LC50 BIOASSAY REPORT
ON
ECO-FLASH

FOR

ALL PETROL S.A.
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ATTENTION
Rafael Ferreyra

BY

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M13-5550

APPROVED: Bob Lamm
DATE: 4/19/13

DRILLING FLUID TOXICITY DATA SHEET
SUSPENDED PARTICULATE PHASE

DEFINITIVE 96 HOUR BIOASSAY
SAMPLE ID NUMBER: 130115-07

TEST CONDUCTED BY:

MUDTECH, INC.
5310 MILWEE
HOUSTON, TEXAS 77092

MUD TYPE: Seawater **SUPPLIER:** ALL PETROL S.A.

MUD COMPOSITION:

ADDITIVE	CONCENTRATION
ECOFLASH	100 ppm in Seawater

TOXICITY RESULTS:

Method Used: Probit Analysis

LC50: 85,790 ppm
95% Confidence Limits: 39,540 ppm to 181,250 ppm

TOXICITY DATA:

TEST CONCENTRATION (ppm SPP)	NUMBER EXPOSED	NUMBER DEAD	ADJUSTED RESPONSE
Seawater Control	60	0	0
10,000	60	0	0
30,000	60	9	9
300,000	60	26	26
500,000	60	60	60
1,000,000	60	60	60

STANDARD REFERENCE TOXICANT: Sodium Dodecyl Sulfate

LC50: 9.89 ppm
95% Limites de la Confianza: 7.70 ppm to 12.34 ppm

The toxicity test was conducted according to EPA, "Drilling Fluids Toxicity Test", Federal Register, Vol. 58, No. 41, March, 1993.

DATE TEST WAS RUN: April 11, 2013 to April 15, 2013

CONCLUSION: 100 ppm ECOFLASH in Seawater met the Minimum EPA Requirement of 30,000 ppm.

Bob Sidner
Lab Manager

INTRODUCTION:

Mudtech Laboratories, Inc was asked to conduct a 96 hour Definitive LC50 Bioassay on **ECOFLASH**. This report presents the test procedures and the results of the bioassay.

The bioassay was conducted at Mudtech Laboratories, Inc, Houston, Texas on April 11, 2013 to April 15, 2013.

All testing was conducted using the guidelines in the protocol specified by the U.S. Environmental Protection Agency.

MATERIALS AND METHODS

SAMPLE DELIVERY:

The sample of **ECOFLASH** to be tested was delivered to Mudtech on January 15, 2013.

Testing was conducted using the guidelines specified by the U.S. Environmental Protection .

TEST PREPARATION:

On April 11, 2013, the **ECOFLASH** sample was prepared for testing using the procedures described in the Drilling Fluid's Toxicity Test, published in the Federal Register.

All glassware used in the test was cleaned according to the methods detailed in the procedure.

A Suspended Particulate Phase (SPP) was prepared. A 1:9 ratio by volume of a **100 ppm ECOFLASH in Seawater mixture** and 20 parts per thousand (ppt) seawater was prepared by stirring one part of **100 ppm ECOFLASH in Seawater mixture** with nine (9) parts of filtered seawater in a clean linear polyethylene bucket for five (5) minutes. The pH of this suspension was adjusted to 8.0 using 6N hydrochloric acid and allowed to settle for one hour. After the settling period, the pH was rechecked to insure it had not changed during the settling period. The supernatant or SPP was slowly decanted into a separate container. The pH and dissolved oxygen were measured and adjusted to acceptable ranges. This SPP was then diluted to test concentrations and used immediately in the toxicity test.

A healthly population of laboratory cultured Mysidopsis bahia (3 to 6 days old) was used as the test organisims. The Mysids were fed live Artemia nauplii every 24 hours. Tests were conducted with five (5) concentrations (three replicates per concentration), a seawater control set, and a standard positive control set of sodium dodecyl sulfate. Twenty (20) Mysids were randomly distributed in each of the concentrations. Tests were performed in 1700 ml crystalization dishes which contained one liter of solution. Filtered seawater with a salinity of 20 ppt was used to make up the solutions for the test concentrations and the control sets. A 14:10 light:dark photoperiod was maintained and aeration was supplied to each test container. Dissolved oxygen,

pH, salinity, and temperature was monitored and maintained within protocol guidelines daily. At the end of 96 hours, the number of surviving Mysids was determined.

REFERENCE TOXICANT:

Sodium dodecyl sulfate (95% pure) was used as the toxicant for the positive control. The test material was prepared by adding one (1) gram of sodium dodecyl sulfate to 100 mls of dionized water in a volumetric flask. Dilutions were prepared by adding 0.1 mls of the stock solution for each ppm desired to one liter of seawater. The Reference toxicant ran concurrent to the definitive bioassay.

Reference toxicant testing ran concurrent to the definitive bioassay test and yielded a 96-hour LC50 of 9.89 with a 95% confidence interval of 7.70 to 12.34.

These results indicate a healthy strain of Mysidopsis *bahia*.

RESULTS

The results of the 96 hour bioassay, the concentration at which 50% of the test organisims respond on **100 ppm ECOFLASH in Seawater** yielded an LC50 of less than 85,790 ppm. The sample met the minimum EPA requirement of 30,000 ppm.

REFERENCES

Finney, D.J., 1971, Probit Analysis, 3rd edition.

Stephen, C.E., 1982, Computer Program for Calculation of LC50 Values, U.S. Environmental Protection Agency, Duluth, Minn.

U.S. Environmental Protection Agency, 1985, Drilling Fluid's Toxicity Test. Federal Register, Vol 50, No. 165, Monday, August 26, 1985, pages 34631-34636.