



## Independent Human Health and Environmental Hazard Assessments of Dispersant Chemicals in Australia, produced by NICNAS and CSIRO

AMSA/National Plan preamble to the three independent reports by:

NICNAS (April 2014)	<i>Chemicals used as oil dispersants in Australia: Stage 1. Identification of chemicals of low concern for human health</i>
NICNAS (October 2014)	<i>Chemicals used as oil dispersants in Australia: Stage 2. Summary report of the human health hazards of oil spill dispersant chemicals</i>
<b>CSIRO (August 2015)</b>	<b><i>A review of the ecotoxicological implications of oil dispersant use in Australian waters.</i></b>

### The Australian National Plan Dispersant Strategy

The Australian National Plan for Maritime Environmental Emergencies has had a longstanding dispersant response strategy that is transparent, fit for purpose and effective, and safe to use for people and the environment. At all stages of dispersant management: acceptance and purchase; storage and transport; and application in spill, the National Plan requires transparency. These requirements, results and processes are all published on the AMSA website. To ensure that Australia has suitable information to undertake all these steps, AMSA has always sought the best independent advice it could find. Most recently AMSA addressed questions of human health hazards and environmental hazards.

### Health hazard assessment by National Industrial Chemicals Notification and Assessment Scheme (NICNAS)

NICNAS comprehensively addressed the question of dispersant health hazard in two stages. The first stage assessment identified 2 of 11 chemicals to be of low concern for human health. The second stage was a more full assessment that concluded that 7 of the 11 chemicals were of no concern. The remaining four were considered hazardous based on Safe Work Australia's Approved Criteria for Classifying Hazardous Substances. Of these three were already in the Safe Work Australia Hazardous Substances Information System (HSIS) and the fourth will be added to HSIS by NICNAS for completeness, now the assessment has been completed.

### Environmental hazard assessment by CSIRO

CSIRO reported on the state of knowledge of the environmental hazards from dispersant use worldwide and within Australian waters. CSIRO noted that modern dispersants are much less toxic than spilled oil. However, their use can increase localised oil toxicity, but this is very short-lived due to the dilution effects and will result in much lower exposure and dosage than without dispersant use. They noted that some areas, some species groups and some organism life-stages are more susceptible to oil and oil/dispersant exposure than others. AMSA has addressed this in National Plan and AMSA policies.

### AMSA response to the independent reports

AMSA has for many years been aware of the chemical constituent of dispersants, and has in place rigorous procedures and safeguards for purchase, storage, handling and use of dispersants, that minimize human exposure at all phases of the dispersant cycle. These were reviewed and revised in light of the new information.

AMSA accepts low environmental toxicity dispersant formulations that are also readily biodegradable. The rigorous requirements for secure storage and transport ensure no inadvertent release. Pre-use assessment through a rigorous and robust expert NEBA also ensures the right dispersant is used on the right oil at the right time in the right location and for the right duration. When specific knowledge gaps cannot be addressed during a response, a precautionary approach is taken to the NEBA and use approval. Any time a dispersant is used, real-time and post-application monitoring occurs to assess its effectiveness and effects.

Any questions or comments, please use the AMSA contact us page on the AMSA website.

Paul Irving, Scientific Coordinator, AMSA. May 2016

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# A review of the ecotoxicological implications of oil dispersant use in Australian waters

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August 2015

Prepared for the Australian Maritime Safety Authority

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## Terms and Definitions

*Acute*: Toxicity that occurs following a short exposure (alternatively lethal effects as per the ANZECC guidelines)

*Alkanes*: linear chains of hydrocarbons. These can be unsubstituted (all carbon and hydrogen in single bonds) or substituted (containing carbon-carbon double bonds)

*Aromatics*: hydrocarbons arranged in a ring. These can have one ring (monoaromatic hydrocarbons) or multiple rings (polyaromatic hydrocarbons)

*Archaea*: the third domain of life, along with the bacteria and the eukaryotes (all multicelled organisms and unicellular organisms with organized chromosomes)

*Bioaccumulation*: the uptake of a contaminant from the environment and storage within the tissues or cells of an organism

*Biodegradation*: the break down of contaminants by microbial organisms

*Container effects*: Artefacts introduced in the laboratory that arise from using a fixed volume and limiting diffusion

*Chronic*: Toxicity that occurs over long exposure periods (alternatively sublethal effects as per the ANZECC guidelines)

*Detritovore*: An animal that eats debris and organic carbon, typically in soil or sediment

*Edema*: An abnormal accumulation of fluid

*Heterocycles*: aromatic compounds with carbon and at least one other element (typically sulphur or nitrogen) in its rings

*Heat shock proteins*: a stress response protein that has roles in protecting and repairing other proteins

*Infauna*: animals that live in sediment or soil.

*Lethal*: Toxicity that causes death

*Multi-xenobiotic resistance protein*: A stress response protein that pumps contaminants out of cells

*Net Environmental Benefit Analysis*: The environmental risk assessment protocol typically used for the selection of oil spill countermeasures including the use of chemical oil dispersants. Chemical dispersion should only be undertaken when it is thought to be a net benefit to the environment relative to natural attenuation (natural recovery) and other spill response options.

*Narcosis*: Also called baseline toxicity. Non-specific toxicity that occurs when contaminants disrupt cell membranes.

*Nauplii*: A juvenile life form of copepods and other crustaceans.

*Phototoxicity*: Toxicity that occurs as a result of or is enhanced by UV light.

*Pycnocline*: Also the thermocline. Warm water is less dense than cold water, so large bodies of water (such as the ocean) typically have (at least) two layers that do not mix. The depth at which they are separated is called the pycnocline.

*Receptor mediated toxicity*: Toxicity that occurs as a result of specific contaminant binding to a specific intracellular ligand.

*Saturated Hydrocarbons*: Those that contain no double bonds between carbons

*Sedimentation*: the process of having particles sink and be incorporated in the sediments

*Sublethal*: toxicity that causes effects other than death. Even though developmental effects may cause mortality of the embryo or early life stage, they are often thought of as sublethal because they affect recruitment, not the maintenance of adult populations.

*Weathering*: the changes in composition that oil undergoes as it is exposed to the environment.

## Abbreviations

<i>AhR</i>	Aryl-hydrocarbon receptor
<i>ANZECC/ARMCANZ</i>	Australian and New Zealand Environment and Conservation Council, Agriculture and Resource Management Council of Australia and New Zealand.
<i>BTEX</i>	Benzene, Toluene, Ethylbenzene, Xylene (monoaromatic hydrocarbons)
<i>CEWAF</i>	Chemically enhanced water accommodated fraction, i.e. chemically dispersed oil prepared following the methodology specified by Singer et al.(2000) as described below, modified to include the addition of dispersant. Importantly, droplets are allowed to collect at the surface before solutions for testing are collected.
<i>CEWSF</i>	Chemically enhanced water soluble fraction, i.e. chemically dispersed oil not prepared using the methodology specified by Singer et al., 2000. As these are often prepared differently in each study, the physical and chemical differences between these and exposures prepared with standard methodology are uncertain.
<i>CYP 1A</i>	Cytochrome p450 1A, the first enzyme in the metabolism of oil in vertebrates.
<i>DOSS</i>	Dioctyl sulfosuccinate
<i>EROD</i>	Ethoxyresorufin-o-deethylase, an enzyme activity assay that can be used to measure Cytochrome p450 1A, the first enzyme in the metabolism of oil in vertebrates
<i>EC<sub>50</sub></i>	The concentration at which 50% of the population is affected
<i>HEWAF</i>	High energy enhanced water accommodated fraction, i.e mechanically dispersed oil
<i>LC<sub>50</sub></i>	The concentration at which 50% of the population is dead
<i>NATA</i>	National Association of Testing Authorities
<i>PAH</i>	Polycyclic aromatic hydrocarbon
<i>TPH</i>	Total petroleum hydrocarbon
<i>WAF</i>	Water accommodated fraction, i.e. oil prepared following the methodology specified by Singer et al., 2000. To briefly summarize, in this preparation, a known quantity of oil is added to seawater and allowed to mix (using a vortex on a stir plate) for 24 hours. The water below the oil at the surface is then collected and used for toxicity testing. This methodology is meant to include the components of oil that naturally dissolve in sea water, but not entrained droplets.
<i>WSF</i>	Water soluble fraction, i.e. chemically dispersed oil not prepared using the methodology specified by Singer et al.( 2000). As these are often prepared differently in each study, the physical and chemical differences between these and exposures prepared with standard methodology are uncertain, but may include entrained droplets and other insoluble components.

## Executive summary

Chemical dispersants are used following oil spills to break up surface slicks. This can prevent oil spills at sea from reaching coastal and shoreline environments and reduce the direct exposure of marine mammal and bird populations to surface oil slicks. However, this dispersion puts planktonic and benthic organisms at a greater exposure risks because the dispersed oil is transported into the water column as small oil droplets that may also eventually impact bottom sediments. As the effectiveness of dispersants typically declines with the weathering of oil which enhances its viscosity, responders need to quickly decide if they are going to apply chemical oil dispersants following a spill. The current decision process is based on a Net Environmental Benefit Analysis (NEBA) to determine whether the benefits of chemical dispersant use outweigh the risks of other active oil spill countermeasures (e.g., physical recovery, in situ burning, etc.) as well as natural recovery (i.e. natural attenuation). To fully evaluate the gains in environmental services or other ecological properties attained from the use of dispersants against potential environmental injuries from their use, NEBA assessors need scientific information. This information should describe the influence that the dispersant has on the rates of oil biodegradation and whether the dispersant adds to the toxicity of the oil, either by making the oil constituents more bioavailable or through toxicity of the dispersant itself.

Oil is normally dispersed by physical weathering processes and biodegradation by bacteria in the aquatic environment. Dispersants can both make the oil more water-soluble and more available to the bacteria, and thus speed up the rates of dispersion and oil degradation, or they can exert toxicity to bacteria or prevent them from attaching to oil droplets, which will slow down the rates of oil degradation. There is also the potential for diauxic growth, where the dispersant is preferentially degraded instead of the spilled oil. The composition of both the type of oil spilled and dispersant used determines which impact occurs. Following the *Deepwater Horizon* oil spill, dispersion of oil with Corexit formulations increased the rates of oil degradation in the Gulf of Mexico. Consideration of factors such as these must be given towards the selection of future dispersants to be used in Australia.

Modern dispersants are much less toxic than oil. However, some studies indicate that they may add to the toxic response of organisms in the water column. Studies comparing the toxicity of oil and dispersed oil need to be carefully designed to ensure that they are accurately measuring the amount of oil and exposure time to which the organisms are exposed by measuring the amount of oil in the water, and not just the amount of oil added to the system. Studies conducted in the last few years suggest that while fish embryos are very sensitive to exposure to oil (and hence to dispersed oil), and that the dispersant does not add appreciably to the toxic response at concentrations expected in spill response operations. Studies conducted with invertebrates suggest that dispersants may increase the toxicity of oil, but these studies are difficult to interpret because oil exposure was not well quantified. Ideally, a dispersant would be chosen that does not increase the toxicity of oil to key species in an area. There is a knowledge gap for the dispersants that would be used in Australia because the toxicity of these dispersants has not been tested in combination with oil and often the most relevant life stages within the regions have not been considered.

The Australian Maritime Safety Authority (AMSA) currently requires that the toxicity of dispersants be tested on a range of species, and that the LC<sub>50</sub> values (lethal concentration to 50% of the species) for fish and crustaceans be above 10 mg/L. However, the effects of these dispersants have not been tested with oil, nor has the impact of dispersion on the rates of oil degradation been tested. While many studies have been conducted on Corexit, the dispersant used in the U.S. following the *Deepwater Horizon* oil spill, the chemical formulations of the dispersants for future use in Australia are anticipated to be different, and thus effects may be different. The impacts may also differ due to differences in the composition/ types of oil spilled, which would influence their potential biodegradation rate. Scientific information regarding risk and benefits associated with chemical dispersant use is needed for decision making under our current Net Environmental Benefit Analysis (NEBA) system for the selection of spill response options.

# 1 Introduction and Background Information on Dispersants and their Use

Oil spills are ecological disasters that can have long-term impacts on the environment. These impacts can range from two years following the *Prestige* oil spill in Northern Spain (Martinez-Gomez et al., 2009) to as much as decades following the *Exxon Valdez* spill or the *Metula* oil spill in Chile (Peterson et al., 2003; Owens et al., 2008). Following these incidents, there is often a huge public outcry that the damage be minimised, both to protect the environment but also to protect the livelihoods of people who depend on coastal resources, e.g. fisheries, aquaculture and tourism. Despite advances in technologies and safety practices, accidents will continue to occur. The growing exploitation of Australia's offshore oil and gas resources and increased shipping traffic near Australia's coasts means that there is a high likelihood of an oil spill of some magnitude in the future. Once an oil spill occurs, response options include mechanical techniques such as containment with booms coupled with physical recovery with oil skimmers, chemical dispersal, or, in rare cases, burning the oil (although this has yet to be used in Australia). Following the use of dispersants in the *Deepwater Horizon* spill in the Gulf of Mexico in 2010 (and to a lesser degree, the Montara well release in the Timor Sea in north-western Australia in 2009), there has been increased interest in the potential ecological risks associated with dispersant use, in part due to media attention on the issue.

Although the ecological health impacts of dispersants has been reviewed previously (e.g. NRC, 2005, 2013; Wise and Wise, 2011), dispersant formulations change frequently, and a substantial amount of new research has been conducted. Most of these reviews were, however, with dispersant formulations commercially available in North America and Europe. This review examines issues associated with products identified for potential future use by Australia. It discusses principles behind the use of dispersants, then reviews new information provided since the 2005 report by the US National Research Council (NRC, 2005), with special attention paid to the studies resulting from the *Deepwater Horizon* incident. The review then addresses the specific risks associated with the use of those chemical dispersants on the Australian Oil Spill Control Agent Register (AMSA, 2011). Recommendations are made for additional testing and research needed to accurately evaluate the "Net Environmental Benefit Analysis" (Coehlo et al., 2013) for use of dispersants locally, i.e. whether the benefits of using a chemical dispersant outweigh the benefits of natural attenuation of oil with consideration of the potential for negative consequences from adding another contaminant to the environment.

When oil is spilled on the sea surface under calm conditions, it will spread out to a thickness of approximately 0.1 mm (NRC, 2005). The oil slick is further spread by current, wind, waves, and by droplets sinking, aggregating and then resurfacing. Oil from sub-surface well blowouts during offshore oil production may be further influenced by the turbulence and buoyancy of natural gas simultaneously released that would promote the formation of a larger surface slick (NRC, 2005). Diffusion of toxic components present in the oil from the surface slick into the water column tends to be slow (in comparison with the dispersed oil).

The objective in using chemical dispersants following an oil spill is to break up oil slicks and increase the amount of oil that enters the water column (NRC, 2005) by the formation of small oil droplets that are dispersed by natural physical processes (e.g. currents) to concentrations below toxicity threshold limits. Furthermore, the formation of oil droplet increases the surface to volume ratio of the oil, increasing its bioavailability to oil degrading microbes. The use of chemical dispersants is intended to allow oil droplets to become so diffuse in the water column that nutrient concentrations no longer limit biodegradation (NRC, 2014). Although this increased concentration of oil in the

water column increases the potential for exposure for benthic and pelagic organisms, it decreases the potential exposure for birds and marine mammals as well as decreasing the amount of oil deposited on shorelines (NRC, 2005). In addition, the visual impact of the spill is significantly reduced.

Surface oil poses an ecological threat to animals that need to cross it to breathe (such as marine mammals), and to sea-dwelling birds. Wind and currents can also push this oil onshore (as was the case with the 1989 *Exxon Valdez* spill in Prince William Sound, Alaska), where it can be deposited in the shoreline sediments and a potential source of contaminants for many years (Atlas and Hazen, 2011). The impacts of oil spills may be greatest in coastal systems that support higher levels of living biomass and where there is less water depth into which the oil can diffuse.

Dispersant formulations are mixtures of chemicals (typically a solvent with at least one surfactant) that reduce the tension at the oil-water interface, causing oil to break up into small droplets and disperse into the water column by wave energy and turbulence (NRC, 2013). Once oil droplets are thus entrained into the water column, they move away from the source oil via turbulent mixing, advection, and diffusion (Fingas, 2011). If there is insufficient mixing, oil droplets will aggregate and resurface (NRC, 2005). Dispersion of oil into small droplet sizes is preferred because this better prevents particle aggregation and resurfacing (NRC, 2005). Oil droplets may be transported further away from the source by sub-surface currents. If these currents are not unidirectional, they can cause further dispersion (NRC, 2005). Oil droplets can also interact with suspended particulates, which will cause enhanced deposition of oil in the bottom sediments (NRC, 2005; Gong et al., 2014; Sorenson et al., 2014).

The efficacy of chemical dispersants is dependent not only on physical oceanographic conditions, but also on the viscosity of the oil or refined product being dispersed. Salinity, temperature, and oil composition (i.e. the amount of saturated hydrocarbons) can also influence dispersant efficacy (Fingas, 2011). Oil viscosity is dependent on evaporation, water washing and emulsification (NRC, 2005). During the process of weathering, the viscosity of the oil changes. Low molecular weight compounds evaporate or dissolve into the underlying waters from an oil slick first, leaving behind resins and asphaltenes. Depending on wind and wave conditions, a foamy "mousse" can form, and these emulsions are not easily dispersed. Photo-oxidation makes oil more polar and can also increase the viscosity of the oil (NRC, 2005). As a consequence, using a chemical dispersant to disperse oil is only a feasible option shortly after a spill.

Although the use of chemical dispersants increases the amount of oil in the water column (relative to what is at the sea surface), it may not increase the concentration of oil, because in open ocean environments, the processes involved in dispersion will rapidly dilute the oil droplets and the soluble components of the oil (NRC, 2005). In fact, in deep waters, dispersants are thought to have minimal ecotoxicological effects because of dilution (NRC, 2005). However, use of dispersants greatly enhances the compound-specific dissolution from oil into water because of the increase in the oil-water surface area (NRC, 2013). It also can increase the solubility of high molecular weight PAH relative to undispersed oil (Wolfe et 1998,a,b, 2001; Couillard et al., 2005).

The ecological trade-offs involved in dispersant use are difficult to predict and that may indeed increase the risk associated with chemical dispersion. It is recommended that assessors perform a Net Environmental Benefit Analysis considering the trade-off between the toxicity of dispersed oil constituents and the benefits of a reduced half-life in the water column and reduced likelihood of oil depositing on shore (Coelho et al., 2013).

Evaluating the net environmental benefit of dispersant use is complicated, not only because of the myriad of social, political, economic and ecological aspects that are involved in responding to an oil spill, but because of the complexities of understanding oil and its dispersion at a systems level. Laboratory studies inherently have artefacts. The inherent difficulty in replicating the ocean at the bench scale (Lee et al., 2013) often results in poor study design. Oil is a complex mixture of

thousands of chemicals (Head et al., 2006), while dispersants are mixtures of additional compounds, and some dispersants are petroleum based, adding to the complexity. As a consequence, studies comparing the toxicity of dispersed and non-dispersed oil can be difficult to interpret. These compounds have different solubilities, and each compound reaches saturation in water differently, both with and without dispersion. Without measuring the total petroleum hydrocarbon (TPH) or total polycyclic aromatic hydrocarbon (PAH) concentrations in the test waters, the amount of oil to which the organism was actually exposed is unquantified, meaning that dose is unknown and the responses to oil cannot be meaningfully compared. If toxicity is expressed relative to loadings, dispersing oil often increases its measured toxicity. However, if the toxicity is related to TPH or total PAH concentrations, these trends are not always observed. The results typically vary from study to study as a result of differences in oil composition (Adams et al., 2014). Also, dispersed oil, when studied in the laboratory, is often created with a 1:10 dispersant to oil ratio (Bejarano et al., 2013). This is higher than the amount of dispersant needed to effectively break up surface slicks following an oil spill. The exact amount of dispersant applied to a given area can be difficult to quantify as dispersants are sprayed from planes, so a 1:10 ratio serves as a worst case scenario (Bejarano et al., 2013).

Currently, the literature recommends using established protocols to develop a water-accommodated fraction (WAF) (a test of water-accommodated oil components) and a chemically enhanced WAF (CEWAF) (the water-accommodated oil in the presence of a dispersant) (Singer et al., 2000) and that TPHs and total PAHs are characterised, so that treatments within a single study and different studies can be objectively compared (Coelho et al., 2013). Without these measurements, the interpretation of additive or interactive effects of oil and dispersant is difficult (Coelho et al., 2013). In addition, it has been pointed out that container effects (i.e. artefacts created by conducting experiments in enclosed spaces) may increase the observed inhibitory effects of dispersants, as they would result in concentrations of oil particles, dissolved components of oil, and dispersant that greatly exceed what would be observed under spill scenarios (Lee et al., 2013a).

The first generation dispersants (used in the late 1960s and early 1970s) were industrial cleaners that had toxicities that greatly exceeded those of the oil itself (EMSA, 2010). The so called second generation dispersants were much less toxic, but were not sufficiently concentrated to allow for the dispersants to be sprayed from planes. Instead, they had to be applied from ships at volumes approaching the spilled oil itself, posing limits to their utility. The current use (or third generation) dispersants are less toxic than oil and the original dispersants and sufficiently concentrated to be effective at a ratio of 1:10 dispersant to oil or less (EMSA, 2010). For instance, recent studies have found that Corexit 9500 is not more acutely toxic in standardized tests than common household cleaning products (Word et al., 2014). These third generation dispersants are covered in this review, as they are the only dispersants being considered for use in Australia.

The current guidelines for deciding whether or not to apply a dispersant from the IMO – (International Maritime Organization (Merlin et al, 2014)) suggest that a number of factors be taken into consideration. These include the characteristics of the oil, the logistics of dispersion, how quickly the oil and dispersant are likely to be diluted, and whether dispersion is going to have a net environmental benefit. IPECA/OGP guidelines also recommend that dispersants are not typically used in environments less than 30m depth. In order to complete the Net Environmental Benefit Analysis, the assessor needs to know about the distribution of biological resources in an area, the value of the resources from both an economic and environmental perspective, and the wind and current patterns that would be moving oil and dispersants in an area. It is not recommended that chemical dispersants be used in biologically sensitive areas. However, it is recommended that the habitats and the reproductive potential of organisms be protected. The example given in the guidelines is of an oil spill in a coral reef environment that abuts a mangrove swamp. The guidelines suggest that to protect the biological integrity of the system, that the oil be dispersed, even though it puts the coral at higher risk of oil exposure. The reasoning behind the recommendation is that if

oil is deposited in the swamp, it will damage both systems for years, as opposed to the dispersed oil posing a short-term risk in a coral reef environment.

If an assessor is going to determine how to best protect the biological integrity of a system, he or she will need to have robust information about both the persistence of the oil and the dispersant in natural systems and the hazards posed by oil, dispersed oil, and dispersants to organisms in that system. It is the availability of that information for the dispersants that would be used in Australia that is the subject of this review.

Conceptual models of how oil is likely to be distributed in the water column and sediments are shown in Figure 1. Figure 1A provides a holistic overview of how dispersed oil interacts with various receptors within the water column. Figure 1B shows more detail of how the oil is likely to be distributed in the open ocean, whereas Figure 1C shows covers near shore environments. Assuming a spill of the same product and treatment with the same agent, the main difference between Figure 1B and Figure 1C would be the residence time of the dispersed oil. In Figure 1B, the oil droplets would be expected to rapidly dissipate. By contrast, oil deposited into sediments (especially anaerobic sediments with limited biodegradation potential) can have a much longer residence time. Following the *Exxon Valdez* oil spill, oil can still be found in sub-surface sedimentary deposits more than 25 years later (Atlas and Hazen, 2011). Furthermore, contaminants in oil distributed in deep oceanic waters are expected to be dispersed and diluted to non-toxic concentrations. This would not necessarily happen in coastal environments with poor flushing (Merlin et al., 2014).

This review focuses on the environmental risks associated with dispersant use in oil spill response. Two main factors are important in determining the net detriments associated with the use of a dispersant: biodegradation and ecotoxicology. Biodegradation (breakdown of compounds by indigenous bacteria) of not only the dispersant but the dispersed oil largely determines the persistence of these toxicants in the water column and the potential for ongoing exposure. Ecotoxicology determines the response of biota in the water column to the dispersant and the dispersed oil.

Due to the recent number of scientific manuscripts recently released on the *Deepwater Horizon* oil spill and the response operations that followed, the circumstances surrounding the spill will be briefly summarized here along with emerging information on the biodegradability and toxicity of the oil spill released including the influence of chemical oil dispersant additions.

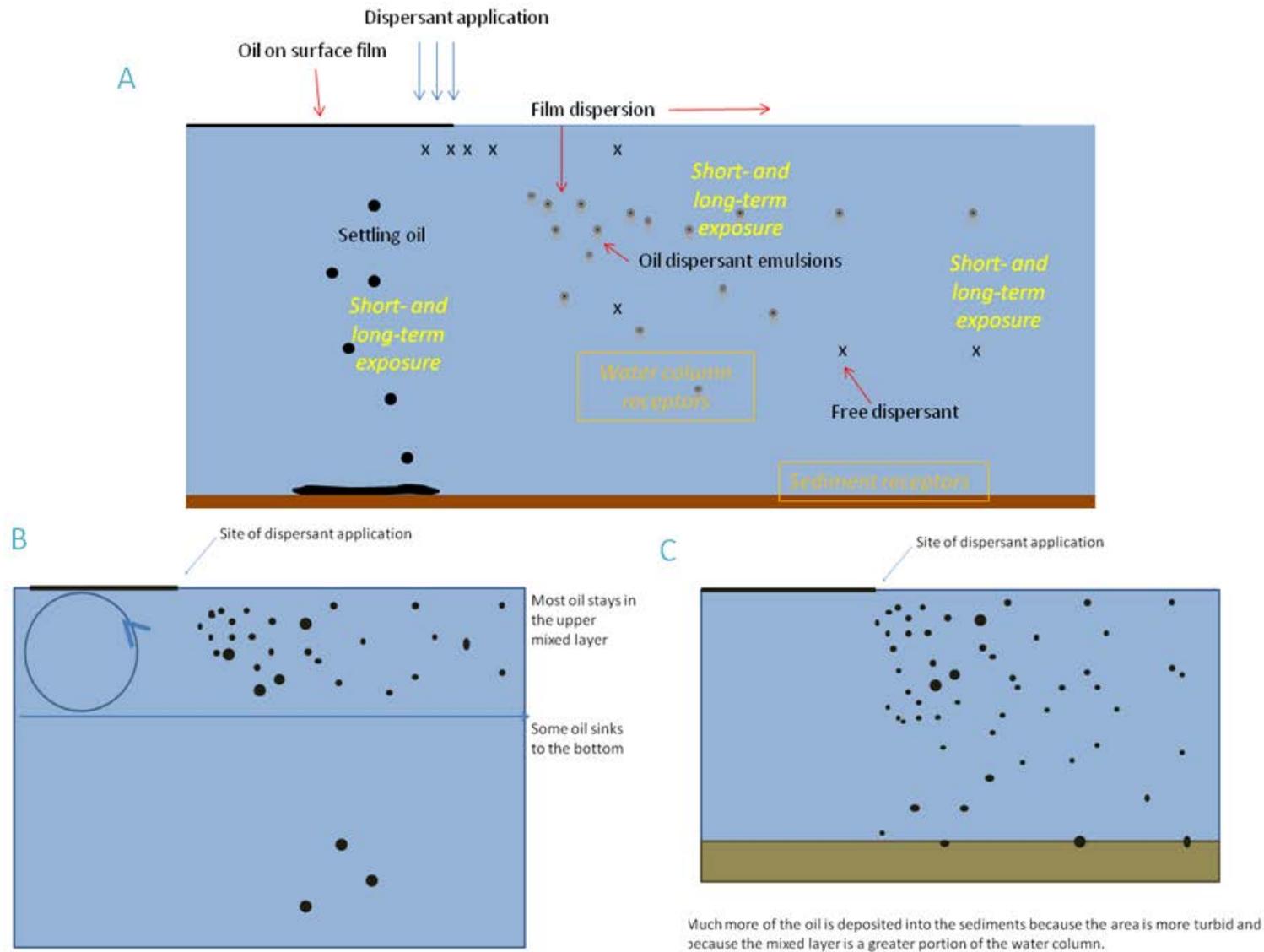


Figure 1. Conceptual model of oil dispersion. A. Overview of processes, B. Dispersion in the open ocean and C. Dispersion in shallower near-shore waters.

## 2 Review of the Recent Literature

### 2.1 Case study: The *Deepwater Horizon* wellhead blowout

In April 2010, the *Deepwater Horizon* wellhead (located in 1500 m deep water in the Gulf of Mexico, about 80 km south of the Louisiana (USA) coastline) exploded, releasing an estimated  $5 \pm 0.5$  million barrels of oil (or  $759 \pm 76$  million litres) (Atlas and Hazen, 2011; Gray et al., 2014). To reduce the amount of oil reaching productive coastal waters and sensitive wetland habitats and to reduce the exposure of volatile components from the oil to spill responders working at the spill site, a decision was made to add chemical oil dispersants (Atlas and Hazen, 2011). Surface and sub-surface additions of 4 million litres and 2.94 million litres, respectively of the chemical dispersants Corexit 9500 or Corexit 9527 (NRC, 2013; Campo et al., 2013; Gray et al., 2014) were made from surface vessels, aircraft and direct injection with the aid of ROVs at the wellhead. The addition of chemical dispersants has been estimated to enhance oil dispersion by 10–29% above that mediated by natural processes. While some of the oil was deposited along the Louisiana, Mississippi, Alabama and Florida coastlines and substantial amounts were degraded at sea or deposited in deep-sea sediments (Valentine and Benfield, 2013) the use of dispersants has been deemed to have provided a net environmental benefit by contributing towards a reduction of 500,000 barrels (or 8 million litres) of oil from being deposited on shore and into coastal ecosystems (NRC, 2013).

Numerous Natural Resources Damage Assessment (NRDA) studies on the ecological impacts of this spill and the application of various oil spill countermeasures, including oil dispersants, are ongoing or completed for upcoming legal proceedings. This review covers the scientific information open to the public as of November 2014.

#### CHARACTERIZATION OF THE SPILL

During the response to the *Deepwater Horizon* spill, water samples were collected below the surface in areas where surface dispersants were used, allowing for direct measures of water concentrations of both oil and dispersant. Analysis of these samples collected at the time of the spill, permitted characterisation of the TPH and total PAH concentrations, and the concentrations of dipropylene glycol *n*-butyl ether, one of the constituents of Corexit that was used as a tracer of that dispersant (Bejarano et al., 2013). Although the benchmark values set by the US Environmental Protection Agency (USEPA) for the protection of aquatic life for TPHs and total PAHs were exceeded, the values for the Corexit tracer were not. The TPH and total PAH concentrations were as high as 5 and 77  $\mu\text{g/L}$  respectively. It was noted that effective dispersion resulted in an average concentration of 10  $\mu\text{g/L}$  of total PAHs at 1 m depth, but only 0.29  $\mu\text{g/L}$  at 10 m depth, suggesting rapid dilution of oil droplets (Bejarano et al., 2013). Following oil dispersion, concentrations of high molecular weight PAHs (including phenanthrene and chrysene) also reached values that cause this cardiac impairment in fish embryos in laboratory studies (Bejarano et al., 2013; Incardona et al., 2004, 2013). The severity of the toxic impact has been shown to vary with both time and oil concentration, with short exposures (2.4 h) to environmentally realistic concentrations of oil have been shown to cause impacts in some studies (Greer et al., 2012). However, it is uncertain whether these exposures would have made a population level impact (reviewed in Fodrie et al., 2014), although some predictions suggest that recruitment of commercially important invertebrate species such as blue crab may have been impacted (Jones et al., 2015). Values greater than the US EPA water quality criteria for total PAHs were relatively rare, with exceedances occurring for 41 of 5733 near-shore

water samples (defined as shoreline to 3 nautical miles), 6 of 481 offshore samples (defined as 3 nautical miles to 200 meters depth) and 70 of 3612 deep-water (collected from water depths greater than 200 m) samples collected as part of the OSAT study (OSAT, 2010). Some water samples collected before the well was capped caused acute toxicity in laboratory based toxicity tests (Echols et al., 2015). These samples with levels above the US EPA criteria were located within 100 km of the wellhead were not detected after 3<sup>rd</sup> August 2010, three weeks after the wellhead was capped and fresh oil was no longer being added to the system. It is uncertain whether the oil concentrations measured in the Gulf of Mexico were elevated for a sufficient duration of exposure to cause toxic impacts.

For sediments in the near-shore environment, 13 of 1136 samples collected as part of the Operational Science Advisory Team (OSAT) study exceeded sediment quality benchmarks set by the US EPA for total PAH and were contaminated with Macondo oil. In the off-shore environment, no sediment samples exceeded guidelines, while in deep water, 7 of 127 samples exceeded criteria. The sediment exceedances were also near the wellhead (typically within 3 km) (OSAT, 2010).

The footprint of Corexit at sea was also surveyed by measuring the concentrations of dioctyl sulfosuccinate (DOSS, which also goes by the trade name Tween) and is the primary surfactant in both formulations of Corexit. Both DOSS and the solvent used in Corexit, dipropylene glycol butyl ether, are known to rapidly degrade in light (Gray et al., 2014; Glover et al., 2014), and Corexit has been shown to be microbially degraded at 5 and 20 degrees (Campo et al., 2013). The US EPA benchmark for the protection of aquatic life is 40 µg/L. At the surface, DOSS was detected in the highest concentrations in stations near the wellhead, although few stations had concentrations above 3 µg/L. It was found to be more persistent in the deep-water oil plume, as its degradation in the sub-surface was minimal. The maximum concentration observed at depth was 9 µg/L (Gray et al., 2014). For the entirety of the OSAT surveys, only one sample was found that exceeded the US EPA guideline for aquatic organisms (Gray et al., 2014). No dispersant water quality exceedances were measured in the OSAT program (OSAT, 2010).

However, more recent studies show that substantial quantities of the oil from the 1100 m dispersed oil plume were ultimately deposited into sediment (Valentine et al., 2014). Dispersed oil forms aggregates with sediment particles, and low viscosity oil, as well as more weathered oils with higher resin and asphaltene are more likely to form aggregates or sorb to sediment particles (Gong et al., 2014, Sorensen et al., 2014). Laboratory based studies have shown that the less labile fraction of oil (frequently referred to as the unresolved complex mixture), are not readily biodegraded, irrespective of the dispersant strategy used (Brakstad et al., 2015). As the labile fraction of the oil degrades, the density of the droplet increases, causing it to sink. In a recent study, the authors used the hopane concentrations in the upper sediment layer to suggest that this oil was from the *DeepWater Horizon* well head blow out. The deposition patterns and presence of some volatile alkanes suggest that this oil was never at the surface. Instead, the authors hypothesise that the oil deposited in deep sea sediments was a results of flocculation of insoluble components of the dispersed oil which flocculated once the more soluble, labile components had been degraded by bacteria (Valentine et al., 2014). The authors estimate that as much as 12% (4-31%) of the deep sea plume was ultimately deposited in sediments (Valentine et al., 2014). Other studies have come to similar conclusions using the isotopic signature of carbon deposited on the sea floor (Chanton et al., 2015), or by using this sections of sediment cores to compare rates the deposition of oil into deep sea sediments in 2010-2011 to other years (Romero et al., 2015).

As noted in a review by Hayworth and Clement (2012), a number of studies in coastal and inland waters have claimed that constituents of the Corexit dispersants, such as propylene glycol, 2-butoxyethanol, and sodium dioctylsulfosuccinate, were environmentally persistent and widely distributed. The conclusion of these studies has been questioned as these compounds are found in many household and industrial products, including as a solvent for lacquers and other coatings (2-butoxyethanol), in brake fluid, antifreeze, cosmetics, pharmaceuticals and synthetic resins

(propylene glycol), and widespread use as an anionic surfactant in pharmaceuticals, cosmetics, and pesticides (sodium dioctyl sulfosuccinate). Follow up investigations on the spatial and temporal distribution of these compounds indicated that they most likely originate from point and non-point terrestrial sources, not from the *Deepwater Horizon* usage (Hayworth and Clement, 2012). This is analogous to the finding of pharmaceutical residues within the aquatic environment as the result of municipal discharges (Fatta-Kassinos et al., 2011).

## 2.2 Impacts of dispersant use on rates of oil degradation

Much of the early literature (as reviewed in NRC, 2005) suggested that the impact of dispersant use on oil biodegradation was likely to be inhibitory, as surfactants may be directly toxic, or may cause indirect toxicity by increasing the solubility of the toxic components of oil; prevent bacteria from adhering to the surfaces of oil particles; and/or be preferentially degraded by bacteria over the oil (i.e. diauxic growth). The results of initial studies on the effects of chemical dispersion on oil degradation were frequently inconsistent limitations in the methods used to quantify oil degradation rates.

A microcosm study was performed by the State of Alaska to examine the rates of oil degradation with the addition of Corexit 9500 in comparison to those of undispersed oil (Davies et al., 2001). In these studies, chemical dispersion was found to stimulate biodegradation. Both dispersed and non-dispersed oil led to a rapid growth in hydrocarbon-degrading bacteria. However, the dispersed oil droplets were rapidly colonised by bacteria, suggesting that degradation rates were higher with dispersion (Davies et al., 2001). Furthermore, studies by Lee et al. (1985) also suggested that dispersed oil may be more quickly degraded due to toxic effects on bacteriophages (Lee et al., 1985) that reduced the “top down” pressure on bacterial populations.

More recent studies have suggested that since oil is degraded at the oil/water interface, dispersion would increase the rate of oil degradation (Atlas and Hazen, 2011). Studies comparing the rates of biodegradation of lightly weathered Alaska North Slope crude oil to that same oil dispersed with Corexit 9500 found a slight increase in biodegradation rates (Prince et al., 2013). Dispersed oil has a half-life in seawater of 11 days, whereas for undispersed oil, the half-life is about 13 days (Prince et al., 2013). Laboratory studies have replicated the rates of degradation of dispersed oil in a deep water plume (Brakstad et al., 2015). The authors found that degradation of labile compounds was faster with 10 µm droplet sizes than with 30 µm droplet sizes, suggesting that chemical dispersion would increase the rates of biodegradation if it decreased the size of oil droplets (Brakstad et al., 2015). However, C<sub>10</sub> to C<sub>25</sub> saturates are not readily bioavailable, and did not degrade appreciably in either treatment (Brakstad et al., 2015). Field-based studies on the degradation rates of dispersed Macondo oil (the oil released by the Deepwater Horizon spill) suggest that the residual oil was efficiently biodegraded (ASM, 2011). Microbial community analysis of water samples inside and outside of the hydrocarbon plume (that had been dispersed with Corexit 9500) indicated that hydrocarbon-degrading bacteria were enriched by the spill (Hazen et al., 2010; Chakraborty et al., 2012). Initially, the gamma proteobacteria (with the genus *Oceanospirillades* dominant) (Rivers et al., 2013) were enriched, followed by *Cycloclasticus* and *Colwellia*, and finally methanotrophs and methylotrophs in succession (Lu et al., 2012). Composition of archaeal species remained unchanged (Chakraborty et al., 2012). Functional enrichment of genes involved in BTEX (monoaromatic hydrocarbons), alkane, cycloalkane and PAH degradation, carbon metabolism, sulfate reduction, nitrate assimilation and metal resistance was also observed (Chakraborty et al., 2012; Rivers et al., 2013).

In summary, the creation of a large number of small oil droplets in the water column increases available the surface area of the oil to natural oil degrading bacteria, which colonize the droplets within a few days (Lessard and Demarco, 2000; MacNaughton et al., 2003). Contrary to common

belief, the background levels of oil degrading bacteria prior to a spill has little influence on the final outcome, due to exponential growth and their ability of bacteria to rapidly adapt to their surrounding environment (NRC, 2014). As dispersed oil will dilute to concentrations in the parts per million range within a few hours of effective dispersant application and to concentrations in the parts per billion range in one or more days depending upon the currents and wind dynamics (Lee et al., 2013); natural levels of biologically available oxygen and nutrients are not depleted and are sufficient to support efficient oil biodegradation (Swannell and Daniel, 1999; Hazen et al., 2010; Prince and Butler, 2014).

Similar findings regarding the potential oil biodegradation rates were also reported for coastal salt marsh sites and beaches. In a recent study, the fate of oil deposited in coastal Louisiana salt marshes was investigated using a combination of analytical chemistry, isotope analysis, next-generation sequencing for community composition, and lipid analysis (Mahmoudi et al., 2013). The results suggested that the oil at these heavily impacted sites (up to 50% of the dissolved organic carbon content was attributed to petroleum hydrocarbons) was rapidly degraded. Changes in microbial community structure over an eighteen month period were attributed to the biodegradation of the residual oil by the bacteria *Rhodobacterales* and *Sphingomordales* and the fungus *Dothideomycetes*. Archeal contributions to these processes were difficult to discern (Mahmoudi et al., 2013). In a separate study on beach samples, the metatranscriptomes and compositional structure of microbial communities collected from heavily oiled Grand Isle, Louisiana were analysed (Lamendella et al., 2014). Contaminated samples had a higher abundance of both bacteria that are known hydrocarbon degraders and of genes with a known function in breaking down hydrocarbons (Lamendella et al., 2014). However, in anaerobic sediments, these processes take place much more slowly (Turner et al., 2014 a,b). While the elevations in alkanes resulting from deposition of DWH oil are expected to persist only through 2015, oil from the Macondo wellhead blowout that was deposited into anaerobic sediments in coastal Louisiana is gradually becoming enriched in high molecular weight PAH, and these may be elevated for decades (Turner et al., 2014 a,b).

Recent studies comparing the capacity of strains of known hydrocarbon-degrading bacteria to break down dispersed oil have reported no evidence of inhibition by Corexit 9500A. These studies were conducted by comparing the loss of TPHs from water collected from the Macondo plume (which had both source oil and dispersant); when strains of known hydrocarbon-degrading bacteria were added versus water samples where heat-killed bacteria were added (Chakraborty et al., 2013). Bacteria isolated from uncontaminated deep-sea environments in the Gulf of Mexico also readily degraded crude oil more rapidly in the presence of Corexit 9500 (Baeleum et al., 2012). Further laboratory studies compared the rates of oil degradation rates of South Louisiana crude oil with and without the addition of Corexit 9500 at both 25°C (the temperature of surface waters) and 5°C (the temperature of the deep-water plume) (Campo et al., 2013). At 25°C, the indigenous bacteria completely metabolised both the oil and dispersant, this metabolism occurring at a faster rate with the addition of Corexit (Campo et al., 2013). Different results were found at 5°C. DOSS was not metabolised completely within 28 days at the lower temperature, and the high molecular weight n-alkanes were persistent in 5°C water. It was hypothesised that this persistence was a result of the n-alkanes crystallizing and becoming less biologically available (Campo et al., 2013).

Some laboratory studies conducted after the *Deepwater Horizon* incident did not support the idea that dispersants increase the rate of biodegradation. For instance, Hamdan and Fulmer (2012) used standardised microbial assays (and microorganisms as well as weathered oil isolated from a beach where *Deepwater Horizon* oil had been stranded) to show that the addition of Corexit reduced microbial activity and inhibited survival of hydrocarbon degrading bacteria viability. However, it has been pointed out that container effects (which occur in confined spaces because they do not allow chemicals to naturally dissipate) may have contributed towards the observed inhibitory effects of dispersants by maintaining elevated concentrations of oil particles, dissolved components of oil, and

dispersant above that observed under actual spill conditions (Lee et al., 2013a). Also, since the United States does not allow the use of dispersants within 3 nautical miles of the shoreline, the relevance of using dispersant on beach samples is questioned.

Dispersants themselves are biodegradable, but the rates of degradation can vary dramatically with the types of surfactant used (NRC, 2013). As a consequence, results obtained for Corexit formulations cannot be simply extrapolated to other dispersants. However, DOSS has been measured in deep sea corals and GOM beaches as long as 45 months after dispersant application ceased, suggesting that the compounds can be persistent in the environment and bioaccumulated (White et al., 2014).

## 2.3 Ecotoxicological impacts of dispersed oil

Environmental toxicology differs from biomedical toxicology in that, for environmental studies, it is the health of populations that we are concerned with, not individuals. We are interested in measuring impacts on growth, reproduction, fitness and survival. Toxicity (the negative effects of environmental contaminants on the health of plants and animals) can be measured using a variety of methods. Often these methods are standardised, and used fixed exposure periods (often 48-72 hours) and defined endpoints. Lethal tests (alternatively defined as acute per the Australian and New Zealand Environment and Conservation Council, Agriculture and Resource Management Council of Australia and New Zealand (ANZECC/ARMCANZ) water quality guidelines (ANZECC/ARMCANZ, 2000)) measure the survival of adult (sexually mature) organisms. Sub-lethal tests (defined as chronic by the ANZECC/ARMCANZ guidelines) measure other endpoints, such as growth, reproduction, larval development or fertilisation success. In general these sub-lethal processes are considered to be less severe with regards to the continued viability of populations than mortality of adults. The results of these toxicity studies are often presented as  $LC_{50}$  or  $EC_{50}$  values, that are the concentrations at which 50 percent of the population does not survive or is otherwise affected, respectively. Compounds with lower  $LC_{50}$  or  $EC_{50}$  values are more toxic. Many studies do not use standardised methods and measure other endpoints, which nevertheless, can still be used to predict the impact of a chemical contaminant on the health of a population.

### 2.3.1 OIL TOXICITY

Oil is a complex mixture of literally thousands of compounds (Head et al., 2006). The mode of toxic action is different for many of these compounds, but will not necessarily change with oil dispersion. Based on the measurement of concentrations during oil spill response operations, and existing toxicity data for oil and various dispersant formulations, the toxicity of dispersed oil at sea is thought to be driven primarily by the toxicity of oil. The modes of toxic action of oil are briefly summarised in the following paragraphs and would cover impacts from both oil and dispersed oil.

Low molecular weight petroleum-derived compounds cause toxicity via narcosis (i.e. interrupt membranes) (NRC, 2005). Toxicity is related to bioavailability. Since lower molecular weight compounds are the most bioavailable, it has been assumed that they drive toxic response. This process can be efficiently modelled (see Di Toro et al., 2000; Di Toro et al., 2007), however, narcosis-based models are under-protective for effects on fish embryos (due to blue sac disease and cardiac impairment, that is caused by higher molecular weight PAHs, as discussed in more detail below). Higher molecular weight compounds (such as multi-ringed PAHs and heterocycles) tend to cause toxicity via receptor-mediated processes and oxidative stress processes. Environmental factors can influence the physico-chemical properties of the oil and thus its mode of action in eliciting toxicity. For example, as a result of natural weathering, low molecular weight compounds will volatilise and leave behind PAHs and heterocycles (NRC, 2005). Furthermore, the bioavailability (and

consequently toxicity) of dissolved, colloidal and particulate oil may differ (NRC, 2005). Oil droplets can cause smothering, and direct contact with these droplets (especially for lipid-rich organisms or tissues) can increase oil bioavailability. Oil droplets can also be ingested (e.g. Almeda et al., 2013; Olsen et al., 2013).

Embryonic and larval stages of organisms are typically the most sensitive to oil, but this can vary depending on differences in permeability of the membrane around the egg (NRC, 2005). Recent studies examining the toxic responses to oil have found that proper cardiac formation in developing fish embryos is very sensitive to disruption via PAH exposure (Marty et al., 1997; Billiard et al., 1999; Carls et al., 1999; Barron et al., 2004; Colavechia et al., 2004; Incardona et al., 2004, 2013, 2014). Crude oil exposure also impairs cardiac excitation-contraction coupling in juvenile and adult fish (Brette et al., 2014). Disruption of normal circulation leads to yolk sac edema (or blue sac disease), and developmental failure. Three-ringed PAHs (such as chrysene and phenanthrene) exposure, as opposed to the classic Ah R agonists that are the known biomarker inducers and potent human carcinogens, seem to have the greatest potential to cause these malformations (Incardona et al., 2004, 2013, 2014). Even fish that survive the initial exposure to oil can have altered atrium/ventricle ratios, which causes decreased burst swimming speed, morphological deformities and, presumably, decreased survival under wild conditions (Hicken et al., 2011; Ingvarsdottir et al., 2012; Mager et al., 2014). Much less research has been done on embryonic exposure in invertebrates to determine whether similar sensitivity is seen.

Exposure to oil can also cause subtle impacts, such as increased oxidative stress, decreased immune competence, and increased physiological costs associated with metabolising oil (Theron et al., 2014). For instance, an increased prevalence of skin lesions was reported in fish collected from the Gulf of Mexico in 2011 that had biliary PAH metabolites matching the source oil from the Macondo wellhead (Murawski et al., 2014). However, as we expect that most exposure to oil following a spill is likely to be short term, this review primarily focuses on changes that are likely to be irreversible (i.e. death, reproductive and/or developmental failure) and manifested following a short exposure (one day or less). For instance, previous studies have shown that even short-term exposures (2.4 hours) to environmentally realistic concentrations of chemically dispersed oil can cause developmental abnormalities (Greer et al., 2013). However, although conclusive evidence that exposure to even low levels of oil cause organism level toxic effects, these are not always manifested at the level of populations because a myriad of factors can either obscure observation of these impacts or prevent these impacts from occurring, including spatial and temporal variability in population sizes, density dependant recruitment, and lagged impacts (Fodrie et al., 2014). Again, since the toxicity of dispersed oil is thought to be largely similar to the toxicity of oil, the effects summarised above would occur following exposure to either.

Fish embryos would be subject to the same mixing forces as oil droplets, and could be expected to have the same behaviour in the water column. Tanaka and Franks (2008) looked at the vertical distribution of Japanese sardine eggs in the water column, and found that although these eggs were positively buoyant, they were mixed through the water column to the pycnocline (a gradient between surface and deep waters- the two generally do not mix). By contrast, neutrally buoyant eggs were mixed below the euphotic zone. They hypothesized that positive buoyancy is advantageous because it keeps eggs in the euphotic zone, which is warmer (allowing for faster development) and food-rich, yet keeps them out of the sea surface microlayer, where they may be more subject to predation (Tanaka and Franks, 2008). Because eggs and oil droplets would be subject to the same mixing forces, there would be a high potential for eggs to be exposed to oil.

Most of the toxicity from oil and dispersant mixtures is associated with the dispersed oil, not from the dispersants themselves (NRC, 2005). Much of the current data on the toxicity of dispersants and dispersed oil is based on acute toxicity data (Bejarano et al., 2013). This may be appropriate for assessing the toxicity of dispersants that exert toxicity via membrane damage and narcosis, but dispersed oil exerts toxicity both by narcosis (which is non-specific) and receptor-mediated pathways

(in which hydrocarbons bind to the aryl hydrocarbon receptor), suggesting that the mortality endpoint of acute tests is not a good endpoint for predicting receptor-mediated effects, such as those that occur during embryonic development. Metabolism, bioaccumulation and photo-enhanced toxicity are also not addressed by these short-term tests (NRC, 2005). There are few studies on the toxicity of dispersants alone, and even fewer of these explore mechanisms, so contrasting the effects of oil, dispersed oil, and dispersant toxicity is difficult. Furthermore, most tests are based on a 48-96 h of exposure to a static concentration that is not observed at sea. The NRC (2005) report suggested frequently changing exposures during toxicity tests to allow for the reduction of TPH to concentrations in test solutions to below the limits of detection by 8h to mimic the natural dissipation of dispersed oil during a spill. In contrast, however, following the 1996 *Sea Empress* spill, where dispersants were used in relatively shallow water, measured TPH concentrations were still above 1mg/L for as much as two days after chemical dispersion (reviewed in EMSA, 2010). The general consensus based measurements of elevated PAH and TPH concentrations in the water column following an oil spill indicate that they dissipate very rapidly (e.g. typically at least ten fold within 24 hours (reviewed in EMSA, 2010, Lee et al., 2013a)).

The following provides an overview of the studies that have been reported recently on the effects of dispersed oil. It focuses primarily on water column testing and coastal organisms. It does not include wildlife, marine mammals and sea birds for which dispersant use is thought to provide a net benefit by reducing the probability of their exposure to surface oil slicks.

## Phytoplankton

A recent review of the impacts of crude oil on phytoplankton, macroalgae and aquatic vascular plants compiled data that had been collected on the relative toxicity of dispersants, dispersed oil and undispersed crude oil (Lewis and Pryor, 2013). Unfortunately, this review did not include many of the dispersants on the Australian schedule. Because of differences in experimental design, and because many of the formulations for which data are available are out of date, few generalisations about the comparative toxicities could be made. Fresh oils were said to be typically more toxic than weathered oils, light oils more toxic than heavy oils, and refined oils more toxic than crude, suggesting that the lower molecular weight components of oil are driving the toxic responses in algae and plants (Lewis and Pryor, 2013). Few conclusions could be drawn about the toxicity of dispersants or dispersed oils.

Recent studies have compared the toxicity of the WAFs, CEWAFs of Bass Strait Crude oil, and the dispersant Slickgone NS (on the Australian register) to the diatom, *Phaeodactylum tricornutum* (Hook and Osborn, 2012). The primary goal of the study was to determine whether dispersant and oil interacted in exerting toxic effects. WAF, CEWAF and dispersant-only exposures were created using standardised protocols, however, each WAF and CEWAF was created separately (as opposed to through serial dilution). Diatoms were continuously exposed to each treatment, and measures were made of membrane damage and growth inhibition daily for 72 h and cells were harvested for gene expression analysis after 96 h. Growth inhibition did not correlate with TPH concentrations (presumably due to saturation and differences in toxicity among differently soluble components). When plotted on the basis of loadings, the diatoms were more sensitive to either the CEWAF (which resulted in a higher TPH concentration because of increased solubility) or the Slickgone dispersant alone than to the WAFs. Membrane damage was studied because it is thought that narcotic compounds in oil damage membranes, and that the surfactants in dispersants would damage membranes as well. Only the dispersant and the dispersed oil caused membrane damage. Exposure to the WAF alone did not. However, the concentration range of TPHs used for the WAF and CEWAF did not overlap, making direct comparisons impossible. Gene expression profiles can be correlated to mode of toxic action, and similar gene expression profiles mean that different chemicals cause toxicity via the same cellular pathways. The gene expression profiles for all three treatments were

highly similar, suggesting that it is the hydrocarbon-based solvent in this dispersant (Slickgone NS), not the surfactant that is responsible for the toxic response (Hook and Osborn, 2012).

### Microzooplankton

In an early study, Lee et al. (1985), examined the rates of Corexit 9527 degradation, with and without Prudhoe Bay Crude oil, in mesocosm experiments. The standing stock of bacteria were found to be highest in the dispersant and oil enclosure, in part because this treatment reduced the numbers of ciliates, appendicularians and flagellates. The reduction in grazers was thought to increase the rates of oil degradation (Lee et al., 1985).

Recent studies suggest that ciliates and other microbial eukaryotes may be more sensitive to dispersed oil than to oil (Ortman et al., 2012; Rico-Martinez et al., 2013, Almeda et al., 2014a). In one experiment, the impacts of glucose, oil, dispersed oil and Corexit 9500 on the microbial composition in mesocosms were compared (Ortman et al., 2012). No description of how the oil, dispersant and dispersed oil were mixed into the mesocosms is provided, and the amount of each compound added was specified on the basis of how much carbon was added only, making the doses in this study difficult to compare to both other studies and field-measured concentrations. However, the size and distribution of oil droplets was measured using a flow cytometer, and these were as high as 10 000 particles/mL for the dispersant only treatment. Biomass (as  $\mu\text{g C/L}$ ) was measured in six microbial taxa over 5 days. Adding oil or glucose increased the abundance of both prokaryotes and ciliates, whereas adding dispersed oil or dispersants increased the abundance of prokaryotes but decreased the abundance of ciliates. It was concluded that dispersant and dispersed oil cause less efficient trophic transfer to mesozooplankton (Ortman et al., 2012).

Rico-Martinez et al. (2013) examined rotifer survival and cyst hatching following exposure to the WSF, CEWSF and Corexit 9500. WSFs were not prepared using a standard method and were instead mixed for 8 h. Both endpoints were more sensitive to dispersed oil than to either oil or Corexit 9500, suggesting that dispersing the oil causes synergistic toxicity. The shortcomings of these studies are discussed in greater detail by Coehlo et al. (2013). In both studies, TPHs were not measured, so any increases in effects may be due simply to an increased dose of petroleum hydrocarbons, and not due to any synergistic effects.

A third study, which also did not follow standardised preparations of WAFs or quantify the amounts of either PAH or TPH in test solutions, compared the toxicity of oil and dispersants to microzooplankton (Almeda et al., 2014a). Based on loadings alone, microzooplankton were more sensitive to dispersant and dispersed oil than to crude oil. Ciliates were found to be the most sensitive taxa to both dispersant and dispersed oil, and toxicity decreased as size increased (Almeda et al., 2014a). Again, the lack of quantitative information regarding the chemical composition of the exposure solutions makes the findings of this work difficult to interpret.

Foraminifera in oiled marsh sites were affected by the spill as well (Brunner et al., 2013). In heavily oiled salt marshes, there were fewer foraminifera and these animals did not burrow as deep in the sediment as in unoiled sites. The authors also observed an increase in dead and deformed foraminifera at highly oiled sites. In lightly oiled sites, there were more foraminifera, perhaps because of an increase in bacterial biomass or a decrease in grazing pressure, but these still did not burrow as deeply into the sediment (Brunner et al., 2013).

### Crustaceans

Recent studies suggest that exposure to dispersants and dispersed oil may cause toxicity to crustaceans under field conditions (Lee et al., 2013b). Copepods were exposed to WAF, CEWAF (at a 1:10 dispersant to oil ratio) dispersed with HiClean or Corexit 9500A, and dispersant for 48 h for acute tests or for the period of nauplii development for chronic tests. The oil preparations were created using standard methods. Mortality, time to metamorphosis, and fecundity were assessed.

Although toxicity was not presented as a function of TPHs or total PAHs, these data are presented in the paper, allowing for some comparison between treatments. When the LC50 values were compared, Corexit was found to be more toxic than HiClean and the CEWAF generated using Corexit was more toxic than the CEWAF generated with HiClean (Lee et al., 2013b). The endpoints for the other treatments were not tested over the same concentration range, making comparison difficult. The TPH and PAH concentrations were highest in the WAF created with Corexit, which could explain the difference in observed toxicity.

Calanoid copepods may also be more sensitive to chemically dispersed oil than to oil alone. A recent study compared the impact of oil dispersed with 4% Dasic Slickgone NS (which is on the Australian register) to mechanically dispersed oil (Hansen et al., 2012). Chemically dispersing the oil decreased the 96 h LC50 1.6 fold on a TPH basis (Hansen et al., 2012). Another recent study by the same group examined the impacts of exposure to mechanically dispersed oil (no dispersant was used) on survival and reproductive parameters in copepods (Olsen et al., 2013) for 96 h. Effects were measured for 21 days after the exposure ended. Reproduction was quantified at the end of the experiment only. The authors noted delayed mortality, with survival lower in the copepods exposed to oil, but the organisms died after the exposure period had ended. They also noted that fewer females were reproducing, but that overall egg production numbers had not changed possibly due to differences in cannibalism amongst treatments (Olsen et al., 2013). Both studies demonstrated that copepods ingest dispersed oil droplets. A third study by the same group has compared the reproductive output of copepods exposed to mechanically dispersed oil to the reproductive output of oil chemically dispersed with Slickgone NS (Hansen et al., 2015). In each exposure scenario, the oil concentrations and resultant PAH concentrations in the copepods were equivalent. However, egg and nauplii production was more affected by exposure to chemically dispersed oil than to mechanically dispersed oil, in part because egg production was suppressed longer following exposure to chemically dispersed oil (Hansen et al., 2015). In copepods exposed to chemically dispersed oil, egg production was lower than controls even once the animals were transferred to clean water. Whereas for mechanically dispersed oil, rates of egg production were higher than controls following transfer to clean water, such that over the duration of the experiment, reproductive output was the same between controls and copepods exposed to mechanically dispersed oil (Hansen et al., 2015).

Cohen et al. (2014) also showed that copepods may be sensitive to dispersants. This study compared the 24 h and 48 h LC50 values and swimming speed of a coastal copepod to WAF, CEWAF and dispersant only prepared via standardised methods. Toxicity was compared on the basis of TPHs. The LC50 was found to be 4.5 mg/L of Corexit, suggesting that copepods may be more sensitive than other taxa. The toxicity of the CEWAF was less than the toxicity of the WAF, suggesting that there were no interactive effects between oil and dispersants (Cohen et al., 2014).

Other studies have shown that copepods (of the genus *Acartia* as well as *Calanus*, described above) can ingest oil droplets and accumulate PAHs from them (Almeda et al., 2013). The studies did not use standardised methods for the preparation of WSF and CEWSF, and reported toxicity on the basis of microlitres of material added to solution, limiting their utility. Animals were exposed to treatments for 48 h, after which survival and egg production was quantified. Dispersants and dispersed oil were more toxic than crude oil alone, but as the study did not quantify TPHs, the results are more difficult to interpret. The study also examined sub-lethal toxicity in microcosms in the presence and absence of rotifers. Exposure to oil caused decreases in both egg production and egg hatching (Almeda et al., 2013). The treatment with rotifers had less of a decrease than the treatment without. It was claimed that these data indicate that rotifers somehow ameliorate the toxic impacts of oil. Alternatively, rotifers could be a superior food item for copepods, increasing the copepods' fitness and resilience to toxicological injury.

Barnacle larvae (*Amphibalanus improvises*) have also been found to ingest dispersed oil particles during laboratory exposures (Almeda et al., 2014b). This study compared mortality and growth rates

in barnacle larvae exposed to oil, dispersed oil, and dispersant. The authors did not use standardized methods to prepare their laboratory exposures nor did they quantify the levels of PAH or TPH in their exposures. They found a roughly 2.5 fold increase in mortality of barnacle larvae exposed to dispersed oil relative to dispersants of crude oil alone. The growth rate of barnacle larvae exposed to dispersed oil is also roughly two thirds of the growth rate of larvae exposed to oil or dispersant alone (Almeda et al., 2014b). Again, these data are difficult to interpret as petroleum concentration information is not provided.

The trends of dispersant increasing sensitivity to oil may be different for crustaceans with different life histories. For example, a recent study examined bioaccumulation of petroleum hydrocarbons (prepared using standard methods for WAF and CEWAF) by the fiddler crab *Uca minax* (a benthic detritivore). After 24 h, the authors found greater uptake (measured as TPHs/g wet weight) of oil without dispersion than was found from dispersed oil (Chase et al., 2013). This may occur because oil may bind to the sediment, whereupon it is ingested, whereas dispersed oil stays in solution. The elimination of TPHs accumulated into the crab tissues does not vary, suggesting that the components of oil that are bioaccumulated in both scenarios are the same or similar (Chase et al., 2013).

## Corals

Corals may be very sensitive to petroleum contamination, as they have thin, lipid-rich tissues and accumulate PAHs efficiently (NRC, 2005). Furthermore, direct contact with oil droplets may cause tissue damage (NRC, 2005). Previous studies have shown that coral symbionts are less efficient at photosynthesis following exposure to produced formation water (containing hydrocarbons), and also bleaching was observed at the highest study concentrations (Jones and Heyward, 2003).

Deep sea corals may have been damaged by oil deposited from the deep sea plume following the Deepwater Horizon well blowout (Fisher et al., 2014). Oil from the 1100 m dispersed oil plume was deposited in deep sea sediments once the more labile fractions had been degraded by bacteria (Valentine et al., 2014). Surveys conducted via ROV indicated that deep sea coral near the plume originating from the wellhead were damaged by this oil deposition (Fisher et al., 2014). As these are deep sea species, they can only be studied by survey, so sublethal impacts (such as delayed or decreased reproduction) can not be determined. Since these species are rare, long lived and slow to reproduce, this damage may have long term, population level impacts (Fisher et al., 2014).

A study conducted before the *Deepwater Horizon* spill compared the toxicity from continuous exposure to the water-soluble fraction of oil, the dispersant Corexit 9527, and oil dispersed with Corexit 9527 (not prepared via standard methods) (Negri and Heyward, 2000). They found that at both a 1:10 and a 1:100 ratio, both CEWSF and dispersant inhibited fertilisation. All treatments, (oil, dispersed oil, dispersant alone, as well as produced formation water), inhibited metamorphosis. When expressed relative to TPHs, the CEWSF made with 1:10 oil to dispersant ratio was more toxic than the WSF or the crude oil made with 1:100 oil to dispersant ratio dispersant (Negri and Heyward, 2000). Corals exposed to Corexit 9527 also showed increased expression of the cellular stress proteins heat shock 70, heat shock 90, and a multi-xenobiotic protein (Venn et al., 2009).

A previous study also examined the toxicity to coral larvae resulting from 2-96 h of continuous exposure to five dispersants (Inipol IP-90, Petrotech PTI-25, Bioreico R-93, Biosolve and Emugal C-100) and dispersed oil (Epstein et al., 2000). Unfortunately, standard methods for WSF preparation were not used and TPH concentrations were not quantified, so all toxicity is on a loadings basis only, and the results are difficult to interpret. However, the observed toxic response to dispersed oil occurred at lower loadings than to oil only, and changes in morphology were observed with both

dispersed oil and dispersants, but not oil alone, suggesting the possibility of different modes of action (Epstein et al., 2000). The water soluble fraction (WSF) of crude oil only caused a decrease in settling rates, whereas all the dispersants tested caused changes in survival at some concentrations and reduced settlement at the lowest concentrations tested. Exposure to dispersed oil caused high mortality and major morphological abnormalities (Epstein et al., 2000).

The toxicity of oil, chemically dispersed oil, and the dispersant Corexit 9500 were compared for two different coral larvae, *Porites astreoides*, a brooder; and *Montastrea faveolata*, a broadcast spawner (Goodbody-Gringley et al., 2013). Coral larvae were exposed to WAF or CEWAF for 48-72 h (prepared as stipulated by the Singer et al., 2000, protocols). Larval settlement, swimming and survival was affected by exposure to both oil and chemically dispersed oil, with substantial changes measured at all doses examined (including sub- $\mu\text{g/L}$  TPH concentrations), making comparisons of relative potency between the two treatments difficult. Sensitivity to Corexit alone was found at 25 mg/L, the lowest concentration tested (Goodbody-Gringley et al., 2013).

### Other invertebrates

In a study comparing the response of four different marine taxa (anemones, prawns, mussels and seagrass) following a 48-h exposure and 72-h recovery period to either Corexit 9527 or Superdispersant -25, the anemones were found to be the most sensitive to both, with EC50 values for morbidity of 15 and 25 mg/L, respectively (Scarlett et al., 2005). The other taxa had EC50 values above 50 mg/L (Scarlett et al., 2005).

In a survey of the literature on the two Corexit formulations, mollusc embryos and certain crustaceans (the mysid *Holmesimysis costata* and the copepod *Pseudocalanus minutes*) were found to be the most sensitive to dispersants, often having LC50 values below 10 mg/L, an order of magnitude below those for other crustaceans (other mysids, an amphipod, and brine shrimp), the next most sensitive species (George-Ares and Clark, 2000).

Invertebrate embryos may experience toxicity from exposure to chemically dispersed oil that is greater than expected from dispersed oil. For instance, a recent study compared the toxicity of four different dispersants, including Finasol OSR51, which could be used in Australia in the future. The WSF and CEWSF were prepared from each of the dispersants using 50 mL of oil and 950 mL of seawater mixed together using an orbital shaker as opposed to standardised methods. The development of larval sea urchins was measured after 48-h continuous exposure. Statistical models that looked at the influence of both the measured surfactant and TPH concentrations on the toxicological impact were used to determine whether the dispersant was contributing significantly to the observed toxicity. While the models found no evidence of synergism, the dispersants contributed significantly towards the toxicity observed (Rial et al., 2014). Finasol OSR51 was the most toxic of the dispersants tested. This study is difficult to interpret, however, as although the authors quantified TPHs and surfactant concentrations, these data are not provided in the paper. Instead, the data are presented as mL added per litre test solution, and the metrics of their statistical models are presented. The authors used dispersant to oil ratios that were as high as 1:2, which far exceeds recommendations for application by producer and what would be used in the environment.

Other studies have measured the impact of oil and dispersed oil and the dispersant Corexit 9500A on sub-lethal endpoints in the nematode *C. elegans*, including growth, reproduction and gene expression (Zhang et al., 2013). Dispersed oil was more toxic than oil or dispersant alone, but these conclusions were based on nominal loadings alone, and TPHs or the concentrations of any oil constituents were not quantified, so the increased response may be a function of decreased dose. Standardised protocols for WAF preparation were not followed in this study.

## Fish embryos

Fish embryos, as described previously, are very sensitive to oil, and consequently, to dispersed oil. As summarised previously, dispersant use may solubilise PAHs such that the concentrations are sufficient to disrupt normal developmental processes. Whether these concentrations were elevated for sufficient time to cause disruptions in normal developmental processes planktonic fish eggs in the Gulf of Mexico is not known, nor can any interactive effects of phototoxicity from these exposures be quantified at this point. There is indirect evidence that exposure to *Deepwater Horizon* oil may have caused toxicity to early life stages of fish. A comparison of the abundances of the most numerous pelagic fishes (black-fin tuna, blue marlin, dolphinfish and sailfish) showed decreased abundance of larval fishes relative to the four years prior (Rooker et al., 2013). Although these findings are equivocal as they were not significantly different from all of the previous years, the trends are consistent across species. For all four species, the habitat occupied by fish early life stages overlapped with surface oil, suggesting that these fish embryos had the potential to be exposed to the spilled and dispersed oil. The authors were also able to use electronic tags to show that blue marlin (the only species for which data were available) avoided the oil-impacted area (Rooker et al., 2013). It is important to note that other oceanographic conditions, such as sea-surface temperature, sea-surface height anomaly (an indication of circulation), chlorophyll  $\alpha$  concentration, salinity and depth were also considered, and may also have contributed to differences in larval abundance (Rooker et al., 2013).

Another recent study demonstrated that developmental defects occurred when embryonic tuna and amberjack were exposed to water samples comparable to those collected during the *Deepwater Horizon* spill response (Incardona et al., 2014). The study authors noted cardiac defects, changes in the development of the eye, and altered development of fin rays. The developmental abnormalities observed caused both acute and delayed mortality (Incardona et al., 2014). Toxicity was inversely proportional to egg size. Although, as summarized previously, these mechanisms of toxic action are well characterized, this was the first study to characterize these impacts in warm water fish with short development times (Incardona et al., 2014).

The impacts may have been considerably less for near-shore organisms. Another recent study used data originally collected for an oyster reef restoration project to compare larval abundances before and after *Deepwater Horizon* in salt marsh tidal creeks (Moody et al., 2013). It was hypothesized that since coastal species often rely on oceanic waters to transport eggs into shore, the *Deepwater Horizon* incident had the potential to decrease recruitment, however, the authors found no overall trends to suggest that the oil spill had any influence on larval abundances (Moody et al., 2013).

In contrast to invertebrates, fish embryos do not seem to be as sensitive to the Corexit formulations alone. For instance, the toxicity of four different chemically dispersed (using Corexit 9500) and undispersed oils to rainbow trout embryo mortality and incidence of blue sac disease were compared (Wu et al., 2012). WAF and CEWAF treatments were created using standardised protocols and exposure solutions were renewed daily. Trout embryos were exposed to treatments for 22 days. The CEWAF was consistently more toxic than the WAF when toxicity was compared on the basis of oil loading. The difference is starkest for the heaviest, least water soluble oils. However, these differences, both amongst the oils and between the dispersed and undispersed oil disappeared once TPHs were measured, suggesting that the observed differences in effects with loading were due to differences in the solubility (and consequently bioavailability) of components of the oil (Wu et al., 2012). Differences in lethal toxicity of the heavy oils also disappeared once the data were normalized to TPH concentration. No increased incidence of Blue Sac disease was observed in embryos exposed to the dispersant-only treatment. The toxicity observed in this study could be explained on the basis of the measured TPH concentrations. There was no evidence that the embryo membranes were damaged following exposure to Corexit 9500 (Wu et al., 2012).

Other studies performed by the same group addressed the question of interactive effects more directly (Adams et al., 2014). Again, WAF and CEWAF treatments were created using standardised protocols and test solutions were renewed every 48 h during exposure. Atlantic herring embryos were exposed for 19 days to treatments, and rainbow trout were exposed for 22 days. In both cases, this time period encompassed most of the fishes' embryonic development. With Atlantic herring embryos, it was again found that the CEWAF was more toxic than the WAF if toxicity was expressed on the basis of loading, but not if expressed on the basis of TPH concentrations (see Figure 2). In additional studies with rainbow trout embryos, toxicity caused by exposure to dispersant alone was shown to act via a different mode of toxic action to dispersant alone. Yolk sac edema was not seen in embryos exposed to Corexit only (Adams et al., 2014). The timing of mortality was also different for oil-exposed and dispersant-exposed embryos. Following exposure to Corexit, mortality occurred in the first 4 days after exposure, whereas following exposure to oil, the mortality occurred in the latter half of the 22-day exposure period (Adams et al., 2014).

### Juvenile and Adult Fish

Following the *Deepwater Horizon* spill, the US EPA conducted a series of experiments comparing the lethal toxicity, measured as 96-h LC50, of Louisiana sweet crude (LSC), 8 different dispersants (none of which are on the Australian register), and LSC dispersed with each of the dispersants to inland silversides (*Menidia beryllina*) and to mysid shrimp (*Americamysis bahia*) (Hemmer et al., 2011). Toxicity was expressed on the basis of TPH, and WAFs and CEWAFs were created using standard methods. They found that the dispersants alone were typically less toxic than the WAFs. The CEWAFs were not notably more toxic than the WAFs for most dispersants (Nokomis 3AA being a notable exception), and some CEWAFs were less toxic than WAFs. These data suggest that the dispersant did not add appreciably to the toxicity that would have been observed from the action of dispersing the oil (Hemmer et al., 2011).

The transcriptomic responses of first-feeding fish larvae (i.e. those that have only recently hatched) to chemically dispersed oil using the dispersant Dasic Slickgone NS (on the Australian register) and mechanically dispersed oil were examined by Olsvik et al. (2012). Cod larvae were exposed to oil for 96 h. Mortality and PAH concentrations in the water correlated better to mixing energy than to type of dispersion. The number of genes with significant changes in expression also increased with increased mixing energy. The chemically and mechanically dispersed oil generated different transcriptomic profiles, suggesting that the modes of toxic action were different for the two dispersion methods (Olsvik et al., 2012).

In a 2005 study, newly hatched mummichogs were exposed to either WAF or CEWAF from Mesa crude oil or mesa crude oil and Corexit 9500, respectively (Couillard et al., 2005). The exposure solutions were prepared using standardized protocols, and the total PAH (but not TPH) were quantified. The study found that chemically dispersed oil had a greater proportion of HMWPAH, even at the same total PAH concentration. The alkylated fraction of PAH was also increased. The authors noted an increase in EROD induction with CEWAF exposure, and hypothesize that exposure to CEWAF may result in more chronic toxic effects than exposure to WAF, even at the same TPAH concentration, because of the greater abundance of HMWPAH (Couillard et al., 2005).

Many studies have used biomarkers to measure exposure to oil, including EROD induction, the first step in the biological metabolism of oil in many animals. To measure the influence of dispersants on oil uptake, juvenile rainbow trout were exposed to either the WAF, CEWAF, (prepared via standard methods) or Corexit 9500 only for 48 h. EROD induction was used to estimate PAH uptake and it was found at greater levels in fish exposed to CEWAF than to WAF if concentrations were based on loading, but when the PAH concentrations were used instead, the EC50s were comparable (Ramachandran et al., 2004). Corexit was not found to induce CYP1A activity or increase the permeability of the gill to EROD inducers.

Another study examined oxidative stress biomarkers in the hearts of juvenile golden grey mullet exposed to an unnamed dispersant, CEWSF, mechanically dispersed WSF or the Water Soluble Fraction (WSF) of crude oil (Milinkovitch et al., 2013). These treatments were not created using standardized methods. Fish were exposed to each treatment for 48 h. Changes were found in these biomarkers following exposure to the oil compounds, but little change following exposure to the dispersant alone. Both of the dispersed oil treatments had increased PAH concentrations relative to the WSF only, and both had increased biomarker response. There is no indication that the dispersant increased the toxic response to the oil (Milinkovitch et al., 2013).

However, not all studies have found dispersants to be comparatively benign. A recent study compared the impacts of HEWAF (high energy mechanical dispersion), CEWAF (prepared using standard methods), and Corexit 9500 on the expression of CYP 1A and growth in larvae and juveniles of the spotted sea trout (*Cynoscion nebulosus*) (Brewton et al., 2013). Larvae were exposed to oil treatments for 96 h, and juveniles were exposed for 72 h. The TPH concentrations in each treatment were roughly comparable. Both oil treatments increased expression of CYP1A, however larval sea trout had a greater response to the chemically dispersed oil (as measured via increased CYP1A expression relative to the HEWAF treatment and decreased growth) and the dispersant (as decreased growth), whereas the juvenile sea trout were most responsive to the HEWAF (as measured via increased CYP1A expression relative to the CEWAF treatment and decreased growth) and dispersant as decreased growth. For the juvenile treatment, differences in growth that were observed at the end of the exposure period but these fish had recovered 20 days later, as differences in growth were no longer observable. Larval growth was only measured at the end of the exposure (Brewton et al., 2013).

Another study exposed European sea bass (*Dicentrarchus labrax*) to oil, oil dispersed with the TOTAL-Fluides dispersant Finasol, or dispersant for 48 h (Claireaux et al., 2013). Exposure to oil was determined by measuring the TPHs in each treatment and by measuring PAHs in the bile. Both the oil and the oil and dispersant treatments increased the levels of PAHs in the bile, and TPHs were significantly increased in both treatments but in the dispersed oil by the greatest amount (Claireaux et al., 2013). Resilience to a physiological challenge (hypoxia and heat stress) was measured before and after exposure, and growth and survival were measured over six months following exposure. Although no differences were found in resilience, both the oil and the dispersed oil decreased growth, and the dispersed oil decreased survival (Claireaux et al., 2013). It is uncertain whether the increased potency of dispersed oil was due to the increased levels of TPHs in this treatment as the TPH ranges in this study did not overlap.

Another recent study looked at the interactive effects of hypoxia and exposure to oil, dispersed oil and chemically dispersed oil to larvae of the sheepshead minnow *Cyprinodon variegatus* (Dasgupta et al., 2015). Exposure to either Corexit 9500 or oil dispersed with corexit 9500 increased the hypoxia induced mortality, whereas exposure to oil alone did not (Dasgupta et al., in press). However, the WAF concentrations used in the study were low and did not overlap with the CEWAF, making the results harder to interpret.

In terms of population level effects, acute toxicity levels field following successful application of dispersants would probably only occur for a limited time because may not be exceeded in field operations for a prolonged period due to rapid dilution of the oil. This hypothesis appears to be supported in the results of numerous case studies that have shown no significant effect on fish populations following dispersant use. Indeed, monitoring efforts linked to the Gulf of Mexico oil spill have indicated no significant losses of juvenile fish or adults as catch rates remained relatively high after the spill compared to the previous four years (Fodrie and Heck, 2011).

## Benthic habitats

Even though oil spills are primarily a surface water and coastal disturbance, they can have impacts on oceanic benthic communities, as some oil is deposited in deep-sea sediments. However, by mass, the amount of oil deposited in sediments is much smaller than the amounts in surface waters. Oil is transported into deep-sea sediments via sedimentation of droplets (which adhere to other particles), direct sinking, and transport via fecal pellets (Montagna et al., 2013; NRC, 2014, Gong et al., 2014, Sorensen et al., 2014). Sediment samples were collected from sites with varying degrees of impact during the response to the *Deepwater Horizon* well blowout. The biodiversity of infaunal organisms in these sediment cores was compared to physical and chemical variables, including levels of oil contamination. Following the blowout, a loss of biodiversity was measured in deep-sea sediments around the wellhead. The most severe losses occurred within 3 km of the wellhead, but reported losses in biodiversity associated with elevated TPH and PAHs typed as MC252 oils were measurable as distant as 17 km (Montagna et al., 2013). However, active seeps were measured in the study area (JAG, 2010), and any contribution of these natural petroleum inputs towards differences in biodiversity is not known. These losses could be correlated with TPH and total PAH concentrations in the sediments (Montagna et al., 2013). In a similar study, an increase in the infaunal nematode to copepod ratio and a decrease in species diversity and abundance was noted in sites closer to the well head (Baguley et al., 2015). The authors noted a decrease in the abundance of infaunal copepods, ostracods and kinorhynchans at the most impacted sites, and a decrease in copepod nauplii at the intermediate sites. The authors also note that at the most impacted sites, the bioturbation depth was shallower (Baguley et al., 2015). The study authors point out that recovery times in the deep sea are likely to be slow (Montagna et al., 2013). The impact on deep sea corals (Fisher et al., 2014) was described previously. Other studies have shown that dispersing the oil can have a protective impact on communities by decreasing the persistence of oil (reviewed in NRC, 2005).

The use of dispersants may decrease the sedimentation rate of oil (Sorensen et al., 2014). The interactions between oil droplets and suspended particles were studied, and chemically dispersed oil was found to be less likely to sorb to sand-sized particles. The heavier oil was more likely to sorb to particles with a preference for higher surface area clay particles than sand (Sorensen et al., 2014). However, even with dispersion, microcosm studies have shown that as the lighter components of oil are biodegraded, the oil droplets sink, so dispersed, heavy oil may be deposited to the sediments (Davies et al., 2001).

## 2.4 Summary of environmental studies

The 2013 NRC report states that chemical dispersion can have a net environmental benefit. When chemical dispersants are used to remove the oil from the surface layer, they can reduce the volume of oil washed ashore and into coastal environments by wind-driven processes. Chemical dispersion can increase the rates of biodegradation because the compounds within oil are made more soluble. However, there can be disadvantages to dispersant use. The increased solubility of oil of dispersed oil can put at increased risk planktonic and benthic organisms that would not normally be in contact with surface oil (NRC, 2013).

Reviews of the literature identified many studies that did not measure TPHs, or did not use overlapping ranges of TPHs in the WAF and CEWAF treatments, making the findings of these studies, at best, difficult to interpret (Bejarano et al., 2014). Nevertheless, the findings of this literature review, which includes the most recent literature, are consistent with those of NRC (2005, 2013) summarised above, namely:

1. Chemical dispersion with Corexit accelerates the process of biodegradation, decreasing the residence time of oil in the environment, and thus its overall ecological impact. This has

been measured both in laboratory and field studies conducted following the *Deepwater Horizon* wellhead blow out. Comparable studies have not been conducted with other dispersants, including those on the Australian register. This is particularly important because different surfactants have different rates of biodegradation (NRC, 2013).

2. Dispersing oil increases the solubility (and hence bioavailability and toxicity) of toxic constituents of oil in the water column. Following the *Deepwater Horizon* spill, the measured TPH and TPAH concentrations in areas where Corexit was used were sometimes above those that have been shown to disrupt normal embryonic development of fish and coral in laboratory studies. However, it is not certain whether the frequency of elevated oil concentrations and the duration of exposure were sufficient to disrupt development of embryonic fishes that would have an overall effect on community structure and populations as a whole. The dispersant concentrations were not above the US EPA benchmark concentration for the protection of aquatic life. These impacts are summarized in Table 1.
3. Laboratory studies indicate that dispersants themselves may be toxic to invertebrate embryos and corals. Thus, these organisms may be at added risk from dispersed oil relative to undispersed oil or mechanically dispersed oil at the same TPH concentration. Whether the duration of exposure likely to be encountered following an oil spill is sufficient to cause lasting impact is uncertain. Copepods may also be more sensitive to chemically than mechanically dispersed oil. However, there is little evidence from laboratory studies that exposure to Corexit added to the toxic response in fish embryos. This data is summarized in Table 2. However, these data are difficult to interpret as few studies quantify TPH concentrations, and the range of TPH used for both the WAF and the CEWAF are different.

As a result of the uncertainty arising from studies that do not characterise the concentrations of oil and dispersed oil well, the long exposure times, and the ambiguities in the literature, the risk of dispersing oil cannot be assessed accurately using existing information, making the Net Environmental Benefit Analysis difficult. As will be described in the next section, this process would be even more difficult for the dispersants on the Australian register because they are less well studied.

These generalisations are, however, drawn chiefly from studies performed with the Corexit formulations. Some studies have been performed with other dispersants, and they are noted in the sections above. Other dispersants (with different chemical formulations) may have different environmental impacts.

**Table 1. List of studies that would predict toxic impact from either dispersed oil or Corexit at the concentrations measured in the Gulf of Mexico (1 mg/L of TPHs, 10 µg/L of total PAHs, 100 µg/L of Corexit) (from Bejarano et al., 2013)<sup>a</sup>**

SPECIES	ENDPOINT	EFFECTS FROM TPHs?	EFFECTS FROM TOTAL PAHs?	EFFECTS FROM COREXIT?	REFERENCE
Atlantic herring	% hatched	Yes	ND <sup>b</sup>	No	Adams et al., 2014
Spotted sea trout	Larval growth; juvenile growth	Yes	ND	No	Brewton et al., 2013
Coral	Larval survival	Yes	ND	Yes	Gringley-Goodbody et al., 2013
Bluefin Tuna Yellowfin Tuna Amberjack	Developmental abnormalities	ND	Yes	No	Incardona et al., 2014
Diatom	Growth inhibition	No	No	ND	Hook and Osborn, 2012
Copepod	Survival and Fecundity	No	ND	No	Lee et al., 2013b
Coral	Fertilization and metamorphosis	Yes	ND	No	Negri and Heyward, 2000
Rainbow trout	Incidence of blue sac disease	Yes	ND	No	Wu et al., 2012
Pacific herring	Incidence of blue sac disease	ND	Yes	No	Carls et al., 1999

<sup>a</sup>Only those studies that presented data as TPHs or total PAHs and using environmentally realistic concentrations of both oil and dispersant are included; <sup>b</sup>ND = not determined

**Table 2. Studies comparing the toxicity of oil (as WAF) and dispersed oil (as CEWAF) on a TPH basis**

SPECIES	ENDPOINT	DISPERSANT USED	IS TOXICITY GREATER FROM CEWAF THAN WAF?	REFERENCE
Atlantic herring	% hatched	Corexit 9500	No	Adams et al., 2014
Spotted sea trout	Larval growth	Corexit 9500	Yes	Brewton et al., 2013
Spotted sea trout	Juvenile growth	Corexit 9500	No	Brewton et al., 2013
Coral	Larval metamorphosis	Corexit 9527	Yes <sup>b</sup>	Negri and Heyward, 2000
Rainbow trout	Severity of blue sac disease	Corexit 9500	No	Wu et al., 2012

<sup>a</sup>Only studies where the data are presented as TPHs and the ranges of TPHs from the WAF and the CEWAF overlap are included

b. Standardised methods for WAF preparation were not used in this study

## 2.5 Dispersants currently licensed or under consideration for use in Australia and current protocols for testing

The current protocols for registering an Oil Spill Control Agent for use in Australia (described in detail by AMSA (2011) and briefly summarised below) use NATA-accredited standardised toxicity tests on a variety of taxa. These tests include lethal and sub-lethal endpoints (detailed in Table 3), and could be easily reproduced for comparisons to data collected elsewhere in the world. To be registered in Australia, the LC<sub>50</sub> values must be greater than 10 mg/L for the fish larvae and crustaceans listed in Table 3, which is considered “slightly toxic” by the US EPA (Hemmer et al., 2011). The results of these tests are coupled with the results of dispersant efficacy tests (performed using Mackay apparatus, reviewed by NRC, 2005) to determine whether the oil spill control agent can be listed for use in Australia.

In other countries, the process of registering a dispersant for potential use is markedly different. For instance, in the UK, toxicity tests must show that dispersed oil is not more toxic than oil alone to prawns and limpets on a TPH basis (EMSA, 2010). France requires that dispersants be more than ten times less toxic than a reference toxicant. They also require that use of dispersants does not impede oil biodegradation (EMSA, 2010). Norway conducts its toxicity tests using diatoms instead of the crustaceans used in other countries (EMSA, 2010). Other EU member nations (including Ireland, Denmark and Germany) have no formalised plan for testing or approving dispersants, and others (including Portugal, Slovenia and Sweden) would not use dispersants in the event of a spill.

The US EPA requires testing for toxicity to fish and crustaceans of dispersants both on their own and mixed with #2 Fuel oil (Hemmer et al., 2011). In addition, following the *Deepwater Horizon* spill and the wide spread use of Corexit 9500A, additional testing was undertaken. The toxicity of the dispersant was tested with Louisiana Sweet Crude, which is a reasonable proxy for Macondo oil and more relevant than #2 fuel oil for estimating the impact of the *Deepwater Horizon* spill (Hemmer et al., 2011). In addition, because there were concerns that Corexit would have endocrine-disrupting or cytotoxic activities (although the known endocrine-active nonylphenol ethoxylates are not currently in use in dispersants (EMSA, 2010)), cell-line assays were also used (Judson et al., 2010). No estrogenic, androgenic, anti-estrogenic, or anti-androgenic activities were found, and cells experienced cytotoxicity at Corexit concentrations higher than 100 ppm, indicating a low potential for toxic effects (Judson et al., 2010).

Most of the information in the literature on the safety of dispersants relates to Corexit formulations, which are not registered for use in Australia. However, the international oil companies that drill and ship oil in Australia are in possession of Corexit 9500, so that, in an emergency, this may be used in Australian waters. For instance, this dispersant formulation was used after the Montara well release (AMSA 2010). The dispersants that have completed the Oil Spill Control Agent (OSCA) 2011 process (discussed in more detail in Section 2.6), the testing required before a dispersant can be used in Australia, include Dasic Slickgone NS and Dasic Slickgone EW. Ardrex 6120 and Slickgone LTSW have transitional approval, and approval will be sought for Finasol OSR 51. The dispersants that would be considered for use in Australia have much less data available for them than does Corexit, which are briefly summarized below. Table 4 shows some of their active ingredients, allowing for some comparison between them and the better-studied dispersant formulations. Additional information is available in the online resources listed in Appendix A. However, these comparisons should be used cautiously until further research is done, as recent studies with pesticides have shown that toxicity cannot always be predicted from their active ingredients (e.g. Beggel et al., 2010).

**Table 3. Toxicity tests currently required as part of the Oil Spill Control Agent Register process (AMSA, 2011)**

REFERENCE	TAXONOMIC GROUP	SPECIES NAME	REGION	END POINT	TEST TYPE
1	Crustacean	<i>Penaeus monodon</i> (Tiger prawn)	Tropical	96-h survival	Lethal
		<i>Allochestes compressa</i> (Amphipod)	Temperate	96-h survival	Lethal
		<i>Melita plumulosa</i> (Amphipod)	Temperate	96-h survival	Lethal
2	Fish larvae	<i>Pagrus auratus</i> (Pink snapper)	Temperate/ Tropical	96-h fish imbalance <sup>a</sup>	Lethal
		<i>Serolia lalandi</i> (Yellowtail kingfish)	Temperate/ Tropical	96-h fish imbalance <sup>a</sup>	Lethal
		<i>Lates calcarifer</i> (Barramundi)	Tropical	96-h fish imbalance <sup>a</sup>	Lethal
		<i>Macquaria novemaculeata</i> (Australian bass)	Temperate	96-h fish imbalance <sup>a</sup>	Lethal
		<i>Acanthopagrus butcheri</i> (Black bream)	Temperate	96-h fish imbalance <sup>a</sup>	Lethal
		<i>Acanthochromis polycanthus</i> (damselfish)	Tropical	96-h fish imbalance <sup>a</sup>	Lethal
3	Algae/ Phytoplankton	<i>Nitzschia closterium</i> (Diatom)	Temperate	72-h growth inhibition	Sub-lethal
		<i>Isochrysis galbana</i> (Flagellate)	Tropical	72-h growth inhibition	Sub-lethal
4	Macroalgae/ seaweeds	<i>Ecklonia radiata</i> (Kelp)	Temperate	72-h germination	Sub-lethal
		<i>Hormosira banksii</i> (Neptune's necklace)	Temperate	72-h germination	Sub-lethal
5	Bivalve larvae	<i>Mimachlamys asperrima</i> (Doughboy scallop)	Temperate	48-h larval development	Sub-lethal
6	Sea urchin larvae	<i>Heliocidaris tuberculata</i> (Sea urchin)	Temperate	72-h larval development or 1-h fertilisation success	Sub-lethal

<sup>a</sup>The fish imbalance test is a humane alternative to a 96-h survival test.

<sup>b</sup> Lethal would be considered acute and sub-lethal would be considered chronic as defined by the ANZECC/ARMCANZ (2000). The test is considered chronic if run for ≥7 days for an early life stage.

**Table 4. The active ingredients of current use dispersants**

NAME	CAS #	TYPE	COREXIT 9500	SLICKGONE LTSW	SLICKGONE NS	SLICKGONE EW	ARDROX 6120	FINASOL 51
Sodium dioctylsulfosuccinate (also dioctyl sulfosuccinate or DOSS)	577-11-7	Surfactant	X	X	X	X		X
Sorbitan, mono-(9Z)-9-octadecenoate	1338-43-8	Surfactant	X	X	X	X		X
Polyoxy-1,2-ethanediyl derivatives of sorbitan, mono-(9Z)-9-octadecenoate	9005-65-6	Surfactant	X	X				
Polyoxy-1,2-ethanediyl derivatives of sorbitan, tri-(9Z)-9-octadecenoate	9005-70-3	Surfactant	X			X		
Ethoxylated fish oil	103991-30-6	Surfactant		X	X			X
Tall oil	8002-26-4	Surfactant			X			
Butyldiglycol; Diethylene glycol monobutyl ether; 2-(2-butoxyethoxy)ethanol	112-34-5	Solvent					X	
Petroleum distillates, hydrotreated light fraction	64742-47-8	Solvent	X		X	X		X
Dipropylene glycol monobutyl ether	29911-28-2	Solvent	X			X		
Propylene glycol	57-55-6	Solvent	X					
2-Butoxyethanol (ethylene glycol mono-n-butyl ether)	111-76-2	Solvent		X				

### 2.5.1 SLICKGONE NS

Slickgone NS was developed to be effective at dispersing North Sea oils (EMSA, 2010). The active ingredients of Slickgone NS include sodium dioctylsulfosuccinate (a surfactant) and mono-(9Z)-9-octadecenoate (also a surfactant) (Table 4). Both surfactants are found in the Corexit 9500 and 9527 formulations. As solvents, Slickgone NS uses ethoxylated fish oil, Tall oil fatty acid (Surfac TOFA2) and petroleum distillates. The product's material safety data sheet (MSDS) states that kerosene is among the product's petroleum-based solvents.

One study examined delayed effects (as % survival and growth of isolated coral colonies) 24 h, 7 days and 50 days following a 24-h exposure to oil and oil dispersed with 6 different dispersants, including Slickgone NS (Shafir et al., 2007). While all dispersed oils showed some toxicity, and dispersion increased the toxicity of oil on a loadings basis, Slickgone NS was the least toxic (Shafir et al., 2007). Unfortunately, this study did not quantify TPHs, so it is uncertain if the toxicity relates to a lack of efficacy or to a lessened severity of the effects of exposure. Since dose is not well quantified, the results have limited applicability to risk assessment.

Toxicity tests performed for the US EPA compared the lethal toxicity of Slickgone NS to fuel oil #2 and dispersed fuel oil #2 (loading ratio of 1:10) to the brine shrimp *Artemia salina* and the mummichog *Fundulus heterclitus*, an estuarine fish. The report from 1987 refers to a US EPA standard method that is not available online. The test may also have been performed using an older formulation of the dispersant. The data are seemingly presented on a loadings basis, and it seems that dispersing the oil greatly enhances its toxicity (e.g. the LC<sub>50</sub> for brine shrimp decreases from 7840 to 101 mg/L with dispersion), but since the data are not presented on the basis of TPH in solution, it is difficult to draw conclusions. These data are presented in Appendix B.

A recent study compared the acute toxicity of eight different dispersants (including Slickgone NS and Corexit 9500A) to North Sea diatoms, three copepods, and an amphipod (Hansen et al., 2014). The diatoms and copepods were roughly equally sensitive to the two different dispersants (EC<sub>50</sub>s for the diatom were 24.4 and 30.4 mg/L, respectively; LC<sub>50</sub>s for the copepods are 11.5 and 6.5; 24.1 and 20.6; 12.3 and 11.8; respectively, depending on species). However, the amphipods were much more sensitive to Slickgone NS than to Corexit 9500A, with a ten day LC<sub>50</sub> to SlickgoneNS being 170 compared to 1006 for Corexit 9500A (Hansen et al., 2014). The other dispersants in the study are not under consideration for use in Australia.

Recent testing performed by Ecotox Services Australasia (ESA, 2012) confirmed generalised trends seen for dispersants (as discussed by George-Ares and Clark, 2000). Crustaceans and algae were the most sensitive, with LC<sub>50</sub> or EC<sub>50</sub> concentrations falling in the range that would be considered moderately toxic by the US EPA (2.6 and 6.4 mg/L, respectively) (ESA, 2012; Hemmer et al., 2011). The toxicity observed in other species would suggest that the dispersant was slightly toxic or practically non-toxic according to the US EPA criteria. No tests were done in conjunction with oil, so any potential interactive effects could not be examined. Tests performed by CEDRE for registration of this dispersant in France found LC50 values of about 50 mg/L for two different species of prawn (Appendix C). The rate of oil biodegradation was found to be slightly higher in the presence of Slickgone NS in the French tests (Appendix C).

### 2.5.2 SLICKGONE EW

The active ingredients of Slickgone EW also include sodium dioctyl sulfosuccinate and mono-(9Z)-9-octadecenoate (Table 4). Both surfactants are found in the Corexit 9500 and 9527 formulations, as well as Slickgone NS. As solvents, Slickgone EW uses dipropylene glycol monobutyl ether (which is also used in the Corexit formulations) and petroleum distillates. The product's MSDS states that

kerosene comprises 40-50% of the product's petroleum-based solvents. The toxicity of this dispersant was tested by Ecotox services in 2009 and 2012. For all the organisms tested, the dispersant was found to have LC<sub>50</sub> or EC<sub>50</sub> values greater than 10 mg/L (ESA, 2009, 2012).

### **2.5.3 ARDROX**

The only information about the active ingredients of Ardrox 6120 is that it contains the solvent butyldiglycol (Table 4). None of the other dispersants contain this ingredient.

Negri and Heyward (2000) refer to an earlier, unpublished study, in which it was found that coral larvae were more sensitive to treatment with Ardrox (exact formulation unspecified) than to the WAF of crude oil. However, as this study is unpublished, it is difficult to infer many conclusions from this observation. A preliminary report on the toxicity of Ardrox 6120 prepared by ESA (2013a) indicates that it may be toxic to crustaceans, with LC<sub>50</sub> values of 9.1 mg/L for tiger prawns and 13 mg/L for amphipods. Fish were unaffected by exposure as high as 20 mg/L. Another report with a slightly different formulation of this dispersant (renamed LT 13002) again indicates that it may be toxic to crustaceans. The LC<sub>50</sub> for this dispersant to copepods was 0.6 mg/L, although for fish, sea urchins, and oysters the LC<sub>50</sub> or EC<sub>50</sub> was above 20 mg/L (ESA, 2013b). Again, no tests were done in conjunction with oil, so any potential interactive effects could not be examined.

### **2.5.4 FINASOL OSR 51**

Finasol 51 has a chemical composition similar to the other modern use dispersants, such as Slickgone formulations and the Corexit formulations, in that it uses sodium dioctylsulfosuccinate and mono-(9Z)-9-octadecenoate (Table 4) as surfactants. It uses ethoxylated fish oil and petroleum distillates as solvents. Recent studies that compare the toxicity of dispersants found that toxicity of Finasol OSR51 (as LC<sub>50</sub> and EC<sub>50</sub>, to amphipods and diatoms, respectively) that was comparable to that of Slickgone NS and Corexit 9500, and had higher LC<sub>50</sub> values (less toxic) to copepods (Abbasova et al., 2005). When the toxicity of crude oil and dispersant plus crude oil were compared, the dispersed oil was less toxic than the crude oil for the crustacean. Dispersion increased the toxicity of oil to diatoms, but the EC<sub>50</sub> had been over 10,000 mg/L to crude oil alone, and dispersion reduced the toxicity to 160 mg/L (Abbasova et al. 2005), a concentration that greatly exceeds what was measured in the Gulf of Mexico. As summarised above, Finasol OSR 51 was also the most toxic of four dispersants tested to sea urchin embryos (Rial et al., 2014).

#### **Data Gaps for Australian Dispersants**

If there was an oil spill in Australian waters, AMSA would currently be required to conduct a quick "Net Environmental Benefit Analysis" to determine whether the ecological risks associated with dispersing the oil outweigh the benefits. While the current process identifies that the dispersants that would be applied in the environment in large volumes, are comparatively non-toxic, it does not make predictions about how the toxicity of oil would change (if at all) when dispersed. An accurate estimation of the risk of dispersed oil to relevant taxa is needed in order to conduct the Net Environmental Benefit Analysis and ensure that the dispersion will lessen the impacts of any oil spill to the ecosystem overall. Since the ecotoxicological risks associated with oil dispersion are specific to the precise formulation of the dispersant, findings for one dispersant will not necessarily carry over to another. By comparison to the well-studied Corexit formulations, there is a paucity of information for the dispersants on the Australian register, so that we cannot accurately assess the risks from any of these dispersants. Because Corexit is not on the Australian register, we cannot expect that the literature coming out of that event is going to help us close that gap as it will better define the risks associated with use of Corexit only. Additional research must be conducted if we are

to fill the gaps and enable quick decision-making regarding the benefits and detriments of oil dispersion.

## 3 Shortcomings with the Existing Data

As shown in the conceptual model in Figure 1, the impact of oil alone will differ from that of chemically dispersed oil. It is assumed that a decision on whether or not to use a dispersant will be driven by issues other than toxicity of the dispersed oil (threats to shorelines and coastal areas, charismatic megafauna, birds, etc). If the dispersant option is taken, it will be necessary to choose a chemical that has low toxicity, does not interfere with biodegradation, and where the toxicity of the dispersed oil product is also minimised. Current testing protocols say little about the risk associated with the use of dispersants following an oil spill, because it cannot be determined from these tests whether the chemical dispersants meaningfully increase the risk of a deleterious toxic response from the oil itself. Under conditions expected during spill response operations, the toxicity of chemically dispersed oil appears to be from components within the oil rather than the dispersant. The majority of toxicity tests have not been conducted with the exposure scenarios that would be encountered during an actual oil spill, nor have they represented the most critical life stages or those species most likely to be exposed to oil during the spill. These protocols have not covered potential changes in trophic level dynamics and the significance of bacterial degradation which would alter the environmental persistence of the residual oil. With these gaps in the existing data, it would be very difficult for AMSA to conduct a Net Environmental Benefit Analysis. Consequently, the consequences of short-term (hours) exposure to oil, dispersed oil, and dispersant to planktonic organisms, the consequences of chronic (days to weeks) exposure to oil, dispersed oil and dispersant to benthic organisms, and the effects of dispersant on the rates of oil degradation should be investigated. For optimum protection of our Commonwealth waters, our NEBA approach requires a database on the potential toxic effects of the products approved for national use against the crude oils and refined products that have a high probability of being spilled within the region.

To date, “Net Environmental Benefit Analysis” is primarily focussed on data generated from short term toxicity tests, which is appropriate for spills that occur in deep or well flushed waters due to the rapid dissipation of oil droplets. However, the results of case studies and long-term chronic toxicity studies must also be considered. If dispersants are to be used in near shore or poorly flushed areas, there may also be damage that occurs following long term exposure to low levels of oil or dispersant. These impacts may have deleterious consequences for valued resources such as coral reefs (tourism) and aquaculture industries (fish-farming, oyster culture, mussel and scallop industries) may yet be significant.

### 3.1 Assessing the potential for dispersants to alter the rates of biodegradation

It has been shown that chemical dispersion of oil can either accelerate or inhibit the rates of oil degradation, which will affect the residence time of oil in the water column. Early reports had conflicting data (NRC, 2005), and subsequent reports indicate that the choice of surfactant may greatly alter microbial processes (NRC, 2013). As a consequence, break down of oil by bacteria may be greatly influenced by the choice of dispersants. None of the dispersants that would be used in Australia have had their influence on the rates of oil degradation studied.

## 3.2 Assessing the toxicity of dispersant in combination with oil

The toxicity of modern use dispersants is less than that of oil for many organisms (Fingas, 2011; Bejarano et al., 2014). However, following an oil spill, assessors would need to predict whether dispersants increase the risk of harm to pelagic and benthic organisms above what would be expected for the risks following exposure to the oil alone. Any interactive effects between the oil and dispersants, if they were to occur, would increase the risk from oil exposure. The toxicity of undispersed oil, mechanically dispersed oil, chemically dispersed oil, and dispersants alone should be compared in side by side bench scale tests. The undispersed oil would represent the risks associated with an untreated spill, the chemically dispersed oil would represent the risks from a spill treated with dispersants, mechanically dispersed oil would represent the risks associated with increasing the solubility of the oil on its own, without any toxicity associated with the dispersant, and the dispersant only treatment would be the toxicity associated with the dispersant sprayed in areas without oil (as would undoubtedly occur given that the dispersant is sprayed from airplanes and the distribution of oil is patchy). For each of these treatments (including the dispersant only treatment), toxicity should be measured against both the loading and the total petroleum hydrocarbons in solution. Any difference in toxicity between mechanically and chemically dispersed oil that could not be explained by the TPH in solution would be indicative of interactive effects between oil and dispersant.

An example of how this information would be used in a real world scenario is as follows: For Corexit 9500A, the literature shows that the dispersant does not add to the toxic response in fish embryos under laboratory conditions (Adams et al., 2014), but it may for coral larvae (Goodbody-Gringley, 2013). As a consequence, the risk associated with using Corexit in an area where fish are spawning is lower than using it in an area where coral are spawning. For the dispersants on the Australian schedule, there is not yet the information necessary to predict what the risk will be, complicating the Net Environmental Benefit Analysis.

## 3.3 Measuring appropriate time scales for each zone of impact

The potential zones of impact are shown in Figure 1. The most common scenario for ecological risk, and the zone where most of the dispersed oil will be distributed if dispersants are used in the open ocean, is into the upper mixed layer of the water column (Figure 1B). Planktonic organisms (such as phytoplankton, zooplankton, and eggs from broadcast spawners), which are unable to propel themselves independently of the currents and hence unable to avoid an oil plume, would be the most vulnerable. Planktonic organisms would be exposed to comparatively high concentrations of dispersed oil for a short time period (hours), but effects of this exposure (such as developmental abnormalities) could manifest over a longer period and either cause outright mortality or a permanent decline in the fitness of the organism. Current tests that measure toxicity over 72 h may overestimate the chemical exposure but underestimate the latent effects. The second likely scenario for ecological risk is sinking of weathered oil droplets and deposition of dispersed oil into the sediments (Figure 1B). This exposure scenario may be particularly relevant for coastal environments. Once oil is deposited in the sediments, it can stay there for a very long time depending on the rates of biodegradation, so there is the potential for chronic exposure to oil. Furthermore, this oil can be ingested by deposit-feeding organisms and potentially passed to higher trophic levels.

### 3.4 The application of toxicity data in Net Environmental Benefit Analysis

Once research studies have been conducted, assessing the contribution of the chosen dispersant to the risk associated with the dispersed oil is recommended as described below. For each toxic endpoint, the data should be plotted against the measured TPH concentration and ensuring that the ranges of TPH tested for each condition overlap (Bejarano et al., 2014). Where similar relationships between chemically dispersed oil and mechanically dispersed oil TPH concentrations and effects are observed, the dispersant would not be predicted to add to the risk from the oil (see Figure 2). However, if the relationships are not the same, the dispersant would add to the risk from the dispersed oil for that species and endpoint. This process is shown in Figure 3, where the toxicity of dispersed crude oil is compared to crude oil on a TPH basis by Hemmer et al. (2011). For most dispersants, the dispersed oil was not more toxic than oil on its own. The two that may confer additional toxicity would be avoided. Ideally, a dispersant that did not increase the ecotoxicological risk to relevant taxa would be chosen. As shown in Figure 2, drawing conclusions about risk using the loading levels alone can be misleading. In figure 2, the same toxicity data are plotted either on the basis of loadings (panel A) or measured hydrocarbon concentration (panel B). Measured on the basis of loadings, the dispersed oil appears to be approximately 100- fold more toxic, when if the measured hydrocarbon concentrations are compared, the toxicity of the two compounds is identical.

In assessing safe concentrations of contaminants in waters or safe dilutions of complex effluents, the common approach is to undertake toxicity tests on at least 8 species from a minimum of four taxonomic groups (ANZECC/ARMCANZ, 2000). The test data are then plotted in a species sensitivity distribution (SSD) (Figure 4). Ideally plot should use either all chronic or all acute data, although estimates of chronic effects can be made from acute data. Comparisons of the SSDs for the different dispersants and dispersed oil products being evaluated would indicate the most sensitive species and how the relative toxicity to particular sentinel species (e.g. corals, fish) changes with the dispersant formulation (Bejarano et al., 2014). A concentration required for a given level of species protection (95, 90, 80%) can be calculated from each SSD. The current protocol for assessing dispersants in Australia (AMSA 2011), where multiple species are tested but only a few are used for the pass fail criteria, does not mimic this process.

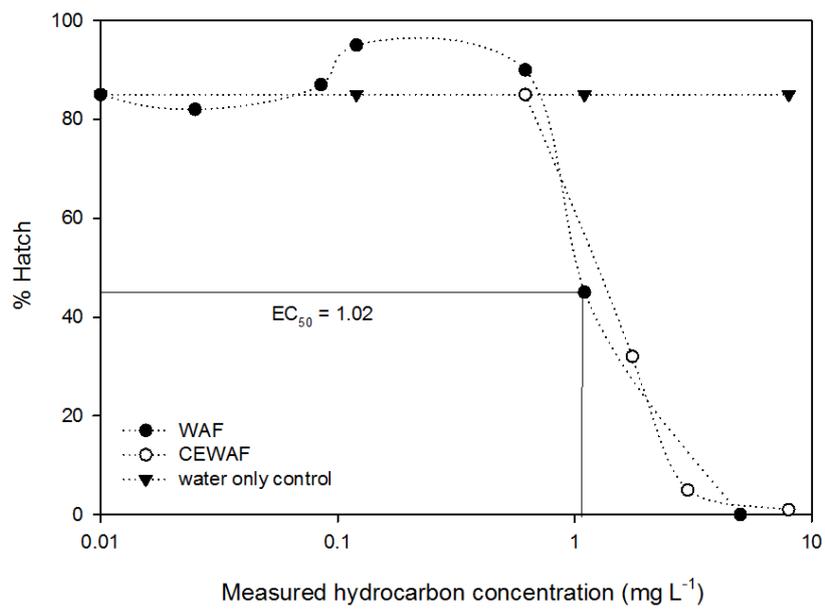
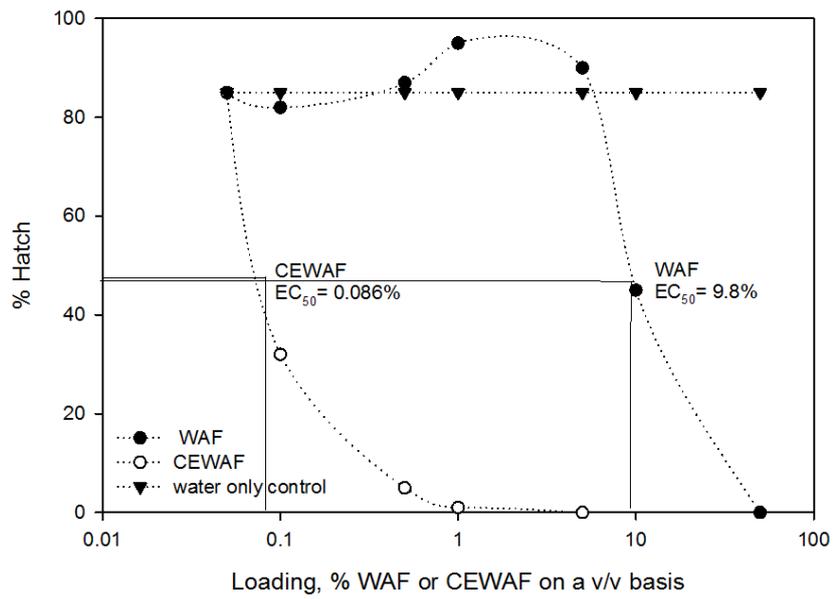


Figure 2. Toxicity of oil and dispersed oil to Atlantic herring embryos plotted against loadings versus against TPH in the water column. (Redrawn with data taken from Adams et al., 2014).

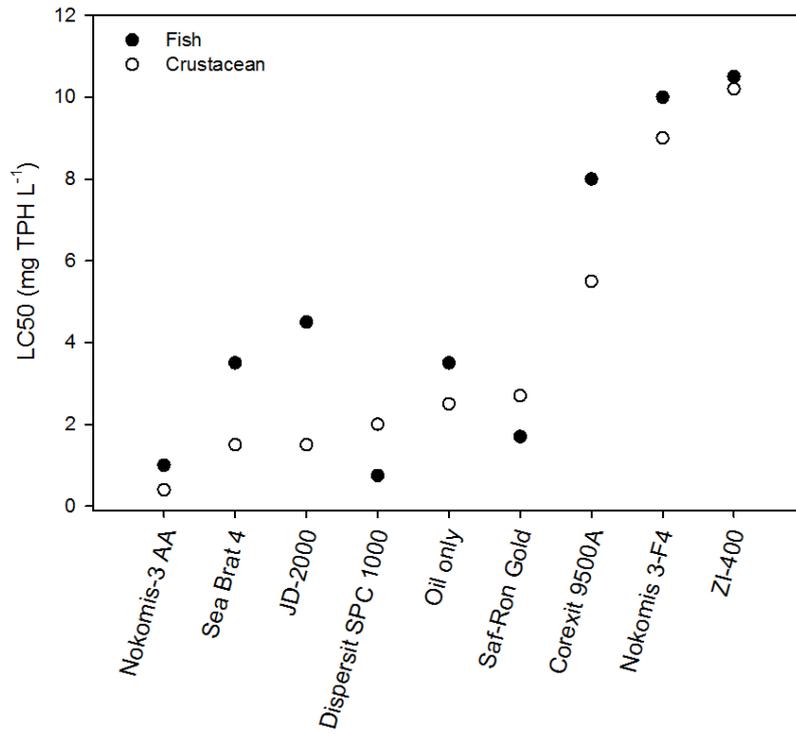


Figure 3. Ranked comparisons of the toxic response of dispersed oil, and oil on a TPH basis (redrawn with data taken from Hemmer et al., 2011)

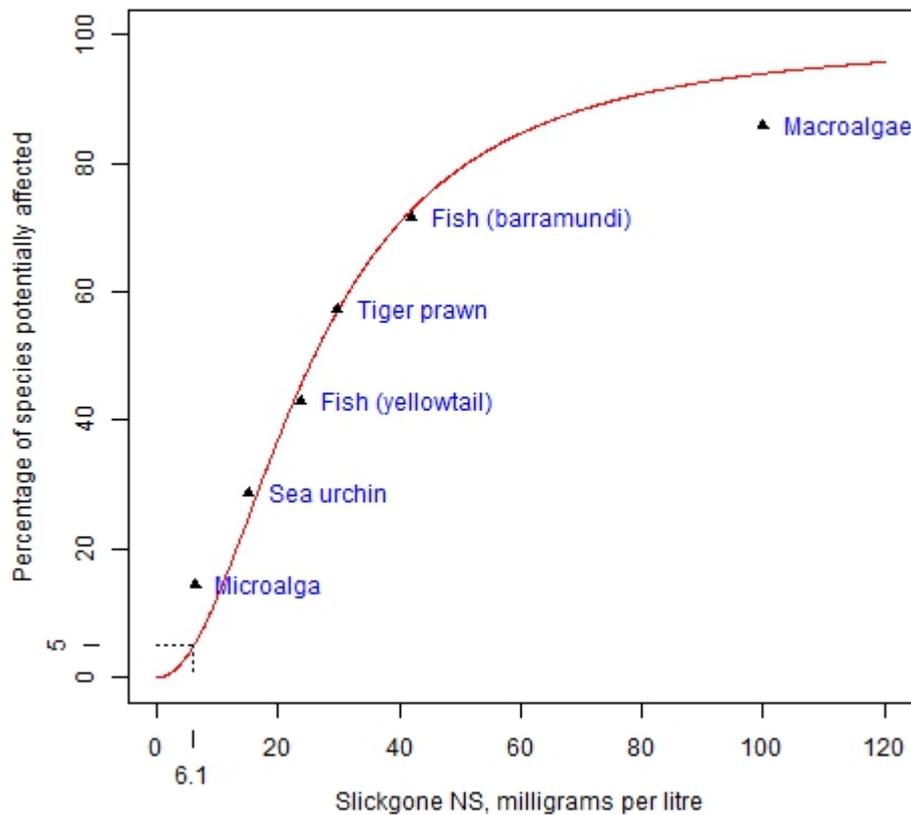


Figure 4. Species sensitivity distribution for Slickgone NS (data provided by ESA). This figure contains a mixture of acute and chronic endpoints .

The above approach is essential for ranking the dispersant to be used, both on its intrinsic toxicity and its toxicity in a mixture with oil. The risk scenario can be readily compared for differing conditions of dispersion and dilution during a Net Environmental Benefit Analysis if this information is compiled.

### Summary

The goal of dispersant use is to reduce environmental impacts caused by surface slicks (e.g., impacts to marine mammals, seabirds, marshes, etc.), rapidly reduce oil toxicity through dilution, and ultimately enhance the biodegradation and removal of oil from the environment. Their use will increase the amount of oil that is distributed in the water column increasing the risk to planktonic, pelagic and benthic organisms. However, used appropriately, they can also protect coastal ecosystems as well as bird and marine mammal populations (NRC 2005, 2013). Furthermore, they may also decrease the residence time of oil in the water column by making it more bioavailable to oil-degrading bacteria. There is generally a net benefit from the use of dispersants on large oil spills as the short-term, transient exposure of dispersed oil to water-column communities has much less of an overall ecological effect than impact of oil that becomes stranded on shorelines where it will typically persist for years. Experts have concluded that oil spills with significant environmental impacts have typically been associated with near-shore or intertidal accumulations of oil (Lewis et al., 1997).

For the most studied dispersant formulation, Corexit 9500, the increased risk for most taxa seems to come from the increased solubility of the toxic components of the oil as opposed to from the dispersant itself. Other dispersant formulations have not been studied well enough to make a similar generalisation. Following a spill, responders need to quickly perform a “Net Environmental Benefit Analysis” to determine whether the potential risks involved in dispersion are counter-balanced by the hazards of inaction, and potentially letting oil wash ashore, where it may persist. However, as described in detail above, substantial data gaps regarding the impacts of oil and dispersants persist, especially for those dispersants that either are registered or are to be registered for use in Australia by AMSA, making risk assessment impossible. These gaps persist because:

- (i) It is not known how dispersants change the biodegradability of oil;
- (ii) It is not known how the very short time periods to which planktonic organisms are exposed to oil is sufficient to cause long term effects;
- (iii) Many literature studies poorly characterize the exposure to oil in their test systems;
- (iv) Few studies address the impacts on corals and deposit feeding organisms, and
- (v) The registered dispersant products approved for use in Australia have not been studied as much as other products used elsewhere in the world.

Research must be carried out to fill data gaps to support science based decision making.

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## Appendix A. Online resources for additional information for the components of current use dispersants

CAS #	NAME	USA NLM OR TOXNET REFERENCE	TOXIPEDIA REFERENCE
577-11-7	Sodium dioctylsulfosuccinate (also dioctyl sulfosuccinate or DOSS)	<a href="http://chem.sis.nlm.nih.gov/chemidplus/rn/577-11-7">http://chem.sis.nlm.nih.gov/chemidplus/rn/577-11-7</a>	<a href="http://www.toxipedia.org/display/toxipedia/Butanedioic+acid%2C+sulfo-%2C+1%2C4-bis%282-ethylhexyl%29+ester%2C+sodium+salt">http://www.toxipedia.org/display/toxipedia/Butanedioic+acid%2C+sulfo-%2C+1%2C4-bis%282-ethylhexyl%29+ester%2C+sodium+salt</a>
1338-43-8	Sorbitan, mono-(9Z)-9-octadecenoate	<a href="http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~xPj8sV:1">http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~xPj8sV:1</a>	<a href="http://www.toxipedia.org/display/toxipedia/Sorbitan%2C+mono-%289Z%29-9-octadecenoate">http://www.toxipedia.org/display/toxipedia/Sorbitan%2C+mono-%289Z%29-9-octadecenoate</a>
9005-65-6	Polyoxy-1,2-ethanediyl derivatives of sorbitan, mono-(9Z)-9-octadecenoate	<a href="http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~xPj8sV:2">http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~xPj8sV:2</a>	<a href="http://www.toxipedia.org/pages/viewpage.action?pageId=6005095">http://www.toxipedia.org/pages/viewpage.action?pageId=6005095</a>
9005-70-3	Polyoxy-1,2-ethanediyl derivatives of sorbitan, tri-(9Z)-9-octadecenoate	<a href="http://chem.sis.nlm.nih.gov/chemidplus/rn/9005-70-3">http://chem.sis.nlm.nih.gov/chemidplus/rn/9005-70-3</a>	<a href="http://www.toxipedia.org/pages/viewpage.action?pageId=6016214">http://www.toxipedia.org/pages/viewpage.action?pageId=6016214</a>
103991-30-6	Ethoxylated fish oil		
8002-26-4	Tall oil	<a href="http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~FLR6lk:1">http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~FLR6lk:1</a>	
112-34-5	Butyldiglycol; Diethylene glycol monobutyl ether;	<a href="http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~sjDCFK:16">http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~sjDCFK:16</a>	<a href="http://www.toxipedia.org/pages/viewpage.action?pageId=6017162">http://www.toxipedia.org/pages/viewpage.action?pageId=6017162</a>
64742-47-8	2-(2-butoxyethoxy)ethanol		
64742-47-8	Petroleum distillates, hydrotreated light fraction	<a href="http://chem.sis.nlm.nih.gov/chemidplus/rn/64742-47-8">http://chem.sis.nlm.nih.gov/chemidplus/rn/64742-47-8</a>	<a href="http://www.toxipedia.org/display/toxipedia/Distillates+%28petroleum%29%2C+hydrotreated+light">http://www.toxipedia.org/display/toxipedia/Distillates+%28petroleum%29%2C+hydrotreated+light</a>
29911-28-2	Dipropylene glycol monobutyl ether	<a href="http://chem.sis.nlm.nih.gov/chemidplus/rn/29911-28-2">http://chem.sis.nlm.nih.gov/chemidplus/rn/29911-28-2</a>	<a href="http://www.toxipedia.org/pages/viewpage.action?pageId=6017088">http://www.toxipedia.org/pages/viewpage.action?pageId=6017088</a>
57-55-6	Propylene glycol	<a href="http://chem.sis.nlm.nih.gov/chemidplus/rn/57-55-6">http://chem.sis.nlm.nih.gov/chemidplus/rn/57-55-6</a>	<a href="http://www.toxipedia.org/display/toxipedia/1%2C2--Propanediol">http://www.toxipedia.org/display/toxipedia/1%2C2--Propanediol</a>
111-76-2	2-Butoxyethanol (ethylene glycol mono-n-butyl ether)	<a href="http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~sjDCFK:7">http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~sjDCFK:7</a>	<a href="http://www.toxipedia.org/pages/viewpage.action?pageId=6017170">http://www.toxipedia.org/pages/viewpage.action?pageId=6017170</a>

# Appendix B. Results of US EPA testing of Dasic Slickgone NS, fuel oil #2, and dispersed fuel oil #2

Data provided by John Belk, Dasic International

United States Testing Company, Inc.

Client: Dasic International Ltd. 07345  
 Winchester Hill, Romsey 12/17/87  
 Hampshire, S051 7Yd, England U.K.

Project: EPA revised Standard Dispersant Toxicity Test

Sample: One (1) sample submitted and identified by Client as:  
 Slickgone NS  
 Sample was low viscosity, oily, yellowish, water miscible liquid stored at ambient temperature.

Procedure: Tests were conducted in accordance with the guidelines Of the U.S. EPA "Standard Dispersant Effectiveness and Toxicity Tests", 40 CFR, Part 300, Vol. 49, NO. 139, July 18, 1984.

Summary:

Toxicity

<u>Test Sample</u>	<u>Species</u>	<u>LC<sub>50</sub> (ppm)</u>
Dispersant	<u>Fundulus heteroclitus</u>	(96hr) 95
	<u>Artemia salina</u>	(48hr) 51
Dispersant & No. 2 Fuel Oil (1:10)	<u>Fundulus heteroclitus</u>	(96hr) 498
	<u>Artemia salina</u>	(48hr) 101
Reference Toxicant (DDS)	<u>Fundulus heteroclitus</u>	(96hr) 11.5
	<u>Artemia salina</u>	(48hr) 17

## Appendix C. Toxicity testing performed by CEDRE

(translated by the authors)

### 2. TOXICITY OF SLICKGONE TO THE GREY PRAWN (*CRANGON CRANGON*)

2.1 Acute toxicity test of the dispersant "Slickgone" to the grey prawn

#### 2.1.1 Source of the specimens

Microchalutage, Concarneau Bay, La Froet-Fouesnant

#### 2.1.2 Sensitivity of the prawns to the nominal doses

(Results from tests from 16-17 April 1991)

Mortality observed after 6 hours exposure, followed by 24 Hours recovery in flowing water:

Nominal Doses (concentrations in mg/l)	Mortality	%
Control	0/30	0.0
20	0/30	0.0
40	0/30	0.0
50	2/30	6.7
60	14/30	46.7
70	15/30	50.0
80	23/30	76.7
100	26/30	86.7
120	28/30	93.3

LC<sub>50</sub> calculated via probit method = 66.7 mg/L

#### 2.1.3 Acute toxicity

(tested 18-19 April 91)

Mortality observed in three tanks after a six hour exposure to a concentration of slickgone 10 times the LC<sub>50</sub> based on nominal dosage (667 mg/L) followed by 24 hours recovery in flowing water

	Tank 1	Tank 2	Tank 3	Control
Mortality (No. Of ind)	1/30	6/30	6/30	0/30

The intertank difference is not significant at a 10% threshold (the test would be rejected at 5)

Mean mortality is 14.4%

### 3. TOXICITY OF SLICKGONE TO WHITE PRAWNS (*Palaemonetes varians*)

#### 3.1. Acute toxicity of Slickgone

##### 3.1.1 Source of specimens

Ets. TYMER, le Croisic



### 3.1.2 Sensitivity of prawns to the nominal doses

Results of tests from 19-20 June 1991. Mortality observed after 6 hours exposure, followed by 24

Hours recovery in flowing water:

Nominal Doses (concentrations in mg/l)	Mortality	%
Control	0/30	0.0
20	0/30	0.0
40	0/30	0.0
50	1/30	3.3
60	11/30	36.7
70	20/30	66.7
80	27/30	90
100	29/30	96.7
120	30/30	100

LC<sub>50</sub> calculated via probit method = 54.0 mg/L

# Report provided by Dasic (John Belk) Appendix D. Biodegradation of oil in the presence of Slickgone NS

## EFFECT OF THE DISPERSANT DASIC SLICKGONE NS ON THE BIODEGADABILITY OF CRUDE OIL

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The trials were conducted according to the protocol described in part IV of the CEDRE document.

### RESULTS

Weight of residual hydrocarbons (mg)

	<u>T1</u>	<u>T2</u>	<u>T3</u>	<u>E</u>
	Oil biodegradation	Oil control	Oil + Dispersant control	Oil+ Dispersant Biodegradation
	101.2	128	137.5	98.2
	98.9	131.1	137.6	106.4
	99.4	133.2	140.2	104
	101.1	131	139.3	104.2
	105.3	131.4	145.4	105.5
Mean	101.2	130.9	140	103.7
SD	2.5	1.9	3.2	3.2

Biodegradation of crude oil  $BT = T2 - T1 = 29.7$  mg

Biodegradation of oil dispersant  $BE = T3 - E = 36.3$  mg

$BE/BT = 1.22$

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