A Consensus on the State of the Knowledge and Research Recommendations on the Fate and Effects of Deep Water Releases of Oil, Dispersants and Dispersed Oil

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ABSTRACT 299967:

American Petroleum Institute (API) and its member companies have initiated a multi-year research program to generate information that can be used in subsea dispersant application decision-making. An important part of this program is the evaluation of biodegradation and toxicity of oil, dispersants and dispersed oil in a deepwater environment. The available scientific literature was reviewed by a panel of international experts in deepwater ecology, toxicology, microbiology, and petroleum chemistry, who summarized the state of the knowledge on these topics and recommended additional studies that would inform subsea dispersants decision-making. The recommended research projects have been funded by API. This paper summarizes findings to-date on toxicity and biodegradation of oil, dispersants and dispersed oil in deep water environments.

INTRODUCTION:

To better understand the fate and effects of oil, dispersed oil, and dispersants in a deep water environment, the American Petroleum Institute (API) initiated a research program to conduct relevant biodegradation and toxicity research to advance the state of the knowledge. This information will be used to inform net environmental benefit analysis (NEBA) associated

with dispersant applications at uncontrolled, deep sea releases of oil. To design this research program, API sought to outline the state of the knowledge, identify areas for future research, and develop research objectives that could be achieved in the next three years.

A workshop was held in October 2012 in Houston, Texas, to develop a framework to meet these objectives. Experts from academia, government, non-governmental organizations, and industry were asked to summarize best practices and existing, reliable data on biodegradation and toxicity of dispersants, oil, and dispersed oil; and reach consensus on test protocols for future research in biodegradation and toxicity of dispersants, oil, and dispersed oil on deep water (or surrogate) species. The attendees were advised that generated data should have strong environmental relevancy to the fate and effects of oil releases in deep sea environments; testing and analyses should be cost effective and timely, with the goal of providing industry and government decision-makers relevant data in the next two to five years; and, recommended test projects should allow technically credible extrapolations over a wide range of geographies and oil spill scenarios.

CHEMISTRY OF OIL AND GAS AT DEPTH[1]:

State of the knowledge

Phase changes are most common with gases, and encompass gas present as bubbles, liquids, dissolved in seawater, or frozen as hydrates. The gas phase plays a major role in regulating the buoyant velocity and the behavior of a subsea oil plume. Additionally, increased pressure creates a phase shift where gas molecules can form clathrate hydrates that decrease oil droplet buoyancy. Dissolution of gases may also alter the toxicity of a solution.

Oil droplet size is determined by release rate, orifice size, gas-to-oil ratio, and the chemical properties of the oil. Changes in oil droplet size play a role in the fate and transport of the oil plume due to changes in buoyancy and greater bioavailability to carbon degrading organisms. To illustrate, a 10-fold decrease in oil droplet diameter decreases rise rates by almost 40-fold.

When considering natural turbulence and biodegradation, droplets less than 100 microns are not expected to rise to the surface. This diameter could be higher for denser oils or oils that interact with suspended sediments. External factors such as dispersant application may also influence oil droplet size. Hydrocarbon degradation begins almost immediately after a spill. During microbial consumption of oil and gas molecules, ethane and propane are degraded initially, followed by methane (Valentine *et al*., 2010; Kessler *et al*., 2011).

Skadsheim (2004) observed that uptake of larger, less water soluble polycyclic aromatic hydrocarbons (PAH) may increase at high pressures in that PAH uptake at 400 atmospheres (atm) was 20-100 percent higher than 1 atm. Uptake increased linearly with the K_{ow} of the PAH compounds. However, no increased uptake was noted at 200 atm, implying limitations to the experiment or the correlation between pressure and increased uptake.

[1] The deep ocean, for this workshop, was defined as those waters greater than 1000 meters (m) in depth, and may be characterized by high pressures and low temperatures.

From this evaluation of the state of the knowledge of the chemistry of oil and gas at depth, several conclusions were made regarding potential research topics, including:

- Pressure affects the solubility of gas molecules and other volatile compounds. This change is non-linear as gases no longer behave according to the ideal gas law at depths around 200 m. Additionally, pressure affects the solubility of other crude oil components.
- A wide range of various analytical chemistry techniques are available which provide methods to obtain whole oil, fixed gases, volatile, semi-volatile, and non-volatile compounds contained in oil. Each technique can also provide isotope differences so that a wide range of questions can be asked of each sample.
- More research is necessary to analyze for chemical dispersant constituents. A method with a low-detection limit exists for dioctyl sodium sulfosuccinate (DOSS), found in Corexit 9500, but no low-detection limit methods exist for the remaining surfactants.

Recommendations for future research: Chemistry

The highest priority issue identified by the workgroup was to understand how the solubility and partitioning of petroleum compounds changes with pressure and temperature. Significant changes in both solubility and partitioning with pressure could influence the design of both biodegradation and toxicity tests. The group's consensus points included:

- To develop an appropriate dosing mechanism, deep water chemistry must be understood before toxicity testing is initiated. This approach will provide information on dosing techniques and chemical analyses required to interpret the results of toxicity tests.
- Single chemical component toxicity testing needs to be conducted both at the surface and under pressure to understand the technical aspects of exposure and response. Once single component chemical testing has been completed and is understood, multiple component testing (with crude oil) could be undertaken. The results can be used to model PAH toxicity levels to determine relationships between increased pressure and toxicity.
- Surrogate toxicity testing employing semi-permeable membranes devices, solid phase micro-extraction, and stir bar testing could be considered. There are challenges with these types of methods as desorption and sorption rates are unknown and the time it takes to reach equilibrium needs to be examined to allow full interpretation and extrapolation of toxicity test data. These exposure methods, however, may not be an effective manner to determine toxicity related to volatiles and gases.
- Concentrations of dispersed oil in water must be as representative as possible during controlled toxicity testing programs. For certain studies, higher concentrations may have to be used (e.g., 1 ppm TPH for biodegradation, 1-10 ppm TPH for toxicity, etc.) to attain lethal exposure conditions.
- Natural seeps should be considered for *in situ* high pressure tests.

Recommendations for future research: Oil droplet and gas bubble sizes

The crude oil release scenarios for a deep water well control event may range from extremely high velocity to relatively low energy releases. The release conditions will depend on the geometry and size of the release point, the temperature and pressure of the reservoir, the flow path from the reservoir to the release point, the amount of associated gas in the oil, and the

properties of the oil. These factors, combined with the addition of dispersants, may influence the oil droplet and gas bubble size distributions. These size distributions, the gas-to-oil ratio (GOR), and the crude oil properties will in turn influence hydrocarbon bioavailability.

The proposed research program cannot test all potential scenarios. Therefore, the research should focus on a well-dispersed crude oil that forms dispersed oil droplets <70 microns in diameter. Further, only reservoirs containing low viscosity oils are likely to flow at high rates. Consequently, the preferred oil for initial testing should be a low-viscosity crude (<5 centipoise [cP] at 60°F). High energy releases of crude oil will likely have high GOR. Thus, test protocols that evaluate the effects of dissolved gases should be representative of this characteristic. Exposure regimes used for both biodegradation and toxicity testing should use plume models and concentrations of dissolved components expected in a likely deep water release scenario. Other research sponsored by API should be consulted to ensure that appropriate metrics are used when defining exposure regimes during biodegradation and toxicity testing.

Recommendations for future research: Formation of gas hydrates

The sequestration of low molecular weight hydrocarbon gases into gas hydrates may influence their bioavailability in deep ocean environments. Gas hydrate formation could influence both the toxicity and biodegradability of a dispersed oil mixture. The hydrate formation issue is complex and the findings from another API research effort could be used to determine if an investigation of the influence of hydrates on biodegradation and toxicity should be pursued.

OIL, GAS, DISPERSANTS AND DISPERSED OIL BIODEGRADATION:

State of the knowledge

Physical weathering.

Crude petroleum hydrocarbons are subject to weathering that affects their composition after leaving the formation location (McGenity *et al.*, 2012). At the surface, evaporation dominates the weathering of the lighter molecules, dissolution of water-soluble components, and photochemical oxidation (Harayama *et al.*, 1999). Petroleum hydrocarbons released below water can dissolve, remain in the water column, or rise to the surface. These in-the-water-column processes are altered in the deep ocean as increased pressure can result in greater solubility, reduced oil droplet sizes and reduced degassing. Therefore, subsurface weathering may result in different petroleum component composition shifts compared to weathering in shallow depths.

Biological processes.

Biological processes in oil degradation include transformation of organic molecules to other forms (such as carbon dioxide and water), incorporation of oil components into cell biomass, and (or) mineralization to constituent nutrients and carbon dioxide or methane. Degradation capability and carbon utilization, and the rates of these processes, depend on local factors including the co-location of petroleum hydrocarbon with oil degrading microbes; the presence of essential micro- and macronutrients; the presence of terminal electron acceptors, and the physical and chemical conditions suitable for microorganism growth.

Co-location of oil and degraders.

Different petroleum components are degraded by a variety of organisms using many potential degradation pathways. Prince *et al.* (2010) compiled a comprehensive list of known petroleum degrading bacteria, which have been found in all diligently-searched environments. Therefore, it is reasonable to expect petroleum degraders to be present with oil released at depth.

The abundance of oil degraders tends to decline with depth. Recent microbial census activities in the deep ocean biosphere show communities are different in composition and abundance from that observed in shallower waters and yet the deep ocean offers a vast range of metabolic potential (Sogin *et al.*, 2006; Amaral-Zettler *et al.*, 2010). In some cases, relatively rare taxa can rapidly reproduce to take advantage of energy sources presented by spilled oil (Hazen *et al.*, 2010). Thus, it is possible that low abundance, widely distributed degrader populations are sufficient to meet the need for co-location degrader populations and oil resulting from natural seeps or an accidental oil release.

Presence of essential nutrients.

Researchers have recognized and described the ability of deep-sea microorganisms to degrade organic materials at slow rates, as limited by nutrient availability (Atlas & Bartha, 1972; Jannasch & Wirsen, 1973). Additionally, the ability of native communities to degrade petroleum hydrocarbons has been intensively studied, showing that temperature, oxygen, and nutrients were indicated as critical factors affecting bioremediation potential (Atlas, 1981; Leahy & Colwell, 1990; Margesin & Schinner, 2001). While oil can certainly be degraded in deep-sea environments, degradation rates may be dependent on local abundance of oxygen and nutrients, which can vary widely, yet are at levels capable of sustaining some level of degradation.

Presence of terminal electron acceptors.

Local redox potential and associated terminal electron acceptors profoundly affect the population of petroleum hydrocarbon-degrading and -utilizing organisms, the pathways used to degrade and incorporate petroleum hydrocarbons, and the rate at which these processes occur. Specifically relevant to the deep ocean environment, energetic yields change with increasing pressure which in turn may affect the processes and rates of petroleum degradation and utilization in deep ocean ecosystems. Thus, rates of heterotrophic degradation for petroleum hydrocarbons in the deep ocean may result in lower energy yield for microbes in most redox phases, and may result in slower population growth and longer lag phases. Thus, a diversity of metabolic processes may bear on petroleum biodegradation and carbon utilization, whether in surface waters or in the deep ocean (Antic *et al.*, 2006; Heijs *et al.*, 2008).

Conditions suitable for the growth of degrading microorganisms.

Co-location of microbes and substrate alone is not a sufficient condition for petroleum degradation. Physical and chemical conditions, such as temperature, pressure, salt concentration, and pH, must be in appropriate ranges to allow growth of the indigenous oil-degrading microorganisms (Daffonchio *et al.*, 2006). The extreme physical conditions of high pressure, cold temperatures, low oxygen and sometimes high salinity found in the deep ocean have not prohibited microbial growth (Prince *et al.*, 2010). When conditions are appropriate, degradation occurs along a number of biochemical pathways specific to the types of microbial communities and petroleum compounds present. Different transformation pathways are utilized for different

petroleum hydrocarbon groups (e.g., linear alkanes, simple aromatic hydrocarbons, polynuclear aromatic hydrocarbons, ashphaltenes, etc.).

Recommendations for future research on biodegradation

There are a number of areas where additional research to support oil spill response decision making may not be necessary. The group recommended preparation of a scientific paper to document the current state of the knowledge on biodegradation, including documenting the scientific consensus supporting the following observations:

- hydrocarbon degraders are ubiquitous in the open ocean;
- microbial populations of hydrocarbon degraders increase rapidly in the presence of hydrocarbons;
- dilute solutions of oil in small droplets biodegrade faster than oil in surface slicks or oil concentrated in sediments and dispersed oil is sufficiently dilute that oxygen and nutrient concentrations are not limiting factors for biodegradation;
- biodegradation rates of various oil types that are currently produced in deep water and which are amenable to chemical dispersion do not vary substantially;
- the gas component and oil droplet behavior at depth is a factor in the process of biodegradation, but research efforts should focus on the dynamics of the degrader populations and their means of accessing oil contained in droplets; and,
- the specific brand of dispersant used is unlikely to have a substantial effect on ultimate rates of oil biodegradation – assuming that different dispersant products are similar in their effectiveness in generating oil droplets of a desired or optimal size distribution.

The report summarizing the state of the knowledge on deepwater biodegradation of dispersants, oil and dispersed oil will also facilitate identification of areas for additional research.

SENSITIVITY OF DEEPWATER ORGANISMS TO DISPERSANTS, OIL AND DISPERSED OIL:

State of the knowledge

Valuable ecosystem components.

Valuable ecosystem components (VECs) are species that are important to deep sea food webs, representative of pelagic and demersal habitats, relatively abundant, and of potential cultural or commercial importance. By focusing on more common species toxicity tests, the likelihood that studies can be conducted with appropriate numbers of test organisms increases.

Within the mesopelagic region, diel vertical migrator (DVM) groups exist that can endure significant changes in temperature, pressure, and dissolved oxygen (DO). The key groups in this category that are VECs and relatively abundant for capture are the calanoid copepods, euphausiids, and myctopid fish. While no one species is found in all of the biogeographic regions, within each group there are families or genera that encompass the spectrum from the polar to the tropic climates.

Mesopelagic invertebrates that are VECs and DVMs range from invertebrates to fish, such as copepods, euphausiids, chaetognaths, squid and lanternfish (Myctophidae). These groups

are abundant, geographically diverse, and have been used in laboratory tests. In many cases, the mesopelagic invertebrates are phylogenetically similar to shallow-water species (e.g., Calanus and Euphausia). In addition, there are oil and dispersed oil sensitivity data on some copepods and euphausiids at surface pressures, providing a comparison point for deep-sea species.

The lanternfish and bristlemouths (Gonostomatidae) are among the most abundant mesopelagic fish and are an important trophic link between the copepods and euphausiids and upper trophic level predators from the mesopelagic, bathypelagic, and demersal community. The two groups are phylogenetically distinct from shallow-water species. Myctophids and gonostomatids DVMs can be captured near the surface at night and are pre-adapted to wide ranges in temperature and pressure. These conditions make them ideal candidates for testing the effects of chemically and physically dispersed oil under varying temperatures and pressures.

For species inhabiting the lower depths of the water column (>1000 m) and near the sea floor, vertical migration is not as prevalent. VECs that represent these communities are also important to determining the effect of oil and dispersant exposure. Possible taxa in this category include amphipods (Lysianassids); decapods (Olophoidae, Sergestidae, Penaeids, and Aristeidae; fish (Sablefish [*Anoplopoma fimria*] and Rattail); and deep sea corals (*Lophelia* spp.). These taxa are not the only VECs, but have secondary characteristics (e.g., species densities, barotolerance, lipid content) that may make them more easily collected or suitable for laboratory exposure. While many of the bathypelagic species are genetically distinct from shallow-water species (e.g., the order Lophogastrida which includes the genus *Gnathophausia*), some species such as Sablefish are cultured and may be a possible test species. Mussels are somewhat barotolerant and may represent a shallow-water surrogate for bioaccumulation testing.

Organisms living in deep water sediments are typically diverse but scarce. Collecting sufficient numbers of deep water infaunal organisms for testing would be challenging. Deep water epifauna include species that can be attracted to bait, generally in high abundance and with hyperbaric traps could be returned to the surface in good abundance. Mobile epifaunal scavengers may provide the best opportunity to test benthic invertebrates.

Deep sea corals are more common than once thought and may occur near cold-seeps. Corals are susceptible to smothering from sediments or drilling muds; however, there are some indications that deep-sea corals may tolerate oil exploration activities in that reef building species have been found to settle and grow on oil and gas platforms. Experiments with corals are feasible and laboratory studies have been conducted under surface conditions with coral fragments collected from trawls and submersibles.

Existing toxicity data for vertically migrating species.

Laboratory research has shown that the stress of pressures from depths will cause significant behavioral, physiological and biochemical changes in marine fish and invertebrates adapted to living in shallower marine environments (i.e., <100 m), ruling out the option of conducting toxicity tests with routine laboratory test species under representative deep sea pressures of and extrapolating the results to deep-sea toxicology. Other studies showed that many deep-sea species would not tolerate toxicity testing at surface conditions due to the behavioral, physiological and biochemical changes they underwent when brought to shallow depths.

However, some deep-sea species will thrive under shallow water conditions, making them suitable for standard laboratory toxicity testing. Toxicity tests with deep-sea species conducted at surface pressures may not resolve all the uncertainty regarding toxicity of oil at depth due to potential changes in exposure conditions at depth and metabolic or physiological changes the organisms undergo to survive extreme pressures; however, such data would be an acceptable first start at benchmarking the relative sensitivity of these species against the wealth of toxicity data available for shallow water species.

As shown in Table 1, some evaluations of oil toxicity to species with vertical ranges exceeding 500 m have been done with physically and chemically dispersed oil or specific PAHs under surface pressures. The acute median lethal concentrations for Water Accommodated Fractions (WAF) and Chemically Enhanced Water Accommodated Fractions (CE-WAF) for species that have a broader vertical range was compared to species that are more strictly shallow water species (Figure 1). It is important to note that all exposures represented in this dataset were exposed at surface pressures. For this overview, data summarized by de Hoop *et al.* (2011) using standard exposures was combined with data from spiked exposures (Gardiner *et al.*, in prep) to allow a broader group of species and a more robust data set to be compared, albeit with an additional variability component. Species with an extended depth range do not appear to be different in their relative sensitivity to physically or chemically dispersed oil. The adaptations that allow these species to move from the surface to depths as low as 4,000 m do not appear to alter their sensitivity to the test preparations at surface conditions.

Table 1. Taxa that Spend Some Time at Depth, Collected and Tested Under Surface Conditions.

Species	Depth Range (m)	Tested Temp	WAF Spiked	CEWAF Spiked	WAF All	$C_{11}H_{10}$	$C_{10}H_8$
Boreogadus saida (Fish: Arctic Cod)	900	2°C	√	√	√	√	√
Hippoglossoides platessoides (Fish: American Plaice)	3,000	NR				√	
Calanus glacialis[1]	1,000	2°C	√	√	√		
Calanus finmarchicus[1]	1,300	2°C	√	√	√		
Calanus hyperboreaus[1]	2,400	2°C			√		
Calanus sinicus[1]	>1,000	8°C	√	√	√		
Paracalanus aculeatus[1]	4,206	8°C	√	√	√		
Pandalus borealis (Shrimp)	1,330	5°C	√	√	√	√	
Anonyx nugax (Amphipod)	1,697	4-10°C	√		√	√	√
Strongylocentrotus droebachiensis (Sea Urchin)	1,200					√	√

Note: (1) Copepod

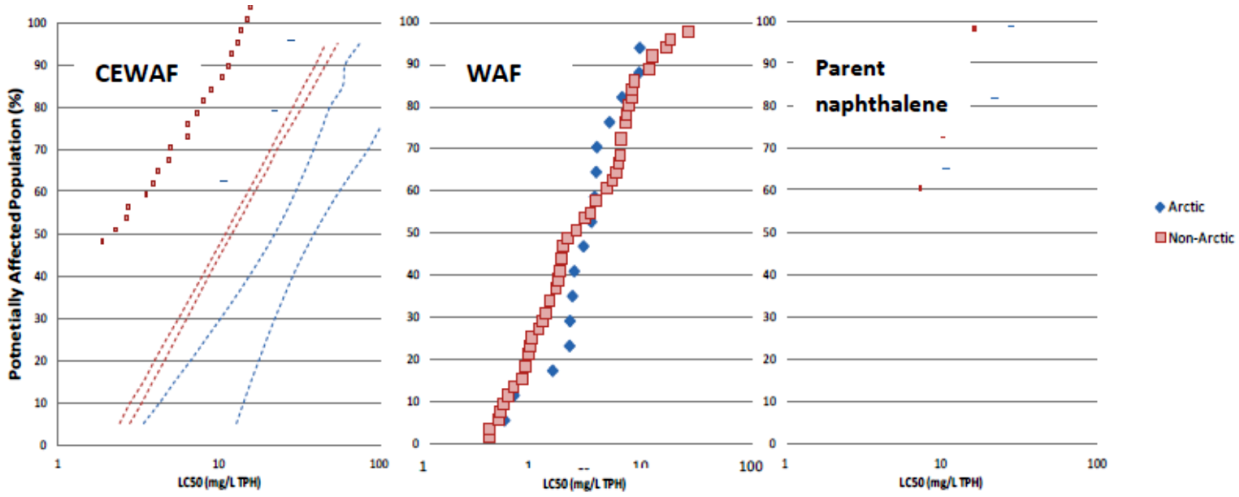

Figure 1. Species Sensitivity Distribution for CEWAF, WAF, and Parent Naphthalene Exposures (Word and Gardiner *in review*)

Current knowledge on oil toxicity for deep-sea species

While no studies to date have been specifically directed at comparing the relative sensitivity of deep water species under deep sea conditions to more temperate species, there are some indications that they may have a comparable sensitivity. Tests with polar species at temperatures representative of cold deep waters did not result in marked difference in sensitivity. Furthermore, species that have a vertical distribution down to 1000 m or more do not appear to be more or less sensitive when tested at surface pressures. Finally, limited studies with shallow-water species exposed to oil at depth appear to be more sensitive under pressure due to exposure change resulting from pressure alone and not an additional toxic response. Many of these studies conducted with species at high pressures were pilot studies with low replication and limited doses and were clearly influenced by pressure-related effects. Additional studies with the use of species adapted to deep sea conditions would presumably remove this source of uncertainty and allow a clearer comparison.

Recommendations for future research on toxicity

Central to toxicity research is the understanding of factors such as temperature, pressure, time, and life stage in the context of conducting toxicity testing representative of deep water conditions. The initial goal of future work should be to determine the relative sensitivity of deep water VEC organisms to petroleum and dispersants compared to shallow water species whose sensitivities have been well characterized. The initial phase of the testing should be conducted with acute exposures to selected, single hydrocarbons (e.g., a representative monoaromatic, diaromatic and polyaromatic hydrocarbon) maintained at constant concentrations to derive comparative response to known chemicals for deep water and shallow water species based on species sensitivity distributions (SSDs) similar to the approach reported by Olsen *et al.* (2011). The availability of acute toxicity data for single hydrocarbons can also be used to support the development of models that link hydrocarbon structure to toxicity thereby advancing a mechanistic framework for predicting toxicity of dispersed oil (Redman *et al.*, 2012).

It's recommended to perform the initial toxicity tests with barotolerant species, with the first set of test conditions set within the following parameters: one atmosphere of pressure; no light; with temperatures, salinities, and DO concentrations appropriate for the test species/life stage. Subsequent testing under pressure may be performed after the initial studies are completed and appropriate pressure dosing and exposure characterization methods have been developed. Results from these studies can be used to refine the effects model to potentially take into account the potential antagonistic effect of pressure on toxicity (Kaminoh *et al.*, 1998).

CONCLUSIONS:

As a result of the literature analysis and scoping workshop, several high priority biodegradation and toxicology projects were recommended and will be funded by API to advance the state of the knowledge regarding dispersants, oil, and dispersed oil in the deep water environment. These projects include:

Determine the Need for High Pressure Laboratory Biodegradation Research. Scientific literature should be reviewed to determine if additional testing is needed to confirm what is already understood regarding oil biodegradation at depth. Several questions should be answered:

- What information is available and what does it indicate regarding biodegradation of dispersants and dispersed oil in deep water? Does additional testing need to be conducted? Under what conditions should these tests be conducted?

Literature & Model Review on Aquatic Toxicity of Gas Molecules at Depth and the Role of Pressure on Hydrocarbon Toxicity. To support subsequent toxicity projects, scientific literature and existing modeling data should be reviewed to determine the potential exposure and toxicity of lightweight hydrocarbons at deep sea depths. Several questions should be addressed:

- Does pressure have a significant effect on the aquatic toxicity of hydrocarbons?
- Under what conditions would C1-C4 hydrocarbons cause significant toxicity to deep sea and shallow water species following a wellhead release? Can existing toxicity models predict the toxicity of C1-C4 hydrocarbons to aquatic organisms?

Understanding Exposure to Oil Components at Depth. There is a need to understand deep water marine species exposure before toxicity test results can be interpreted. This research should be divided into two parts. The first one should address the following questions:

- Using available exposure modeling knowledge, what are the predicted exposure concentrations of dissolved hydrocarbon constituents given several deep water release scenarios of a representative oil? Do WAF/CE-WAF protocols used in laboratory toxicity testing need to be modified to reflect the modeled exposure concentrations occurring following a deep water release? What are the current limitations and assumptions in exposure modeling that can best benefit from further studies or additional data?

Once these initial questions have been answered, during the next research phase testes under pressure will be designed and carried out.

Species Sensitivity Distribution (SSD) Check for Deep Sea Species. Testing new marine species at 1 atm with a single test chemical may generate repeatable and interpretable data and results may be readily compared to the existing database. This research should include acute, lethal toxicity tests at 1 atm using constant single test chemical exposures with at least three deep sea species. These test will answer the following questions:

- Where deep sea species fit on the existing SSD curves relative to other previously investigated species tested at 1 atm? Which potential endpoints could be used for sub-lethal or chronic toxicity endpoints and rates of recovery from sublethal exposures?

Toxicity of Continuous and Spiked Exposures to Crude Oil for Deep Sea Species. Once there is confidence with test species and test systems using single chemical exposures, testing would be conducted with mixtures (i.e., WAFs and CE-WAFs) at 1 atm and under pressures representative of the deep sea environment. The following questions will be answered:

- Where LC50s for these deep sea species fit among existing SSD curves for WAFs, CE-WAFs and dispersant alone? How LC50s for deep sea species change with pressure and what is the extent of any change in SSDs for WAFs and CE-WAFs and dispersant alone.

Test if Pressure Changes Affect LC50s and Species Position on Single Chemical SSD. Testing new species at pressure with a single test chemical may generate the most repeatable and interpretable data and may be readily compared to the existing database as well as data previously generated at 1 atm. This research would conduct acute, lethal toxicity tests at high pressure using a single test chemical on a representative deep sea species. The following questions will be answered:

- What is the extent of biochemical/physiological changes that might affect uptake or metabolism at pressure? What is the significance of any observed shift in LC50s with respect to where this would put deep sea species on existing SSDs for species tested at 1 atm and when tested at pressure?

The API research group has developed a three phase testing program, to be conducted over the next two to three years. Contracts were awarded and work has began in the following projects: *Determining the Need for High Pressure Laboratory Biodegradation Research*; *Understanding Exposure to Oil Components at Depth Literature & Model Review on Aquatic Toxicity of Gas Molecules at Depth;* and *The Role of Pressure on Hydrocarbon Toxicity*. A key component of each of the projects is the development of a paper suitable for publication in a peer-reviewed journal. The research papers that are developed as part of this project will be submitted for publication to ensure wide distribution after undergoing the rigorous examination afforded by the peer-reviewed process. Additionally, every attempt will be made to present the research findings at internationally-recognized symposia.

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