USE OF BRINE SHRIMP (*ARTEMIA*) IN DISPERSANT TOXICITY TESTS: SOME CAVEATS[1]

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ABSTRACT: *Several Latin American countries currently use* Artemia *to evaluate the aquatic toxicity of dispersants. Test methods used to evaluate dispersant toxicity to* Artemia *are not uniform. The study reported here demonstrates how varying* Artemia *test conditions can significantly affect toxicity results for the dispersant Corexit® 9500.*

The type of seawater used in Artemia *toxicity tests affects 48 hour LC_{50} values for Corexit 9500 (lethal concentration for 50% of test organisms). Nominal LC_{50} values ranged from 35 to 147 ppm when natural seawater was used. Nominal LC_{50} values ranged from 29 to 39 ppm when a synthetic seawater prepared from Crystal Sea® Marinemix was used. Greater toxicity was observed when synthetic (reconstituted) seawater was prepared according to the U. S. Environmental Protection Agency (USEPA, 1987)* Artemia *dispersant test guideline. Observed nominal LC_{50} values ranged from 8.4 to 14 ppm.*

Age of the Artemia *nauplii is another test variable that can significantly affect toxicity results. The 48 hour nauplii showed greater toxicity to Corexit 9500 than 24 hour old nauplii. In tests using two types of synthetic seawater (Coral Reef Red Sea Salt® and Crystal Sea® Marinemix at 20 °C, 20 ppt salinity), nominal LC_{50} values ranged from 29 to 68 ppm for 24 hour old nauplii; 48 hour old nauplii had LC_{50} values ranging from 9 to 27 ppm. Greater toxicity was also observed in 48 hour nauplii under different salinity and temperature (Red Sea, 25 °C, 33 to 35 ppt salinity). The LC_{50} values were 33 and 1.6 ppm for 24 and 48 hour nauplii respectively.*

Introduction

Several Latin American countries (Argentina, Brazil and Venezuela) use *Artemia franciscana* (*salina*) to evaluate the aquatic toxicity of dispersants. Test methods used to evaluate dispersant toxicity to *Artemia* are not uniform and lead to markedly different toxicity outcomes. Toxicity results can impact regulatory approval of a dispersant if aquatic toxicity limits are set for dispersants. We conducted laboratory studies in New Jersey (E tests, Tables 3 to 5) and Sao Paulo (T tests, Tables 3 to 5) to evaluate the toxicity of the dispersant Corexit® 9500 to *Artemia.* The studies reported here demonstrate how varying *Artemia* test conditions can significantly affect toxicity results for Corexit® 9500.

Materials and methods

Seawater sources and test organisms. Natural seawater was collected from Manasquan Inlet, New Jersey. Reconstituted seawater was prepared according to USEPA (1987). Two types of artificial seawater were prepared from commercial products, Crystal Sea® Marinemix (Forty Fathoms®) and Coral Reef Red Sea Salt®. Salinities ranged from approximately 20 to 35‰ for cyst hydration and laboratory tests.

The San Francisco Bay *Artemia franciscana* (*salina*) strain (Argent Chemical Laboratories, Redmond, Washington) was used in the ExxonMobil tests. The Macau (Brazil) *Artemia franciscana* (*salina*) strain was used in the Sao Paulo tests. San Francisco Bay cysts were hatched in natural seawater. Macau cysts were hatched in artificial seawater made from Crystal Sea® Marinemix. Temperatures during hatching were approximately 20°C (ExxonMobil tests) and 25 to 28 °C (TECAM tests). The cyst density was approximately 0.6 to 2.0 grams of cysts per Liter of seawater. Hatching was accomplished using separatory funnels, aerated to keep the cysts in continuous suspension. The nauplii (larval stages) were approximately 24 to 48 hours old at study initiation.

Toxicity test solutions and procedures. Test solutions were prepared according to USEPA (1987). Stock solutions (1000 ppm) of Corexit® 9500 were prepared by adding 0.55 mL of dispersant to 550 mL of dilution water and mixed at less than 10,000 rpm for 5 seconds. Individual treatments were then prepared by adding the appropriate amount of stock solution to the dilution water. Reference toxicant studies were performed concurrently with the dispersant studies. Sodium dodecyl sulfate (SDS) was used as a reference toxicant.

Table 1. Water Quality Parameters for ExxonMobil Tests.

Dilution Medium (Seawater Type)	Temperature °C	Salinity ‰	D.O. ppm	pH
EPA Guideline, Reconstituted Seawater	19.0-20.4	20.0-21.0	7.5-7.7	7.9-8.8
EPA Guideline, Reconstituted Seawater (Aged)	20.5-20.9	20.0	7.6-7.7	8.2
Crystal Sea® Marinemix	20.8-20.9	20.0	7.2-7.7	8.3-8.4
Natural Seawater	20.1-20.9	21.0	7.3-7.7	8.0-8.6

Table 2. Water Quality Parameters for TECAM Tests.

Dilution Medium (Seawater Type)	Temperature °C	Salinity ‰	D.O. ppm	pH
Crystal Sea® Marinemix	19.7-20.3	20.0-22.0	5.2-6.1	7.9-8.0
Coral Reef Red Sea Salt®	19.7-20.3	20.0-21.0	4.3-6.5	7.8-8.0
Coral Reef Red Sea Salt®	22.0-25.0	25.0	4.2-6.1	7.8-8.3
Coral Reef Red Sea Salt®	24.7-25.5	33.0-35.0	4.1-5.9	8.0-8.2

Table 3. SDS Acute Toxicity Data.

Date	Lab[1]	Seawater Type	LC50 (ppm)[2] (Confidence interval)
24 Hour Old *Artemia*			
1/23/01	E	EPA Guideline, Reconstituted Seawater	**37** (CNC)
1/23/01	E	Natural Seawater	**28** (CNC)
2/21/01	E	EPA Guideline, Reconstituted Seawater (Aged)	**27 (23-32)**
2/21/01	E	Natural Seawater	**26** (CNC)
2/28/01	E	Crystal Sea® Marinemix	**46** (CNC)
2/28/01		Natural Seawater	**19 (16-21)**
4/25/01	T	Crystal Sea® Marinemix	15 (13-16)
4/25/01	T	Coral Reef Red Sea Salt®	14 (2.9-38)
1/16/01	E	Natural Seawater	**43** (CNC)
48 Hour Old *Artemia*			
4/25/01	T	Crystal Sea® Marinemix	5.1 (4.6-5.8)
4/25/01	T	Coral Reef Red Sea Salt®	7.7 (7.0-8.6)

[1]E = ExxonMobil Biomedical Sciences, Inc., T = TECAM Tecnologia Ambiental Ltda
[2]Bold values = analytical results; other values are nominal; CNC = Could Not Calculate

Table 4. Corexit® 9500 Acute Toxicity Data for 24 Hour old Artemia.

Date	LAB[1]	Seawater Type temperature/salinity	LC50 (ppm)[2] (Confidence interval)
1/23/01	E	EPA Guideline, Reconstituted Seawater 20°C/20‰	9.4 (3.5-19) **4.1 (0.1-24)**
2/21/01	E	EPA Guideline, Reconstituted Seawater 20°C/20‰	14 (CNC) **9.3 (CNC)**
2/21/01	E	EPA Guideline, Reconstituted Seawater (aged) 20°C/20‰	8.4 (CNC) **4.3 (CNC)**
5/23/00	E	Crystal Sea® Marinemix 20°C/20‰	39 (33-48)
2/28/01	E	Crystal Sea® Marinemix 20°C/20‰	29 (17-59) **21 (8.2-95)**
5/2/01	E	Crystal Sea® Marinemix 20°C/20‰	29 (CNC)
5/7/01	T	Coral Reef Red Sea Salt® 25°C/33‰	33 (29-38)
4/25/01	T	Coral Reef Red Sea Salt® 20°C/20‰	68 (CNC)
5/23/00	E	Natural Seawater 20°C/20‰	147 (CNC)
1/16/01	E	Natural Seawater 20°C/20‰	35 (22-86) **24 (13-117)**

[1]E = ExxonMobil Biomedical Sciences, Inc., T = TECAM Tecnologia Ambiental Ltda
[2]Bold values = analytical results; other values are nominal; CNC = Could Not Calculate

Table 5. Corexit® 9500 Acute Toxicity Data for 48 Hour old Artemia.

Date	LAB[1]	Seawater Type temperature/salinity	LC50 (ppm)[2] (Confidence interval)
5/2/01	E	Crystal Sea® Marinemix 20°C/20‰	13 (CNC)
4/25/01	T	Crystal Sea® Marinemix 20°C/20‰	9.1 (0.2-23)
12/6/00	T	Coral Reef Red Sea Salt® 25°C/35‰	1.6 (1.2-2.3)
1/24/01	T	Coral Reef Red Sea Salt® 25°C/25‰	2.1 (1.4-3.2)
1/24/01	T	Coral Reef Red Sea Salt® 22°C/25‰	3.8 (2.7-5.3)
4/25/01	T	Coral Reef Red Sea Salt® 20°C/20‰	27 (2.7-227)

[1] E = ExxonMobil Biomedical Sciences, Inc., T = TECAM Tecnologia Ambiental Ltda
[2]CNC = Could Not Calculate

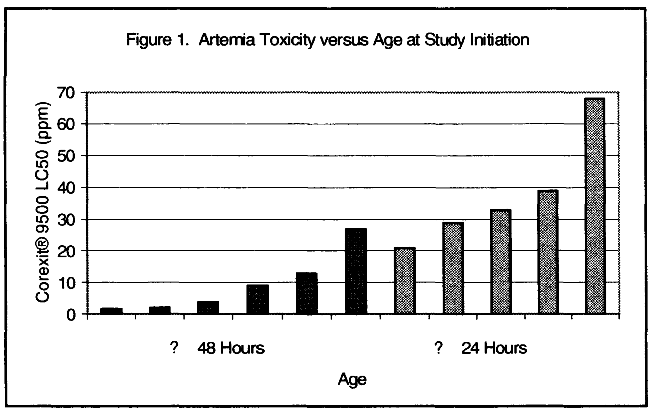

FIGURE 1 *Artemia* Toxicity versus Age at Study Initiation. Figure 1 shows that the age of *Artemia* at test initiation (x-axis) has a major influence on the toxicity results (LC_{50} values) for Corexit® 9500 (y-axis). *Artemia* nauplii that are 24 hours old generally show lower toxicity (greater (LC_{50} values) than 48 hour old *Artemia* nauplii.

The test procedures generally followed those provided in USEPA (1987). Exposures were conducted in 80 x 40 mm glass crystallizing dishes containing 100 mL of solution. Five concentrations and a test water control were used for each study. Five replicates (ExxonMobil tests) or three replicates (TECAM tests) containing twenty nauplii each were used per concentration. Mortality and abnormal behavior were monitored at 24 and 48 hours.

Water quality. Water quality values are shown in Tables 1 and 2. Dissolved oxygen (D.O.), pH, and temperature were measured in each concentration at initiation and in a composite of the replicates of each concentration at termination. Dissolved oxygen was determined with a YSI probe and meter (Yellow Springs Instrument Company, Ohio, USA) or an Orion model 820 meter (Orion Research, Inc., Boston, Massachusetts, USA). The pH was measured with model 520A or 230A Orion meters. Temperatures were measured with a glass mercury thermometer. Salinity was measured with a refractometer.

Sample analysis. In some tests, water samples were analyzed for an anionic surfactant component of Corexit® 9500 or the SDS reference toxicant, as appropriate using a Dionex 4500i Ion Chromatograph system. Water samples were acidified and extracted/concentrated using C18 solid phased extraction cartridges. The anionic analytes were eluted with methanol. The extracts were analyzed by ion chromatography with suppressed conductivity detection.

Data analysis. For each test, LC_{50} values and associated confidence intervals were determined with either a Binomial Method (Stephan, 1977), trimmed Spearman-Karber (Hamilton et al., 1977) or a Probit procedure of SAS (Finney, 1971, SAS Institute Inc., 1989). The LC_{50} values were based on the nominal Corexit® 9500 and SDS concentrations. In some tests, analytical concentrations of Corexit 9500 and SDS were determined and measured LC_{50} values are also included.

Results

Table 3 provides aquatic toxicity results for SDS. Tables 4 and 5 provide aquatic toxicity results for Corexit 9500. The data show that seawater type and nauplii age at test initiation have a major influence on toxicity results. The guideline (USEPA, 1987) reconstituted seawater produced consistently lower LC_{50} values for Corexit 9500 but the reference toxicant (SDS) results were not correspondingly lower. The results of the SDS reference toxicant studies showed that the age of the organisms had the greatest influence on their sensitivity. Figure 1 shows the greater toxicity (lower LC_{50} values) observed in 48 hour nauplii compared to 24 hour nauplii.

Discussion

Artemia is a convenient and inexpensive laboratory test organism since it can be readily obtained by hatching the dried cysts in seawater. For many years, *Artemia* was the standard invertebrate test organism for dispersants in the United States (Tarzwell, 1969; McCarthy, et al. 1973). The U. S. Environmental Protection Agency later revised the dispersant toxicity test protocols, replacing *Artemia* with the mysid, *Mysidopsis bahia* (Federal Register, 1994). In Brazil, toxicity tests with *Artemia* and another mysid species *M. juniae* are required for dispersant registration (IBAMA 2000, 2001)

Artemia tests can result in highly variable toxicity results for a given test material. Numerous test parameters such as geographic origin of the cyst, hatching conditions, and nauplii age must be carefully controlled in order to generate reliable and reproducible results (Vanhaecke et al., 1981). George-Ares and Clark (2000) provide further discussions on testing conditions that can modify aquatic toxicity results for dispersants.

Sorgeloos et al. (1978) reported that *Artemia* strains from different geographic origins have different sensitivities to chemicals. In addition, *Artemia* larval stages can vary significantly in their response to chemicals (Sorgeloos et al., 1978). Our results show that nauplii age can significantly affect toxicity results for Corexit® 9500. In tests using both *Artemia* strains, the 48 hour nauplii showed greater toxicity to Corexit 9500 than did 24 hour old nauplii.

Our data indicate that the type of seawater used in *Artemia* tests (24 hour nauplii) modifies the toxicity results for Corexit 9500. Nominal LC_{50} values ranged from 35 to 147 ppm when natural seawater was used. Nominal LC_{50} values ranged from 29 to 39 ppm when Crystal Sea® Marinemix was used. Comparisons were made between Crystal Sea® Marinemix and Coral Reef Red Sea Salt® under similar conditions of nauplii age, temperature (20 C°), and salinity (20 ppt.). Since two *Artemia* strains were tested, the data are insufficient to make conclusions regarding relative toxicity for tests run in Crystal Sea® Marinemix compared to Coral Reef Red Sea Salt®. Greatest toxicity was observed for 24 hour nauplii when synthetic (reconstituted) seawater was used (USEPA, 1987). Observed nominal LC_{50} values ranged from 8.4 to 14 ppm. Reconstituted seawater was not used in tests conducted with 48 hour old *Artemia*.

Depending on the test material, seawater type has also been shown to affect toxicity results in the sea urchin fertilization assay (Neiheisel and Young, 1992; Jonczyk, et al. 2001). For example, the mean EC_{50} value (concentration causing a specific effect in 50% of test organisms) for SDS in sea urchin tests was significantly higher (less toxic) in the artificial seawater HW Marinemix® than in the Forty Fathoms® and Instant Ocean® artificial seawaters (Neiheisel and Young, 1992). In another sea urchin fertilization study, EC_{50} values for copper sulfate in Instant Ocean® were significantly higher than those for copper sulfate in natural seawater (Jonczyk, et al. 2001).

Conclusions

Varying *Artemia* test conditions can significantly affect toxicity results for the dispersant Corexit® 9500. Toxicity results in turn can impact regulatory approval of a dispersant if aquatic toxicity limits are set for dispersants.

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Biography

Dr. George-Ares is program leader for oil spill research at ExxonMobil Biomedical Sciences, Inc. She is senior author of several peer-reviewed papers on dispersants and the ExxonMobil Dispersant Guidelines. She serves as an officer for the American Society for Testing and Materials Committee on Hazardous Substances and Oil Spill Response.

[1] Opinions expressed in this paper are solely those of the authors and do not necessarily represent the views of other organizations.

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