Operational Science Advisory Team (OSAT)

Gulf Coast Incident Management Team

Summary Report for Sub-Sea and Sub-Surface Oil and Dispersant Detection: Ecotoxicity Addendum

Prepared for

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Executive Summary

The purpose of this report addendum is to provide the Federal On-Scene Coordinator (FOSC) for the Deepwater Horizon MC252 Spill of National Significance with information on the remaining toxicity of released oil and dispersant to representative water column and sediment-dwelling organisms at the time the samples were collected. This information is intended to inform the FOSC regarding transition of nearshore activities from the emergency response phase to the long term recovery and restoration phase.

Extensive collections of water and sediment samples for ecotoxicity testing and chemical analysis were conducted by multiple federal entities and BP (a responsible party) during the Response to the Deepwater Horizon MC252 Spill of National Significance (DWH oil spill). Beginning in early May 2010, sampling and monitoring operations were conducted in both surface and subsurface environments of the Gulf of Mexico to locate any oil and/or dispersant-related constituents from the DWH oil spill and associated Response. This report addendum provides an assessment of the distribution of the samples and results of toxicity tests and chemical analyses. Results presented in this addendum build on the information provided in the *Summary Report for Sub-Sea and Sub-Surface Oil and Dispersant Detection: Sampling and Monitoring* prepared by the Operational Science Advisory Team (OSAT) released on 17 December 2010. These data are presented to address significant toxicity to benthic inverterbrates in the nearshore zone - the sole remaining indicator for the presence of potentially actionable subsurface oil not addressed in that earlier report.

Key Findings

- With respect to the indicators considered in the OSAT 2010 report, the results discussed in this addendum are consistent with the OSAT conclusions that "no exceedances of EPA's dispersant benchmarks were observed" and that "since 3 August 2010 (last day with potentially recoverable oil on the ocean surface), <1% of water samples and ~1% of sediment samples exceeded EPA's aquatic life benchmarks for polycyclic aromatic hydrocarbons (PAHs)". In addition, results of the toxicity tests support the conclusions of the OSAT report regarding the distribution of actionable (i.e. amenable to removal actions) oil and dispersant-related constituents.

- Petroleum odors and/or oily sheens were noted in <1% of the collected samples (1 sheen, 5 odors based on field notes). Total polycyclic aromatic hydrocarbons (PAHs) were detected at >1 µg/g (>1,000 ppb) in 1% of sediment locations and >1 µg/L (>1 ppb) in <1% of water locations in the nearshore zone. Dispersant markers were detected in 1.5% of the collected sediment and 34% of the water samples.

- A total of 3,548 toxicity tests were conducted during the DWH oil spill and associated Response, making this the most extensive testing program ever conducted to characterize the effects of an oil spill in the marine environment. Overall, statistically significant effects were reported in 10% of the toxicity tests.

- Based on evaluation of analytical chemistry results for the toxicity samples collected after 3 August 2010, 1% of sediment locations and <1% of water locations exceeded EPA's chronic aquatic benchmark for PAHs. None of the water or sediment pre-impact samples exceeded the benchmark.

- None of the concentrations of dispersant-related constituents found in the sediment and water samples collected after 3 August 2010 in the nearshore zone exceeded EPA's chronic aquatic benchmarks.

- Statistically significant effects were observed at 31% of the 104 pre-impact locations. Seventy-nine of the pre-impact locations were resampled after 3 August 2010, and 32% of the locations (24 sediment and 2 water locations) showed significant effects.

- Of the locations sampled after 3 August 2010, 18 showed significant effects and had chemical fingerprints characteristic of MC252 oil. Fifty-five locations (51 sediment and 4 water locations) that showed significant effects were not characteristic of MC252 oil.

- Out of a total of 647 nearshore locations sampled after 3 August 2010, 451 locations were not fingerprinted due to low total PAH concentrations. Of these locations, 145 locations showed significant effects (total PAH concentrations <0.32 μg/g [320 ppb] in sediment, <0.03 μg/L [0.03 ppb] in water), and 306 locations did not show significant effects (total PAH concentrations <0.58 μg/g [580 ppb] in sediment, <0.18 μg/L [0.18 ppb] in water).

- These data were collected to determine the presence or absence of potentially actionable oil and not to develop empirical relationships between oil constituents and toxicity. While representative of conditions in the Gulf of Mexico after 3 August 2010, the skewed distribution and narrow range of concentrations of constituents measured in this data set do not support development of empirical relationships with toxicity.

Based on evaluation of the extensive dataset of toxicity test results and associated chemical analyses, the sampling conducted during the Response is adequate for decision-making by the FOSC regarding when to transition nearshore activities from the emergency response phase to long-term recovery and restoration phase. In some of the locations with significant effects in the toxicity tests, MC252 oil was present at levels above EPA's chronic aquatic benchmark for PAHs. Additional locations that contained MC252 oil did not show significant toxicity test effects. Further locations were not fingerprinted due to low total

PAH concentrations. In some samples, other contaminants (e.g., metals, biogenic or pyrogenic sources of PAHs) or physicochemical characteristics (e.g., ammonia, grain size, carbon content) may have contributed to or caused significant effects. Statistically significant effects in laboratory tests may or may not be biologically or ecologically relevant. Long-term chronic effects on aquatic life are being assessed as part of the injury assessment conducted through the Natural Resources Damage Assessment (NRDA) process and Gulf of Mexico Research Institute (GRI)-funded research. Several factors should be considered in interpreting the findings of this report for any purpose besides assessment of actionable oil, including variations in detection limits and sampling methods, assumptions used to calculate chronic aquatic life ratios and fingerprinting of the samples.

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Section 1: Introduction

The purpose of this report addendum is to provide the Federal On-Scene Coordinator (FOSC) for the Deepwater Horizon MC252 Spill of National Significance with information on the remaining toxicity of released oil and dispersant to representative water column and sediment-dwelling organisms at the time the samples were collected. This information is intended to inform the FOSC regarding the transition of nearshore activities from the emergency response phase to the long term recovery and restoration phase.

During the Deepwater Horizon MC252 Spill of National Significance (DWH oil spill), oil and gas were discharged from the wellhead approximately 5,000 feet (1500 meters) below the sea surface for 87 days until the well was successfully capped on 15 July 2010. After the National Incident Command (NIC) directive of 3 August 2010 (which approximately coincided with the last visual observations of oil on the ocean surface), the response initiated a comprehensive sampling and monitoring program to locate and identify potentially actionable oil in the sub-surface environment. Substantial quantities of dissolved and dispersed oil were deposited in the sub-surface environment of the Gulf of Mexico ("sub-surface" refers in this report to both the water column and the bottom sediments) as a result of naturally occurring physical processes, the use of drilling muds during relief well drilling activities, and dispersant use as a response option.

Beginning in early May 2010, sampling and monitoring operations were conducted in both surface and sub-surface environments of the Gulf of Mexico to locate any oil and/or dispersant-related constituents from the DWH oil spill and associated Response. A multitude of state and federal agencies, BP contractors, academics, and non-governmental organizations performed pre-impact (defined in this report as the period between the rig explosion and shoreline oiling) sampling and numerous sampling programs throughout the Response. A summary report prepared by the Operational Science Advisory Team (OSAT) on 17 December 2010 included an assessment of the qualitative and quantitative analytical data collected in the nearshore, offshore, and deep-water zones during the Response. The OSAT report '*provides an assessment of the distribution of actionable (i.e. amenable to removal actions) oil and dispersant-related constituents that remain in the water column and/or bottom sediments and provides a summary of sampling results to inform decision makers on further oil removal operations*' (OSAT 2010, pg. 1).

As stated in the OSAT report, the results of toxicity tests conducted on various benthic and pelagic species were not included in the report, and would be the subject of an addendum to the report. Toxicity to benthic invertebrates in the nearshore zone is one of the specific indicators established by the Unified Area Command (UAC) to define the presence or

absence of potentially actionable oil. This report addendum summarizes the toxicity tests and associated chemical data developed during the DWH oil spill and associated Response. Additionally, to provide a synopsis of all toxicity data generated during the DWH oil spill and associated Response, results of toxicity tests conducted on samples collected in the deepwater and offshore zones are also summarized in this document.

The intent of the sampling and monitoring program discussed in the OSAT report and this report addendum was to assess the presence of oil and dispersant-related constituents and not for assessing long-term ecological impacts in sub-surface environments. Additional work is underway to fully evaluate the acute and chronic impacts of the DWH oil spill and associated Response as part of the Natural Resource Damage Assessment (NRDA) process and Gulf of Mexico Research Initiative (GRI)-funded independent research.

Section 2: Sample Collection and Testing

Ecotoxicity tests evaluate the effects of environmental samples on the survival, growth, reproduction, or metabolism of test organisms. Toxicity testing using animals representing different trophic levels and taxonomic groups can indicate whether samples contain substances that are toxic under laboratory conditions. Responses in test samples are compared to laboratory control samples that represent "ideal" conditions for the test species. Different test organisms vary in their sensitivity to individual contaminants, and different responses may be observed in short-term vs. longer-term exposures. Some test organisms are also sensitive to factors such as sediment grain size, ammonia, or salinity. The benefit of toxicity testing using environmental samples is that it provides an integrated assessment of adverse effects in whole samples. However, determining the definitive cause of observed responses in laboratory toxicity tests using whole environmental samples is not possible without significant further effort to fractionate the samples to rule out alternative causes. Toxicity tests were conducted during the Response as part of an integrated evaluation of the impacts on aquatic life in the immediate context of a removal action.

Toxicity tests with benthic and pelagic species were conducted in the laboratory by multiple agencies (U.S. Environmental Protection Agency [EPA] Regions 4 and 6, U.S. Geological Survey [USGS]) and BP contractors using water and sediment samples collected in the nearshore zone (waters inshore of the 3 nautical miles state waters boundary) (Map 7.1). The sampling zone designations used in this report are consistent with those identified in the OSAT report (OSAT 2010). Raw data (e.g., collection date, location coordinates, sample depth, test conditions and results) for all tests discussed in this document are available at www.restorethegulf.gov and GeoPlatform.gov (http://www.geoplatform.gov/gulfresponse/).

In this report, the term “location” is used to identify a point in time and space where a sample was collected for testing/analysis, while “test” is used to define an evaluation of a sample using a particular organism, exposure time, and set of endpoints. A unique location is defined by the following data: sample coordinates, collection date, media type, and sample depth (for water samples). Only locations that included paired toxicity test results and chemical analyses were evaluated. Each data provider conducted quality assurance evaluations of their own data. Data providers evaluated test results and identified significant endpoints using standard statistical analysis programs.

Results from a toxicity test were considered “significant” if effects on any test endpoint (e.g., survival, growth, fertilization, development, biomass, fecundity) were observed in the field-collected samples in relation to the untreated laboratory controls. As a conservative approach, for each location, any statistical difference ($p \leq 0.05$) between the field-collected

samples and untreated laboratory controls for any endpoint in any toxicity test was considered a significant effect. Statistically significant effects in laboratory tests may or may not be biologically or ecologically relevant.

Multiple toxicity tests and chemical analyses were performed on samples collected from the same location. As a result, the following approach was developed to calculate the number of locations and tests. To calculate number of locations evaluated for a time period or endpoint, the dataset was sorted by sample chemical analysis results, and then by toxicity test results. To calculate number of tests evaluated for a time period or endpoint, the dataset was sorted by toxicity test endpoint, and then by sample chemical analysis results. Chemical analysis results were not available at 8 locations with toxicity data, so these data were not included in the assessment, but all data can be found at www.restorethegulf.gov and www.geoplatform.gov/gulfresponse/.

2.1 Nearshore Zone Samples

A total of 1,609 toxicity tests (Table 1) were conducted with eleven test species (Table 2) on samples collected at 751 locations in the nearshore zone (Figure 1, Map 7.2). Pre-impact sediment toxicity tests were conducted at 104 locations by the EPA and USGS beginning on 2 May 2010 (Map 7.3). Toxicity test samples were collected after 3 August 2010 at 647 locations by the EPA, USGS, and BP based on the extent of oil at surface (from ships, aircraft, satellites, and *in situ* sampling and observations) and from knowledge of the nearshore physical oceanography (i.e., movement of water and sediments) (Strategic Plan, UAC 2010). Samples for chemical analysis were collected at all 647 locations.

Table 1. Summary of toxicity tests conducted on sediment and water samples collected in the nearshore zone.

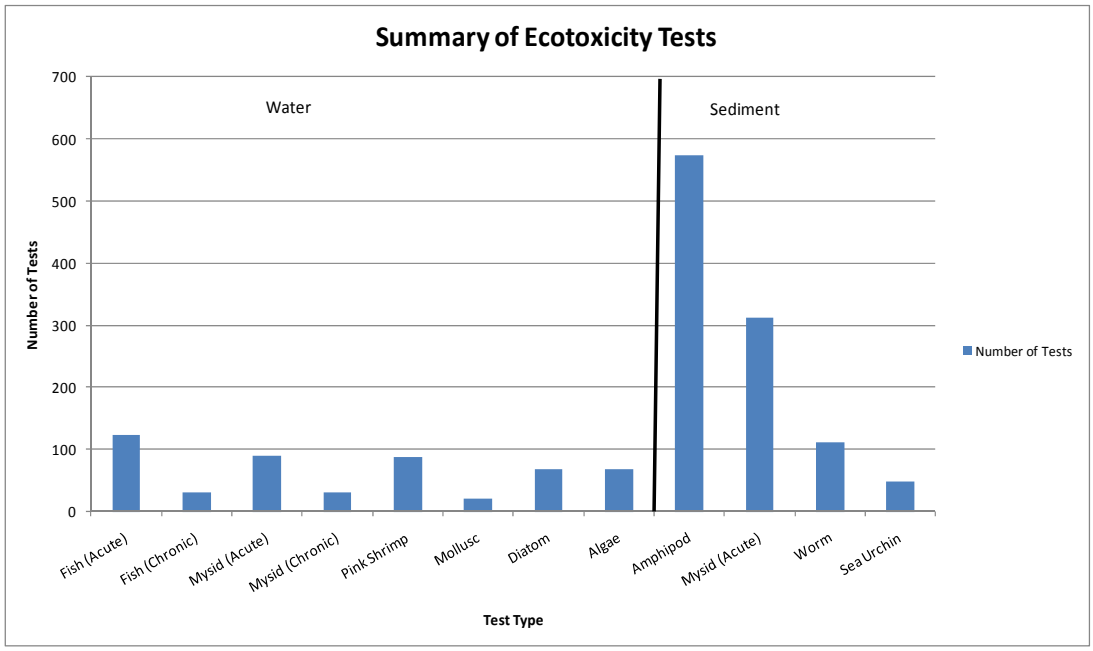
Sample Type	Test Type	Duration	Endpoint	Number of Tests
Water	Fish	96 hrs	Survival	126
		7 days	Survival, Growth, Biomass	36
	Mysid	96 hrs	Survival	93
		7 days	Survival, Growth, Fecundity, Biomass	30
	Pink Shrimp	7 days	Survival	88
	Sea Urchin	120 min	Fertilization	2
	Mollusc	48 hrs	Survival, Embryo Development	20
	Diatom	96 hrs	Growth	68
	Algae	96 hrs	Growth	68

Sediment	Amphipod	96 hrs	Survival, Growth	74
		10 days	Survival, Growth, Reburial	505
	Worm	10 days	Survival	112
	Mysid	48 hrs	Survival	256
		96 hrs	Survival, Growth	65
	Sea Urchin	60 min, 48 hrs	Fertilization, Embryo Development	66

Table 2. List of species evaluated in toxicity tests with water column and sediment samples collected in the offshore and nearshore zones.

Scientific Name	Common Name
Americamysis bahia (also known as *Mysidopsis bahia*)	Mysid shrimp
Arbacia punctulata	Sea urchin
Crassostrea gigas	Pacific oyster (Mollusc)
Cyprinodon variegatus	Sheepshead minnow
Dunaliella tertiolecta	Algae
Farfantepanaeus duorarum	Pink shrimp
Leptocheirus plumulosus	Amphipod
Menidia beryllina	Inland silversides fish
Mytilus galloprovincialis	Mussel (Mollusc)
Neanthes arenaceodentata	Polychaete worm
Skeletonema costatum	Diatom

Figure 1. Summary of sediment and water column toxicity tests performed on nearshore samples collected by the EPA, USGS, and BP.



Water samples were collected in the field using sampling devices (e.g., GO-FLO bottles) that can be deployed in closed position, then opened at the desired sample depth, filled and closed, before the bottle is returned to the surface. In shallow water, samples were collected by dipping the sample container in the water to a sufficient depth to collect below the surface, while minimizing disturbance of bottom sediments. Sediment in the deeper portions of the nearshore zone was collected using grab samplers (e.g., Ponar, Van Veen). Sediment in shallow water was collected using push core tubes or scoops. After collection, overlying water was removed, and sediment samples were collected from the top 1-3 cm of the grab samples to capture recent accumulation. All collected samples were held on ice at 4 °C from collection and during transport under Chain-of-Custody to the respective laboratories for toxicity testing and chemical analysis. Sampling and toxicity test procedures are outlined in Quality Assurance Project Plans prepared by each data provider.

Tests were performed in accordance with EPA (1994; 1995, 2002a, b) and ASTM (E724, E1367, E1563, E1611) guidance. Toxicity tests were conducted on water samples following salinity adjustment, if necessary to achieve test conditions appropriate for the test species. Whole sediment toxicity tests were conducted with the amphipod, worm and mysid following sample homogenization and sieving. Mysids would be exposed to chemical constitutents leaching out of the sediment into the overlying water, while amphipods and worms are burrowers in the sediment. Porewater samples were evaluated in the sea urchin tests, and were extracted from the sediment and centrifuged at 1200 g for 20 min. to remove suspended particulates from the supernatant before use. The toxicity tests conducted by all entities were performed to determine if there was a significant difference between the undiluted surface water, porewater, or whole sediment sample (100% exposure) and the associated laboratory control. In some of the tests, additional dilutions were evaluated as part of the study design.

One of the criteria established by EPA (1995, 2002a, b) and ASTM E1367 for toxicity test acceptability relates to control survival. Each test method contains specific test acceptability criteria (e.g., 90% control survival for acute toxicity tests, 80% control survival for chronic toxicity tests) which define minimum acceptable control performance for each endpoint, statistical resolution, and test conditions. Test acceptability criteria were used to evaluate laboratory performance. Positive control samples were used to demonstrate sensitivity of test organisms to contaminants. Test acceptability criteria were evaluated by all data providers, and data used in this report met the established criteria.

Pre-impact toxicity tests (183 tests) were conducted on samples collected at 104 locations in the five potentially affected coastal states (Alabama, Florida, Louisiana, Mississippi, Texas) by considering the location of sensitive natural resources and availability of historical data. Pre-impact toxicity tests included: 1) the 10-day *Leptocheirus plumulosus* (burrowing amphipod) sediment survival test, 2) the 10-day *Neanthes arenanceodentata*

(polychaete worm) survival test, 3) the 96-hour *Menidia beryllina* (inland silverside fish) surface water survival test, 4) the 7-day *Cyprinodon variegatus* (sheepshead minnow) surface water survival test, 5) the 48-hour and 96-hour *Americamysis bahia* (mysid shrimp, formerly known as *Mysidopsis bahia*) surface water and sediment survival tests, and 6) 30-min and 48-hour *Arbacia punctulata* (sea urchin) sediment porewater fertilization and embryological tests.

Toxicity testing efforts continued through the Response, with 1426 additional tests conducted on samples collected after 3 August 2010 as part of the Strategic Plan (UAC 2010) using a variety of species at 647 locations (Map 7.4). During this time, 79 pre-impact locations were revisited by the EPA and USGS, and a number of new locations were added. Locations sampled by the EPA, USGS, and BP included targeted sites where oil had been observed previously during the Response. Surface water and sediment toxicity tests with additional species and endpoints were included in the later efforts.

Nearshore toxicity tests with water column samples collected after 3 August 2010 included: 1) 96-hour *Americamysis bahia* survival test, 2) 7-day *Americamysis bahia* survival, growth, and fecundity test, 3) 96-hour *Menidia beryllina* survival tests, 4) 7-day *Menidia beryllina* survival and growth test, 5) 7-day *Farfantepanaeus duorarum* (pink shrimp) survival test, 6) 48-hour *Crassostrea gigas* (oyster) survival and development test, 7) 48-hour *Mytilus galloprovincialis* (mussel) survival and development test, 8) 96-hour *Skeletonema costatum* (marine diatom) toxicity test, and 9) 96-hour *Dunaliella tertiolecta* (marine algae) toxicity test. Sediment tests included: 1) 96-hour and 10-day *Leptocheirus plumulosus* survival and reburial test, 2) 10-day *Neanthes arenanceodentata* survival test, 3) 96-hour *Americamysis bahia* whole sediment survival test, and 4) 60-min and 48-hour *Arbacia punctulata* (sea urchin) fertilization and embryological test.

Additional toxicity tests were conducted in the offshore and deepwater zones. These results will be identified and summarized in subsequent sections of the report. The results of the toxicity tests for samples collected in these zones were included to assist in interpretation of toxicity to benthic invertebrates in the nearshore zone.

Section 3: Data Evaluation and Interpretation

Toxicity test results and associated analytical data for samples collected by all entities discussed in this report are accessible from http://www.restorethegulf.gov/, GeoPlatform.gov (http://www.geoplatform.gov/gulfresponse/), and other sources. Summary reports of toxicity test procedures and results from EPA and USGS as part of the DWH oil spill and associated Response have been compiled (EPA Region 4, 2010; USGS 2011b).

3.1 Approach Used to Evaluate Data

Toxicity test results from all data providers were compiled in a centralized database. Integration of the data sets involved standardization of terminology and endpoints. Once the master database was compiled, the next step in the evaluation process was to review the toxicity test results on an individual species-specific basis to look for trends in significance/non-significance with respect to time, spatial distribution, and among data providers.

As an initial approach, analytical chemistry results were evaluated for associations with toxicity test endpoints. Appendices A and B present the results of rank correlations between the measured chemical parameters and toxicity test endpoints. Correlations are presented for detected analytes only; all non-detected analytes are not included in these Appendices. Of particular focus during this evaluation were the concentrations of oil and dispersant-related constituents and the association between these compounds and the toxicity test endpoints in the nearshore zone.

3.2 EPA's Chronic Aquatic Benchmark for PAHs

As summarized in the OSAT (2010) report, benchmark values have been developed by the EPA[1, 2] to evaluate for potential adverse effects to aquatic life. To calculate screening values for the Response, a total of 41 oil-related organic compounds were assessed jointly through a mixture approach because they can have a cumulative effect on aquatic organisms (See Appendix A.3 in OSAT 2010). The oil-related compounds reviewed by this process include 7 volatile organic compounds, 16 parent polycyclic aromatic hydrocarbons (PAHs) and 18 alkylated homologues of the parent PAHs. The individual compounds are given potency divisors, which are used in calculating the cumulative toxicity of the mixture of compounds in each sample – hereafter referred to as the chronic aquatic life ratio. For sediment samples, the amount of total organic carbon (TOC) in the sediment is factored into the

[1] http://www.epa.gov/bpspill/water-benchmarks.html
[2] http://www.epa.gov/bpspill/sediment-benchmarks.html

calculation because organic matter can bind PAHs, reducing their effective toxicity. If the alkylated PAHs were not measured, an "alkylation multiplier" was used (See Appendix A.3 in OSAT 2010). To assess the potential hazard to aquatic organisms, the chronic aquatic life ratios for each hydrocarbon component are summed and compared to a hazard index value of 1. A value >1 indicates that the sample has the potential to cause an acute or chronic effect to aquatic organisms based on literature-derived screening values (EPA 2003). Appendix A.3 to the OSAT (2010) report describes the calculations in more detail and includes a complete list of the compounds used in the assessment and their divisors as well as a discussion of the development of the multipliers and the uncertainty associated with their use with regard to the DWH oil spill data.

The chronic aquatic life ratio calculations presented in this report addendum (Figures 2 and 3) include a subset of the results compiled in the OSAT (2010) report and additional analytical chemistry results not available at the time the OSAT report was prepared. For calculations of the chronic aquatic life ratios, values below detection limits were treated as 0 rather than using other approaches, such as one-half the detection limit. It is noted that this approach has the potential to underestimate concentrations. The results presented in Figures 2 and 3 include the chemical analyses associated with the toxicity tests summarized in Figures 3.2 and 3.3 in the OSAT (2010) report, as well as the results of additional chemical analyses and fingerprinting. None of the locations referenced in Figures 2 and 3 were resampled (each data point is a unique location). Only sample locations that included paired toxicity test results and chemical analyses were evaluated. None of the pre-impact samples (water or sediment) exceeded EPA's chronic aquatic benchmark for PAHs (hazard index <1) (Table 3). Six locations sampled after 3 August 2010 (5 sediment and 1 water) exceeded EPA's chronic aquatic benchmark for PAHs (Map 7.5).

3.3 Fingerprinting of Toxicity Samples

Nearshore sediment samples that exceeded the EPA's chronic aquatic benchmark for PAHs (Map 7.5) and had at least one significant toxicity endpoint were reviewed to assess the likelihood that the oil-related exceedances resulted from MC252 oil (Appendix C). Prior fingerprinting assessments posted on GeoPlatform.gov and reported by USGS (2010, 2011a) were incorporated into this review. Additional characterizations were performed on sediment samples that showed significant effects in the toxicity tests and had total PAH concentrations >0.32 µg/g (320 ppb) and water samples that showed significant effects with total PAH concentrations >0.03 µg/L (0.03 ppb). Characterizations performed on samples from locations that did not show significant effects had total PAH concentrations >0.58 µg/g (580 ppb) in sediment and 0.18 µg/L (0.18 ppb) in water. Characterizations were also performed by the USGS (2010, 2011a) on all of their collected samples. Evaluations of the detected PAHs and their distribution 'fingerprint' were conducted on

samples from 196 nearshore locations to determine whether the sample was "characteristic" or "not characteristic" of MC252 oil (Appendix C). If examination of the sample yielded insufficient data to make a determination or suggested that MC252 oil was present and mixed with other sources, the sample was considered "indeterminate" and the sample was identified as characteristic of MC252 oil (Map 7.6). Samples that contain low concentrations of PAHs may not yield sufficient information to determine whether they are characteristic of MC252 oil.

Figure 2. Summary of chronic aquatic life ratios for nearshore sediment samples collected by the EPA, USGS, and BP after August 3, 2010. The horizontal dotted line represents the EPA's chronic aquatic benchmark of 1.

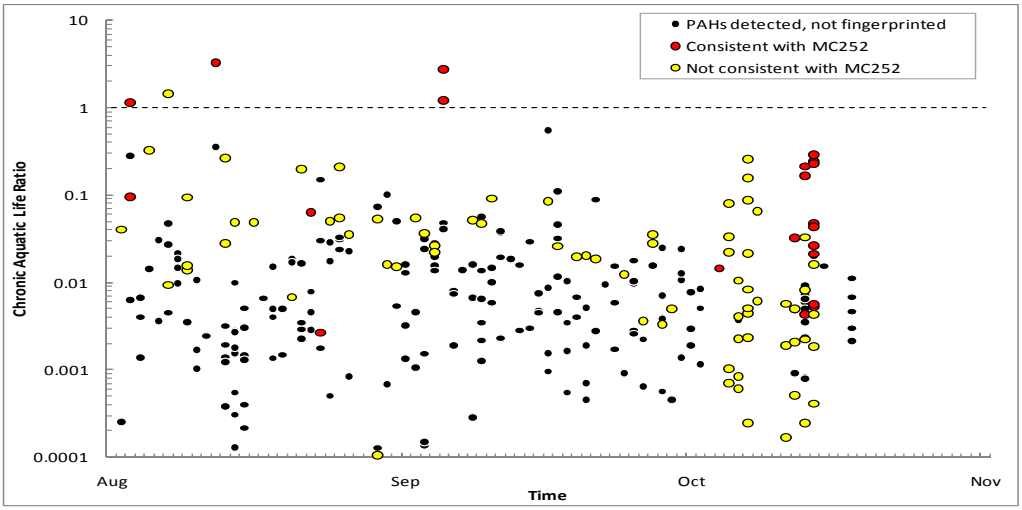


Figure 3. Summary of chronic aquatic life ratios for nearshore water samples collected by the EPA, USGS, and BP after August 3, 2010. The horizontal dotted line represents the EPA's chronic aquatic benchmark of 1.

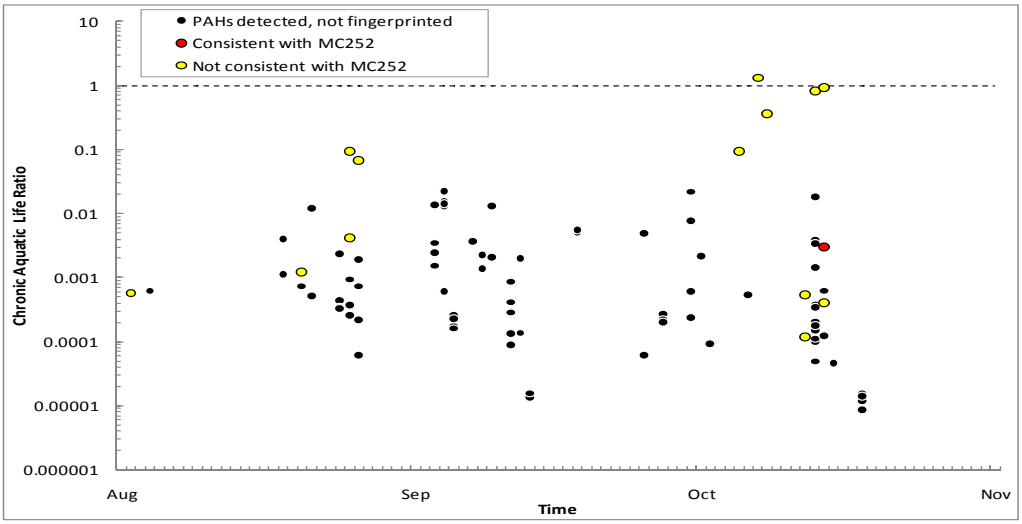


Table 3. Summary of EPA's chronic aquatic benchmark for PAHs for water and sediment sample locations collected during the DWH oil spill and associated Response.

Sample Type		Total # of Locations	Hazard Index ≥1	Hazard Index <1
Water	Pre-Impact	9	0	9
	Post 3 Aug 2010	181	1	180
Sediment	Pre-Impact	95	0	95
	Post 3 Aug 2010	466	5	461

Based on review of the detected compounds and chromatographic profiles for 196 nearshore locations, 42 locations were identified as characteristic of MC252 oil or indeterminate. Of these locations, 43% (18 out of 42 locations) showed significant effects in the toxicity tests. The remaining fingerprinted locations had predominantly pyrogenic signatures or trace levels of individual PAHs, but were not characteristic of MC252 oil.

3.4 Evaluation of Pre-Impact Samples

Collection of pre-impact samples increases our ability to assess the likelihood that MC252 oil caused or contributed to significant effects in toxicity tests. There are numerous other sources of oil-related compounds and other non-hydrocarbon contaminants within the Gulf of Mexico in addition to MC252 oil. For example, the National Research Council (2003) estimated that 140,000±60,000 tonnes (approximately one million barrels) of petroleum hydrocarbons enter the Gulf of Mexico from natural seeps each year. On an average annual basis, the Gulf also receives an additional 10,400 tonnes (approx. 74,000 barrels) of oil spilled from oil production operations, transportation accidents such as tanker leaks, and other sources such as unburned fuel. The quantity of oil entering the Gulf each year from other sources is spread over a very large area and water volume. A three-year study of the source of PAH compounds detected along the Louisiana coast determined that approximately 50% of the sources were from petrogenic (crude oil) sources, 36% were from pyrogenic (e.g. combustion or engine exhaust) sources, and 14% were from biogenic or diagenetic sources (Iqbal et al. 2008).

In addition to the available literature on petroleum hydrocarbon contributions to the Gulf of Mexico system from natural seeps and man-made sources, EPA's National Coastal Assessment (NCA) survey[3] has been monitoring sediment and water quality, benthic community condition, and sediment toxicity at approximately 190 locations per year in the coastal Gulf states. As summarized in the National Coastal Condition Report III (NCCR

[3] http://www.epa.gov/emap/nca/html/data/index.html

2008, 2005), the NCA program is a collaborative effort among the EPA, NOAA, the U.S. Fish and Wildlife Service, and the U.S. Department of Agriculture. Additional data were collected under the NCA in 2009 and 2010, but the report summarizing the results is not yet available. Sediment data collected from 2000 – 2006 as part of the NCA program using the amphipod, *Ampelisca abdita,* showed that selected sites in each of the states had survival <80% in 10-day sediment toxicity tests, with the percent of sites showing impact ranging from 1% in Louisiana (2 out of 159 sites) to 23% in Florida. A number of the sites showing toxicity in Florida may be associated with naturally high levels of hydrogen sulfide in the sediments rather than anthropogenic contamination (NCCR 2005). Sediment contaminants in the reported 190 locations included a variety of metals (e.g., arsenic, nickel, mercury, silver, cadmium, copper) which exceeded sediment ERL guidelines (an indicator of toxicity) in 28% of the estuarine area, and pesticides or polychlorinated biphenyls (PCBs), which exceeded guidelines in 14% of the area (NCCR 2005). Polycyclic aromatic hydrocarbons (PAHs) rarely exceeded the guidelines in the Gulf Coast estuaries (NCCR 2005).

As part of the DWH oil spill response, the EPA and USGS collected pre-impact sediment samples from 104 locations for toxicity testing (183 tests) and chemical analyses in the nearshore zone prior to the oil making landfall (Map 7.3). Sampling locations were selected based on NOAA trajectory modeling of areas likely to be impacted by MC252 oil. Based on data from 183 tests, statistically significant effects (test vs. control) were observed in 30% (31 out of 104 locations) of the pre-impact locations (Map 7.3), demonstrating that samples were toxic prior to oil making landfall.

3.5 Evaluation of Samples Collected After 3 August 2010

Sediment and water samples from 647 nearshore locations (1,426 toxicity tests) were collected after 3 August 2010 by EPA, USGS, and BP (Maps 7.7 and 7.8). Samples were evaluated as part of the Strategic Plan (UAC 2010) to assess the '*presence of oil that could be removed to prevent, minimize, or mitigate damage to the public health or welfare (hereinafter referred to as "actionable oil")*'. As part of this assessment, 79 pre-impact locations were revisited by the EPA and USGS. Parallel samples were also collected at 49 coastal locations by USGS and BP.

Nineteen percent of the sediment and water samples collected after 3 August 2010 (267 tests at 218 locations) show a significant effect compared to their associated controls. Greater effects were seen in sediment samples than in water samples. At locations where multiple test organisms were evaluated, significant effects were observed in more than one test at 18% (33 out of 185 locations) of the sediment locations and 13% (4 out of 31 locations) of the water locations.

The majority of the primary test endpoints (percent survival, percent normal development of sea urchin) were greater than 80% survival (Figure 4).

3.5.1 Re-Evaluation of Pre-Impact Locations

Based on the toxicity results of the pre-impact samples coupled with historical surveys (NCCR 2008, 2005), evaluation of the likelihood that MC252 oil caused or contributed to significant effects in toxicity tests should consider potential contributions from other factors. Seventy-nine of the 104 pre-impact locations were resampled after the well was capped as part of the toxicity evaluation (Appendix D). Twenty-four pre-impact locations showed significant effects in any test, and 25 locations had significant effects after 3 August 2010 (Map 7.9). Of the reevaluated pre-impact locations, 14 locations changed in their designation from significant to not significant and 15 locations changed from not significant to significant. As summarized in Figure 5 for the amphipod, mysid and sea urchin results from the same paired set of samples (e.g. samples collected at the same location prior to and following well capping), toxicity results were generally consistent among the samples, with no apparent increase in toxicity in the samples associated with DWH oil spill and associated Response (Appendix D).

Figure 4. Results of toxicity tests with nearshore samples collected after 3 August 2010.

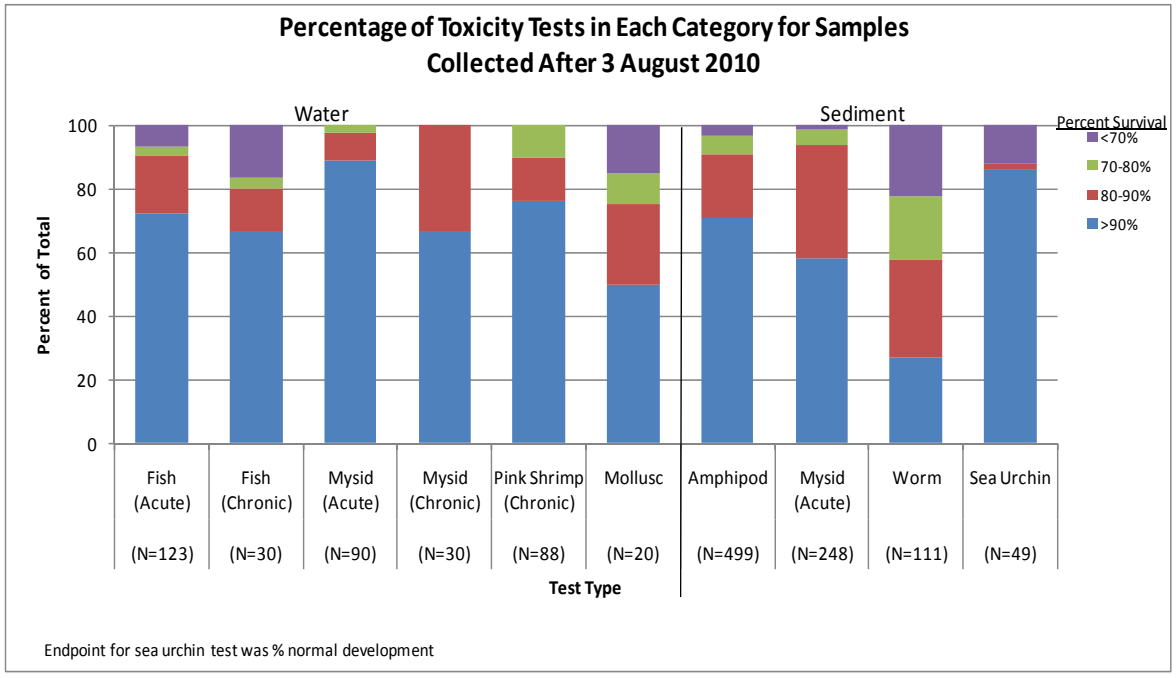
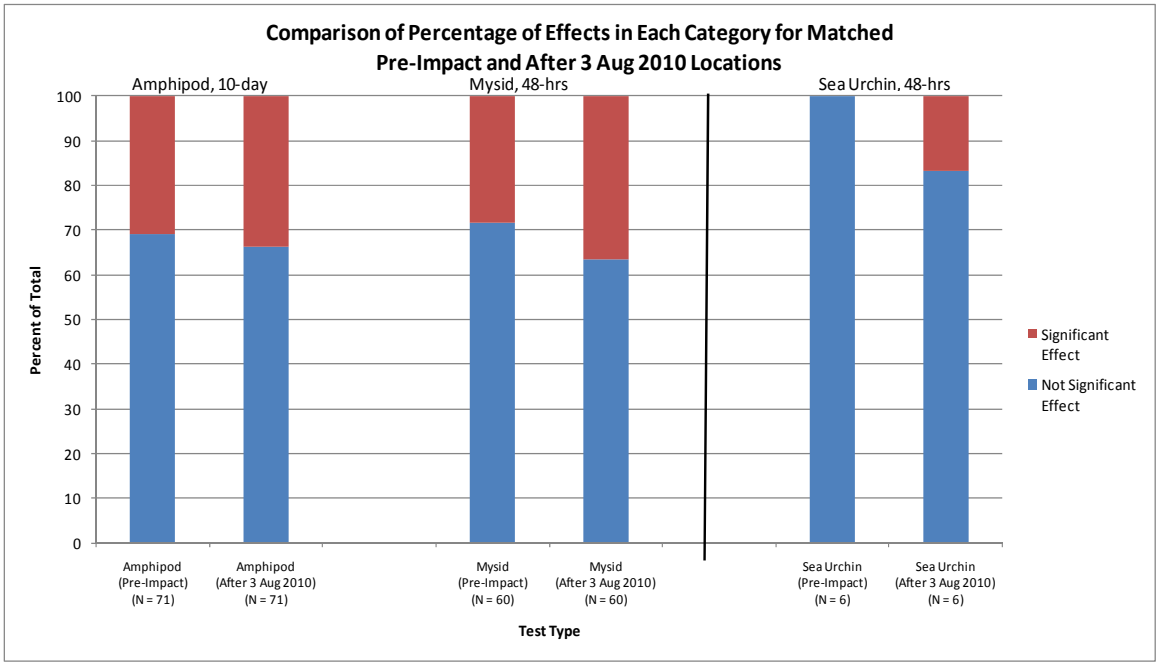


Figure 5. Results of toxicity tests with nearshore samples collected at the same locations.



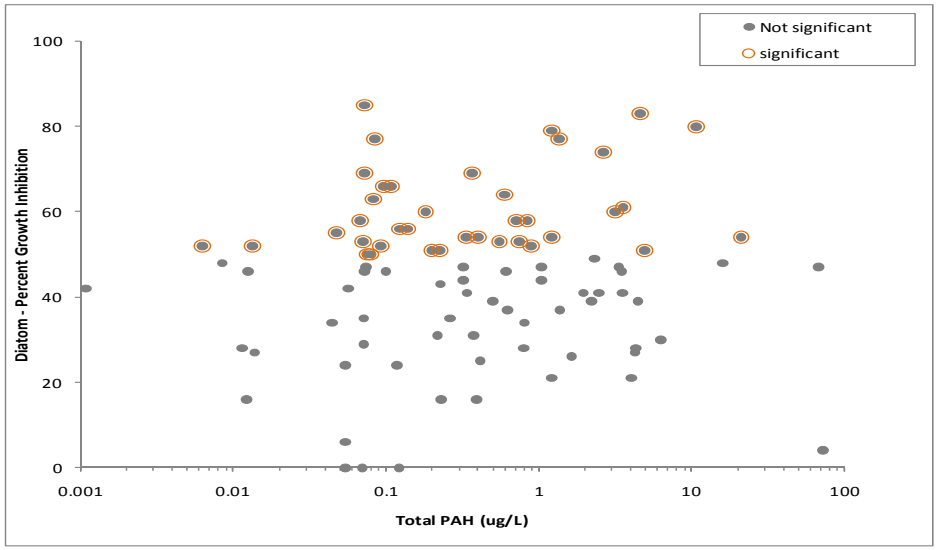
3.6 In-Slick Monitoring

Samples were collected prior to 3 August 2010 in the offshore and deep water zones underneath or adjacent to surface oil slicks to evaluate dispersant effectiveness. These samples were collected as part of the SMART protocols described in the *Special Monitoring of Applied Response Technologies* developed by the U.S. Coast Guard (USCG), National Oceanic and Atmospheric Administration (NOAA), US Environmental Protection Agency (EPA), Centers for Disease Control and Prevention (CDC), and the Minerals Management Service (MMS) (2006).

Water samples for toxicity testing were collected at 1 m and 10 m depths underneath oil slicks prior to and following dispersant application (335 tests conducted at 99 locations). Samples were also collected from reference (remote from spill influence) and background (adjacent but outside the slick) locations. Statistically significant effects were observed in 13% of the tests (43 out of 335 tests), with the majority of the effects associated with the effects on diatoms (40 tests) and the remainder associated with the mysid (3 tests). A very weak correlation ($r = 0.24$) is observed between diatom effects and Total PAH concentrations (an indicator of oil presence, Figure 6) or individual oil constituents. Reductions in mean diatom cell growth were highly variable among the samples (effects observed in some reference samples, and an inconsistent response beneath oil slicks), suggesting that this test may not be a reliable indicator of effects from MC252 oil. No

significant mortality was observed in the fish and limited effects were observed in the mysid tests (3 out of 99 tests).

Figure 6. Distribution of toxicity test results for diatoms with total polycyclic aromatic hydrocarbon concentrations for samples collected as part of on-water slick monitoring.

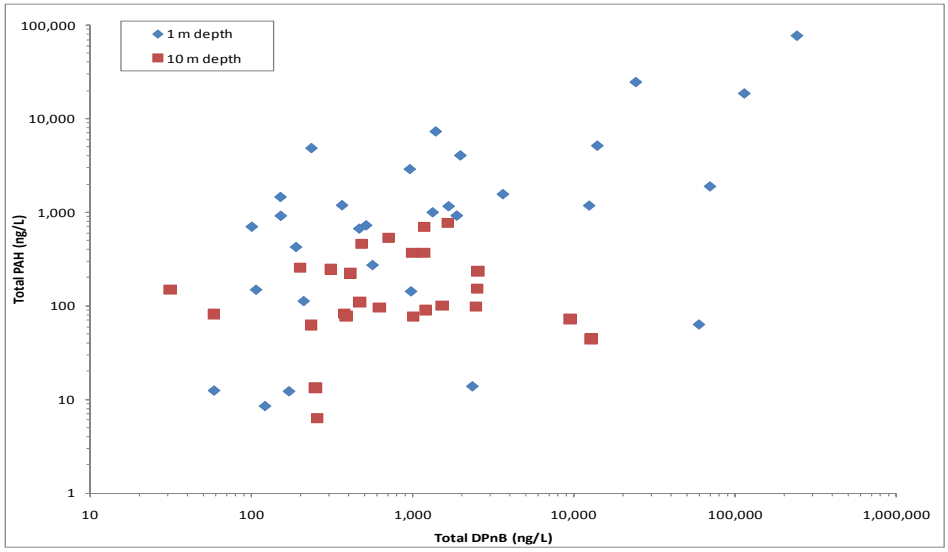


Water samples were analyzed for hydrocarbons, dispersant marker dipropylene glycol n-butyl ether (DPnB), and metals. Seventeen of the locations exceeded EPA's chronic aquatic benchmark for PAHs, including 2 locations outside slicks. The maximum level of detected DPnB in the samples was 240 µg/L (cf. EPA screening level of 1 mg/L). There was a correlation between the concentration of DPnB in the surface water samples (1 m depth) and the measured total PAH (r = 0.55 - Figure 7). Higher levels of DPnB were seen in the 1 meter samples than in the 10 meter samples, a pattern consistent with the hydrocarbon levels. The co-occurrence of Total PAH and dispersant marker concentrations in water at 10 m depth suggests that dispersants were effective at dispersing oil into the water column.

3.7 Integrated Toxicity Assessment

Multiple lines of evidence were used to evaluate whether the observed effects in the water and sediment tests could be attributed to the DWH oil spill and associated Response. The association between toxicity and chemistry was evaluated using each unique combination of toxicity and chemistry collected at a location. For example, if both sediment and water were evaluated at a location, this would result in two evaluation units for that location.

Figure 7. Association between total PAHs and dispersant marker for on water in-slick sample locations collected during the DWH oil spill and associated Response.



3.7.1 Relationship Between Toxicity and Chemical Results

As an initial assessment of potentially actionable oil, the relationship between toxicity and chemical analyses in the nearshore zone were evaluated using correlations calculated for all test endpoints and chemical constituents (e.g., individual PAHs, total PAH, EPA chronic aquatic life ratio, individual metals) (see Appendix A). Evaluation of relationships between toxicity and each of the chemical constituents show no significant correlations (see Appendix B). For example, the relationship between percent survival and total PAHs and EPA chronic aquatic life ratios for the amphipod toxicity test is shown in Figure 8. Similar weak correlations are also seen between toxicity and individual PAHs, sub-groups of PAH compounds (e.g., alkylated hydrocarbons, low molecular weight hydrocarbons, high molecular weight hydrocarbons), dispersant constituents, and metals (see Appendices A and B).

The Spearman non-parametric rank correlation method was used because the measured parameters and toxicity endpoint values are not normally distributed. Toxicity (response variable) and concentrations of chemical constituents (explanatory variables) both exhibited highly skewed distributions (e.g., many locations with no significant toxicity in any tests [429 out of 647 locations], most percent survival > 80% - see Figure 4, low concentrations of chemical constituents).

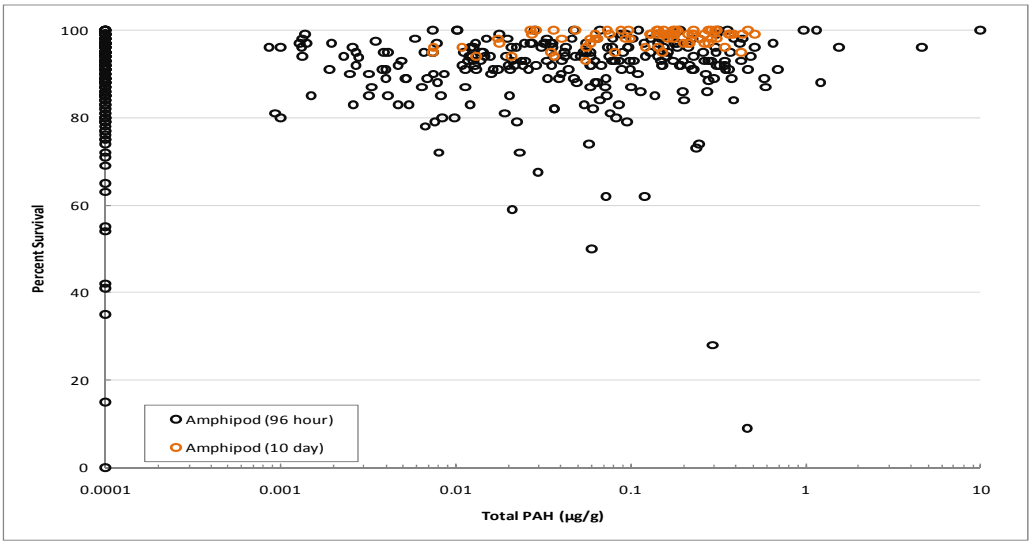
Correlations presented in Appendix A are based on concentrations of each parameter above their detection limit (i.e., non-detect results excluded). This approach, although known to result in a bias in the correlations, was utilized to assess any possible association between parameters and toxicity endpoints. These analyses were unable to identify any

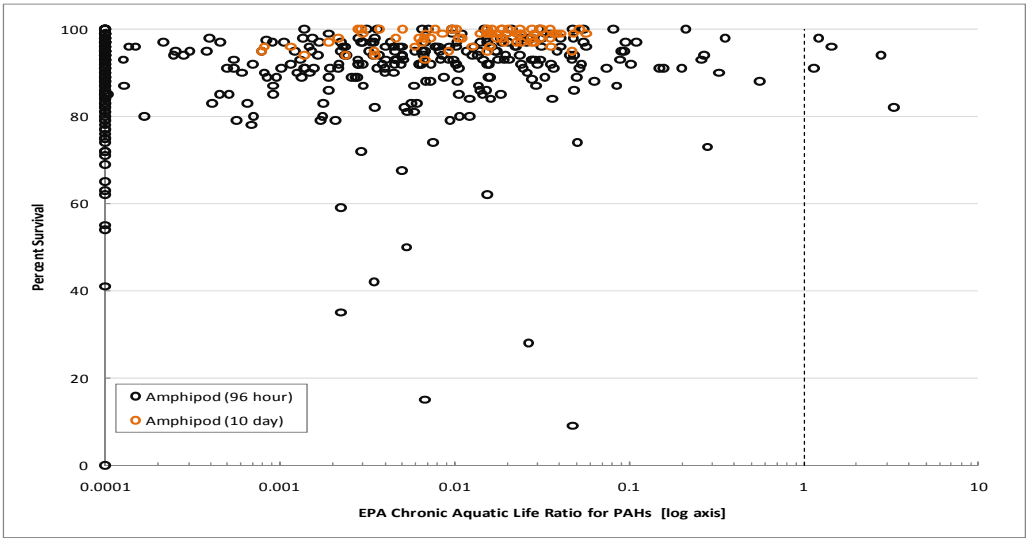
factor which was a significant contributor to observed toxicity, including MC252 oil and dispersant-related constituents providing support to the OSAT (2010) conclusions regarding actionable oil. It should be noted that while useful for addressing actionable oil, the skewed distributions coupled with the narrow range of both response and explanatory variables do not support development of empirical relationships with toxicity.

3.7.2 Dispersant Analyses

Dispersant constituents were analyzed in 55% of the nearshore samples collected after 3 August 2010 (359 out of 647 locations) (Map 7.10). The dispersant constituents that were analyzed included dipropylene glycol n-butyl ether (DPnB), propylene glycol, and dioctylsulfosuccinate sodium salt (DOSS). Only 68 samples (7 sediment and 61 water samples) had detectable concentrations of one of the dispersant constituents (Table 4). None of the concentrations of dispersant-related constituents found in the sediment and water samples collected in the nearshore zone exceeded EPA's chronic aquatic benchmarks (see Appendix A.1 of OSAT 2010 report, UAC 2010). DPnB was the most commonly analyzed and detected constituent (range of detection limits for sediment 0.00034 to 0.52 µg/g [0.34 to 520 ppb], for water 0.0014 to 1.0 µg/L [0.0014 to 1.0 ppb]) and was found in 62 (1 sediment and 61 water samples) of the 286 samples.

Figure 8. Distribution of amphipod results with total polycyclic aromatic hydrocarbons and EPA chronic aquatic life ratio for samples collected after 3 August 2010. The horizontal dotted line represents the EPA's chronic aquatic benchmark of 1.





Significant effects were observed in 25% (17 out of 68 samples) of the samples with detectable levels of the dispersant constituents DPnB and propylene glycol. No detectable levels of DOSS (range of detection limits 2.45 to 20 µg/L) were found in these samples. Evaluation of relationships between toxicity test results and dispersant constituent concentrations alone and relative to total PAHs are presented in Appendix B. DPnB was detected in water samples at concentrations <0.3 µg/L (cf. EPA screening level of 1 mg/L). Propylene glycol was detected in sediment samples at 1 µg/g or less (cf. EPA screening level of 500,000 µg/L). In addition to the very low levels of detected dispersant constituents, Total PAH concentrations in the samples showing significant effects were less than 0.05 µg/L in water and 0.01 µg/g in sediment, well below the EPA's chronic aquatic benchmark for PAHs.

Table 4. Summary of dispersant analyses for nearshore water and sediment sample locations collected during the DWH oil spill and associated Response.

Sample Type		Total # of Locations	Detected	Not Detected	Not Analyzed
Water	Pre-Impact	9	0	9	0
	Post 3 Aug 2010	181	61	117	3
Sediment	Pre-Impact	95	0	19	76
	Post 3 Aug 2010	466	7	174	285

3.7.3 Integrated Data Evaluation

Assessment of the contribution of MC252 oil to the observed effects in the samples collected after 3 August 2010 was first evaluated by calculating correlations between toxicity test endpoints and chemical constituents (Appendices A and B). No relationship

between measured chemical constituents and effects in the toxicity tests was apparent. Building on this initial assessment, the spatial extent and distribution of the co-occurrence of significant effects and MC252 oil was examined by 1) identifying locations with significant effects in at least one endpoint, 2) evaluating the chromatographic fingerprints of the samples with calculated Total PAHs >0.32 μg/g (320 ppb) in sediment, <0.03 μg/L (0.03 ppb) in water for samples with significant effects in toxicity tests and calculated Total PAHs >0.58 μg/g (580 ppb) in sediment, >0.18 μg/L (0.18 ppb) in water for samples with no significant effects for consistency with MC252 oil, and 3) calculating EPA's chronic aquatic life ratios for PAHs for each of these samples (Figure 9). The distribution and range of concentrations of constituents measured in this data set were representative of conditions in the Gulf of Mexico after 3 August 2010, but do not support development of empirical relationships with toxicity.

For a number of locations (218 out of 647 locations), significant effects were observed in the toxicity tests (Figure 9) (Map 7.11). Samples from 18 of these locations contained oil characteristic of MC252 oil, and 55 locations were not characteristic. The remaining 145 locations were not fingerprinted due to the low levels of TPAHs detected (<0.32 μg/g (320 ppb) in sediment, <0.03 μg/L (0.03 ppb) in water). The chronic aquatic life ratios for samples showing significant effects in any of the three categories (characteristic, not characterized, and not characteristic) ranged from 0 to 3.29 (median 0 to 0.004, mean 0.0004 to 0.36). This indicates that the distribution is skewed towards low concentrations relative to EPA's chronic aquatic benchmark for PAHs.

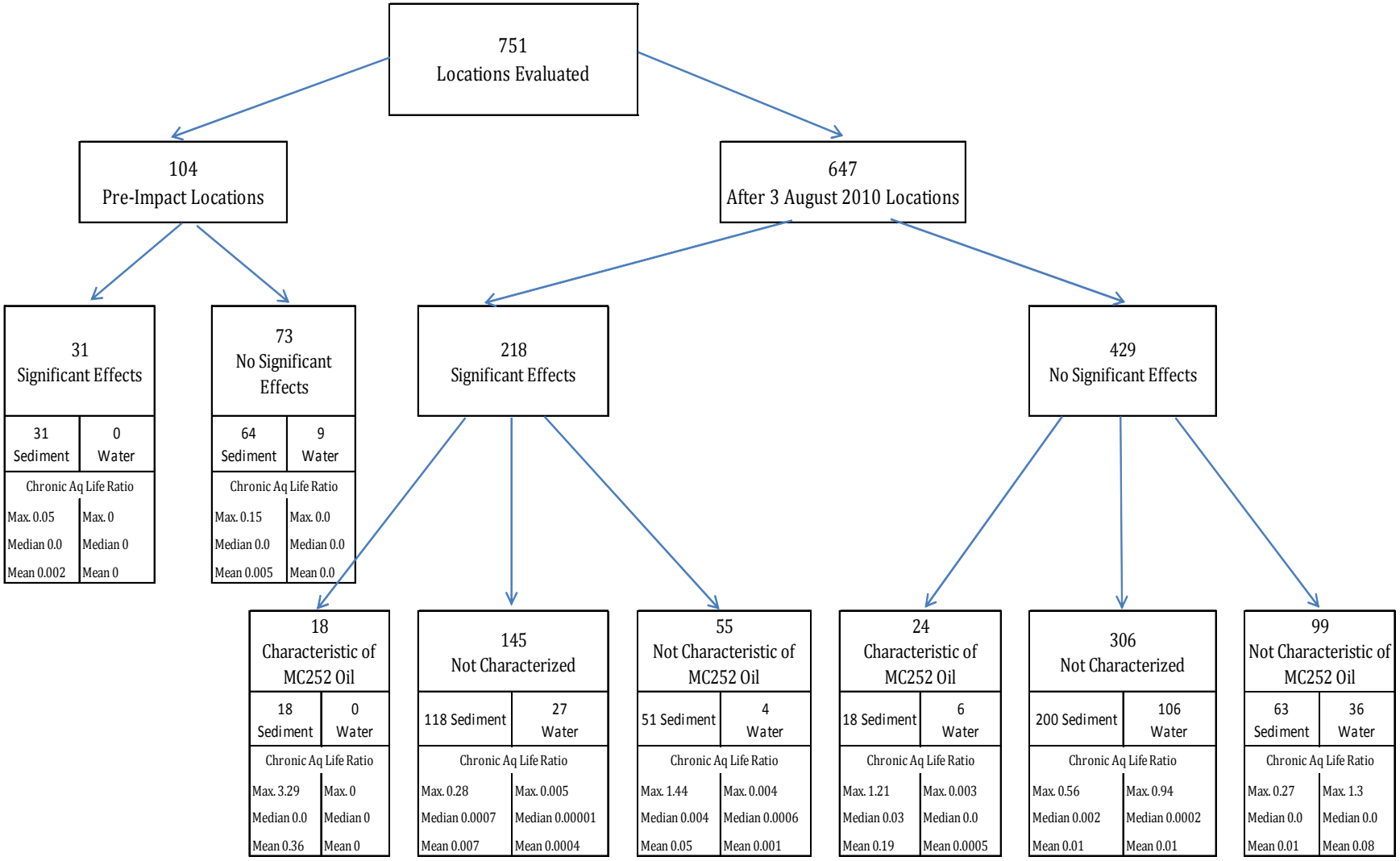
For the remaining locations sampled after 3 August 2010 (429 out of 647 locations), no significant effects were observed in the toxicity tests (Figure 9). Samples from 24 of these locations with no significant effects were fingerprinted as containing oil characteristic of MC252 oil, while samples from 99 locations were not characteristic, and samples from the remaining 306 locations were not fingerprinted. The chronic aquatic life ratios for samples showing no significant effects in any of the three categories ranged from 0 to 1.21 (median 0 to 0.03, mean 0.0005 to 0.19). This indicates that the distribution is skewed towards low concentrations relative to EPA's chronic aquatic benchmark for PAHs.

Results of the pre-impact tests show there are significant effects in nearshore sediment and water that are not related to MC252 oil (Figure 9). The chronic aquatic life ratios for samples showing significant effects ranged from 0 to 0.05 (median 0, mean 0.002). The chronic aquatic life ratios for samples not showing significant effects ranged from 0 to 0.15 (median 0, mean 0.005).

Other factors contributing to the significant effects observed in the toxicity tests may have included metals, other PAH sources, other oil constituents, and sediment and water quality characteristics. Grain size was identified as a contributing factor in worm tests conducted

by EPA Region 4 (EPA 2010). A similar pattern with grain size was observed in the BP amphipod tests. Ammonia was the primary contaminant in three of the sea urchin fertilization and embryological development tests based on toxicity identification evaluations conducted by the USGS (USGS 2011b).

Figure 9. Integrated evaluation of nearshore toxicity test data from the DWH oil spill and associated Response.



Section 4: Additional Toxicity Tests

Additional toxicity tests were conducted in the field and laboratory with samples from the deep water zone (region seaward of the 200-m bathymetric contour [approximately the continental shelf break]) and the offshore zone (region between the state water's boundary and the 200 m bathymetric contour, which delineates the continental shelf break) to guide DWH oil spill response actions. Raw data (e.g., collection date, location coordinates, sample depth, test conditions and results) for these additional tests are available at www.restorethegulf.gov and http://www.geoplatform.gov/gulfresponse/.

Screening toxicity tests were conducted during the Response with water and sediment samples collected in the deep water zone using the commercially available rotifer toxicity test (RotoxKit M™) and Microtox® procedures. Rotifer tests were conducted shipboard on freshly collected samples during subsurface dispersant application as mandated by the *Dispersant Monitoring and Assessment Directive* issued 10 May 2010 by the USCG and EPA to BP. Water samples were collected at depth using a rosette containing a Niskin bottle array. The RotoxKit M test kits and protocol were used for the rotifer (*Brachionus plicatilis*) tests. Of the 1,047 samples evaluated with rotifer, 2% (22 samples) showed mortality of greater than 20%.

Microtox tests were conducted at an onshore laboratory with shipboard-frozen sediment and water samples from locations in the deep water zone as outlined in the *Strategic Plan for Sub-Sea and Sub-Surface Oil and Dispersant Detection, Sampling, and Monitoring* issued 13 November 2010 by the Deepwater Horizon MC252 Response Unified Area Command (Strategic Plan). Sediment samples were taken from the top 3 cm of sediment collected from the sea floor with multicorers. Water samples used for toxicity testing were collected from the supernatant water overlying the sediment in the core tube. The Microtox analyzer and protocol were used for the photoluminescent marine bacteria (*Vibrio fischeri*) tests. Of the 557 Microtox tests performed, <1% of the sediment tests (2 out of 227 tests) had EC50 values less than 1,000 mg/L and 1.5% of the supernatant tests (5 out of 330 tests) showed decreases in detectable light emission in undiluted samples.

Section 5: Conclusions

The OSAT 2010 report concluded that sampling was adequate to address the presence and distribution of sub-surface oil and dispersants (with the exception of very shallow nearshore areas where submerged tar mats may be present) (UAC 2010). The results of the toxicity tests summarized in this addendum support the conclusions of the OSAT report regarding the absence of actionable (i.e. amenable to removal actions) oil and dispersant-related constituents in the nearshore zone.

A conservative approach was utilized in this report addendum to evaluate the toxicity tests and associated analytical chemistry data generated by the EPA, USGS, and BP. Extensive evaluation of the data using a variety of approaches and statistical analyses determined that:

- A total of 3,548 toxicity tests were conducted during the DWH oil spill and associated Response, making this the most extensive testing program ever conducted to characterize the effects of an oil spill in the marine environment. Overall, statistically significant effects were reported in 10% of the toxicity tests.
- Of the 1,426 toxicity tests conducted on samples (including 907 sediment and 519 water tests) collected after 3 August 2010 in the nearshore zone from 647 locations, 19% (267 tests) showed statistically significant effects compared to their associated controls.
- Based on evaluation of analytical chemistry results for the toxicity samples collected after 3 August 2010, 1% of sediment locations (5 out of 466 locations) and <1% of water locations (1 out of 181 locations) exceeded EPA's chronic aquatic benchmark for PAHs. None of the water or sediment pre-impact samples exceeded the benchmark.
- None of the concentrations of dispersant-related constituents found in the sediment and water samples collected after 3 August 2010 in the nearshore zone exceeded EPA's chronic aquatic benchmarks.
- Statistically significant effects were observed in 31% of the 104 pre-impact locations in the near shore zone. Seventy-nine of the pre-impact locations were resampled after 3 August 2010, and 32% of those locations (24 sediment and 2 water locations) show significant effects.
- Of the 466 sediment locations sampled after 3 August 2010 in the nearshore zone, 18 showed significant effects and had chemical fingerprints characteristic of MC252 oil.

Fifty-five locations (51 sediment and 4 water locations) that showed significant effects were not characteristic of MC252 oil.

- Out of a total of 647 nearshore locations sampled after 3 August 2010, 451 locations were not fingerprinted due to low total PAH concentrations. Of these locations, 145 locations showed significant effects (total PAH concentrations <0.32 μg/g [320 ppb] in sediment, <0.03 μg/L [0.03 ppb] in water), and 306 locations did not show significant effects (total PAH concentrations <0.58 μg/g [580 ppb] in sediment, <0.18 μg/L [0.18 ppb] in water).

- These data were collected to determine the presence or absence of potentially actionable oil in the nearshore zone and not to develop empirical relationships between oil constituents and toxicity. While representative of conditions in the Gulf of Mexico after 3 August 2010, the skewed distribution and narrow range of concentrations of constituents measured in this data set do not support development of empirical relationships with toxicity.

Based on evaluation of the extensive dataset of toxicity test results and associated chemical analyses, the sampling conducted during the Response was adequate for decision-making by the FOSC regarding when to transition nearshore activities from the emergency response phase to the long-term recovery and restoration phase. MC252 oil was present in some of the samples showing significant effects in the toxicity tests. Additional locations that contained MC252 oil did not show significant toxicity test effects. Further locations were not fingerprinted due to low total PAH concentrations. In some samples, other contaminants (e.g. metals, ammonia, other PAH sources) may be contributing to significant effects observed. In EPA Region 4 (2010), for example, grain size was identified as a factor in the significant effects observed in the worm tests. Ammonia was the primary contaminant in three of the sea urchin fertilization and embryological development tests, based on toxicity identification evaluation (USGS 2011b). Long-term chronic effects to the environment are being assessed as part of the injury assessment conducted through the Natural Resources Damage Assessment (NRDA) process.

This Ecotoxicity Addendum to the OSAT (2010) report provides an assessment of the effects of field-collected samples on water column and sediment-dwelling organisms in the nearshore zone. This information is intended to support decisions on further oil removal operations in that area. Addressing other questions, including the long-term environmental impacts of the DWH oil spill, is beyond the scope of this report.

Ecotoxicity information was evaluated as one indicator of the presence of actionable MC252 oil. The OSAT is confident that the quality of these data is sufficient to address the presence or absence of MC252 oil within the context of an operational emergency response

evaluation. However, several factors should be considered in interpreting the findings of this document for any other purpose besides assessment of actionable oil.

- Sample detection limits varied. Because some analytes had very high detection limits, the treatment of "below detection" results (calculating them as zeros rather than using other approaches, such as one-half the detection limit) has the potential to underestimate concentrations.
- The chronic aquatic life ratios used in the Chronic Aquatic Benchmark for PAHs are estimates. Since some calculations did not include contribution of volatile organic compounds, ratios may be underestimated in some circumstances. Some analyses did not include alkylated compounds. The use of a multiplier for these samples to adjust total concentrations could underestimate or overestimate concentrations. The use of benchmarks alone may be insufficient to predict toxicity, cover all possible effects and modes of action, or life stages.
- Many TOC values used in calculating the Chronic Aquatic Benchmark for PAHs for sediments are estimates. Actual sample results were often not used to interpret concentrations relative to benchmarks. Although a conservative TOC concentration was applied, this could underestimate or overestimate exceedances of sediment quality benchmarks for individual samples.
- Determining whether MC252 oil is present in any sample is dependent on the chemical analyses performed and can be confounded by the presence of multiple hydrocarbon sources.
- Variability in sample collection procedures may influence the chemical analyses and toxicity test results.

Section 6: References

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Section 7: Maps

Appendices

Appendix A: Correlation Matrix of Toxicity Test Endpoints with Chemical Analytes

Appendix B: Distribution Plots of Toxicity Test Endpoints with TPAH and EPA's Chronic Aquatic Benchmark for PAHs

Appendix C: Sample Fingerprinting

Appendix D: Toxicity Re-Evaluation of Pre-Impact Samples

Appendix E: List of Acronyms

Appendix F: OSAT Membership