

Bioassay Test Report
96 hour Acute Static LC50 Test
SuperAll #38®
Menidia beryllina

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Prepared for:
SuperAll Products, L.L.P
P. O. Box 2954
Spring, TX 77383

Prepared by:
South Central laboratories
P. O. Box 390
Morgan City, LA 70381
(303) 385-1855

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1.0 Sample Information

PRODUCT.....SuperAll #38
TEST OIL.....High Aromatics No. 2 Fuel Oil
CONTROL NUMBER.....1290

2.0 Test Information

TYPE OF TESTS.....96 Hour Acute Static LC50
PROCEDURE FOLLOWED.....59 FR No. 178
TEST ORGANISMS.....*Menidia beryllina*
DATE AND TIME INITIATED.....03/15/96 at 17:05
DATE AND TIME COMPLETED.....03/19/96 at 17:00

3.0 Test Summary

This toxicity test consisted of 3 test systems. *Menidia beryllina* larvae, 7 days old, were exposed to SuperAll #38 diluted with laboratory prepared seawater. Additionally, *M. beryllina* larvae were exposed to a high aromatics No. 2 fuel oil and No. 2 fuel mixed with SuperAll #38 at a ratio of 10:1. Each test system consisted of one test species, five test concentrations and one control solution. Each concentration was prepared in duplicate and each test chamber was populated with ten test organisms. Mortality was the end point of interest and was recorded daily in each replicate of each test concentration.

4.0 Materials and Methods

The procedures followed in conducting this test were those stated in 59 Federal Register pages 47461 thru 47464. The materials used in the course of this test conform to the specifications as stated in the reference cited above. All glassware and other apparatus that comes in contact with test material was washed in accordance with the reference cited above.

4.1 Test Chambers

The test chambers used for this test were 1200 ml Pyrex® beakers and were filled with 1000 ml of test solution. All of the test chambers were covered with Plexiglas®. Since dissolved oxygen remained above 60% of saturation during the course of the tests, aeration of the test chambers was not necessary.

4.2 Control Media

Reconstituted laboratory prepared seawater (prepared following procedure outlines in 58 FR page 47462) was used as a performance control and diluent in this test.

4.3 Organism Acquisition

Menidia beryllina larvae, 7 days old, were purchased from a commercial bioassay organism supplier. The sensitivity of the test organisms was documented by performing a Standard Reference Toxicant test. The toxicant used in this test was Docechyl sodium sulfate. The SRT test was conducted simultaneously on the same lot of *M. beryllina* larvae. The results of the SRT test are a LC50 of 6.75 mg/L. These are expected results and suggest that the silverside minnow larvae used in these biomonitoring tests were in good health prior to the test.

5.0 Experimental Conditions

The test solutions prepared for these tests were warmed to 25+1oC. The test solution temperature in the environmental chamber was continuously monitored and recorded. The light/dark cycle in the environmental chamber was maintained at 14 hours light and 10 hours dark for the duration of the test. The dissolved oxygen concentrations in the test solutions prepared for each test were high enough that aeration was not required.

6.0 Experimental Procedure

Two replicates of each a control and five SuperAll concentrations, No. 2 fuel oil and a 10:1 mixture of No. 2 fuel oil and SuperAll #38 were prepared in 2500 ml glass jars and their dissolved oxygen and pH were measured. The five test concentrations were 1000 ppm, 500 ppm, 100 ppm, 50 ppm, and 10 ppm.

After the test solutions were prepared and water quality measurements made, the six jars were placed on an Erbach shaker (Model No.) and shaken on the high setting for five minutes.

Each test beaker was filled with 1000 ml of test solution and randomly placed in the environmental chamber. Seven day old *Menidia beryllina* larvae were randomly distributed in groups of ten to the test chambers. Survival by the test organisms was recorded after 24 hours. Dissolved oxygen and pH were measured daily in the control and each test concentration. After 96 hours of exposure, the number of surviving test organisms in each replicate test chamber of the test was recorded and this test was terminated

7.0 Results

Raw biological data appears in Appendix A. The LC50 for SuperAll #38, the lethal concentration to 50% of the organisms, is 913.56 ppm with an upper 95%

concentration value of 1299.0 ppm and a lower 95% confidence value of 642.5 ppm. The LC50 was calculated using the Trimmed Spearman-Kärber method. Mortality was not monotonically increasing, therefore, the data was smoothed using Abbotts Equation prior to analysis by Spearman-Kärber.

The LC50 of both the No. 2 fuel oil and fuel oil/SuperAll mixture is less than 10 ppm. Excessive mortality in the upper concentrations in both test systems made formal statistical analysis impossible.

8.0 References

1. Standard Methods for the Examination of Water and Wastewater, 17th edition.
2. Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms, fourth edition. U. S. EPA, Environmental Monitoring and Support Laboratory Cincinnati, OH 45268. EPA/600/4-90/027.
3. Federal Register, Volume 59, No. 178, pages 47461-47464.
4. Hamilton, M A., R. C. Russo, and R. V. Thurston, 1977. "Trimmed Spearman-Kärber Method for Estimating Median Lethal Concentrations in Toxicity Bioassays". Environ Sci. Technol. 11(7): 714-719; Correction 12(4): 417 (1978).

9.0 Laboratory Certification

The data presented in this report were derived by the methods described in the "Materials and Methods" section of this report.

Data collected during this experiment was transcribed in SCL Laboratory Notebook No. BS003 and was checked for accuracy by two persons.

South Central Laboratories, Inc.
Blair H. Spencer
Laboratory Director