

96- HOUR ACUTE TOXICITY TEST ON

De-Oil-It Industrial Grade Concentrate

Prepared for:

TRINIDAD TANK AND FUEL SERVICES
LIMITED

Test Conducted by:

POWER TECH PETRO ENVIRONMENTAL
COMPANY LIMITED

#22 DAL KEITH ROAD, COCOYEA
Tel/Fax: (868) 221-1522, Tel: 653-1070
Email: powertechlab@gmail.com



Power TECH

Petro Environmental Company Limited

#62 Union Road, Marabella, Trinidad, W.I.

Tel./Fax.: (868) 658-2267

1st June, 2012

Trinidad Tank & Fuel Services Limited

Trinidad Tank and Fuel Building

1A 1st Avenue South, Western Main Road,

Chaguanas, Trinidad.

Mr. John Collier

Power Tech Petro Environmental Company Limited has conducted the LC 50 96 –hour Acute Toxicity Test (USEPA/600/4-90/027/F) on a sample of De-Oil-It Industrial Grade Concentrate and is pleased to submit the results.

The sample collected showed an LC 50 96-hour Acute Toxicity using estuarine mysid *Metamysodopsis insularis*, as **0.001 %** or **10.0 mg/L**.

A full report is attached for your perusal.

Regards,

Prepared by:

Ms. Donelle Hamilton

BSc. Biochemistry / Biology

Biochemist

Reviewed by:

Senior Laboratory Technician

**POWER TECH. PETRO
ENVIRONMENTAL CO. LTD.**

Test Sample: De-Oil-It Industrial Grade Concentrate

Environment: Swamp Water

Results Required: 96-hour Acute Toxicity

Job Description: Acute Toxicity tests were conducted at Power Tech Petro Environmental Company Limited on a sample of De-Oil-It Industrial Grade Concentrate.

Summary: In determining the relative toxicity of this sample to aquatic organisms, an acute test was conducted to estimate its median Lethal Concentration (LC50). The (LC50) refer to the concentration (mg/l) or percent (%) of the Water Sample that is lethal to 50% of the test population over a 96- hour period.

Procedure: Upon receipt of the sample, it was logged into a chain of custody and assigned a sample identification number. The sample was refrigerated at 4 C until the test was initiated. Toxicology testing in the laboratory follows strict guidelines documented in our Laboratory Quality Assurance Procedure.

The acute toxic effect of the chemical product to the mysid shrimp *Metamysidopsis insularis*, was determined using the standard 96-hour toxicity test. The mysids used are indigenous to Trinidad and were maintained in swamp water of salinity 3.01‰ at a temperature of 28.3 °C under a 12:12 photoperiod. Three (3) to five (5) day old juvenile mysids were used for the investigation and were collected and maintained under the same environmental condition as the adults.

The pH of the stock solution was measured and adjusted if necessary. Test solutions were prepared by accurately mixing stock solution with dilution water. A total of twenty (20) organisms were exposed to each test concentration. One (1) test concentration was used and daily water quality measurements were made.

The health and sensitivity of the test organisms were determined by the use of a reference toxicant to measure the reproducibility of the test. This quality assurance procedure indicated that the species used were of an acceptable standard. Water quality measurements of the test solutions remained within the acceptable limits for the duration of the test.

Organisms used for testing were 5-day old mysid shrimp. The dilution water used was water from their natural habitat of salinity $3.01 \pm 2\text{‰}$ at a temperature of $28.3 \pm 2^{\circ}\text{C}$. The concentration was calculated from the observed mortality. The LC50 (Table 1) was determined using Probit, Trimmed Spearman or ICp analysis and is used as a measured of the toxicity of the chemical product.

TOXICITY TEST METHOD

96-hr Acute Toxicity U.S.EPA 600/4-90/027F Mortality (no movement)

END OF TEST

DATE TEST STARTED (TIME)	21 st May,2012					
DATE TEST ENDED (TIME)	25 th May,2012					
MODE OF ANALYSIS	STATIC					
SPECIES	<i>Metamysidopsis insularis</i>					
COMMON NAME	Estuarine Mysid Shrimp					
SOURCE	Power Tech					
MEAN	2.27					
AGE	3-5DAYS					
Volume of solution per chamber/cm ³	200					
Volume of test per chamber/cm ³	250					
Concentration of Produced Water used %	Neat (100%)					
Concentration of Produced Water used/%	100	10	1	0.1	0.01	Control
No. of Animals per chamber	20					
No. of replicates per concentration of Produced Water	One (1)					
Total No. of Animals per concentration	20					
Total No. of Animals per control	20					
Food Frequency	Not fed					
Type of food	Brine Shrimp					
Acclimation temperature / °C	28.3 ± 2					
Test temperature °C	28.3 ± 2					
Dilution water	Natural water from habitat					
pH of dilution water	7.53 ± 0.5					
Salinity ‰	3.01 ± 2					

The specific concentration of the test substance that was lethal to 50 % (LC 50) of the species in the concentration series after 96hrs was calculated from the above mortalities.

RESULTS

The data in Table 1 represents the LC50-96hr values for the sample, to the mysid shrimp, *Metamysidopsis insularis*.

The toxicity of the De-Oil-It Industrial Grade Concentrate to mysids was **0.001% or 10 mg/L**.

TABLE 1: Showing the LC50-96hr of the laboratory- Prepared sample using *Metamysidopsis insularis*.

LABORATORY PREPARED SAMPLE	LC50-96hr	
	± 95% Confidence Intervals	
MH-4 INDUSTRIAL CLEANER	% PRODUCT	mg/L
	0.001 (0.000-0.020)	10 (0.0- 200.0)

The pH of the stock solution prepared was 7.53; hence no pH adjustments were necessary.

ADDITIONAL DATA AND OBSERVATIONS DURING 96-hour test.

[A] Range Finding Test:

Concentration of De-Oil-It Industrial Grade Concentrate = 1%

Date: 18th - 19th May 2012 (24hours)

Concentration MH-4 (%)	Number Exposed	Mortality
100	5	5
10	5	5
1	5	3
0.1	5	2
0.01	5	2

[B] Definitive Test:

Concentration of De-Oil-It Industrial Grade Concentrate = 0.01%

Date: 21st -25th May 2012 (96 hours)

Concentration MH-4 (%)	Number Exposed	Mortality
Control	20	01
0.01	20	13
0.1	20	16
1	20	17
10	20	18
100	20	20

The statistical analyses used on the data were performed using the following methods:

1. USEPA Probit Analysis Program – used for calculating Lethal Concentration (LC) and Exposure Concentration (EC) values version 1.5.
2. Trimmed Spearman- Karber (TSK) Program, Version 1.5.

References:

U.S.EPA (1995) - Methods for Measuring the Acute Toxicity of Effluents and Receiving Water to Freshwater and Marine Organisms. U.S. Environmental Protection Agency, Office of Research, Office of Research and Development, Cincinnati, Ohio 4th Edition, Edited by Weber C.I. , EPA-600/4-90-027.

USEPA (1991)- Ecological Monitoring Research Division, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio, 45628.

DE OIL IT

EPA PROBIT ANALYSIS PROGRAM
USED FOR CALCULATING LC/EC VALUES
Version 1.5

De Oil It

Conc.	Number Exposed	Number Resp.	Proportion		
			Observed	Responding	Predicted
			Proportion	Adjusted for	Proportion
			Responding	Controls	Responding
Control	20	1	0.0500	0.0000	0.0502
0.0100	20	13	0.6500	0.6315	0.6268
0.1000	20	16	0.8000	0.7894	0.7625
1.0000	20	17	0.8500	0.8421	0.8655
10.0000	20	18	0.9000	0.8947	0.9327
100.0000	20	20	1.0000	1.0000	0.9704

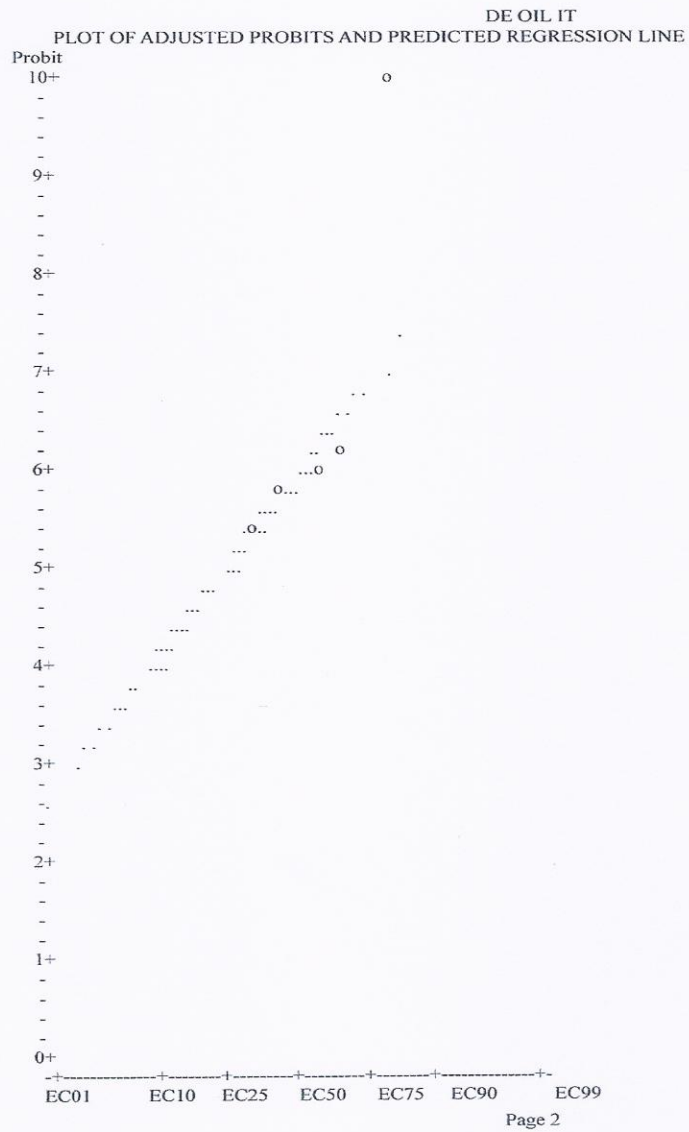
Chi - Square for Heterogeneity (calculated) = 1.178
Chi - Square for Heterogeneity
(tabular value at 0.05 level) = 7.815

Mu = -2.827580
Sigma = 2.558307

Parameter	Estimate	Std. Err.	95% Confidence Limits	
Intercept	6.105255	0.185011	(5.742633,	6.467876)
Slope	0.390884	0.129585	(0.136896,	0.644871)
Spontaneous Response Rate	0.050238	0.048840	(-0.045488,	0.145963)

Estimated LC/EC Values and Confidence Limits

Point	Exposure Conc.	95% Confidence Limits	
		Lower	Upper
LC/EC 1.00	0.000	0.000	0.000
LC/EC 5.00	0.000	0.000	0.000
LC/EC 10.00	0.000	0.000	0.000
LC/EC 15.00	0.000	0.000	0.000
LC/EC 50.00	0.001	0.000	0.020
LC/EC 85.00	0.667	0.081	20.106
LC/EC 90.00	2.826	0.395	612.371
LC/EC 95.00	24.020	2.224	179578.703
LC/EC 99.00	1329.869	33.454	12913356800.000



DISCUSSION

The objective of an acute toxicity test with effluents and receiving waters is to identify discharges of toxic effluents in acutely toxic amounts. The Second Schedule of the Environmental Management Authority's Water Pollution Rules 2001 suggests that toxicity levels of all solutions/chemicals being released into the inland surface water, coastal nearshore, marine offshore or environmentally sensitive areas/ground water should display no acute toxic effect (NATE). The 96-hour acute toxicity value (LC50)for De Oil It Industrial Grade Concentrate was 10ml/L. The test was acceptable since there was 90% survival of the control, while the sensitivity of the test was increased due to the use of duplicate test chambers for each concentration of V008 used and the use of the reference toxicant NaCl.

RECOMMENDATION

For disposal purposes it is recommended that the chemical is diluted to >10 000 times its concentration. This is necessary since a 0.01% starting concentration of De Oil It gave an acute toxic effect of 10ml/L. The direct release of this chemical into the receiving environment will present itself as an environmental pollutant.

POWER TECH
PETRO ENVIRONMENTAL

CHAIN OF CUSTODY

62 Union Road, Marabella, Trinidad, W.I.

Tel/Fax: (868) 658-2267

NO.:

347

LABORATORY

[illegible]

SAMPLED BY: John Colley BLOCK LETTERS [Signature] SIGNATURE

May 10 2012
DATE