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Critical Review

ISSUES AND CHALLENGES WITH OIL TOXICITY DATA AND IMPLICATIONS FOR THEIR USE IN DECISION MAKING: A QUANTITATIVE REVIEW

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Abstract: Aquatic toxicity considerations are part of the net environmental benefit analysis and approval decision process on the use of dispersants in the event of an offshore oil spill. Substantial information is available on the acute toxicity of physically and chemically dispersed oil to a diverse subset of aquatic species generated under controlled laboratory conditions. However, most information has been generated following standard laboratory practices, which do not realistically represent oil spill conditions in the field. The goal of the present quantitative review is to evaluate the use of standard toxicity testing data to help inform decisions regarding dispersant use, recognizing some key issues with current practices, specifically, reporting toxicity metrics (nominal vs measured), exposure duration (standard durations vs short-term exposures), and exposure concentrations (constant vs spiked). Analytical chemistry data also were used to demonstrate the role of oil loading on acute toxicity and the influence of dispersants on chemical partitioning. The analyses presented here strongly suggest that decisions should be made, at a minimum, based on measured aqueous exposure concentrations and, ideally, using data from short-term exposure durations under spiked exposure concentrations. Available data sets are used to demonstrate how species sensitivity distribution curves can provide useful insights to the decision-making process on dispersant use. Finally, recommendations are provided, including the adoption of oil spill–appropriate toxicity testing practices. *Environ Toxicol Chem* 2014;9999:1–11. © 2013 SETAC

Keywords: Chemical dispersants Oil spill Species sensitivity distribution curves Aquatic toxicity

INTRODUCTION

The debate on the use of dispersants in oil spill response, revived after the Deepwater Horizon oil spill in the Gulf of Mexico in 2010, has been a recurring issue since at least 1970. Dispersants are considered in spill response because their application may mitigate the environmental impacts of oil by moving it from the water surface to the top few meters of the water column, promoting dilution and enhancing oil biodegradation. This not only reduces the likelihood of oil exposures to marine wildlife with long life spans (seabirds, marine mammals, and turtles) but also reduces the amount of oil that might reach shoreline habitats, which would recover relatively slowly, compared with the water column community, from oil impacts and cleanup activities [1,2].

As with all response techniques, the decision to use dispersants should be carefully considered, while taking into account oil characteristics, sea and weather conditions, and environmental sensitivities. Significant environmental and economic benefits may be achieved by using dispersants, particularly when other at-sea response techniques are limited by weather conditions, or when responses are constrained by the availability and adequacy of alternate oil recovery options. In certain situations, dispersants may provide the only means of removing significant quantities of surface oil. However, an important consideration is the relative net environmental impact of using dispersants versus the impacts of oil persisting on the water surface or stranding on shorelines. Consequently, aquatic toxicity considerations are an integral part of net environmental benefit analysis (NEBA) and the dispersant approval decision process. The outcome of the NEBA process shows that, under most circumstances, the rates of mixing and dilution in open waters that are $\geq$3 nm offshore or $\geq$10 m in depth are sufficient to minimize the potential toxic effects of dispersed oil on marine communities [1,2]. At these depths and distances from shore, the short-term and decreasing risks to aquatic organisms (particularly entrained fauna and flora) from chemically dispersed oil are usually much less than the risks to wildlife, shallow water marine life, and shoreline communities if oil slicks persist on the water surface. These analyses have been used to support the establishment of preapproved areas for dispersant use for offshore oil spills in a number of countries, including the United States, United Kingdom, France, and Australia (e.g., Addassi et al. [3]).

However, data on the toxicity of chemically dispersed oil are often difficult to interpret and apply, causing confusion among the public, policy makers, and environmental management authorities. Although many dispersant formulations used before the 1970s were merely solvents and emulsion breakers containing harsh chemical constituents that contributed to the toxicity of dispersed oil (e.g., persistent hydrocarbon distillates or kerosene and similar solvents), new-generation dispersants have been specifically formulated for reduced toxicity using materials common in food additives and personal care products. These surfactants enhance dispersant effectiveness and reduce environmental toxicity by replacing the toxic and persistent hydrocarbon distillates with more water-soluble and lower-toxicity formulations (e.g., propylene glycol, di(propylene glycol)butyl ether, dioctyl sodium sulfosuccinate) [1,4,5]. Consequently, changes in dispersant formulations over time, combined with the variety of experimental laboratory designs

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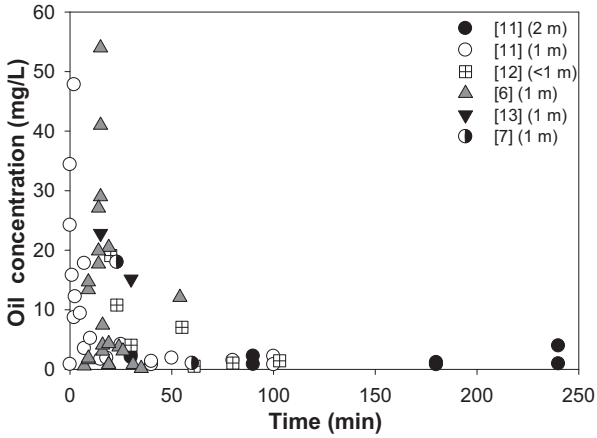

Figure 1. The relationship between measured oil concentrations in the upper water column (≤2 m) versus time from field trials at sea. Data represent oil concentrations from physically ([11], black circles) and chemically enhanced dispersion of oil ([11], white circles; [6,7,12,13]).

and laboratory practices used to generate toxicity data, have made interpreting this information challenging. Thus, our main goal is to provide a quantitative synthesis and review of the currently available toxicity data on physically and chemically dispersed oil and to facilitate the understanding and interpretation of these data. Whereas the information presented here is targeted to a technical audience, we also illustrate the applicability of these data in the decision process for offshore dispersant use.

Issues and challenges with oil toxicity data

For the purpose of the present review, a clear distinction is made between physically and chemically dispersed oil. Physically dispersed oil refers to oil that has been naturally dispersed or entrained in the water column (e.g., by currents, wind, and waves in open waters), or aqueous exposure media prepared by means of physical processes (e.g., by controlled mixing energy, such as vortex). Chemically dispersed oil refers to oil that has been treated chemically to enhance its dissolution and partitioning into the water column, or aqueous exposure media prepared by means of additions of dispersants.

Field trials. Numerous field trials at sea have contributed to our understanding of the fate and behavior of physically versus chemically dispersed oil slicks [6–13]. These field trials have shown that under open ocean conditions, both physically and chemically enhanced dispersed oils are subject to rapid dilution caused by advection by currents and to water mixing within the water column, resulting in oil concentrations declining rapidly over time (Figure 1). Consequently, the initially high, spiked oil concentrations typically observed within the top few meters of the water column (up to 54 mg/L) decline within minutes to hours (≤4 h) to concentrations typically 1 mg/L or less [6,7,11–13]. As a result, exposures of water column biota to chemically dispersed oil from offshore use of dispersants is short and often limited to the few top meters of the water column, mostly within the footprint of the treated slick. The dynamic nature of field conditions make the realistic design and execution of laboratory or field-based toxicological tests challenging. Nevertheless, decades of laboratory testing and field research have generated a wealth of toxicological information of potential use in NEBA and offshore dispersant use decision making.

Field toxicity studies. Despite the costs, complexities, and legal issues associated with conducting field toxicity testing of controlled oil releases, several efforts have been successfully implemented to study the effect of chemically dispersed oil on water column biota [14]. In 1996, 2 surface oil releases were performed off Norway, one of which was dispersed with Corexit 9500 [9]. Water samples collected at 1-m and 5-m depth from control and treated areas were used for toxicity testing with 4 species (the marine rotifer *Brachionus plicatilis*, the bioluminescent bacteria *Vibrio fischeri*, oyster *Crassostrea gigas* larvae, and the copepod *Acartia tonsa*) [14]. The rotifer and oyster larvae tests were not successful because of stresses of conducting standard laboratory testing aboard a ship at sea, but exposure–response relations were characterized with bioluminescent bacteria and copepod tests using water samples collected 1 h after dispersant treatment at 1 m depth beneath the treated slick (total petroleum hydrocarbon [TPH] concentrations ≤22 mg/L). These field toxicity efforts showed the potential usefulness of the bioluminescent test as a rapid screening assay to generate near real-time results (<15 min) of potential use in spill response decision making [14].The copepod test data were highly variable, providing inconsistent and unreliable information as a rapid field test [14].

A mesocosm study [15] used a variety of caged organisms to characterize potential toxic effects from exposures to physically or chemically enhanced dispersed oil under simulated near shore exposure conditions (7–16 mg TPH/L and 48–68 mg TPH/L, respectively). Of several species tested (*Uca* spp., *Cyprinodon variegatus*, *Crassostrea virginica*, *Palaemonetes pugio*, *Littorina littorea*), only snails in the intertidal zone showed lethal and sublethal effects caused by aqueous exposures to either physically or chemically dispersed oil, possibly from the combined effect of increased soluble fractions and direct impacts from oil smothering. An important finding of that study was that, compared with the chemically dispersed treatment, physically dispersed oil caused greater shoreline and intertidal oiling and related toxic impacts [15]. Although other field studies have provided useful assessments of dispersant used in oil spill response and characterized the environmental fate of the oil, and in some cases characterized impacts on biota [1,4,16,17], they have not provided sufficiently detailed exposure–response data to allow for direct comparisons with laboratory toxicity test data.

Although these field toxicity tests are useful in documenting the potential toxicological effects from chemical dispersion of oil, most of the currently available toxicity data on chemically dispersed oils (aqueous exposures) have been generated under controlled laboratory test conditions. Most such tests use exposure regimens that are not representative of dynamic field conditions with respect to dilution, and the lack of correspondence between exposure and toxic response make application of laboratory test data challenging for real-world decision making. However, NEBA assessments and spill response decisions regarding dispersant use have been made with the understanding that laboratory data represent an unrealistic, worst-case aqueous exposure, and provide conservative metrics of lethal and nonlethal exposure concentrations for field organisms [1,2,18,19].

Laboratory toxicity studies: issues and challenges. Toxicity testing data from laboratory studies have facilitated our understanding of the effects of oil and oil constituents on a diverse number of aquatic species. This information has been reviewed by the US National Academy of Sciences, the American Petroleum Institute, the National Oceanic and Atmospheric Administration, and other institutions and scientists facilitating the development of useful benchmarks that help inform decision makers [1,2,20–24]. However, shortcomings

are inherent in the use of standard laboratory toxicity data to assess the effects to water column biota from chemically treated oil spills, as well as to support management decisions on the offshore use of dispersants.

Consequently, the main objective of the present quantitative review is to facilitate a technical understanding of toxicity data while addressing some of the outstanding issues and challenges with existing data. Although not the primary intent of this quantitative review, a section is included to illustrate the potential use of existing information by managers and decision makers. The data presented here come from a recently developed acute toxicity database (DTox, Bejarano A.C., et al., unpublished data), which through a rigorous process compiles quantitative information on the aquatic toxicity of dispersants, and physically and chemically dispersed oil from both peer-reviewed and gray literature. A detailed list of references used in these analyses is found in the Supplemental Data section.

Comparison of physically and chemically dispersed oil on the basis of measured versus nominal concentrations. Reporting toxicity in terms of the actual aqueous concentration of petroleum hydrocarbons in the exposure media is important to facilitate comparison across studies of all types, including laboratory, mesocosm, and field studies. As demonstrated below and stated elsewhere [2,25–28], acute toxicity of chemically enhanced water accommodated fraction (CEWAF) can be grossly overpredicted when reporting nominal concentrations or oil loadings (e.g., mg oil/L water), leading to the erroneous perception that chemically enhanced dispersed oil is more acutely toxic than physically dispersed oil.

The present review of the toxicity of oil (median lethal concentration [LC50]; median effective concentration [EC50]; primarily from 96-h aqueous exposures, but including aqueous exposures from 1 h to 96 h) that had been chemically dispersed with Corexit 9527 or Corexit 9500 (CEWAF), and oil physically or mechanically dispersed (water accommodated fraction [WAF]), reveals large discrepancies between studies reporting measured versus nominal aqueous exposure concentrations (329 WAF-CEWAF paired-data for individual species from 36 independent studies; Figure 2). Although these studies were performed using different species, a range of experimental conditions (exposure conditions and duration), and some reporting various measured analytical endpoints (TPH typically the C10–C36 hydrocarbon range; total hydrocarbon content (THC) typically the C6–C36 hydrocarbon range; volatile organic compounds typically the C6–C9 hydrocarbon range), these data are useful in allowing a comparison of acute toxicity values between CEWAF and WAF exposures. These data also allow for comparisons between toxicity reported as measured concentrations versus nominal concentration/loading rates.

In studies using the dispersant Corexit 9527 and reporting measured concentrations (as either TPH or THC), 89% of paired WAF-CEWAF data had CEWAF LC50|EC50 values greater than or equal to WAF values (lower or equal toxicity). In cases in which CEWAF LC50|EC50 values were less than WAF values (greater toxicity), the fold-difference (the degree of change in relative toxicity of CEWAF vs WAF) between values ranged from 1.62 to 1.76 and were generally within the degree of repeatability for standard acute toxicity tests [29]. By contrast, 80% of paired-data reporting nominal concentrations or loading rates had CEWAF LC50|EC50 values between 1.1 and >1000 fold smaller (greater toxicity) than WAF values.

A similar pattern was observed for oil chemically dispersed with Corexit 9500, for which a larger number of records were available. Most studies with reported measured concentrations

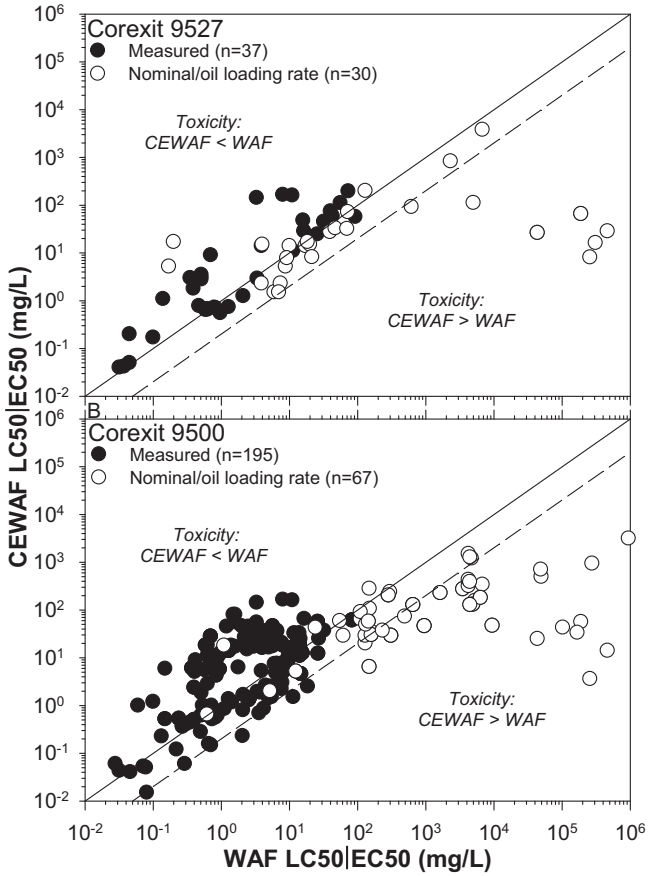

Figure 2. Comparison of the acute toxicity (median lethal concentration [LC50] and median effective concentration [EC50]; primarily 96-h exposures) of water accommodated fraction (WAF) or chemically enhanced water accommodated fraction (CEWAF) dispersed with Corexit 9527 (**A**) and Corexit 9500 (**B**) between studies reporting effects concentrations as measured (black circles) or nominal (including oil loading rates; white circles). Data represent paired WAF-CEWAF observations for each species within each study, with paired WAF-CEWAF observations reported based on the same analytes (total petroleum hydrocarbon [TPH] or total hydrocarbon content [THC]). The solid line represents the 1:1 line (equal toxicity), whereas the dashed line represents a 5-fold toxicity difference (increased toxicity).

(78% of paired-data) had CEWAF LC50|EC50 values greater than or equal to measured WAF values (lower or equal toxicity). In cases in which CEWAF LC50|EC50 values were less than WAF values (greater toxicity), these were between 1.55-fold and 8.09-fold smaller, but most (76%) were within 3-fold of WAF values. By contrast, 93% of paired-data reporting nominal concentrations or loading rates had CEWAF LC50|EC50 values between 1.2 and greater than 1000-fold smaller (greater toxicity) than WAF values.

As shown here, concentrations reported on a nominal basis can greatly overestimate the toxicity of CEWAF relative to that of WAF. Because oil is a mixture of compounds with different solubilities and volatilities, nominal concentrations or oil loadings do not accurately represent the aqueous exposure media [23,26–28,30] and, consequently, are not reliable metrics of toxicity. When data based on nominal concentrations are brought into the dispersant approval discussion without consideration of the true differences in exposure conditions, dispersant use is discouraged because it appears to make the oil accommodated in water more toxic. Consequently, nominal toxicity data should not be used because these data are not

sufficiently detailed or accurate to support decision making regarding potential toxic effects of dispersed oil (see also Coelho et al. [27] and Singer et al. [28]). Although some studies have found a positive correlation between nominal and measured concentrations for oil toxicity tests [31,32], most studies have not made these comparisons, rendering toxicity data from nominal aqueous exposure studies of limited scientific value. Furthermore, dispersants are designed to alter the interaction between petroleum hydrocarbons and water by effectively increasing the rates at which soluble oil constituents and small oil droplets enter the water phase, further negating comparisons of nominal concentrations or loading rates between physically dispersed oil (WAF) and chemically dispersed oil (CEWAF) [26–28]. The same criticism regarding nominal concentrations has been raised in the past [2,25–28], and it is not limited only to oil spill research, but also to other areas in the aquatic toxicology field [29,33].

The relationship between exposure duration and toxicity. As previously discussed, the highly dynamic nature of the open ocean influences the behavior of physically and chemically dispersed oil such that exposure concentrations in the water column are short lived and rapidly diluted via advection and water column mixing. These dynamic conditions have seldom been adequately replicated in the laboratory, with some notable exceptions (i.e., short acute exposures) [32,34,35]. Consequently, standard acute toxicity tests that use continuous aqueous exposure conditions for extended periods (e.g., 96 h) are not representative of these episodic, short exposure conditions (minutes to hours) that are seen in offshore waters [25,28]. If used to assess potential real world risks, such laboratory data can lead to an overestimation of potential field exposure because laboratory effects occur at much lower concentrations than those from dynamic field exposure conditions. Yet, in most toxicity tests with dispersants, WAF or CEWAF have been traditionally performed with constant exposures ranging from 24 h to 96 h, and these data are commonly brought into discussions during NEBA and dispersant approval decisions.

To demonstrate the importance of exposure duration on toxicity, a comparison of paired toxicity data between 96 h and shorter exposures (24 h, 48 h, or 72 h) for the same species was made using 55 paired-data for individual species from 7 independent studies (combined dispersant, WAF and CEWAF data; LC50|EC50 data reported as TPH or THC; Figure 3). This comparison shows that most short exposures (80%) produced toxicity values (LC50|EC50) that were up to 1.5-fold greater (less toxicity) than those derived from 96-h exposures. The same might be expected when comparing 24 h or longer data versus toxicity data derived from short exposures (1–8 h), which are the exposure conditions most representative of an oil spill setting. However, comparisons with 8-h or shorter exposures were only possible for a handful of studies [34–38], reporting at least 3 exposure durations (Figure 4). Simple log-log linear regressions (data not shown) were used to estimate effect concentrations for 3-h exposures and resulting values compared with values from 96-h and 24-h exposures. These comparisons showed that LC50|EC50 values from 3-h exposures were between 3-fold and 981-fold greater (less toxicity) than 96-h exposure values (most observations $\leq$60-fold greater), and between 2-fold and 62-fold greater than 24-h exposure values (most observations $\leq$12-fold greater). Related studies have also shown a negative decay relationship between time and toxicity endpoints [22,32,39] such that there are greater differences in effect concentrations at time steps under 24-h exposures, compared with time steps above 24 h, where toxicity values tend to stabilize. Consequently, toxicity data from the more commonly tested standard exposure durations should be used with caution and, when possible, in combination with other data sources to make informed decisions regarding potential impacts of dispersant use that are not overly conservative.

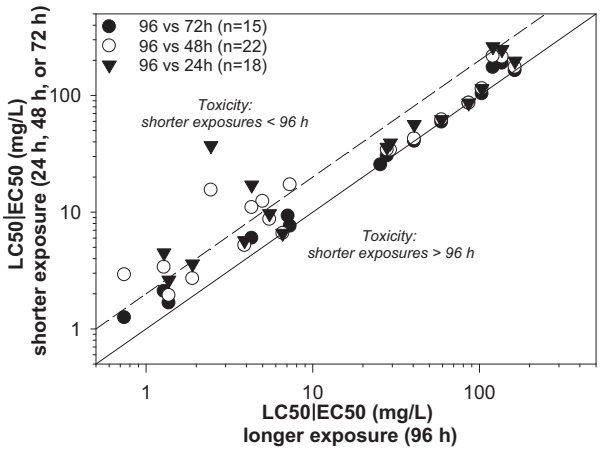

Figure 3. Comparison of acute toxicity data (median lethal concentration [LC50] and median effective concentration [EC50]) for exposure media (dispersants, water accommodated fraction [WAF] or chemically enhanced water accommodated fraction [CEWAF]; measured total petroleum hydrocarbon [TPH] or total hydrocarbon content [THC] concentrations) between studies reporting effects concentrations as a function of exposure duration (24 h, 48 h, 72 h, and 96 h). Data represent paired observations of effects concentrations at 96 h versus a shorter exposure (24 h, 48 h, or 72 h) for the same species within each study. The solid line represents the 1:1 line (equal toxicity), whereas the dashed line represents a 2-fold toxicity difference (decreased toxicity).

As demonstrated here, studies that perform tests under short-term regimens are necessary to derive toxicity metrics relevant to oil spill exposure scenarios. In addition, short exposures may allow gathering of additional sublethal data, as shown by Greer et al. [34]. In that study, exposure of Atlantic herring (*Clupea harengus*) embryos to CEWAF for 2.4 h induced blue-sac disease and reduced the percentage of normal embryos at hatch. This type of sublethal effect information is important because it relates ecological effects to exposure durations and concentrations that better mimic environmentally realistic conditions and can have significant impacts for exposed populations.

Adhering to standard toxicity practices to adequately replicate dynamic field conditions has been encouraged for many years to facilitate decision processes on dispersant use. As a result, the Chemical Response to Oil Spills Ecological Effects Research Forum was formed to address the acute toxicity to several aquatic species of aqueous exposures to oil under conditions expected to be encountered in the field after dispersant use [25]. These studies used a modeled exposure regimen based on expected dilution rates of a dispersed oil plume in open, offshore waters [40]. A number of participating testing laboratories evaluated exposure–response relationships between simulated, real-world exposures and standard, acute toxicity laboratory tests (revised in Nuclear Regulatory Commission [2]), showing that exposure duration was a significant factor in assessing toxicity of dispersed oil.

Comparison of constant versus spiked exposure conditions. Unlike constant static exposures, WAF and CEWAF toxicity data generated from spiked exposures better simulate the exposure regimens observed during oil spills: short-term, acute exposures of rapidly declining concentrations of toxic oil

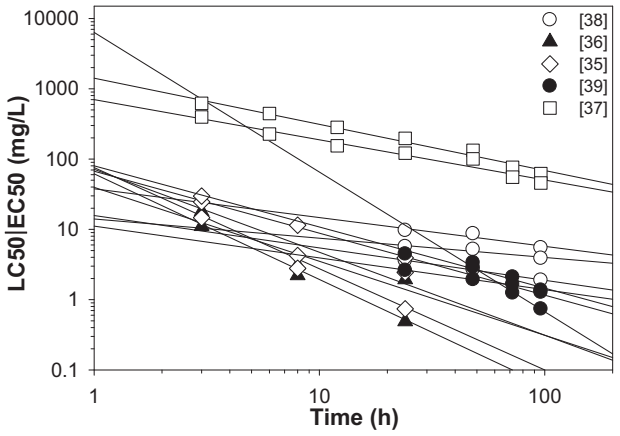

Figure 4. Correlation between the acute toxicity data (median lethal concentration [LC50] and median effective concentration [EC50]) of exposure media (water accommodated fraction [WAF] or chemically enhanced water accommodated fraction [CEWAF]; measured total petroleum hydrocarbon [TPH] or total hydrocarbon content [THC] concentrations) and exposure duration for selected studies reporting toxicity in at least 3 exposure durations. The solid lines represent simple log-log regressions between LC50|EC50 values and exposure duration.

constituents. To demonstrate the effect of exposure conditions on toxicity values, a review of the toxicity (LC50|EC50; primarily from 96-h aqueous exposures) of WAF and CEWAF (chemically dispersed with Corexit 9527 or Corexit 9500) was performed focusing on studies reporting measured values (THC) of acute toxicity for the same species, but under different exposure conditions (constant vs spiked). The present review resulted in 58 constant-spiked paired-data for individual species from 10 independent studies (Figure 5). This comparison shows that constant exposures produced toxicity values much lower than those of spiked tests for both WAF and CEWAF for the same exposure duration. Seventy-three percent of WAF toxicity values (LC50|EC50) from constant exposures were 7-fold lower (greater toxicity) than those from spiked tests. Similarly, 80% of

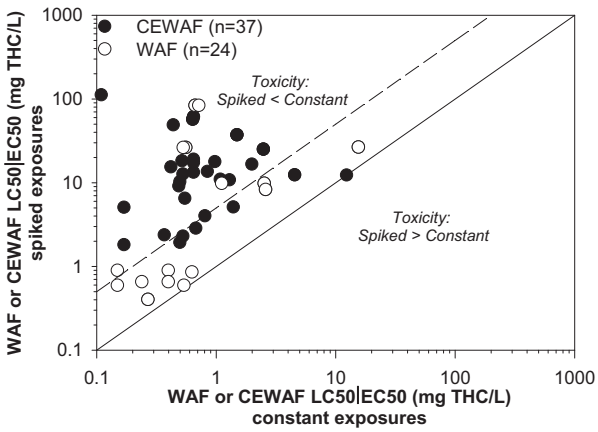

Figure 5. Comparison of the acute toxicity (median lethal concentration [LC50] and median effective concentration [EC50]; primarily 96-h exposures) of water accommodated fraction (WAF; white circles) and chemically enhanced water accommodated fraction (CEWAF; black circles) between constant and spiked exposures. Data represent paired constant-spiked observations for each species within each study. The solid line represents the 1:1 line (equal toxicity), and the dashed line represents a 5-fold toxicity difference (lower toxicity).

CEWAF toxicity values (LC50|EC50) from constant exposures were between 5-fold and 27-fold lower (greater toxicity) than those from spiked tests. The largest fold-difference for CEWAF between these 2 types of tests was 107. This analysis and previous studies suggest that decisions regarding dispersant net environmental benefit and regulatory approvals for offshore dispersant use may be more appropriate if they are based on toxicity data that are derived from realistic declining aqueous exposures (e.g., spiked flow-through tests, or at least short duration constant exposures) because these test exposures are a better approximation to the conditions likely observed during a typical offshore oil spill [25,26,41–43].

The effect of oil loading on toxicity. Although no consensus has been reached about which method of preparation is a better analog for field conditions, when preparing individual WAF or CEWAF, direct additions of oil (oil loading) may provide a better method than relying on serial dilutions, because in the latter the resulting exposure media is influenced by oil partitioning rates in the stock media [2,23,25,28,44–46]. Studies have reported a good correlation between oil addition rates used for each test concentration and the expected concentration of oil fractions that were measured in the test concentration preparations (volatile compounds: benzene, toluene, ethylbenzene, xylene [BTEX]; polycyclic aromatic hydrocarbons [PAH]; and TPH), primarily in WAF [25,26,44–47].

Because dispersants enhance the rate of partitioning of oil fractions into the water column, CEWAF can be prepared with lower bulk oil loadings to achieve similar aqueous exposure concentrations as WAF. To demonstrate the extent to which dispersants can change oil partitioning rates and influence acute toxicity, chemistry data from several studies [25,44–46] were used to calculate the relative contribution of individual PAHs in the aqueous exposure media to the overall toxic potential of the PAH mixture [2,23]. This was done using the Equilibrium Partitioning Benchmark Toxic Unit approach [48], which assumes additive toxicity of soluble PAHs. Under this approach, samples with toxic unit values greater than 1 exceeded concentrations acceptable for the protection of aquatic organisms [48]. For the purpose of this analysis, toxic units were calculated using only analytes that were commonly measured in 100% CEWAF and WAF across studies (BTEX, and PAHs C0–C4 naphthalene, C0–C2 fluorene, C0–C4 phenanthrene, fluoranthene, pyrene, C0–C3 chrysene).

Using this approach and regardless of the oil source, the first exceedance of the toxic unit in CEWAF occurred at an oil loading of 357 mg/L, whereas the first toxic unit exceedance in WAF occurred at an oil loading almost 10 times higher (3470 mg/L; Figure 6). In all cases, WAF had smaller toxic unit values than CEWAF at comparable oil loadings. Despite the limited availability of analytical chemistry data, these comparisons can provide information complementary to that generated through standard toxicity tests; hence the importance of performing analytical chemical analyses on samples from the exposure media. Furthermore, these analyses are in agreement with studies indicating that oil loading can influence toxicity values and that dispersants can alter the rate of oil partitioning into test media, allowing lesser oil loadings for CEWAF to achieve the same exposure concentrations as greater oil loadings for WAF test media [23,30,49]. These findings also support the recommendation that individual oil loadings for each test concentration are preferable over serial dilutions when preparing oil exposure media [28]. Although an effort was made to generate information on the influence of serial dilutions from high oil loadings, and the effect of different dispersant to oil

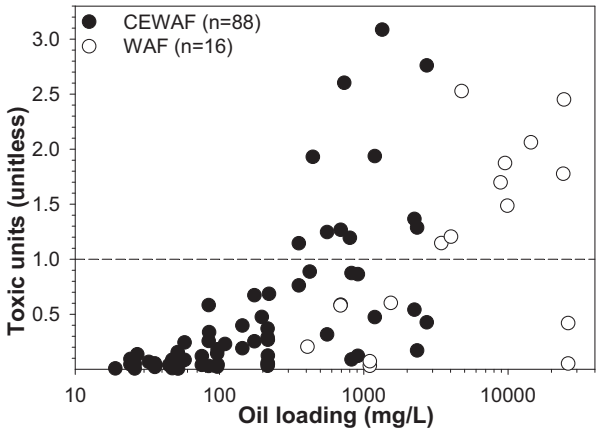

Figure 6. Comparison of toxic units (TU) for a subset of aromatic analytes (BTEX, C0–C4 naphthalene, C0–C2 fluorene, C0–C4 phenanthrene, fluoranthene, pyrene, C0–C3 chrysene) in water accommodated fraction (WAF; white circles) and chemically enhanced water accommodated fraction (CEWAF; black circles) prepared from various oil loadings. The dashed line represents the TU above which aromatic concentrations in the mixture are unacceptable for the protection of aquatic organisms.

ratios on PAH toxic units in the aqueous exposure media, analytical chemistry results were not available to support these detailed analyses.

One of the criticisms of the currently available data is that most studies report effects concentrations on the basis of TPH or THC. These analyte groups are not considered a single chemical, but rather a combination of chemicals with similar, but not identical, physical and chemical properties, for which little toxicity data has been published for the individual components. Consequently, and as discussed by Di Toro et al. [21], the composition of the mixture and concentration of each analyte is important in determining the toxicity of a hydrocarbon mixture. Because this composition is greatly influenced by the oil/water partitioning coefficients for each component, it is important not to perform serial dilutions from a single stock preparation [50]. Although these analyte groups constituted a significant fraction of the oil and are common and rapidly achievable metrics of oil content in the exposure media, interpretation of toxicity data would benefit from better characterizations of the bioavailable fractions that are understood to drive acute toxic responses, such as PAHs [22,51–53]. As demonstrated in the present analysis, the toxic unit approach [21,48] provides additional information to assessments of potential acute toxicity for complex mixtures such as crude oils and petroleum products. Consequently, a detailed chemical characterization of the aqueous exposure media, including perhaps recommended analytes [25], is highly desirable for all future toxicity tests with petroleum products to allow comprehensive toxicity assessments and related analyses.

The use of toxicity data in decision making

Although we have acknowledged the technical challenges and limitations with interpretations and applications of existing toxicity data (see Barron et al. [54] for other issues not covered here, specifically photo-induced toxicity), one must recognize that, within the proper scientific context, these data can provide useful insights for decision making on the use of dispersants in response to offshore oil spills.

A tool that has gained acceptance among ecological risk assessors, and chemical and oil spill practitioners in particular, is the use of species sensitivity distributions (SSDs) [39,55–57]. Species sensitivity distributions are cumulative distributions of laboratory-derived toxicological endpoints (e.g., LC50, EC50; typically from the same exposure duration) for species used in testing programs that allow for comparisons of the relative sensitivities of several species to the same chemical [58]. Species sensitivity distributions can be useful to establish scientifically based concentrations (toxic benchmarks) that are assumed to be protective of a range of species (e.g., Bejarano et al. [57]). This approach can be extended to protect untested species (including keystone species) under the assumption that their sensitivity is within the range of sensitivities captured by the tested species. Consequently, well-crafted and representative SSDs (with a minimum of 5 species per SSD) can facilitate and support the decision-making process on dispersant use and can help evaluate potential risks to specific aquatic resources (e.g., resources of special interest, or threatened endangered species) from a proposed action (e.g., chemical dispersion of oil) in the absence of toxicity data for the species of interest.

As emphasized earlier, the use of measured concentrations to assess toxic responses is essential and, consequently, SSDs were developed based on standard test endpoints (LC50|EC50) with reported measured (THC, including C6–C36 carbon chains) aqueous exposure concentrations in which each point on an SSD represents the geometric mean of toxicity values for individual species. Species sensitive distributions were developed following the procedures outlined in Bejarano and Farr [39]. For the purpose of the analyses in the present review, the fifth percentile of the probability curve was used as the toxic benchmark, which is assumed to be a conservative concentration that protects 95% of the species on the SSD (Hazard Concentration 5 [HC5]).

Species sensitive distributions are highly useful in the decision-making process, because these may facilitate comparisons of the sensitivity among distinct taxonomic groups or life stages (Figure 7) for various species or functional ecological groups of interest (e.g., phytoplankton, zooplankton, filter feeders, fishes). Most of the currently available toxicity data (LC50|EC50) from which SSDs can be developed are from fish

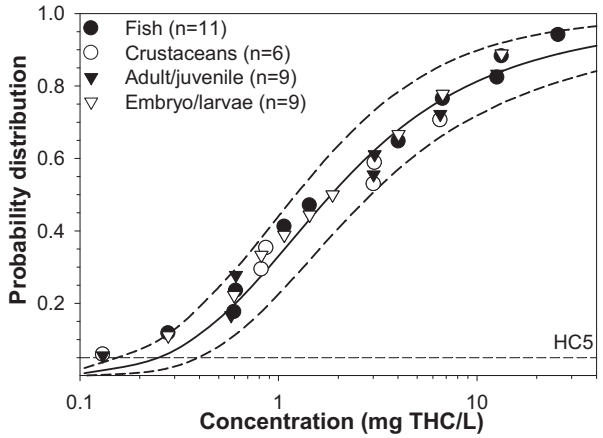

Figure 7. Species sensitivity distributions (SSD) of chemically enhanced water accommodated fraction (CEWAF) and water accommodated fraction (WAF) toxicity data (96 h median lethal concentration [LC50] and median effective concentration [EC50] data; measured total hydrocarbon content [THC] concentrations) from constant exposures comparing taxonomic groups (circles) and life stages (triangles). The curved lines represent the mean estimated SSD (solid) and its 95% confidence interval (dashed), and the horizontal line represents the 5th percentile of the probability curve (HC5; dashed).

and crustacean test species, with the latter being generally more sensitive to contaminant exposures. The SSD of fish and crustacean test species shows that 3 of the 6 crustacean species for which 96-h LC50|EC50 CEWAF and WAF data (constant exposures and measured THC concentrations) were available fell within the bottom half of the SSD, with the kelp forest mysid (*Holmesimysis costata*) being the most sensitive among 16 species. This is not surprising because the standard test species and life stages used by federal and state agencies for regulatory purposes focus on those species known to be more sensitive to a broad range of chemical toxicants [59–62]. However, uncertainties exist regarding the relative sensitivity of many untested species, which also need to be considered. Therefore, toxicity tests of CEWAF and WAF under realistic exposure conditions with a wider number of species, particularly crustacean species, which lack a strong detoxification system compared with fish, may help reduce uncertainties regarding the protection of untested species (e.g., tropical, polar, deepwater), sensitive species of other taxa (e.g., coral larvae), and other marine species. Similarly, early life stages are often more sensitive than the adults of the same species [59–62], but wide variability in the sensitivity of life stages exists when considering several aquatic species (see also Singer et al. [63]). In both of these examples, 96-h HC5 values, based on LC50 and EC50 data (0.28 mg/L for fish and crustaceans; 0.29 mg/L for early life stages), appear to be conservative enough to protect all of the other species for which toxicity data are available and possibly all but 5% of untested species for extended and worst-case exposure conditions. Interestingly, these concentrations are similar to the 96-h consensus value (0.5 mg/L) used for ecological risk assessments of dispersed oil [2].

As discussed here, test exposure conditions have a direct impact on the reported toxicity data and derived toxicity benchmarks and, consequently, decisions made using constant aqueous exposures may lead to overestimation of risks for real-world exposure conditions. To demonstrate the impact of the exposure test concentrations (constant static vs spiked) on the development of potential toxic benchmarks, SSDs were developed using data generated through these 2 types of exposure conditions (Figure 8). These data include all CEWAF and WAF toxicity values (LC50|EC50) from studies reporting measured concentrations (TPH and THC, including C6–C36 carbon chains) for 96-h aqueous exposures (crustaceans and fish combined). Under the assumption that the HC5 (based on LC50 and EC50 data) represents a concentration protective of 95% of the species on the SSD, constant exposures would produce an HC5 of 0.35 mg THC/L (95% confidence interval 0.21–0.55 mg THC/L), which is 8-fold lower than the HC5 derived from an SSD with spiked data (2.66 mg THC/L, 95% confidence interval 2.01–3.47 mg THC/L). However, the use of a conservative approach (e.g., HC5s based on constant exposures; HC1s based on spiked exposures; lower 95% confidence interval HC5 based on spiked exposures with a safety factor of 10) might be considered when decision makers are concerned about the presence of a particular species with little or no laboratory toxicity data available (sensitive species or life stages, including threatened and endangered species, and species of regional significance) in areas in which the use of dispersants are being considered. Another benefit of using SSDs is that this approach allows for verification of consensus values commonly used in ecological risk assessments, where levels of concern have been developed for sensitive life stages, adult fish, and adult invertebrates as a function of exposure duration [2]. In the present study, a benchmark value of 2.66 mg THC/L for a short exposure (using spiked data as a surrogate) was within the same order of magnitude as the low to medium level of concern (1–5 mg/L) for sensitive life stages in the water column exposed to chemically dispersed oil for 3 h or less used in oil spill response ecological risk assessments [2].

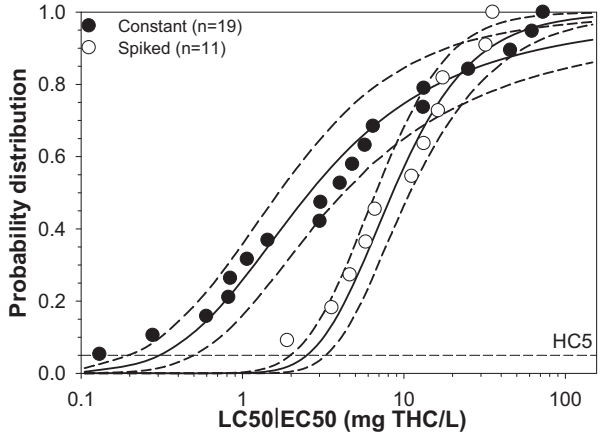

Figure 8. Comparison of species sensitivity distributions (SSD) between constant (black circles) and spiked (white circles) exposures using chemically enhanced water accommodated fraction (CEWAF) and water accommodated fraction (WAF) toxicity data (96-h median lethal concentration [LC50] and median effective concentration [EC50]; crustaceans and fish combined) from studies reporting measured total hydrocarbon content [THC] concentrations. The curved lines represent the mean estimated SSD (solid) and their 95% confidence interval (dashed), and the horizontal line represents the fifth percentile of the probability curve (HC5; dashed).

Characterizing the risk of exposure to potentially toxic levels of physically or chemically dispersed oil to species of special interest is not an easy task because toxicity information for many species is limited or not readily available. For example, the white and back abalone (*Haliotis sorenseni* and *H. cracherodii*, respectively) are endangered marine gastropods found in intertidal and nearshore habitats (<6 m depth) along the California coast. In the event that dispersants were to be used to mitigate the impacts of an offshore oil spill during the reproductive season of abalone, larvae entrained in the upper few meters of the water column may be exposed to a chemically dispersed oil plume of an offshore treated oil slick. Chemically enhanced water accommodated fraction toxicity data are not currently available for these 2 abalone species, but limited data (EC50 growth and development endpoints) are available for red abalone (*H. rufescens*) [64,65], which may serve as a suitable surrogate. With abalone as an example, SSDs were developed using all available CEWAF and WAF toxicity data (LC50|EC50; measured THC concentrations) for crustaceans and fish larvae from spiked exposures (Figure 9). Because of data limitations, data for 48-h and 96-h aqueous exposures were combined. Under the assumption that the HC5 (based on LC50 and EC50 data) represents a concentration protective of 95% of larvae stages of marine species (including white and back abalone), spiked exposures would produce an HC5 of 0.64 mg THC/L (95% confidence interval 0.40–0.97 mg THC/L). Note that red abalone larvae are toward the upper end of the curve (probability distribution, 0.8). Similar assumptions and analyses also can be made for species with limited toxicity data (e.g., coral larvae) for which suitable surrogates are not available. However, validation of these proposed benchmarks is recommended before their use in regional NEBAs or risk assessments.

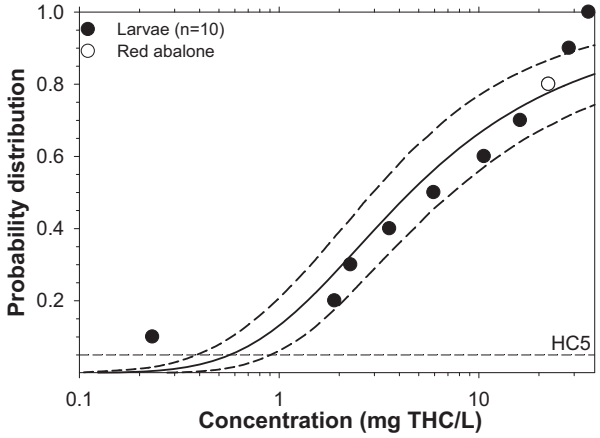

Figure 9. Species sensitivity distributions (SSD) for larvae of several species using chemically enhanced water accommodated fraction (CEWAF) and water accommodated fraction (WAF) toxicity data (48 and 96 h median lethal concentration [LC50] and median effective concentration [EC50]; crustaceans and fish combined) from studies reporting measured total hydrocarbon content (THC) concentrations. The curved lines represent the mean estimated SSD (solid) and its 95% confidence interval (dashed), and the horizontal line represents the fifth percentile of the probability curve (HC5; dashed).

Discussion and recommendations

Comparison of toxicity data across studies is challenging because not all studies are designed under similar experimental conditions, yet the test results are commonly pooled when regulatory or oil spill response decisions are to be made. Comparisons are further complicated by differences in the chemical composition of source oils used in toxicity testing [31,34,66], the temperature at which these test are conducted and temperate tolerance of the tested species [50,67–69], the degree of mixing in preparing WAF and CEWAF solutions, and other procedural details that influence the partitioning of oil constituents into the aqueous exposure media [25,27,28,50,70]. In addition, studies are often not performed under environmentally realistic solar lighting, which could potentially overlook the contribution of photo-induced toxicity to the overall toxicity of oil [2,53,54,71,72]. Despite these challenges, efforts have been made to standardize oil toxicity testing procedures [25–28,73–75], with modifications for situations with longer oil persistence [67], to generate information useful and applicable to oil spills. Consequently, toxicity data of high scientific value for dispersant use decision making should be generated through experiments that incorporate environmentally realistic oil spill exposure conditions in their design (see also Coelho et al. [27]). Hence, test standardization and full characterization of the aqueous exposure concentrations and oil components is recommended not only to generate useful information, but also to allow for comparisons across studies and to reduce uncertainty in toxicity data.

A major uncertainty identified as part of this quantitative review deals with the relative contribution of oil droplets to the overall toxicity of oil (not reviewed quantitatively, given limitations in available published data). Aquatic organisms in the water column are exposed under both physical and chemical dispersion of oil to 2 types of oil contact: 1) dissolved oil fractions that are taken up into the organism across cell membranes and cause toxicity by narcosis, and 2) oil droplets of a range of sizes that can be ingested or coat body appendages and integument. Dispersants increase the concentration of entrained small oil particles (generally $<70\,\mu m$ in diameter) in the aqueous exposure media relative to their concentration in physically dispersed oil [2,76]. Although the use of dispersants is well known to increase the concentration of oil droplets in the water column, the contribution of droplets to the overall toxicity of oil has been seldom studied in parallel with standard toxicity testing. However, studies have found that although oil particles can coat the feeding apparatus of many aquatic species [77], or can be ingested by pelagic invertebrates [77–79], toxicological effects have been repeatedly and quantitatively characterized as primarily the result of exposures to the dissolved oil fractions (namely bioavailable PAHs) [22,51,80,81], or factors other than oil droplet size [76]. Although studies have provided valuable insights on the acute and sub-acute toxicity of oil droplets on fish species [51,80,81], the specific role of increased concentrations of small oil droplets on toxicity continues to be a gap in our understanding, and it should be further investigated.

Uncertainties also exist in the current understanding of the sensitivity to physically and chemically dispersed oil for many untested marine species or species in extreme warm or cold environments, including sensitive life stages and species of special concern. Recent efforts have been undertaken to better understand the relative sensitivity of cold-water species to oil components and to physically and chemically dispersed oil [50,56]. In both of these studies, cold-water species fell within the range of sensitivities of commonly tested species, mostly of temperate climates. Although these studies partially addressed the relative sensitivity of non-commonly tested cold-water species, similar research is also needed to answer related questions on sensitivities for untested species or those found in deep water environments. Based on issues identified in the present review, a need exists for increased toxicity testing of species common among those known sensitive taxa (e.g., crustaceans), as well as testing with different life stages of the same species.

The SSDs have been shown to provide useful information of potential significance for decisions makers involved in oil spill response decision making. Although SSDs have been used in the field of environmental toxicology and ecological risk assessment for at least a decade [58], these have not been broadly used in chemical or oil spill situations to help decide appropriate response options. However, and as demonstrated here and elsewhere [55,57], SSDs can provide scientifically defensible benchmark values for dispersant use decision making and related oil spill issues, even when the SSDs are based on standard exposure regimens rather than real world, dynamic exposure tests. With an awareness of the conservatism built into standard testing approaches, derived benchmarks can be applied with a high level of confidence for dispersant use decisions.

Although this quantitative literature review focused on in vivo studies, a large body of emerging literature has been published on in vitro studies related to oil spill impacts and the use of dispersants [82–90]. Although in vitro endpoints can serve as early warning systems of potential sublethal or lethal effects, their usefulness in dispersant use decision making, or in ecological risk assessments related to oil spills (or for other environmental risk assessments), has not been carefully evaluated (e.g., ecological significance of observed effects) or proven reliable (see Nuclear Regulatory Commission [30]). In vitro studies are becoming integral elements of the regulatory framework for human health assessments [30,91], where efforts have been made to standardize practices and procedures;

however, their advancement and adoption for ecological risk assessments and regulatory decision making are evolving. Similar efforts should be made to ensure that in vitro endpoints are generated under realistic oil spill exposure conditions (concentrations and exposure duration) and can be related to effects of ecological significance.

A general recommendation emerging from the analyses in the present review is that toxicity data used in oil spill response decision making should be based on experimental designs and toxicological results that best represent typical field conditions of oil spills, or at a minimum based on the best available science (e.g., measured concentrations). Researchers must present their toxicological findings in the context of the conditions and environmental concentrations of oil spills. For example, studies have reported toxicity values for TPHs in CEWAF in the less than 0.1 mg/L to 170 mg/L range (Figure 2). By comparison, monitoring data collected during the Deepwater Horizon oil spill [57] revealed a maximum TPH concentration of 2 mg/L at 1 m depth approximately 30 min after chemical dispersion of a weathered oil slick at the surface. Monitoring data from subsea dispersant applications at the wellhead during the same incident showed concentrations in the 0.03 mg TPH/L to 0.07 mg TPH/L range at sampling points close to the source [92]. Although risks to water column species clearly are associated with surface spills, from exposure to both physically and chemically dispersed oil, interpreting scientific findings within the proper context may assist regulatory decisions on promoting response options that provide the greatest environmental benefits.

Standard testing has generated useful information that has helped inform decisions on the offshore use of chemical dispersants. However, and as shown in the present quantitative review, selection of the best available toxicity data needs to consider those key outstanding issues discussed here to adequately support future decisions on dispersant use. Furthermore, studies on the toxicity of both physically and chemically dispersed oil would greatly benefit from the adoption of existing recommendations, including environmentally realistic experiment designs and chemical characterization of the aqueous exposure media. Adoption of better toxicity testing practices is a necessary step critically needed to generate information of great scientific value to future decisions.

SUPPLEMENTAL DATA

References. (20 KB DOCX).

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