# Comparative Toxicity of Eight Oil Dispersant Products on Two Gulf of Mexico Aquatic Test Species

**U.S. Environmental Protection Agency**
**Office of Research and Development**

## U.S.EPA/ORD Contributors

**National Health and Environmental Effects Research Laboratory**
Michael J. Hemmer, Mace G. Barron and Richard M. Greene

## 1. Introduction

Large quantities of Louisiana sweet crude oil have been released into the Gulf of Mexico since the explosion of the Deepwater Horizon oil exploration platform on April 20, 2010. As part of the integrated response effort to mitigate the impact of the oil in the environment, the decision was made to use dispersants listed on the U.S. Environmental Protection Agency's (EPA) National Contingency Plan (NCP) Product Schedule (EPA 2010a). Dispersants are being applied offshore on the surface as well as underwater at the source of the leak. The EPA conducted independent studies to assess the relative acute toxicity of eight dispersants on the NCP Product Schedule.

This report summarizes results of the first phase of testing obtained from acute toxicity tests conducted with eight oil dispersants using two Gulf of Mexico aquatic species: (1) the mysid shrimp, *Americamysis bahia*, an aquatic invertebrate, and (2) the inland silverside, *Menidia beryllina*, a small estuarine fish. These species are standard test organisms used in a variety of EPA toxicity test methods. The eight dispersants tested were Corexit 9500A, Dispersit SPC1000, JD-2000, Nokomis 3-AA, Nokomis 3-F4, Saf-Ron Gold, Sea Brat #4 and ZI-400. The tests were conducted using an established contract testing laboratory and in compliance with the Good Laboratory Practice regulations as provided in EPA 40CFR160 (USEPA, 40CFR Part 160). The approach described herein utilized consistent test methodologies within a single laboratory which provided a means to assess acute toxicity estimates across dispersants and independently evaluate the NCP Product schedule toxicity information. The next phases of this study will examine the acute toxicity of Louisiana sweet crude oil and dispersant-sweet crude oil mixtures on mysids and *Menidia* – the results will be reported separately at a later date.

## 2. Test Methods

The acute toxicity test methods followed, with slight modification, the requirements specified in U.S. Environmental Protection Agency's 62 FR 15576, Appendix C of Part 300 – *Swirling Flask Dispersant Effectiveness Test, Revised Standard Dispersant Toxicity Test and Bioremediation Agent Effectiveness Test* (USEPA, 1997) and the EPA Test Method 821-R-02-012, *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms* (USEPA, 2002). Specific modifications are shown in Appendix A.

The exposure concentration range for each dispersant was chosen to bracket the estimated median lethal concentration (LC50) values reported in the NCP Product Schedule. The LC50 is defined as the concentration of a substance causing mortality in 50% of test organisms for a specified time interval, in this case, 48-hours for the mysid test and 96-hours for the silverside test. The commercially available statistical software package, CETIS® was used for the calculation of LC50 values using an automated decision tree adapted from EPA for selection of the appropriate statistical method (CETIS, 2009; USEPA, 1994). Point estimate procedures used to calculate LC50 values included linear regression methods, the non-parametric Spearman-Karber method and the binomial method. A qualitative comparison was made between LC50 values for the eight dispersants tested as well as with those available in the NCP Product Schedule. Note that the reproducibility of static acute tests among laboratories using the same species/toxicant combination has been reported to generally fall within a factor of 3.5 among laboratories when using nominal concentrations (unmeasured treatment concentrations) for both freshwater and marine species (USEPA, 1981). 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.

## 3. Results - Mysid Toxicity Tests

### 3.1 Mysid Testing Schedule

Following the first round of eight acute toxicity tests, dispersant LC50s were greater than the highest concentration tested for four of the eight dispersants. Definitive acute toxicity tests were repeated using higher test concentrations for JD-2000, Saf-Ron Gold, Sea Brat #4 and ZI-400.

### 3.2 Mysid Test Acceptability

Control performance (without dispersant) met all criteria for an acceptable exposure in each 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 ppm) in the Nokomis 3-AA exposure at 24 hours, which was measured at 56% of saturation. As dissolved oxygen levels were >60% at other time points in the test and the toxicity was clearly dose related, the departure observed in the 56 ppm concentration at 24 hours was not considered to have had a negative impact on the exposure with Nokomis 3-AA.

3.3 Mysid Toxicity Results

In the first series of acute toxicity tests, LC50 values and 95% confidence intervals were successfully determined for Corexit 9500A, Dispersit SPC 1000, Nokomis 3-AA and Nokomis 3-F4 and in the second series of acute tests, LC50s were calculated for JD-2000, Saf-Ron Gold, Sea Brat #4 and ZI400. Test results are summarized in Table 1.

The LC50 values for dispersant acute tests with mysids ranged from 12 ppm for Dispersit SPC1000 to 788 ppm for JD-200 (Table 1). EPA uses a five-step scale of toxicity categories to classify pesticides based on their acute toxicity to aquatic organisms: LC50 values of >100 ppm are considered practically nontoxic; >10 to 100 ppm as slightly toxic; > 1 to 10 ppm as moderately toxic; LC50s of 0.1 to 1 ppm as highly toxic and LC50s < 0.1 ppm as very highly toxic (USEPA, 2010b). Using this toxicity classification, Corexit 9500A, Dispersit SPC1000, 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 non-toxic to mysids (Table 1).

Based on comparison of LC50 values and 95% confidence intervals across the eight dispersants tested in the present study, the rank order toxicity (most to least toxic) of the dispersants to mysids was: (1) Dispersit SPC1000, (2) Nokomis 3-AA, (3) Nokomis 3-F4, Corexit 9500A, (4) ZI-400, Sea Brat #4, (5) Saf-Ron Gold, and (6) JD-2000.

Factor ratios were used to compare LC50s derived for the same species/dispersant combination from different laboratories. The factor ratios between LC50 values determined in this 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 1). As an example, using information from Table 1, the factor ratio for Corexit 9500A was determined as 42/32.2 = 1.3. 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 inter-laboratory variability (USEPA, 1981). 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 to their reported NCP LC50 values.

3.4 Mysid Reference Toxicant Test

A 48-hr acute toxicity test was conducted with the standard reference toxicant, sodium dodecyl sulfate (SDS), to evaluate the relative sensitivity of the mysids used in the series of dispersant toxicity tests. The mysids tested with SDS were from the same population and age range used for dispersant testing. The 48-hr LC50 and 95% confidence interval calculated for SDS was 23 ppm [19-26 ppm] which was consistent with the reported NCP LC50 values for SDS.

## 4. Results - *Menidia* Toxicity Tests

4.1 *Menidia* Testing Schedule

Following the first round of acute toxicity tests, dispersant LC50s were determined to be greater than the highest concentration tested for two of the eight dispersants. Definitive acute toxicity tests were repeated using higher test concentrations for Corexit 9500A and JD-2000.

4.2 *Menidia* Test Acceptability

Control performance met all criteria for an acceptable exposure in each of the eight dispersant tests conducted ($\geq$ 90%). All water quality parameters were within ranges specified in the test protocol for *Menidia beryllina*.

4.3 *Menidia* Toxicity Results

In the first series of acute tests, LC50 values and 95% confidence intervals were successfully determined for Dispersit SPC 1000, Nokomis 3-AA, Nokomis 3-F4, Saf-Ron Gold, Sea Brat #4 and ZI-400. In the second series of repeat acute tests, an LC50 was calculated for Corexit 9500A but not for the dispersant JD-2000. These data are summarized in Table 2. In the case of JD-2000, 20% mortality was observed in the highest concentration tested of 5,600 ppm, followed by no mortality observed in the next two highest exposure concentrations which indicated an LC50 > 5,500 ppm. At the highest concentration, solid material was observed at the bottom of the replicate test vessels suggesting saturation of the dispersant may have been achieved.

The LC50 values for dispersant acute toxicity tests with *Menidia* ranged from 2.9 ppm for Dispersit SPC1000 to 130 ppm for Corexit 9500A; the LC50 for JD 2000 exceeded the highest test concentration of 5,600 ppm. Using the EPA toxicity classification, Dispersit SPC1000 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 non-toxic to inland silversides.

Based on comparison of LC50 values and 95% confidence intervals, the rank order toxicity (most to least toxic) of the dispersants to *Menidia* were: (1) Disersit SPC1000, (2) Nokomis 3-F4, Nokomis 3-AA, ZI-400, (3) Saf-Ron Gold, (4) Sea Brat #4, (5) Corexit 9500A, and (6) JD-2000.

The factor ratios calculated for Dispersit SPC1000, 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 inter-laboratory variability. The factor ratios of 5.2 and 13.8 for Corexit 9500A and JD-2000 indicate that the LC50 values reported for Corexit 9500A and JD-200 in the NCP Product Schedules would be considered different (i.e., lower) from the LC50 values 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 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.

4.4 *Menidia* Reference Toxicant Test

A 96-hr acute toxicity test was conducted with the reference toxicant SDS to evaluate the relative sensitivity of the *Menidia* used in the series of dispersant toxicity tests. The *Menidia* tested with SDS were from the same population and age range used for dispersant testing. The

96-hr LC50 and 95% confidence interval calculated for SDS was 9.5 ppm [8.7-10 ppm] which was consistent with the reported NCP LC50 values for SDS. It should be noted that during the last 24 hours of the test, the temperature dropped to 22$^{o}$C, which was 2 degrees below the acceptable criteria and thus invalidated the test. However, there was no difference in mortality counts between the 72-hour and the 96-hour observations suggesting the temperature change had no negative impact on the test or the final calculated LC50.

**5.0 Conclusions**

The present study provided an independent, quantitative assessment of acute toxicities of eight dispersants to two aquatic species inhabiting Gulf of Mexico waters. Toxicity was determined as the LC50 derived from standard short term acute tests using standard test species, specifically the Gulf mysid, *Americamysis bahia*, and the inland silverside, *Menidia beryllina*. In general, the toxicity values (i.e., LC50s) for mysids ranged over nearly two orders of magnitude and for *Menidia* over three orders of magnitude. Given the expected range of inter-laboratory variability, the results of the present study were consistent with test results reported in the NCP Product Schedule, with the exception of two dispersants for each test species which yielded higher LC50s (i.e., lower toxicity) than reported in the NCP. The rank order toxicity of the eight dispersants was generally similar to the information provided in the NCP Product Schedule. For both test species, Dispersit SPC1000 was the most toxic and JD-2000 the least toxic. The other six dispersants varied in relative toxicity to mysids and *Menidia*, with LC50 values ranging from 20 to 130 ppm. Overall, the dispersants were classified as being slightly toxic to practically non-toxic to both test species, with the exception that Dispersit SPC1000 would be considered moderately toxic to *Menidia*. Corexit 9500A, the dispersant currently applied offshore at the surface and underwater, falls into the slightly toxic category for mysids and the practically non toxic category for *Menidia*.

Short-term acute toxicity tests using consistent methodologies and test organisms provide important and fundamental information on oil spill dispersants and other toxicants. The next phase of testing will examine the acute toxicity of Louisiana sweet crude oil and dispersant-sweet crude oil mixtures on mysids and *Menidia*. The comparative toxicity analysis of dispersants, sweet crude oil and dispersant-sweet crude oil mixtures on standard aquatic test species will provide improved understanding of potential toxicological effects associated with this oil spill.

## 6.0 References

CETIS. 2009. Comprehensive Environmental Toxicity Information System: Users Manual. Tidepool Scientific Software, McKinleyville, CA.

USEPA. 40 CFR, Part 160. Federal Insecticide, Fungicide, and Rodenticide Act. Good Laboratory Practices Standards; Final Rule. Office of the Federal Register, National Archives and Records Administration. U.S. Government Printing Office, Washington, D.C.

USEPA. 1981, Results: Interlaboratory Comparison- Acute Toxicity Tests Using Estuarine Animals. February 1981. EPA-600/4-81-003. U.S. Environmental Protection Agency, Gulf Breeze, Florida.

USEPA. 1994. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine organisms, 2nd ed. Environmental Monitoring Systems Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH. EPA/600/4-51/003.

USEPA. 1997. Swirling Flask Dispersant Effectiveness Test, Revised Standard Dispersant Toxicity Test, and Bioremediation Agent Effectiveness Test. Part 300, Appendix C. EPA 62 FR 15576. U.S. Environmental Protection Agency, Washington, D.C. April 1, 1997.

USEPA. 2002. Methods for Measuring the Acute Toxicity of Effluent and Receiving Waters to Freshwater and Marine Organisms. 5th Edition, October 2002. EPA-821-R-02-012. U.S. Environmental Protection Agency, Washington, D.C.

USEPA. 2010a. http://www.epa.gov/emergencies/content/ncp/product_schedule.htm

USEPA. 2010b. http://www.epa.gov/oppefed1/ecorisk_ders/toera_analysis_eco.htm#Ecotox. Accessed June 23, 2010.

Table 1. Results of mysid 48-hr static acute toxicity tests with eight dispersants. LC50 values (ppm), 95% confidence intervals [in brackets] and the toxicity classification of dispersant LC50s derived in the present study. NCP Product Schedule listing of dispersant LC50s and 95% confidence intervals [in brackets] shown in right column for comparison.

| **Dispersant** | **This Study LC50 (ppm) [95% CI]** | **Toxicity Category[1]** | **NCP Product Schedule LC50 (ppm) [95% CI][d]** |
|---|---|---|---|
| Dispersit SPC 1000 | 12 [10-14][a] | Slightly Toxic | 16.6 [14.1-19.6] |
| Nokomis 3-AA | 30 [27-34][b] | Slightly Toxic | 20.2 [17.4-22.8] |
| Corexit 9500A | 42 [38-47][c] | Slightly Toxic | 32.2 [26.5-39.2] |
| Nokomis 3-F4 | 42 [38-47][c] | Slightly Toxic | 32.2 [28.4-36.5] |
| ZI -400 | 55 [50-61][b] | Slightly Toxic | 21.0 [17.9-24.5] |
| Sea Brat #4 | 65 [57-74][a] | Slightly Toxic | 14.0 [±10.4] |
| Saf-Ron Gold | 118 [104-133][b] | Practically Non-Toxic | 63.0[e] [52.9-75.1] |
| JD-2000 | 788 [627-946][a] | Practically Non-Toxic | 90.5[e] [76.1-108] |

[1]Toxicity classification per USEPA 2010 applied to results of present study
[a]Estimated by linear regression method
[b]Estimated by Spearman-Karber method
[c]Estimated by binomial method
[d]Values as reported in NCP Product Schedule documentation by manufacturer
[e]Classified as slightly toxic according to values provided in NCP Product Schedule

Table 2. Results of *Menidia* 96-hr static acute toxicity tests with eight dispersants. LC50 values (ppm), 95% confidence intervals [in brackets] and the toxicity classification of dispersant LC50s derived in the present study. NCP Product Schedule listing of dispersant LC50s and 95% confidence intervals [in brackets] shown in right column for comparison.

| **Dispersant** | **This Study LC50 (ppm) [95% CI]** | **Toxicity Category[1]** | **NCP Product Schedule LC50 (ppm) [95% CI][d]** |
|---|---|---|---|
| Dispersit SPC 1000 | 2.9 [2.5-3.2][b] | Moderately Toxic | 3.5 [3.1-4.0] |
| Nokomis 3-F4 | 19 [16-21][b] | Slightly Toxic | 29.8 [24.0-35.4] |
| Nokomis 3-AA | 19 [17-21][b] | Slightly Toxic | 34.2 [29.2-37.95] |
| ZI -400 | 21 [18-23][b] | Slightly Toxic | 31.8 [28.7-35.1] |
| Saf-Ron Gold | 44 [41-47][b] | Slightly Toxic | 29.4 [25.2-34.3] |
| Sea Brat #4 | 55 [49-62][b] | Slightly Toxic | 30.0 [±16.2] |
| Corexit 9500A | 130 [122-138][b] | Practically Non-Toxic | 25.2[e] [13.6-46.6] |
| JD-2000 | >5,600 | Practically Non-Toxic | 407 [330-501] |

[1]Toxicity classification per USEPA 2010 applied to results of present study
[b]Estimated by Spearman-Karber method
[d]Values as reported in NCP Product Schedule documentation by manufacturer
[e]Classified as slightly toxic according to values provided in NCP Product Schedule

**Appendix A**

| **Test parameter** | **Specified in SubPart J Appendix C (USEPA 1997)** | **Method used in present study, and specified in USEPA 2002** |
|---|---|---|
| Photoperiod and light intensity | *24 hr light<br>*higher intensity light | *16 hr light/8 hr dark<br>*Moderate intensity light |
| 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 |
| Mysid age | *5-7 day old larvae | *1 to 6 day old; all within 24 hr same age |
| Toxicant stock solution preparation for mysid test | *Blender 10,000 rpm<br>*gas tight syringes | *Top stirring at 70% vortex<br>*graduated glass pipettes |
| Mysid test solution mixing | *no specification | *short term gentle mixing following stock addition |
| Mysid additions to test chambers | *no specification | *impartial, two at a time |
| *Menidia* age | *7 day old larvae | *9-14 day old, all within 24 hr same age |
| *Menidia* test solution mixing | *test jars on shaker platform | *same procedure as for mysids |
| Dilution Water | *Natural Seawater Prefered | *Salinity adjusted, 20 µm filtered natural seawater |