CERTIFICATE OF ANALYSIS

Triad Forensics
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Reported To:	3 Tier Technologies, LLC 2302 Mercator Dr. Suite 102 Orlando, FL 32807	**Case Number:** **Date Received:** **Date Reported:**	TFL-10-0721 7/21/2010 08/25/2010
Product:	SOIL-RX	**Test Method:**	U.S. EPA Appendix C to Part 300
Lot Number:	10050119018	**Test Name:**	Swirling Flask Dispersant Effectiveness Test, Revised Standard Dispersant Test, and Bioremediation Agent Effectiveness Test

Overview:

In addition to gravimetric analysis, normal alkanes as well as the more highly resistant and toxic polynuclear aromatic hydrocarbons (PAHs), were monitored using High Resolution Gas Chromatography Mass Spectrometry (GC/MS) to quantify changes in the oil composition resulting from biodegradation during a 28-day effectiveness study for SOIL-Rx®[1]. The bioremediation effectiveness test was performed using a Shimadzu GC-2010 with AOC-20i auto sampler. The GC/MS was operated in the selective ion-monitoring mode (SIM mode) at a scan rate greater than 1.5 scans per second using Helium as the carrier gas. All other instrument settings and analytical procedures follow U.S. EPA Method 8270 and the configuration and calibration section of reference text A; see below. Concurrent microbiological analyses were performed to determine and monitor the viability of the microbial cultures being studied. ASCLD-LAB International approved laboratory notebooks were maintained to document the chemicals, media, surrogates, stock solutions, standards, and reagents used to perform the 28-day effectiveness study. Effectiveness testing was conducted using triplicate samples of the following: control[2], nutrient[3], and product.[4] Control and treatment flasks were sampled three times over a 28-day period: day 0, day 7, and day 28.

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[1] Manufactured by 3 Tier Technologies

[2] Control samples contain approximately 0.5 grams of reference oil and brought to a final volume of 1000-mL with non-sterile seawater

[3] Nutrient samples contain approximately 0.5 grams of reference oil, 10-mL of N&P Salt solution, 2-mL solutions 2-5, and brought to a final volume of 1000-mL with non-sterile seawater.

[4] Product samples contain approximately 0.5 grams of reference oil, 10-mL of N&P Salt solution, 2-mL of solutions 2-5, and brought to a final volume of 1000-mL with 1:10 diluted Soil-Rx®/non-sterile seawater solution.

The effectiveness study was performed using the test methodologies described in the following texts:

A. EPA Appendix C to Part 300 – *Swirling Flask Dispersant Effectiveness Test, Revised Standard Dispersant Test, and Bioremediation Agent Effectiveness Test.*

B. 40 CFR part 300 - *National Oil and Hazardous Substances Pollution Contingency Plan*

C. L.T. McCarthy, Jr., I. Wilder, and J.S. Dorrier. *Standard Dispersant Effectiveness and Toxicity Tests.* EPA Report EPA–R2–73–201 (May 1973).

Every attempt was made to comply with all requirements and quality guidelines for conducting effectiveness testing. In the referenced test methods, certain resources, chemicals, or equipment were no longer available. As a result, equivalent substitutes were used to facilitate rigorous testing and evaluation of the bioremediation product studied. The equivalency of substitute chemicals was determined by comparative assessment of the mass spectra data[5] for both the original and all potential substitute chemicals. Substitutions of equipment mentioned in the reference texts were made after consulting with the device manufacturer and examining equipment and/or software manuals.

Independent Laboratory Results:

Data Table 1: Bioremediation Agent Effectiveness Test Data

Sample Description	CONTROL			NUTRIENT			SOIL-RX® PRODUCT		
Day of Collection	Day 0	Day 7	Day 28	Day 0	Day 7	Day 28	Day 0	Day 7	Day 28
Gravimetric Results (mg)*	496.20	458.24	456.24	491.03	487.03	454.52	497.65	482.93	347.64
Microbial Viability ad Activity (MPN/g)	<3.0	<3.0	<3.0	<3.0	3.6	7.4	43	>1100	>1100
GC/MS Results Total Mean Alkanes (ppm)**	34908.9	35206.2	34287.8	34863.3	32894.1	32610.0	34993.0	24446.0	17597.0
GC/MS Results Total Mean Aromatics (ppm)**	6051.3	5720.9	5892.7	6047.4	5745.8	5205.1	6069.0	3219.2	1600.1
Percent Reduction in Total Mean Alkanes after 28 Days	1.779%			6.463%			49.713%		
Percent Reduction in Total Mean Aromatics after 28 Days	2.621%			13.928%			73.635%		

* Gravimetric results are averages of the three replicate studies performed on each sample (Control, Nutrient, and Product) for each sample day (Day 0, Day 7, Day 28).

** GC/MS results of averaged total aromatic compounds of the three replicate studies performed on each sample (Control, Nutrient, and Product) for each sample day (Day 0, Day 7, Day 28). Total of nine samples per day sampled (Three for Control, Nutrient, and Product on Day 0, three for Control, Nutrient, and Product on Day 7, and three for Control, Nutrient, and Product on Day 28).

[5] Obtained by a serial dilution and three point calibration curve

Effectiveness testing for the control, nutrient, and product samples was conducted in triplicate. Minor qualitative changes were noted for the oil fraction in the nutrient and control sample flasks for days 7 and 28. However, no observable changes in either color or turbidity were noted for the nutrient and control samples during the 28-day test period. For all three of the product flasks, significant qualitative changes in the sample solution's turbidity, coloration, and volume of the oil fraction were noted. The qualitative changes to the oil fraction layer in each of the samples were verified quantitatively using U.S. EPA Methods 1664 (gravimetric) and 8270 (Characterization of Volatile Organic Compounds).

Upon termination of the effectiveness study, a significant reduction in hydrocarbons was documented for the product samples containing the bioremediation agent in Soil-Rx®. The microbial viability and activity was maximal for the Soil-Rx® product samples assayed on day 7 and day 28. The data obtained from testing those samples indicates that a strong correlation exists between Soil-Rx® microbial activity and hydrocarbon compound reduction. The product samples indicate that the microbes in the Soil-Rx® product are highly effective in reducing hydrocarbon compounds in a time period of less than 28 days.

Laboratory Statement of Qualifications (SOQ):

Triad Forensics Laboratory (TFL) is compliant with ISO/IEC 17025:2005, EPA, FDA, NELAC, AOAC, and ASCLD-LAB International accreditation guidelines for independent testing laboratories. We are audited and conduct external proficiency tests at least twice annually to maintain all accreditations and certifications. All laboratory equipment and instrumentation are calibrated in accordance with ISO/IEC 17025 guidelines. TFL is certified by, registered with, and operates in the State of North Carolina.

END OF LABORATORY REPORT

Reported By: _____

Lauren A. Stainback
Forensic Laboratory Director,
Member AOAC, APHA, and NELAC
ASCLD-LAB Legacy Auditor

Date Signed: August 25, 2010

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Reported To:	3 Tier Technologies, LLC 2302 Mercator Dr. Suite 102 Orlando, FL 32807	**Case Number:** **Date Received:** **Date Reported:**	TFL-10-0913A 6/5/2010 6/7/2010
Product:	Soil Rx®	**Test Method:**	EPA 2000.0
Lot Number:	10050119018	**Test Name:**	24 Hour Acute Toxicity

Methodology Overview:

The freshwater organisms, *Pimephales promelas,* selected for this study were purchased from a local fishery and were certified to be free of parasitic and bacterial pathogens. Approximately 160 live animals were purchased and transported 15 miles to the laboratory on 6/1/2010. Once the animals arrived at the laboratory, they were placed in a holding tank of simulated freshwater for observation. Their physical health and size were satisfactory according to EPA method 2000.0. The physical and chemical characteristics of the holding water tank were recorded at least once daily and the results are provided as attachments to this document. All aquarium tanks were cleaned using the procedure described in EPA 2000.0 Sections 5.2.2-5.2.3. All organisms were fed 0.007 grams Aqueon Tropical Flakes fish food one time per day.

After a five-day observation period, approximately 20-24 healthy organisms were selected for each test of 0% (control), 12.5%, 18.75%, 25%, 50% and 100% dilute effluent toxicant. Dilute Soil Rx® solution was prepared by the manufacturers instructions by diluting 1 part Soil Rx® solution to 10 parts deionized water. 22 Selected organisms were then placed into aquarium tanks (including 1 bubble air hose, 1 filter, and 1 glass thermometer) that had been spiked with reagent grade chemicals to create simulated freshwater having the physical and chemical composition as described in EPA method 2000.0 section 7.2.3.1 and Table 7. Physical and chemical results for all pre- and post-toxicant water effluents were tested. The results are included as attachments.

After the organisms acclimated to the new environment, the desired level of dilute toxicant was added to the water. The time was noted at the beginning of the study and each study had duration of 24 hours. Some tests were terminated before 24 hours of toxicant exposure time due to greater than 90% organism mortality. Studies in which all organisms expired were noted and all data have been tabulated and are provided as attachments.

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Starting Live Organism Qty.	Methodology	Effluent Dilutions in %	Final Live Organism Qty Post 24 Hour Toxicant Exposure
22	Fathead minnow, *Pimephales promelas, Acute Toxicity Tests with Standard Synthetic Freshwater Effluents and Receiving Waters*	12.5	22
22	Fathead minnow, *Pimephales promelas, Acute Toxicity Tests with Standard Synthetic Freshwater Effluents and Receiving Waters*	18.75	11
22	Fathead minnow, *Pimephales promelas, Acute Toxicity Tests with Standard Synthetic Freshwater Effluents and Receiving Waters*	25	0
22	Fathead minnow, *Pimephales promelas, Acute Toxicity Tests with Standard Synthetic Freshwater Effluents and Receiving Waters*	50	0
22	Fathead minnow, *Pimephales promelas, Acute Toxicity Tests with Standard Synthetic Freshwater Effluents and Receiving Waters*	100	0

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Independent Laboratory Results:

Total organism survival was 100% after 24 hours of exposure at 12.5% dilute Soil Rx® solution. LD50 was reached at 18.75% dilute Soil Rx® solution after 24 hours of exposure. All organisms expired within 12 hours of exposure to 25%, 50%, and 100% dilute Soil Rx® solutions.

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Reported By _____
Lauren A. Stainback, Forensic Laboratory Director

Date Signed 06/07/2010