



Drilling Fluids Toxicity Test Report

prepared for
ProOne, Inc.

Client Contact: Tim Wagner

Lab-prepared Sample: EE USA Lab #: **M-270-12**
3% Bio-Stable Downhole Drilling Fluid in Generic #7
Additive Received April 26, 2012.

Mysidopsis bahia Acute, Static 96-hour Toxicity Test.
US EPA, March 1993, FR 58(41): 12507-12512.

This test was initiated at 1138 on May 4, 2012.
96-Hour LC50: 464,800 ppm SPP. **PASS.**
95% Confidence Interval: 437,200 – 494,100 ppm SPP.


Report Date: May 9, 2012

by

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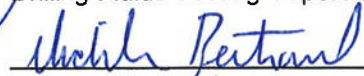
This report contains three pages plus five appendices, A - F. This report must not be reproduced in part, only in whole. The results and conclusions presented in this report apply only to the sample(s) tested. All results included in this report are from a valid test.



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Drilling Fluids Testing Supervisor

05-09-12

DATE



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05-10-12

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TEST OVERVIEW

A 96-hour static definitive toxicity test (DT) was conducted by Environmental Enterprises USA, Inc. (EE USA) using an additive sample supplied by ProOne, Inc. (Appendix E). Testing was conducted with the suspended particulate phase (SPP) of a Generic No. 7 drilling fluid sample prepared at EE USA with 3% Bio-Stable Downhole Drilling Fluid. Test organisms were cultured at EE USA and 6-days old when this test was initiated. This document presents methods, materials, and results of this testing. The DT was conducted from May 4 - 8, 2012 at the laboratory of EE USA.

MATERIALS AND METHODS

Mysids were cultured and maintained in synthetic seawater prepared with hw-MARINEMIX + Bio-Elements and Crystal Sea Marinemix Bioassay Laboratory Formula sea salts (80:20) and deionized water and adjusted to 23 +/- 2 parts per thousand (ppt) salinity and 23 +/- 2°C. Mysids were acclimated to a salinity of 20 +/- 1 ppt and a temperature of 20 +/- 1°C prior to test initiation. Synthetic seawater used as dilution water was maintained at 20 +/- 1 ppt salinity and 20 +/- 1°C. Test organisms were fed 125 – 250 µl of a standardized suspension of less than 24-hour old *Artemia* nauplii twice daily by replicate. The standard suspension is equal to 0.05 grams wet weight strained *Artemia* per ml synthetic seawater.

Standard reference toxicant (SRT) testing in synthetic seawater yielded a 96-hour LC50 of 728 ppm with a 95% confidence interval of 711 to 745 ppm as determined by Trimmed Spearman-Kärber analysis. Potassium Chloride, 99.0-100.5% purity, Sigma Chemical, Lot #021M0113V, was used as the SRT. The SRT test was initiated April 30, 2012. Appendix D contains a *M. bahia* SRT control chart.

The additive sample was received at EE USA on April 26, 2012. The Generic No. 7 drilling fluid was prepared at EE USA on April 25, 2012. Generic No. 7 drilling fluid was spiked with 3% Bio-Stable Downhole Drilling Fluid and hot-rolled at 150°F for 16 hours prior to SPP preparation (Appendix F). SPP was prepared as described by EPA. The sample was mixed for 15 minutes with a high-shear mixer and then the mud/seawater slurry (1 part homogenized mud: 9 parts synthetic seawater) was prepared. Mud/seawater slurries with WBM are prepared in single mixing containers of various sizes. While mixing, the pH of the slurry was measured and adjusted to +/- 0.2 su of the synthetic seawater with 6N HCl acid. After settling for one hour, the SPP was carefully poured into a clean container. WBM SPPs include everything except settled solids – sand, bit grit, etc. and are usually very turbid with relatively high total suspended and total dissolved solids. The temperature, dissolved oxygen (DO), salinity, and pH of the SPP were measured and recorded. If necessary, the pH of the SPP was adjusted to 7.8 +/- 0.1 su with 6N HCl acid. If the DO of the SPP was less than 65% of saturation or 4.9 ppm, it was aerated until it was at least 4.9 ppm or for a maximum of five minutes.

A 96-hour range-finding test (RFT) with neat Generic No. 7 drilling fluid (M-269-12) indicated that the LC50 of the base mud used in these tests was greater than 1,000,000 ppm SPP (Appendix A). A range-finding test with Generic No. 7 drilling fluid (200 ml total volume) spiked with 3% Bio-Stable Downhole Drilling Fluid was completed prior to the DT (Appendix B). The RFT results indicated that the 96-hour LC50 should be between 500,000 and 1,000,000 ppm SPP. For the DT, Generic No. 7 drilling fluid (600 ml total volume) was spiked with 3% Bio-Stable Downhole Drilling Fluid and set with six treatments, five SPP concentrations (13, 22, 36, 60, and 100%) and a negative control. Each treatment included three replicates of 20 mysids each and surviving mysids were counted and recorded daily in each replicate of the control. Surviving mysids were counted daily in the SPP treatments unless they were obscured by high turbidity. After 96 hours, each replicate of each treatment was terminated and the final number of surviving organisms recorded. This test was aerated. At 24-hour intervals, temperature & pH, DO, and salinity were measured in each treatment (Appendix C). Each meter was calibrated prior to use. Daily calibration data are documented in EE USA's pH, DO & Conductivity Calibration and Verification Logs.

RESULTS

The 96-hour exposure to 13, 22, 36, 60, and 100% SPP treatments resulted in an LC50 of 464,800 ppm SPP with a 95% confidence interval of 437,200 to 494,100 ppm SPP as determined by Probit analysis. Concurrent 96-hour control survival was 100% (Appendix C).

REFERENCES

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