISPERSANT USE**

Prior to the DWH, many studies were done on the toxicity of dispersants (primarily Corexit 9527 and 9500) and dispersed oil (<sup>1, 53</sup> and references therein; <sup>54-56</sup>). Although studies have filled critical data gaps in the knowledge and understanding on the effects of dispersants (for example<sup>57, 58</sup>), the experience from the DWH clearly showed that many of the data gaps identified earlier<sup>1, 53</sup> still persist. In this section we build upon the NRC recommendations for additional studies based on the state of knowledge prior and after the DWH. However, an independent effort should focus on reviewing and evaluating knowledge gaps and gains from past spills (controlled or accidental) involving the use of dispersants.

### **General Data Gaps**

Significant advances in the understanding of dispersant efficacy have been gained since the recommendations of the NRC and subsequent reports. However, all the recommendations regarding fate and effects are still relevant. Specific data gaps include: photo-enhanced toxicity; relative contribution of dissolved and particulate oil phases to toxicity; interaction of dispersed oil with sediment particles and effects to benthic fauna; tests with representative species, sensitive species and different life stages; toxicity tests that addresses delayed effects; exposures through different routes; toxicity from pathways other than narcosis (e.g., oxidative products, receptor-mediated pathways associated dissolved fractions, and smothering by oil droplets); and long-term effects on population and communities.

Comparing oil/dispersant toxicity across studies can be a challenge. Not only the preparation of WAFs and CEWAFs has differed over the years (e.g., differences in mixing energies, settling times, media treatments- filtered vs. unfiltered), but also have exposure conditions (static vs. flow-thru, closed vs. open systems, constant vs. spiked), and chemical analysis of exposure media (nominal vs. measured, particulates vs. dissolved phases, TPAH vs. TPH). Consequently, making comparative use of the existing toxicity data is almost impossible. Efforts should continue to support standardization methods and procedures (e.g., CROSERF or similar) that would allow greater comparability and reproducibility of toxicological data, and a more certain use of experimental data as scientific decision tools in future spills.

Toxicity testing under constant exposures (e.g., LC<sub>50</sub> tests) does not realistically and adequately assess the risk to aquatic receptors. Under field conditions, organisms are likely exposed to multiple stressors at any given time, which could result in additive, synergistic, or antagonistic effects. But dynamic environments are expected to dilute and mix the water column, resulting in rapidly declining exposure concentrations. However, constant exposures tests may serve as conservative estimates of toxicity. The traditional constant exposure durations in standard LC<sub>50</sub> (48 or 96 hours) tests should be compared to the much shorter (a few hours) and rapidly declining exposures experienced by marine organisms when oil is dispersed in open waters.

Analyses of biological effects following an oil spill have not typically focused on the effects from chronic exposures to extremely low concentrations, or have explored the potential of changes in behavioral responses (e.g., olfactory, time-response to stimuli) as indicators of exposure. These endpoints are relevant as these can lead to measurable effects at the population and community levels (e.g., increased predation; subtle changes in trophic structure and links), and should be considered in future spills.

Although chemical analyses used in spill response typically follow recommended protocols, standardization of such techniques throughout the response should be considered. Standardization of such procedures extends to the separation of dissolved vs. particulate oil phases, the use of chemical signatures, analysis of a whole suite of PAHs (beyond the 16 priority PAHs), as well as analysis of TPHs, and dispersant indicators. Efforts should also discuss acceptable method detection limits.

### **Data Gaps from the DWH**

Temporal and spatial sampling intensity throughout the duration of the spill response should be considered when evaluating and interpreting short and long-term effects to aquatic receptors. Although several thousand samples were collected for the detection and characterization of oil constituents, sampling efforts specific to dispersants and dispersed oil were limited, and varied substantially over space and time.

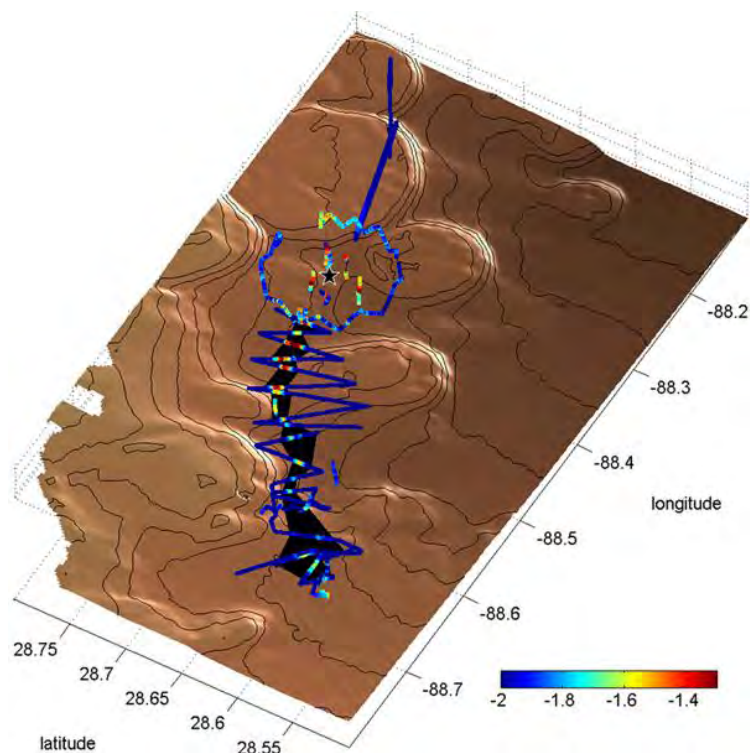
The effects of low temperature and high pressures on both physically and chemically dispersed oil and dispersants are not well understood, and therefore their fate and effects in deep waters constitute a significant data gap. Although much information was gained from the DWH on the effect of dispersants on droplet size distribution at depth, future studies should focus on the correlation between oil droplet size distribution and oil constituent bioavailability and toxicity, particularly on the toxicological effects resulting from exposures to dissolved vs. particulate oil.

Another question that remains unanswered is the fate and effects of oil at depth if injection of dispersants at the wellhead had not occurred.

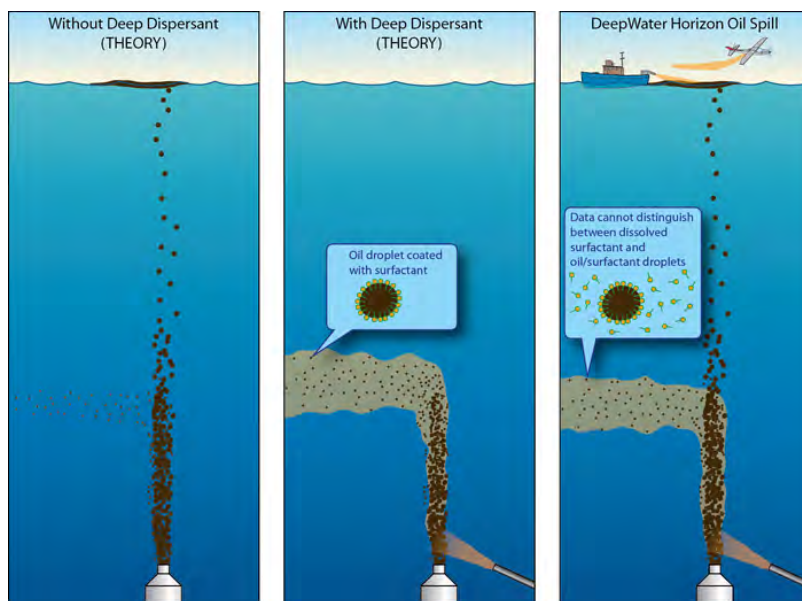
Most toxicity testing of dispersant and dispersed oil during the DWH response focused on 2 or 3 species, which have limited capabilities when characterizing risks to several hundred likely receptors. In addition, these tests did not sufficiently address potential differences in sensitivity to organisms living in the water-column in the Gulf of Mexico. Furthermore, the toxicity testing conducted during the response did not address the potential effects of dispersants and dispersed oil to deepwater species inhabiting areas where low temperatures can inhibit or reduce biodegradation and affect uptake and depuration kinetics. Sediment sampling of offshore deepwater bottoms was relatively limited, and so were the toxicity testing of these samples. Thus, these efforts may have not adequately quantified the impacts of subsurface injection of dispersants on these habitats, though assessments can use the state of knowledge from other spills (e.g., IXTOC, Sea Empress, Montero).

Limited *in-situ* testing was available to assess adverse effects to aquatic receptors. Rotifer toxicity tests, which are logistically simple to perform, were conducted onboard ships and used as a decision tool during subsurface application of dispersants. However, these tests are considerably less sensitive than tests performed with early life stages of fish or crustaceans. Tests species amenable to field testing aboard ship aside from rototox should be explored in the near future.

There were no studies on the photo-induced toxicity of chemically dispersed oil at the water surface. Studies should consider the increased toxicity of some PAHs in the presence of UV light by including exposures to natural sunlight or ultraviolet light. Also, most of the toxicity assessments conducted during the response were confined to PAHs (either total PAHs or comparisons versus benchmarks), and did not take into account other oil-related constituents (e.g., diesel range organics, normal alkanes, isoparaffins, heterocycles and unresolved complex mixtures) which may also contribute to the overall toxicity of dispersed oils.



**Figure 3.** A 35 km long oil plume at ~1000-1200 m depth near the DWH wellhead (indicated by black star) discovered using mass spectrometry and fluorescence data to detect monoaromatic petroleum hydrocarbon concentrations (from<sup>42</sup>).



**Figure 4.** Ultrahigh resolution mass spectrometry and liquid chromatography were used to identify and quantify the surfactant DOSS in deepwater during and after DWH oil flow. The first two panels show the general theories of the fate of oil with and without dispersant application at the wellhead. The third panel suggests that dispersant remained in the deep waters plume, associated with oil and gas phases, and that dissolved surfactant could not be distinguished from surfactant coating oil droplets (from<sup>47</sup>).



## REFERENCES

1. NRC *Oil Spill Dispersants: Efficacy and Effects*; National Academies Press: Washington DC, 2005; p 377.
2. Schmidt, C. W., Between the devil and the deep blue sea: dispersants in the Gulf of Mexico. *Environmental Health Perspectives* **2010**, *118*, (8), A338-A344.
3. Kintisch, E., An audacious decision in crisis gets cautious praise. *Science* **2010**, *329*, (5993), 735.
4. Lin, C.-Y.; Tjeerdema, R. S., Crude oil, oil, gasoline and petrol. In *Encyclopedia of Ecology, Volume 1: Ecotoxicology*, Jorgensen, S. E.; Fath, B. D., Eds. Oxford, UK, 2008; pp 797-805.
5. Singer, M.; George, S.; Lee, I.; Jacobson, S.; Weetman, L.; Blondina, G.; Tjeerdema, R.; Aurand, D.; Sowby, M., Effects of dispersant treatment on the acute aquatic toxicity of petroleum hydrocarbons. *Archives of Environmental Contamination and Toxicology* **1998**, *34*, (2), 177-187.
6. Lin, C. Y.; Anderson, B. S.; Phillips, B. M.; Peng, A. C.; Clark, S.; Voorhees, J.; Wu, H. D. I.; Martin, M. J.; McCall, J.; Todd, C. R., F. Hsieh, D. Crane, M. R. Viant, M. L. Sowby and R. S. Tjeerdema, Characterization of the metabolic actions of crude versus dispersed oil in salmon smolts via NMR-based metabolomics. *Aquatic Toxicology* **2009**, *95*, (3), 230-238.
7. Van Scoy, A. R.; Yu Lin, C.; Anderson, B. S.; Philips, B. M.; Martin, M. J.; McCall, J.; Todd, C. R.; Crane, D.; Sowby, M. L.; Viant, M. R., nd R. S. Tjeerdema, Metabolic responses produced by crude versus dispersed oil in Chinook salmon pre-smolts via NMR-based metabolomics. *Ecotoxicology and Environmental Safety* **2010**, *73*, (5), 710-717.
8. Singer, M. M.; Smalheer, D. L.; Tjeerdema, R. S.; Martin, M., Toxicity of an oil dispersant to the early life stages of four California marine species. *Environmental toxicology and chemistry* **1990**, *9*, (11), 1387-1395.
9. Singer, M. M.; Smalheer, D. L.; Tjeerdema, R. S.; Martin, M., Effects of spiked exposure to an oil dispersant on the early life stages of four marine species. *Environmental toxicology and chemistry* **1991**, *10*, (10), 1367-1374.
10. Singer, M. M.; Aurand, D.; Bragins, G. E.; Clark, J. R.; Coelho, G. M.; M.L., S.; Tjeerdema, R. S., Standardization of preparation and quantification of water-accommodated fractions of petroleum for toxicity testing. *Marine Pollution Bulletin* **2000**, *40*, 1007-1016.
11. OSAT *Operational Science Advisory Team. Unified Area Command. Summary report for sub-sea and sub-surface oil and dispersant detection: Sampling and monitoring*; December 17, 2010.
12. JAG *Review of R/V Brooks McCall Data to Examine Subsurface Oil*; National Incident Command Joint Assessment Group, June 20, 2010: 2010; p 58.
13. JAG *Review of Preliminary Data to Examine Subsurface Oil In the Vicinity of MC252#1* National Incident Command Joint Assessment Group, July 20, 2010: 2010; p 73.
14. JAG *Measurement of Concentration and Size Distribution of Surface and Subsurface Small Particles Using LISST-100X at the Deepwater Horizon Spill Site*; National Incident Command Joint Assessment Group, unpublished report 2011; p 85.
15. Benner, J. R. A.; El Said, K. R.; Jester, E. L. E.; Flurer, R. A.; Boyd, B. L.; Gamble, B.; Gratz, S.; Mulligan, K. J.; Heitkemper, D. T.; Burrows, D. G. *Investigation of Corexit® 9500 Dispersant in Gulf of Mexico Seafood Species*; Gulf Coast Seafood Laboratory, U.S. Food and Drug Administration 2010; p 22.
16. USEPA *Procedures for the Derivation of Equilibrium Partitioning Sediment Benchmarks (ESBs) for the Protection of Benthic Organisms: PAH Mixtures. EPA-600-R-02-013*; United States Environmental Protection Agency: Washington, DC., 2003; p 175.
17. BenKinney, M.; Brown, J.; Mudge, S.; Russell, M.; Nevin, A.; Huber, C. In *Monitoring Effects of Aerial Dispersant Application during the MC252 Deepwater Horizon Incident* International Oil Spill Conference, Portland, Oregon, 2011; Portland, Oregon, 2011; p 7.
18. Hemmer, M. J.; Barron, M. G.; Greene, R. M. *Comparative Toxicity of Eight Oil Dispersant Products on Two Gulf of Mexico Aquatic Test Species*; U.S. Environmental Protection Agency Office of Research and Development: 2010; p 11.

19. Judson, R. S.; Martin, M. T.; Reif, D. M.; Houck, K. A.; Knudsen, T. B.; Rotroff, D. M.; Xia, M.; Sakamuru, S.; Huang, R.; Shinn, P.; Austin, C. P.; R.J., K.; Dix, D. J., Analysis of eight oil spill dispersants using rapid, in vitro tests for endocrine and other biological activity. *Environmental Science and Technology* **2010**, *44*, 5971-5978.
20. BP Dispersant Studies of the Deepwater Horizon Oil Spill Response Volume 3; 2010; p 32.
21. BP Dispersant Studies of the Deepwater Horizon Oil Spill Response Interim Report on Surface Application Evaluations Conducted May 17-27, 2010 2010; p 16.
22. Hemmer, M. J.; Barron, M. G.; Greene, R. M. *Comparative Toxicity of Louisiana Sweet Crude Oil (LSC) and Chemically Dispersed LSC to Two Gulf of Mexico Aquatic Test Species*; U.S. Environmental Protection Agency Office of Research and Development: 2010; p 13.
23. OSAT Operational Science Advisory Team. *Unified Area Command. Summary Report for Sub-Sea and Sub-Surface Oil and Dispersant Detection: Toxicity Addendum*; July 8, 2011; p 35.
24. USEPA *Methods for Measuring the Acute Toxicity of Effluent and Receiving Waters to Freshwater and Marine Organisms. 5th Edition, October 2002. EPA-821-R-02-012. U.S. Environmental Protection Agency, Washington, D.C.*; 2002.
25. Hamdan, L. J.; Fulmer, P. A., Effects of COREXIT® EC9500A on bacteria from a beach oiled by the Deepwater Horizon spill. *Aquatic Microbial Ecology* **2011**, *63*, 101-109.
26. SETAC In Meeting Program, *Gulf Oil Spill SETAC Focused Topic Meeting*, Society of Environmental Toxicology and Chemisitry, Pensacola, FL, 2011; Pensacola, FL, 2011; p 51.
27. Felder, D. L.; Camp, D. K., *Gulf of Mexico Origin, Waters, and Biota: Biodiversity*. Texas A&M University Press 2009; Vol. 1.
28. Hamdan, L. J.; Fulmer, P. A., Effects of COREXIT® EC9500A on bacteria from a beach oiled by the Deepwater Horizon spill. *Aquatic Microbial Ecology* **2011**, *63*, (2), 101-109.
29. Widger, W. R.; Golovko, G.; Martinez, A.; Ballesteros, E.; Howard, J.; Xu, Z.; Pandya, U.; Fofanov, V.; Rojas, M.; Bradburne, C.; Hadfield, T.; Olson, N. A.; Santarpia, J. L.; Fofanov, Y., Longitudinal metagenomic analysis of the water and soil from Gulf of Mexico beaches affected by the Deep Water Horizon oil spill. *Nature Precedings* **2011**.
30. Lindstrom, J. E.; Braddock, J. F., Biodegradation of petroleum hydrocarbons at low temperature in the presence of the dispersant Corexit 9500. *Marine Pollution Bulletin* **2002**, *44*, 739 – 747.
31. Hu, C.; Weisberg, R. H.; Liu, Y.; Zheng, L.; Daly, K. L.; English, D. C.; Zhao, J.; Vargo, G. A., Did the northeastern Gulf of Mexico become greener after the Deepwater Horizon oil spill? *Geophysical Research Letters* **2011**, *38*, (9).
32. Rabalais, N. N.; Turner, R. E.; Wiseman, W. J., Gulf of Mexico Hypoxia, A.K.A. "the Dead Zone". *Annual Review of Ecology and Systematics* **2002**, *33*, (1), 235-263.
33. National Oceanic and Atmospheric Administration Links Between Gulf Hypoxia and the Oil Spill. [http://response.restoration.noaa.gov/dwh.php?entry\\_id=812](http://response.restoration.noaa.gov/dwh.php?entry_id=812).
34. Chen, J.; Denison, M. S., The Deepwater Horizon oil spill: environmental fate of the oil and the toxicological effects on marine organisms. *The Journal of Young Investigators* **2011**, *21*, (6), 84 - 95.
35. Graham, W. M.; Condon, R. H.; Carmichael, R. H.; D'Ambra, I.; Patterson, H. K.; Linn, L. J.; Hernandez Jr, F. J., Oil carbon entered the coastal planktonic food web during the Deepwater Horizon oil spill. *Environmental Research Letters* **2010**, *5*, (4), 045301.
36. Mascarelli, A., After the oil. *Nature* **2010**, *467*, 22 - 24.
37. Schrope, M., Deep Wounds: Impacts of GoM Oil Spill. *Nature* **2011**, *472*, 152 - 154.
38. Greer, C. D. Toxicity of Chemically Dispersed Crude Oil to Herring Embryos. Queen's University, Kingston, Ontario, Canada, 2011.
39. Milinkovitch, T.; Ndiaye, A.; Sanchez, W.; Le Floch, S.; Thomas-Guyon, H., Liver antioxidant and plasma immune responses in juvenile golden grey mullet (*Liza aurata*) exposed to dispersed crude oil. *Aquatic Toxicology* **2011**, *101*, (1), 155-164.
40. Bue, B. G.; Sharr, S.; Seeb, J. E., Evidence of damage to pink salmon populations inhabiting Prince William Sound, Alaska, two generations after the Exxon Valdez oil spill. *Transactions of the American Fisheries Society* **1998**, *127*, (1), 35-43.

41. Carls, M. G.; Rice, S. D.; Hose, J. E., Sensitivity of fish embryos to weathered crude oil: Part I. Low-level exposure during incubation causes malformations, genetic damage, and mortality in larval pacific herring (*Clupea pallasii*). *Environmental Toxicology and Chemistry* **1999**, *18*, (3), 481-493.
42. Camilli, R.; Reddy, C. M.; Yoerger, D. R.; Van Mooy, B. A. S.; Jakuba, M. V.; Kinsey, J. C.; McIntyre, C. P.; Sylva, S. P.; Maloney, J. V., Tracking hydrocarbon plume transport and biodegradation at Deepwater Horizon. *Science* **2010**, *330*, (6001), 201-204.
43. Diercks, A. R.; Asper, V. L.; Highsmith, R. C.; Woolsey, M.; Lohrenz, S. E.; McLetchie, K.; Gossett, A.; Lowe, M.; Joung, D.; McKay, L., The discovery of deep oil plumes at the Deepwater Horizon oil spill site. In *American Geophysical Union, Fall Meeting 2010*, San Francisco, California, USA, 2010.
44. Thibodeaux, L. J.; Valsaraj, K. T.; John, V. T.; Papadopoulos, K. D.; Pratt, L. R.; Pesika, N. S., Marine oil fate: knowledge gaps, basic research, and development needs; a perspective based on the Deepwater Horizon spill. *Environmental Engineering Science* **2011**, *28*, (2), 87-93.
45. Haddad, R.; Murawski, S. *Analysis of Hydrocarbons in Samples Provided from the Cruise of the R/V WEATHERBIRD II, May 23–26, 2010*; Silver Spring, Maryland, 2010; pp 1 - 14.
46. Reddy, C. M.; Arey, J. S.; Seewald, J. S.; Sylva, S. P.; Lemkau, K. L.; Nelson, R. K.; Carmichael, C. A.; McIntyre, C. P.; Fenwick, J.; Ventura, G. T.; Van Mooy, B. A. S.; Camilli, R., Composition and fate of gas and oil released to the water column during the Deepwater Horizon oil spill. *Proceedings of the National Academy of Sciences* **2011**.
47. Kujawinski, E. B.; Kido Soule, M. C.; Valentine, D. L.; Boysen, A. K.; Longnecker, K.; Redmond, M. C., Fate of dispersants associated with the Deepwater Horizon oil spill. *Environmental Science and Technology* **2011**, *45*, 1298-1306.
48. Bandara, U. C.; Yapa, P. D.; Xie, H., Fate and transport of oil in sediment laden marine waters. *Journal of Hydro-environment Research* **2011**.
49. Ramseur, J. L. *Deepwater Horizon Oil Spill: The Fate of the Oil*; Library of Congress: Washington, DC, 2011.
50. Hazen, T. C.; Dubinsky, E. A.; DeSantis, T. Z.; Andersen, G. L.; Piceno, Y. M.; Singh, N.; Jansson, J. K.; Probst, A.; Borglin, S. E.; Fortney, J. L.; Stringfellow, W. T.; Bill, M.; Conrad, M. E.; Tom, L. M.; Chavarria, K. L.; Alusi, T. R.; Lamendella, R.; Joyner, D. C.; Spier, C.; Baelum, J.; Auer, M.; Zemla, M. L.; Chakraborty, R.; Sonnenthal, E. L.; D'Haeseleer, P.; Holman, H. Y.; Osman, S.; Lu, Z.; Van Nostrand, J. D.; Deng, Y.; Zhou, J.; Mason, O. U., Deep-sea oil plume enriches indigenous oil-degrading bacteria. *Science* **2010**, *330*, (6001), 204-8.
51. Kessler, J. D.; Valentine, D. L.; Redmond, M. C.; Du, M.; Chan, E. W.; Mendes, S. D.; Quiroz, E. W.; Villanueva, C. J.; Shusta, S. S.; Werra, L. M.; Yvon-Lewis, S. A.; Weber, T. C., A persistent oxygen anomaly reveals the fate of spilled methane in the deep Gulf of Mexico. *Science* **2011**, *331*, (6015), 312-5.
52. Valentine, D. L.; Kessler, J. D.; Redmond, M. C.; Mendes, S. D.; Heintz, M. B.; Farwell, C.; Hu, L.; Kinnaman, F. S.; Yvon-Lewis, S.; Du, M.; Chan, E. W.; Garcia Tigreros, F.; Villanueva, C. J., Propane respiration jump-starts microbial response to a deep oil spill. *Science* **2010**, *330*, (6001), 208-11.
53. NRC, *Oil in the Sea III: Inputs, Fates, and Effects*. National Academies Press: Washington, DC, 2003.
54. Scarlett, A.; Galloway, T. S.; Canty, M.; Smith, E. L.; Nilsson, J.; Rowland, S. J., Comparative toxicity of two oil dispersants, superdispersant 25 and corexit 9527, to a range of coastal species. *Environmental toxicology and chemistry* **2005**, *24*, (5), 1219-1227.
55. Clark, J. R.; Bragin, G. E.; Febbo, R. J.; Letinski, D. J. In *Toxicity of physically and chemically dispersed oils under continuous and environmentally realistic exposure conditions: Applicability to dispersant use decisions in spill response planning, Proceedings of the 2001 International Oil Spill Conference*, Tampa, Florida, 2001; American Petroleum Institute, Washington, D.C.: Tampa, Florida, 2001; pp 1249-1255
56. George-Ares, A.; Clark, J., Aquatic toxicity of two Corexit® dispersants. *Chemosphere* **2000**, *40*, (8), 897-906.

57. Anderson, B. S.; Arenella-Parkerson, D.; Phillips, B. M.; Tjeerdema, R. S.; Crane, D., Preliminary investigation of the effects of dispersed Prudhoe Bay Crude Oil on developing topsmelt embryos, *Atherinops affinis*. *Environmental Pollution* **2009**, *157*, (3), 1058-1061.
58. Ramachandran, S. D.; Hodson, P. V.; Khan, C. W.; Lee, K., Oil dispersant increases PAH uptake by fish exposed to crude oil. *Ecotoxicology and Environmental Safety* **2004**, *59*, (3), 300-308.