Critical Evaluation of CROSERF Test Methods for Oil Dispersant Toxicity Testing under Subarctic Conditions

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EXECUTIVE SUMMARY

The Alaska Region Oil and Hazardous Substance Pollution Contingency Plan requires that decisions regarding chemical dispersants use in oil spill response in Alaska consider the potential impacts of chemically dispersed oil, including the toxicity to aquatic organisms. This review critically evaluates the aquatic organism toxicity testing protocols developed by the Chemical Response to Oil Spills: Ecological Research Forum (CROSERF) for applicability to assessing chemical dispersant toxicity under subarctic conditions. CROSERF was established as a working group of industry, government, and university scientists to coordinate and disseminate research on chemical oil spill dispersants. CROSERF participants developed aquatic toxicity testing protocols during 1994 to 2000 with the foremost objective of standardizing test methods and reducing inter-laboratory variability. A number of refinements are recommended to adapt the CROSERF protocols for testing with subarctic species under conditions of expected longer oil persistence. Recommendations were focused on providing toxicity test data most relevant to risk management decisions regarding dispersant use in subarctic environments, rather than the primary CROSERF objective of standardizing procedures. Recommended refinements of the CROSERF protocols include (1) testing both a fresh and moderately weathered oil under conditions of moderate mixing energy, (2) testing both Corexit 9500 and 9527 using a high dispersant:oil ratio, (3) preparing toxicity test solutions using variable dilutions rather than variable loading, (4) using static exposures in open chambers, (5) increasing the duration of tests from 4 days to 7 days to allow assessment of delayed mortality, (6) quantifying approximately 40 polycyclic aromatic hydrocarbons (PAHs) and their alkyl homologs (i.e., predominant petroleum PAHs) in the toxicity test solutions, (7) testing Pacific herring larvae, an urchin species, and a calanoid copepod under subarctic conditions of temperature and salinity, (8) assessing the potential for photoenhanced toxicity by incorporating a limited exposure to sunlight or simulated natural sunlight, and (9) incorporating a bioaccumulation endpoint by measuring PAH accumulation in copepod tissue. Refinements in the preparation of oil dosing solutions, exposure and light regimes, and analytical chemistry should increase the utility of the test results for interpreting the toxicity of chemically dispersed oil and making risk management decisions regarding dispersant use under subarctic conditions.

INTRODUCTION

Research performed to date has not revealed a consistent trend in the relative toxicity of chemically dispersed and physically dispersed oil, with toxicity dependent on the specific dispersant and oil evaluated, the test organism, and the oil exposure regime (e.g., Anderson et al., 1985; Fucik et al., 1995; DeCola, 1999; Singer et al., 1999; Clark et al., 2001; Fingas, 2002). It is well established that chemically dispersed oil is substantially more toxic than dispersant treatment alone for current generation chemical dispersants such as Corexit 9500 and 9527 (ExxonMobil, Houston, TX).   There is no clear trend in the toxicity of chemically dispersed fresh oil compared to chemically dispersed weathered oil (Anderson, 1985; Singer et al., 1999; Clark et al., 2001; Rhoton et al., 2001). However with only a few exceptions (Anderson, 1985; Rhoton et al., 2001; Barron et al., 2003), comparisons of the toxicity of chemical dispersions of fresh and weathered oil have not been performed with species and test conditions (e.g., temperature, salinity) that occur in subarctic marine environments. As discussed below, dispersant efficacy and toxicity is dependent on temperature, salinity, mixing energy, dispersant application, and test species/toxicity endpoint (Fingas, 2002). Thus any laboratory toxicity test results must be viewed relative to the conditions used to prepare and expose aquatic organisms to water accommodated fractions of dispersed oil (WAF). Currently, the Unified Plan for Alaska (ARRT, 1999) allows dispersant use only when conventional response activities (e.g., mechanical recovery) are not feasible or are likely to be inadequate in containing an oil spill. Decisions concerning dispersant use must be based on an evaluation of the potential impacts of the chemically dispersed oil versus not dispersing the oil (ARRT, 1999). The toxicity of chemically dispersed oil to aquatic organisms is a critical consideration in the evaluation of chemical dispersant use in oil spill response.

The Chemical Response to Oil Spills: Ecological Research Forum (CROSERF) is a working group that was established to coordinate and disseminate research on chemical oil spill dispersants. CROSERF participants included researchers at several universities, petroleum companies, and government agencies. The CROSERF working group developed aquatic toxicity testing protocols during 1994 to 2000 with the foremost objective of standardizing test methods and reducing inter-laboratory variability. CROSERF toxicity test methods and protocols have been published in various scientific and technical publications, with the most recent compilation presented in the proceedings of the 2001 International Oil Spill Symposium (e.g., Clark et al., 2001; Singer et al., 2001a,b; Rhoton et al., 2001). The protocols include standardized methods for preparing WAF of physically dispersed and chemically dispersed oil products and weathered oil. The test protocols also include methods for exposing early life stages of aquatic organisms to dispersed oil, with the objective of standardizing as many test parameters as possible.

The objective of this paper is to critically evaluate CROSERF toxicity test methods for their applicability to assessing the toxicity of chemically dispersed oil under subarctic conditions. Whereas CROSERF participants have published extensively on the toxicity test methods and results, an independent critical assessment of the test protocols has not been previously presented. Refinements in the CROSERF toxicity testing protocols are recommended, rather than developing a subarctic dispersed oil testing program 'from scratch'. This recommendation is made with the recognition of the considerable effort and thought invested in the existing

CROSERF protocols. Recommendations were focused on providing toxicity test data most relevant to risk management decisions regarding dispersant use in subarctic environments, rather than the primary CROSERF objective of standardizing procedures. While focused on Alaska relevant test conditions and species, the recommendations for altering the CROSERF WAF preparation and testing protocols should be considered for chemical dispersant testing in general.

OVERVIEW OF CROSERF PROCEDURES

The CROSERF toxicity testing protocols were established to standardized WAF preparation, laboratory exposures to aquatic organisms, and analytical chemistry measurements used to determine the acute toxicity of physically dispersed and chemically dispersed oil. The procedures were adapted from early 'slow stir' WAF preparation methods (e.g., Anderson, 1985) and standardized toxicity testing protocols from the U.S. Environmental Protection Agency and American Society for Testing and Materials. The typical CROSERF WAF preparation includes an initial 0.45 um seawater filtration, a geometric series of increasing oil to water loading rates, and 18 hours of slow stirring of each water-oil mixture in a sealed carboy with a 20 to 25% headspace. The oil-water mixture is allowed to settle for 6 hours then the aqueous phase WAF is used in 4 day acute toxicity tests. WAF of chemically dispersed oil is prepared at a moderate mixing energy using a 1:10 dispersant to oil ratio.

CROSERF participants have standardized methods for conducting static renewal exposures with aquatic organisms, but recommend a short duration flow-through testing regime in airtight test vessels. The CROSERF objective of the flow-through test regime was to characterize toxicity from exposure to a decreasing concentration that was considered more representative of environmental mixing and dilution during an oil spill, compared to standard static renewal procedures. Airtight test chambers were developed to reduce losses of volatile organic compounds (VOCs) such as monoaromatic hydrocarbons (MAHs). CROSERF test endpoints have included mortality or impaired mobility after 4 days from initiation of oil exposure with a variety of standard Gulf of Mexico and Pacific Coast species. CROSERF recommendations also include analytical verification of toxicity tests solutions by measuring VOCs and total petroleum hydrocarbons (TPH).

RECOMMENDED REFINEMENTS TO CROSERF PROCEDURES

The toxicity of chemically dispersed oil to aquatic organisms is a critical consideration in the evaluation of chemical dispersant use in oil spill response. An independent critical assessment of the CROSERF toxicity test protocols was performed because the data generated from these methods are likely to be used by risk managers in assessing the potential impacts of chemically dispersed oil. Also, the applicability of CROSERF test methods to subarctic conditions was evaluated because of the limited available toxicity data on chemically dispersed oil to subarctic species, and the likelihood of additional testing in the future.

A number of refinements are recommended to adapt the protocols for testing with subarctic species under conditions of expected longer oil persistence. Refinements are recommended in the WAF preparation, exposure and light regimes, and analytical chemistry to increase the utility of the test results for making risk management decisions regarding the toxicity of chemically dispersed oil and dispersant use under subarctic conditions. Table 1 summarizes CROSERF WAF preparation and testing procedures and recommended changes based on this evaluation.

Oil Weathering

The most important determinant of dispersant efficacy is oil composition, which is directly affected by weathering (Fingas, 2002). CROSERF test protocols have included evaluating the toxicity of both fresh and weathered oil. Based on the method for quantifying petroleum exposure, weathered oil has been reported as similar, more toxic, or less toxic than fresh oil in CROSERF reports (e.g., Clark et al., 2001; Rhoton et al., 2001; Singer et al., 2001a).

Testing of both fresh and a moderately weathered Alaska North Slope crude oil (ANS) is recommended to evaluate the range of possible oil weathering that may be encountered under possible dispersant use scenarios. Singer et al. (2001a) used an oil weathering procedure of distillation (topping) to a vapor phase temperature of 200 $^\circ$C under controlled conditions in the laboratory to simulate evaporative weathering. This topping procedure is equivalent to 0.5 to 1 day under temperate conditions (Neff et al., 2000), and thus represents a reasonable 'worst case' scenario for the degree of oil weathering that may occur prior to dispersant use under subarctic conditions. Oil weathering under subarctic conditions may occur at a substantially slower rate than in temperate environments (Payne et al., 2001).

Chemical Dispersant Application and Mixing Energy

Dispersant efficacy can be dependent on the type of chemical dispersant used, and efficacy generally increases with increasing dispersant application and increasing energy used to mix the oil, dispersant, and water (Fingas, 2002). For example, efficacy tests with Corexit 9500 on ANS indicated that dispersant effectiveness was directly proportional to the dispersant:oil ratio in the range of 1:10 to 1:50 (DeCola, 1999). Dispersants are known to increase the entrainment and dissolution of oil components into the water column, and increase the volume of water that is impacted during a spill (French McCay and Payne, 2001). The toxicity of chemically dispersed oil is expected to be similar to physically dispersed oil in cases of poor dispersant efficacy. The reported CROSERF tests have used a 1:10 dispersant to oil ratio (e.g., Clark et al., 2001; Rhoton et al., 2001; Singer et al., 2001b), which represents the maximum likely dispersant application during a spill. Field applications of dispersants are likely to be lower (e.g., 1:20 or 1:50; Davies et al., 1998) but toxicity testing of a high dispersant:oil ratio ensures that maximum dispersion of oil under the laboratory test conditions.

Consistent with the CROSERF approach, a 1:10 dispersant:oil ratio is recommended for assessing dispersant toxicity under subarctic conditions because it provides the greatest potential to detect differences in the toxicity of chemically dispersed and physically dispersed oil. Testing various dispersant:oil ratios is important in evaluating dispersant efficacy, but determining the

toxicity of a single high ratio provides a means to maximize exposures and should provide a conservative estimate of toxicity. Only Corexit 9500 has been tested for toxicity under subarctic conditions using CROSERF protocols (Rhoton et al., 2001), although Barron et al. (2003) tested Corexit 9527 with high mixing energy mixing and other non-standard CROSERF test conditions. Testing of both Corexit 9500 and 9527 is recommended because both dispersants are available for use in Alaska.

CROSERF protocols use moderate mixing energy (e.g., 20 to 25% vortex) in preparing chemically dispersed WAF, but differ by investigator regarding the mixing energy of the physically dispersed oil. Singer et al. (2001b) recommend low mixing energy (<5% vortex) for physically dispersed oil to limit entrainment of particulate oil greater than 1 μm, but acknowledged that the use of WAF preparations of differing energy results in less comparable results. Blenkinsopp et al. (1996) evaluated repeatability of mixing procedures and also recommended a minimal vortex to ensure reproducibility in the chemical composition of WAF. In contrast, the same moderate mixing energy for producing physically and chemically dispersed oil was used by Clark et al. (2001), because it was considered to be more representative of environmental conditions during an oil spill. The Clark et al. (2001) CROSERF procedure of the same moderate mixing energy (20 to 25% vortex) in preparing WAF of both physically dispersed and chemically dispersed oil is recommended. We also agree with the CROSERF procedure of an 18 hour mixing time followed by a 6 hour settling time for WAF of both physically dispersed and chemically dispersed oil. Singer et al. (2000) considered a 20 to 25% vortex to be representative of field conditions that did not produce identified dispersion or emulsification of Prudhoe Bay crude.

Water Accommodated Fractions of Oil

Two general approaches are available for producing a series of toxicity test solutions of decreasing concentration of either chemically or physically dispersed oil: variable loading and variable dilution (Fig. 1). Variable loading uses a series of decreasing concentrations of applied oil, whereas variable dilution using the same oil to water ratio and a series of increasing water dilutions. In both procedures, a series of decreasing concentrations of WAF is generated and tested to define the toxicity of the dispersed oil. Also, in both procedures the recommended dispersant:oil ratio of 1:10 is maintained in the chemically dispersed WAF.

Singer et al. (2001b) have argued for using variable loading in the CROSERF test protocols because of the more realistic oil:water ratios and the approach allows the loading rate to be determined (e.g., 0.01 to 10 g oil per L). One concern is that the variable loading approach results in compositional changes in the aqueous phase oil as illustrated in Figure 1. For example, the composition of the toxic components and the potency of the oil are different in each of the tested WAFs produced using variable loading. This may be problematic in interpreting and comparing the toxicity of different oil products because the estimated measure of toxicity (e.g., median lethal concentration; LC50) is confounded with changes in both concentration and potency, as well as the applied loading rate. For example, Rhoton et al. (2001) and Singer et al. (2000) have shown that the proportion of MAHs relative to TPH increases with increasing oil loading rate. Blenkinsopp et al. (1996) recommended against serial dilution because it

underestimated concentrations of some MAHs, but this recommendation was made in the context of extrapolating WAF toxicity to a specified loading rate. Clark et al. (2001) has stated that while loading rate has merit, it was not a relevant measure of exposure for interpreting the toxicity of chemically dispersed oil. Also, loading rate did not correlate well with the toxicity of chemically dispersed oil in the Clark et al. (2001) CROSERF tests.

Although the merits of WAF preparation procedures are a matter of scientific debate, a variable dilution approach is recommended for preparing WAF for dispersed oil testing. The variable dilution will provide a consistent chemical composition in a test series for each oil-dispersant combination and provides some economies in analytical costs by reducing the need to analyze the composition of every tested concentration. The variable dilution approach will also provide greater comparability of the toxicity of chemically and physically dispersed oil by standardizing the oil:water ratio used in preparing WAF. Various CROSERF tests have used a maximum of 25 g oil per L of water, whereas Blenkinsopp et al. (1996) have recommended that a maximum loading rate of 10 g/L. To ensure that test concentrations exceed median lethal concentrations for physically dispersed oil, a maximum loading rate of 25 g/L is recommended for testing under subarctic conditions. This loading is equivalent to a 1:40 oil to water ratio, which is substantially lower than typical non-CROSERF WAF preparations (1:10 ratio) used to make serial dilutions (e.g., Anderson, 1985; Barron et al., 1999). Other CROSERF procedures for preparing WAF are considered appropriate, including preparing WAF in the dark in sealed containers using a fixed head space to water volume of approximately 20% (Singer et al., 2000).

An alternative approach to the loading rate concept should also be considered in future testing based on the surface to volume ratio of oil and oil layer thickness during WAF preparation. For example in field experiments with ANS, the thickness of oil plumes ranged from 0.015 to 2.5 mm over two days of environmental weathering, with a mean thickness of 0.8 mm (Davies et al., 1998). This information could be used in developing appropriate maximum oil to water ratios.

Analytical Chemistry

Various approaches have been used to quantify oil exposure and toxicity to aquatic organisms, including quantifying the amount of oil added to the initial WAF mixture (i.e., loading rate), and measuring the total concentration of aqueous phase petroleum (TPH) or specific oil components in the WAF. CROSERF protocols recommend measuring TPH and specific volatile organic compounds (VOCs), which are comprised of some mono-aromatics (MAHs) and alkanes with 6 to 9 carbons. CROSERF protocols have not recommended measuring polycyclic aromatic hydrocarbons (PAHs), although Singer et al. (2000) identified the analysis of polycyclic aromatic hydrocarbons (PAHs) as an optional analytes. PAHs are considered to be determinants of both the acute and chronic toxicity of spilled oil, and are standard analytes used to assess the source, persistence, and toxicity of spilled oil (e.g., Neff and Burns, 1996; French, 2002). We recommend measuring the standard suite of approximately 40 PAHs including the alkyl homologs (e.g., Short and Harris, 1996), as well as TPH because it quantifies total petroleum exposure and is particularly relevant in low PAH oils (Barron et al., 1999).

TPH and PAH concentrations should be measured at test initiation and test end to quantify average exposure concentrations. Additionally, PAHs should be quantified in tissues of selected test species to quantify bioaccumulation potential and hydrocarbon persistence (see below). MAHs should only be considered an optional analytical chemistry measurement because, as discussed below, they are not persistent in spilled oil and are no longer routinely measured during oil spill events. Evaporative losses of volatiles during oils spills can be extensive, thus a focus on VOC measurements is not merited. For example in experimental slicks in the North Sea, ANS weathered for 5 hours had an approximately 30% loss of volume from evaporation, and a lighter oil (Forties blend crude oil) lost approximate 30% volume within 2 hours primarily via evaporation (Davies et al., 1998).

Test Species and Life Stage

A variety of aquatic organisms have been tested using CROSERF protocols, with most reports on the early life stages of temperate species (e.g., Gulf of Mexico and Pacific crustaceans and fish). Only one CROSERF report (Rhoton et al., 2001) has evaluated the toxicity of a subarctic species. Rhoton et al. (2001) determined that tanner crab larvae were more sensitive to ANS than a standard Gulf of Mexico fish species (*Menidia berylina*), and had similar sensitivity as a standard test species of mysid shrimp. Perkins (ADEC, 2000) summarized some relevant criteria in selecting test species for evaluating the toxicity of chemically dispersed oil in Alaska, including organism availability and practicability of testing, economic/social value, ecological relevance, and susceptibility to oil (e.g., high exposure and sensitivity). Perkins (ADEC, 2000) noted that most Alaskan marine species were either (1) not commercially available and thus required field collections, (2) were of large size and not conducive to CROSERF recommended test chambers, and/or (3) could only be obtained for a relatively limited time each year. Because of these concerns, Perkins (ADEC, 2000) recommended testing the topsmelt (a non-Alaskan fish) and a species of Alaskan echinoderm (purple or green sea urchin species), or developing cultures of Alaskan mysids or copepods.

A variety of Alaskan marine species have been evaluated for the sensitivity to ANS or Prudhoe Bay crude oil under non-CROSERF test conditions including salmon, flounder, amphipods, crabs, shrimp, shellfish, copepods, herring, and sand lance (Rice et al., 1976; Anderson, 1985; Moles, 1998; Duesterloh et al., 2002; Barron et al., 2003). Based on available information and experience, three Alaskan species are recommended for evaluating dispersed oil toxicity under subarctic conditions: a calanoid copepod, a sea urchin species, and larval Pacific herring. These species are known to be sensitive to petroleum, are important ecologically and/or economically, and can be maintained under the static test conditions recommended below (EPA, 1995; Duesterloh et al., 2002; Barron et al., 2003).

Ecological relevance and known sensitivity should outweigh logistical concerns with collection and rearing, as long as high laboratory and control survival can be achieved. Collection and testing have been previously demonstrated for both herring and copepods (e.g., Carls et al., 1999; Deusterloh et al., 2002; Barron et al., 2003), and standardized tests with purple sea urchins have been developed (EPA, 1995). Herring were considered an excellent candidate test species in ADEC (2000), with the caveat of their seasonal availability. Herring larvae are recommended

over herring embryos because of similar sensitivity, and more rapid bioaccumulation and toxicity responses. As noted by Perkins (ADEC, 2000), sea urchins occur in Alaska, are sensitive to oil, and an Alaskan species may be more available than alternative subarctic species. A calanoid copepod is also recommended because of the capacity of copepods such as *Calanus marshallae* to bioaccumulate petroleum compounds, making them ideal for assessing and comparing the bioavailability of chemically and physically dispersed oil.

Oil Exposure Regime

The oil exposure regime in a dispersed oil toxicity test must be balanced between environmental realism (i.e., persistence of spilled oil in the environment), concerns for chronic toxicity (i.e., potential for sublethal and/or delayed effects), and the practicability and costs of testing. Two CROSERF protocols are available, each with a total duration of four days (e.g., Singer et al., 2001): static-renewal exposure in open test chambers and flow-through exposures in airtight test chambers. The static-renewal exposure consists of the addition of newly prepared WAF to an open glass test chamber every 24 hours; the chamber is aerated as necessary to maintain dissolved oxygen levels. The static-renewal regime results in an initial spike then declining concentrations of oil every 24 hours, which CROSERF participants conclude is not representative because it overestimates the potential environmental toxicity of oil. The oil exposure regime considered most representative of oil toxicity by CROSERF participants is a flow-through exposure with clean seawater that results in dilution of the initial WAF to non-detectable levels within 8 hours (half-life ~ 2 hr; Clark et al., 2001). Various CROSERF reports state that the duration of oil exposure in the flow-through regime is most representative of the persistence of spilled oil in the environment, but quantitative support and literature citations for this assertion are generally not provided in the available reports (e.g., Singer et al., 1999; Singer et al., 2000; Clark et al., 2001; Rhoton et al., 2001; Singer et al., 2001a,b). ARRT (1999) noted that marine field experiments that indicated of disappearance of dispersed oil in 5 to 12 hours. However, Fingas (2002) has recently highlighted the need for caution in interpreting previous field experiments with dispersants because of the concern for inadequate tracking and sampling of the dispersed phase plume, which can have a different trajectory than the oil slick.

The short duration of the CROSERF flow-through exposure protocol requires reassessment for its relevancy to subarctic conditions where evaporative losses are expected to be substantially slower than in temperate environments (e.g., Payne et al., 1991). For example, aqueous phase oil was present in some intertidal areas during the Exxon Valdez oil spill (EVOS) for weeks rather than hours. Total PAH concentrations in the water column of Snug Harbor, Herring Bay, and Northwest Bay (Knight Island and Eleanor Island, Alaska) ranged between 1 and 10 µg/L for 2 to over 5 weeks after EVOS (Neff and Burns, 1996; Short and Harris, 1996). Large spills in temperate environments can also persist for days rather than hours, although this is considered to be less likely. For example, a toxic plume of oil persisted in the North Cape, Rhode Island fuel oil spill for multiple days (>10 µg/L aqueous phase PAHs for over 6 days; French, 1998). Additionally, aqueous phase petroleum is expected to be generally more persistent when spilled oil is chemically dispersed than when it is only physically dispersed (French McCay and Payne, 2001). In conclusion, the CROSERF flow-through protocol may be representative of most spills

in temperate environments, but they do appear to adequately represent the spills of greatest concern in subarctic environments.

An additional concern is the CROSERF protocol of using airtight chambers in the flow-through exposure regime. These test chambers were designed to minimize test variability, but artificially elevate concentrations of MAHs (e.g., Rhoton et al., 2001). Wave tank experiments and EVOS measurements indicate the majority of MAHs are lost within the first 24 to 48 hours of a spill of ANS (Payne et al., 1991; Wolfe et al., 1994). As previoiusly noted, North Sea field trials have shown rapid and extensive evaporative losses of volatile compounds of both ANS and a ligher crude oil (Davies et al., 1998). Use of airtight chambers without the prescribed CROSERF flow-through dilution would likely result in persistent MAH exposure and thus are not recommended.

An alternative oil exposure regime consisting of a static exposure in open chambers is recommended over the two CROSERF test protocols. A static exposure regime provides an intermediate duration of oil exposure because test concentrations are not renewed, but they are not diluted. Persistence of oil in the static test chambers will be determined at least in part by the properties of the oil (solubility, henry's law constant) and test conditions (e.g., salinity and temperature representative of subarctic environments), rather than manipulated dilution or renewal regimes. Testing of each species under two test durations is recommended to bracket the range of environmental exposures to dispersed oil: 8 hr and 7 d. Although longer oil exposures are plausible, 7 days is considered sufficient to detect delayed effects, hydrocarbon concentrations will have declined to non-detectable levels within 7 days, and longer exposures may lead to artifacts caused by declining organism health in the test system unrelated to oil exposure. Anderson (1985) has previously noted the importance of a latency period in determining the toxicity of chemically dispersed oil to Pacific herring larvae. Both Calanoid copepods and Pacific herring larvae have been shown to have high control survival under static conditions and water quality was not impaired (Deusterloh et al., 2002; Barron et al., 2003). Mortality should also be monitored daily to allow reporting of LC50s at standard reporting times of 2 and 4 days. If possible, delayed mortality should also be assessed in the 8 hr exposure, by transfering test organisms to clean water.

Salinity and Temperature

Dispersant effectiveness generally increases with increasing salinity and increasing temperature (Fingas, 2002; Moles et al., 2002). Temperature and salinity effects on the toxicity of chemically dispersed oil are largely unknown because toxicity tests are usually performed at the preferred temperature and salinity of each test species. There have been only a few toxicity tests of chemically dispersed oil under subarctic conditions of salinity and temperature. Rhoton et al. (2001) assessed the toxicity of Prudhoe Bay crude oil dispersed with Corexit 9500 using tanner crab larvae and CROSERF protocols. Using non-CROSERF methods and subarctic conditions, Anderson (1985) reported the toxicity of Prudhoe Bay crude oil dispersed with either 'dispersant E' or Corexit 9527, and Barron et al. (2003) evaluated the toxicity of ANS dispersed with Corexit 9527. Dispersant use under subarctic conditions may occur over a range of salinities and temperatures, thus dispersant testing should encompass conditions that reflect potential environmental exposures. We recommend testing at two different temperature-salinity regimes reflective of a range of subarctic conditions: 10 $^{\circ}$C at 15 $^{\circ}/_{\circ\circ}$ and 3 $^{\circ}$C at 32 $^{\circ}/_{\circ\circ}$.

Light Regime

Recent research indicates that aqueous phase petroleum is phototoxic, with toxicity increasing 2 to greater than 100 fold for fresh and weathered crude oils and fuel oils in the presence of the ultraviolet radiation (UV) that occurs in aquatic habitats (e.g., Pellitier et al., 1997; Little et al., 2000; Duesterloh et al., 2002). The vast majority of oil toxicity tests have been performed under fluorescent lighting (minimal UV), and only one study has investigated the photoenhanced toxicity of chemically dispersed oil. Barron et al. (2003) observed a 2 to 450-fold increase in the toxicity of weathered ANS chemically dispersed with Corexit 9527 in 5 hour sunlight exposures compared to chemically dispersed ANS tested only under fluorescent lighting. The growing body of evidence for the photoenhanced toxicity of oil, and its likely ecological relevance in aquatic environments (Barron et al., 2000; Barron and Ka'aihue, 2000) indicates that a UV light regime should be incorporated into a chemical dispersant toxicity testing program.

The toxicity of chemically dispersed oil can not be adequately evaluated without an assessment of the potential for photoenhanced toxicity. A limited UV exposure is a recommended addition to the CROSERF protocols, and has been successfully evaluated with both calanoid copepods and Pacific herring larvae using non-CROSERF methods (Deusterloh et al., 2002; Barron et al., 2003). A brief (e.g., less than 8 hour) exposure to either sunlight or simulated sunlight should be included that is equivalent to 5 to 10% of the clear sky UVA at a latitude of 60° N on the summer solstice. This equates to a UV dose of 2 to 5 $mW*hr*cm^2$ UVA (320-400 nm) and 0.02 to 0.05 $mW*hr*cm^2$ UVB (280-320 nm) (Barron and Ka'aihue, 2001). The UV light regime should be applied after 24 hours of initiation of WAF exposure to allow initial bioaccumulation of phototoxic petroleum hydrocarbons and heterocycles.

Test Endpoints

CROSERF test endpoints are generally focused on mortality, although morbidity (e.g., impaired mobility) has been assessed in some tests. An assessment of petroleum bioaccumulation has not been previously recommended or reported in tests using CROSERF protocols. Recommended changes to the CROSERF protocols include addition of an assessment of delayed mortality and morbidity by extending the test duration to 7 days, quantification of PAHs in test solutions (e.g., LC50s and no effect concentrations based on petrogenic PAHs in WAF), assessment of PAH bioaccumulation in copepods. The bioaccumulation assessment could be restricted to a calanoid copepod because of the rapid and extensive uptake of PAHs by these species. For example, Duesterloh et al. (2002) report a bioconcentration factor (tissue:water) of 8000 for total PAHs in *C. marshallae*. The bioaccumulation assessment should be performed at 1 and 7 days following oil exposure to provide an estimate of both uptake and elimination of PAHs. More comprehensive bioaccumulation sampling would yield more definitive estimates of uptake and persistence (e.g., Barron et al., 1990), but would be an expensive addition to the recommended testing regimes.

CONCLUSIONS AND RECOMMENDATIONS

The CROSERF protocols have substantial merit in meeting the objectives of standardizing toxicity testing procedures and reducing test variability. Blenkinsopp et al. (1996) reported on variability in WAF chemistry, and interlaboratory testing has been performed, although the results are not currently available. A number of refinements are recommended to adapt the protocols for testing with subarctic species under conditions of expected longer oil persistence. Refinements in the WAF preparation, exposure and light regimes, and analytical chemistry should increase the utility of the test results for interpreting the toxicity of chemically dispersed oil and making risk management decisions regarding dispersant use under subarctic conditions. Specific protocols should be developed and peer reviewed prior to initiating a chemical dispersant testing program under subarctic conditions.

Key elements of the program should include:

X   testing both a fresh and moderately weathered oil under conditions of moderate mixing energy (20-25% vortex).

X   testing both Corexit 9500 and 9527 using a high dispersant:oil ratio (1:10)

X   preparing toxicity test solutions using variable dilutions rather than variable loading

X   using static exposures in open chambers

X   testing oil exposures of 8 hr and 7 days to encompass a range of environmental exposure scenarios and allow assessment of delayed mortality

X   quantifying approximately 40 PAHs and alkyl homologs in WAF

X   testing Pacific herring larvae, a subarctic sea urchin species, and a calanoid copepod under a range of subarctic conditions of temperature and salinity

X   assessing the potential for photoenhanced toxicity by incorporating a limited exposure to sunlight or simulated natural sunlight (e.g., <8 hour exposure to 5-10% of clear sky UVA at latitude 60° N).

X   incorporating a bioaccumulation endpoint by measuring PAH bioaccumulation in copepod tissue at 1 d and 7 days from initiation of static oil exposures

Additional investigation should include assessing the toxicity and photoenhanced toxicity of the dispersed oil plume in either field trials or mesocosm studies.

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Table 1. CROSERF toxicity test procedures and recommended refinements.		
Parameter	**CROSERF Procedure[1]**	**Recommendations**
Oil weathering	<fresh <weathered (20%)[2]	<fresh <weathered (20%)
Oil loading	<variable (e.g., 0.01 –25 g/L)	<25 g/L
Dispersant (dispersant:oil)	<Corexit 9500 and/or 9527 (1:10)	<test both Corexit 9500 and 9527 (1:10)
<Mixing energy/time	<18 hr moderate energy < (slow stir WAF)[3]	<same energy for physically and chemically dispersed oil
Test concentrations	<variable loading	<variable dilutions
<Oil exposure regime	<4 d static-renewal; open chamber <$\leq$8 hr flow-through; airtight chamber	<static: open chamber < 8 hr and 7 d
Analytical chemistry[4]	<TPH + VOCs (GC/FID)	<TPH (GC/FID or GC/MS) <PAHs (GC/MS SIM)
<Species/life stage	<temperate species[5]	<calanoid copepod <subarctic sea urchin larvae <Pacific herring larvae
<Temperature; salinity	<6-8 ^{o}C; 31.5 $^o/_{oo}$ (Tanner crab)	<10 ^{o}C; 15 $^o/_{oo}$ < 3 ^{o}C; 32 $^o/_{oo}$
<Light regime	<laboratory (fluorescent)	<incorporate limited UV
<Toxicity endpoint	<impaired mobility <death	<also delayed impairment/death
<Bioaccumulation	<not measured	<tissue PAHs (GC/MS SIM) at 1 d and 7 d in copepods

1. Source: ADEC (2000), Singer et al. (2000); Clark et al. (2001), Rhoton et al. (2001), Singer et al., 2001a).
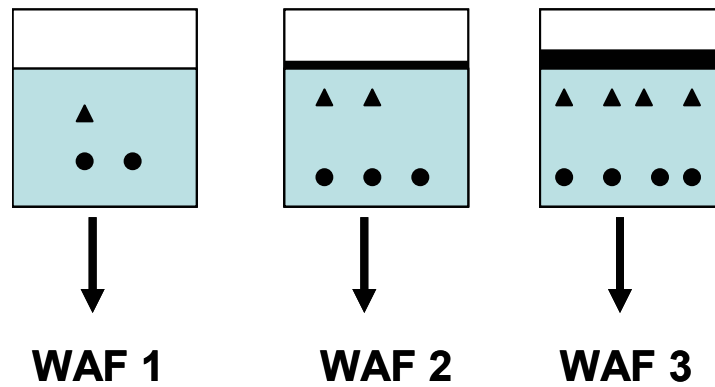2. Distillation to 200 C (Singer et al., 2001b).
3. Approximately 18 hour mix time, 20-25% vortex. Use Clark et al. (2001) modification of standard CROSERF mixing energy protocol for physically dispersed oil, followed by 6 hour settling time.
4. TPH: total petroleum hydrocarbons; VOC: volatile organic compounds (<10 carbon alkanes and MAHs); PAHs: polycyclic aromatic hydrocarbons; GC: gas chromatography; FID: flame ionization detection; MS: mass spectrometry; SIM: single ion mode.
5. ADEC (2000) recommended topsmelt (a non-Alaskan fish) and purple sea urchin for a testing under subarctic testing.

Figure 1. Illustration of variable loading and variable dilution approaches for preparing water accommodated fractions (WAF) of oil (black fill) and water (grey fill). Triangles and circles represent different concentrations of aqueous phase petroleum compounds.