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Environmental Toxicology and Chemistry, Vol. 30, No. 10, pp. 2244–2252, 2011
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Printed in the USA
DOI: 10.1002/etc.619

COMPARATIVE TOXICITY OF EIGHT OIL DISPERSANTS, LOUISIANA SWEET CRUDE OIL (LSC), AND CHEMICALLY DISPERSED LSC TO TWO AQUATIC TEST SPECIES

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(*Submitted* 5 *January* 2011; *Returned for Revision* 7 *February* 2011; *Accepted* 16 *June* 2011)

Abstract—The present study describes the acute toxicity of eight commercial oil dispersants, South Louisiana sweet crude oil (LSC), and chemically dispersed LSC. The approach used consistent test methodologies within a single laboratory in assessing the relative acute toxicity of the eight dispersants, including Corexit 9500A, the predominant dispersant applied during the DeepWater Horizon spill in the Gulf of Mexico. Static acute toxicity tests were performed using two Gulf of Mexico estuarine test species, the mysid shrimp (*Americamysis bahia*) and the inland silversides (*Menidia beryllina*). Dispersant-only test solutions were prepared with high-energy mixing, whereas water-accommodated fractions of LSC and chemically dispersed LSC were prepared with moderate energy followed by settling and testing of the aqueous phase. The median lethal concentration (LC50) values for the dispersant-only tests were calculated using nominal concentrations, whereas tests conducted with LSC alone and dispersed LSC were based on measured total petroleum hydrocarbon (TPH) concentrations. For all eight dispersants in both test species, the dispersants alone were less toxic (LC50s: 2.9 to >5,600 μl/L) than the dispersant–LSC mixtures (0.4–13 mg TPH/L). Louisiana sweet crude oil alone had generally similar toxicity to *A. bahia* (LC50: 2.7 mg TPH/L) and *M. beryllina* (LC50: 3.5 mg TPH/L) as the dispersant–LSC mixtures. The results of the present study indicate that Corexit 9500A had generally similar toxicity to other available dispersants when tested alone but was generally less toxic as a mixture with LSC. Environ. Toxicol. Chem. 2011;30:2244–2252. © 2011 SETAC

Keywords—Oil dispersants Corexit 9500A *Menidia* Mysids South Louisiana sweet crude

INTRODUCTION

An estimated 4.9 million barrels of South Louisiana sweet crude (LSC) oil was released into the northern Gulf of Mexico between April 20 and July 15, 2010 as a result of the explosion and collapse of the Deepwater Horizon (DWH) oil exploration platform (www.restorethegulf.gov). During the catastrophe, a number of recovery strategies to contain the oil were employed, including direct capture of oil from the wellhead (17%), surface skimming (3%), booming and burning (5%), and the application of chemical oil dispersants (8%) (www.restorethegulf.gov). The use of dispersants in oil spill response involves tradeoffs between the direct effects of oil to bays, estuaries, and beaches and effects of dispersants and dispersed oil to pelagic and deep sea environments [1–3]. To mitigate the environmental impact of floating oil on sensitive shoreline habitats along the northern Gulf coast, the decision was made to apply Corexit 9500A, an oil dispersant listed on the U.S. Environmental Protection Agency's (U.S. EPA) National Contingency Plan (NCP) Product Schedule (http://www.epa.gov/emergencies/content/ncp/product_schedule.htm). Approximately 4,059,854 L of Corexit 9500A was applied to floating oil offshore, and an additional 2,914,767 L was injected directly into the oil and gas plume at the wellhead 1,544 m below the surface (www.restorethegulf.gov).

The U.S. Clean Water Act and Oil Pollution Act of 1990 requires the maintenance of a federal NCP for response to oil spills that identifies specific commercial products used for control of oil discharges and the quantities and water bodies in which the products may be used. These products consist of dispersants, surface washing agents, surface collecting agents, bioremediation agents, and other miscellaneous oil spill control agents. Under the NCP, the U.S. Environmental Protection Agency has statutory responsibility for obtaining toxicity and efficacy information from the manufacturers before placing a dispersant on the National Product Schedule. Toxicity data requirements and test procedures are stipulated under 62 FR11576, Appendix C, of Part 300, which consists of static acute toxicity tests conducted with the dispersant product and a separate test with no. 2 fuel oil using two estuarine test species [4]. The National Product Schedule acts as a preapproval mechanism, allowing the Federal On-Scene Coordinator working with state and local governments to respond quickly to a spill situation using the best available technology.

Currently, 15 dispersants are listed on the National Product Schedule (http://www.epa.gov/emergencies/content/ncp/product_schedule.htm). Although the exact compositions of most commercially available oil dispersants are proprietary, they typically contain a high percentage of one or more uncharged or charged anionic surfactants of different solubility. Surfactants are amphiphatic molecules possessing both hydrophilic and hydrophobic groups that act to decrease tension between the water and oil interface, stimulating the development of small oil-surfactant micelles less than 100 μM [5,6]. The greater surface area to volume of the droplets enhances entrainment of the micelles in the water column while also increasing the oil's availability to physical and microbial degradation. Inclusion of solvents such as petroleum distillates in dispersant mixtures assists in dissolving surfactants through reduction in viscosity.

Here we report a two-phase toxicity study to determine the hazards of eight commercial dispersant products using two Gulf of Mexico aquatic species. Phase 1 included acute toxicity tests performed with each of the dispersants in the absence of oil. The second phase of testing consisted of acute toxicity tests

All Supplemental Data may be found in the online version of this article.

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Published online 15 July 2011 in Wiley Online Library (wileyonlinelibrary.com).

conducted with LSC oil only and chemically dispersed LSC using each of the eight oil dispersants. The approach used consistent test methodologies within a single laboratory, which provided a means to assess and compare independently acute toxicity estimates of the dispersants, LSC, and dispersant–oil mixtures.

MATERIALS AND METHODS

Study design

In the present study, the acute toxicity of eight dispersants, LSC, and dispersant–LSC mixtures was examined using two Gulf of Mexico aquatic species: the mysid shrimp, *Americamysis bahia*, an aquatic invertebrate, and the inland silverside, *Menidia beryllina*, a small estuarine fish. These species are standard test organisms used in a variety of U.S. EPA toxicity test methods. Larval *A. bahia* were supplied from in-house cultures maintained by the contract testing laboratory using filtered natural seawater. Larval *M. beryllina* were purchased from Aquatic Biosystems, shipped overnight to the testing laboratory and held a minimum of 2 d before testing. Culture and holding conditions for both species were 25°C and 20 parts per thousand salinity.

The static acute toxicity test methods followed U.S. EPA Test Method 821/R-02-012 [7], with slight modifications as described (Table 1). *Americamysis bahia* were 24 to 48 h old and *M. beryllina* 11 or 14 d old at test initiation. All organisms for a given exposure were within 24 h of the same age. Three replicates were conducted for each exposure concentration. Test organisms were randomly assigned across exposure and control treatments, with each replicate receiving 10 animals, for a total of 30 animals per treatment level. One-liter beakers containing 1 L test solution were maintained in 25°C temperature-controlled water baths under a photoperiod of 16:8 h light:dark. All test vessels were aerated continuously (100 bubbles/min). The duration of the acute tests was 48 h for *A. bahia* and 96 h for *M. beryllina*. Temperature was monitored continuously using maximum–minimum thermometers; salinity and dissolved oxygen were measured once a day. All tests were conducted under contract with Smithers-Viscient Laboratory and performed in compliance with the Good Laboratory Practice regulations as provided in U.S. EPA 40 CFR 160 [8].

Nonweathered LSC oil, lot no. WP 681, was purchased from RT Corporation in 500-ml amber bottles and shipped directly to the testing laboratory. Selection of eight commercially available oil dispersants for testing was based on listing in the National Contingency Plan Product Schedule, product availability, and adequate production capacity. Liquid concentrates of Corexit 9500A (Nalco), Dispersit SPC 1000 (U.S. Polychemical), JD-2000 (GlobeMark Resources), Nokomis 3-AA, and Nokomis 3-F4 (Mar-Len Supply), Saf-Ron Gold (Sustainable Environmental Technologies), Sea Brat #4 (Alabaster), and ZI-400 (Z.I. Chemicals) were shipped directly from each manufacturer to the contract testing laboratory. They were logged into their test material center and maintained according to good laboratory practice and chain-of-custody requirements.

Dispersant-only tests

Exposure concentrations for use in acute toxicity tests with dispersants alone were prepared, with slight modification as listed in Table 1, following the requirements specified in U.S. EPA 62 FR 15576, Appendix C of Part 300 [4]. In brief, stock solutions were prepared by adding 1.1 ml dispersant to 110 ml seawater. The solutions were mixed using a top stirrer (TAMCO) equipped with a stainless steel blade at speeds providing a 70% vortex. An appropriate aliquot of stock was removed from the area between the mixing vessel wall and edge of the vortex and placed directly in the dilution water within an exposure beaker. Each exposure solution was mixed by stirring before introducing test organisms. The exposure concentration range for each dispersant was chosen to bracket the estimated median lethal concentration (LC50) values reported in the NCP Product Schedule (Table 2).

LSC and dispersant–LSC tests

Assessment of oil-only and dispersant–oil toxicity was determined using water-accommodated fractions (WAFs) of LSC or chemically enhanced water-accommodated fractions (CE-WAFs) of dispersant–LSC as described later. South Louisiana sweet crude WAFs were prepared following the methods of the Chemical Response to Oil Spills: Ecological Effects Research Forum [9] with the variable dilution modification described by Barron and Ka'aihue [10]. In brief, glass aspirator bottles with hose bibs at the base were each fitted with a length of silicone tubing and a hose clamp. Each bottle was filled with 19 L seawater, leaving a 20% headspace above the liquid, placed on a magnetic stir plate, and a stir bar was added. South Louisiana sweet crude was added at 25 g/L seawater, using a long tube attached to a glass funnel to reduce production of air bubbles in the surface slick. The stir plate was adjusted to obtain an oil vortex of 25% of the total volume of seawater, which provided a similar mixing energy in each WAF preparation. The

Table 1. List of modifications to test procedures specified under Appendix C of 62 CFR Part 300 Appendix C [4] for conducting acute toxicity tests with dispersants alone using the mysid shrimp, *Americamysis bahia*, and inland silverside, *Menidia beryllina*

Test parameter	Specified in SubPart J Appendix C	Method used in present study
Photoperiod and light intensity	Constant light conditions 2,000 lumens/m^2	16:8 h light:dark 1,000 lumens/m^2
Glassware cleaning	Hexane immersion	Acetone rinse
Reference toxicant test	Two species simultaneously	Staggered tests
Rangefinder tests	Prior to definitive test	Use NCP data to define test concentrations
Age at test start:		
Mysid	5–7-d-old larvae	3–4 d old; all within 24 h same age
Menidia	7-d-old larvae	9–14 d old; all within 24 h same age
Stock solution preparation	Blender 10,000 rpm	Top stirring at 70% vortex
Mysid test solution mixing	Not specified	Short term gentle mixing following stock addition
Menidia test solution mixing	Test jars on shaker platform	Same procedure as for mysids
Loading of test organisms	Not specified	Impartial, two at a time
Dilution water	Natural seawater preferred	20-μm filtered natural seawater, salinity adjusted

Table 2. Nominal exposure concentrations of dispersant preparations used in acute toxicity tests with *Americamysis bahia* and *Menidia beryllina*

	Definitive test concentrations (μl/L)	
Dispersant	Mysid shrimp (*Americamysis bahia*)	Inland silverside (*Menidia beryllina*)
Corexit 9500A	100, 56, 32, 18, 10	320, 180, 100, 56, 32
Dispersit SPC 1000	56, 32, 18, 10, 5.6	10, 5.6, 3.2, 1.8, 1.0
JD-2000	3,200, 1,800, 1,000, 560, 320	5,600, 3,200, 1,800, 1,000, 560
Nokomis 3-AA	56, 32, 18, 10, 5.6	100, 56, 32, 18, 10
Nokomis 3-F4	100, 56, 32, 18, 10	100, 56, 32, 18, 10
Saf-Ron Gold	1,000, 560, 320, 180, 100	100, 56, 32, 18, 10
Sea Brat 4	320, 180, 100, 56, 32	100, 56, 32, 18, 10
ZI-400	320, 180, 100, 56, 32	100, 56, 32, 18, 10

bottles were securely covered; the solutions were mixed for 18 h then allowed to settle for 6 h. The WAF (aqueous phase) was removed from the bottom without disturbing the oil slick remaining on the surface. The WAF was remixed after removal, and 2 L WAF was used for analysis of TPH, with the remaining volume available to prepare the test solutions. In a secondary series of acute tests conducted only with *M. beryllina*, two WAFs using 50 and 100 g LSC/L seawater were prepared following the procedures described previously.

The method for preparing each of the eight dispersant/LSC CE-WAFs followed the LSC WAF procedure, with the addition of each dispersant at a ratio of 1:10 dispersant:oil (2.5 g/L) after the 25% oil vortex was established. Mixing and settling times followed the oil-only procedures described previously. Dispersant manufacturers have generally recommended application rates using dispersant-to-oil ratios between 1:50 to 1:10, depending on oil type and sea conditions. A ratio of 1:10 is the standard recommendation for dispersant toxicity testing, because it maximizes the effect of the dispersant on oil in the CE-WAF [10]. Two rounds of testing of the dispersant–oil mixtures were necessary to ensure that test concentrations bracketed the CE-WAF LC50 and met test condition requirements for both *A. bahia* and *M. beryllina*.

Separate oil-only WAFs were used to prepare test solutions for *A. bahia* and *M. beryllina* with LSC. For dispersant–oil testing, each CE-WAF was divided and used to prepare solutions for both *A. bahia* and *M. beryllina* tests. Natural filtered seawater adjusted to 20 parts per thousand with laboratory well water was used for all static acute tests. Larval *A. bahia* and *M. beryllina* were treated with dilutions of LCS WAF or dispersant/oil CE-WAF plus an untreated seawater control. Six concentrations (plus control) of the oil-only WAF were tested, with the highest exposure level being 100% WAF. Each of the dispersant/oil CE-WAF tests was performed with six to eight exposure concentrations plus an untreated seawater control treatment to bracket the median lethal concentration (Table 3).

Reference toxicity tests

Two sets of 48-h (*A. bahia*) and 96-h (*M. beryllina*) acute toxicity tests were conducted with the standard reference toxicant, sodium dodecyl sulfate (SDS; Sigma-Aldrich), to evaluate the relative sensitivity of the test organisms over the course of the two-phase study. The organisms tested with SDS were from the same populations and age range used in both phases of the toxicity tests conducted with dispersants, LSC, and dispersant–LSC mixtures.

Chemical analysis

A 1-L sample was collected from each WAF, and two replicate 1-L samples were collected from each CE-WAF for analysis of C9-C32 total petroleum hydrocarbons (TPH), the standard method for quantifying oil in water (Fig. S1). Each sample was extracted with methylene chloride, reduced to 1 ml, and analyzed by gas chromatography-flame ionization detection. Analysis was conducted on an HP5890 GC (Hewlett-Packard) following EPA SW-846, Method 8015B-DRO. Additional gas chromatography-flame ionization detection analyses were performed on the CE-WAFs of Corexit 9500A and JD-2000 to provide tentative identification of single high-level chemical peaks in the chromatograms. The peaks were identified as nonpetroleum hydrocarbon constituents in Corexit 9500A and JD-2000 consisting of ethoxylated mono- and trioleates and were removed from the calculation of their

Table 3. Measured concentrations, visual observations, and percent exposure concentrations of dispersant–oil chemically enhanced water accommodated fractions (CE-WAFs) and Louisiana sweet crude (LSC) WAF preparations used in acute toxicity tests with *Americamysis bahia* and *Menidia beryllina*

			Definitive test concentrations (% CE-WAF)	
Dispersant-LSC CE-WAF or LSC-WAF	CE-WAF visual observations	Measured TPH in 100% CE-WAF or LSC-WAF (mg/L)	Mysid shrimp (*Americamysis bahia*)	Inland silverside (*Menidia beryllina*)
9500A	Very dark brown	44.6	50, 25, 13, 6.3, 3.1, 1.6	100, 50, 25, 13, 6.3, 3.1
Dispersit SPC 1000	Cloudy beige	400	3.1, 1.6, 0.8, 0.4, 0.2 0.1, 0.05	6.3, 3.1, 1.6, 0.8, 0.4, 0.2, 0.1, 0.05
JD-2000	Slightly cloudy with oil particulates	6.8	100, 50, 25, 13, 6.3, 3.1	100, 50, 25, 13, 6.3, 3.1
Nokomis 3-AA	Slightly cloudy beige	87	3.1, 1.6, 0.8, 0.4, 0.2 0.1, 0.05	6.3, 3.1, 1.6, 0.8, 0.4, 0.2, 0.1, 0.05
Nokomis 3-F4	Dark cloudy brown	1,600	3.1, 1.6, 0.8, 0.4, 0.2 0.1, 0.05	3.1, 1.6, 0.8, 0.4, 0.2, 0.1, 0.05, 0.025
Saf-Ron Gold	Cloudy pearlescent white	57 (mysid) 63 (*Menidia*)	50, 25, 13, 6.3, 3.1, 1.6	6.3, 3.1, 1.6, 0.8, 0.4, 0.2
Sea Brat 4	Slightly cloudy, brown tint	86	6.3, 3.1, 1.6, 0.8, 0.4, 0.2	13, 6.3, 3.1, 1.6, 0.8, 0.4
ZI-400	Very cloudy brown	1,800	6.3, 3.1, 1.6, 0.8, 0.4, 0.2, 0.1, 0.05	3.1, 1.6, 0.8, 0.4, 0.2, 0.1, 0.05, 0.025
LSC-Only	Clear	4.4 (mysid) 5.1 (*Menidia*)	100, 50, 25, 13, 6.3, 3.1	100, 50, 25, 13, 6.3, 3.1

respective TPH values. Final CE-WAF concentrations of TPH were determined as the average of the two replicate measured values and reported as milligrams TPH per liter.

Statistical analysis

The commercially available statistical software package CETIS (Tidepool Scientific Software) was used to calculate LC50 values, using an automated decision tree adapted from the U.S. EPA for selection of the appropriate statistical method [11,12]. The LC50 is defined as the concentration of a substance causing death in 50% of test organisms for a specified interval; in this case, 48 h for the *A. bahia* tests and 96 h for the *M. beryllina* tests. Procedures used to calculate LC50 values and 95% confidence intervals included linear regression methods, the nonparametric Spearman-Karber and Trimmed Spearman-Karber methods, and the binomial method. The LC50 values are reported as parts per million in microliters per liter for the dispersant-only tests and milligrams TPH per liter for tests with LSC and dispersant–LSC mixtures.

RESULTS

SDS reference toxicity

The LC50 values (and 95% confidence intervals) for the reference toxicant SDS were 23 mg/L (19–26 mg/L) and 18 mg/L (15–21 mg/L) for the 48-h *A. bahia* tests and 9.5 mg/L (8.7–10 mg/L) and 10 mg/L (8.6–12 mg/L) for the 96-h *M. beryllina* tests conducted during phases 1 and 2, respectively. During the last 24 h of the SDS test with *M. beryllina* conducted during phase 1, the temperature dropped to 22°C, which was 2° below the acceptable criteria. However, no difference was seen in mortality counts between the 72-h and the 96-h observations, suggesting that the temperature change had no negative impact on the test or the final calculated LC50. For both species, the results of the reference toxicant testing were similar between phases 1 and 2, indicating similar sensitivity of test organisms over the course of the study.

Dispersant-only toxicity

Americamysis bahia. Control performance (without dispersant) met all criteria for an acceptable exposure in each *A. bahia* test (≥90% survival). All water quality parameters were within ranges specified in the protocol, with the exception of dissolved oxygen for the high test concentration (56 µl/L) in the Nokomis 3-AA exposure at 24 h, which was measured at 56% of saturation. Because dissolved oxygen levels were greater than 60% at other time points in the test and the toxicity was clearly dose related, the departure observed in the 56 µl/L concentration at 24 h was not considered to have had a negative impact on the exposure with Nokomis 3-AA. The LC50 values for dispersant acute tests with *A. bahia* ranged from 12 µl/L for Dispersit SPC 1000 to 788 µl/L for JD-2000 (Fig. 1; Table 4). Examples of representative concentration–response curves for exposures conducted with Corexit 9500A are presented in Figure 2. The U.S. EPA uses a five-step scale of toxicity categories to classify pesticides based on their acute toxicity to aquatic organisms (Table 5; http://www.epa.gov/oppefed1/ecorisk_ders/toera_analysis eco.htm#Ecotox). Using this toxicity classification, Corexit 9500A, Dispersit SPC 1000, Nokomis-3AA, Nokomis 3-F4, Sea Brat 4, and ZI-400 would be classified as slightly toxic, whereas JD-2000 and Saf-Ron Gold would be classified as practically nontoxic to *A. bahia.*

Menidia beryllina. All water quality parameters were within ranges specified in the test protocol, and *M. beryllina* control performance met all criteria for an acceptable exposure in each of the eight dispersant tests conducted (≥90% survival). The LC50 values for dispersant acute toxicity tests with *M. beryllina* ranged from 2.9 µl/L for Dispersit SPC 1000 to 130 µl/L for

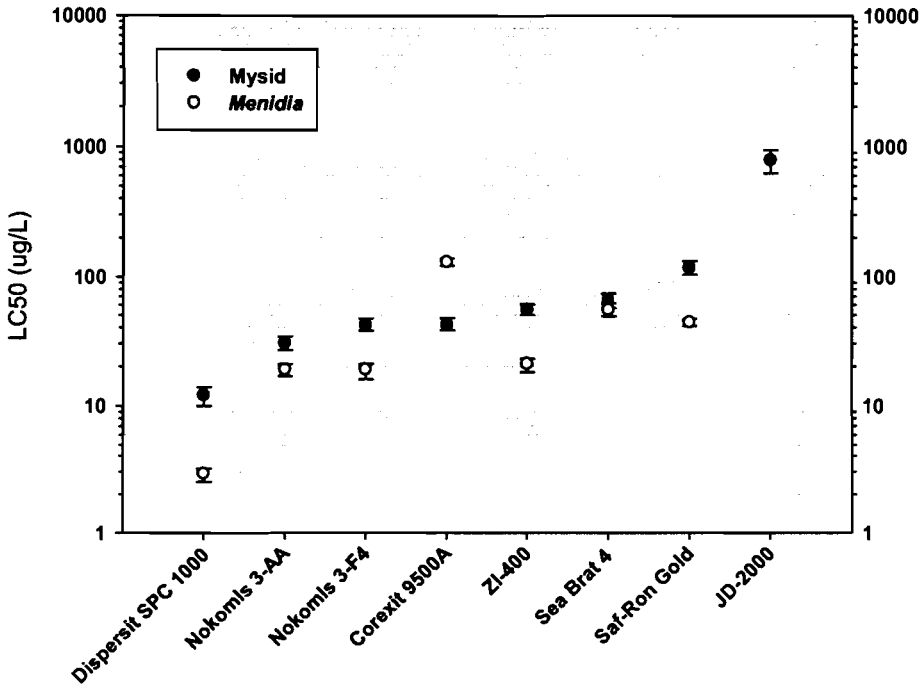

Fig. 1. Median lethal concentrations (LC50s) and 95% confidence intervals from *Americamysis bahia* 48-h and *Menidia beryllina* 96-h acute toxicity tests conducted with eight oil dispersants. Dispersants are listed in decreasing order of toxicity based on results with *A. bahia.* For *M. beryllina,* the LC50 for the dispersant JD-2000 exceeded the highest concentration tested of 5,600 µl/L (not shown).

Table 4. National Contingency Plan (NCP) Product Schedule listing of dispersants and results of *Americamysis bahia* 48-h and *Menidia beryllina* 96-h static acute toxicity tests with eight dispersants derived in the present study[a]

	Mysid shrimp (*Americamysis bahia*)		Inland silverside (*Menidia beryllina*)	
Dispersant	NCP Product Schedule LC50 (μl/L) [95% CI]	Present study LC50 (μl/L) [95% CI]	NCP Product Schedule LC50 (μl/L) [95% CI]	Present study LC50 (μl/L)[b] [95% CI]
Corexit 9500A	32.2 [26.5–39.2]	42[c] [38–47]	25.2 [13.6–46.6]	130 [122–138]
Dispersit SPC 1000	16.6 [14.1–19.6]	12[d] [10–14]	3.5 [3.1–4.0]	2.9 [2.5–3.2]
JD-2000	90.5 [76.1–108]	788[d] [627–946]	407 [330–501]	>5,600 [NA]
Nokomis 3-AA	20.2 [17.4–22.8]	30[b] [27–34]	34.2 [29.2–37.95]	18 [16–21]
Nokomis 3-F4	32.2 [28.4–36.5]	42[c] [38–47]	29.8 [24.0–35.4]	19 [16–21]
Saf-Ron Gold	63.0 [52.9–75.1]	118[b] [104–133]	29.4 [25.2–34.3]	44 [41–47]
Sea Brat 4	14.0 [±10.4]	65[d] [57–74]	30.0 [±16.2]	55 [49–62]
ZI-400	21.0 [17.9–24.5]	55[b] [50–61]	31.8 [28.7–35.1]	21 [18–23]

[a] Median lethal concentration (LC50, μl/L) values and 95% confidence intervals in brackets.
[b] Estimated by Spearman-Karber method.
[c] Estimated by binomial method.
[d] Estimated by linear regression method.

Corexit 9500A (Fig. 1). The LC50 for JD-2000 exceeded the highest test concentration of 5,600 μl/L and was not retested. Using the U.S. EPA toxicity classification, Dispersit SPC 1000 would be considered moderately toxic, whereas Nokomis-3AA, Nokomis 3-F4, Saf-Ron Gold, Sea Brat 4, and ZI-400 would be classified as slightly toxic, and Corexit 9500A and JD-2000 as practically nontoxic to *M. beryllina*. Based on comparison of LC50 values and 95% confidence intervals, the rank order toxicity (most to least toxic) of the dispersants to *M. beryllina* was Dispersit SPC 1000 > Nokomis 3-AA, Nokomis 3-F4, ZI-400 > Saf-Ron Gold > Sea Brat 4 > Corexit 9500A > JD-2000.

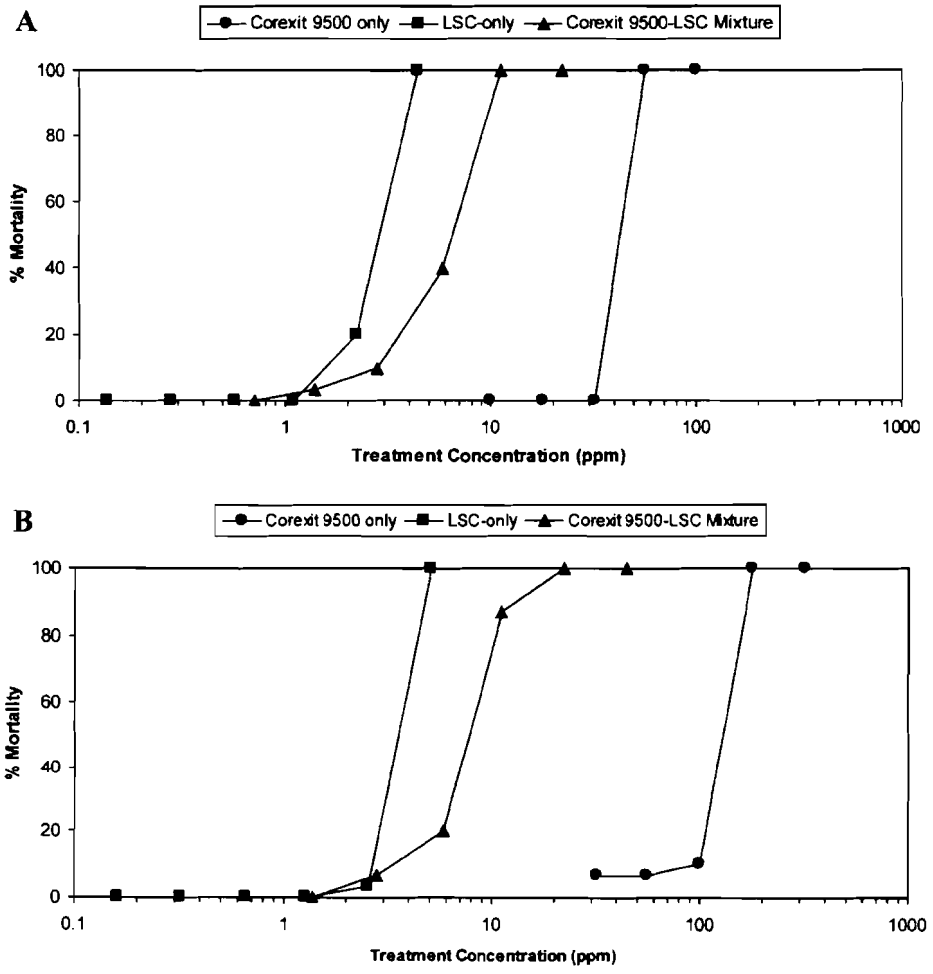

Fig. 2. Concentration–response curves for mysid shrimp, *Americamysis bahia* (**A**), and inland silversides, *Menidia beryllina* (**B**), exposed to Corexit 9500A-only, Louisiana sweet crude (LSC) water-accommodated fractions (WAF), and Corexit 9500A–LSC chemically enhanced WAFs.

Table 5. U.S. Environmental Protection Agency five-step scale of toxicity categories used to classify chemicals based on their acute toxicity

LC50 (μl/L or mg/L)	Toxicity classification
> 100	Practically nontoxic
>10 to 100	Slightly toxic
>1 to 10	Moderately toxic
0.1 to 1.0	Highly toxic
<0.1	Very highly toxic

LC50 = median lethal concentration.

LSC-only toxicity

Americamysis bahia and M. beryllina. Control performance met all criteria for an acceptable exposure in each test (≥90% survival) for both *A. bahia* and *M. beryllina* exposures. All water quality parameters in all treatments were within ranges specified in the protocol for each species. The measured TPH concentration in the LSC WAF used for the acute mysid test was 4.4 mg/L, resulting in a calculated LC50 value of 2.7 mg/L and corresponding 95% confidence interval of 2.5 to 3.0 mg/L (Fig. 3). The measured TPH concentration in LSC WAF used for the acute test with *M. beryllina* was 2.9 mg/L where mortality did not exceed 7% in the highest concentration tested, consisting of 100% WAF. When *M. beryllina* was retested under identical conditions, no mortality occurred in the 100% WAF having a measured TPH of 2.3 mg/L. A secondary series of acute tests conducted using WAFs prepared with loading rates of 50 g and 100 g LSC/L seawater resulted in higher TPH concentrations of 5.4 and 5.1 mg/L, respectively. Using these WAF preparations, calculated LC50s and 95% confidence intervals of 3.5 mg/L (3.4–3.7 mg/L) and 4.05 mg/L (3.8–4.3 mg/L) were determined for *M. beryllina* exposed to LSC (Fig. 3). Using the U.S. EPA toxicity classification, LSC oil would be classified as moderately toxic to both *A. bahia* and *M. beryllina.*

Dispersant–LSC toxicity

The measured TPH in 100% CE-WAF of the dispersant–LSC mixtures used for both *A. bahia* and *M. beryllina* acute tests ranged from 6.8 to 1,800 mg/L (Table 3). Visual inspection of the CE-WAFs reflected the range of aqueous phase TPH in these mixtures.

Americamysis bahia. Control performance met all criteria for an acceptable exposure (≥90% survival) in all tests with *A. bahia.* A definitive LC50 was determined for the initial *A. bahia* test with Saf-Ron Gold CE-WAF, and all water quality parameters were within ranges specified in the protocol for all treatments. Initial mysid tests with CE-WAFs of Dispersit SPC 1000, Nokomis 3-AA, Nokomis 3-F4, and ZI-400 had LC50s less than the lowest concentration tested, which required a second round of testing. A second round of testing was also required for Corexit 9500A, JD-2000, and Sea Brat 4 because of temperature or dissolved oxygen deviations from protocols. All water quality parameters were within ranges specified in the test protocol for *A. bahia,* and definitive LC50 values were determined for each of the seven dispersant–oil CE-WAFs tested. The LC50 values for dispersant–oil acute toxicity tests with *A. bahia* ranged from 0.39 mg TPH/L for Nokomis 3-AA CE-WAF to 9.7 mg TPH/L for the ZI-400 CE-WAF (Fig. 3; Table 6). Using the U.S. EPA toxicity classification, oil–dispersant mixtures using Nokomis 3-AA would be considered highly toxic, whereas Corexit 9500A, Dispersit SPC 1000, JD-2000, Nokomis 3-F4, Saf-Ron Gold, Sea Brat 4, and ZI-400 would be classified as moderately toxic to *A. bahia.*

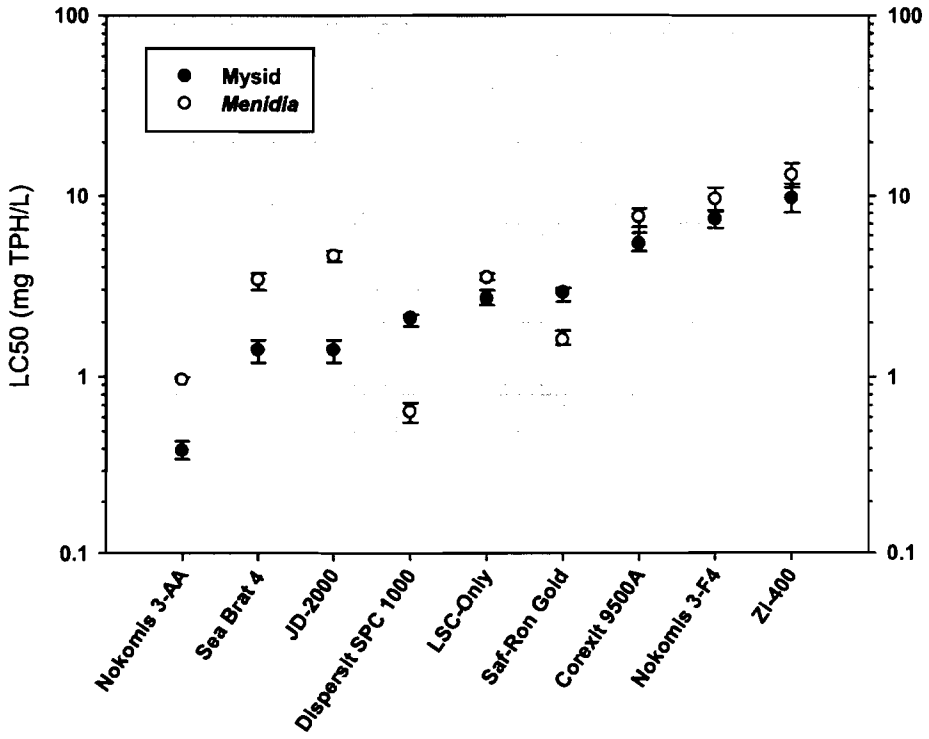

Fig. 3. Median lethal concentrations (LC50s) and 95% confidence intervals from mysid shrimp, *Americamysis bahia,* 48-h and inland silversides, *Menidia beryllina,* 96-h acute toxicity tests conducted with eight dispersant–oil chemically enhanced water-accommodated fractions (CE-WAFs) and Louisiana sweet crude (LSC)-only WAFs. Dispersant–oil mixtures and LSC are listed in decreasing order of toxicity based on results with *A. bahia.* TPH = total petroleum hydrocarbon.

Table 6. Results of *Americamysis bahia* 48-hr and *Menidia beryllina* 96-h static acute toxicity tests with eight dispersant-oil mixture chemically enhanced-water accommodated fractions (CE-WAFs) and Louisiana sweet crude oil-only WAF derived in the present study[a]

	LC50 (mg TPH/L)[b] [95% CI]	
Dispersant CE-WAF	Mysid shrimp (*Americamysis bahia*)	Inland silverside (*Menidia beryllina*)
Corexit 9500A	5.4 [4.9–6.7]	7.6 [6.2–8.5]
Dispersit SPC 1000	2.1 [1.9–2.2]	0.64 [0.56–0.72]
JD-2000	1.4 [1.2–1.6]	4.6 [4.3–4.9]
Nokomis 3-AA	0.39 [0.35–0.44]	0.96 [0.96–1.0]
Nokomis 3-F4	7.4 [6.6–8.3]	9.6 [8.2–11.2]
Saf-Ron Gold	2.9 [2.6–3.1]	1.6 [1.5–1.8]
Sea Brat 4	1.4 [1.2–1.6]	3.4 [3.0–3.7]
ZI-400	9.7 [8.1–11.7]	13.1 [11.2–15.3]
Louisiana sweet crude oil-only WAF	2.7 [2.5–3.0]	3.5 [3.4–3.7]

[a] Median lethal concentration (LC50) values and 95% confidence intervals (CI) in brackets calculated using Spearman-Karber method.
[b] Measured mg total petroleum hydrocarbons/L.

Menidia beryllina. Control performance met all criteria for an acceptable exposure (≥90% survival) in all tests with *M. beryllina*. Initial tests with CE-WAFs of Dispersit SPC 1000, Nokomis 3-AA, Nokomis 3-F4, Saf-Ron Gold, and ZI-400 had LC50s less than the lowest concentration tested, which required a second round of tests to be conducted. Second-round testing was also required for Corexit 9500A, JD-2000, and Sea Brat 4 because of temperature or dissolved oxygen deviations from protocols. The LC50 values for dispersant–oil acute toxicity tests with *M. beryllina* ranged from 0.64 for Dispersit SPC 1000 to 13.1 mg TPH/L for ZI-400 (Fig. 3). Using the U.S. EPA toxicity classification, oil–dispersant mixtures using Dispersit SPC 1000 or Nokomis 3-AA would be considered highly toxic, whereas Corexit 9500A, JD-2000, Nokomis 3-F4, Saf-Ron Gold, and Sea Brat 4 would be classified as moderately toxic, with ZI-400 as slightly toxic to *M. beryllina*.

DISCUSSION

Companies that manufacture dispersants are required to submit information on the toxicity and percentage effectiveness of their products to the U.S. EPA contingent to listing their products on the NCP Product Schedule. As a result, the toxicity information from these tests was generated by a variety of commercial testing laboratories over an extended period. To ensure the most accurate data were available to make informed decisions on the least toxic, most efficient dispersants to combat the DWH spill, U.S. EPA's Office of Research and Development conducted independent toxicity studies using both cell-based in vitro assays and whole animal in vivo tests. The in vitro assays were based on U.S. EPA's ToxCAST program and focused on the toxicity and potential endocrine effects of the dispersants alone [13]. The in vivo acute tests presented here included dispersants, LSC, and dispersant–LSC mixtures conducted by a single contract laboratory under U.S. EPA oversight. Of the 15 dispersants on the NCP product schedule, eight were chosen for testing based on known toxicity and efficacy information, product availability for immediate testing, and availability of sufficient dispersant quantities to respond to the spill. In these studies, LSC was selected as the reference oil, because it was considered more relevant and representative of conditions occurring in the Gulf of Mexico than testing no. 2 fuel oil as specified by the NCP. Thus, our approach provided an independent, consistent, and quantitative assessment of acute toxicities of dispersants, LSC oil, and eight dispersant–LSC mixtures to two aquatic species inhabiting Gulf of Mexico waters.

An important component of the present study included assessing the accuracy of information on dispersant toxicity the manufacturers provided to the NCP. A qualitative comparison was made between LC50 values for the eight dispersants tested as well as with those available in the NCP Product Schedule. The reproducibility of static acute tests among laboratories using the same species/toxicant combination has been reported to fall generally within a factor of 3.5 among laboratories when using nominal concentrations (unmeasured treatment concentrations) for both freshwater and marine species [14]. Given the use of whole organisms in these tests, some variation in response attributable to differences in parameters such as culture and acclimation conditions, stock populations, or variable water quality is expected and acceptable. Factor ratios were used to compare LC50s derived for the same species/dispersant combination from different laboratories. The factor ratios between dispersant LC50 values determined in the present study and NCP-reported LC50 values were calculated as a ratio by dividing the higher of the two LC50 values by the lower LC50 value for each of the eight dispersants, respectively (Table 4). As an example, using information for *A. bahia* from Table 4, the factor ratio for Corexit 9500A was determined as 42/32.2 = 1.3. For *A. bahia*, the factor ratios calculated for Corexit 9500A, Dispersit SPC1000, Nokomis 3-AA, Nokomis 3-F4, Saf-Ron Gold, and ZI-400 were less than or equal to 2.6, which was considered within normal interlaboratory variability [14]. Results for JD-2000 and Sea Brat 4 showed lower toxicities (i.e., higher LC50s), with factor ratios of 8.7 and 4.6, respectively, compared with their reported NCP LC50 values. With *M. beryllina*, the factor ratios calculated for Dispersit SPC 1000, Nokomis 3-AA, Nokomis 3-F4, Saf-Ron Gold, Sea Brat 4, and ZI-400 were less than or equal to 1.83, which was considered within normal interlaboratory variability. The factor ratios of 5.2 and 13.8 for Corexit 9500A and JD-2000 indicate that the LC50 values reported for these dispersants in the NCP Product Schedule would be considered different from (that is, lower than) the LC50 values for *M. beryllina* determined in the present study. Possible explanations for the 13.8-fold difference between the reported NCP LC50 for JD-2000 and the highest exposure concentration tested for *M. beryllina* in the present study may be attributable to batch-to-batch variability in the manufacturing process, instability of the stored product over time, or a change in the product formulation.

Corexit 9500A was the predominant dispersant used in response to the DWH event, with an estimated 6,974,621 liters used in the Gulf of Mexico in both surface and subsurface applications (www.restorethegulf.gov). Initially, small amounts of a predecessor dispersant, Corexit 9527, were applied during early response efforts until locally available stocks had been depleted. Previous studies reported by Singer et al. [6] indicate no significant difference in acute toxicity to marine organisms between the two Corexit formulations. This suggests the possibility that unique toxicological effects occurring through exposure to Corexit 9527 during the early period of the spill were probably negligible. In experiments with dispersants alone, we found the toxicity of Corexit 9500A to be essentially equivalent to most of the dispersants tested. The exceptions were the increased sensitivity of *M. beryllina* exposed to Dispersit SPC 1000 and the lack of toxicity observed in both species exposed to JD-2000. Without specific information on product formulations, surmising why the acute toxicity of these two dispersants, especially JD-2000, differed from the other dispersants by approximately an order of magnitude was difficult. With the exception of Corexit 9500A, little or no toxicity information with aquatic species is available for the remaining seven dispersants. Inspection of LC50 values for Corexit 9500 referenced in George-Ares and Clark [15] for the *A. bahia* and *M. beryllina* are from unpublished laboratory toxicity reports and identical to the values listed on the NCP Product Schedule, suggesting that these values were submitted to the U.S. EPA NCP. However, a comparative toxicity study conducted with Corexit 9500 and *A. bahia*, *M. beryllina*, and the sheepshead minnow (*Cyprinodon variegatus*) using 96-h acute static-renewal tests reported similar LC50s of 32, 79, and 180 mg/L, respectively [16]. In other studies using static-renewal exposures, 96-h LC50s (95% CL) were reported for Corexit 9500A of 21 mg/L (18.6–23.5 mg/L) for *A. bahia*, 79.3 mg/L (70.5–81.1 mg/L) for *M. beryllina* [17], and 35.9 mg/L (32.2–41.3 mg/L) for *A. bahia* [18], which are consistent with the results of the present study. Our results, together with reported values, suggest that *A. bahia* are marginally more sensitive than *M. beryllina* to Corexit 9500A, although the dispersant alone is considered only slightly toxic to both species using U.S. EPA toxicity categories.

Second-generation dispersants such as Corexit 9500A are typically less toxic than oil alone or dispersed oil [5,15], suggesting that dispersant toxicity during a spill response is of secondary concern to the inherent toxicity of the oil or dispersed oil. This observation was substantiated in the present study, in which LSC showed higher toxicity (lower LC50s) to mysids than the eight dispersants tested alone, with similar effects observed for *M. beryllina*. Anderson et al. [19] reported comparable 96-h LC50s (95% CL) for static tests conducted with adult *M. beryllina* of 5.5 mg/L (3.3–9.4 mg/L) and adult *Americamysis (Mysidopsis) almyra*, a sympatric species with *A. bahia*, of 8.7 mg/L (7.2–10.7 mg/L), suggesting little difference in sensitivity to LSC between larval and adult life stages of these species. Further, most of the dispersant–LSC mixtures we tested were within the same order of magnitude of toxicity as LSC alone, indicating a lack of additive or synergistic effect of the dispersants on LSC toxicity. Therefore, the principal concern associated with the use of dispersants is not the toxicity associated with a dispersant alone, but rather with dispersed oil and the increased bioavailability of toxic oil constituents dissipated over a larger spatial area of the water column.

Measured TPH concentrations were found to vary over a wide range for the eight 100% CE-WAF stock solutions used in preparation of the test concentrations (Table 3). Interestingly, the two dispersant–LSC stock solutions demonstrating the highest measured TPH, ZI-400 (1800 mg TPH/L) and Nokomis 3-F4 (1600 mg TPH/L), generated the lowest overall acute toxicity values for both *A. bahia* and *M. beryllina*. However, this trend did not extend to the other six dispersant/LSC mixtures tested, and whether dispersant-specific constituents were responsible, or whether the occurrence was a function of the low dilution factors used in preparation of the ZI-400 and Nokomis 3-F4 exposure concentrations, is unclear.

Some speculation has been made on whether the *A. bahia* and *M. beryllina* specified by the NCP for use in the acute toxicity studies of dispersants and dispersed oil serve as adequate surrogates capable of protecting the biological and geographical diversity of species potentially impacted by an oil spill event. Both species were selected for inclusion in the U.S. EPA acute and chronic test methods based on their ease of culture, availability of life stages, and relative sensitivity to a wide range of chemical contaminants. Inspection of reported values for a variety of dispersants, oils, and chemically dispersed oils indicate that both *A. bahia* and *M. beryllina* are generally representative of most aquatic vertebrate and invertebrate species tested [5,15]. However, only a small number of species of limited diversity have been routinely used in testing. Expansion of test species for organisms at risk to oil exposures should be considered based on habitat and geographic location to ensure that adequate information is available for hazard assessment should dispersant application become an option. Unique to the DWH spill was the first-time use of deepwater dispersant injection into the oil plume 1,544 meters below the surface of the Gulf of Mexico. Because of our limited knowledge of life histories for most deep-water pelagic and benthic animals, little is known of the impact on these organisms caused by the DWH event. Unfortunately, because of biological and logistical constraints such as animal availability and extreme environmental conditions, the routine use of deep-water organisms for dispersant and oil toxicity studies are not logistically or economically feasible. These difficulties coupled with the increasing pressure for deep-water oil exploration will force our continued reliance on surrogate test species.

Short-term acute toxicity tests using consistent methodologies and test organisms provided important and fundamental information to the U.S. EPA, allowing the continued use of Corexit 9500A during the DWH disaster. The comparative toxicity analysis of dispersants, sweet crude oil, and dispersant–sweet crude oil mixtures on standard aquatic test species provides an improved understanding of acute toxicological effects associated with dispersant use and helps inform future decision making.

SUPPLEMENTAL DATA

Fig. S1. Representative spectra from gas chromatography-flame ionization detection analysis. (48 KB PDF).

Acknowledgement—The authors acknowledge the data contributions of Lee Sayers, Susan Shepherd, Alison Fournier, and Marjorie Dix of Smithers Viscient, Wareham, MA (contract EP-10W001502). We also thank our U.S. EPA Office of Research and Development colleagues John Macauley for contract management and Michael Lewis for valuable advice. The present study is contribution number 1412 from the U.S. EPA, Gulf Ecology Division, Gulf Breeze, FL. The views expressed in this article are those of the authors and do not necessarily reflect the views and policies of the U.S. EPA. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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