King Fahd University of Petroleum & Minerals
Research Institute
Center for Environment & Water

FINAL REPORT

A REPORT ON THE EVALUATION OF OIL SPILL EATER II (OSE- II)

Prepared for

RMC ConstructionCompany
Al-Khobar, Saudi Arabia

Safar 1436 H
December 2014 G

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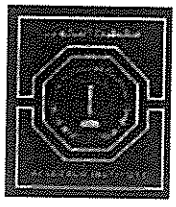

King Fahd University of Petroleum & Minerals
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SUMMARY

The RMC Company, Al-Khobar, Saudi Arabia, has requested the Research Institute of the King Fahd University of Petroleum & Minerals, Dhahran (KFUPM/RI) to evaluate the technical and analytical aspects of an oil spill bioremediation product named "Oil Spill Eater II' (OSE II). This product was developed in 1989 by Sky Blue Chems Company in USA and is now owned by OSEI Corporation, Dallas, Texas, USA.

As a part of this assignment, KFUPM/RI evaluated of the technical and analytical reports regarding use of OSE II product to treat oil spills in rivers and sea. These reports were evaluated based on theoretical, operational, and technical aspects and chemical tests conducted on the product related to synthetic spill experiments. Utilization of the OSE II

in other parts of the world is also taken into account and conclusions were drawn about the suitability and applicability of the spilled oil bioremediation product for introduction in Saudi Arabia.

The product contains enzymes and micronutrient additives needed for bacterial growth. The product is diluted 50 times by v/v with water and applied on the contaminated area where a spill had occurred. The sequence of processes consists of dilution of the product, spray dispersal and suspension of oil followed by degradation of oil into fragments and gases. During this process, the enzymes degraded the higher molecular weight petroleum hydrocarbons whereas microbes from the environment further degraded the oil. During this process, chemolithoautotrophs get nutrients from supplemented material, water, and energy from the degradation of oil.

Based on our evaluation, the OSE II product can be considered as an innovative addition for the biological treatment of spilled oil. The product is an economical solution to an oil spill of different origin with low operational cost and high treatment efficiencies. It is very effective for a wide range of oil spill remediation. This product can be used locally for the treatment of spilled oil in environments including river water, seawater and contaminated soil.

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SECTION 1 INTRODUCTION

This report has been prepared in response to a request from the RMC Company, Al-Khobar, Saudi Arabia (Appendix A). A product evaluation and certification request was received from the RMC Company to evaluate the technical/analytical reports and brochures regarding their product named Oil Spill Eater II (OSE II), from OSEI Company USA. After evaluation of the reports, KFUPM/RI is required to suggest its suitability regarding environmental compliance in Saudi Arabia.

The manufacturer claims that the OSE II product is a mixture of enzyme and nutrients additives to be used after 50-x dilution on the spilled oil. The procedure requires a single treatment to clean oil spillage. The test reports provided to us contained material on the toxicity testing, efficacy reports on bioremediation treatments carried out by various companies and universities e.g., U.S EPA, NELAC, NETAC-University of Pittsburg Applied Research Center, Chemical Analysis Inc., (Legal and Expert Witness),South West Research Institute, San Antonio, Texas USA , Canadian Efficacy Test Report, Material Safety Data Sheet (MSDS) and US EPA NCP listing. In addition to these reports, literature was also searched for third party verification.

As a part of this assignment, KFUPM/RI performed the evaluation of these technical and analytical reports regarding use of OSE II products to treat oil spills. These reports were evaluated on the basis of theoretical and technical aspects as well as chemical test conducted on the product. Utilization of the OSE II product in other parts of the world was also considered and final conclusion was reached based on literature evaluation about the suitability and applicability of the product to remediate spill oil from different sources.

SECTION 2 OBJECTIVES

The main objective of this evaluation is to provide the assessment of the OSE II for the remediation of spilled oil in rivers and seawater and the decision to introduce this product in Saudi Arabia.

SECTION 3 EVALUATIONS OF REPORTS

3.1 EVALUATION OF TOXICTY TESTING REPORTS.

3.1.1 Evaluation of Department of Labor, OSHAS, Alaska, USA Report

The document presented for toxicological concern is a letter from Department of Labor, Occupational Safety and Health, Labor Standards and Safety Division, Alaska, USA. The letter was issued on August 23, 1989 (given in the Appendix B 2.1). The review of the MSDS provided for the product does not show any special toxicological concerns with the ingredients that would pose a significant health problem with the application of the product on spilled oil.

Other toxicity testing done by the Florida Western University in simulated open water field test showed no acute or chronic toxicity for a seven day test (LC_{50} > 2500 ppm). These finding are available at http://www.nbiap.vt.edu/brag/brasym95/kavanaugh95.htm. (Accessed on July 25, 2014)

More than twenty toxicity tests were performed on the OSE II and it made through the Tier III level, as reviewed by the 31 Scientist Panel and the Panel moved it to the Tier IV level.

3.1.2 Evaluation of ECOTOX Services Australia Report

The documents presented for toxicological evaluation are the toxicity testing reports from ECOTOX Services Australia (an ISO 17025 accredited contract laboratory services as accredited by NATA) which are given in Appendix B 2.2. These tests were performed on the request of the CMTA International Pty Ltd, Australia (a local distributor of the OSE II product in Australia). These tests were performed in order to qualify the product for use in Australia.

These tests were performed following the ASTM, APHA and USEPA standard methods on different organisms. The tests were the Milky Oyster Larval Development tests using *Saccostrea echinata* and the Mussel Toxicity test on *Mytilus galloprovincialis,* Acute Survival tests on Juvenile Copepod *-Parvocalanus crassirostris* and Juvenile *Melita plumulosa*, and Fish Imbalance test on barramundi *Lates calcarifer*.

The results given in the Appendix provide the detailed statistical data and information on EC 10, EC 50, with No Observable Effective Concentration-NOEC and Lowest Observable Effective Concentration-LOEC. These results indicate that the product qualifies under the toxicity test standards set by the Australian authorities.

Based on the toxicity and efficacy criteria, the CMTA International Pty Ltd, Australia applied to the Australian Maritime Safety Authority (AMSA, Australia) to register the OSE II in the listing of the National Plan Oil Spill Control Agents (OSCA). The request was accepted by the AMSA and the CMTA was given the approval through a letter issued on August – September 2013 (included in Appendix 2.2).

3.1.3 Evaluation of Enviro System Division of Resource Analysts, Inc. Hampton report

The toxicity tests of the OSE II performed on *Mysidopsis Bahia* by the Enviro System Division of Resource Analysts, Inc. Hampton, New Hampshire in Gulf Breeze, Florida in March, 1990. These tests were performed for Acute Toxicity testing for 96 hours and Chronic Toxicity testing for seven days measuring LC50.

The results given in the Appendix B 2.3 provide detailed information on the LC 50 for a duration of four and seven days. Twenty Mysids were randomly distributed among a single replicate. The number of surviving organisms and the occurrence of sub lethal effects (loss of equilibrium, erratic swimming, loss of reflex, excitability,

discoloration, or change in behavior were determined visually and recorded regularly after, 24, 48,72 and 96 hours. The LC 50 for the acute test was greater than 1900 and up to 10,000 mg/L. This value is higher than Environmental Canada's cutoff value of 1000 mg/L and proved the that OSE II is non-toxic. The LC 50 for chronic toxicity was measured for seven days and it was found to be 2500 mg/L. This value reflects that the OSE II is non-toxic even if the specie is exposed for seven days.

3.1.4 Evaluation of Enviro System Division of Resource Analysts, Inc. Humpton Report

This toxicity test was requested by the US EPA and is a continuation of above mentioned test. The Enviro System Division of Resource Analysts, Inc. Hampton, New Hampshire performed this test on *Artemia salina* (marine invertebrate) in October, 1990. These tests were performed to compare toxicity of fuel oil as compared to the OSE II mixed with the Fuel Oil.

The complete experimental design and detail is given in the Appendix 2.3. Twenty organisms were randomly distributed to each of 5 replicates of each treatment. The number of surviving organisms was recorded regularly at the beginning and after, 24, 48, 72 and 96 hours. The fuel oil and mix of fuel oil with OSE II concentration varied from 0 (control), To 10, 25, 40, 60 and 100 mg/L.

The exposure of the *Artemia salina* to the test substances resulted in the following 48 hours median lethal concentrations (LC 50) was : OSE II > 100 mg/L, Fuel Oil12.6 mg/L and 1:10 mixture of OSE II and Fuel Oil is 29.4 mg/L. The results showed that fuel oil toxicity is reduced 100 folds when it is mixed with the OSE II as compared to the fuel oil alone. This result confirms that the OSE II is non-toxic and render fuel oil non-toxic after some hours of action.

3.1.5 Evaluation of Environmental Technology Center, Ontario, Canada Report

Environment Canada performed five toxicity tests on the product OSE II in 2001. These tests comprised *Daphnia magna*, Microtox test, *Onchrhynchus mykiss and Photobacterium phosphoreum* for various time spans.

The complete experimental design and detail is given in the Appendix B 2.4. The *Daphnia magna* and Microtox test proved to be insensitive since the exposure of the organisms for 48 hours showed LC 50 > 10, 000 m/L. It was also observed that *Onchrhynchus mykiss* when exposed to 96 hours showed LC 50 > 10, 000 mg/L. *Photobacterium phosphoreum* was exposed to different time intervals. It was observed that when *Photobacterium phosphoreum* was exposed to 30 minutes an LC 50 of 5109 mg/L was determined. The LC 50 of 5474 mg/L of the OSE II was observed when *Photobacterium phosphoreum* was exposed to 15 minutes, and the LC 50 of 7952 mg/L was determined when the organism was exposed for less than 8 minutes. This shows an increase of LC 50 with a decrease of exposure time.

3.1.6 Evaluation of Bio-Aquatic Testing Inc. Report

Bio-aquatic Testing Inc. Carrollton, Texas carried out a Toxicity Test to demonstrate that the OSE II rapidly detoxifies hydrocarbons once the OSE II is

applied. This Toxicity Test was set up with the Physical Engineer of the City of Plano, Texas in December 1991. The test summary is given in Appendix B 2.5

Half a gallon (approx.. 2 L) of gasoline was poured a concrete surface, where the OSE II (pre-diluted 100times) was immediately applied. The treated gasoline was allowed to set for two (2) minutes after which time two (2) gallons of fresh water were used to wash this effluent into a catch basin. At the end of test, spilled and treated water was collected and sent to the Bio-Aquatic Laboratory.

The Bio-Aquatic Laboratory performed a Static 48 Definitive Toxicity Test using Fathead Minnows (*Pimphales promeas*). The LC 50 was 9,300 mg/L which is a relatively low toxicity level.

This test showed that the OSE II when applied to a mineral fuel rapidly reduces toxicity. This detoxifying action of the OSE II limits the toxicity of a spill to marine organisms, and will allow naturally occurring bacteria to rapidly attack this detoxified spill. The rapid detoxification of a spill shows that the OSE II is a beneficial application as a first response cleanup for an oil spill.

3.1.7 Evaluation of NETAC Efficacy and Toxicity Testing Report

These tests were performed in collaboration with the EPA and the NETAC on the OSE II for the EPA NCP protocol development in 1992 and the report was released in 2003.

The EPA performed two separate tests, 48 hour and a 96 hour exposure tests, on two different species, *Mysidopsis bahia*, and *Menidia beryllina*. The *Mysidopsis bahia* tests also contained a static renewal LC50 for 48 hours and 96 hours with the OSE II, and a 7 day toxicity test as well.

The US EPA's first toxicity test of the OSE II was on *Mysidopsis bahia* for 48 and 96 hours of exposure. The 48 hour exposure toxicity test showed the OSE II's toxicity value to be between 5,661 to 7,927 for an average of 6,698. The 96 hour exposure toxicity test showed the OSE II's toxicity value to be between 3,125 to 6,250 for an LC 50 of 5,970. These two tests carried out by the US EPA demonstrated the OSE II to be practically non toxic.

The US EPA static renewal LC 50 with the OSE II and the *Mysidopsis bahia* was > 5,700 for the 48 hour exposure, and >5,700 for the 96 hr as well. The EPA values for the OSE II with this species for both exposure times established that the OSE II is practically non toxic. The US EPA went on to perform a seven (7) day toxicity test with the OSE II and *Mysidopsis bahia*. The LC 50 was 2,225 to 3,133, for an LC 50 value of 2,500 which for a seven (7) day toxicity test indicates non toxicity.

The US EPA also performed toxicity tests on a second species *Menidia beryllina*. The first test on this species was for an exposure time of 48 hours, and the LC 50 value was 6,250 to 12,500 for an LC 50 value of 8,839. The second test with the *Menidia beryllina* was for the exposure time of 96 hours, and the value was between 6,250 and 12,500 as well for an LC 50 of 8,839. These two tests by the US EPA demonstrates that the OSE II is practically non toxic .

3.1.8 Evaluation of Toxicity Testing by the OSEI Corps. for South Korean Government

The OSEI Corporation performed a Toxicity Test for the Korean Government involving minnows (*Pimephales promelas*) in June 2008. The test was endorsed by Huther & Associates, Inc, Denton Texas. The complete report is given in the appendix B 2.6. The Acute Toxicity Test was performed on *Pimephales promelas* for 24 hours. The OSE II was applied at 20% and the LC 50 value for this test was found to be 707.11 mg/L, which conforms to the Korean Government Standards. The extrapolated test value for the OSE II application concentration of 2% instead of 20%, would have seen LC 50 to be over 1337.11 mg/L which demonstrates the OSE II to be practically non toxic.

3.2 EVALUATION OF FIELD TEST REPORTS

3.2.1 Evaluation of EPA and NETAC Efficacy Testing Report

The USEPA and National Environmental Technology Center (NETAC), University of Pittsburg, conducted a one and a half year study of the OSE II on different components of oil in shake flasks. The results of 21 days experiment showed a significant decrease in the concentrations of pristine, C18, phytane, C30, total-n-paraffins, fluorene, phenanthrene, chrysene and total aromatics. These results are available in the July 1993 issue of the *Evaluation methods manual for oil spill response bioremediation agents* (see Appendix B 2.7, Report submitted on July 22, 1993). The product is more effective on polycyclic aromatic hydrocarbon (PAH) than others. BAH is one of the most resilient components of oil as compared to paraffin.

This study shows the efficacy of the product in remediation of oil spills under model conditions.

3.2.2 Evaluation of Second US EPA and NETAC Bioremediation Test Report.

The second USEPA NETAC tests were carried out in February 2001. These test were more through and used different procedures for testing the kinetics of bioremediation. These tests were performed in three different sets or groups and present a comparison of statistical difference of remediation of control (no treatment-Group 1) with nutrient control (Dr. Venosa's Media-Group 2) and the product the OSE II (Group 3).

The tests were performed for 28 days on a sample of oil containing 69 analytes (components which naturally occur in oil) and samples were collected on day 0, 7 and 28. The raw data show that during the first 7 day the OSE II reduced oil concentration by 15 % compared to both controls. On days 28 the oil reduced by the OSE II was more than 50 % compared to both controls. (Appendix B 2.8)

These data were further subjected to Analysis of Variance (ANOVA) statistical test. The raw data showed more than 15 % and 50 % reduction in oil components on days 7 and 28 respectively and one way ANOVA and two way ANOVA calculations on F-statistic for interaction indicates that group differences exist for one or more days. On pair wise protected LSD mean separation among the groups clearly indicates

the existence of three groups. The T-grouping letter indicates that the product mean values (Group 3) at day 7 and day 28 are significantly different from those of nutrient group and non nutrient group (Groups 1 & 2). These tests indicate at least in terms of total aromatic degradation, the statistically significant difference between the mean of the product and the mean of the non-nutrient control.

3.2.3 Evaluation of Bio Aquatic Testing Report.

These tests were performed by Bio Aquatic Testing, Texas, USA Laboratory (an TCEQ-NELAP and LDEQ-NELAP accredited lab). These tests were performed in 2009 and were more through compared to the NELAC test described earlier. These tests were performed in three different sets or groups and present a comparison of statistical difference of remediation of Control (Oil + Seawater-Group 1) with Nutrient (Oil + Seawater + EPA nutrient-Group 2) and the Product the OSE II (Oil + Seawater + the OSE II-Group 3).

The tests were performed for 28 days on a sample of oil ANS 521 being naturally degraded and samples were collected on day 0, day 7 and day 28. The raw data clearly indicate that the OSE II is very effective during 28 days in reducing the oil compared to both controls.

These data were also subjected to different statistical analysis including Andrerson-Darling Goodness of Fit test, Pearson correlation coefficient, Dunnett's test ability to detect statistically significant differences (control and treatments) and multiple factor Analysis of Variance (ANOVA) model. The raw data was normalized with different non biodegradable markers such as C_2 or C_3-phenanthrene, C_2-chrysene or C_{30} 17α(H), 21β (H)-hopane and recovery surrogate on GCMS analysis with 5 α–androstane and d_{10}-phenanthrene for aliphatic and aromatic components respectively. The calculations were performed on the data collected for the degradation of oil components (gravimetrically and GCMS analysis) on the samples collected on day 0, 7 and 28 respectively. The details of experimental procedures and statistical analysis are given in Appendix 2.5.

The calculation on non transformed and ranked transformed surrogate adjusted alkane data with General Linear ANOVA Model and Dunnett multiple comparison tests between treatments and control showed that at least one significant difference between one or more days at a chosen (α) alpha level of 0.05 exists. It also demonstrated significant reduction on day 28 for treatment compared to control. Although the analysis of the surrogate adjusted data with ANOVA and Dunnett's test did not show a significant effect, the data upon rank transformation achieved the desired linearity showing Day 7 and day 28 product results to be significantly less than the respective controls.

The Tukey's test on untransformed alkane data showed a significant difference between the day 28 the OSE II results and day 28 nutrient results, indicating that the product is more effective than nutrients control alone. This also applies on the data obtained on aromatic components of the oil where untransformed data showed significant difference between the product and the nutrient treatment on day 28 compared to day 0 and day 7.

3.2.4 Evaluation of Texas A & M University Report

The General Land Office for the State of Texas asked Texas A&M University to perform a study on 13 bioremediation products listed in the EPA National Contingency Plan (NCP) for oil spills.

The efficacy tests were performed using the EPA/NETAC guidelines protocol for bioremediation agents.

The test was performed on oil and grease, aliphatic, and aromatic components of the oil and the plate counts on the numbers of hydrocarbon degraders grown or colonized during the test. The report was released on October 12, 1995 (Appendix B 2.9).

The study showed that out of 13 products tested the OSE II performed well in degrading oil and grease. This was determined by measuring the production of extractable material such as biomass and or metabolite. The degradation of aliphatic fraction was more extensive on day 28 by the OSE II by the than nutrient control.

These results also revealed that the OSE II degraded the aliphatic fraction of the oil up to 54 % and polar aromatic fraction only 21 %. The results thus showed that the OSE II is more efficient in degrading the aliphatic part of the oil as compared to the aromatic component.

It was also observed that the microbial counts were higher in number when treated with the OSE II (4.07×10^7 cell counts) on day 28 as compared to other products of the Group (1×10^6).

All these findings lead to a general conclusion that the OSE II is an efficient biodegrading agent for oil and grease, aliphatic, and aromatic components of the oil.

3.2.5 Evaluation of RECIPROCITY- TEST Report

The Reciprocity Test was developed jointly by the NETAC and the USEPA to verify the hydrocarbon mineralization to CO_2 and water. These tests were performed on the OSE II to see the efficacy of the product in consuming oxygen to produce carbon dioxide by degrading hydrocarbons.

The efficacy test was performed by the Chemical Analysis Inc. Research and Consultation, Legal and Expert Witness using the EPA/NETAC guidelines protocol for bioremediation agents and the experimental setup is given in the Appendix B 2.10.

The OSE II, 1 part to 100 part of Alaskan seawater was applied at a ratio of 1 is to 1000 part per million Alaskan Prudhoe Bay crude oil. The test was compared with two other products. It was observed that one of the products, which the USEPA claimed outperformed the other products, had an oxygen uptake of 280 and 460 mg/L in 10 and 30 days respectively. The other product had an oxygen uptake of 40 and 440 mg/L in 10 and 30 days respectively. The OSE II had an oxygen uptake of 520 and 810 mg/L in 10 and 30 days respectively. This indicates that the OSE II consumes

more oxygen (almost double) and produces more carbon dioxide after 10 and 30 days as compared to two other products as well as degrades more hydrocarbons.

3.2.6 Evaluation of University of Alaska, Fairbanks, Alaska Report.

These test were performed by The University of Alaska, Fairbanks, Alaska on the request of the OSEI Inc. The tests were performed to compare biodegradation of oil by natural microbes and the OSE II product. Since crude oil contain aliphatic and aromatic compounds, these tests were performed on two model components, hexadecane and naphthalene an aliphatic and aromatic hydrocarbon respectively. The report is given in the Appendix B 2.11.

The tests were conducted on a consortium of microbes collected from Prince Willium Sound, Alaska using Alaska seawater as mineral nutrients alone and various dilutions of the OSE II, ranging from 1/50, 1/500, 1/1000 to 1/10.

The results of the treatments on hexadecane showed that 1/500 dilution of the OSE II transformed 50 % of the component to CO_2 compared to 16, 19.3 43.7 and 0% for nutrients only, at 1/50, 1/1000 and 1/10 dilution of the OSE II respectively.

The tests performed on naphthalene showed that 1/500 dilution of the OSE II transformed 46 % of the naphthalene to CO_2 compared to 3, 29 and 27 0% for nutrients only, at 1/50 and 1/1000 dilution of the OSE II respectively.

These tests showed that hexadecane and recalcitrant naphthalene compounds can be degraded with microbial consortium and seawater alone but in the presence of the OSE II (1/500 dilution) the degradation is much faster than with only nutrients seawater (3.1 times for hexadecane and 15.33 times for naphthalene and respectively)

3.2.7 Evaluation of Southwest Research Institute Report

The Southwest Research Institute, San Antonio, Texas also performed tests and residual weight tests on the OSE II. The test was performed on South African crude oil from Megaborg oil tanker spilled off the coast of Galveston, Texas. The report given in the Appendix B 2.12 (August 3, 1990).

The experiment was carried out on 600 ml seawater, 6 mL Megaborg oil and 6 mL of the OSE II product. Samples were collected at 48, 72, 96 and 216 hours. The control was 600 mL sea water and 6 mL Megaborg oil. The Megaborg oil contains 1,070,000 mg/L Total Resolvable Petroleum Hydrocarbons (TRPH).

The results showed a 95% reduction in TPH (chemical reduction) and 94.7 residual weight reduction (physical reduction) in 216 hours.

This report clearly shows that the OSE II is an effective bioremediation product that decreases the chemical components of crude oil and effectively biodegrades the physical components.

3.2.8 Evaluation of Southwest Research Institute Report

The Southwest Research Institute, San Antonio, Texas also performed tests on the BTEX and the OSE II. These tests were performed on the request of the OSEI Inc., and guided by the procedure provided by the client. The clients also provided all components. The report is presented in Appendix B 2.13 (March 14, 1990).

The different components were mixed as mentioned in the experimental protocol and four different solutions were prepared. The final composition of fourth solution contained aromatics (benzene, toluene, ethyl benzene and xylene-BTEX) 5% v, the OSE additive 0.05 % v and Florida Seawater 94.95 %v. The resultant solution was allowed to stir for 96 hours and the volume of the BTEX aromatic content was measured.

The results of the analysis showed an overall decrease of 32 %v in the BTEX content. This implies that a 2000:1 dilution reduces 32% of the BTEX and extrapolation showed 64 % reduction in 1000:1 dilution and 98% reduction when diluted to 100:1. The application the OSE II at a very dilute concentration level showed a very cost effective way to degrade aromatic (BTEX) components of the crude oil.

3.2.9 Evaluation of Literature Reporting on the OSE II

In order to investigate the authenticity of the reports presented for evaluation by the client, literature search was also conducted to collect some reports or research articles published in peer reviewed journals.

One article entitled "*Oil spill bioremediation agents- Canadian Efficacy Test Protocols*" reported oil spill bioremediation agents (OSGAs). Thirteen commercial OSBAs were tested over a two year period during the development of screening protocols to evaluate the hydrocarbon degradation efficacy of the OSBAs under various conditions of warm fresh water and cold marine water [1].

These products were tested on the TPH and the PAH using warm fresh water and cold marine water. The OSE II was also included in the thirteen products screened as the OSBA. This report also highlight the efficiency of the OSE II in bioremediation of the TPH and the PAH under cold seawater condition.

In "Literature review on the use of commercial bioremediation agents for cleanup of oil-contaminated estuarine environment' published by the US EPA in July 2004 [2], the OSE II was also included in 33 ASBAs product screening. This report also highlights the efficacy of the OSE II in remediation of oil contaminated estuarine environment as reported in peer-reviewed literature.

This indicates that the product is efficient in bioremediation of oil spills and the supporting material is authentic.

SECTION 4 CONCLUSIONS

The literature provided by the RMC includes brochures, memoranda and official reports from well-reputed universities, government organizations of America, Canada and Australia, and ISO, ILAC certified laboratories.

The literature include reports from the USEPA NCP, National Environmental Technology Applications Center, University of Pittsburg, Texas A & M University, University of Alaska, and from Chemical Analysis Inc., Southwest Research Institute, Texas, USA. CEW, KFUPM/RI also included literature from peer-reviewed journals. Based on the critical review of the referenced literature the following conclusions can be drawn:

- The OSE II is an enzyme and nutrient additive developed for the efficient bioremediation of spill oil.

- The OSE II is an innovative bioremediation agent which uniquely employs a single very efficient and economical treatment for oil spill.

- A comprehensive acute and chronic toxicity testing carried out on fresh water and sea water, single and multi-cellular organisms such as *Photobacterium phosphoreum Saccostrea echinata, Mytilus galloprovincialis, Parvocalanus crassirostris, Melita plumulosa, Lates calcarifer, Mysidopsis Bahia, Artemia salina, Menidia beryllina, Daphnia magna, Onchrhynchus mykiss, and Pimphales promeas* showed very high LC 50 and did not show any health related concerns. These tests also demonstrated that the product is environmentally safe.

- The product was subjected to various stringent experimental trials and it proved its efficacy for biodegradation of petroleum hydrocarbon to an extent of more than 90 %.

- As suggested in the literature (experimental trials, reports, brochures, etc.), the product is effective in the treatment of a wide range of petroleum hydrocarbons, crude oil, aliphatic, aromatics and is more effective in degrading polycyclic aromatic hydrocarbons than others.

- The economical aspect of the process is shown due to the needed use of a very dilute product (1/50) applied in 1:1 ratio on oil spilled.

- Based on its efficiency in treatment of spilled oil, the OSE II is included in the National Contingency Plan of the United States, Canada and Australia. Moreover, it is recommended in more than one hundred countries for the treatment of oil spill in fresh water, sea water and contaminated land.

Based on our evaluation, this product can be recommended for the treatment of different types of oil spills in sea and on land in Saudi Arabia.

SECTION 5 REFERENCES

[1] Blenkinsopp, S., G. Sergy, Z. Wang, M.F. Fingas, J. Foght, and D.W.S. Westlake (1995). Oil spill bioremediation agents-Canadian efficacy test protocols, Oil spill conference 1995, Long beach, California, USA, pp. 91-96

[2] Zhu, X., A.D. Venosa, and M.T. Suidan (2004). Literature review on the use of commercial bioremediation agents for cleanup of oil-contaminated estuarine environment, US EPA/600/R-04/075 July 2004.

14A03

APPENDICES

APPENDIX A
REQUEST FOR THE OSE II EVALUATION

OSE II Product Literature Review

Atif M. Al Hassan [atif@rmcholding.com]

Sent: Tuesday, May 13, 2014 12:09 PM

To: ecw@kfupm.edu.sa

Cc: SHEMSI AHSAN MUSHIR; oseicorp@msn.com

Attachments: ; ; OSE Information.pdf (6 MB)[Open as Web Page]; 9-OSEI%20Manual_OSHA.pdf (237 KB)[Open as Web Page]; OSE II Safety Datasheet.pdf (841 KB)[Open as Web Page]

Dear Dr. Bukhari,

Thank you very much for meeting us at your office in the university and it is a pleasure meeting you and Dr. Shemsi.

We would kindly like to request you to send us your proposal for reviewing OSE II product literature, in the meantime I am attaching you some basic information to start with and Mr. Steven Pedigo will furnish you with all the technical data and test certificates required for your ready reference, please feel free to contact him directly in case you need any information he is in CC (oseicorp@msn.com).

Thanks & kind regards

Atif M. Al Hassan

Executive Manager

RMC Company

Al-Meflah Building, 4A,

King Abdulaziz Street, 7th Cross,

Al-Khobar, Saudi Arabia

Tel: +966 13 895 5252, Fax: 893 8989

Mobil : +966 503 819 526

E-mail: atif@rmcholding.com

APPENDIX B
SUPPORTING MATERIALS

APPENDIX B
SECTION 2.1

EVALUATION OF DEPARTMENT OF LABOR, OSHAS, ALASKA, USA REPORT

STEVE COWPER, GOVERNOR

DEPARTMENT OF LABOR

OCCUPATIONAL SAFETY AND HEALTH
LABOR STANDARDS AND SAFETY DIVISION

3301 EAGLE STREET, SUITE 303
P.O. BOX 107022
ANCHORAGE, ALASKA 99510-7022
PHONE: (907) 264-2597

August 23 1989

North Country Investment
2522 Arctic Blvd.
Anchorage, Alaska 99503

Corporate Office as of Oct. 1996:
OSEI, CORP.
13127 Chandler Drive
Dallas, Texas 75243

Attn: Steve Kacz

Dear Mr. Kacz:

An inquiry was made to this office concerning Sky Blue Chems "Oil Spill Eater." Specifically, we were asked to assess whether or not the use of this product would pose any health concerns by reason of the properties of the constituents.

Upon review of the material safety data sheet and other documents, we see no special toxicological concern with the ingredients that would pose a significant health concern with its application as described.

We would appreciate knowing in advance of any field tests or uses of this product.

Sincerely,

Dennis L. Smythe
Chief of Compliance

cc: Ron Biggers

MATERIAL SAFETY DATA SHEET
MAY BE USED TO COMPLY WITH
OSHA's HAZARD COMMUNICATION STANDARD,
29 CFR 1910 1200 STANDARD MUST BE
CONSULTED FOR SPECIFIC REQUIREMENTS

U S DEPARTAMENT OF LABOR
OCCUPATIONAL SAFETY AND HEALTH ADMINISTRATION
(NON-MANDATORY FORM)
FORM APPROVED
OMB No 1218-0072

IDENTITY (AS USED ON LABEL AND LIST) BIODERRA	NOTE: BLANK SPACES ARE NOT PERMITTED IF ANY ITEM IS NOT APPLICABLE, OR NO INFORMATION IS AVAILABLE THE SPACE MUST BE MARKED TO INDICATE THAT.

SECTION I

MANUFACTURER'S NAME OIL SPILL EATER INTERNATIONAL	EMERGENCY TELEPHONE NUMBER (972) 669-3390
ADDRESS (NUMBER, STREET CITY STATE AND ZIP CODE) 13127 CHANDLER DRIVE	TELEPHONE NUMBER FOR INFORMATION SAME FAX (972) 644-8359
DALLAS TEXAS 75243	*DATE PREPARED* APRIL 30, 2003
	SIGNATURE OF PREPARER (OPTIONAL)

SECTION II - HAZARDOUS INGREDIENTS/IDENTITY INFORMATION

HAZARDOUS COMPONENTS (SPECIFIC CHEMICAL IDENTITY COMMON NAME(s)	OSHA PEL	ACGIH TLV	OTHER LIMITES RECOMMENDED	% (OPTIONAL)
NO HAZARDOUS COMPONENTS (BIODERRA)	NO TLV	NO TLV	NONE	
H2O	NO TLV	NO TLV	NONE	
NITROGEN	NO TLV	NO TLV	NONE	
MOLASSES	NO TLV	NO TLV	NONE	
NON IONIC SURFACTANT	NO TLV	NO TLV	NONE	
SUGAR	NO TLV	10 mg PER CUBIC mm DRY	NONE	
PROTEASE	NO TLV	NO TLV	NONE	
PHOSPHORUS	NO TLV	NO TLV	NONE	
YEAST	NO TLV	NO TLV	NONE	
AMYLASE	NO TLV	NO TLV	NONE	
ANIONIC SURACTANT	NO TLV	NO TLV	NONE	
MALT	NO TLV	NO TLV	NONE	

SECCTION III - PHYSICAL/CHEMICAL CHARACTERISTICS

BOILING POINT	214° F *	SPECIFIC GRAVITY (H2O = 1) A 20°C	1 05
VAPOR PRESSURE (mm Hg)		MELTING POINT	0° F
VAPOR DENSITY (AIR = 1)	1 1	EVAPORATION RATE (BUTYL ACETATE = 1)	

SOLUBILITY IN WATER 100%

APPEARANCE AND ODOR AMBER WITH THE SMALL OF SOME FERMENT.

SECTION IV - FIRE AND EXPLOSION HAZARD DATA

FLASH POINT (METHOD USED)* FIRE IN EXCESS - 7000°F - RETARDANT	FLAMMABLE LIMITS NON FLAMMABLE	LEL NA	UEL NA

EXTINGUISHING MEDIA
*NONE - FIRE RETARDANT *METHOD - ASTM D-56*

SPECIAL FIRE FIGHTING PROCEDURES
NONE - FIRE RETARDANT

UNUSUAL FIRE AND EXPLOSION HAZARDS
NONE

SECTION V - REACTIVITY DATA			
STABILITY	UNSTABLE		CONDITIONS TO AVOID *TEMPERATURE ABOVE 120°F CAN REDUCE ENZYME ACTIVITY, AVOID*
	STABLE	X	ACIDIC CONDITIONS BELOW 3 5 P
INCOMPATIBILITY (MATERIALS TO AVOID)			STRONG BASES OVER 11 7 STRONG BASES OVER 11.7
HAZARDOURS DECOMPOSITION OR BY PRODUCTS			NONE (BY-PRODUCTS CO2 AND WATER)
HAZARDOURS POLYMERIZATION	MAY OCCUR		CONDITIONS TO AVOID
	WILL NOT OCCUR	X	

SECTION VI - HEALTH HAZARD DATA			
ROUTE(s) OF ENTRY	INHALATION? NON – TOXIC	SKIN? NON – TOXIC	INGESTION? TOXIC IF MORE THAN ONE QUART INJESTED.
HEALTH HAZARDS (ACUTE AND CHRONIC)			TOXICITY TESTS – INHALATION, SKIN SENSATIZATION, OCCULAR, AND INGESTION SHOW VIRTUALLY NO TOXICITY
CARCINOGENICITY NONE	NTP? NO LISTING	ARC MONOGRAPHS? NONE	OSHA REGULATED? NO
SIGNS AND SIMPTOMS OF EXPOSURE			N/A
MEDICAL CONDITIONS *GENERALLY AGGRAVATED BY EXPOSURE*			NONE
EMERGENCY AND FIRST AID PROCEDURES			WASH EYES THOROUGHLY. USE GOOD HYGENIC PRACTICES.

SECTION VII - PRECAUTIONS FOR SAFE HANDLING AND USE
STEPS TO BE TAKEN IN CASE MATERIAL IS RETEASED OR SPILLED CAN BE WASHED INTO SEWER SYSTEMS, OR ABSORBED BY EARTH.
WASTE DISPOSAL METHOD NO SPECIAL DISPOSAL.
PRECAUTIONS TO BE TAKEN IN HANDLING AND STORING HANDLING – ONE. DO NOT STORE WHERE TEMP. EXCEEDS 120°F/5 YEAR SHELF LIFE
OTHER PRECAUTIONS NONE

SECTION VIII - CONTROL MEASURES		
RESPIRATORY PROTECTION (SPECIFY TYPE) NONE REQUIRED.		
VENTILATION	LOCAL EXHAUST NOT REQUIRED	SPECIAL NONE
	MECHANICAL (GENERAL) *NOT REQUIRED*	OTHER *NONE*
PROTECTIVE GLOVES NOT REQUIRED	EYE PROTECTION NOT REQUIRED	
OTHER PROTECTIVE CLOTHING OR EQUIPMENT NONE		
WORK/HYGIENIC PRACTICES USE GOOD NORMAL HYGENIC PRACTICES.		

APPENDIX B
SECTION 2.2

EVALUATION OF ECOTOX SERVICES AUSTRALIA REPORT

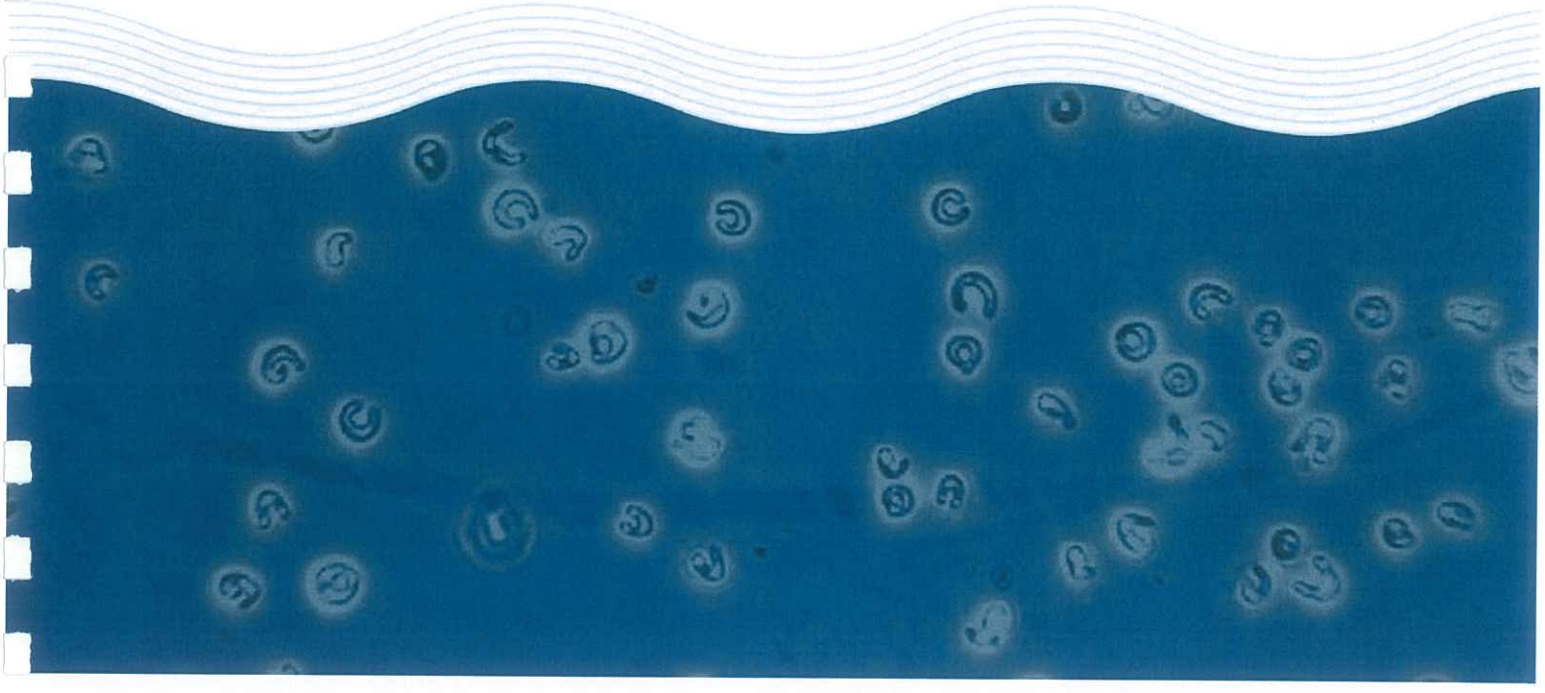
ecotox
SERVICES AUSTRALASIA

Toxicity Assessment of Oil Spill Eater II

CMTA

Test Report

August 2013



ecotox
SERVICES AUSTRALASIA

Toxicity Assessment of Oil Spill Eater II

CMTA

Test Report

August 2013

ECOTOX Services Australasia Pty Ltd ABN>45 094 714 904
unit 27/2 chaplin drive lane cove nsw 2066 T>61 2 9420 9481 F>61 2 9420 9484 W>www.ecotox.com.au

Toxicity Test Report: TR1083/1

This document is issued in accordance with NATA's accreditation requirements

Client:	CMTA 158 Garretts Rd Longford VIC 3851	**ESA Job #:** **Date Sampled:** **Date Received:**	PR1083 Not supplied 19 August 2013
Attention:	Joel Farhadian	**Sampled By:**	Client
Client Ref:	Not supplied	**ESA Quote #:**	PL1083_q01

Lab ID No.:	**Sample Name:**	**Sample Description:**
6232	Oil Spill Eater II	Chemical received at room temperature in apparent good condition

Test Performed:	48-hr larval development test using the milky oyster *Saccostrea echinata*
Test Protocol:	ESA SOP 106 (ESA 2011), based on APHA (1998) and Krassoi (1995)
Test Temperature:	The test was performed at 29±1°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	The highest test concentration of 20mg/L was prepared by adding a weighed aliquot of sample 6232 'Oil Spill Eater II' into filtered seawater (FSW). The remaining test concentrations were achieved by serially diluting the highest test concentration with FSW. A FSW control was tested concurrently with the prepared sample.
Source of Test Organisms:	Field collected from Mackay, QLD.
Test Initiated:	20 August 2013 at 1800h

Sample 6232: *Oil Spill Eater II* **Concentration (mg/L)**	**% Normal larvae (Mean ± SD)**	*Vacant*	*Vacant*
FSW Control	72.0 ± 2.2		
1.3	73.3 ± 4.6		
2.5	73.8 ± 2.1		
5.0	74.0 ± 3.7		
10.0	72.0 ± 4.3		
20.0	23.3 ± 16.7 *		
48-hr IC10 = 11.0 (10.0-11.9)mg/L			
48-hr EC50 = 16.5 (16.0-17.1)mg/L			
NOEC = 10.0mg/L			
LOEC = 20.0mg/L			

*Significantly lower percentage of normal larvae compared with the FSW Control (Steel's Many-One Rank Test, 1-tailed, P=0.05)

Toxicity Test Report: TR1083/1

QA/QC Parameter	Criterion	This Test	Criterion met?
FSW Control mean % normal	≥70%	72.0%	Yes
Reference Toxicant within cusum chart limits	13.1-18.8µg Cu/L	15.2µg Cu/L	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 3 September 2013

Results are based on the samples in the condition as received by ESA.

NATA Accredited Laboratory Number: 14709

This document is issued in accordance with NATA's accreditation requirements. Accredited for compliance with ISO/IEC 17025. NATA is a signatory to the APLAC mutual recognition arrangement for the mutual recognition of the equivalence of testing, calibration and inspection reports. This document shall not be reproduced except in full.

Citations:

APHA (1998) Standard Methods for the Examination of Water and Wastewater. 20th Ed. American Public Health Association, American Water Works Association and the Water Environment Federation, Washington, DC.

ESA (2011) SOP 106 – *Bivalve Larval Development Test.* Issue No. 10. Ecotox Services Australasia, Sydney, NSW.

Krassoi, R (1995) Salinity adjustment of effluents for use with marine bioassays: effects on the larvae of the doughboy scallop Chlamys asperrimus and the Sydney rock oyster *Saccostrea commercialis*. *Australasian Journal of Ecotoxicology*, 1: 143-148.

Toxicity Test Report: TR1083/2

This document is issued in accordance with NATA's accreditation requirements

Client:	CMTA 158 Garretts Rd Longford VIC 3851	**ESA Job #:**	PR1083
		Date Sampled:	Not supplied
		Date Received:	19 August 2013
Attention:	Joel Farhadian	**Sampled By:**	Client
Client Ref:	Not supplied	**ESA Quote #:**	PL1083_q01

Lab ID No.:	Sample Name:	Sample Description:
6232	Oil Spill Eater II	Chemical received at room temperature in apparent good condition

Test Performed:	48-hr larval development test using the mussel *Mytilus galloprovincialis*
Test Protocol:	ESA SOP 106 (ESA 2011), based on APHA (1998) and USEPA (1996)
Test Temperature:	The test was performed at 20±1°C.
Deviations from Protocol:	The test was extended to 72 hours.
Comments on Solution Preparation:	The highest test concentration of 20mg/L was prepared by adding a weighed aliquot of sample 6232 'Oil Spill Eater II' into filtered seawater (FSW). The remaining test concentrations were achieved by serially diluting the highest test concentration with FSW. A FSW control was tested concurrently with the prepared sample.
Source of Test Organisms:	Farm-reared, Mercury Passage, TAS
Test Initiated:	26 August 2013 at 1545h

Sample 6232: *Oil Spill Eater II*	*Vacant*	*Vacant*
Concentration (mg/L) / **% Normal larvae (Mean ± SD)**		
FSW Control: 75.8 ± 4.4		
1.3: 72.5 ± 1.3		
2.5: 77.8 ± 7.0		
5.0: 75.3 ± 5.8		
10.0: 77.8 ± 5.0		
20.0: 75.3 ± 5.3		
72-hr EC10 = >20.0mg/L **72-hr EC50 = >20.0mg/L** **NOEC = 20.0mg/L** **LOEC = >20.0mg/L**		

Toxicity Test Report: TR1083/2

QA/QC Parameter	Criterion	This Test	Criterion met?
FSW Control mean % normal	≥70%	75.8%	Yes
Reference Toxicant within cusum chart limits	7.3-17.2µg Cu/L	7.5µg Cu/L	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 3 September 2013

Results are based on the samples in the condition as received by ESA.

NATA Accredited Laboratory Number: 14709

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Citations:

APHA (1998) *Standard Methods for the Examination of Water and Wastewater*. 20th Ed. American Public Health Association, American Water Works Association and the Water Environment Federation, Washington, DC, USA.

ESA (2011) *Bivalve Larval Development Test.* Issue No. 10. Ecotox Services Australasia, Sydney, NSW

USEPA (1996) *Bivalve acute toxicity test (embryo larval) OPPTS 850.1055. Ecological Effects Test Guidelines.* United States Environmental Protection Agency. Prevention, Pesticides and Toxic Substances. EPA/712/C-96/137.

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Toxicity Test Report: TR1083/3

Client:	CMTA 158 Garretts Rd Longford VIC 3851	**ESA Job #:**	PR1083
		Date Sampled:	Not supplied
		Date Received:	19 August 2013
Attention:	Joel Farhadian	**Sampled By:**	Client
Client Ref:	Not supplied	**ESA Quote #:**	PL1083_q01

Lab ID No.:	Sample Name:	Sample Description:
6232	Oil Spill Eater II	Chemical received at room temperature in apparent good condition

Test Performed:	48-hr acute survival test using the copepod *Parvocalanus crassirostris*
Test Protocol:	ESA SOP 124 (2012)
Test Temperature:	The test was performed at 27±1°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	The highest test concentration of 20mg/L was prepared by adding a weighed aliquot of sample 6232 'Oil Spill Eater II' into filtered seawater (FSW). The remaining test concentrations were achieved by serially diluting the highest test concentration with FSW. A FSW control was tested concurrently with the prepared sample.
Source of Test Organisms:	In house culture
Age of Test Organisms:	<7 days old
Test Initiated:	14 November 2013 at 1300h

Sample 6232: *Oil Spill Eater II*		*Vacant*	*Vacant*
Concentration (mg/L)	**% Survival (Mean ± SD)**		
FSW Control	95.0 ± 10.0		
1.3	95.0 ± 10.0		
2.5	100 ± 0.0		
5.0	90.0 ± 11.6		
10.0	95.0 ± 10.0		
20.0	90.0 ± 11.6		
48-hr IC10 = >20.0mg/L			
48-hr EC50 = >20.0mg/L			
NOEC = 20.0mg/L			
LOEC = >20.0mg/L			

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean % survival	≥80.0%	95.0%	Yes
Reference Toxicant within cusum chart limits	4.4-30.5µg Cu/L	10.0µg Cu/L	Yes

Toxicity Test Report: TR1083/3

Test Report Authorised by: Dr Rick Krassoi, Director on 25 November 2013

Results are based on the samples in the condition as received by ESA. This document shall not be reproduced except in full.

Citations:

ESA (2012) *SOP 124 – Acute toxicity test using the copepod Gladioferens imparipes*. Issue No. 1. Ecotox Services Australasia, Sydney, New South Wales.

Toxicity Test Report: TR1083/4

This document is issued in accordance with NATA's accreditation requirements

Client:	CMTA 158 Garretts Rd Longford VIC 3851	**ESA Job #:** **Date Sampled:** **Date Received:**	PR1083 Not supplied 19 August 2013
Attention:	Joel Farhadian	**Sampled By:**	Client
Client Ref:	Not supplied	**ESA Quote #:**	PL1083_q01

Lab ID No.:	**Sample Name:**	**Sample Description:**
6232	Oil Spill Eater II	Chemical received at room temperature in apparent good condition

Test Performed:	96-hr acute toxicity test using the amphipod *Melita plumulosa*
Test Protocol:	ESA SOP 108 (ESA 2011), based on USEPA (2002) and Department of Transport and Communications (1990)
Test Temperature:	The test was performed at 20±1°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	The highest test concentration of 20mg/L was prepared by adding a weighed aliquot of sample 6232 'Oil Spill Eater II' into filtered seawater (FSW). The remaining test concentrations were achieved by serially diluting the highest test concentration with FSW. A FSW control was tested concurrently with the prepared sample.
Source of Test Organisms:	In-house culture, originally sourced from Hawkesbury River, NSW
Test Initiated:	14 November 2013 at 1230h

Sample 6232: *Oil Spill Eater II* **Concentration (mg/L)**	**% Unaffected (Mean ± SD)**	*Vacant*	*Vacant*
FSW Control	95.0 ± 10.0		
1.3	95.0 ± 10.0		
2.5	100 ± 0.0		
5.0	90.0 ± 11.6		
10.0	100 ± 0.0		
20.0	100 ± 0.0		

96-hr EC10 = >20.0mg/L
96-hr EC50 = >20.0mg/L
NOEC = 20.0mg/L
LOEC = >20.0mg/L

Toxicity Test Report: TR1083/4

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean % unaffected	≥90.0%	95.0%	Yes
Reference Toxicant within cusum chart limits	69.6-456.4µg Cu/L	140.8µg Cu/L	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 25 November 2013

Results are based on the samples in the condition as received by ESA.

NATA Accredited Laboratory Number: 14709

This document is issued in accordance with NATA's accreditation requirements. Accredited for compliance with ISO/IEC 17025. NATA is a signatory to the APLAC mutual recognition arrangement for the mutual recognition of the equivalence of testing, calibration and inspection reports. This document shall not be reproduced except in full.

Citations:

Department of Transport and Communications (1990) Guidelines for Acceptance of Oil Spill Dispersants in Australian Waters. Pollution Prevention Section, Department of Transport and Communications, Canberra ACT.

ESA (2011) SOP 108 – *Amphipod Acute Toxicity Test.* Issue No 8. Ecotox Services Australasia, Sydney, NSW.

USEPA (2002) Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Fifth Edition. United States Environmental Protection Agency, Office of Research and Development, Washington DC, EPA/600/4-90/027F.

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Toxicity Test Report: TR1083/5

Client:	CMTA	**ESA Job #:**	PR1083
	158 Garretts Rd	**Date Sampled:**	Not supplied
	Longford VIC 3851	**Date Received:**	19 August 2013
Attention:	Joel Farhadian	**Sampled By:**	Client
Client Ref:	Not supplied	**ESA Quote #:**	PL1083_q01

Lab ID No.:	**Sample Name:**	**Sample Description:**
6232	Oil Spill Eater II	Chemical received at room temperature in apparent good condition

Test Performed:	96-hr fish imbalance toxicity test using barramundi *Lates calcarifer*
Test Protocol:	ESA SOP 117 (ESA 2012), based on USEPA (2002)
Test Temperature:	The test was performed at 25±2°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	The highest test concentration of 20mg/L was prepared by adding a weighed aliquot of sample 6232 'Oil Spill Eater II' into filtered seawater (FSW). The remaining test concentrations were achieved by serially diluting the highest test concentration with FSW. A FSW control was tested concurrently with the prepared sample.
Source of Test Organisms:	Hatchery reared, SA
Test Initiated:	14 November 2013 at 1500h

Sample 6232: *Oil Spill Eater II*		*Vacant*	*Vacant*
Concentration (mg/L)	**% Unaffected (Mean ± SD)**		
FSW Control	95.0 ± 10.0		
1.3	100 ± 0.0		
2.5	85.0 ± 19.2		
5.0	100 ± 0.0		
10.0	90.0 ± 11.6		
20.0	95.0 ± 10.0		
96-hr EC10 = >20.0mg/L			
96-hr EC50 = >20.0mg/L			
NOEC = 20.0mg/L			
LOEC = >20.0mg/L			

Toxicity Test Report: TR1083/5

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean % unaffected	≥80.0%	95.0%	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 25 November 2013

Results are based on the samples in the condition as received by ESA. This document shall not be reproduced except in full.

Citations:

ESA (2012) SOP 117 –*Freshwater and Marine Fish Imbalance Test.* Issue No 9. Ecotox Services Australasia, Sydney, NSW

USEPA (2002) Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Fifth edition EPA-821-R-02-012. United States Environmental Protection Agency, Office of Research and Development, Washington FC, USA

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Toxicity Test Report: TR1083/6

Client:	CMTA 158 Garretts Rd Longford VIC 3851	**ESA Job #:** **Date Sampled:** **Date Received:**	PR1083 Not supplied 19 August 2013
Attention:	Joel Farhadian	**Sampled By:**	Client
Client Ref:	Not supplied	**ESA Quote #:**	PL1083_q01

Lab ID No.:	**Sample Name:**	**Sample Description:**
6232	Oil Spill Eater II	Chemical received at room temperature in apparent good condition

Test Performed:	96-hr fish imbalance toxicity test using Australian Bass *Macquaria Novemaculeata*
Test Protocol:	ESA SOP 117 (ESA 2012), based on USEPA (2002)
Test Temperature:	The test was performed at 20±2°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	The highest test concentration of 20mg/L was prepared by adding a weighed aliquot of sample 6232 'Oil Spill Eater II' into filtered seawater (FSW). The remaining test concentrations were achieved by serially diluting the highest test concentration with FSW. A FSW control was tested concurrently with the prepared sample.
Source of Test Organisms:	Hatchery reared, SA
Test Initiated:	8 November 2013 at 1200h

Sample 6232: *Oil Spill Eater II* **Concentration (mg/L)**	**% Unaffected (Mean ± SD)**	*Vacant*	*Vacant*
FSW Control	95.0 ± 10.0		
1.3	93.3 ± 11.6		
2.5	100 ± 0.0		
5.0	100 ± 0.0		
10.0	95.0 ± 10.0		
20.0	80.0 ± 20.0		

96-hr IC10 = 15.7mg/L*
96-hr EC50 = >20.0mg/L
NOEC = 20.0mg/L
LOEC = >20.0mg/L

*95%confidence limits are not reliable

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SERVICES AUSTRALASIA

Toxicity Test Report: TR1083/6

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean % unaffected	≥80.0%	95.0%	Yes
Reference Toxicant within cusum chart limits	58.3-3473.8µg Cu/L	347.6µg Cu/L	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 25 November 2013

Results are based on the samples in the condition as received by ESA. This document shall not be reproduced except in full.

Citations:

ESA (2012) SOP 117 –*Freshwater and Marine Fish Imbalance Test.* Issue No 9. Ecotox Services Australasia, Sydney, NSW

USEPA (2002) Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Fifth edition EPA-821-R-02-012. United States Environmental Protection Agency, Office of Research and Development, Washington FC, USA

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SERVICES AUSTRALASIA

Statistical Printouts for the Milky Oyster Larval Development Tests

Bivalve Larval Development Test-Proportion Normal

Start Date:	20/08/2013 18:00	Test ID:	PR1083/01	Sample ID:	Oil Spill Eater II
End Date:	22/08/2013 18:00	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 106	Test Species:	SE-Saccostrea echinata
Comments:					

Conc-mg/L	1	2	3	4
FSW Control	0.7200	0.7400	0.6900	0.7300
1.3	0.7200	0.7900	0.6800	0.7400
2.5	0.7600	0.7200	0.7500	0.7200
5	0.7600	0.7000	0.7800	0.7200
10	0.7800	0.7200	0.6800	0.7000
20	0.4600	0.1900	0.2200	0.0600

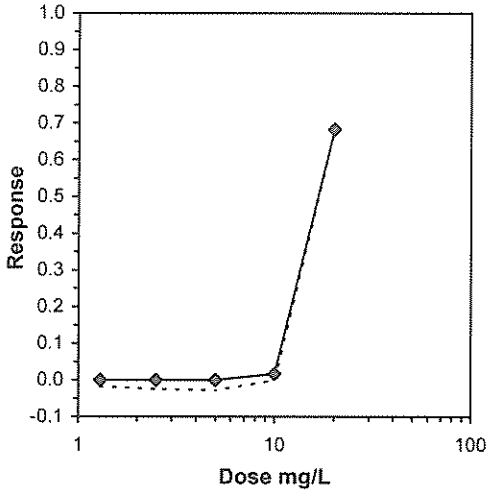
			Transform: Arcsin Square Root					Rank	1-Tailed	Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
FSW Control	0.7200	1.0000	1.0134	0.9803	1.0357	2.359	4			0.7325	1.0000
1.3	0.7325	1.0174	1.0283	0.9695	1.0948	5.070	4	19.00	10.00	0.7325	1.0000
2.5	0.7375	1.0243	1.0331	1.0132	1.0588	2.272	4	21.00	10.00	0.7325	1.0000
5	0.7400	1.0278	1.0364	0.9912	1.0826	4.025	4	20.50	10.00	0.7325	1.0000
10	0.7200	1.0000	1.0141	0.9695	1.0826	4.832	4	16.50	10.00	0.7200	0.9829
*20	0.2325	0.3229	0.4830	0.2475	0.7454	42.321	4	10.00	10.00	0.2325	0.3174

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.791823	0.916	0.475743	7.130866
Bartlett's Test indicates unequal variances (p = 1.05E-03)	20.41248	15.08627		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	10	20	14.14214	
Treatments vs FSW Control				

Log-Logit Interpolation (200 Resamples)

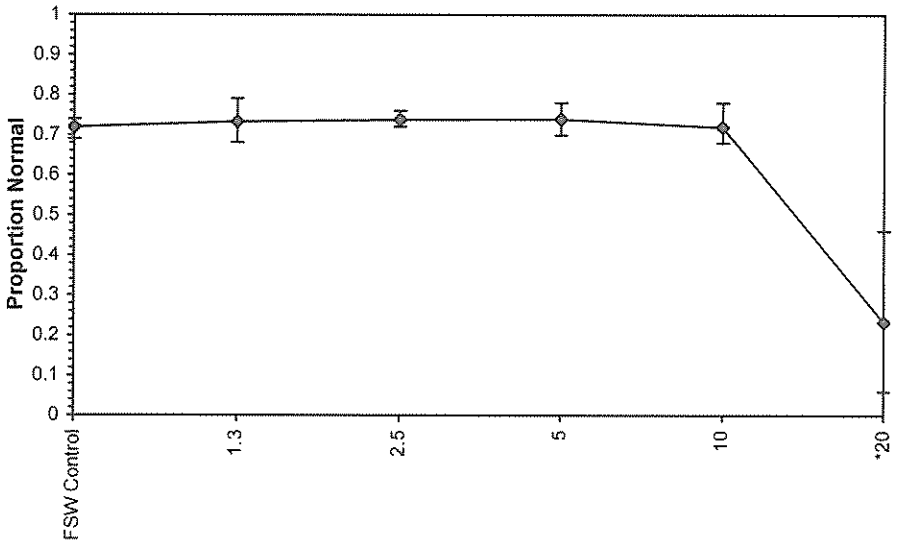
Point	mg/L	SD	95% CL(Exp)		Skew
IC05	10.395	0.623	6.739	10.906	-2.2999
IC10	10.988	0.303	10.037	11.886	0.3357
IC15	11.579	0.389	10.443	12.867	0.7568
IC20	12.176	0.507	10.793	14.080	0.9655
IC25	12.784	0.647	11.168	15.266	1.0823
IC40	14.752				
IC50	16.275				



Bivalve Larval Development Test-Proportion Normal

Start Date:	20/08/2013 18:00	Test ID:	PR1083/01	Sample ID:	Oil Spill Eater II
End Date:	22/08/2013 18:00	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 106	Test Species:	SE-Saccostrea echinata
Comments:					

Dose-Response Plot



Reviewed by:_____

Bivalve Larval Development Test-Proportion Normal

Start Date:	20/08/2013 18:00	Test ID:	PR1083/01	Sample ID:	Oil Spill Eater II
End Date:	22/08/2013 18:00	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 106	Test Species:	SE-Saccostrea echinata
Comments:					

Auxiliary Data Summary

Conc-mg/L	Parameter	Mean	Min	Max	SD	CV%	N
FSW Control	% Normal	72.00	69.00	74.00	2.16	2.04	4
1.3		73.25	68.00	79.00	4.57	2.92	4
2.5		73.75	72.00	76.00	2.06	1.95	4
5		74.00	70.00	78.00	3.65	2.58	4
10		72.00	68.00	78.00	4.32	2.89	4
20		23.25	6.00	46.00	16.68	17.57	4
FSW Control	pH	8.30	8.30	8.30	0.00	0.00	1
1.3		8.10	8.10	8.10	0.00	0.00	1
2.5		8.10	8.10	8.10	0.00	0.00	1
5		8.10	8.10	8.10	0.00	0.00	1
10		8.10	8.10	8.10	0.00	0.00	1
20		8.10	8.10	8.10	0.00	0.00	1
FSW Control	Salinity ppt	34.80	34.80	34.80	0.00	0.00	1
1.3		34.30	34.30	34.30	0.00	0.00	1
2.5		34.40	34.40	34.40	0.00	0.00	1
5		34.50	34.50	34.50	0.00	0.00	1
10		34.50	34.50	34.50	0.00	0.00	1
20		34.50	34.50	34.50	0.00	0.00	1
FSW Control	DO %	99.30	99.30	99.30	0.00	0.00	1
1.3		98.70	98.70	98.70	0.00	0.00	1
2.5		97.50	97.50	97.50	0.00	0.00	1
5		97.20	97.20	97.20	0.00	0.00	1
10		96.80	96.80	96.80	0.00	0.00	1
20		97.20	97.20	97.20	0.00	0.00	1

Bivalve Larval Development Test-Proportion Normal

Start Date:	20/08/2013 18:00	Test ID:	PR1083/01	Sample ID:	Oil Spill Eater II
End Date:	22/08/2013 18:00	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 106	Test Species:	SE-Saccostrea echinata
Comments:					

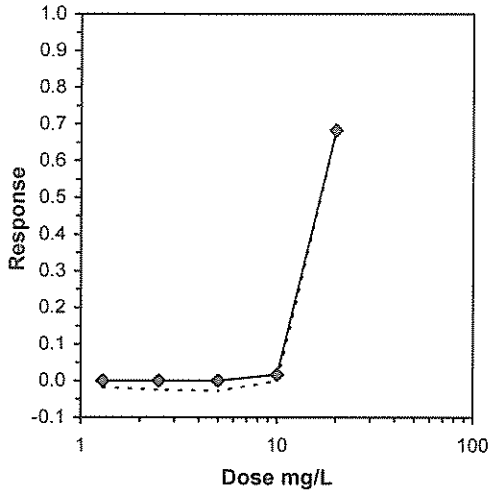
Conc-mg/L	1	2	3	4
FSW Control	0.7200	0.7400	0.6900	0.7300
1.3	0.7200	0.7900	0.6800	0.7400
2.5	0.7600	0.7200	0.7500	0.7200
5	0.7600	0.7000	0.7800	0.7200
10	0.7800	0.7200	0.6800	0.7000
20	0.4600	0.1900	0.2200	0.0600

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
FSW Control	0.7200	1.0000	1.0134	0.9803	1.0357	2.359	4			112	400
1.3	0.7325	1.0174	1.0283	0.9695	1.0948	5.070	4	19.00	10.00	107	400
2.5	0.7375	1.0243	1.0331	1.0132	1.0588	2.272	4	21.00	10.00	105	400
5	0.7400	1.0278	1.0364	0.9912	1.0826	4.025	4	20.50	10.00	104	400
10	0.7200	1.0000	1.0141	0.9695	1.0826	4.832	4	16.50	10.00	112	400
*20	0.2325	0.3229	0.4830	0.2475	0.7454	42.321	4	10.00	10.00	307	400

Auxiliary Tests					Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)					0.791823	0.916	0.475743	7.130866
Bartlett's Test indicates unequal variances (p = 1.05E-03)					20.41248	15.08627		
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**				
Steel's Many-One Rank Test	10	20	14.14214					
Treatments vs FSW Control								

Trimmed Spearman-Karber

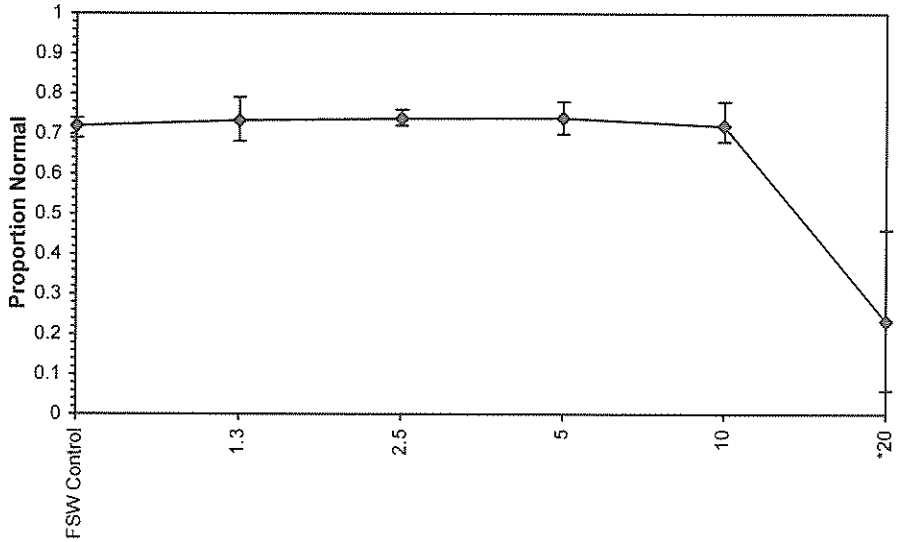
Trim Level	EC50	95% CL	
0.0%			
5.0%			
10.0%			
20.0%			
Auto-31.7%	16.536	15.962	17.132



Bivalve Larval Development Test-Proportion Normal

Start Date:	20/08/2013 18:00	Test ID:	PR1083/01	Sample ID:	Oil Spill Eater II
End Date:	22/08/2013 18:00	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 106	Test Species:	SE-Saccostrea echinata
Comments:					

Dose-Response Plot



Reviewed by:_____

Bivalve Larval Development Test-Proportion Normal

Start Date:	20/08/2013 18:00	Test ID:	PR1083/01	Sample ID:	Oil Spill Eater II
End Date:	22/08/2013 18:00	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 106	Test Species:	SE-Saccostrea echinata
Comments:					

Auxiliary Data Summary

Conc-mg/L	Parameter	Mean	Min	Max	SD	CV%	N
FSW Control	% Normal	72.00	69.00	74.00	2.16	2.04	4
1.3		73.25	68.00	79.00	4.57	2.92	4
2.5		73.75	72.00	76.00	2.06	1.95	4
5		74.00	70.00	78.00	3.65	2.58	4
10		72.00	68.00	78.00	4.32	2.89	4
20		23.25	6.00	46.00	16.68	17.57	4
FSW Control	pH	8.30	8.30	8.30	0.00	0.00	1
1.3		8.10	8.10	8.10	0.00	0.00	1
2.5		8.10	8.10	8.10	0.00	0.00	1
5		8.10	8.10	8.10	0.00	0.00	1
10		8.10	8.10	8.10	0.00	0.00	1
20		8.10	8.10	8.10	0.00	0.00	1
FSW Control	Salinity ppt	34.80	34.80	34.80	0.00	0.00	1
1.3		34.30	34.30	34.30	0.00	0.00	1
2.5		34.40	34.40	34.40	0.00	0.00	1
5		34.50	34.50	34.50	0.00	0.00	1
10		34.50	34.50	34.50	0.00	0.00	1
20		34.50	34.50	34.50	0.00	0.00	1
FSW Control	DO %	99.30	99.30	99.30	0.00	0.00	1
1.3		98.70	98.70	98.70	0.00	0.00	1
2.5		97.50	97.50	97.50	0.00	0.00	1
5		97.20	97.20	97.20	0.00	0.00	1
10		96.80	96.80	96.80	0.00	0.00	1
20		97.20	97.20	97.20	0.00	0.00	1

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Statistical Printouts for the Mussel Toxicity Tests

Bivalve Larval Development Test-Proportion Normal

Start Date:	26/08/2013 15:45	Test ID:	PR1083/01	Sample ID:	Oil Spill Eater II
End Date:	29/08/2013 15:45	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 106	Test Species:	MG-Mytilus galloprovincialis
Comments:					

Conc-mg/L	1	2	3	4
FSW Control	0.8200	0.7400	0.7200	0.7500
1.3	0.7300	0.7200	0.7400	0.7100
2.5	0.8500	0.7400	0.8200	0.7000
5	0.8300	0.6900	0.7400	0.7500
10	0.7800	0.7900	0.8300	0.7100
20	0.7300	0.7400	0.8300	0.7100

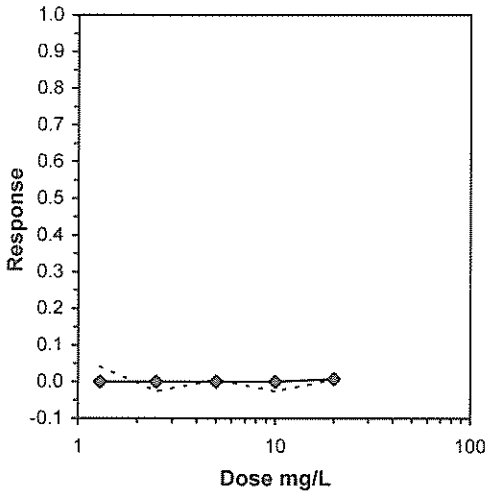
			Transform: Arcsin Square Root					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
FSW Control	0.7575	1.0000	1.0572	1.0132	1.1326	4.942	4				0.7580	1.0000
1.3	0.7250	0.9571	1.0189	1.0021	1.0357	1.419	4	0.887	2.410	0.1041	0.7580	1.0000
2.5	0.7775	1.0264	1.0832	0.9912	1.1731	7.771	4	-0.601	2.410	0.1041	0.7580	1.0000
5	0.7525	0.9934	1.0523	0.9803	1.1458	6.545	4	0.114	2.410	0.1041	0.7580	1.0000
10	0.7775	1.0264	1.0813	1.0021	1.1458	5.501	4	-0.558	2.410	0.1041	0.7580	1.0000
20	0.7525	0.9934	1.0520	1.0021	1.1458	6.090	4	0.120	2.410	0.1041	0.7525	0.9927

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.942211	0.916	0.356552	-0.59913
Bartlett's Test indicates equal variances (p = 0.30)	6.045919	15.08627		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs FSW Control	20	>20			0.094079	0.124016	0.002221	0.003735	0.704366	5, 18

Log-Logit Interpolation (200 Resamples)

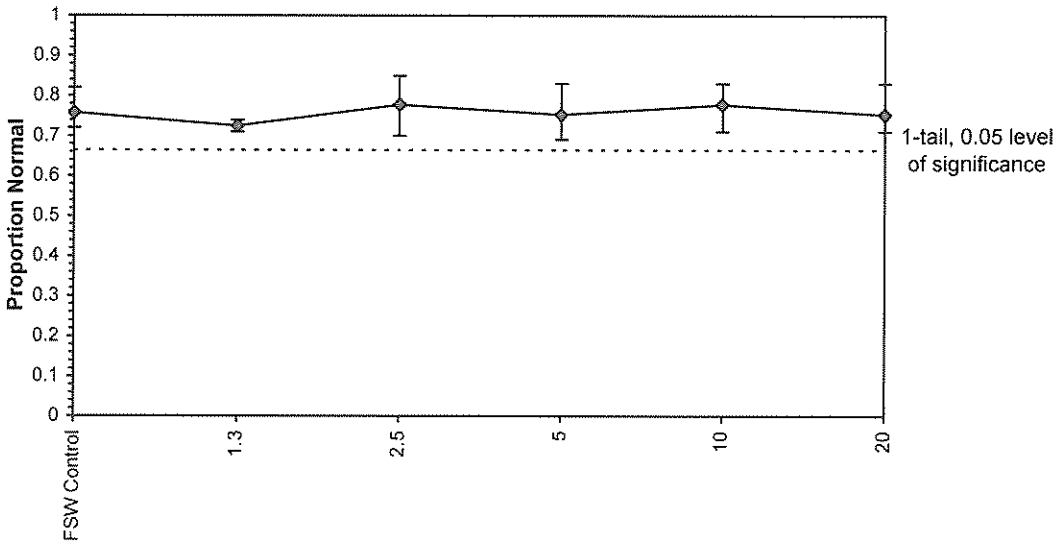
Point	mg/L	SD	95% CL(Exp)	Skew
IC05	>20			
IC10	>20			
IC15	>20			
IC20	>20			
IC25	>20			
IC40	>20			
IC50	>20			



Bivalve Larval Development Test-Proportion Normal

Start Date:	26/08/2013 15:45	Test ID:	PR1083/01	Sample ID:	Oil Spill Eater II
End Date:	29/08/2013 15:45	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 106	Test Species:	MG-Mytilus galloprovincialis
Comments:					

Dose-Response Plot



Bivalve Larval Development Test-Proportion Normal

Start Date:	26/08/2013 15:45	Test ID:	PR1083/01	Sample ID:	Oil Spill Eater II
End Date:	29/08/2013 15:45	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 106	Test Species:	MG-Mytilus galloprovincialis
Comments:					

Auxiliary Data Summary

Conc-mg/L	Parameter	Mean	Min	Max	SD	CV%	N
FSW Control	% Normal	75.75	72.00	82.00	4.35	2.75	4
1.3		72.50	71.00	74.00	1.29	1.57	4
2.5		77.75	70.00	85.00	6.95	3.39	4
5		75.25	69.00	83.00	5.80	3.20	4
10		77.75	71.00	83.00	4.99	2.87	4
20		75.25	71.00	83.00	5.32	3.06	4
FSW Control	pH	8.20	8.20	8.20	0.00	0.00	1
1.3		8.20	8.20	8.20	0.00	0.00	1
2.5		8.20	8.20	8.20	0.00	0.00	1
5		8.20	8.20	8.20	0.00	0.00	1
10		8.20	8.20	8.20	0.00	0.00	1
20		8.20	8.20	8.20	0.00	0.00	1
FSW Control	Salinity ppt	34.20	34.20	34.20	0.00	0.00	1
1.3		34.30	34.30	34.30	0.00	0.00	1
2.5		34.30	34.30	34.30	0.00	0.00	1
5		34.30	34.30	34.30	0.00	0.00	1
10		34.40	34.40	34.40	0.00	0.00	1
20		34.30	34.30	34.30	0.00	0.00	1
FSW Control	DO %	99.00	99.00	99.00	0.00	0.00	1
1.3		99.90	99.90	99.90	0.00	0.00	1
2.5		99.70	99.70	99.70	0.00	0.00	1
5		99.70	99.70	99.70	0.00	0.00	1
10		99.40	99.40	99.40	0.00	0.00	1
20		99.20	99.20	99.20	0.00	0.00	1

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Statistical Printouts for the Juvenile Copepod Tests

Marine Copepod Acute Test-48-hr Survival

Start Date:	14/11/2013 13:00	Test ID:	PR1083/25	Sample ID:	Oil Spill Eater II
End Date:	16/11/2013 12:10	Lab ID:	6232	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 124	Test Species:	PC- Parvocalanus crassirostris
Comments:					

Conc-mg/L	1	2	3	4
FSW Control	1.0000	0.8000	1.0000	1.0000
1.3	1.0000	1.0000	1.0000	0.8000
2.5	1.0000	1.0000	1.0000	1.0000
5	0.8000	0.8000	1.0000	1.0000
10	1.0000	0.8000	1.0000	1.0000
20	0.8000	1.0000	0.8000	1.0000

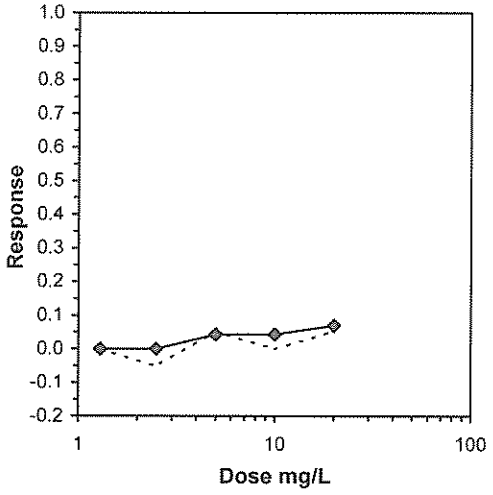
			Transform: Arcsin Square Root					Rank	1-Tailed	Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
FSW Control	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4			0.9667	1.0000
1.3	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4	18.00	10.00	0.9667	1.0000
2.5	1.0000	1.0526	1.3453	1.3453	1.3453	0.000	4	20.00	10.00	0.9667	1.0000
5	0.9000	0.9474	1.2262	1.1071	1.3453	11.212	4	16.00	10.00	0.9250	0.9569
10	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4	18.00	10.00	0.9250	0.9569
20	0.9000	0.9474	1.2262	1.1071	1.3453	11.212	4	16.00	10.00	0.9000	0.9310

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.840894	0.916	-0.67177	-0.98034
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	20	>20		
Treatments vs FSW Control				

Log-Logit Interpolation (200 Resamples)

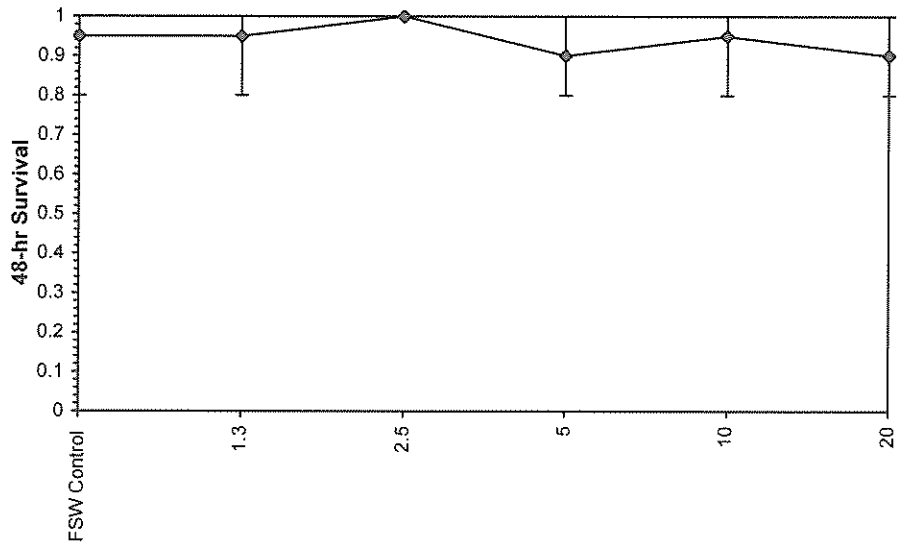
Point	mg/L	SD	95% CL(Exp)	Skew
IC05	12.297			
IC10	>20			
IC15	>20			
IC20	>20			
IC25	>20			
IC40	>20			
IC50	>20			



Marine Copepod Acute Test-48-hr Survival

Start Date:	14/11/2013 13:00	Test ID:	PR1083/25	Sample ID:	Oil Spill Eater II
End Date:	16/11/2013 12:10	Lab ID:	6232	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 124	Test Species:	PC- Parvocalanus crassirostris
Comments:					

Dose-Response Plot



Marine Copepod Acute Test-48-hr Survival

Start Date:	14/11/2013 13:00	Test ID:	PR1083/25	Sample ID:	Oil Spill Eater II
End Date:	16/11/2013 12:10	Lab ID:	6232	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 124	Test Species:	PC- Parvocalanus crassirostris
Comments:					

Auxiliary Data Summary

Conc-mg/L	Parameter	Mean	Min	Max	SD	CV%	N
FSW Control	% Survival	95.00	80.00	100.00	10.00	3.33	4
1.3		95.00	80.00	100.00	10.00	3.33	4
2.5		100.00	100.00	100.00	0.00	0.00	4
5		90.00	80.00	100.00	11.55	3.78	4
10		95.00	80.00	100.00	10.00	3.33	4
20		90.00	80.00	100.00	11.55	3.78	4
FSW Control	pH	8.30	8.30	8.30	0.00	0.00	1
1.3		8.30	8.30	8.30	0.00	0.00	1
2.5		8.30	8.30	8.30	0.00	0.00	1
5		8.30	8.30	8.30	0.00	0.00	1
10		8.30	8.30	8.30	0.00	0.00	1
20		8.40	8.40	8.40	0.00	0.00	1
FSW Control	DO %	110.60	110.60	110.60	0.00	0.00	1
1.3		101.10	101.10	101.10	0.00	0.00	1
2.5		101.40	101.40	101.40	0.00	0.00	1
5		101.50	101.50	101.50	0.00	0.00	1
10		101.10	101.10	101.10	0.00	0.00	1
20		101.30	101.30	101.30	0.00	0.00	1
FSW Control	Salinity ppt	35.50	35.50	35.50	0.00	0.00	1
1.3		35.50	35.50	35.50	0.00	0.00	1
2.5		35.50	35.50	35.50	0.00	0.00	1
5		35.50	35.50	35.50	0.00	0.00	1
10		35.50	35.50	35.50	0.00	0.00	1
20		35.60	35.60	35.60	0.00	0.00	1

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Statistical Printouts for the Juvenile *Melita plumulosa* Tests

Amphipod Acute Toxicity Test-96 hr survival

Start Date:	14/11/2013 12:30	Test ID:	PR1083/22	Sample ID:	Oils Spill Eater II
End Date:	18/11/2013 13:00	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 108	Test Species:	ML-Melita Plumulosa
Comments:					

Conc-mg/L	1	2	3	4
FSW Control	1.0000	1.0000	0.8000	1.0000
1.3	0.8000	1.0000	1.0000	1.0000
2.5	1.0000	1.0000	1.0000	1.0000
5	1.0000	0.8000	1.0000	0.8000
10	1.0000	1.0000	1.0000	1.0000
20	1.0000	1.0000	1.0000	1.0000

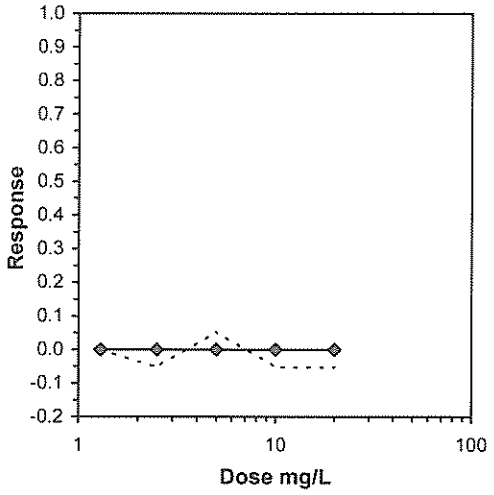
			Transform: Arcsin Square Root					Rank	1-Tailed	Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
FSW Control	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4			0.9667	1.0000
1.3	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4	18.00	10.00	0.9667	1.0000
2.5	1.0000	1.0526	1.3453	1.3453	1.3453	0.000	4	20.00	10.00	0.9667	1.0000
5	0.9000	0.9474	1.2262	1.1071	1.3453	11.212	4	16.00	10.00	0.9667	1.0000
10	1.0000	1.0526	1.3453	1.3453	1.3453	0.000	4	20.00	10.00	0.9667	1.0000
20	1.0000	1.0526	1.3453	1.3453	1.3453	0.000	4	20.00	10.00	0.9667	1.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.829814	0.916	-0.99267	0.896104
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	20	>20		
Treatments vs FSW Control				

Log-Logit Interpolation (200 Resamples)

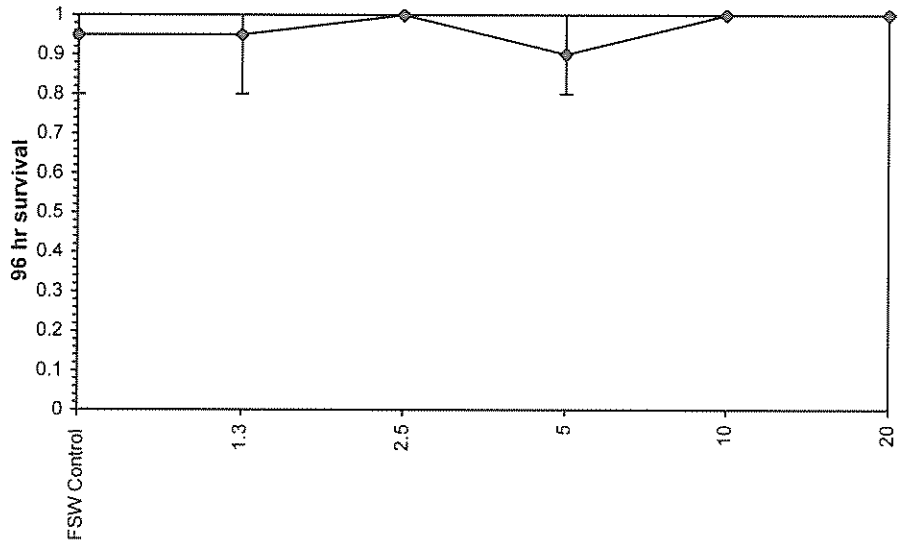
Point	mg/L	SD	95% CL(Exp)	Skew
IC05	>20			
IC10	>20			
IC15	>20			
IC20	>20			
IC25	>20			
IC40	>20			
IC50	>20			



Amphipod Acute Toxicity Test-96 hr survival

Start Date:	14/11/2013 12:30	Test ID:	PR1083/22	Sample ID:	Oils Spill Eater II
End Date:	18/11/2013 13:00	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 108	Test Species:	ML-Melita Plumulosa
Comments:					

Dose-Response Plot



Amphipod Acute Toxicity Test-96 hr survival

Start Date:	14/11/2013 12:30	Test ID:	PR1083/22	Sample ID:	Oils Spill Eater II
End Date:	18/11/2013 13:00	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 108	Test Species:	ML-Melita Plumulosa
Comments:					

Auxiliary Data Summary

Conc-mg/L	Parameter	Mean	Min	Max	SD	CV%	N
FSW Control	% Non-immobilised	95.00	80.00	100.00	10.00	3.33	4
1.3		95.00	80.00	100.00	10.00	3.33	4
2.5		100.00	100.00	100.00	0.00	0.00	4
5		90.00	80.00	100.00	11.55	3.78	4
10		100.00	100.00	100.00	0.00	0.00	4
20		100.00	100.00	100.00	0.00	0.00	4
FSW Control	pH	8.30	8.30	8.30	0.00	0.00	1
1.3		8.30	8.30	8.30	0.00	0.00	1
2.5		8.30	8.30	8.30	0.00	0.00	1
5		8.30	8.30	8.30	0.00	0.00	1
10		8.30	8.30	8.30	0.00	0.00	1
20		8.40	8.40	8.40	0.00	0.00	1
FSW Control	DO %	110.60	110.60	110.60	0.00	0.00	1
1.3		101.10	101.10	101.10	0.00	0.00	1
2.5		101.40	101.40	101.40	0.00	0.00	1
5		101.50	101.50	101.50	0.00	0.00	1
10		101.10	101.10	101.10	0.00	0.00	1
20		101.30	101.30	101.30	0.00	0.00	1
FSW Control	Salinity ppt	35.50	35.50	35.50	0.00	0.00	1
1.3		35.50	35.50	35.50	0.00	0.00	1
2.5		35.50	35.50	35.50	0.00	0.00	1
5		35.50	35.50	35.50	0.00	0.00	1
10		35.50	35.50	35.50	0.00	0.00	1
20		35.60	35.60	35.60	0.00	0.00	1

ecotox
SERVICES AUSTRALASIA

Statistical Printouts for the Fish Imbalance Tests

Fish Imbalance Test-96 hr Imbalance

Start Date:	14/11/2013 15:00	Test ID:	PR1083/20	Sample ID:	Oils Spill Eater II
End Date:	18/11/2013 16:30	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 117	Test Species:	LT-Lates calcarifer
Comments:					

Conc-%	1	2	3	4
FSW Control	1.0000	1.0000	0.8000	1.0000
1.3	1.0000	1.0000	1.0000	1.0000
2.5	1.0000	0.8000	0.6000	1.0000
5	1.0000	1.0000	1.0000	1.0000
10	0.8000	1.0000	1.0000	0.8000
20	1.0000	1.0000	0.8000	1.0000

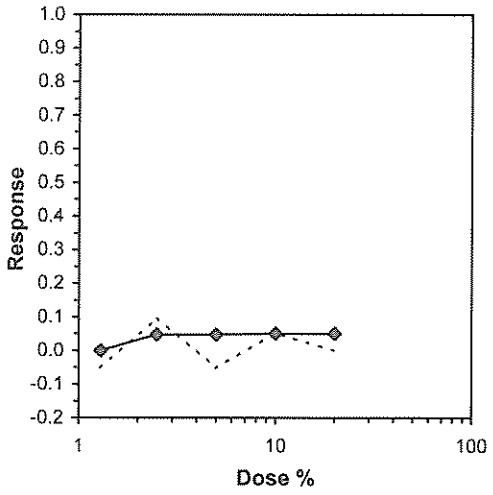
			Transform: Arcsin Square Root					Rank	1-Tailed	Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
FSW Control	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4			0.9750	1.0000
1.3	1.0000	1.0526	1.3453	1.3453	1.3453	0.000	4	20.00	10.00	0.9750	1.0000
2.5	0.8500	0.8947	1.1759	0.8861	1.3652	19.221	4	17.00	10.00	0.9286	0.9524
5	1.0000	1.0526	1.3453	1.3453	1.3453	0.000	4	20.00	10.00	0.9286	0.9524
10	0.9000	0.9474	1.2262	1.1071	1.3453	11.212	4	16.00	10.00	0.9250	0.9487
20	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4	18.00	10.00	0.9250	0.9487

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.926986	0.916	-0.75635	0.717947
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	20	>20		5
Treatments vs FSW Control				

Log-Logit Interpolation (200 Resamples)

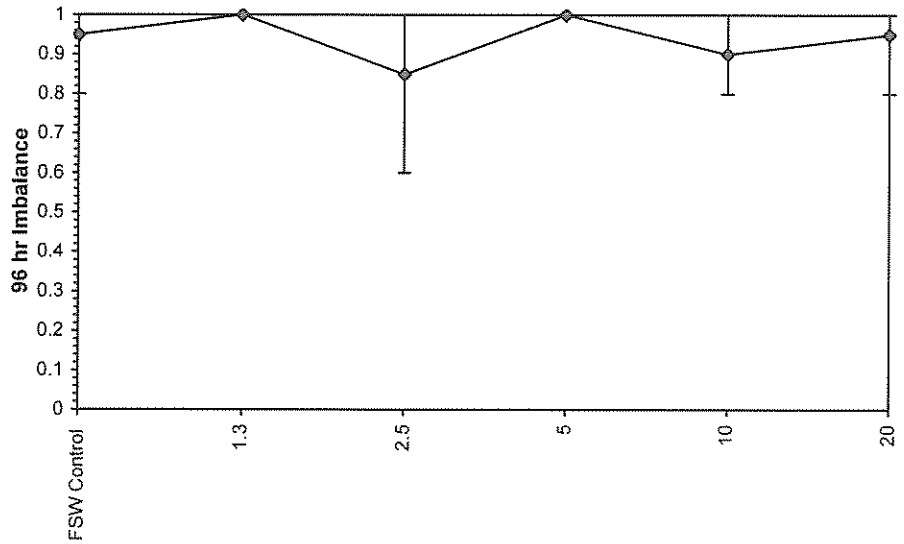
Point	%	SD	95% CL(Exp)	Skew
IC05	7.9248			
IC10	>20			
IC15	>20			
IC20	>20			
IC25	>20			
IC40	>20			
IC50	>20			



Fish Imbalance Test-96 hr Imbalance

Start Date:	14/11/2013 15:00	Test ID:	PR1083/20	Sample ID:	Oils Spill Eater II
End Date:	18/11/2013 16:30	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 117	Test Species:	LT-Lates calcarifer
Comments:					

Dose-Response Plot



Fish Imbalance Test-96 hr Imbalance

Start Date:	14/11/2013 15:00	Test ID:	PR1083/20	Sample ID:	Oils Spill Eater II
End Date:	18/11/2013 16:30	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 117	Test Species:	LT-Lates calcarifer
Comments:					

Auxiliary Data Summary

Conc-%	Parameter	Mean	Min	Max	SD	CV%	N
FSW Control	% Un-affected	95.00	80.00	100.00	10.00	3.33	4
1.3		100.00	100.00	100.00	0.00	0.00	4
2.5		85.00	60.00	100.00	19.15	5.15	4
5		100.00	100.00	100.00	0.00	0.00	4
10		90.00	80.00	100.00	11.55	3.78	4
20		95.00	80.00	100.00	10.00	3.33	4
FSW Control	pH	8.30	8.30	8.30	0.00	0.00	1
1.3		8.30	8.30	8.30	0.00	0.00	1
2.5		8.30	8.30	8.30	0.00	0.00	1
5		8.30	8.30	8.30	0.00	0.00	1
10		8.30	8.30	8.30	0.00	0.00	1
20		8.40	8.40	8.40	0.00	0.00	1
FSW Control	Salinity ppt	35.50	35.50	35.50	0.00	0.00	1
1.3		35.50	35.50	35.50	0.00	0.00	1
2.5		35.50	35.50	35.50	0.00	0.00	1
5		35.50	35.50	35.50	0.00	0.00	1
10		35.50	35.50	35.50	0.00	0.00	1
20		35.60	35.60	35.60	0.00	0.00	1
FSW Control	DO %	110.60	110.60	110.60	0.00	0.00	1
1.3		101.10	101.10	101.10	0.00	0.00	1
2.5		101.40	101.40	101.40	0.00	0.00	1
5		101.50	101.50	101.50	0.00	0.00	1
10		101.10	101.10	101.10	0.00	0.00	1
20		101.30	101.30	101.30	0.00	0.00	1

Fish Imbalance Test-96 hr Imbalance

Start Date:	8/11/2013 12:00	Test ID:	PR1083/21	Sample ID:	Oils Spill Eater II
End Date:	12/11/2013 10:30	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 117	Test Species:	MN-Macquaria novemaculeata
Comments:					

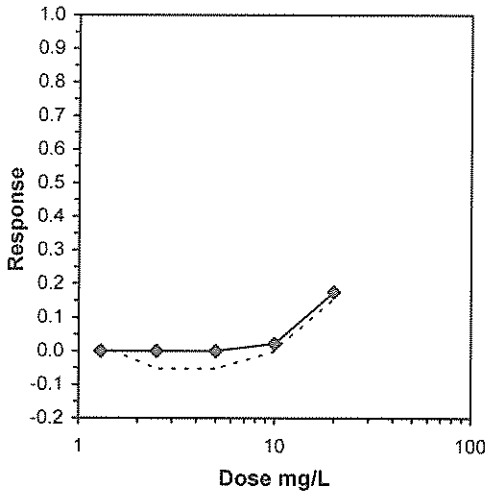
Conc-mg/L	1	2	3	4
FSW Control	1.0000	0.8000	1.0000	1.0000
1.3	0.8000	1.0000	1.0000	
2.5	1.0000	1.0000	1.0000	
5	1.0000	1.0000	1.0000	
10	1.0000	1.0000	0.8000	1.0000
20	0.8000	0.6000	1.0000	

			Transform: Arcsin Square Root					Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Mean	N-Mean
FSW Control	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4	0.9708	1.0000
1.3	0.9333	0.9825	1.2659	1.1071	1.3453	10.861	3	0.9708	1.0000
2.5	1.0000	1.0526	1.3453	1.3453	1.3453	0.000	3	0.9708	1.0000
5	1.0000	1.0526	1.3453	1.3453	1.3453	0.000	3	0.9708	1.0000
10	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4	0.9500	0.9785
20	0.8000	0.8421	1.1128	0.8861	1.3453	20.637	3	0.8000	0.8240

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.861842	0.905	-0.54281	0.656
Equality of variance cannot be confirmed				

Log-Logit Interpolation (200 Resamples)

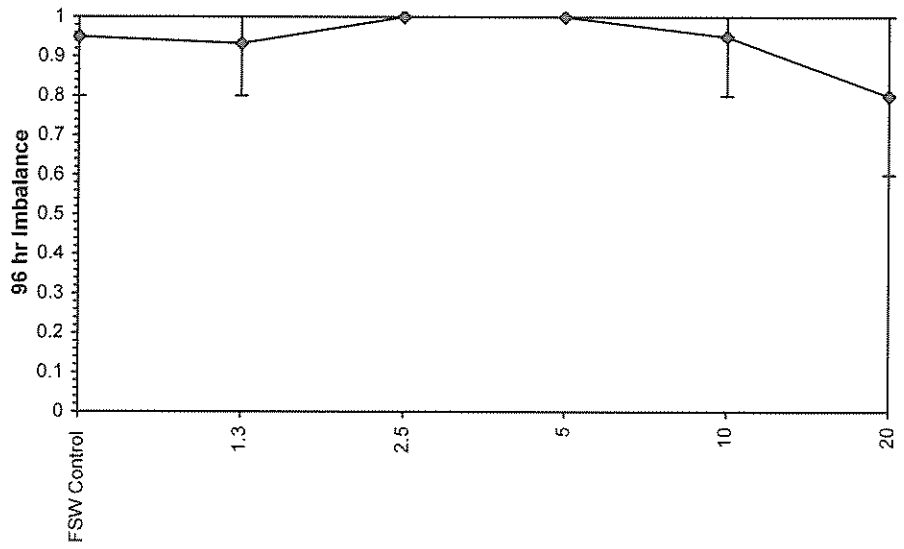
Point	mg/L	SD	95% CL(Exp)	Skew
IC05	12.372			
IC10	15.727			
IC15	18.604			
IC20	>20			
IC25	>20			
IC40	>20			
IC50	>20			



Fish Imbalance Test-96 hr Imbalance

Start Date:	8/11/2013 12:00	Test ID:	PR1083/21	Sample ID:	Oils Spill Eater II
End Date:	12/11/2013 10:30	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 117	Test Species:	MN-Macquaria novemaculeata
Comments:					

Dose-Response Plot



Fish Imbalance Test-96 hr Imbalance

Start Date:	8/11/2013 12:00	Test ID:	PR1083/21	Sample ID:	Oils Spill Eater II
End Date:	12/11/2013 10:30	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 117	Test Species:	MN-Macquaria novemaculeata
Comments:					

Auxiliary Data Summary

Conc-mg/L	Parameter	Mean	Min	Max	SD	CV%	N
FSW Control	% Un-affected	95.00	80.00	100.00	10.00	3.33	4
1.3		93.33	80.00	100.00	11.55	3.64	3
2.5		100.00	100.00	100.00	0.00	0.00	3
5		100.00	100.00	100.00	0.00	0.00	3
10		95.00	80.00	100.00	10.00	3.33	4
20		80.00	60.00	100.00	20.00	5.59	3
FSW Control	pH	8.10	8.10	8.10	0.00	0.00	1
1.3		8.20	8.20	8.20	0.00	0.00	1
2.5		8.20	8.20	8.20	0.00	0.00	1
5		8.20	8.20	8.20	0.00	0.00	1
10		8.20	8.20	8.20	0.00	0.00	1
20		8.20	8.20	8.20	0.00	0.00	1
FSW Control	Salinity ppt	35.30	35.30	35.30	0.00	0.00	1
1.3		35.50	35.50	35.50	0.00	0.00	1
2.5		35.40	35.40	35.40	0.00	0.00	1
5		35.40	35.40	35.40	0.00	0.00	1
10		35.30	35.30	35.30	0.00	0.00	1
20		35.20	35.20	35.20	0.00	0.00	1
FSW Control	DO %	98.30	98.30	98.30	0.00	0.00	1
1.3		99.60	99.60	99.60	0.00	0.00	1
2.5		99.50	99.50	99.50	0.00	0.00	1
5		99.80	99.80	99.80	0.00	0.00	1
10		100.70	100.70	100.70	0.00	0.00	1
20		101.70	101.70	101.70	0.00	0.00	1

Australian Government
Australian Maritime Safety Authority

23 September 2013

Peter Jackson
CMTA International Pty Ltd
158 Garretts Road
Longford
Vic 3851

Dear Peter Jackson

I am pleased to convey that your application for Listing in the National Plan Oil Spill Control Agent (OSCA) Register for the product Oil Spill Eater II has been accepted.

Oil Spill Eater II (OSEII) will be listed as a Bioremediation Agent – Biological (or OBA) Oil Spill Control Agent on the AMSA National Plan website at:
http://www.amsa.gov.au/environment/maritime-environmental-emergencies/national-plan/General-Information/control-agents/list/index.asp

We will also include in the listing links to the MSDS, ecotoxicology reports and other publishable material that you provided as part of your application as these are the information of most significance to likely users under the National Plan.

We also have an option of providing a hyperlink from the OSCA Register page to a product website, and if you wish to take advantage of this, please provide a suitable link to Paul Irving, who I understand you have already been working with during the application process.

Yours sincerely

Toby Stone
GENERAL MANAGER
MARINE ENVIRONMENT DIVISION

Level 5, 82 Northbourne Ave, Braddon ACT 2612
GPO Box 2181, Canberra, ACT 2601
+61 (0)2 6279 5073

APPENDIX B
SECTION 2.3

EVALUATION OF ENVIRO SYSTEM DIVISION OF RESOURCE ANALYSTS, INC. HAMPTON REPORT

OIL SPILL EATER INTERNATIONAL, CORP.

P.O. Box 515429
Dallas, Texas 75251
Ph: (972) 669-3390
Fax: (469) 241-0896
Email oseicorp@msn.com
URL www.osei.us

MARINE TOXICITY TEST SUMMARY
18 Toxicity Tests

OSEI Corporation, i *"Oil Spill Eater II"* is virtually non-toxic, presents the following toxicity tests on salt water , fresh water species, as well as land based species. These tests were performed by the US EPA, Environment Canada, for the South Korea government, and by industry:

The **MYSIDOPSIS BAHIA (or Mysid)** is one of the more sensitive marine organisms found in the oceans. LC50's (Lethal Concentration) is the level in which there is mortality with 50% of the species being tested. The lethal concentration calculated for OSEII on the Mysid was calculated once 10% of the test species showed equilibrium problems or mortality. At 96 hours, only 10% of the test species showed equilibrium problems or mortality at a calculated level of 2100 mg/L or 2,100 parts per million. This shows OSEII to have a low toxicity level, and had a true LC50 been performed the toxicity level would have been even lower.

The **MUMMICHOG (Fundulus Heteroclitus)** a somewhat larger organism (1 to 1.5 inches long) was tested to see how toxic OSEII was to it. 5,258 mg/L was established. 5,285 parts per million shows a very little toxicity for the Mummichog when exposed to Oil Spill Eater II.

OSEI Corporation had two (2) fresh water toxicity tests run also. Environmental Canada, the U.S. EPA's equivalent in Canada, performed a toxicity test on rainbow trout. Rainbow trout are very sensitive fresh water species. The LC50 was greater than 10,000 mg/L. This shows OSEII to have virtually no toxicity in fresh water as well as salt water.

The other fresh water test was run on fathead minnows for the physical engineer in Plano, Texas, USA. We were attempting to prove that hydrocarbons which have had

OSEII applied to them and then washed in the storm drain would not add any toxicity to the storm drain.

Environment Canada performed toxicity tests with OSE II

Two gallons of gasoline was poured onto a low area in a commercial business parking lot, and OSEII was applied, allowed to set 3 minutes, and then washed to another low area for collection.

Approximately 1 ••• gallons of runoff was collected and taken to the lab where a 48 hour fathead minnow survival test was initiated. The resulting LC50 test was 9,300 mg/L which shows that gasoline which has had OSEII applied to it is rendered virtually non-toxic.

This helped alleviate the physical engineer's concerns for adding anything toxic to the storm drain and ultimately to a creek, river or lake.

This test shows that using OSEII would help reduce the toxicity to storm drains from rain water runoff. If OSEII is used periodically to clean the parking lot allowing the site to stay within its NPDES permitted discharge levels.

Sincerely,
Steven Pedigo
Chairman

SP/eem99 OIL SPILL EATER INTERNATIONAL, CORP.

SUMMARY
EPA/NETAC TOXICITY TEST
MYSIDOPSIS BAHIA

The Environmental Protection Agency in Gulf Breeze, Florida tested OIL SPILL EATER II Concentrate, for toxicity using a sensitive species named "Mysidopsis Bahia". This test was in conjunction with Efficacy Tests performed by the EPA and NETAC.

The LC50 for the acute (96 hr.) test was greater than 1,900 and up to 10,000 mg/L which shows OSE II to be virtually non-toxic.

The EPA allowed the use of Inipol during the Valdez Spill and Inipol's LC50 was 135 mg/L which would seem to OSEI, Corp to be somewhat toxic considering Environmental Canada's cut off is 1,000 mg/L.

A second LC50 was performed at 7 days to see if there was any problem with chronic toxicity. The LC50 was 2,500 mg/L, which once again shows OSE II to be virtually non-toxic even when the species was exposed in a closed environment for 7 days. It would be extremely difficult for a species to be exposed to OSE II for 7 days in an open system due to currents, wind and tidal actions.

This 3rd party, U.S. EPA Toxicity Test absolutely proves OSE II is virtually non-toxic.

By: Steven R. Pedigo
Chairman/OSEI, Corp.

SRP/AJL100

OIL SPILL RESPONSE BIOREMEDIATION AGENTS
EVALUATION METHODS VALIDATION TESTING
DISCUSSION OF RESULTS

The following data are provided for the oil spill response bioremediation agent producer as a means to begin to assess how this bioremediation agent may behave in response to an oil spill in the environment.

The Tier II 96-hour toxicity test data was conducted with Mysidopsis bahia test species. Mortality was the single measure response, therefore, survival data were used to calculate the 96-hour LC50. LC50 is the lowest concentration effecting 50% mortality of the test organism during a 96 hour exposure period. Sub-lethal and lethal responses were noted at concentrations between 1,000-10,000 mg/L (> 1,900 mg/L) following acute exposure of M.bahia to your bioremediation product.

Oil Spill Eater II was shown to cause a statistically significant reduction ($p = 0.05$) in the survival of Mysidopsis when animals were exposed during a chronic estimator test for a 7 day period. In general, 7 day exposure (2,500 mg/L) correlated well with values calculated following the 96 hour exposure (> 1,900 mg/L)..NETAC101

TIER II TOXICITY DATA
TABLE 1

ACUTE TOXICITY VALUES FOR 96 HOUR LC_{50} – MYSIDOPSIS BAHIA

LC = Lethal concentration of product that will cause the death of 50% of the test species population within a defined exposure time.
a = LC50 presented as a range of test concentrations since data were from 96-hour acute range-finding test.
b = LC50 presented as a single, numerical value since data were from a definitive 96-hour acute toxicity test.
ND = Not Determined

TABLE 2

CHRONIC TOXICITY VALUES FOR 7 DAY LC_{50} – ***MYSIDOPSIS BAHIA***

NOEC = No Observable Effect Concentration
LOEC = Lowest Observable Effect Concentration
CI = Confidence Interval
NE = No Effect
Fecundity = Egg Production

As we indicated prior and to better understand the data presented above we are including a copy of the Evaluation Methods Manual. The Statistical Method Summary is found in Section 4, Method #8, page 40, of the manual and is intended to help a scientist understand the basis of the experimental objectives developed for this test.

Max. Test
Concentration
(mg/L)
Confidence
Interval

(95%)
96 hour LC50
(mg/L)
Product
1,000-10,000[a]
>1,900[b]
Oil Spill
Eater II
10,000
ND
7 Day LC50
(mg/L)
(95% CI)
Endpoints
(mg/L)
Effects
Measurement
Product

NOEC LOEC

5,700
NE
1,900
1,900
1,900
633
Survival
Growth
Fecundity
2,500(mg/L)
(2,225-3,313)

Oil Spill
Eater II.NETAC102
Static Acute Toxicity of
Oil Spill Eater II, Batch 329,

To the Mysid, Mysidopsis bahia
Study Completed
March 9, 1990
Performing Laboratory
EnviroSystems Division

Resource Analysts, Incorporated
P.O. Box 778
One Lafayette Road
Hampton, New Hampshire 03842

I. SUMMARY

The acute toxicity of Oil Spill Eater II, batch 329 to the mysid, Mysidopsis bahia, is described in this report. The test was conducted for Incorporated for 96 hours during March 5-9, 1990 at the EnviroSystems Division of Resource Analysts, Inc. in Hampton, New Hampshire. It was conducted by Jeanne Magazu, Peter Kowalski, Robert Boeri, and Timothy Ward.

The test was performed under static conditions with five concentrations of test substance and a dilution water control at a mean temperature of 19.5°C. The dilution water was filtered natural seawater collected from the Atlantic Ocean at Hampton, New Hampshire. Aeration was not required to maintain dissolved oxygen concentrations above an acceptable level. Nominal concentrations of Oil Spill Eater II were: 0 mg/L (control), 1 mg/L, 10 mg/L, 100 mg/L, 1,000 mg/L, and 10,000 mg/L. Nominal concentrations were used for all calculations.

Mysids used in the test were less than 5 days old at the start of the test. They were produced at Resource Analysts, Inc. and acclimated under test conditions for their entire life. All mysids were in good condition at the beginning of the study.

Exposure of mysids to the test substance resulted in a 96 hour LC50 of 2,100 mg/L Oil Spill Eater II, with a 95 percent confidence level of 100 – 10,000 mg/L. The 96 hour no observed effect concentration is estimated to be 100 mg/L.

IV. METHODS AND MATERIALS

TEST SUBSTANCE:

Oil Spill Eater II (EnviroSystems Sample Number 2351E) was delivered to EnviroSystems on March 5, 1990. It was contained in a 500 ml plastic bottle that was labeled with the following information: Oil Spill Eater II, Batch 329. The sample was supplied by Incorporated. Prior to use the test material was stored at room temperature. Nominal concentrations were added to test media on a weight/vol basis and are reported as mg/L.

DILUTION WATER:

Water used for acclimation of test organisms and for all toxicity testing was seawater collected from the Atlantic Ocean at EnviroSystems in Hampton, New Hampshire. Water was adjusted to a salinity of 11-17 ppt (parts per thousand) and stored in 500-gallon polyethylene tanks, where it was aerated.

TEST ORGANISM:

Juvenile mysids employed as test organisms were from a single source and were identified using an approximate taxonomic key. They were produced and acclimated at the Resource Analysts, Inc. facility for their entire life. During acclimation mysids were not treated for disease and they were free of apparent sickness, injuries, and abnormalities at the beginning of the test. Mysids were fed newly hatched Artemia salina nauplii (EnviroSystems lot number BS01) once or twice daily before the test.

TOXICITY TESTING:

The definitive toxicity test was performed during March 5-9, 1990. It was based on procedures of the U.S. Environmental Protection Agency (1986, 1987). The test was conducted at a target temperature of 20 ± 2°C with five concentrations of test substance and a dilution water control. A stock solution was prepared by combining 20.0 g of test substance with 2,000 ml of dilution water. The stock solution was added directly to dilution water contained in the test vessels without the use of a solvent. Nominal concentrations of the test material were: 0 mg/L, 10 mg/L, 100 mg/L, 1,000 mg/L, and 10,000 mg/L.

Twenty mysids were randomly distributed among a single replicate of each treatment. The test was performed in 2 liter glass dishes (approximately 25 cm in diameter and 8 cm deep) that contained 1.0 liter of test solution (water depth was approximately 4 cm). Test vessels were randomly arranged in an incubator during the 96 hour test. A 16 hour light and 8 hour dark photoperiod was automatically maintained with cool-white fluorescent lights that provided a light intensity of 40 $eEs^{-1}m^{-2}$. Aeration was not required to maintain dissolved oxygen concentrations above acceptable levels. Mysids were fed newly hatched Artemia salina nauplii once per day during the test.

The number of surviving organisms and the occurrence of sublethal effects (loss of equilibrium, erratic swimming, loss of reflex, excitability, discoloration, or change in behavior) were determined visually and recorded initially and after 24, 48, 72, and 96 hours. Dead test organisms were removed when first observed. Dissolved oxygen (YSI Model 57 meter; instrument number PRL-3), pH (Beckman model pHI 12 meter; instrument number PRL-4), salinity (Labcomp SCT meter, instrument number PRL-6), and temperature (ASTM mercury thermometer; thermometer number 2211) were measured and recorded daily in each test chamber that contained live animals.

STATISTICAL METHODS:

Results of the toxicity test were interpreted by standard statistical techniques. Computer methods (Stephan, 1983) were used to calculate the 96 hour median lethal concentration (LC50). The no observed effect level is the highest tested concentration at which 90% or more of the exposed organisms were unaffected.

V. RESULTS

No insoluble material was observed in any test vessel during the test. Biological and water quality data generated by the acute toxicity test are presented in Table 1 and Appendix A, respectively. One hundred percent survival occurred in the control exposure.

The dose – response curve for organisms exposed to the test substance for 96 hours is presented in Figure 1. Exposure of mysids to the Oil Spill Eater II, batch 329, resulted in a 96 hour LC50 of 2,100 mg/L, with a 95 percent confidence interval of 100 – 10,000 mg/L. The 96 hour no observed effect concentration is estimated to be 100 mg/L.

Table 1. Survival data from toxicity test

Nominal Concentration	Number Alive					Number Affected				
(mg/L)	0hr	24hr	48hr	72hr	96hr	0hr	24hr	48hr	72hr	96hr
0 (control)	1	10	10	10	10	0	0	0	0	0
1	1	10	10	9	9	0	0	0	0	0
10	1	10	10	9	9	0	0	0	0	0
100	1	10	10	10	9	0	0	0	0	0

1,000 1 10 9 9 8 8 0 0 0 0 0
10,000 1 10 0 0 0 0 0 - - - -

TOXICITY TEST
FOR ARTEMIA SALINA

To gain acceptance on the U.S. EPA's National Contingency Plan List, we were requested to perform an additional Toxicity Test on Artemia Salina using EPA's Standard Dispersant Toxicity Test.
OSE II Concentrate was presented to the laboratory, but the laboratory refers to the product as a Dispersant instead of OSE II throughout the write-up, since it was a Dispersant Toxicity Test. The Test proved that OSE II Concentrate is once again virtually non-toxic. This particular test proved OSE II helps to detoxify oil. The fuel oil had a higher toxicity rate than did the fuel and OSE II, which shows OSE II to immediately starts reducing the toxicity of hydrocarbons once OSE II is applied. The fuel oils toxicity was 12.4 ppm, and the fuel oil and with OSE II applied showed a drop in the fuel oils toxicity to 29.4, over a 100 percent reduction of the toxicity of the fuel oil. This shows real value in utilizing OSE II since the toxicity of the spilled contaminant would be reduced immediately lesoning the impact of a spill to the associated environment and marine species.
OSE II gained acceptance to the EPA's National Contingency Plan once this test was presented to the EPA.

By: Steven R. Pedigo
Chairman, OSEI, Corp.

Standard Dispersant Toxicity Test with the

OSE II, Batch #9820 and Artemia salina

Authors
Timothy J. Ward
Robert L. Boeri
Performing Laboratory
EnviroSystems Division
Resource Analysts, Incorporated
P.O. Box 778
One Lafayette Road
Hampton, New Hampshire 03842
October, 1990

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IV. INTRODUCTION

The objective of the study was to determine the acute toxicity of the dispersant – Batch # 9820, No. 2 fuel oil, and a 1:10 mixture of dispersant and oil to *Artemia salina*, a marine invertebrate. The report contains sections that describe the methods and materials employed in the study, and the results of the investigation. The report also contains an appendix that presents the water quality data collected during the tests.

V. METHODS AND MATERIALS

TEST SUBSTANCE:

The dispersant – Batch # 9820 (EnviroSystems Sample Number 2591E) was delivered to EnviroSystems on August 17, 1990. It was contained in two 1,000 ml plastic bottles that were labeled with the following information: "Batch # 9820". The No. 2 fuel oil (EnviroSystems Sample Number 2599E) was delivered to EnviroSystems on August 28, 1990. It was contained in a 1,000 ml plastic bottle that was labeled with the following information: "# 2 fuel oil".

DILUTION WATER:

Water used for hatching and acclimation of test organisms and for all toxicity testing was formulated at EnviroSystems in Hampton, New Hampshire. Water was diluted to a salinity of 20 parts per thousand and stored in polyethylene tanks where it was aerated.

TEST ORGANISM:
Juvenile *Artemia salina* employed as test organisms were from a single source and were identified using an appropriate taxonomic key. *Artemia salina* used in the test were produced from an in-house culture and were 24 hours old at the start of the test. Prior to testing, *Artemia salina* were maintained in 100% dilution water under static conditions. During acclimation *Artemia salina* were not treated for disease and they were free of apparent sickness, injuries, and abnormalities at the beginning of the test. They were not fed before or during the tests.
TOXICITY TESTING:
Screening tests with the test substances were conducted during October 1 to 3, 1990. The definitive toxicity tests were performed with the dispersant, No. 2 fuel oil, a 1:10 mixture of dispersant and oil, and the standard toxicant, dodecyl sodium sulfate during October 3 to 5, 1990, according to procedures of the U.S. EPA (1984). The tests were conducted at a target temperature of 20 ± 1°C with five concentrations of each test substance and a dilution water control.

The dispersant and oil stock solutions were prepared by combining 550 ml of sea water and 0.55 ml of test substance in a glass blender jar and mixing the solution at 10,000 rpm for 5 seconds. The combined dispersant and oil stock solution was prepared by mixing 550 ml of sea water at 10,000 rpm and adding 0.5 ml of oil and 0.05 ml of dispersant. This combined mixture was then mixed for 5 seconds. Nominal concentrations of each test material were: 0 mg/L (control), 10 mg/L, 25 mg/L, 40 mg/L, 60 mg/L, and 100 mg/L. Media in each test vessel was added at the beginning of the test and not renewed.
Twenty *Artemia salina* were randomly distributed to each of 5 replicates of each treatment. The tests were performed in 250 ml glass Carolina culture dishes that contained 100 ml of test solution (water depth was approximately 2.5 cm). Test vessels were randomly arranged in an incubator during the 48 hour test. A 24 hour light and 0 hour dark photoperiod was maintained below the dishes. Aeration was not required to maintain dissolved oxygen concentrations above acceptable levels. *Artemia salina* were not fed during the tests.
The number of surviving organisms was determined visually and recorded initially and after 24 and 48 hours. Dead test organisms were removed when first observed. Dissolved oxygen (YSI Model 57 meter; instrument number PRL-18), pH (Beckman model pHI 12 meter; instrument number PRL-4), salinity (Refractometer, instrument number PRL-6), and temperature (ASTM mercury thermometer; thermometer number 2211) were measured and recorded at the beginning and end of each test in one test chamber of each concentration.
STATISTICAL METHODS:
Results of the toxicity test were interpreted by standard statistical techniques (Stephen, 1983). The binomial method was used to calculate the median lethal concentration (LC50) values.

VI. RESULTS

All test vessels containing dispersant appeared clear throughout the test and all test vessels containing oil or oil and dispersant had an oil slick on the surface of the test media throughout the

test. Biological and water quality data generated by the acute toxicity tests are presented in Table 1 and Appendix A, respectively. Ninety-nine percent survival occurred in the control exposure. The 48 hour LC50 for *Artemia salina* exposed to the reference toxicant dodecyl sodium sulfate is 38.7 mg/L.

The 24 and 48 hour LD50s from the three toxicity tests are presented in Table 2. The 48 hour LC50s for *Artemia salina* exposed to the test substances are: dispersant/OSE II - >100 mg/L, No. fuel oil – 12.6 mg/L (95% confidence interval = 10.0 – 25.0 mg/L), and a 1:10 mixture of dispersant/OSE II and

No. 2 fuel oil – 29.4 mg/L (95% confidence interval = 25.0 – 40.0 mg/L).

Table 1. Survival data from toxicity tests

Number Alive

Nominal Concentration (mg/L)	Dispersant/OSE II rep.	0hr	24hr	48hr	No. 2 fuel oil 0hr	24hr	48hr	Oil + Dispersant/OSE II 0hr	24hr	48hr
0 (control)	1	20	20	20	20	20	20	20	20	20
	2	20	20	19	20	20	19	20	20	20
	3	20	20	20	20	20	20	20	20	20
	4	20	20	20	20	20	20	20	20	20
	5	20	20	20	20	20	20	20	20	20
10	1	20	19	17	20	20	17	20	20	19
	2	20	20	17	20	20	19	20	20	18
	3	20	20	20	20	20	12	20	18	18
	4	20	20	19	20	20	9	20	20	17
	5	20	19	18	20	18	10	20	20	16
25	1	20	20	16	20	18	0	20	19	19
	2	20	19	17	20	19	3	20	18	15
	3	20	20	18	20	19	2	20	20	16
	4	20	19	12	20	20	2	20	20	17
	5	20	19	15	20	20	0	20	19	14
40	1	20	19	16	20	20	0	20	19	0
	2	20	20	14	20	19	0	20	20	0
	3	20	20	19	20	20	0	20	20	0
	4	20	20	15	20	18	0	20	14	0
	5	20	20	17	20	17	0	20	18	2
60	1	20	19	18	20	18	0	20	18	0
	2	20	19	16	20	19	0	20	19	0
	3	20	19	19	20	16	0	20	19	0
	4	20	20	17	20	19	0	20	16	0
	5	20	20	16	20	14	1	20	16	1
100	1	20	20	18	20	13	0	20	20	0
	2	20	20	18	20	8	0	20	20	0
	3	20	19	13	20	9	0	20	20	0
	4	20	20	19	20	10	0	20	20	0
	5	20	20	16	20	8	0	20	20	0

VII. REFERENCES

Stephen, C.E. 1983. Computer program for calculation of LC50 values. Personal communication.

U.S. EPA. 1984. Revised Standard Dispersant Toxicity Test. Federal Register, Volume 49, Number 139, Wednesday, July 18, 1984, pages 29204 to 29207.

Appendix A. WATER QUALITY DATA FROM TOXICITY TESTS

I. Summary

The acute toxicity of the dispersant – Batch #9820, No. 2 fuel oil, and a 1:10 mixture of dispersant/OSE II and No. 2 fuel oil to *Artemia salina*, is described in this report. The test was conducted for OSEI corp for 48 hours during October 3 to 5, 1990, at the EnviroSystems Division of Resource Analysts, Inc. in Hampton, New Hampshire.

The test was performed under static conditions with five concentrations of each test substance and a dilution water control at a temperature of 20 ± 1◦C. The dilution water was sea water adjusted to a salinity of 20 parts per thousand. Aeration was not employed to maintain dissolved oxygen concentrations above an acceptable level. Nominal concentrations of all three test substances were: 0 mg/L (control), 10 mg/L, 25 mg/L, 40 mg/L, 60 mg/L and 100 mg/L. Nominal concentrations were used for all calculations.

Artemia salina used in the test were 24 hours old at the start of the test and they were all in good condition at the beginning of the study. Exposure of *Artemia salina* to the test substances resulted in the following 48 hours median lethal concentrations (LC50): dispersant/OSE II >100 mg/L, No. 2 fuel oil – 12.6 mg/L (95% confidence interval = 10.0- 25.0 mg/L), and a 1:10 mixture of dispersant/OSE II and No. 2 fuel oil-29.4 mg/L (95% confidence interval = 25.0 – 40.0 mg/L).

SUMMARY
ENVIRONMENT CANADA'S TOXICITY TEST

Environmental Canada performs five (5) Toxicity Tests for determining if a product could gain approval for use in Canada. The level that is considered toxic is 1,000

APPENDIX B
SECTION 2.4

EVALUATION OF ENVIRONMENTAL TECHNOLOGY CENTER, ONTARIO, CANADA REPORT

U.S. EPA. 1984. Revised Standard Dispersant Toxicity Test. Federal Register, Volume 49, Number 139, Wednesday, July 18, 1984, pages 29204 to 29207.

Appendix A. WATER QUALITY DATA FROM TOXICITY TESTS

I. Summary

The acute toxicity of the dispersant – Batch #9820, No. 2 fuel oil, and a 1:10 mixture of dispersant/OSE II and No. 2 fuel oil to *Artemia salina*, is described in this report. The test was conducted for OSEI corp for 48 hours during October 3 to 5, 1990, at the EnviroSystems Division of Resource Analysts, Inc. in Hampton, New Hampshire.

The test was performed under static conditions with five concentrations of each test substance and a dilution water control at a temperature of 20 ± 1°C. The dilution water was sea water adjusted to a salinity of 20 parts per thousand. Aeration was not employed to maintain dissolved oxygen concentrations above an acceptable level. Nominal concentrations of all three test substances were: 0 mg/L (control), 10 mg/L, 25 mg/L, 40 mg/L, 60 mg/L and 100 mg/L. Nominal concentrations were used for all calculations.

Artemia salina used in the test were 24 hours old at the start of the test and they were all in good condition at the beginning of the study. Exposure of *Artemia salina* to the test substances resulted in the following 48 hours median lethal concentrations (LC50): dispersant/OSE II >100 mg/L, No. 2 fuel oil – 12.6 mg/L (95% confidence interval = 10.0- 25.0 mg/L), and a 1:10 mixture of dispersant/OSE II and No. 2 fuel oil-29.4 mg/L (95% confidence interval = 25.0 – 40.0 mg/L).

SUMMARY
ENVIRONMENT CANADA'S TOXICITY TEST

Environmental Canada performs five (5) Toxicity Tests for determining if a product could gain approval for use in Canada. The level that is considered toxic is 1,000

mg/L or less. A product that exceeds this level is deemed acceptable. The higher the number the less toxic.
Oil Spill Eater II Concentrate, tested at 10,000 mg/L – on Rainbow Trout (**Oncorhynchus mykiss)** which shows OSE II is virtually non-toxic and far exceeds the level deemed to toxic by Environment Canada.
Rainbow Trout is one of the most sensitive fresh water organisms to test.

Environment Canada tested OSE II on water fleas (Dahnia magna) as well the LC 50 was > than 10,000 ppm million showing that OSE II would not be toxic to intertidal zone species.

The next three (3) test Environment Canada performed is interesting since it is tests to see if a product would adversely effect single celled bacteria living in intertidal zones. The reason it is interesting is the fact that Environment Canada performed the same efficacy test on OSE II as the US EPA established with NETAC to determine if products could remediate oil, so a product could then be placed on the US EPA National contingency Plan approved list. This test also determined the number of bacteria OSE II/a product could colonize/enhance/grow as well. If a product enhances or grows bacteria then there is little chance it will be toxic to bacteria, so to perform a bacteria toxicity test is interesting. Environment Canada's test was performed on bacteria photobacterium phosphoreum for .5 (30 minutes), the LC 50 for this time was 5209 mg/l for .25 (15 minutes) which had an LC 50 of 5474 mg/l and .083 (4.98 minutes) which had an LC 50 of 7952 mg/l. These varied timed toxicity test further shows OSE II is non toxic to even single celled bacteria, therefore the likely hood of being toxic to any species would be minimal, since single celled bacteria are more susceptible to toxins than larger species.

OSE II proved that even with third party testing by a Foreign Government, OSE II is virtually non-toxic.

By: Steven R. Pedigo
Chairman/OSEI, Corp.121

Environment Canada
Conservation and PotetionEmergencies Science Division
River Road Environmental Technology Centre
3439 River Road

Ottawa, Ontario K1A 0H3
May 17, 1993 4808-13-7

Steven R. Pedigo, Chairman,
OSEI Corporation
5545 Harvest Hill
Suite 1116
Dallas, TX 75230
U.S. A.

Dear Mr. Pedigo,

Thank-you for participating in the development of Environment Canada's draft guidelines for assessing the toxicity and effectiveness of oil spill bioremediation agents (OSBAs).

The Tier I toxicity testing is now complete. Our preliminary screening has indicated that the *Daphnia magna* test and the Microtox test were either insensitive or erratic. Therefore, we do not consider these particular tests useful for OSBA evaluation. Comments on the toxicity of your product will thus be limited to those obtained using the 96-hour Rainbow Trout acute lethality test. 'Oil Spill Eater II' had a rainbow trout 96-hour LC50 of greater than 10,000 mg of application solution per litre of water. There was, however, a 23% mean fish mortality at this concentration. Also note that between 24 and 96 hours of exposure to the product, sublethal effects were present. The fish were noted to surface, be on their side, turn dark, exhibit rapid breathing and no swimming. These sublethal effects should be of concern. The effectiveness test analyses are still being performed. You will be notified as soon as those results are available.

If your product meets both the effectiveness and toxicity criteria it will be placed on our Standard List of Oil Spill Bioremediation Agents. Placement on this list is not an indication that the product will be used in the event of an oil spill. The list and test results are public information. They may be provided to oil spill response personnel to enable them to make informed decisions.

Please take note that the placement of a product on our Standard List does not constitute an approval or certification or licensing of your product for use in Canada. Your product may be required to comply with the New Substances Notification Regulations (NSNR) for biotechnology products under the Canadian Environmental Protection Act (CEPA). For information on the draft regulations, please contact the Chief of the New Substances Division at (819) 997-4336 or at the following address: Chief, New Substances Division, CCB, Environmental Canada, P.V.M. 14th Floor, Ottawa, Ontario, K1A 0H3, CANADA.

Sincerely,
Merv Fingas
Chief, Emergencies Science Division

ENVIRONMENT CANADA
TIER I TOXICITY TESTING
FOR EVALUATION OF DRAFT OSBA GUIDELINES

The testing was performed as follows. An application solution of the OSBA was prepared based on instructions provided by the manufacturer/supplier. The highest strength of solution tested was 10,000 mg of application solution per litre of water (approx. a 1:100 dilution). For products in which solids are normally added to the water, suspensions comprised of 10,000 mg of product/combined product per litre of water were prepared for use in the toxicity tests. (If several solids were to be added, they were combined in the appropriate ratio). This initial screening concentration was tested in triplicate. If this concentration was toxic to greater than 50% of the organisms, lower concentrations were tested. Sub-lethal effects on the behavior and/or appearance of the organisms were also made. The toxicity of the product in water was assessed using each of the following three biological test methods, developed and standardized by Environment Canada for these and other applications:

Oil Properties
Brochure
Spilltox

Chemical Synonyms PPA Instruments Tanker Spills

Spilltox

OILSPILL EATER II

Aliases
OSEII

Species Latin Name
Test Length (h)

Test Endpoint	Qualifier	Toxicity Value	Units of Measurement
Daphnia magna	>	10000	mg/L
48			
LC50			
Oncorhynchus mykiss	>	10000	mg/L
96			
LC50			
Photobacterium phosphoreum	=	5109	mg/L
.5			
IC50			
Photobacterium phosphoreum			
.25			
IC50			

=

5474
mg/L

Photobacterium phosphoreum
.083
IC50

=

7952
mg/L

Environment Canada, 1990a. **Biological test method: acute lethality test using rainbow trout.** Environment Canada, Conservation and Protection, Ottawa, Ontario. Report EPS 1/RM/9, 51 pp.
Environment Canada, 1990b. **Biological test method: acute lethality test using *Daphnia* spp.** Environment Canada, Conservation and Protection, Ottawa, Ontario. Report EPS 1/RM/11, 57 pp.
Environment Canada, 1992. **Biological Test method: toxicity test using luminescent bacteria (*Photobacterium phosphoreum*).** Environment Canada, Conservation and Protection, Ottawa, Ontario. Report EPS
1/RM/24, 61 pp.
May 17, 1993123 OIL SPILL EATER INTERNATIONAL, CORP.

TOXICITY TEST SUMMARY USINGCITGO GASOLINE, OIL SPILL EATER II AND FATHEAD MINNOWS

APPENDIX B
SECTION 2.5

EVALUATION OF BIO-AQUATIC TESTING INC. REPORT

TCEQ NELAP Certified

LDEQ LELAP Certified

Bio-Aquatic Testing
2501 Mayes Rd
Suite 100
Carrollton, TX 75006
(972) 242-7750

Bioremediation Agent Effectiveness Test

Oil Spill Eater II

Oil Spill Eater International, Corp.

June 25, 2009

Prepared by: _____ 6/26/2009
Vice President **Date**

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Appendix III Gravimetric Results Statistical Analysis Computer Printouts

EXECUTIVE SUMMARY

Bio-Aquatic Testing, Inc. located at 2501 Mayes Rd. Suite 100 Carrollton, Texas 75006 was contracted by Oil Spill Eater International, Corp. (OSEI) to test effectiveness of their bioremediation product, Oil Spill Eater II, using Environmental Protection Agency (EPA) protocol listed in 40 CFR Chapter 1 (7-1-99) Pt. 300 Appendix C, Item 4.0. The test protocol calls for application of products onto ANS 521 oil. The product was applied to test flasks according to manufacturer's specifications. Samples were sacrificed on Day 0, Day 7, and Day 28 of the test period. Day 0 and Day 7 samples were sampled for microbiological analysis and then frozen at –10° C until GC/MS results were known for the Day 28 samples. Each replicate of product and control were tested for continued microbiological viability over time, reduction in weight via gravimetric analysis, and reduction in alkane and/or aromatic constituents via Gas Chromatography/Mass Spectroscopy (GC/MS). The product was deemed effective if the data showed the GC/MS product results for Day 28 treatments to be statistically less than the Day 28 controls and Day 28 treatments to be statistically less than Day 0 treatments.

GC/MS data for Days 0, 7, and 28, were consolidated and analyzed with the Minitab Statistical program 13.3. Data was analyzed for a significant difference between controls and treatments (products) using a General Linear ANOVA Model with Dunnett's and/or Tukey's means comparison test. GC/MS analysis showed significant reduction of both alkane and aromatic constituents of the test oil as indicated by the statistically significant difference between the Day 28 controls and Day 28 treatments as well as between Day 0 control and Day 28 treatments. Day 7 results also showed a statistically significant reduction of treatments as compared to controls.

The surrogate compounds, d-10 phenanthrene and 5-α androstane showed recovery percentages which indicates the test meets acceptability criteria and is considered valid.

Microbiological results showed continued viability of the oil-eating microorganisms over time. Day 7 and Day 28 gravimetric analysis showed a statistically significant reduction from the controls to the treatments.

Based on the parameters of this test, the product should be included on the NCP list of approved bio-remediation products.

BIOREMEDIATION AGENT EFFECTIVENESS TEST USING OSEI CORP. PRODUCT "Oil Spill Eater II"

Introduction

The bioremediation agent effectiveness testing protocol is designed to determine a product's ability to biodegrade oil by quantifying changes in the oil composition resulting from biodegradation. The protocol quantifies the disappearance of saturated hydrocarbons and polynuclear aromatic hydrocarbons (PAHs) as well as weight loss. The protocol also tests for microbial activity over time to ascertain continued viability of oil degrading microorganisms.

Summary of Method

The protocol calls for gas chromatography/mass spectrophotometry and gravimetric analyses to quantify saturated hydrocarbons and PAHs, and determine weight loss respectively. The sample preparation procedure extracts the oil phase into dicloromethane (DCM), with a subsequent distillation to 1-3-mL using a K-D apparatus and Snyder column. To effectively accomplish the goals of the testing protocol, it is necessary to normalize the concentration of the various analytes in oil to a non-biodegradable marker, either C_2- or C_3 – phenanthrene, C_2 –chrysene, or hopane. The test method targets the relatively easy to degrade normal alkanes and the more resistant and toxic PAHs. It normalizes their concentrations to C_2 or C_3 phenanthrene, C_2 –chrysene, or $C_{30}17\alpha$(H), 21β (H)-hopane on an oil weight basis (mg marker/kg oil, mg target analyte/kg). The analytical technique uses a high-resolution gas chromatography/mass spectrophotometer (GC/MS) because of its high degree of chemical separation and spectral resolution. GC/MS has long been used to study the weathering and fate of oil spilled into the environment. For quantitative analyses, the instrument is operated in the selective ion detection mode (SIM) at a scan rate of greater than 1.5 scans per second to maximize the linear quantitative range and precision of the instrument. The sample preparation method does not exclude analysis of selected samples by GC/MS in the full scanning mode of operation to qualitatively assess changes in the oil not accounted for by the SIM approach. Gravimetric analysis is used to support the GC/MS analysis by measuring weight loss of samples over time as compared to controls by drying the extracted samples using nitrogen a blowdown technique.

Performed concurrently with the chemical analysis described above is a microbiological analysis. The microbiological analysis is performed to determine and monitor the viability of relative concentrations of the microbial cultures being studied. Using this method, continued viability is measured over time by comparing serial dilutions of microorganisms, to determine statistical significance between treatments and controls.

MATERIALS AND METHODS

The following methods* were obtained from 40 CFR Chapter 1 (7-1-99) Pt. 300 Appendix C, item 4.0 Bioremediation Effectiveness test, as submitted by the Environmental Protection Agency. Some modifications were made to these methods as discussed below.

The procedure consists of an experimental orbital shaker flask setup using 250-mL Erlenmeyer flasks labeled with unique identifiers using the following treatment design:

Table 1.
*Details from these methods can be found in the aforementioned 40 CFR Chapter 1 (7-1-99) Pt. 300 Appendix C, item 4.0. A copy is available upon request.

Treatment	Number of samples at sampling times			Total number of analytical determinations		
	Day 0	Day 7	Day 28	ANALYSES		
				Microbial counts	Gravimetric	GC/MS
Control	3	3	3	9	9	9
Nutrient	3	3	3	9	9	9
Oil Spill Eater II	3	3	3	9	9	9

Number of replicates per treatment or control per sampling event - 3
Number of replicates per treatment or control – 9
Total replicates - 27
Control - Oil + Seawater
Nutrient – Oil + Seawater + EPA Nutrient
Oil Spill Eater II – Oil + Seawater + Product

Using sterile technique, each appropriately labeled replicate flask has 100-mL of seawater added. The seawater obtained was from the Gulf of Mexico by faculty at LSU. Each flask is placed on a balance and the weight recorded. Approximately one half-gram (0.5 g) of artificially weathered oil (Alaska North Slope 521)* is then added to each flask while still on the balance and the weight recorded again.

*The ANS 521 oil was obtained from John Haines of the Environmental Protection Agency's Environmental Monitoring and Support Laboratory, Cincinnati, Ohio, 45268

The control flasks were prepared by adding oil to the natural seawater.

The nutrient flasks were prepared as instructed in 40 CFR Chapter 1 (7-1-99) Pt. 300 Appendix C.

The product mix was prepared according to the manufacturer's instructions. The product was applied to each oil + product flask at a ratio of 10:1 (V/V).

After preparing all treatments and controls, three replicates of each treatment and control were shaken on an orbital shaker at 190 – 200 rpm and incubated at 20° C until sacrificed for the Day 0, 7, and 28 analyses. At each sampling (sacrifice) day, a 0.5-mL aliquot was set aside for microbiological analysis and the remaining solution is prepared for chemical analysis.

A phosphate buffer solution was made from a recipe obtained from Jan Kurtz of the Microbial Ecology Branch of the Environmental Protection Agency's Gulf Breeze Ecology Division. A 0.5-mL aliquot from each replicate was added to a test tube containing 4.5-mL of a sterile phosphate buffer for the microbiological analysis. Aseptic technique was then used to make serial dilutions down to a 10^{-8} dilution. Microtiter plates were prepared by adding 1.75-mL of Bushnell-Haas broth into to each well. Six replicates per dilution are used per treatment or control giving a total of forty-eight wells, (48) per treatment or control. Each of the wells was inoculated with 0.1-mL of solution from each of the serial dilutions made from the original aliquot of 0.5-mL of sample. 20 µl of sterile No. 2 fuel oil was then carefully placed on top of the solution in each well. Each microtiter plate was then incubated for fourteen (14) days at 20° C. At the conclusion of the fourteen-day incubation period, 100 µl of p-iodotetrazolium violet dye was added to each well and the results were recorded after at least 45 minutes to 2 hours of reaction time. Appearance of a pink to purple color constituted a positive test (continued microbial viability).

Each replicate sacrificed was extracted with an initial volume of 50-mL dichloromethane (DCM) for the chemical analysis. The sample was first extracted three times with 10-mL aliquots of the DCM. The remaining 20-mL was used to rinse the separatory funnel and added to the first 30-mL of extract. Just prior to the initial extraction, each replicate is spiked with 100 µl of a surrogate-recovery standards stock solution. This stock solution was made up of 500 mg/L 5α-androstane and d_{10}-phenanthrene. The separatory funnel was then capped and shaken vigorously for approximately thirty seconds to insure good mixing between phases. After mixing, the separatory funnel was allowed to sit for up to three hours to insure the greatest amount of separation between phases. This was done because of the presence of thick emulsions caused by microbiological activity. After a period of up to three hours, a 10-mL aliquot of the extract is poured into a 40-mL amber vial with a Teflon™ lined cap, and taped with Teflon™ tape. The samples were then stored in a 4° C walk-in refrigerator until retrieval for gravimetric analysis. The extraction was completed by filtering the remaining 40ml of DCM through a glass filter containing 20 grams of anhydrous sodium sulfate (Na_2SO_4) and into a 250-ml flat-bottom distillation flask. The Na_2SO_4 was rinsed with DCM until all traces of oil were removed from the funnel. The 250-ml flat-bottom distillation flask was placed on a Rotovap distillation unit until a volume of 10-ml was attained. Approximately 50-ml of hexane was added to the DCM extract and distilled to a volume of 10-ml. Another 50-ml of hexane was added to the hexane extract and distilled down to a final volume of 10-ml. A 1-ml aliquot of the final extract was removed and prepared for analysis on the GC/MS.

The gravimetric analysis was accomplished by first weighing an empty 40-mL vial and recording the weight. The 10-mL aliquot of extract was then placed in the vial, weighed and concentrated to dryness using a nitrogen gas blowdown technique. The remaining sample was then weighed and subjected to nitrogen blowdown for another ten to fifteen minutes. This was repeated once more to insure that the weight had changed no more than 5% weight difference between the second and third blowdown. If there was greater than a 5% difference, the sample was subjected a final blowdown to insure complete dryness. Weights were recorded after each blowdown, and then subjected to statistical analysis discussed below.

*The GC/MS analysis was subcontracted to Louisiana State University-IES, 42 Atkinson Hall, Baton Rouge, Louisiana, 70803.

STATISTICAL METHODS

GC/MS Data

Surrogate-adjusted data or rank-transformed surrogate adjusted data were analyzed using the Minitab™ 13.3 program. The computer program, unlike many others, is powerful enough to analyze unbalanced sets (uneven replication) of data using a general linear multiple factor ANOVA model. The probability of a type I error (α) was set apriori to 0.05.

Data sets were first analyzed for normality using the Anderson-Darling Goodness of Fit test. This test compares plot points with the normal theoretical distribution. Minitab calculates the statistic, above which there is a danger of non-normality. This is then compared to the chosen (preset by program), alpha (α) level of 0.01. For least-squares estimation, Minitab calculates a Pearson correlation coefficient. If the distribution fits the data well, then the plot points on a probability plot will fall on a straight line. The correlation measures the strength of the linear relationship between the X and Y variables on a probability plot. The correlation will range between 0 and 1, with higher values indicating a better fitting distribution.

Data passing a formal test for normality may not, strictly speaking, come from a normal distribution. Data that has sufficient linearity as shown by the passing results of a formal test for normality, may have attributes that weaken the ANOVA and Dunnett's test's ability to detect statistically significant differences between treatments (Zar, 1984).

Routine transformations were not amenable to non-normal data so an acceptable procedure for multiple-comparison ANOVA was found by using the rank-transformation test (Helsel, 1993). This technique first rank transforms the data and subjects it to the same multiple factor ANOVA test. This allows for an acceptable multiple comparison non-parametric test. After the program calculated the "F" and "P" statistics, the data were automatically subjected to Dunnett's means comparison test for comparison between treatments and controls.

Tables below give the final adjusted P-Values. Values of less than 0.05 (chosen α) indicate statistical significance. The T-Value is a ratio of the Difference of Means and Standard Error of Difference and indicates the degree and direction of the difference.

Microbiological Data

Microbiological data was analyzed with the Environmental Protection Agency's Most Probable Number Calculator, designed by the Risk Reduction Engineering Laboratory, Cincinnati, Ohio. This program calculates the most probable number (mpn) per mL with Salama correction for bias, and a Spearman-Karber Estimate. The program is based on the number of positive reactions in each of six replicates per serial dilution made. Confidence limits are included in the output of the program.

Gravimetric Data

Gravimetric data were analyzed with a simple two sample t-test available on the Minitab™ 13.3 program which compares the Day 0, 7, or 28 control means with their respective treatment means for statistical significance. The calculated p-Value is then compared to the chosen alpha (α) level of 0.05, as in the ANOVA analysis above. If the calculated value exceeds the 0.05, there is no statistical significance.

RESULTS AND DISCUSSION

GC/MS Data

Results of the statistical analysis for the surrogate-adjusted data are reported and discussed below. Results for transformed data, if transformations were necessary, are discussed last, preceded by the non-transformed data. Actual data (raw followed by surrogate-adjusted) are presented in the Appendices. GC/MS spectra appear in APPENDIX I along with computer printouts of the Minitab™ ANOVA analysis discussed below, which appear in APPENDIX II.

OSEI CORP. "OIL SPILL EATER II" Product Solution

Surrogate-Adjusted Alkane Data

Preliminary analysis of surrogate-adjusted alkane data for normality (fig.1) showed the raw data to be non-normal with an Anderson-Darling P-statistic of 0.000. This is below the selected α-level of 0.01 and indicates the data are not normally distributed. Further visual evidence of the data's non-linearity can be seen in the probability plot for residuals of the data (fig.2). The data were rank-transformed and reanalyzed for normality (fig.3) giving an Anderson-Darling statistic of 0.585, well above the chosen α-level of 0.01. The probability plot for the residuals (fig. 4) of the data still show a small degree of non-linearity which can slightly lower the ANOVA and Dunnett's test ability to detect a statistical difference between treatments and controls. More on this subject is discussed in the conclusions.

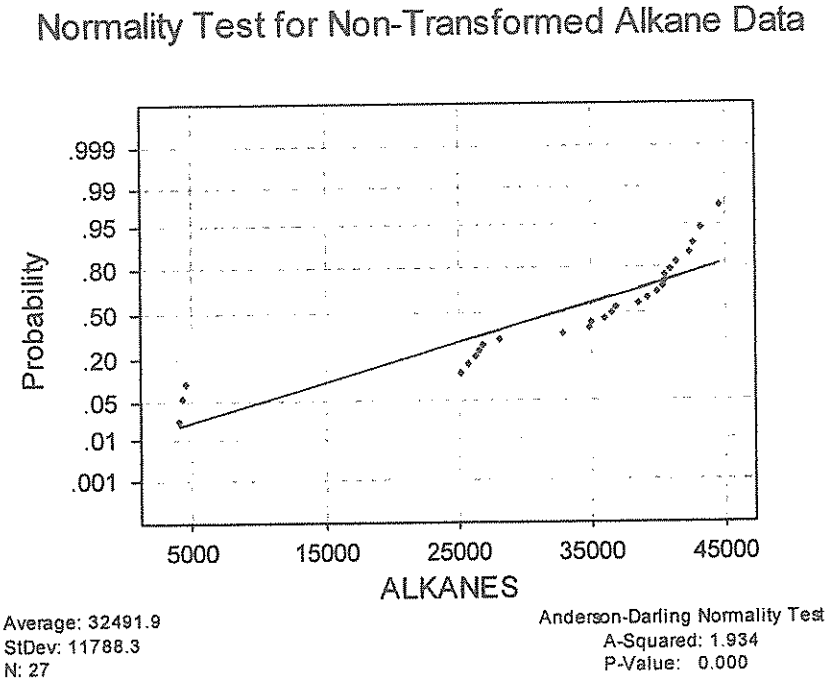

Figure 1. – Anderson-Darling test for normality showing non-linearity of surrogate adjusted alkane data.

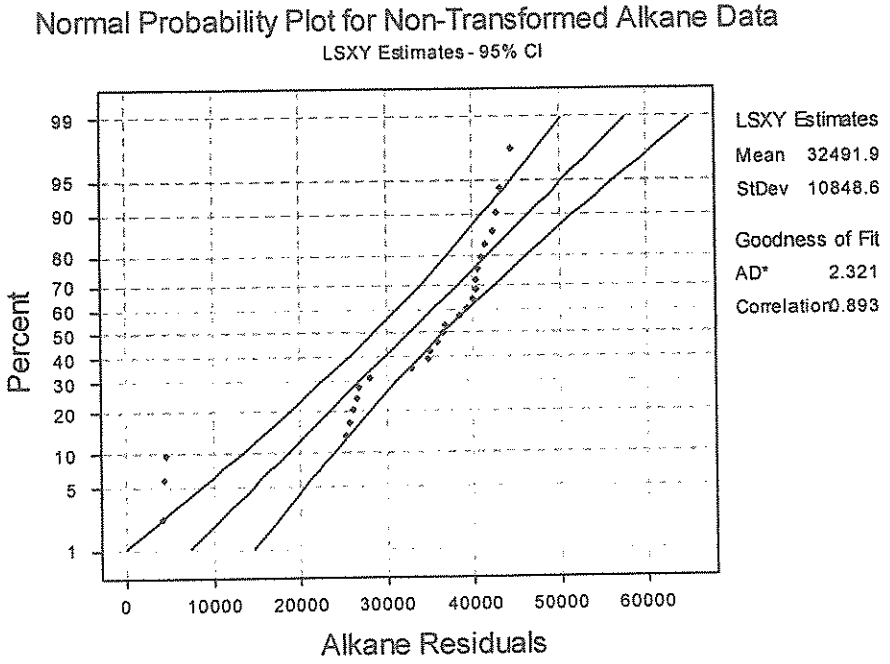

Figure 2. – Probability plot of the surrogate-adjusted alkane residuals showing further evidence of non-linearity.

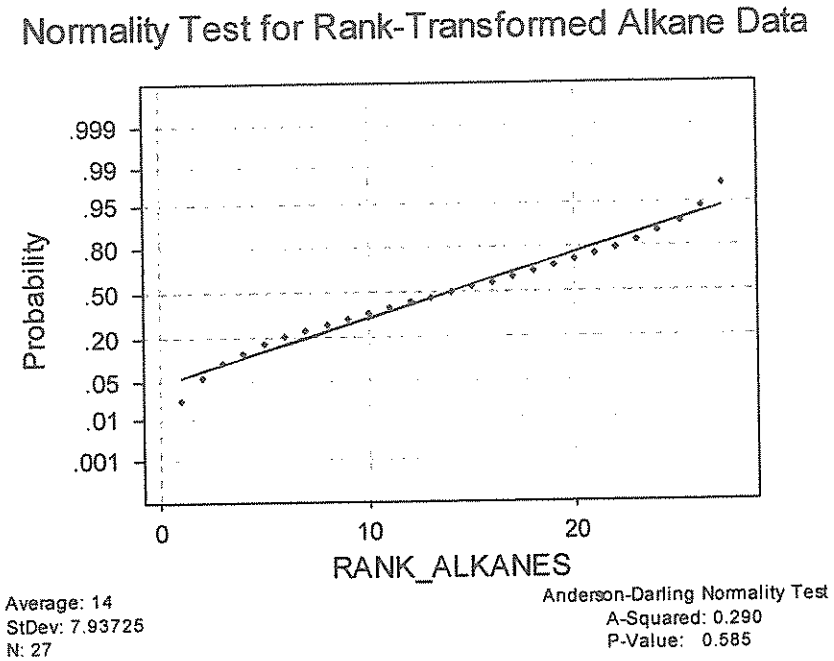

Figure 3. - Anderson-Darling test for normality showing improved linearity of the rank transformed surrogate-adjusted alkane data.

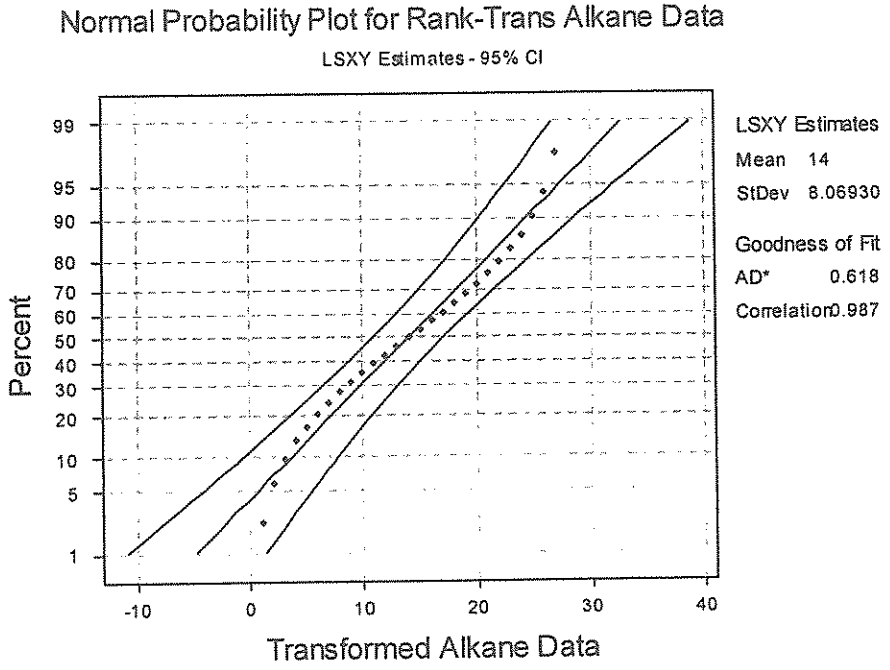

Figure 4. – Probability plot of the rank-transformed surrogate-adjusted alkane residuals showing improved linearity.

Non-transformed and rank-transformed surrogate-adjusted alkane data were analyzed with the General Linear ANOVA Model and Dunnett's multiple comparison tests between treatments and controls. P-statistics calculated for the F-test in the ANOVA table for non-transformed and transformed treatment main effects, and treatment/day interactions are all under the chosen alpha (α) level of 0.05 indicating at least one significant difference between one or more treatments over one or more days.

Adjusted P-values for non-transformed and transformed data Oil Spill Eater II Days 7 and 28 are shown to be significantly less than the Day 0 controls (Table 3). Adjusted P-values for non-transformed and transformed Oil Spill Eater II data, Days 7 and 28 are shown to be significantly less than the Day 7 controls (Table 4). Both transformed and non-transformed product data on Day 28 statistically demonstrated significantly more reduction than the Day 28 control (Table 5).

The Nutrient control behaved in the same manner as the product, showing the same significant differences between the Days 7 and 28 results from the Day 0, Day 7, and Day 28 controls. However, using Tukey's pairwise means comparison method on non-transformed data, the Day 28 Oil Spill Eater II product is also significantly less than the Nutrient alone (Table 6).

Table 2. ANOVA on non-transformed alkane Data

ANOVA non-transformed data

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Day	2	1746813937	1746813937	873406968	697.73	0.000
Treatment	2	1082517417	1082517417	541258708	432.39	0.000
Treatment*Day	4	761225884	761225884	190306471	152.03	0.000
Error	18	22531957	22531957	1251775	----------	----------
Total	26	3613089194	----------	----------	----------	----------

ANOVA on rank transformed alkane data

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Day	2	1178.00	1178.00	589.00	182.79	0.000
Treatment	2	298.67	298.67	149.33	46.34	0.000
Treatment*Day	4	103.33	103.33	25.83	8.02	0.001
Error	18	58.00	58.00	3.22	----------	----------
Total	26	1638.00	----------	----------	----------	----------

Table 3. Dunnett's test results using the Day 0 control as the control level vs. all other treatments and controls (all interactions). Note - non = non-transformed data, trans = transformed data

Treatment	Day	Difference of Means		T-Value		Adjusted P-Value	
		NON	TRANS	NON	TRANS	NON	TRANS
Nutrient	0	-2600	-5.00	-2.85	-3.41	0.0597	0.0094
Oil Spill Eater II	0	-1439	-2.00	-1.58	-1.36	0.5103	0.3439
Control	7	-3920	-8.33	-4.29	-5.69	0.0029	0.0001
Nutrient	7	-8354	-13.33	-9.15	-9.10	0.0000	0.0000
Oil Spill Eater II	7	-16854	-19.33	-18.45	-13.19	0.0000	0.0000
Control	28	-7373	-12.33	-8.07	-8.41	0.0000	0.0000
Nutrient	28	-16663	-18.33	-18.24	-12.51	0.0000	0.0000
Oil Spill Eater II	28	-38896	-23.33	-42.58	-15.92	0.0000	0.0000

Table 4. Dunnett's test results using the Day 7 control as the control level vs. all other treatments and controls (all interactions). Note - non = non-transformed data, trans = transformed data

Treatment	Day	Difference of Means		T-Value		Adjusted P-Value	
		NON	TRANS	NON	TRANS	NON	TRANS
Control	0	3920	8.33	4.26	5.69	0.0029	1.0000
Nutrient	0	1319	3.33	1.44	2.27	0.5977	0.9999
Oil Spill Eater II	0	2480	6.33	2.72	4.32	0.0772	1.0000
Nutrient	7	-4435	-5.00	-4.85	-3.41	0.0009	0.0094
Oil Spill Eater II	7	-12934	-11.00	-14.16	-7.51	0.0000	0.0000
Control	28	-3453	-4.00	-3.78	-2.73	0.0086	0.0376
Nutrient	28	-12743	-10.00	-13.95	-6.82	0.0000	0.0000
Oil Spill Eater II	28	-34977	-15.00	-38.29	-10.23	0.0000	0.0000

Table 5. Dunnett's test results using the Day 28 control as the control level vs. all other treatments and controls (all interactions). Note - non = non-transformed data, trans = transformed data

Treatment	Day	Difference of Means		T-Value		Adjusted P-Value	
		NON	TRANS	NON	TRANS	NON	TRANS
Control	0	7373	12.33	8.07	8.415	1.0000	1.0000
Nutrient	0	4773	7.33	5.22	5.003	1.0000	1.0000
Oil Spill Eater II	0	5934	10.33	6.50	7.050	1.0000	1.0000
Control	7	3453	4.00	3.78	2.729	1.0000	1.0000
Nutrient	7	-981	-1.00	-1.07	-0.682	0.4720	0.6528
Oil Spill Eater II	7	-9481	-7.00	-10.38	-4.776	0.0000	0.0005
Nutrient	28	-9290	-6.00	-10.17	-4.094	0.0000	0.0022
Oil Spill Eater II	28	-31523	-11.00	-34.51	-7.505	0.0000	0.0000

Table 6. Tukey's pairwise means comparison results between the Day 28 Nutrient and the Day 28 OIL SPILL EATER II non-transformed alkane data.

Treatment	Day	Difference of Means	T-Value	Adjusted P-Value
Oil Spill Eater II	28	-22234	-24.34	0.0000

Surrogate-adjusted Aromatic Data

Preliminary analysis of surrogate-adjusted aromatic data for normality (fig.5) showed the raw data to be non-normal with an Anderson-Darling P-statistic of 0.000. This is below the selected α-level of 0.01 and indicates the data are not normally distributed. Further visual evidence of the data's non-linearity can be seen in the probability plot for residuals of the data (fig.6). The data were rank-transformed and reanalyzed for normality (fig.7) giving an Anderson-Darling statistic of 0.585, well above the chosen α-level of 0.01. The probability plot for the residuals (fig. 6) of the data still show a small degree of non-linearity which can slightly lower the ANOVA and Dunnett's test ability to detect a statistical difference between treatments and controls. More on this subject is discussed in the conclusions.

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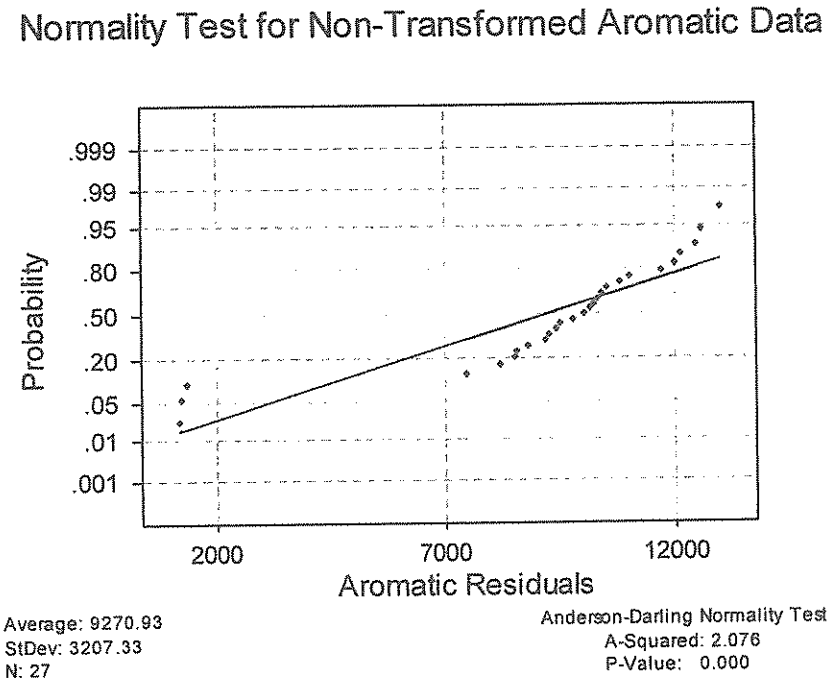

Figure 5. - Anderson-Darling test for normality showing non-linearity of the surrogate adjusted aromatic data.

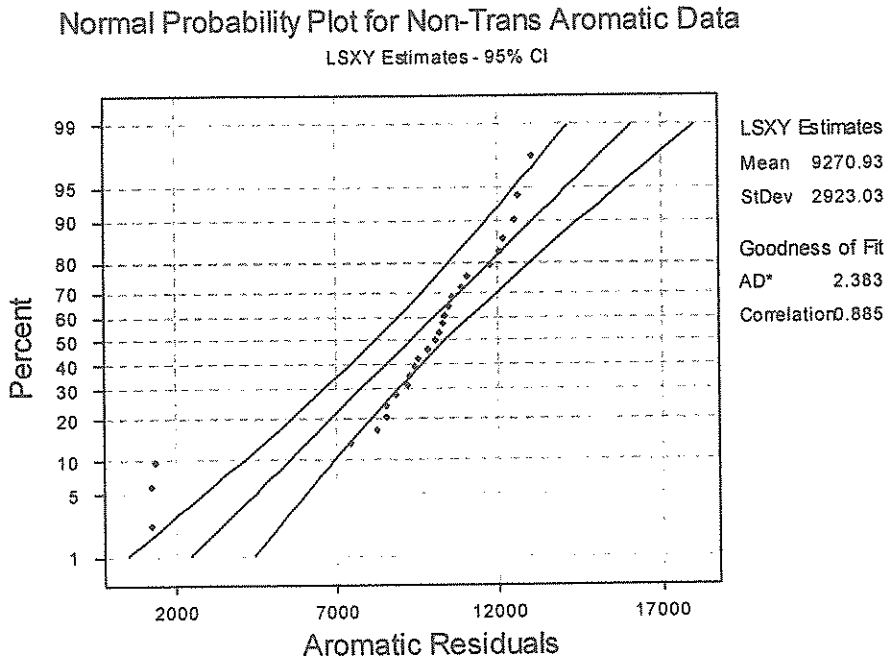

Figure 6. – Probability plot of the surrogate-adjusted aromatic residuals showing further evidence of non-linearity.

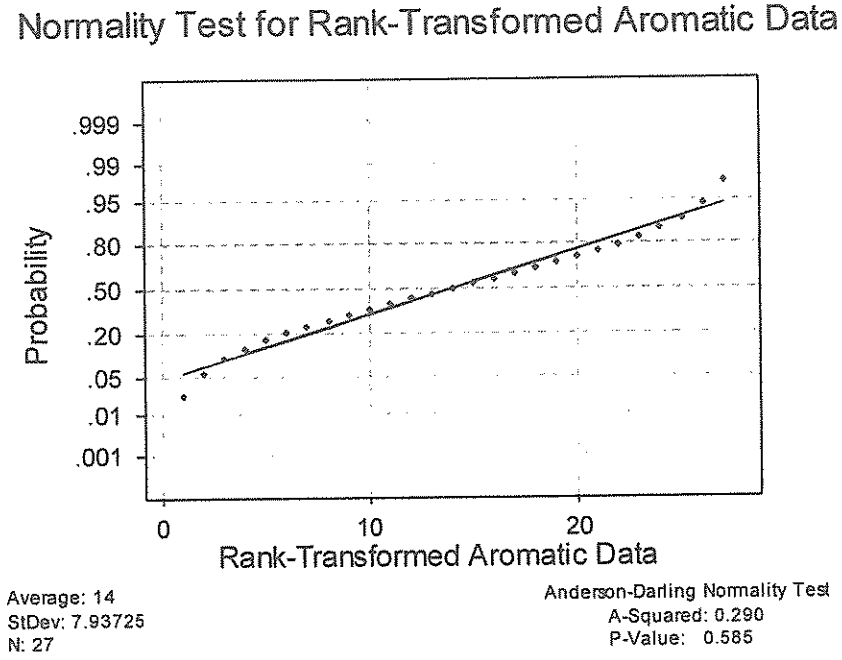

Figure 7. - Anderson-Darling test for normality showing improved linearity of the rank transformed surrogate-adjusted aromatic data.

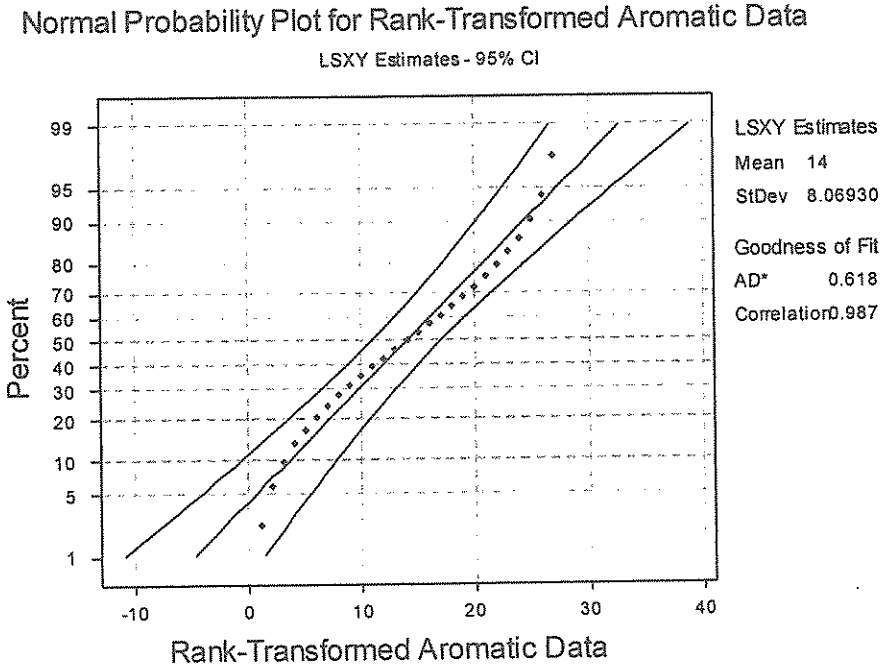

Figure 8. – Probability plot of the rank-transformed surrogate-adjusted aromatic residuals showing improved linearity.

Non-transformed and rank-transformed surrogate-adjusted aromatic data were analyzed with the General Linear ANOVA Model and Dunnett's multiple comparison tests between treatments and controls. P-statistics calculated for the F-test in the ANOVA table for non-transformed and transformed treatment main effects, and treatment/day interactions are all under the chosen alpha (α) level of 0.05 indicating at least one significant difference between one or more treatments over one or more days.

Adjusted P-values for non-transformed and transformed data Oil Spill Eater II Days 7 and 28 are shown to be significantly less than the Day 0 controls (Table 8). Adjusted P-values for non-transformed and transformed product data, Days 7 and 28 are shown to be significantly less than the Day 7 controls (Table 9). Both transformed and non-transformed product data on Day 28 statistically demonstrated significantly more reduction than the Day 28 control (Table 10).

The Nutrient control behaved in a similar manner as the product up to Day 28, showing the same significant differences between the Day 7 and 28 results from Day 0 and Day 7, but not the Day 28 controls. This indicates that nutrient alone is not as effective as the product in reducing aromatics. Using Tukey's pairwise means comparison method on non-transformed data; the Day 28 Oil Spill Eater II product is also significantly less than the Nutrient alone, reinforcing the previous statement (Table 11).

Table 7. ANOVA on Surrogate-adjusted Aromatic Data

ANOVA

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Day	2	122630081	122630081	61315041	142.02	0.000
Treatment	2	60150172	60150172	30075086	69.66	0.000
Treatment*Day	4	76909629	76909629	19227407	44.54	0.000
Error	18	7770989	7770989	431722	----------	----------
Total	26	267460872	----------	----------	----------	----------

ANOVA on rank transformed aromatic data

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Day	2	1102.89	1102.89	551.44	67.37	0.000
Treatment	2	194.00	194.00	97.00	11.85	0.001
Treatment*Day	4	193.78	193.78	48.44	5.92	0.003
Error	18	147.33	58.00	3.22	----------	----------
Total	26	1638.00	----------	----------	----------	----------

Table 8. Dunnett's test using Day 0 control as the control level vs. all other treatments and controls (all interactions). Note - non = non-transformed data, trans = transformed data

Treatment	Day	Difference of Means		T-Value		Adjusted P-Value	
		NON	TRANS	NON	TRANS	NON	TRANS
Nutrient	0	350	1.33	0.65	0.571	0.9772	0.9716
Oil Spill Eater II	0	719	2.67	1.34	1.142	0.9971	0.9974
Control	7	-1080	-4.33	-2.01	-1.855	0.1364	0.1753
Nutrient	7	-1537	-7.67	-2.87	-3.282	0.2880	0.0123
Oil Spill Eater II	7	-3364	-16.33	-6.27	-6.992	0.0000	0.0000
Control	28	-1902	-9.67	-3.54	-4.138	0.0071	0.0020
Nutrient	28	-2497	-12.67	-4.66	-5.422	0.0007	0.0001
Oil Spill Eater II	28	-10168	-19.33	-18.95	-8.276	0.0000	0.0000

Table 9. Dunnett's test using Day 7 Control as the control level vs. all other treatments and controls (all interactions). Note - non = non-transformed data, trans = transformed data

Treatment	Day	Difference of Means		T-Value		Adjusted P-Value	
		NON	TRANS	NON	TRANS	NON	TRANS
Control	0	1080	4.33	2.01	1.855	0.9997	0.9995
Nutrient	0	1430	5.67	2.67	2.426	1.0000	0.9999
Oil Spill Eater II	0	1799	7.00	3.35	2.997	1.0000	1.0000
Nutrient	7	-457	-3.33	-0.85	-1.427	0.5756	0.3186
Oil Spill Eater II	7	-2283	-12.00	-4.26	-5.137	0.0016	0.0002
Control	28	-821	-5.33	-1.53	-2.283	0.2788	0.0862
Nutrient	28	-1417	-8.33	-2.64	-3.567	0.0445	0.0068
Oil Spill Eater II	28	-9088	-15.00	-16.94	-6.421	0.0000	0.0000

Table 10. Dunnett's test using Day 28 control as the control level vs. all other treatments and controls (all interactions). Note - non = non-transformed data, trans = transformed data

Treatment	Day	Difference of Means		T-Value		Adjusted P-Value	
		NON	TRANS	NON	TRANS	NON	TRANS
Control	0	1902	9.67	3.54	4.138	1.0000	1.0000
Nutrient	0	2251	11.00	4.20	4.709	1.0000	1.0000
Oil Spill Eater II	0	2651	12.33	4.88	5.280	1.0000	1.0000
Control	7	821	5.33	4.53	2.283	0.9985	0.9999
Nutrient	7	364	2.00	0.68	0.856	0.9788	0.9872
Oil Spill Eater II	7	-1462	-6.67	-2.73	-2.854	0.0379	0.0294
Nutrient	28	-596	-3.00	-1.11	-1.284	0.4554	0.3778
Oil Spill Eater II	28	-8266	-9.67	-15.41	-4.138	0.0000	0.0020

Table 11. Tukey's pairwise means comparison results between the Day 28 Nutrient and the Day 28 OIL SPILL EATER II non-transformed aromatic data.

Treatment	Day	Difference of Means	T-Value	Adjusted P-Value
Oil Spill Eater II	28	-7671	-14.30	0.0000

Microbiological Analysis Data

The following tables show the most probable number calculated by EPA's most probable number calculator Version 4.04. The data show the continued viability of the organisms through 28 days.

Table 12. Micro Results, MPN (per mL)

Treatments	Day 0	Day 7	Day 28
Control Rep# 1	7,968	8,406	9,843
Control Rep #2	8,179	8,072	10,136
Control Rep #3	7,647	8,724	9,549
Nutrient Rep #1	8,493	1,832,536	7,274,655
Nutrient Rep #2	7,647	2,015,665	7,967,738
Nutrient Rep #3	7,852	2,115,255	7,646,602
Oil Spill Eater II Rep# 1	8,724	7,274,655	182,054,230
Oil Spill Eater II Rep# 2	8,406	7,967,738	175,038,856
Oil Spill Eater II Rep# 3	8,972	7,646,602	197,910,169

Gravimetric Data

The following tables show the P-Values calculated by the two-sample t-test of the Minitab™ program. Table 13 shows the calculated values for Day 28 controls the Day 28 product, and the p-value of the comparison is lower than the chosen alpha (α) level of 0.05 and therefore indicate statistical significance. A computer printout of the analyses can be seen in APPENDIX III. Table 14 shows that the calculated values for the Day 28 controls and both the Day 7 and Day 28 nutrient are both statistically significant.

Table 13. P-Values calculated by the two-sample t-test for product (OIL SPILL EATER II) and the controls

Treatments	Day	Treatment Weight Means (mg)	T-test Scores	p-value
Controls	0	0.099	-2.79	0.966
Oil Spill Eater II	0	0.100		
Controls	7	0.093	1.04	0.187
Oil Spill Eater II	7	0.077		
Controls	28	0.082	42.25	0.000
Oil Spill Eater II	28	0.015		

Table 14. P-Values calculated by the two-sample t-test for the nutrient and the controls

Treatments	Day	Treatment Weight Means (mg)	T-test Scores	p-value
Controls	0	0.099	1.36	0.154
NUTRIENT	0	0.101		
Controls	7	0.093	10.07	0.005
NUTRIENT	7	0.079		
Controls	28	0.082	33.84	0.000
NUTRIENT	28	0.048		

Conclusions

Our conclusions will begin with a discussion of the GC/MS due to its relative importance in judging the tested product effective. A discussion of the microbiological results and gravimetric results will follow.

GC/MS Data

OSEI Corp.Product (Oil Spill Eater II)

Surrogate-adjusted Alkane Data

Surrogate-adjusted alkane Oil Spill Eater II data was shown to be non-normal and had to be rank-transformed to attain an acceptable degree of linearity. Analysis of the surrogate-adjusted data with ANOVA and Dunnett's test did however show the product treatments at Day 7 and 28 to be significantly less than Day 0, 7, and 28 controls. The extreme non-linearity of the non-transformed data makes the results of the ANOVA and Dunnett's test less reliable. The data, upon rank-transformation, achieved the desired linearity showing Day 7 and 28 product results to be significantly less that the respective Day 0, Day 7 and Day 28 controls. Based on this parameter the product appears to be effective.

Surrogate-adjusted alkane nutrient data was shown to be non-normal and had to be rank-transformed to attain an acceptable degree of linearity. Analysis of the surrogate-adjusted data with ANOVA and Dunnett's test did however show the nutrient treatments at Day 7 and 28 to be significantly less than their respective controls. The non-linearity of the non-transformed data may make the results of the ANOVA and Dunnett's test less reliable, however. The data, upon rank-transformation, achieved the desired linearity showing Day 7 and Day 28 nutrient results to be significantly less than the respective Day 0, 7, and 28 controls. Based on this parameter the nutrient treatment alone appears to be effective.

Tukey's test on untransformed alkane data showed a significant difference between the Day 28 Oil Spill Eater II results and Day 28 Nutrient results, indicating that the product seems more effective than nutrient treatment by itself.

Surrogate-adjusted Aromatic Data

Surrogate-adjusted aromatic Oil Spill Eater II data was shown to be non-normal and had to be rank-transformed to attain an acceptable degree of linearity. Analysis of the surrogate-adjusted data with ANOVA and Dunnett's test did however show the product treatments at Day 7 and 28 to be significantly less than Day 0, 7, and 28 controls. The extreme non-linearity of the non-transformed data makes the results of the ANOVA and Dunnett's test less reliable. The data, upon rank-transformation, achieved the desired linearity showing Day 7 and 28 product results to be significantly less that the respective Day 0, Day 7 and Day 28 controls. Based on this parameter the product appears to be effective.

Surrogate-adjusted aromatic nutrient data was shown to be non-normal and had to be rank-transformed to attain an acceptable degree of linearity. Analysis of the surrogate-adjusted data with ANOVA and Dunnett's test did however show the nutrient treatments at Day 7 to be significantly less than the Day 0 and Day 7 controls. The non-linearity of the non-transformed data may make the results of the ANOVA and Dunnett's test less reliable, however. The data, upon rank-transformation, achieved the desired linearity showing Day 7 nutrient results to be significantly less than the respective Day 0, and Day 7, but not the Day 28 controls. Based on this parameter the nutrient treatment alone is not as effective as the product after 28 days and is not significantly than the control alone.

Tukey's test on the aromatic data also showed a significant difference between the Day 28 Oil Spill Eater II results and Day 28 Nutrient results, indicating that the product seems to be more effective than nutrient treatment.

Microbiological Results

OSEI Corp. Product (Oil Spill Eater II)

The microbiological results speak for themselves. They show a definite continued microbiological viability over time for the product treatments. Similar to the product treatment, the nutrient treatments show a definite continued microbiological viability over time also.

Gravimetric Results

OSEI Corp.Product (Oil Spill Eater II)

Gravimetric results showed statistical significance between products and controls by Day 28. This tends to support the bulk of the data seen in both GC/MS analysis and microbiological analysis. Gravimetric results showed statistical significance between the Nutrient and the control on Day 7 and Day 28. This data tends to support the bulk of the data in both GC/MS analysis and microbiological analysis.

Discussion on Surrogate Recovery – QA/QC

The purpose of incorporating surrogate recovery percentages into the raw data is to check the efficiency of extraction techniques and in most cases is a valid quality control check. The acceptable range of surrogate recovery percentages is given in the cited Federal Register document titled Environmental Protection Agency, (EPA) Pt. 300, Appendix C, page 237, as 70%-120%. Percentage recoveries for product and controls for Day 0, Day 7 and Day 28 are given in Table 15 below.

Table 15. Surrogate recovery percentages.

Treatment		Day 0	Day 7	Day 28
Control Rep #1	5-Alpha Andorstane	0.90	0.82	0.82
	Phenanthrene-d10	0.94	0.77	0.79
Control Rep #2	5-Alpha Andorstane	0.86	0.90	0.76
	Phenanthrene-d10	0.81	0.94	0.77
Control Rep #3	5-Alpha Andorstane	0.78	0.87	0.87
	Phenanthrene-d10	0.78	0.90	0.83
NUT Rep #1	5-Alpha Andorstane	0.86	0.99	0.85
	Phenanthrene-d10	0.88	0.74	0.96
NUT Rep# 2	5-Alpha Andorstane	0.90	0.96	0.77
	Phenanthrene-d10	0.94	0.71	0.95
NUT Rep# 3	5-Alpha Andorstane	0.88	0.89	0.84
	Phenanthrene-d10	0.96	0.72	0.95
Oil Spill Eater II Rep #1	5-Alpha Andorstane	0.85	0.90	0.90
	Phenanthrene-d10	0.89	0.77	0.71
Oil Spill Eater II Rep# 2	5-Alpha Andorstane	0.83	0.87	0.92
	Phenanthrene-d10	0.85	0.76	0.73
Oil Spill Eater II Rep# 3	5-Alpha Andorstane	0.91	0.86	0.90
	Phenanthrene-d10	0.94	0.79	0.71

Statistical Analysis

Lastly, we feel that the nature of the data may reduce the ANOVA and Dunnett's means comparison test to detect a legitimate statistical effect between treatments and controls. Before the data can be subjected to the ANOVA analysis, it must pass a "normality" test where a calculated P-value is compared to a chosen alpha (α) level (usually 0.01). ANOVA has reduced power to detect a significant statistical difference when analyzing non-normal data (Zar, 1984). However, data that passes a formal test for normality is not necessarily from a "normal distribution" strictly speaking. A test for normality looks for linearity, which is only one aspect of the assumptions of normality. The data may also be skewed to the left or right as indicated by measurement of the median, may have 'heavy tails" in the distribution or may contain outliers. Normality after all, is usually a matter of degrees and not just whether the data are, or are not normally distributed. If data are not normal in the strictest sense, we feel the test's ability to detect subtle but significant statistical differences may be compromised to some degree.

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APPENDIX I

CONTROL, REPLICATE 1 Testing Date: Day 0 Initial Oil Weight: 629 mg Final Extracted Volume: 10 mL		
	Concentration (ng/mL)	Surrogate Corrected (ng/mL)
Alkane Analyte:		
nC-10 Decane	ND	ND
nC-11 Undecane	ND	ND
nC-12 Dodecane	ND	ND
nC-13 Tridecane	ND	ND
nC-14 Tetradecane	13.4	15
nC-15 Pentadecane	97	101
nC-16 Hexadecane	474	471
nC-17 Heptadecane	1,175	1,205
Pristane	748	829
nC-18 Octadecane	1692	1669
Phytane	969	1075
nC-19 Nonadecane	2112	2347
nC-20 Eicosane	2467	2764
nC-21 Heneicosane	2356	2652
nC-22 Docosane	2561	2845
nC-23 Tricosane	2502	2802
nC-24 Tetracosane	2478	2753
nC-25 Pentacosane	2044	2271
nC-26 Hexacosane	2112	2347
nC-27 Heptacosane	1693	2003
nC-28 Octacosane	1579	1755
nC-29 Nonacosane	1503	1815
nC-30 Triacontane	2126	2305
nC-31 Hentriacontane	2465	2737
nC-32 Dotriacontane	2148	2387
nC-33 Tritriacontane	1301	1445
nC-34 Tetratriacontane	1006	1118
nC-35 Pentatriacontane	631	800
Total Alkanes	*38415*	*43687*
Aromatic Analyte:		
Naphthalene	ND	ND
C1-Naphthalenes	4.8	5.1
C2-Naphthalenes	45	49
C3-Naphthalenes	179	191
C4-Naphthalenes	333	344
Fluorene	17	15
C1-Fluorenes	174	166
C2-Fluorenes	441	469
C3- Fluorenes	631	684
Dibenzothiophene	199	212
C1-Dibenzothiophenes	635	605
C2-Dibenzothiophenes	1218	1208
C3- Dibenzothiophenes	1095	1059
Phenanthrene	359	381
C1-Phenanthrenes/Anth	1078	1142
C2-Phenanthrenes	1630	1735
C3-Phenanthrenes	1126	1191
C4-Phenanthrenes	655	628
Anthracene	ND	ND
Fluoranthene	9.4	10.0
Pyrene	6.6	7.1
C1- Pyrenes	174	165
C2- Pyrenes	259	264
C3- Pyrenes	217	231
C4- Pyrenes	134	143
Naphthobenzothiophene	89	89
C-1 Naphthobenzothiophenes	319	339
C-2 Naphthobenzothiophenes	372	395
C-3 Naphthobenzothiophenes	290	309
Benzo (a) Anthracene	9.5	10.1
Chrysene	105	112
C1- Chrysenes	171	182
C2- Chrysenes	234	249
C3- Chrysenes	228	243
C4- Chrysenes	144	153
Benzo (b) Fluoranthene	15	16
Benzo (k) Fluoranthene	ND	ND
Benzo (e) Pyrene	32	34
Benzo (a) Pyrene	ND	ND
Perylene	ND	ND
Indeno (1,2,3 - cd) Pyrene	ND	ND
Dibenzo (a,h) anthracene	ND	ND
Benzo (g,h,i) perylene	ND	ND
Total Aromatics	*12316*	*12996*
% Surrogate Recovery		
5 Alpha Androstane	0.90	1.00
Phenanthrene-d-10	0.84	1.00

CONTROL, REPLICATE 2 Testing Date: Day 0 Initial Oil Weight: 625 mg Final Extracted Volume: 10 mL		
	Concentration (ng/mL)	Surrogate Corrected (ng/mL)
Alkane Analyte:		
nC-10 Decane	ND	ND
nC-11 Undecane	ND	ND
nC-12 Dodecane	ND	ND
nC-13 Tridecane	ND	ND
nC-14 Tetradecane	10.6	11.6
nC-15 Pentadecane	95	100
nC-16 Hexadecane	456	453
nC-17 Heptadecane	1215	1413
Pristane	722	849
nC-18 Octadecane	1726	2018
Phytane	946	1101
nC-19 Nonadecane	2153	2446
nC-20 Eicosane	2466	2796
nC-21 Heneicosane	2512	2851
nC-22 Docosane	2592	3025
nC-23 Tricosane	2538	3007
nC-24 Tetracosane	2529	3006
nC-25 Pentacosane	2093	2631
nC-26 Hexacosane	2209	2685
nC-27 Heptacosane	1845	2253
nC-28 Octacosane	1561	1888
nC-29 Nonacosane	1324	1649
nC-30 Triacontane	2065	2402
nC-31 Hentriacontane	2339	3090
nC-32 Dotriacontane	1661	1956
nC-33 Tritriacontane	1076	1268
nC-34 Tetratriacontane	1006	1170
nC-35 Pentatriacontane	430	668
Total Alkanes	*32304*	*44539*
Aromatic Analyte:		
Naphthalene	ND	ND
C1-Naphthalenes	4.8	5.8
C2-Naphthalenes	28	35
C3-Naphthalenes	165	204
C4-Naphthalenes	264	313
Fluorene	15	19
C1-Fluorenes	123	164
C2-Fluorenes	305	389
C3- Fluorenes	349	431
Dibenzothiophene	144	178
C1-Dibenzothiophenes	405	504
C2-Dibenzothiophenes	822	1015
C3- Dibenzothiophenes	672	839
Phenanthrene	257	317
C1-Phenanthrenes/Anth	822	1015
C2-Phenanthrenes	1121	1384
C3-Phenanthrenes	805	1142
C4-Phenanthrenes	365	438
Anthracene	ND	ND
Fluoranthene	7.3	9.0
Pyrene	5.6	6.9
C1- Pyrenes	138	170
C2- Pyrenes	183	226
C3- Pyrenes	170	210
C4- Pyrenes	96	119
Naphthobenzothiophene	79	97
C-1 Naphthobenzothiophenes	239	295
C-2 Naphthobenzothiophenes	281	347
C-3 Naphthobenzothiophenes	208	304
Benzo (a) Anthracene	8.2	10.2
Chrysene	85	105
C1- Chrysenes	144	178
C2- Chrysenes	184	228
C3- Chrysenes	178	217
C4- Chrysenes	101	125
Benzo (b) Fluoranthene	13	16
Benzo (k) Fluoranthene	ND	ND
Benzo (e) Pyrene	29	36
Benzo (a) Pyrene	ND	ND
Perylene	ND	ND
Indeno (1,2,3 - cd) Pyrene	ND	ND
Dibenzo (a,h) anthracene	ND	ND
Benzo (g,h,i) perylene	ND	ND
Total Aromatics	*8933*	*11028*
% Surrogate Recovery		
5 Alpha Androstane	0.80	1.00
Phenanthrene-d-10	0.81	1.00

CONTROL REPLICATE 3 Testing Date: Day 0 Initial Oil Weight: 646 mg Final Extracted Volume: 10 mL		
	Concentration (ng/mL)	Surrogate Corrected (ng/mL)
Alkane Analyte:		
nC-10 Decane	ND	ND
nC-11 Undecane	ND	ND
nC-12 Dodecane	ND	ND
nC-13 Tridecane	ND	ND
nC-14 Tetradecane	9.7	12
nC-15 Pentadecane	84	106
nC-16 Hexadecane	407	527
nC-17 Heptadecane	1140	1489
Pristane	631	869
nC-18 Octadecane	1680	2126
Phytane	860	1126
nC-19 Nonadecane	2016	2647
nC-20 Eicosane	2345	2879
nC-21 Heneicosane	2177	2701
nC-22 Docosane	2537	2936
nC-23 Tricosane	2391	2866
nC-24 Tetracosane	2259	2690
nC-25 Pentacosane	1911	2321
nC-26 Hexacosane	1935	2345
nC-27 Heptacosane	1518	1947
nC-28 Octacosane	1371	1830
nC-29 Nonacosane	1245	1698
nC-30 Triacontane	1886	2045
nC-31 Hentriacontane	1904	2441
nC-32 Dotriacontane	1429	1827
nC-33 Tritriacontane	1129	1481
nC-34 Tetratriacontane	769	1012
nC-35 Pentatriacontane	465	668
Total Alkanes	*33256*	*42610*
Aromatic Analyte:		
Naphthalene	ND	ND
C1-Naphthalenes	3.8	4.8
C2-Naphthalenes	33	41
C3-Naphthalenes	166	203
C4-Naphthalenes	256	307
Fluorene	13	17
C1-Fluorenes	116	149
C2-Fluorenes	263	357
C3- Fluorenes	336	434
Dibenzothiophene	116	152
C1-Dibenzothiophenes	423	542
C2-Dibenzothiophenes	768	1011
C3- Dibenzothiophenes	635	814
Phenanthrene	221	286
C1-Phenanthrenes/Anth	745	885
C2-Phenanthrenes	1017	1284
C3-Phenanthrenes	705	905
C4-Phenanthrenes	327	419
Anthracene	ND	ND
Fluoranthene	6.6	7.7
Pyrene	4.6	6.2
C1- Pyrenes	136	174
C2- Pyrenes	159	204
C3- Pyrenes	141	180
C4- Pyrenes	89	114
Naphthobenzothiophene	58	74
C-1 Naphthobenzothiophenes	205	267
C-2 Naphthobenzothiophenes	230	295
C-3 Naphthobenzothiophenes	209	297
Benzo (a) Anthracene	6.3	8.1
Chrysene	68	87
C1- Chrysenes	113	145
C2- Chrysenes	169	217
C3- Chrysenes	144	185
C4- Chrysenes	101	129
Benzo (b) Fluoranthene	10.8	14
Benzo (k) Fluoranthene	ND	ND
Benzo (e) Pyrene	23	30
Benzo (a) Pyrene	ND	ND
Perylene	ND	ND
Indeno (1,2,3 - cd) Pyrene	ND	ND
Dibenzo (a,h) anthracene	ND	ND
Benzo (g,h,i) perylene	ND	ND
Total Aromatics	*6020*	*10282*
% Surrogate Recovery		
5 Alpha Androstane	0.78	1.00
Phenanthrene-d-10	0.72	1.00

CONTROL STATISTICS FOR SURROGATE CORRECTED DATA Testing Date: Day 0						
	Replicate #1	Replicate #2	Replicate #3			
Alkane Analyte:	Conc (ng/mL)	Conc (ng/mL)	Conc (ng/mL)	Average	Stdev	%RSD
nC-10 Decane	ND	ND	ND	ND	ND	ND
nC-11 Undecane	ND	ND	ND	ND	ND	ND
nC-12 Dodecane	ND	ND	ND	ND	ND	ND
nC-13 Tridecane	ND	ND	ND	ND	ND	ND
nC-14 Tetradecane	15	12	12	13	1.7	13
nC-15 Pentadecane	101	119	106	109	9.2	8.4
nC-16 Hexadecane	471	633	527	544	82.2	15.1
nC-17 Heptadecane	1205	1413	1489	1369	147.3	10.8
Pristane	829	849	869	849	20.0	2.4
nC-18 Octadecane	1669	2015	2126	1937	234.3	12.1
Phytane	1075	1101	1126	1101	25.5	2.3
nC-19 Nonadecane	2347	2446	2647	2480	153.1	6.2
nC-20 Eicosane	2764	2796	2879	2813	58.3	2.1
nC-21 Heneicosane	2652	2851	2701	2735	103.8	3.8
nC-22 Docosane	2845	3025	2936	2935	90.0	3.1
nC-23 Tricosane	2802	3007	2866	2891	103.3	3.6
nC-24 Tetracosane	2753	3006	2690	2816	165.4	5.9
nC-25 Pentacosane	2271	2631	2321	2408	195.1	8.1
nC-26 Hexacosane	2347	2685	2345	2459	186.0	8.0
nC-27 Heptacosane	2057	2253	1947	2086	155.7	7.5
nC-28 Octacosane	1755	1888	1830	1758	69.0	3.9
nC-29 Nonacosane	1815	1649	1698	1649	146.1	8.9
nC-30 Triacontane	2045	2402	2045	2270	195.1	8.6
nC-31 Hentriacontane	2737	3090	2441	2756	269.0	10.0
nC-32 Dotriacontane	2387	1956	1827	2056	253.1	14.1
nC-33 Tritriacontane	1445	1268	1481	1265	117.1	9.4
nC-34 Tetratriacontane	1118	1170	1012	1099	81.1	7.3
nC-35 Pentatriacontane	800	668	668	668	86.1	12.9
Total Alkanes	*43687*	*44539*	*42610*	*43278*	*1202*	*2.8*
Aromatic Analyte:	Conc (ng/mL)	Conc (ng/mL)	Conc (ng/mL)	Average	Stdev	%RSD
Naphthalene	ND	ND	ND	ND	ND	ND
C1-Naphthalenes	5.1	5.8	4.8	5.2	0.5	11
C2-Naphthalenes	49	35	41	42	6.5	16
C3-Naphthalenes	191	204	203	199	7	3.5
C4-Naphthalenes	344	313	307	321	20	6.3
Fluorene	15	19	17	16	1.1	6.1
C1-Fluorenes	166	164	149	160	10	11
C2-Fluorenes	469	389	357	405	57	17
C3- Fluorenes	684	431	434	473	79	15
Dibenzothiophene	212	178	152	180	30	17
C1-Dibenzothiophenes	605	504	542	551	55	10
C2-Dibenzothiophenes	1208	1015	1011	1078	109	10
C3- Dibenzothiophenes	1059	839	814	891	127	15
Phenanthrene	381	317	286	331	48	15
C1-Phenanthrenes/Anth	1142	1015	885	1014	129	13
C2-Phenanthrenes	1735	1284	1284	1434	229	16
C3-Phenanthrenes	1191	1142	905	1080	153	14
C4-Phenanthrenes	628	438	419	495	66	13
Anthracene	ND	ND	ND	ND	ND	ND
Fluoranthene	10.0	9.0	7.7	8.9	1.2	13
Pyrene	7.1	6.9	6.2	6.8	0.6	8.6
C1- Pyrenes	165	170	174	170	7.5	4.3
C2- Pyrenes	264	226	204	231	25	11
C3- Pyrenes	231	210	180	207	28	13
C4- Pyrenes	143	119	114	125	16.5	12
Naphthobenzothiophene	95	87	74	85	10.1	11
C-1 Naphthobenzothiophenes	339	295	267	300	36	12
C-2 Naphthobenzothiophenes	395	347	295	346	50	15
C-3 Naphthobenzothiophenes	309	264	267	273	31	11
Benzo (a) Anthracene	10.1	10.2	8.1	9.5	1.2	12
Chrysene	112	105	87	102	14	14
C1- Chrysenes	182	178	145	168	20	12
C2- Chrysenes	249	228	217	231	16	7.0
C3- Chrysenes	243	217	185	215	29	13
C4- Chrysenes	153	125	129	136	15.9	11
Benzo (b) Fluoranthene	16.4	15.8	14.9	15	1.3	8.2
Benzo (k) Fluoranthene	ND	ND	ND	ND	ND	ND
Benzo (e) Pyrene	35.6	30.1	23	29.5	6.4	22
Benzo (a) Pyrene	ND	ND	ND	ND	ND	ND
Perylene	ND	ND	ND	ND	ND	ND
Indeno (1,2,3 - cd) Pyrene	ND	ND	ND	ND	ND	ND
Dibenzo (a,h) anthracene	ND	ND	ND	ND	ND	ND
Benzo (g,h,i) perylene	ND	ND	ND	ND	ND	ND
Total Aromatics	*11035*	*10282*	*10282*	*11435*	*1401*	*12*

NUTRIENT, REPLICATE 1 Testing Date: Day 0 Initial DS Weight: 629 mg Final Extracted Volume: 10 mL		
	Concentration (ng/mL)	Surrogate Corrected (ng/mL)
Alkane Analyte:		
nC-10 Decane	ND	ND
nC-11 Undecane	ND	ND
nC-12 Dodecane	ND	ND
nC-13 Tridecane	ND	ND
nC-14 Tetradecane	14.65	17
nC-15 Pentadecane	88	109
nC-16 Hexadecane	428	497
nC-17 Heptadecane	1154	1289
Pristane	554	644
nC-18 Octadecane	1579	1837
Phytane	1097	1171
nC-19 Nonadecane	2311	2311
nC-20 Eicosane	2368	2673
nC-21 Heneicosane	2052	2263
nC-22 Docosane	2478	2883
nC-23 Tricosane	2958	3050
nC-24 Tetracosane	2672	3107
nC-25 Pentacosane	2220	2602
nC-26 Hexacosane	2239	2594
nC-27 Heptacosane	1419	1647
nC-28 Octacosane	1751	2036
nC-29 Nonacosane	1309	1586
nC-30 Triacontane	1697	2101
nC-31 Hentriacontane	1576	1832
nC-32 Dotriacontane	1153	1377
nC-33 Tritriacontane	1139	1369
nC-34 Tetratriacontane	466	542
nC-35 Pentatriacontane	554	656
Total Alkanes	*34655*	*40344*
Aromatic Analyte:		
Naphthalene	ND	ND
C1-Naphthalenes	6.2	7.1
C2-Naphthalenes	39	44
C3-Naphthalenes	177	202
C4-Naphthalenes	335	375
Fluorene	16	16
C1-Fluorenes	168	179
C2-Fluorenes	315	356
C3- Fluorenes	403	461
Dibenzothiophene	141	160
C1-Dibenzothiophenes	595	674
C2-Dibenzothiophenes	819	930
C3- Dibenzothiophenes	781	889
Phenanthrene	305	353
C1-Phenanthrenes	849	958
C2-Phenanthrenes	1238	1406
C3-Phenanthrenes	924	1065
C4-Phenanthrenes	404	459
Anthracene	ND	ND
Fluoranthene	7.7	8.6
Pyrene	7.6	8.9
C1- Pyrenes	128	145
C2- Pyrenes	185	205
C3- Pyrenes	166	188
C4- Pyrenes	125	142
Naphthobenzothiophene	73	83
C-1 Naphthobenzothiophene	248	282
C-2 Naphthobenzothiophene	281	318
C-3 Naphthobenzothiophene	297	335
Benzo (a) Anthracene	5.5	6.3
Chrysene	85	94
C1- Chrysenes	153	170
C2- Chrysenes	198	213
C3- Chrysenes	148	168
C4- Chrysenes	122	139
Benzo (b) Fluoranthene	15	17
Benzo (k) Fluoranthene	ND	ND
Benzo (e) Pyrene	35	39
Benzo (a) Pyrene	ND	ND
Perylene	ND	ND
Indeno (1,2,3 - cd) Pyrene	ND	ND
Dibenzo (a,h) anthracene	ND	ND
Benzo (g,h,i) perylene	ND	ND
Total Aromatics	*8549*	*10652*
% Surrogate Recovery		
5 Alpha Androstane	0.88	1.00
Phenanthrene d-10	0.85	1.00

NUTRIENT, REPLICATE 2 Testing Date: Day 0 Initial DS Weight: 649 mg Final Extracted Volume: 10 mL		
	Concentration (ng/mL)	Surrogate Corrected (ng/mL)
Alkane Analyte:		
nC-10 Decane	ND	ND
nC-11 Undecane	ND	ND
nC-12 Dodecane	ND	ND
nC-13 Tridecane	ND	ND
nC-14 Tetradecane	16	18
nC-15 Pentadecane	81	99
nC-16 Hexadecane	464	516
nC-17 Heptadecane	1124	1249
Pristane	674	748
nC-18 Octadecane	1650	1807
Phytane	952	1005
nC-19 Nonadecane	2075	2388
nC-20 Eicosane	2368	2626
nC-21 Heneicosane	2426	2694
nC-22 Docosane	2764	3059
nC-23 Tricosane	2781	3030
nC-24 Tetracosane	2873	3183
nC-25 Pentacosane	2128	2583
nC-26 Hexacosane	2166	2431
nC-27 Heptacosane	1563	1737
nC-28 Octacosane	1771	1958
nC-29 Nonacosane	1463	1614
nC-30 Triacontane	1691	2066
nC-31 Hentriacontane	1521	1699
nC-32 Dotriacontane	1421	1579
nC-33 Tritriacontane	1163	1281
nC-34 Tetratriacontane	493	548
nC-35 Pentatriacontane	599	669
Total Alkanes	*36354*	*40436*
Aromatic Analyte:		
Naphthalene	ND	ND
C1-Naphthalenes	7.0	8.4
C2-Naphthalenes	49	58
C3-Naphthalenes	208	241
C4-Naphthalenes	411	458
Fluorene	16	19
C1-Fluorenes	179	199
C2-Fluorenes	384	419
C3- Fluorenes	481	499
Dibenzothiophene	163	173
C1-Dibenzothiophenes	613	652
C2-Dibenzothiophenes	1033	1077
C3- Dibenzothiophenes	895	1027
Phenanthrene	356	387
C1-Phenanthrenes	1212	1370
C2-Phenanthrenes	1299	1382
C3-Phenanthrenes	1023	1087
C4-Phenanthrenes	497	555
Anthracene	ND	ND
Fluoranthene	7.7	8.1
Pyrene	8.1	8.6
C1- Pyrenes	169	185
C2- Pyrenes	220	234
C3- Pyrenes	205	218
C4- Pyrenes	140	149
Naphthobenzothiophene	62	67
C-1 Naphthobenzothiophene	309	327
C-2 Naphthobenzothiophene	345	368
C-3 Naphthobenzothiophene	271	285
Benzo (a) Anthracene	6.3	6.5
Chrysene	152	159
C1- Chrysenes	175	188
C2- Chrysenes	245	261
C3- Chrysenes	194	207
C4- Chrysenes	146	155
Benzo (b) Fluoranthene	18	18
Benzo (k) Fluoranthene	ND	ND
Benzo (e) Pyrene	36	38
Benzo (a) Pyrene	ND	ND
Perylene	ND	ND
Indeno (1,2,3 - cd) Pyrene	ND	ND
Dibenzo (a,h) anthracene	ND	ND
Benzo (g,h,i) perylene	ND	ND
Total Aromatics	*11289*	*12011*
% Surrogate Recovery		
5 Alpha Androstane	0.90	1.00
Phenanthrene d-10	0.84	1.00

NUTRIENT, REPLICATE 3 Testing Date: Day 0 Initial DS Weight: 618 mg Final Extracted Volume: 10 mL		
	Concentration (ng/mL)	Surrogate Corrected (ng/mL)
Alkane Analyte:		
nC-10 Decane	ND	ND
nC-11 Undecane	ND	ND
nC-12 Dodecane	ND	ND
nC-13 Tridecane	ND	ND
nC-14 Tetradecane	14	16
nC-15 Pentadecane	84	99
nC-16 Hexadecane	445	519
nC-17 Heptadecane	1209	1398
Pristane	632	718
nC-18 Octadecane	1606	1823
Phytane	976	1109
nC-19 Nonadecane	1950	2292
nC-20 Eicosane	2308	2672
nC-21 Heneicosane	2513	2856
nC-22 Docosane	2857	3020
nC-23 Tricosane	2889	3067
nC-24 Tetracosane	2895	3063
nC-25 Pentacosane	2122	2422
nC-26 Hexacosane	2184	2291
nC-27 Heptacosane	1453	1635
nC-28 Octacosane	1656	1882
nC-29 Nonacosane	1577	1783
nC-30 Triacontane	1918	2176
nC-31 Hentriacontane	1726	1981
nC-32 Dotriacontane	1251	1421
nC-33 Tritriacontane	1283	1435
nC-34 Tetratriacontane	525	587
nC-35 Pentatriacontane	601	683
Total Alkanes	*36015*	*40975*
Aromatic Analyte:		
Naphthalene	ND	ND
C1-Naphthalenes	8.1	8.4
C2-Naphthalenes	46	48
C3-Naphthalenes	234	244
C4-Naphthalenes	475	489
Fluorene	18	19
C1-Fluorenes	189	197
C2-Fluorenes	389	405
C3- Fluorenes	576	600
Dibenzothiophene	176	184
C1-Dibenzothiophenes	634	661
C2-Dibenzothiophenes	1038	1082
C3- Dibenzothiophenes	979	1010
Phenanthrene	323	335
C1-Phenanthrenes	1082	1106
C2-Phenanthrenes	1388	1456
C3-Phenanthrenes	1127	1174
C4-Phenanthrenes	563	584
Anthracene	ND	ND
Fluoranthene	8.7	9.6
Pyrene	9.0	9.4
C1- Pyrenes	176	185
C2- Pyrenes	244	264
C3- Pyrenes	203	211
C4- Pyrenes	149	156
Naphthobenzothiophene	70	74
C-1 Naphthobenzothiophene	317	331
C-2 Naphthobenzothiophene	380	395
C-3 Naphthobenzothiophene	270	281
Benzo (a) Anthracene	5.9	6.1
Chrysene	97	91
C1- Chrysenes	214	223
C2- Chrysenes	254	265
C3- Chrysenes	174	181
C4- Chrysenes	151	158
Benzo (b) Fluoranthene	20	21
Benzo (k) Fluoranthene	ND	ND
Benzo (e) Pyrene	30	31
Benzo (a) Pyrene	ND	ND
Perylene	ND	ND
Indeno (1,2,3 - cd) Pyrene	ND	ND
Dibenzo (a,h) anthracene	ND	ND
Benzo (g,h,i) perylene	ND	ND
Total Aromatics	*11893*	*12497*
% Surrogate Recovery		
5 Alpha Androstane	0.88	1.00
Phenanthrene d-10	0.86	1.00

NUTRIENT STATISTICS FOR SURROGATE CORRECTED DATA Testing Date: Day 0							
	Replicate #1	Replicate #2	Replicate #3	Average	Stdev	%STD
Alkane Analyte:	Conc (ng/mL)	Conc (ng/mL)	Conc (ng/mL)	Average	Stdev	%STD
nC-10 Decane	ND	ND	ND	ND	ND	ND
nC-11 Undecane	ND	ND	ND	ND	ND	ND
nC-12 Dodecane	ND	ND	ND	ND	ND	ND
nC-13 Tridecane	ND	ND	ND	ND	ND	ND
nC-14 Tetradecane	17	18	16	17	0.8	4.6
nC-15 Pentadecane	109	99	99	99	5.4	5.5
nC-16 Hexadecane	497	516	519	510	9	1.9
nC-17 Heptadecane	1289	1249	1398	1312	78	5.9
Pristane	644	748	718	704	53	7.5
nC-18 Octadecane	1837	1807	1823	1844	29	1.6
Phytane	1171	1005	1109	1095	69	6.3
nC-19 Nonadecane	2311	2388	2292	2330	37	1.7
nC-20 Eicosane	2673	2626	2672	2648	26	1.0
nC-21 Heneicosane	2263	2694	2856	2604	261	9.5
nC-22 Docosane	2883	3059	3020	2987	93	3.1
nC-23 Tricosane	3050	3059	3067	3059	14	0.5
nC-24 Tetracosane	3107	3183	3063	3121	65	2.1
nC-25 Pentacosane	2602	2583	2422	2536	113	4.5
nC-26 Hexacosane	2594	2431	2291	2439	153	6.3
nC-27 Heptacosane	1647	1737	1635	1673	56	3.3
nC-28 Octacosane	2036	1958	1882	1992	77	3.9
nC-29 Nonacosane	1586	1614	1783	1661	89	5.4
nC-30 Triacontane	2101	2066	2176	2115	55	2.6
nC-31 Hentriacontane	1832	1699	1981	1838	135	7.4
nC-32 Dotriacontane	1377	1579	1421	1459	105	7.2
nC-33 Tritriacontane	1369	1281	1435	1340	83	6.2
nC-34 Tetratriacontane	542	548	587	559	26	4.6
nC-35 Pentatriacontane	656	669	683	669	13	2.0
Total Alkanes	*40344*	*40436*	*40975*	*40585*	*313*	*0.8*
Aromatic Analyte:	Conc (ng/mL)	Conc (ng/mL)	Conc (ng/mL)	Average	Stdev	%STD
Naphthalene	ND	ND	ND	ND	ND	ND
C1-Naphthalenes	7.1	8.4	8.4	8.0	0.8	10
C2-Naphthalenes	44	58	48	48	4	7.9
C3-Naphthalenes	202	241	244	229	24	10
C4-Naphthalenes	375	458	489	440	59	14
Fluorene	16	19	19	18	1.8	10
C1-Fluorenes	179	199	197	192	9	4.7
C2-Fluorenes	356	419	405	394	32	8.1
C3- Fluorenes	461	499	600	520	69	13
Dibenzothiophene	160	173	184	172	12	7.0
C1-Dibenzothiophenes	674	652	661	662	11	1.7
C2-Dibenzothiophenes	930	1077	1082	1030	86	8.4
C3- Dibenzothiophenes	889	1027	1010	979	69	7.1
Phenanthrene	353	387	335	358	27	7.6
C1-Phenanthrenes	958	1370	1106	1145	216	19
C2-Phenanthrenes	1406	1382	1456	1415	38	2.7
C3-Phenanthrenes	1065	1087	1174	1109	64	5.8
C4-Phenanthrenes	459	555	584	533	63	12
Anthracene	ND	ND	ND	ND	ND	ND
Fluoranthene	8.6	8.1	9.6	8.7	0.8	8.3
Pyrene	8.9	8.6	9.4	9.0	0.4	4.6
C1- Pyrenes	145	185	185	172	23	13
C2- Pyrenes	205	234	264	234	30	13
C3- Pyrenes	188	218	211	206	15	7.5
C4- Pyrenes	142	149	156	149	7.0	4.7
Naphthobenzothiophene	83	67	74	75	8.3	11
C-1 Naphthobenzothiophene	282	327	331	313	27	8.7
C-2 Naphthobenzothiophene	318	368	395	361	39	11
C-3 Naphthobenzothiophene	335	285	281	300	29	9.7
Benzo (a) Anthracene	6.3	6.5	6.1	6.3	0.2	3.9
Chrysene	94	159	91	115	38	33
C1- Chrysenes	170	188	223	194	27	14
C2- Chrysenes	213	261	265	246	29	12
C3- Chrysenes	168	207	181	185	20	11
C4- Chrysenes	139	155	158	151	10	6.6
Benzo (b) Fluoranthene	17.4	17.5	20.6	18	1.8	10
Benzo (k) Fluoranthene	ND	ND	ND	ND	ND	ND
Benzo (e) Pyrene	39	38	31	36	4.3	12
Benzo (a) Pyrene	ND	ND	ND	ND	ND	ND
Perylene	ND	ND	ND	ND	ND	ND
Indeno (1,2,3 - cd) Pyrene	ND	ND	ND	ND	ND	ND
Dibenzo (a,h) anthracene	ND	ND	ND	ND	ND	ND
Benzo (g,h,i) perylene	ND	ND	ND	ND	ND	ND
Total Aromatics	*10652*	*12011*	*12497*	*11720*	*843*	*7.2*

PRODUCT, REPLICATE 1 Testing Date: Day 6 Initial Oil Weight: 810 mg Final Extracted Volume: 10 mL		
Alkane Analysis:	Concentration (ng/mg)	Surrogate Corrected (ng/mg)
nC-10 Decane	ND	ND
nC-11 Undecane	ND	ND
nC-12 Dodecane	ND	ND
nC-13 Tridecane	ND	ND
nC-14 Tetradecane	12.50	14.7
nC-15 Pentadecane	84	99
nC-16 Hexadecane	432	509
nC-17 Heptadecane	1284	1505
Pristane	695	815
nC-18 Octadecane	1916	2254
Phytane	914	1076
nC-19 Nonadecane	2547	2761
nC-20 Eicosane	2918	3433
nC-21 Heneicosane	2936	3442
nC-22 Docosane	3132	3673
nC-23 Tricosane	3127	3679
nC-24 Tetracosane	2866	3419
nC-25 Pentacosane	2272	2673
nC-26 Hexacosane	2162	2679
nC-27 Heptacosane	1882	2215
nC-28 Octacosane	1291	1695
nC-29 Nonacosane	1389	1911
nC-30 Triacontane	1262	1485
nC-31 Hentriacontane	1030	941
nC-32 Dotriacontane	799	941
nC-33 Tritriacontane	715	841
nC-34 Tetratriacontane	339	387
nC-35 Pentatriacontane	497	585
Total Alkanes	*36761*	*43237*
Aromatic Analysis:		
Naphthalene	ND	ND
C1-Naphthalenes	ND	ND
C2-Naphthalenes	47	53
C3-Naphthalenes	172	192
C4-Naphthalenes	344	388
Fluorene	18.7	21.6
C1-Fluorenes	146	164
C2-Fluorenes	342	385
C3- Fluorenes	624	691
Dibenzothiophene	150	170
C1-Dibenzothiophenes	497	526
C2-Dibenzothiophenes	1011	1139
C3- Dibenzothiophenes	992	1092
Phenanthrene	278	312
C1-Phenanthrenes	647	692
C2-Phenanthrenes	1252	1468
C3-Phenanthrenes	1540	1169
C4-Phenanthrenes	648	581
Anthracene	ND	ND
Fluoranthene	6.6	7.6
Pyrene	5.7	6.4
C1- Pyrenes	159	178
C2- Pyrenes	252	283
C3- Pyrenes	188	203
C4- Pyrenes	118	130
Naphthobenzothiophene	76	85
C-1 Naphthobenzothiophene	271	305
C-2 Naphthobenzothiophene	336	357
C-3 Naphthobenzothiophene	224	251
Benzo (a) Anthracene	10.1	11.3
Chrysene	82	92
C1- Chrysenes	180	203
C2- Chrysenes	211	237
C3- Chrysenes	189	213
C4- Chrysenes	137	154
Benzo (b) Fluoranthene	13.6	15.7
Benzo (k) Fluoranthene	ND	ND
Benzo (e) Pyrene	23.9	26.9
Benzo (a) Pyrene	ND	ND
Perylene	ND	ND
Indeno (1,2,3 - cd) Pyrene	ND	ND
Dibenzo (a,h) anthracene	ND	ND
Benzo (g,h,i) perylene	ND	ND
Total Aromatics	*10445*	*11735*
% Surrogate Recovery		
5 Alpha Androstane	0.85	1.00
Phenanthrene d-10	0.85	1.00

PRODUCT, REPLICATE 2 Testing Date: Day 6 Initial Oil Weight: 810 mg Final Extracted Volume: 10 mL		
Alkane Analysis:	Concentration (ng/mg)	Surrogate Corrected (ng/mg)
nC-10 Decane	ND	ND
nC-11 Undecane	ND	ND
nC-12 Dodecane	ND	ND
nC-13 Tridecane	ND	ND
nC-14 Tetradecane	10.2	12.3
nC-15 Pentadecane	73	91
nC-16 Hexadecane	402	484
nC-17 Heptadecane	1166	1406
Pristane	584	704
nC-18 Octadecane	1666	2022
Phytane	855	1032
nC-19 Nonadecane	2068	2515
nC-20 Eicosane	2786	3280
nC-21 Heneicosane	2402	3002
nC-22 Docosane	3043	3639
nC-23 Tricosane	3076	3766
nC-24 Tetracosane	3139	3782
nC-25 Pentacosane	1966	2389
nC-26 Hexacosane	1835	2329
nC-27 Heptacosane	1647	2219
nC-28 Octacosane	1665	1987
nC-29 Nonacosane	1199	1454
nC-30 Triacontane	1187	1439
nC-31 Hentriacontane	1169	1405
nC-32 Dotriacontane	712	859
nC-33 Tritriacontane	663	799
nC-34 Tetratriacontane	314	378
nC-35 Pentatriacontane	464	555
Total Alkanes	*34363*	*41491*
Aromatic Analysis:		
Naphthalene	ND	ND
C1-Naphthalenes	ND	ND
C2-Naphthalenes	45	57
C3-Naphthalenes	182	214
C4-Naphthalenes	351	413
Fluorene	19.1	19.1
C1-Fluorenes	151	177
C2-Fluorenes	404	476
C3- Fluorenes	563	665
Dibenzothiophene	148	170
C1-Dibenzothiophenes	497	547
C2-Dibenzothiophenes	1049	1234
C3- Dibenzothiophenes	872	1025
Phenanthrene	269	316
C1-Phenanthrenes	721	1053
C2-Phenanthrenes	1269	1595
C3-Phenanthrenes	1667	1278
C4-Phenanthrenes	465	535
Anthracene	ND	ND
Fluoranthene	6.1	7.2
Pyrene	5.3	6.3
C1- Pyrenes	147	173
C2- Pyrenes	242	284
C3- Pyrenes	195	229
C4- Pyrenes	108	127
Naphthobenzothiophene	69	75
C-1 Naphthobenzothiophene	340	384
C-2 Naphthobenzothiophene	368	396
C-3 Naphthobenzothiophene	247	266
Benzo (a) Anthracene	6.9	10.5
Chrysene	70	83
C1- Chrysenes	149	176
C2- Chrysenes	192	229
C3- Chrysenes	162	226
C4- Chrysenes	133	157
Benzo (b) Fluoranthene	16.4	16.2
Benzo (k) Fluoranthene	ND	ND
Benzo (e) Pyrene	23.7	26
Benzo (a) Pyrene	ND	ND
Perylene	ND	ND
Indeno (1,2,3 - cd) Pyrene	ND	ND
Dibenzo (a,h) anthracene	ND	ND
Benzo (g,h,i) perylene	ND	ND
Total Aromatics	*10784*	*12782*
% Surrogate Recovery		
5 Alpha Androstane	0.83	1.00
Phenanthrene d-10	0.85	1.00

PRODUCT, REPLICATE 3 Testing Date: Day 6 Initial Oil Weight: 810 mg Final Extracted Volume: 10 mL		
Alkane Analysis:	Concentration (ng/mg)	Surrogate Corrected (ng/mg)
nC-10 Decane	ND	ND
nC-11 Undecane	ND	ND
nC-12 Dodecane	ND	ND
nC-13 Tridecane	ND	ND
nC-14 Tetradecane	11.4	12.8
nC-15 Pentadecane	79	97
nC-16 Hexadecane	434	495
nC-17 Heptadecane	1214	1354
Pristane	653	761
nC-18 Octadecane	1662	2149
Phytane	1048	1150
nC-19 Nonadecane	2147	2360
nC-20 Eicosane	3105	3413
nC-21 Heneicosane	2903	3212
nC-22 Docosane	3311	3639
nC-23 Tricosane	3403	3800
nC-24 Tetracosane	3287	3525
nC-25 Pentacosane	1966	2182
nC-26 Hexacosane	3625	2224
nC-27 Heptacosane	1868	1856
nC-28 Octacosane	1577	1733
nC-29 Nonacosane	1333	1497
nC-30 Triacontane	1239	1340
nC-31 Hentriacontane	1355	1434
nC-32 Dotriacontane	814	864
nC-33 Tritriacontane	859	902
nC-34 Tetratriacontane	395	324
nC-35 Pentatriacontane	449	484
Total Alkanes	*36993*	*40863*
Aromatic Analysis:		
Naphthalene	ND	ND
C1-Naphthalenes	ND	ND
C2-Naphthalenes	47	50
C3-Naphthalenes	190	209
C4-Naphthalenes	332	363
Fluorene	19.1	20.2
C1-Fluorenes	171	182
C2-Fluorenes	412	435
C3- Fluorenes	631	671
Dibenzothiophene	168	177
C1-Dibenzothiophenes	455	484
C2-Dibenzothiophenes	1170	1254
C3- Dibenzothiophenes	966	1045
Phenanthrene	331	341
C1-Phenanthrenes	1009	1057
C2-Phenanthrenes	1485	1595
C3-Phenanthrenes	1529	1152
C4-Phenanthrenes	657	516
Anthracene	ND	ND
Fluoranthene	6.6	7.4
Pyrene	5.5	5.6
C1- Pyrenes	143	163
C2- Pyrenes	214	257
C3- Pyrenes	173	180
C4- Pyrenes	117	124
Naphthobenzothiophene	74	79
C-1 Naphthobenzothiophene	276	287
C-2 Naphthobenzothiophene	329	350
C-3 Naphthobenzothiophene	248	262
Benzo (a) Anthracene	9.3	9.9
Chrysene	77	82
C1- Chrysenes	165	170
C2- Chrysenes	211	205
C3- Chrysenes	173	164
C4- Chrysenes	159	169
Benzo (b) Fluoranthene	15.2	15.2
Benzo (k) Fluoranthene	ND	ND
Benzo (e) Pyrene	23.6	25
Benzo (a) Pyrene	ND	ND
Perylene	ND	ND
Indeno (1,2,3 - cd) Pyrene	ND	ND
Dibenzo (a,h) anthracene	ND	ND
Benzo (g,h,i) perylene	ND	ND
Total Aromatics	*11406*	*12135*
% Surrogate Recovery		
5 Alpha Androstane	0.91	1.00
Phenanthrene d-10	0.84	1.00

PRODUCT STATISTICS FOR SURROGATE CORRECTED DATA Testing Date: Day 6							
	Replicate #1	Replicate #2	Replicate #3				
Alkane Analysis:	Conc (ng/mg)	Conc (ng/mg)	Conc (ng/mg)	Average	Stdev	%RSD	
nC-10 Decane	ND	ND	ND	ND	ND	ND	
nC-11 Undecane	ND	ND	ND	ND	ND	ND	
nC-12 Dodecane	ND	ND	ND	ND	ND	ND	
nC-13 Tridecane	ND	ND	ND	ND	ND	ND	
nC-14 Tetradecane	14.7	12.3	12.8	13.2	1.3	10	
nC-15 Pentadecane	99	91	97	96	4.4	4.6	
nC-16 Hexadecane	509	484	495	496	21	4.4	
nC-17 Heptadecane	1505	1406	1354	1446	100	10	
Pristane	815	704	761	761	55	7.5	
nC-18 Octadecane	2254	2022	2149	2141	111	5.2	
Phytane	1076	1032	1150	1102	41	3.7	
nC-19 Nonadecane	2761	2515	2360	2545	202	7.9	
nC-20 Eicosane	3433	3280	3413	3375	84	2.5	
nC-21 Heneicosane	3442	3002	3212	3219	220	6.8	
nC-22 Docosane	3673	3639	3639	3647	23	0.6	
nC-23 Tricosane	3679	3766	3800	3748	63	1.7	
nC-24 Tetracosane	3419	3782	3525	3575	187	5.2	
nC-25 Pentacosane	2673	2389	2182	2481	257	11	
nC-26 Hexacosane	2679	2329	2224	2411	185	7.7	
nC-27 Heptacosane	2215	2219	1856	2097	209	10	
nC-28 Octacosane	1695	1987	1733	1738	160	9.3	
nC-29 Nonacosane	1911	1454	1497	1621	201	12.4	
nC-30 Triacontane	1485	1439	1340	1419	73	5.2	
nC-31 Hentriacontane	1572	1405	1434	1470	86	5.1	
nC-32 Dotriacontane	941	859	864	888	41	4.6	
nC-33 Tritriacontane	941	799	902	881	77	10	
nC-34 Tetratriacontane	387	378	324	363	34	9.5	
nC-35 Pentatriacontane	585	555	484	541	51	10	
Total Alkanes	*43237*	*41491*	*40863*	*41730*	*1372*	*3.3*	
Aromatic Analysis:	Conc (ng/mg)	Conc (ng/mg)	Conc (ng/mg)	Average	Stdev	%RSD	
Naphthalene	ND	ND	ND	ND	ND	ND	
C1-Naphthalenes	ND	ND	ND	ND	ND	ND	
C2-Naphthalenes	53	57	50	53	3.5	6.5	
C3-Naphthalenes	192	214	209	205	13.7	6.7	
C4-Naphthalenes	388	413	363	388	25	6.5	
Fluorene	21	19	20	20.2	0.8	4.0	
C1-Fluorenes	164	177	182	174	9.4	5.4	
C2-Fluorenes	385	476	435	432	46	10	
C3- Fluorenes	624	665	671	654	53	8.2	
Dibenzothiophene	150	170	177	166	14	8.7	
C1-Dibenzothiophenes	526	547	484	519	36	6.9	
C2-Dibenzothiophenes	1139	1234	1254	1209	63	5.2	
C3- Dibenzothiophenes	1092	1025	1045	1054	34	3.3	
Phenanthrene	312	316	341	323	16	4.8	
C1-Phenanthrenes	692	1053	1057	934	73	8.0	
C2-Phenanthrenes	1468	1595	1595	1553	110	7.2	
C3-Phenanthrenes	1169	1278	1152	1200	69	5.7	
C4-Phenanthrenes	581	535	516	544	97.2	3.0	
Anthracene	ND	ND	ND	ND	ND	ND	
Fluoranthene	7.6	7.2	7.4	7.4	0.2	2.8	
Pyrene	6.4	6.3	5.6	6.1	0.5	8.0	
C1- Pyrenes	178	173	163	171	10.7	6.2	
C2- Pyrenes	283	284	257	208	26	11	
C3- Pyrenes	203	229	180	204	29.3	9.5	
C4- Pyrenes	130	127	124	127	2.9	2.3	
Naphthobenzothiophene	85	75	79	80	3.9	4.8	
C-1 Naphthobenzothiophene	305	384	287	392	11	3.0	
C-2 Naphthobenzothiophene	357	396	350	368	11.5	3.2	
C-3 Naphthobenzothiophene	251	266	262	260	20.2	7.6	
Benzo (a) Anthracene	11.3	10.5	9.9	10.6	0.7	7.0	
Chrysene	92	83	82	86	5.7	6.7	
C1- Chrysenes	203	176	170	183	17	10	
C2- Chrysenes	237	229	205	224	17	7.9	
C3- Chrysenes	213	226	164	201	31.5	16	
C4- Chrysenes	154	157	169	160	7.8	4.8	
Benzo (b) Fluoranthene	18.2	16.2	15.2	16.5	1.5	9.1	
Benzo (k) Fluoranthene	ND	ND	ND	ND	ND	ND	
Benzo (e) Pyrene	29.9	27.8	25.3	27	1.3	4.9	
Benzo (a) Pyrene	ND	ND	ND	ND	ND	ND	
Perylene	ND	ND	ND	ND	ND	ND	
Indeno (1,2,3 - cd) Pyrene	ND	ND	ND	ND	ND	ND	
Dibenzo (a,h) anthracene	ND	ND	ND	ND	ND	ND	
Benzo (g,h,i) perylene	ND	ND	ND	ND	ND	ND	
Total Aromatics	*11735*	*12582*	*12135*	*12185*	*429*	*3.5*	

Bio-Aquatic, Inc.
9ST-11 Product

4/29/2009

CONTROL, REPLICATE 1
Testing Date: Day 7
Initial Oil Weight: 530 mg
Final Extracted Volume: 10 mL

Alkane Analytes	Concentration (ng/mL)	Surrogate Corrected (ng/mL)
nC-10 Decane	ND	ND
nC-11 Undecane	ND	ND
nC-12 Dodecane	ND	ND
nC-13 Tridecane	ND	ND
nC-14 Tetradecane	10.0	12.2
nC-15 Pentadecane	71	86
nC-16 Hexadecane	367	447
nC-17 Heptadecane	1066	1291
Pristane	574	695
nC-18 Octadecane	1549	1889
Phytane	835	1019
nC-19 Nonadecane	1813	2211
nC-20 Eicosane	2149	2621
nC-21 Heneicosane	2382	2906
nC-22 Docosane	2533	2846
nC-23 Tricosane	2359	2516
nC-24 Tetracosane	2264	2761
nC-25 Pentacosane	1960	2370
nC-26 Hexacosane	1960	2401
nC-27 Heptacosane	1653	2018
nC-28 Octacosane	1431	1745
nC-29 Nonacosane	1504	1835
nC-30 Triacontane	1241	1514
nC-31 Hentriacontane	1047	2049
nC-32 Dotriacontane	887	1709
nC-33 Tritriacontane	1054	1224
nC-34 Tetratriacontane	397	485
nC-35 Pentatriacontane	258	315
Total Alkanes	*31567*	*38434*
Aromatic Analytes		
Naphthalene	ND	ND
C1-Naphthalenes	ND	ND
C2-Naphthalenes	22	28
C3-Naphthalenes	132	171
C4-Naphthalenes	166	203
Fluorene	12.6	15.6
C1-Fluorenes	111	144
C2-Fluorenes	274	356
C3- Fluorenes	351	455
Dibenzothiophene	110	142
C1-Dibenzothiophenes	307	403
C2-Dibenzothiophenes	711	924
C3- Dibenzothiophenes	676	881
Phenanthrene	260	335
C1-Phenanthrenes	738	959
C2-Phenanthrenes	1060	1378
C3-Phenanthrenes	701	910
C4-Phenanthrenes	339	439
Anthracene	ND	ND
Fluoranthene	5.4	7.0
Pyrene	5.8	7.5
C1- Pyrenes	84	109
C2- Pyrenes	149	194
C3- Pyrenes	134	174
C4- Pyrenes	83	108
Naphthobenzothiophene	56	73
C-1 Naphthobenzothiophenes	189	259
C-2 Naphthobenzothiophenes	215	279
C-3 Naphthobenzothiophenes	175	227
Benzo (a) Anthracene	12.9	16.8
Chrysene	73	94
C1- Chrysenes	113	163
C2- Chrysenes	197	217
C3- Chrysenes	133	172
C4- Chrysenes	99	128
Benzo (b) Fluoranthene	9	12
Benzo (k) Fluoranthene	ND	ND
Benzo (e) Pyrene	72	39
Benzo (a) Pyrene	ND	ND
Perylene	ND	ND
Indeno (1,2,3 - cd) Pyrene	ND	ND
Dibenzo (a,h) anthracene	ND	ND
Benzo (g,h,i) perylene	ND	ND
Total Aromatics	*7638*	*10179*
% Surrogate Recovery		
5 Alpha Androstane	0.92	1.00
Phenanthrene d-10	0.73	1.00

CONTROL, REPLICATE 2
Testing Date: Day 7
Initial Oil Weight: 530 mg
Final Extracted Volume: 10 mL

Alkane Analytes	Concentration (ng/mL)	Surrogate Corrected (ng/mL)
nC-10 Decane	ND	ND
nC-11 Undecane	ND	ND
nC-12 Dodecane	ND	ND
nC-13 Tridecane	ND	ND
nC-14 Tetradecane	11.3	13.5
nC-15 Pentadecane	79	93
nC-16 Hexadecane	331	394
nC-17 Heptadecane	1154	1378
Pristane	724	864
nC-18 Octadecane	1316	1566
Phytane	1025	1139
nC-19 Nonadecane	2444	2716
nC-20 Eicosane	2753	3059
nC-21 Heneicosane	2916	3240
nC-22 Docosane	3147	3497
nC-23 Tricosane	2928	3255
nC-24 Tetracosane	2858	3175
nC-25 Pentacosane	2539	2256
nC-26 Hexacosane	2173	2414
nC-27 Heptacosane	1827	2007
nC-28 Octacosane	1521	1690
nC-29 Nonacosane	1458	1620
nC-30 Triacontane	1117	1242
nC-31 Hentriacontane	1488	1652
nC-32 Dotriacontane	853	956
nC-33 Tritriacontane	878	973
nC-34 Tetratriacontane	333	370
nC-35 Pentatriacontane	239	265
Total Alkanes	*35998*	*39996*
Aromatic Analytes		
Naphthalene	ND	ND
C1-Naphthalenes	ND	ND
C2-Naphthalenes	27	29
C3-Naphthalenes	159	159
C4-Naphthalenes	256	251
Fluorene	14.1	15.6
C1-Fluorenes	145	155
C2-Fluorenes	358	351
C3- Fluorenes	466	455
Dibenzothiophene	146	155
C1-Dibenzothiophenes	569	534
C2-Dibenzothiophenes	968	1025
C3- Dibenzothiophenes	836	878
Phenanthrene	315	305
C1-Phenanthrenes	964	1005
C2-Phenanthrenes	1372	1400
C3-Phenanthrenes	953	1014
C4-Phenanthrenes	366	393
Anthracene	ND	ND
Fluoranthene	5.9	6.3
Pyrene	5.5	5.9
C1- Pyrenes	112	119
C2- Pyrenes	171	183
C3- Pyrenes	147	156
C4- Pyrenes	94	100
Naphthobenzothiophene	60	64
C-1 Naphthobenzothiophenes	224	238
C-2 Naphthobenzothiophenes	243	258
C-3 Naphthobenzothiophenes	200	213
Benzo (a) Anthracene	13.1	13.8
Chrysene	70	74
C1- Chrysenes	131	139
C2- Chrysenes	186	177
C3- Chrysenes	146	155
C4- Chrysenes	91	97
Benzo (b) Fluoranthene	9	9
Benzo (k) Fluoranthene	ND	ND
Benzo (e) Pyrene	21	23
Benzo (a) Pyrene	ND	ND
Perylene	ND	ND
Indeno (1,2,3 - cd) Pyrene	ND	ND
Dibenzo (a,h) anthracene	ND	ND
Benzo (g,h,i) perylene	ND	ND
Total Aromatics	*9713*	*10333*
% Surrogate Recovery		
5 Alpha Androstane	0.93	1.00
Phenanthrene d-10	0.64	1.00

CONTROL, REPLICATE 3
Testing Date: Day 7
Initial Oil Weight: 530 mg
Final Extracted Volume: 10 mL

Alkane Analytes	Concentration (ng/mL)	Surrogate Corrected (ng/mL)
nC-10 Decane	ND	ND
nC-11 Undecane	ND	ND
nC-12 Dodecane	ND	ND
nC-13 Tridecane	ND	ND
nC-14 Tetradecane	11.4	13.9
nC-15 Pentadecane	75	85
nC-16 Hexadecane	371	419
nC-17 Heptadecane	1064	1223
Pristane	857	898
nC-18 Octadecane	1696	1909
Phytane	976	1121
nC-19 Nonadecane	2117	2433
nC-20 Eicosane	2589	2953
nC-21 Heneicosane	2869	3295
nC-22 Docosane	2945	3385
nC-23 Tricosane	3083	3482
nC-24 Tetracosane	3059	3482
nC-25 Pentacosane	1814	2095
nC-26 Hexacosane	1913	2199
nC-27 Heptacosane	1567	1851
nC-28 Octacosane	1305	1501
nC-29 Nonacosane	1262	1586
nC-30 Triacontane	1231	1416
nC-31 Hentriacontane	1538	1766
nC-32 Dotriacontane	897	1035
nC-33 Tritriacontane	822	1056
nC-34 Tetratriacontane	460	518
nC-35 Pentatriacontane	294	364
Total Alkanes	*34307*	*39427*
Aromatic Analytes		
Naphthalene	ND	ND
C1-Naphthalenes	ND	ND
C2-Naphthalenes	24	27
C3-Naphthalenes	159	173
C4-Naphthalenes	246	273
Fluorene	12.4	13.8
C1-Fluorenes	127	141
C2-Fluorenes	208	231
C3- Fluorenes	492	513
Dibenzothiophene	139	154
C1-Dibenzothiophenes	507	563
C2-Dibenzothiophenes	1024	1149
C3- Dibenzothiophenes	760	867
Phenanthrene	255	284
C1-Phenanthrenes	795	887
C2-Phenanthrenes	1204	1355
C3-Phenanthrenes	856	969
C4-Phenanthrenes	492	514
Anthracene	ND	ND
Fluoranthene	6.7	7.4
Pyrene	8.6	9.2
C1- Pyrenes	117	130
C2- Pyrenes	161	201
C3- Pyrenes	156	173
C4- Pyrenes	122	135
Naphthobenzothiophene	65	70
C-1 Naphthobenzothiophenes	229	254
C-2 Naphthobenzothiophenes	278	309
C-3 Naphthobenzothiophenes	223	248
Benzo (a) Anthracene	16.8	18.7
Chrysene	63	70
C1- Chrysenes	143	159
C2- Chrysenes	197	207
C3- Chrysenes	179	199
C4- Chrysenes	103	115
Benzo (b) Fluoranthene	8.3	10
Benzo (k) Fluoranthene	ND	ND
Benzo (e) Pyrene	22	24
Benzo (a) Pyrene	ND	ND
Perylene	ND	ND
Indeno (1,2,3 - cd) Pyrene	ND	ND
Dibenzo (a,h) anthracene	ND	ND
Benzo (g,h,i) perylene	ND	ND
Total Aromatics	*8439*	*10053*
% Surrogate Recovery		
5 Alpha Androstane	0.87	1.00
Phenanthrene d-10	0.50	1.00

CONTROL
STATISTICS FOR SURROGATE CORRECTED DATA
Testing Date: Day 7

Alkane Analytes	Replicate #1 Corr. (ng/mL)	Replicate #2 Corr. (ng/mL)	Replicate #3 Corr. (ng/mL)	Average	Stdev	%RSD
nC-10 Decane	ND	ND	ND	ND	ND	ND
nC-11 Undecane	ND	ND	ND	ND	ND	ND
nC-12 Dodecane	ND	ND	ND	ND	ND	ND
nC-13 Tridecane	ND	ND	ND	ND	ND	ND
nC-14 Tetradecane	12	13	13.9	13.1	0.9	7.2
nC-15 Pentadecane	86	93	85	88	4.4	5.0
nC-16 Hexadecane	447	394	419	420	26.5	6.3
nC-17 Heptadecane	1291	1378	1223	1297	78.1	6.0
Pristane	695	864	898	819	107.3	13.1
nC-18 Octadecane	1909	1566	1909	1795	198.3	11.0
Phytane	1019	1139	1121	1093	64.1	5.9
nC-19 Nonadecane	2211	2716	2433	2453	253.1	10.3
nC-20 Eicosane	2621	3059	2953	2878	226.8	7.9
nC-21 Heneicosane	2906	3240	3295	3147	201.1	6.4
nC-22 Docosane	2846	3497	3385	3243	340.1	10.5
nC-23 Tricosane	2516	3255	3482	3084	423.3	13.7
nC-24 Tetracosane	2761	3175	3482	3139	362.1	11.5
nC-25 Pentacosane	2370	2256	2095	2240	147.7	6.6
nC-26 Hexacosane	2401	2414	2199	2338	120.1	5.1
nC-27 Heptacosane	2018	2007	1851	1942	122.1	6.3
nC-28 Octacosane	1745	1690	1501	1645	123.1	7.5
nC-29 Nonacosane	1835	1620	1586	1681	124.1	8.5
nC-30 Triacontane	1514	1242	1416	1390	136.1	10.1
nC-31 Hentriacontane	2049	1652	1766	1756	199.1	11.3
nC-32 Dotriacontane	1709	956	1035	1233	421.1	12.1
nC-33 Tritriacontane	1224	973	1056	1085	128.1	11.8
nC-34 Tetratriacontane	485	370	518	457	78.1	17.1
nC-35 Pentatriacontane	315	265	364	315	50.1	15.9
Total Alkanes	*38484*	*35998*	*39427*	*37970*	*757*	*1.9*
Aromatic Analytes	Corr. (ng/mL)	Corr. (ng/mL)	Corr. (ng/mL)	Average	Stdev	%RSD
Naphthalene	ND	ND	ND	ND	ND	ND
C1-Naphthalenes	ND	ND	ND	ND	ND	ND
C2-Naphthalenes	28	29	27	28	0.7	2.4
C3-Naphthalenes	171	159	173	168	7.1	4.2
C4-Naphthalenes	203	251	273	242	35.1	14.5
Fluorene	15.6	15	13.8	14.8	0.9	6.2
C1-Fluorenes	144	155	141	147	7.1	4.8
C2-Fluorenes	356	351	231	313	70.1	22.4
C3- Fluorenes	455	455	513	474	32.1	6.8
Dibenzothiophene	142	155	154	150	7.2	4.8
C1-Dibenzothiophenes	403	534	563	500	49.1	9.8
C2-Dibenzothiophenes	924	1025	1149	1033	113.1	11.0
C3- Dibenzothiophenes	881	878	867	875	7.1	0.8
Phenanthrene	335	305	284	314	27.1	8.6
C1-Phenanthrenes	959	1005	887	950	59.1	6.3
C2-Phenanthrenes	1378	1400	1355	1378	22.1	1.6
C3-Phenanthrenes	910	1014	969	964	52.1	5.4
C4-Phenanthrenes	439	393	514	449	61.1	13.6
Anthracene	ND	ND	ND	ND	ND	ND
Fluoranthene	7.0	6.3	7.4	6.9	0.6	8.3
Pyrene	7	6	8	6.5	0.8	13.1
C1- Pyrenes	122	119	130	124	5.8	4.7
C2- Pyrenes	194	183	201	193	9.2	5.0
C3- Pyrenes	174	156	173	168	10.2	6.1
C4- Pyrenes	108	100	135	114	18.5	16.2
Naphthobenzothiophene	73	64	70	69	4.6	6.6
C-1 Naphthobenzothiophenes	259	238	254	250	10.8	4.3
C-2 Naphthobenzothiophenes	279	258	309	282	26	9.1
C-3 Naphthobenzothiophenes	227	213	248	229	17.7	7.7
Benzo (a) Anthracene	12.9	13.8	18.7	15	3.1	20
Chrysene	94	74	70	80	12.2	15
C1- Chrysenes	163	139	159	153	9.5	6.2
C2- Chrysenes	217	177	207	200	20.8	10
C3- Chrysenes	172	155	199	175	22	13
C4- Chrysenes	128	97	115	113	15.4	14
Benzo (b) Fluoranthene	11.7	9.4	10.4	10.5	1.2	11
Benzo (k) Fluoranthene	ND	ND	ND	ND	ND	ND
Benzo (e) Pyrene	39	23	24	29	3.1	12
Benzo (a) Pyrene	ND	ND	ND	ND	ND	ND
Perylene	ND	ND	ND	ND	ND	ND
Indeno (1,2,3 - cd) Pyrene	ND	ND	ND	ND	ND	ND
Dibenzo (a,h) anthracene	ND	ND	ND	ND	ND	ND
Benzo (g,h,i) perylene	ND	ND	ND	ND	ND	ND
Total Aromatics	*10179*	*10333*	*10053*	*10188*	*188*	*1.8*

NUTRIENT, REPLICATE 1 Testing Date: Day 7 Initial Oil Weight: 510 mg Final Extracted Volume: 10 mL		
	Concentration (ng/mL)	Surrogate Corrected (ng/mL)
Alkane Analytes		
nC-10 Decane	ND	ND
nC-11 Undecane	ND	ND
nC-12 Dodecane	ND	ND
nC-13 Tridecane	ND	ND
nC-14 Tetradecane	12	12
nC-15 Pentadecane	87	87
nC-16 Hexadecane	439	434
nC-17 Heptadecane	1297	1290
Pristane	624	624
nC-18 Octadecane	617	1622
Phytane	1694	1622
nC-19 Nonadecane	920	920
nC-20 Eicosane	2005	2005
nC-21 Heneicosane	2361	2370
nC-22 Docosane	2478	2481
nC-23 Tricosane	2567	2693
nC-24 Tetracosane	2574	2660
nC-25 Pentacosane	2575	2684
nC-26 Hexacosane	2105	2127
nC-27 Heptacosane	2180	2202
nC-28 Octacosane	1838	1857
nC-29 Nonacosane	1640	1697
nC-30 Triacontane	1582	1689
nC-31 Hentriacontane	1639	1655
nC-32 Dotriacontane	1678	1695
nC-33 Tritriacontane	1268	1299
nC-34 Tetratriacontane	1079	1081
nC-35 Pentatriacontane	466	470
	1241	1255
Total Alkanes	*36427*	*36795*
Aromatic Analytes		
Naphthalene	ND	ND
C1-Naphthalenes	ND	ND
C2-Naphthalenes	41	55
C3-Naphthalenes	139	188
C4-Naphthalenes	269	391
Fluorene	19	19
C1-Fluorenes	103	168
C2-Fluorenes	269	350
C3- Fluorenes	351	474
Dibenzothiophene	131	176
C1-Dibenzothiophenes	460	622
C2-Dibenzothiophenes	740	1002
C3- Dibenzothiophenes	618	839
Phenanthrene	245	333
C1-Phenanthrenes	678	918
C2-Phenanthrenes	994	1345
C3-Phenanthrenes	719	972
C4-Phenanthrenes	267	361
Anthracene	271	366
Fluoranthene	6.2	8.4
Pyrene	6.2	8.4
C1- Pyrenes	81	109
C2- Pyrenes	142	192
C3- Pyrenes	16	22
C4- Pyrenes	4.3	5.8
Naphthobenzothiophene	66	89
C-1 Naphthobenzothiophenes	201	271
C-2 Naphthobenzothiophenes	158	213
C-3 Naphthobenzothiophenes	2.1	2.8
Benzo (a) Anthracene	79	107.3
Chrysene	44	59
C1- Chrysenes	119	161
C2- Chrysenes	138	187
C3- Chrysenes	2.1	2.8
C4- Chrysenes	ND	ND
Benzo (b) Fluoranthene	10.7	14
Benzo (k) Fluoranthene	3.2	4.4
Benzo (e) Pyrene	21	29
Benzo (a) Pyrene	4.4	6.0
Perylene	1.3	1.8
Indeno (1,2,3 - cd) Pyrene	ND	ND
Dibenzo (a,h) anthracene	ND	ND
Benzo (g,h,i) perylene	ND	ND
Total Aromatics	*7450*	*10057*
% Surrogate Recovery		
5 Alpha Androstane	0.99	1.00
Phenanthrene d-10	0.74	1.00

NUTRIENT, REPLICATE 2 Testing Date: Day 7 Initial Oil Weight: 510 mg Final Extracted Volume: 10 mL		
	Concentration (ng/mL)	Surrogate Corrected (ng/mL)
Alkane Analytes		
nC-10 Decane	ND	ND
nC-11 Undecane	ND	ND
nC-12 Dodecane	ND	ND
nC-13 Tridecane	ND	ND
nC-14 Tetradecane	10.1	11
nC-15 Pentadecane	70	73
nC-16 Hexadecane	361	376
nC-17 Heptadecane	909	947
Pristane	474	493
nC-18 Octadecane	1529	1535
Phytane	758	800
nC-19 Nonadecane	1706	1870
nC-20 Eicosane	1858	2015
nC-21 Heneicosane	1966	2048
nC-22 Docosane	2108	2193
nC-23 Tricosane	2009	2115
nC-24 Tetracosane	1966	2068
nC-25 Pentacosane	1831	1887
nC-26 Hexacosane	1598	1698
nC-27 Heptacosane	1625	1695
nC-28 Octacosane	1507	1600
nC-29 Nonacosane	1397	1456
nC-30 Triacontane	1556	1639
nC-31 Hentriacontane	1703	1820
nC-32 Dotriacontane	1295	1379
nC-33 Tritriacontane	1215	1256
nC-34 Tetratriacontane	660	672
nC-35 Pentatriacontane	1513	1554
Total Alkanes	*31529*	*32833*
Aromatic Analytes		
Naphthalene	ND	ND
C1-Naphthalenes	ND	ND
C2-Naphthalenes	63	69
C3-Naphthalenes	137	163
C4-Naphthalenes	264	372
Fluorene	11.1	16
C1-Fluorenes	99	149
C2-Fluorenes	238	336
C3- Fluorenes	293	413
Dibenzothiophene	113	160
C1-Dibenzothiophenes	339	478
C2-Dibenzothiophenes	643	905
C3- Dibenzothiophenes	508	708
Phenanthrene	205	288
C1-Phenanthrenes	627	884
C2-Phenanthrenes	887	1221
C3-Phenanthrenes	564	764
C4-Phenanthrenes	242	341
Anthracene	231	329
Fluoranthene	5.4	7.6
Pyrene	6.7	9.0
C1- Pyrenes	80	113
C2- Pyrenes	113	167
C3- Pyrenes	17	24
C4- Pyrenes	3.8	5.3
Naphthobenzothiophene	55	78
C-1 Naphthobenzothiophenes	169	237
C-2 Naphthobenzothiophenes	180	253
C-3 Naphthobenzothiophenes	2.6	4.0
Benzo (a) Anthracene	87.0	122.5
Chrysene	33	46
C1- Chrysenes	105	164
C2- Chrysenes	137	193
C3- Chrysenes	2.4	3.4
C4- Chrysenes	ND	ND
Benzo (b) Fluoranthene	13	18
Benzo (k) Fluoranthene	3.9	5.1
Benzo (e) Pyrene	23	32
Benzo (a) Pyrene	4.7	6.6
Perylene	1.5	2.1
Indeno (1,2,3 - cd) Pyrene	ND	ND
Dibenzo (a,h) anthracene	ND	ND
Benzo (g,h,i) perylene	ND	ND
Total Aromatics	*6527*	*9193*
% Surrogate Recovery		
5 Alpha Androstane	0.68	1.00
Phenanthrene d-10	0.71	1.00

NUTRIENT, REPLICATE 3 Testing Date: Day 7 Initial Oil Weight: 510 mg Final Extracted Volume: 10 mL		
	Concentration (ng/mL)	Surrogate Corrected (ng/mL)
Alkane Analytes		
nC-10 Decane	ND	ND
nC-11 Undecane	ND	ND
nC-12 Dodecane	ND	ND
nC-13 Tridecane	ND	ND
nC-14 Tetradecane	12	13
nC-15 Pentadecane	67	75
nC-16 Hexadecane	336	327
nC-17 Heptadecane	894	1137
Pristane	494	555
nC-18 Octadecane	1442	1570
Phytane	769	878
nC-19 Nonadecane	1730	1987
nC-20 Eicosane	1949	2179
nC-21 Heneicosane	2061	2248
nC-22 Docosane	2237	2370
nC-23 Tricosane	2383	2660
nC-24 Tetracosane	2355	2680
nC-25 Pentacosane	1988	1884
nC-26 Hexacosane	1720	1948
nC-27 Heptacosane	1620	1715
nC-28 Octacosane	1369	1638
nC-29 Nonacosane	1183	1339
nC-30 Triacontane	1491	1679
nC-31 Hentriacontane	1654	2560
nC-32 Dotriacontane	1147	1366
nC-33 Tritriacontane	942	1058
nC-34 Tetratriacontane	482	549
nC-35 Pentatriacontane	880	1010
Total Alkanes	*30858*	*34818*
Aromatic Analytes		
Naphthalene	ND	ND
C1-Naphthalenes	ND	ND
C2-Naphthalenes	54	75
C3-Naphthalenes	168	217
C4-Naphthalenes	343	476
Fluorene	12	17
C1-Fluorenes	118	163
C2-Fluorenes	276	383
C3- Fluorenes	353	489
Dibenzothiophene	130	181
C1-Dibenzothiophenes	422	586
C2-Dibenzothiophenes	641	889
C3- Dibenzothiophenes	700	972
Phenanthrene	291	404
C1-Phenanthrenes	728	1010
C2-Phenanthrenes	988	1370
C3-Phenanthrenes	706	981
C4-Phenanthrenes	265	368
Anthracene	273	378
Fluoranthene	4.8	6.6
Pyrene	5.5	7.7
C1- Pyrenes	79	110
C2- Pyrenes	107	151
C3- Pyrenes	18	26
C4- Pyrenes	3.8	5.3
Naphthobenzothiophene	61	84
C-1 Naphthobenzothiophenes	182	253
C-2 Naphthobenzothiophenes	178	247
C-3 Naphthobenzothiophenes	2.6	3.6
Benzo (a) Anthracene	82.7	115.2
Chrysene	34	47
C1- Chrysenes	121	168
C2- Chrysenes	142	197
C3- Chrysenes	2.6	3.6
C4- Chrysenes	ND	ND
Benzo (b) Fluoranthene	12	17
Benzo (k) Fluoranthene	3.7	5.1
Benzo (e) Pyrene	18	39
Benzo (a) Pyrene	4.6	6.7
Perylene	1.6	2.2
Indeno (1,2,3 - cd) Pyrene	ND	ND
Dibenzo (a,h) anthracene	ND	ND
Benzo (g,h,i) perylene	ND	ND
Total Aromatics	*7512*	*10434*
% Surrogate Recovery		
5 Alpha Androstane	0.69	1.00
Phenanthrene d-10	0.72	1.00

NUTRIENT STATISTICS FOR SURROGATE CORRECTED DATA Testing Date: Day 7						
	Replicate #1	Replicate #2	Replicate #3			
Alkane Analytes	Conc (ng/mL)	Conc (ng/mL)	Conc (ng/mL)	Average	Mean	S.E.M.
nC-10 Decane	0	ND	ND	ND	ND	ND
nC-11 Undecane	0	ND	ND	ND	ND	ND
nC-12 Dodecane	0	ND	ND	ND	ND	ND
nC-13 Tridecane	0	ND	ND	ND	ND	ND
nC-14 Tetradecane	12	11	13	12	12	0.6
nC-15 Pentadecane	87	73	75	78	8.0	4.0
nC-16 Hexadecane	434	376	327	396	33	8.4
nC-17 Heptadecane	1290	947	1137	1105	152	14
Pristane	624	493	555	557	65	12
nC-18 Octadecane	1622	1535	1570	1576	126	7.6
Phytane	895	800	878	857	84	7.3
nC-19 Nonadecane	2035	1870	1987	1964	78	4.0
nC-20 Eicosane	2375	2015	2179	2190	165	9.2
nC-21 Heneicosane	2361	2048	2248	2219	227	10
nC-22 Docosane	2823	2193	2370	2462	338	9.8
nC-23 Tricosane	2695	2115	2660	2490	289	12
nC-24 Tetracosane	2664	2068	2680	2451	284	14
nC-25 Pentacosane	2127	1887	1884	1966	223	13
nC-26 Hexacosane	2292	1948	1948	2045	197	8.7
nC-27 Heptacosane	1867	1695	1715	1759	68	6.0
nC-28 Octacosane	1667	1599	1638	1635	55	3.7
nC-29 Nonacosane	1606	1456	1339	1434	136	9.9
nC-30 Triacontane	1855	1639	1679	1658	36	1.7
nC-31 Hentriacontane	1693	1629	2560	1958	306	31
nC-32 Dotriacontane	1295	1379	1366	1288	17	0.9
nC-33 Tritriacontane	1251	1256	1058	1188	114	16
nC-34 Tetratriacontane	479	672	549	566	55	16
nC-35 Pentatriacontane	1253	1554	1010	1154	135	12
Total Alkanes	*36795*	*32833*	*34818*	*34815*	*1961*	*5.7*
Aromatic Analytes	Conc (ng/mL)	Conc (ng/mL)	Conc (ng/mL)	Average	Mean	S.E.M.
Naphthalene	ND	ND	ND	ND	ND	ND
C1-Naphthalenes	ND	ND	ND	ND	ND	ND
C2-Naphthalenes	55	69	75	66	9.8	15
C3-Naphthalenes	188	163	217	189	16	7.9
C4-Naphthalenes	391	372	476	413	56	13
Fluorene	19	16	17	17	1.5	8.6
C1-Fluorenes	169	149	163	160	14.4	9.0
C2-Fluorenes	350	336	383	356	24	6.8
C3- Fluorenes	474	413	489	459	40	8.8
Dibenzothiophene	176	160	181	172	11	6.5
C1-Dibenzothiophenes	622	478	586	562	75	13
C2-Dibenzothiophenes	1002	905	889	932	61	6.6
C3- Dibenzothiophenes	839	708	972	840	97	11
Phenanthrene	333	288	404	342	34.3	7.5
C1-Phenanthrenes	918	884	1010	937	66	7.0
C2-Phenanthrenes	1345	1221	1370	1311	69	5.1
C3-Phenanthrenes	972	764	981	906	106	12
C4-Phenanthrenes	361	341	368	356	25	7.1
Anthracene	366.01	329.20	378.27	357	25	7.0
Fluoranthene	8.4	7.6	6.6	7.5	0.9	12
Pyrene	8	8	8	8.0	0.4	4.6
C1- Pyrenes	109	113	110	111	1.9	1.7
C2- Pyrenes	192	167	151	170	14.1	8.3
C3- Pyrenes	22	24	26	24	1.8	7.5
C4- Pyrenes	6	5	5	5	0.3	5.1
Naphthobenzothiophene	89	78	84	84	5.8	7.0
C-1 Naphthobenzothiophenes	271	237	253	253	17.0	6.7
C-2 Naphthobenzothiophenes	213	253	247	238	21	9.0
C-3 Naphthobenzothiophenes	3	4	4	3.6	0.5	12
Benzo (a) Anthracene	107.3	122.5	115.2	115	7.5	6.6
Chrysene	59	46	47	51	7.3	14
C1- Chrysenes	161	144	168	158	12.3	7.8
C2- Chrysenes	187	193	197	192	5.2	2.7
C3- Chrysenes	3	3	4	3	0.4	13
C4- Chrysenes	ND	ND	ND	ND	ND	ND
Benzo (b) Fluoranthene	14.6	18.1	17.2	17	1.5	12
Benzo (k) Fluoranthene	4.4	5.1	5.1	4.9	0.4	8.8
Benzo (e) Pyrene	28	32	39	33	3.2	11
Benzo (a) Pyrene	6.6	6.6	6.7	6.6	0.4	6.4
Perylene	1.8	2.1	2.2	2.0	0.2	10
Indeno (1,2,3 - cd) Pyrene	ND	ND	ND	ND	ND	ND
Dibenzo (a,h) anthracene	ND	ND	ND	ND	ND	ND
Benzo (g,h,i) perylene	ND	ND	ND	ND	ND	ND
Total Aromatics	*10097*	*9193*	*10434*	*9908*	*638*	*6.4*

PRODUCT, REPLICATE 1 Testing Date: Day 7 Initial Oil Weight: 510 mg Final Extracted Volume: 10 mL		
	Concentration (ng/mL)	Surrogate Corrected (ng/mL)
Alkane Analyte:		
nC-10 Decane	ND	ND
nC-11 Undecane	ND	ND
nC-12 Dodecane	ND	ND
nC-13 Tridecane	ND	ND
nC-14 Tetradecane	6.9	7.7
nC-15 Pentadecane	81	88
nC-16 Hexadecane	320	355
nC-17 Heptadecane	879	967
Pristane	487	541
nC-18 Octadecane	1298	1429
Phytane	672	748
nC-19 Nonadecane	1514	1682
nC-20 Eicosane	1672	1858
nC-21 Heneicosane	1680	1878
nC-22 Docosane	1756	1957
nC-23 Tricosane	1723	1914
nC-24 Tetracosane	1732	1924
nC-25 Pentacosane	1465	1628
nC-26 Hexacosane	1539	1709
nC-27 Heptacosane	1309	1429
nC-28 Octacosane	842	855
nC-29 Nonacosane	897	758
nC-30 Triacontane	723	803
nC-31 Hentriacontane	967	1074
nC-32 Dotriacontane	823	846
nC-33 Tritriacontane	881	856
nC-34 Tetratriacontane	356	328
nC-35 Pentatriacontane	314	349
Total Alkanes	*23061*	*25623*
Aromatic Analyte:		
Naphthalene	ND	ND
C1-Naphthalenes	ND	ND
C2-Naphthalenes	2.2	2.6
C3-Naphthalenes	109	141.5
C4-Naphthalenes	138	178.9
Fluorene	13	17.5
C1-Fluorenes	110	142.8
C2-Fluorenes	286	382.0
C3- Fluorenes	277	383.5
Dibenzothiophene	147	183.2
C1-Dibenzothiophenes	362	478.4
C2-Dibenzothiophenes	685	889.9
C3- Dibenzothiophenes	575	744.1
Phenanthrene	259	326.5
C1-Phenanthrenes	729	948.1
C2-Phenanthrenes	950	1234.1
C3-Phenanthrenes	427	567.6
C4-Phenanthrenes	218	288.4
Anthracene	266	345.1
Fluoranthene	8.6	9.6
Pyrene	6.1	7.9
C1- Pyrenes	81	105.5
C2- Pyrenes	181	131.7
C3- Pyrenes	2.7	3.5
C4- Pyrenes	1.9	2.5
Naphthobenzothiophene	73	95.2
C-1 Naphthobenzothiophenes	187	242.6
C-2 Naphthobenzothiophenes	10.0	13.0
C-3 Naphthobenzothiophenes	1.1	1.4
Benzo (a) Anthracene	45	58.0
Chrysene	26	33.6
C1- Chrysenes	100	130.0
C2- Chrysenes	128	165.8
C3- Chrysenes	5.5	7.1
C4- Chrysenes	ND	ND
Benzo (b) Fluoranthene	14	18.2
Benzo (k) Fluoranthene	ND	ND
Benzo (e) Pyrene	20.6	26.8
Benzo (a) Pyrene	7.0	ND
Perylene	ND	ND
Indeno (1,2,3 - cd) Pyrene	2.4	4.4
Dibenzo (a,h) anthracene	ND	ND
Benzo (g,h,i) perylene	ND	ND
Total Aromatics	*5334*	*6328*
% Surrogate Recovery		
5 Alpha Androstane	0.99	1.00
Phenanthrene-d-10	0.77	1.00

PRODUCT, REPLICATE 2 Testing Date: Day 7 Initial Oil Weight: 510 mg Final Extracted Volume: 10 mL		
	Concentration (ng/mL)	Surrogate Corrected (ng/mL)
Alkane Analyte:		
nC-10 Decane	ND	ND
nC-11 Undecane	ND	ND
nC-12 Dodecane	ND	ND
nC-13 Tridecane	ND	ND
nC-14 Tetradecane	7.6	8.8
nC-15 Pentadecane	64	73
nC-16 Hexadecane	313	360
nC-17 Heptadecane	872	1002
Pristane	507	577
nC-18 Octadecane	1331	1530
Phytane	672	773
nC-19 Nonadecane	1441	1657
nC-20 Eicosane	1676	1929
nC-21 Heneicosane	1706	2004
nC-22 Docosane	1839	2216
nC-23 Tricosane	1917	2294
nC-24 Tetracosane	1957	2369
nC-25 Pentacosane	1569	1828
nC-26 Hexacosane	1551	1817
nC-27 Heptacosane	1342	1545
nC-28 Octacosane	1036	1189
nC-29 Nonacosane	652	750
nC-30 Triacontane	878	1011
nC-31 Hentriacontane	976	1123
nC-32 Dotriacontane	769	887
nC-33 Tritriacontane	551	633
nC-34 Tetratriacontane	369	424
nC-35 Pentatriacontane	321	369
Total Alkanes	*20493*	*23153*
Aromatic Analyte:		
Naphthalene	ND	ND
C1-Naphthalenes	ND	ND
C2-Naphthalenes	2.6	3.1
C3-Naphthalenes	94	119.9
C4-Naphthalenes	141	185.4
Fluorene	14.0	18.4
C1-Fluorenes	89	118.7
C2-Fluorenes	284	387.8
C3- Fluorenes	311	408.8
Dibenzothiophene	139	169.8
C1-Dibenzothiophenes	435	572.4
C2-Dibenzothiophenes	644	845.8
C3- Dibenzothiophenes	556	729.6
Phenanthrene	263	345.1
C1-Phenanthrenes	709	961.2
C2-Phenanthrenes	931	1290.7
C3-Phenanthrenes	455	599.6
C4-Phenanthrenes	253	333.3
Anthracene	279	367.4
Fluoranthene	6.0	7.6
Pyrene	6.0	7.9
C1- Pyrenes	89	113.0
C2- Pyrenes	96	126.8
C3- Pyrenes	3.9	5.1
C4- Pyrenes	1.9	2.6
Naphthobenzothiophene	75	98.1
C-1 Naphthobenzothiophene	185	243.1
C-2 Naphthobenzothiophene	13	16.9
C-3 Naphthobenzothiophene	1.2	1.6
Benzo (a) Anthracene	51.1	67.2
Chrysene	31	40.9
C1- Chrysenes	119	155.1
C2- Chrysenes	115	150.7
C3- Chrysenes	4.0	5.3
C4- Chrysenes	ND	ND
Benzo (b) Fluoranthene	12.3	16.2
Benzo (k) Fluoranthene	ND	ND
Benzo (e) Pyrene	18	23.8
Benzo (a) Pyrene	ND	ND
Perylene	3.1	4.0
Indeno (1,2,3 - cd) Pyrene	ND	ND
Dibenzo (a,h) anthracene	ND	ND
Benzo (g,h,i) perylene	ND	ND
Total Aromatics	*6483*	*8539*
% Surrogate Recovery		
5 Alpha Androstane	0.87	1.00
Phenanthrene-d-10	0.76	1.00

PRODUCT, REPLICATE 3 Testing Date: Day 7 Initial Oil Weight: 510 mg Final Extracted Volume: 10 mL		
	Concentration (ng/mL)	Surrogate Corrected (ng/mL)
Alkane Analyte:		
nC-10 Decane	ND	ND
nC-11 Undecane	ND	ND
nC-12 Dodecane	ND	ND
nC-13 Tridecane	ND	ND
nC-14 Tetradecane	5.9	6.5
nC-15 Pentadecane	53	59
nC-16 Hexadecane	275	324
nC-17 Heptadecane	783	909
Pristane	403	510
nC-18 Octadecane	1128	1378
Phytane	607	766
nC-19 Nonadecane	1389	1615
nC-20 Eicosane	1540	1791
nC-21 Heneicosane	1574	1831
nC-22 Docosane	1774	2062
nC-23 Tricosane	1723	2006
nC-24 Tetracosane	1571	1845
nC-25 Pentacosane	1314	1529
nC-26 Hexacosane	1260	1561
nC-27 Heptacosane	1151	1262
nC-28 Octacosane	592	652
nC-29 Nonacosane	654	709
nC-30 Triacontane	703	817
nC-31 Hentriacontane	858	998
nC-32 Dotriacontane	636	736
nC-33 Tritriacontane	496	579
nC-34 Tetratriacontane	321	373
nC-35 Pentatriacontane	302	416
Total Alkanes	*21647*	*25171*
Aromatic Analyte:		
Naphthalene	ND	ND
C1-Naphthalenes	ND	ND
C2-Naphthalenes	2.3	2.9
C3-Naphthalenes	96	124.2
C4-Naphthalenes	106	139.3
Fluorene	13.9	18.4
C1-Fluorenes	83	118.2
C2-Fluorenes	214	270.9
C3- Fluorenes	271	342.6
Dibenzothiophene	118	149.9
C1-Dibenzothiophenes	355	461.6
C2-Dibenzothiophenes	639	859.2
C3- Dibenzothiophenes	568	756.4
Phenanthrene	256	298.3
C1-Phenanthrenes	514	777.6
C2-Phenanthrenes	878	1168.6
C3-Phenanthrenes	411	519.8
C4-Phenanthrenes	249	314.7
Anthracene	269	334.7
Fluoranthene	8.6	7.5
Pyrene	6.0	6.3
C1- Pyrenes	76	95.7
C2- Pyrenes	102	129.4
C3- Pyrenes	3.4	4.3
C4- Pyrenes	1.6	2.3
Naphthobenzothiophene	65	83.6
C-1 Naphthobenzothiophenes	157	199.2
C-2 Naphthobenzothiophenes	10.2	13.5
C-3 Naphthobenzothiophenes	1.2	1.5
Benzo (a) Anthracene	49	62.9
Chrysene	31	39.4
C1- Chrysenes	108	138.1
C2- Chrysenes	113	143.2
C3- Chrysenes	4.2	5.6
C4- Chrysenes	ND	ND
Benzo (b) Fluoranthene	14	17.4
Benzo (k) Fluoranthene	ND	ND
Benzo (e) Pyrene	20	25.9
Benzo (a) Pyrene	ND	ND
Perylene	2.8	3.5
Indeno (1,2,3 - cd) Pyrene	ND	ND
Dibenzo (a,h) anthracene	ND	ND
Benzo (g,h,i) perylene	ND	ND
Total Aromatics	*5883*	*7460*
% Surrogate Recovery		
5 Alpha Androstane	0.88	1.00
Phenanthrene-d-10	0.79	1.00

PRODUCT STATISTICS FOR SURROGATE CORRECTED DATA Testing Date: Day 7						
	Replicate #1	Replicate #2	Replicate #3			
Alkane Analyte:	Conc (ng/mL)	Conc (ng/mL)	Conc (ng/mL)	Average	Stdev	%RSD
nC-10 Decane	ND	ND	ND	ND	ND	ND
nC-11 Undecane	ND	ND	ND	ND	ND	ND
nC-12 Dodecane	ND	ND	ND	ND	ND	ND
nC-13 Tridecane	ND	ND	ND	ND	ND	ND
nC-14 Tetradecane	8	9	7	7.7	1.1	15
nC-15 Pentadecane	88	73	59	73	5.5	8.1
nC-16 Hexadecane	355	360	324	346	19.8	5.7
nC-17 Heptadecane	967	1002	909	959	47	4.9
Pristane	541	577	510	543	33.6	6.2
nC-18 Octadecane	1429	1530	1323	1427	103	7.2
Phytane	748	773	766	762	14	1.8
nC-19 Nonadecane	1682	1657	1615	1651	34	2.1
nC-20 Eicosane	1858	1929	1791	1859	69	3.7
nC-21 Heneicosane	1878	2004	1831	1904	124	6.4
nC-22 Docosane	1957	2216	2062	2077	133	6.4
nC-23 Tricosane	1914	2294	2006	2071	148	7.2
nC-24 Tetracosane	1924	2369	1845	2046	136	6.6
nC-25 Pentacosane	1628	1828	1529	1661	153	9.2
nC-26 Hexacosane	1709	1817	1561	1701	118	6.9
nC-27 Heptacosane	1429	1543	1262	1411	91	6.5
nC-28 Octacosane	855	1189	652	899	167	18
nC-29 Nonacosane	758	750	709	747	15	2.0
nC-30 Triacontane	803	1011	817	877	116	13
nC-31 Hentriacontane	1074	1123	998	1064	64	6.1
nC-32 Dotriacontane	846	887	736	785	191	17
nC-33 Tritriacontane	856	633	579	623	49	6.4
nC-34 Tetratriacontane	328	424	373	375	48	11
nC-35 Pentatriacontane	349	369	416	378	36	9.4
Total Alkanes	*25623*	*26183*	*25171*	*25326*	*1607*	*6.1*
Aromatic Analyte:	Conc (ng/mL)	Conc (ng/mL)	Conc (ng/mL)	Average	Stdev	%RSD
Naphthalene	ND	ND	ND	ND	ND	ND
C1-Naphthalenes	ND	ND	ND	ND	ND	ND
C2-Naphthalenes	3	4	3	3.2	0.5	15
C3-Naphthalenes	142	110	124	125	16	13
C4-Naphthalenes	177	155	139	157	26	16
Fluorene	17	18	18	17	1.0	5.8
C1-Fluorenes	145	137	118	126	16	12
C2-Fluorenes	359	368	271	333	51	15
C3- Fluorenes	364	408	343	305	39	13
Dibenzothiophene	195	176	149	167	17.3	10
C1-Dibenzothiophenes	478	572	462	504	59	11.8
C2-Dibenzothiophenes	850	847	809	845	43	4.8
C3- Dibenzothiophenes	744	795	756	749	48.6	6.1
Phenanthrene	395	348	256	327	20.3	7.7
C1-Phenanthrenes	945	961	772	895	102	11
C2-Phenanthrenes	1234	1291	1169	1231	93	7.7
C3-Phenanthrenes	568	600	509	559	49	7.1
C4-Phenanthrenes	309	333	315	319	27	8.7
Anthracene	345	307	305	319	21	6.7
Fluoranthene	8.6	7.8	7.5	8.0	0.5	6.7
Pyrene	6	8	7.4	6.9	1.2	17
C1- Pyrenes	105	113	98	105	8.7	8.3
C2- Pyrenes	127	127	129	127	2.4	1.9
C3- Pyrenes	4	5	4	4.3	0.6	15
C4- Pyrenes	3	3	2	2.4	0.1	5.5
Naphthobenzothiophene	95	88	84	89	7.7	8.7
C-1 Naphthobenzothiophenes	243	248	169	220	27.1	12
C-2 Naphthobenzothiophenes	13	17	15	15	2.0	13
C-3 Naphthobenzothiophenes	1	2	2	1.5	0.1	6.6
Benzo (a) Anthracene	59	67	62	62	4.0	7.4
Chrysene	39	41	38	39	4.2	11
C1- Chrysenes	130	150	128	141	13.7	9.7
C2- Chrysenes	159	161	143	153	11.5	7.5
C3- Chrysenes	7	6	6	6.9	1.0	15
C4- Chrysenes	ND	ND	ND	ND	ND	ND
Benzo (b) Fluoranthene	18.2	18.2	17.4	17.9	1.0	5.7
Benzo (k) Fluoranthene	ND	ND	ND	ND	ND	ND
Benzo (e) Pyrene	26.8	23.8	25.9	25	1.2	4.7
Benzo (a) Pyrene	ND	ND	ND	ND	ND	ND
Perylene	4.4	4.6	3.5	4.8	0.4	11
Indeno (1,2,3 - cd) Pyrene	ND	ND	ND	ND	ND	ND
Dibenzo (a,h) anthracene	ND	ND	ND	ND	ND	ND
Benzo (g,h,i) perylene	ND	ND	ND	ND	ND	ND
Total Aromatics	*8336*	*8539*	*7460*	*8072*	*551*	*6.8*

CONTROL, REPLICATE 1 Testing Date: Day 28 Initial Oil Weight: 625 mg Final Extracted Volume: 10 mL		
	Concentration (ng/mL)	Surrogate Corrected (ng/mL)
Alkane Analyte:		
nC-10 Decane	ND	ND
nC-11 Undecane	ND	ND
nC-12 Dodecane	ND	ND
nC-13 Tridecane	ND	ND
nC-14 Tetradecane	11.3	14
nC-15 Pentadecane	78	95
nC-16 Hexadecane	429	512
nC-17 Heptadecane	1085	1321
Pristane	610	744
nC-18 Octadecane	1659	2022
Phytane	853	1053
nC-19 Nonadecane	1629	2053
nC-20 Eicosane	1805	2343
nC-21 Heneicosane	2111	2875
nC-22 Docosane	2351	2987
nC-23 Tricosane	2175	2854
nC-24 Tetracosane	2375	2938
nC-25 Pentacosane	1874	2641
nC-26 Hexacosane	1817	2315
nC-27 Heptacosane	1576	1821
nC-28 Octacosane	1378	1566
nC-29 Nonacosane	1244	1517
nC-30 Triacontane	1012	1234
nC-31 Hentriacontane	1062	1466
nC-32 Dotriacontane	677	859
nC-33 Tritriacontane	533	669
nC-34 Tetratriacontane	296	362
nC-35 Pentatriacontane	397	447
Total Alkanes	29466	36934
Aromatic Analyte:		
Naphthalene	ND	ND
C1-Naphthalenes	ND	ND
C2-Naphthalenes	36	46
C3-Naphthalenes	135	171
C4-Naphthalenes	271	343
Fluorene	15	19
C1-Fluorenes	137	174
C2-Fluorenes	290	367
C3- Fluorenes	338	428
Dibenzothiophene	129	161
C1-Dibenzothiophenes	463	586
C2-Dibenzothiophenes	639	808
C3- Dibenzothiophenes	693	876
Phenanthrene	270	270
C1-Phenanthrenes	681	852
C2-Phenanthrenes	1091	1287
C3-Phenanthrenes	661	795
C4-Phenanthrenes	418	526
Anthracene	302	382.6
Fluoranthene	6	7.9
Pyrene	5	5.9
C1- Pyrenes	96	122
C2- Pyrenes	135	171
C3- Pyrenes	5	6
C4- Pyrenes	3	3
Naphthobenzothiophene	69	80
C-1 Naphthobenzothiophenes	189	239
C-2 Naphthobenzothiophenes	165	208
C-3 Naphthobenzothiophenes	5	6
Benzo (a) Anthracene	61	78.6
Chrysene	44	56
C1- Chrysenes	124	157
C2- Chrysenes	145	183
C3- Chrysenes	81	102
C4- Chrysenes	102	129
Benzo (b) Fluoranthene	19	13
Benzo (k) Fluoranthene	ND	ND
Benzo (e) Pyrene	3	3
Benzo (a) Pyrene	ND	ND
Perylene	ND	ND
Indeno (1,2,3 - cd) Pyrene	ND	ND
Dibenzo (a,h) anthracene	ND	ND
Benzo (g,h,i) perylene	ND	ND
Total Aromatics	7633	9536
% Surrogate Recovery		
5 Alpha Androstane	0.80	1.00
Phenanthrene d-10	0.78	1.00

CONTROL, REPLICATE 2 Testing Date: Day 28 Initial Oil Weight: 650 mg Final Extracted Volume: 10 mL		
	Concentration (ng/mL)	Surrogate Corrected (ng/mL)
Alkane Analyte:		
nC-10 Decane	ND	ND
nC-11 Undecane	ND	ND
nC-12 Dodecane	ND	ND
nC-13 Tridecane	ND	ND
nC-14 Tetradecane	9.65	11.4
nC-15 Pentadecane	74	87
nC-16 Hexadecane	351	401.9
nC-17 Heptadecane	900	1034
Pristane	633	791
nC-18 Octadecane	1422	1671
Phytane	685	805
nC-19 Nonadecane	1443	1846
nC-20 Eicosane	1679	2206
nC-21 Heneicosane	1983	2186
nC-22 Docosane	1825	2402
nC-23 Tricosane	1823	2530
nC-24 Tetracosane	1953	2544
nC-25 Pentacosane	1702	2245
nC-26 Hexacosane	1710	2264
nC-27 Heptacosane	1463	1932
nC-28 Octacosane	1245	1456
nC-29 Nonacosane	1314	1729
nC-30 Triacontane	1209	1578
nC-31 Hentriacontane	1201	1580
nC-32 Dotriacontane	912	1239
nC-33 Tritriacontane	565	745
nC-34 Tetratriacontane	299	397
nC-35 Pentatriacontane	455	533
Total Alkanes	24571	34963
Aromatic Analyte:		
Naphthalene	ND	ND
C1-Naphthalenes	ND	ND
C2-Naphthalenes	37	49
C3-Naphthalenes	115	151
C4-Naphthalenes	279	351
Fluorene	11	14
C1-Fluorenes	140	182
C2-Fluorenes	245	319
C3- Fluorenes	324	431
Dibenzothiophene	107	139
C1-Dibenzothiophenes	387	502
C2-Dibenzothiophenes	695	903
C3- Dibenzothiophenes	542	704
Phenanthrene	189	246
C1-Phenanthrenes	677	879
C2-Phenanthrenes	965	1253
C3-Phenanthrenes	519	674
C4-Phenanthrenes	401	521
Anthracene	249.66	323
Fluoranthene	5.1	6.7
Pyrene	5.3	6.8
C1- Pyrenes	85	117
C2- Pyrenes	136	177
C3- Pyrenes	6	8
C4- Pyrenes	3	3
Naphthobenzothiophene	57	74
C-1 Naphthobenzothiophenes	188	242
C-2 Naphthobenzothiophenes	159	247
C-3 Naphthobenzothiophenes	4	5
Benzo (a) Anthracene	55.9	71.5
Chrysene	37	48
C1- Chrysenes	109	141
C2- Chrysenes	132	171
C3- Chrysenes	102	132
C4- Chrysenes	121	158
Benzo (b) Fluoranthene	13	16
Benzo (k) Fluoranthene	ND	ND
Benzo (e) Pyrene	3	4
Benzo (a) Pyrene	ND	ND
Perylene	ND	ND
Indeno (1,2,3 - cd) Pyrene	ND	ND
Dibenzo (a,h) anthracene	ND	ND
Benzo (g,h,i) perylene	ND	ND
Total Aromatics	7130	9269
% Surrogate Recovery		
5 Alpha Androstane	0.78	1.00
Phenanthrene d-10	0.77	1.00

CONTROL, REPLICATE 3 Testing Date: Day 28 Initial Oil Weight: 650 mg Final Extracted Volume: 10 mL		
	Concentration (ng/mL)	Surrogate Corrected (ng/mL)
Alkane Analyte:		
nC-10 Decane	ND	ND
nC-11 Undecane	ND	ND
nC-12 Dodecane	ND	ND
nC-13 Tridecane	ND	ND
nC-14 Tetradecane	9.65	11
nC-15 Pentadecane	64	87
nC-16 Hexadecane	390	447.7
nC-17 Heptadecane	1155	1325
Pristane	609	700
nC-18 Octadecane	1539	1884
Phytane	843	968
nC-19 Nonadecane	1690	2172
nC-20 Eicosane	2346	2991
nC-21 Heneicosane	2317	2848
nC-22 Docosane	2355	2787
nC-23 Tricosane	2345	2899
nC-24 Tetracosane	2367	2721
nC-25 Pentacosane	1947	2235
nC-26 Hexacosane	1855	2169
nC-27 Heptacosane	1757	1992
nC-28 Octacosane	1561	1794
nC-29 Nonacosane	1483	1981
nC-30 Triacontane	1236	1422
nC-31 Hentriacontane	1285	1664
nC-32 Dotriacontane	945	1090
nC-33 Tritriacontane	716	921
nC-34 Tetratriacontane	382	439
nC-35 Pentatriacontane	395	447
Total Alkanes	31509	36867
Aromatic Analyte:		
Naphthalene	ND	ND
C1-Naphthalenes	ND	ND
C2-Naphthalenes	46	54
C3-Naphthalenes	135	163
C4-Naphthalenes	216	263
Fluorene	13	15
C1-Fluorenes	119	143
C2-Fluorenes	291	351
C3- Fluorenes	352	458
Dibenzothiophene	124	159
C1-Dibenzothiophenes	379	457
C2-Dibenzothiophenes	591	905
C3- Dibenzothiophenes	629	747
Phenanthrene	231	278
C1-Phenanthrenes	831	1001
C2-Phenanthrenes	1253	1509
C3-Phenanthrenes	626	674
C4-Phenanthrenes	371	447
Anthracene	286.37	359
Fluoranthene	6.4	7.7
Pyrene	5.7	6.8
C1- Pyrenes	100	120
C2- Pyrenes	160	193
C3- Pyrenes	5	6
C4- Pyrenes	2	3
Naphthobenzothiophene	69	78
C-1 Naphthobenzothiophenes	207	249
C-2 Naphthobenzothiophenes	167	189
C-3 Naphthobenzothiophenes	4	5
Benzo (a) Anthracene	50.5	58.1
Chrysene	38	44
C1- Chrysenes	136	164
C2- Chrysenes	165	199
C3- Chrysenes	150	121
C4- Chrysenes	124	149
Benzo (b) Fluoranthene	13.3	16
Benzo (k) Fluoranthene	ND	ND
Benzo (e) Pyrene	3	3
Benzo (a) Pyrene	ND	ND
Perylene	ND	ND
Indeno (1,2,3 - cd) Pyrene	ND	ND
Dibenzo (a,h) anthracene	ND	ND
Benzo (g,h,i) perylene	ND	ND
Total Aromatics	5138	6805
% Surrogate Recovery		
5 Alpha Androstane	0.87	1.00
Phenanthrene d-10	0.83	1.00

CONTROL STATISTICS FOR SURROGATE CORRECTED DATA Testing Date: Day 28						
	Replicate #1	Replicate #2	Replicate #3			
	Corrected (ng/mL)	Corrected (ng/mL)	Corrected (ng/mL)	Average	Stdev	%RSD
Alkane Analyte:						
nC-10 Decane	ND	ND	ND	ND	ND	ND
nC-11 Undecane	ND	ND	ND	ND	ND	ND
nC-12 Dodecane	ND	ND	ND	ND	ND	ND
nC-13 Tridecane	ND	ND	ND	ND	ND	ND
nC-14 Tetradecane	14	11	11	12	1	11
nC-15 Pentadecane	95	87	87	89	5	6
nC-16 Hexadecane	512	402	448	454	34	7.5
nC-17 Heptadecane	1321	1034	1325	1227	164	13
Pristane	744	791	700	745	25	3.4
nC-18 Octadecane	2022	1671	1884	1859	64.34	4.4
Phytane	1053	805	968	942	74	7.8
nC-19 Nonadecane	2053	1846	2172	2024	169	8.4
nC-20 Eicosane	2343	2206	2991	2513	352	14
nC-21 Heneicosane	2875	2186	2848	2636	318	8.6
nC-22 Docosane	2987	2402	2787	2659	226	6.9
nC-23 Tricosane	2854	2530	2899	2761	85	3.3
nC-24 Tetracosane	2938	2544	2721	2734	170.99	6.5
nC-25 Pentacosane	2641	2245	2235	2373	114	5.3
nC-26 Hexacosane	2315	2264	2169	2249	32.56	1.5
nC-27 Heptacosane	1821	1932	1992	1915	21.30	1.1
nC-28 Octacosane	1566	1456	1794	1605	120	7.2
nC-29 Nonacosane	1517	1729	1981	1742	111	6.8
nC-30 Triacontane	1234	1578	1422	1412	172	13
nC-31 Hentriacontane	1466	1580	1664	1570	74	4.7
nC-32 Dotriacontane	859	1239	1090	1063	191	18
nC-33 Tritriacontane	669	745	921	778	88	12
nC-34 Tetratriacontane	362	397	439	399	39	10
nC-35 Pentatriacontane	447	533	447	476	51	11
Total Alkanes	36934	34963	36494	35797	776	2.2
Aromatic Analyte:	Corrected (ng/mL)	Corrected (ng/mL)	Corrected (ng/mL)	Average	Stdev	%RSD
Naphthalene	ND	ND	ND	ND	ND	ND
C1-Naphthalenes	ND	ND	ND	ND	ND	ND
C2-Naphthalenes	46	49	54	49	4	8.9
C3-Naphthalenes	171	151	163	162	10	6.3
C4-Naphthalenes	343	351	263	319	46	15
Fluorene	19	14	15	16	2.5	16
C1-Fluorenes	174	182	143	166	20	12
C2-Fluorenes	367	319	351	345	24	7.1
C3- Fluorenes	428	431	458	439	20	4.5
Dibenzothiophene	161	139	159	147	7	4.8
C1-Dibenzothiophenes	586	503	457	515	66	12.7
C2-Dibenzothiophenes	808	903	905	872	79	8.8
C3- Dibenzothiophenes	876	704	747	776	88	8.2
Phenanthrene	270	246	278	265	19	7.1
C1-Phenanthrenes	852	879	1001	911	76	8.3
C2-Phenanthrenes	1287	1253	1509	1349	144	11
C3-Phenanthrenes	795	674	745	738	77	10
C4-Phenanthrenes	526	521	447	498	44	8.9
Anthracene	382.6	323.5	359.9	355	30	8.6
Fluoranthene	7.9	6.7	7.7	7.4	0.63	8.4
Pyrene	5.9	6.8	7	6.9	0.5	7.3
C1- Pyrenes	122	117	120	120	2.6	2.2
C2- Pyrenes	171	177	193	180	11.7	6.5
C3- Pyrenes	6	8	6	7	1	15
C4- Pyrenes	3	3	3	3	0.3	11
Naphthobenzothiophene	80	74	78	77	2.6	3.4
C-1 Naphthobenzothiophenes	239	242	249	243	5.5	2.3
C-2 Naphthobenzothiophenes	208	247	189	214	30	14
C-3 Naphthobenzothiophenes	6	5	6	6	0.7	12
Benzo (a) Anthracene	78.6	71.5	58.1	72	4.4	6.1
Chrysene	56	48	44	49	6.1	12
C1- Chrysenes	157	141	164	154	11.5	7.7
C2- Chrysenes	183	171	199	184	13.95	7.4
C3- Chrysenes	102	132	121	118	15.2	13
C4- Chrysenes	129	158	149	145	15.0	10
Benzo (b) Fluoranthene	13.3	16.4	16.1	15.7	1.74	11
Benzo (k) Fluoranthene	ND	ND	ND	ND	ND	ND
Benzo (e) Pyrene	3.2	3.7	3.4	3.4	0.13	3.8
Benzo (a) Pyrene	ND	ND	ND	ND	ND	ND
Perylene	ND	ND	ND	ND	ND	ND
Indeno (1,2,3 - cd) Pyrene	ND	ND	ND	ND	ND	ND
Dibenzo (a,h) anthracene	ND	ND	ND	ND	ND	ND
Benzo (g,h,i) perylene	ND	ND	ND	ND	ND	ND
Total Aromatics	9636	9269	9805	9634	272	2.9

NUTRIENT, REPLICATE 1 Testing Date: Day 28 Initial Oil Weight: 610 mg Final Extracted Volume: 10 mL		
	Concentration (ng/mL)	Surrogate Corrected (ng/mL)
Alkane Analyte:		
nC-10 Decane	ND	ND
nC-11 Undecane	ND	ND
nC-12 Dodecane	ND	ND
nC-13 Tridecane	ND	ND
nC-14 Tetradecane	6.69	6
nC-15 Pentadecane	57	57
nC-16 Hexadecane	499	586
nC-17 Heptadecane	865	1018
Pristane	533	627
nC-18 Octadecane	1123	1321
Phytane	739	869
nC-19 Nonadecane	1270	1494
nC-20 Eicosane	1551	1824
nC-21 Heneicosane	1551	1871
nC-22 Docosane	1689	1987
nC-23 Tricosane	1651	1942
nC-24 Tetracosane	1544	1838
nC-25 Pentacosane	1391	1637
nC-26 Hexacosane	1235	1563
nC-27 Heptacosane	1140	1341
nC-28 Octacosane	1126	1324
nC-29 Nonacosane	1076	1265
nC-30 Triacontane	970	1093
nC-31 Hentriacontane	853	1008
nC-32 Dotriacontane	629	716
nC-33 Tritriacontane	71	84
nC-34 Tetratriacontane	301	354
nC-35 Pentatriacontane	187	231
Total Alkanes	**22272**	**26202**
Aromatic Analyte:		
Naphthalene	ND	ND
C1-Naphthalenes	ND	ND
C2-Naphthalenes	55	57
C3-Naphthalenes	128	142
C4-Naphthalenes	242	267
Fluorene	14	15
C1-Fluorenes	132	136
C2-Fluorenes	278	299
C3- Fluorenes	357	351
Dibenzothiophene	155	165
C1-Dibenzothiophenes	280	306
C2-Dibenzothiophenes	613	661
C3- Dibenzothiophenes	741	792
Phenanthrene	158	208
C1-Phenanthrenes	677	705
C2-Phenanthrenes	1099	1136
C3-Phenanthrenes	851	855
C4-Phenanthrenes	377	393
Anthracene	352	365
Fluoranthene	5	5.0
Pyrene	5	4.6
C1- Pyrenes	192	197
C2- Pyrenes	222	241
C3- Pyrenes	3	3
C4- Pyrenes	3	3
Naphthobenzothiophene	81	84
C-1 Naphthobenzothiophenes	160	167
C-2 Naphthobenzothiophenes	198	204
C-3 Naphthobenzothiophenes	0	0
Benzo (a) Anthracene	81	84.9
Chrysene	39	41
C1- Chrysenes	143	145
C2- Chrysenes	147	153
C3- Chrysenes	2	2
C4- Chrysenes	ND	ND
Benzo (b) Fluoranthene	ND	ND
Benzo (k) Fluoranthene	ND	ND
Benzo (e) Pyrene	2	2
Benzo (a) Pyrene	2	2
Perylene	2	2
Indeno (1,2,3 - cd) Pyrene	ND	ND
Dibenzo (a,h) anthracene	ND	ND
Benzo (g,h,i) perylene	ND	ND
Total Aromatics	**6218**	**6580**
% Surrogate Recovery		
5 Alpha Androstane	0.95	1.00
Phenanthrene-d-10	0.95	1.00

NUTRIENT, REPLICATE 2 Testing Date: Day 28 Initial Oil Weight: 529 mg Final Extracted Volume: 10 mL		
	Concentration (ng/mL)	Surrogate Corrected (ng/mL)
Alkane Analyte:		
nC-10 Decane	ND	ND
nC-11 Undecane	ND	ND
nC-12 Dodecane	ND	ND
nC-13 Tridecane	ND	ND
nC-14 Tetradecane	9	9
nC-15 Pentadecane	69	69
nC-16 Hexadecane	357	483
nC-17 Heptadecane	708	882
Pristane	449	576
nC-18 Octadecane	1058	1379
Phytane	594	771
nC-19 Nonadecane	1319	1713
nC-20 Eicosane	1464	1901
nC-21 Heneicosane	1402	1821
nC-22 Docosane	1600	2084
nC-23 Tricosane	1501	1949
nC-24 Tetracosane	1391	1856
nC-25 Pentacosane	1313	1785
nC-26 Hexacosane	1478	1919
nC-27 Heptacosane	1253	1341
nC-28 Octacosane	1080	1418
nC-29 Nonacosane	997	1182
nC-30 Triacontane	827	1074
nC-31 Hentriacontane	812	1055
nC-32 Dotriacontane	645	838
nC-33 Tritriacontane	69	89
nC-34 Tetratriacontane	289	376
nC-35 Pentatriacontane	208	271
Total Alkanes	**20652**	**26756**
Aromatic Analyte:		
Naphthalene	ND	ND
C1-Naphthalenes	ND	ND
C2-Naphthalenes	66	70
C3-Naphthalenes	116	124
C4-Naphthalenes	249	265
Fluorene	13	14
C1-Fluorenes	169	169
C2-Fluorenes	348	367
C3- Fluorenes	446	439
Dibenzothiophene	153	171
C1-Dibenzothiophenes	420	442
C2-Dibenzothiophenes	957	1050
C3- Dibenzothiophenes	753	792
Phenanthrene	156	195
C1-Phenanthrenes	769	869
C2-Phenanthrenes	1309	1372
C3-Phenanthrenes	973	1025
C4-Phenanthrenes	344	362
Anthracene	269.39	280
Fluoranthene	5.0	5.3
Pyrene	4.5	4.6
C1- Pyrenes	129	126
C2- Pyrenes	216	230
C3- Pyrenes	3	3
C4- Pyrenes	3	3
Naphthobenzothiophene	56	61
C-1 Naphthobenzothiophenes	151	151
C-2 Naphthobenzothiophenes	248	261
C-3 Naphthobenzothiophenes	0	0
Benzo (a) Anthracene	60.3	59.8
Chrysene	34	36
C1- Chrysenes	153	152
C2- Chrysenes	153	161
C3- Chrysenes	2	2
C4- Chrysenes	ND	ND
Benzo (b) Fluoranthene	ND	ND
Benzo (k) Fluoranthene	ND	ND
Benzo (e) Pyrene	3	3
Benzo (a) Pyrene	2.49	3
Perylene	2.72	3
Indeno (1,2,3 - cd) Pyrene	ND	ND
Dibenzo (a,h) anthracene	ND	ND
Benzo (g,h,i) perylene	ND	ND
Total Aromatics	**6962**	**8434**
% Surrogate Recovery		
5 Alpha Androstane	0.77	1.00
Phenanthrene-d-10	0.95	1.00

NUTRIENT, REPLICATE 3 Testing Date: Day 28 Initial Oil Weight: 518 mg Final Extracted Volume: 10 mL		
	Concentration (ng/mL)	Surrogate Corrected (ng/mL)
Alkane Analyte:		
nC-10 Decane	ND	ND
nC-11 Undecane	ND	ND
nC-12 Dodecane	ND	ND
nC-13 Tridecane	ND	ND
nC-14 Tetradecane	7	8
nC-15 Pentadecane	46	57
nC-16 Hexadecane	347	413
nC-17 Heptadecane	691	823
Pristane	435	518
nC-18 Octadecane	1043	1241
Phytane	692	824
nC-19 Nonadecane	1268	1524
nC-20 Eicosane	1514	1802
nC-21 Heneicosane	1543	1851
nC-22 Docosane	1725	2047
nC-23 Tricosane	1702	2027
nC-24 Tetracosane	1548	2200
nC-25 Pentacosane	1454	1779
nC-26 Hexacosane	1470	2116
nC-27 Heptacosane	1502	1389
nC-28 Octacosane	1147	1365
nC-29 Nonacosane	1029	1238
nC-30 Triacontane	799	951
nC-31 Hentriacontane	1018	1212
nC-32 Dotriacontane	532	633
nC-33 Tritriacontane	76	91
nC-34 Tetratriacontane	260	295
nC-35 Pentatriacontane	189	227
Total Alkanes	**22313**	**26563**
Aromatic Analyte:		
Naphthalene	ND	ND
C1-Naphthalenes	ND	ND
C2-Naphthalenes	84	87
C3-Naphthalenes	161	169
C4-Naphthalenes	324	341
Fluorene	12	12
C1-Fluorenes	116	121
C2-Fluorenes	279	294
C3- Fluorenes	371	390
Dibenzothiophene	115	121
C1-Dibenzothiophenes	420	442
C2-Dibenzothiophenes	845	899
C3- Dibenzothiophenes	722	771
Phenanthrene	216	227
C1-Phenanthrenes	706	768
C2-Phenanthrenes	1095	1153
C3-Phenanthrenes	1054	1057
C4-Phenanthrenes	377	397
Anthracene	304.65	321
Fluoranthene	5.9	6.2
Pyrene	6.4	6.7
C1- Pyrenes	154	110
C2- Pyrenes	171	180
C3- Pyrenes	3	4
C4- Pyrenes	3	3
Naphthobenzothiophene	66	69
C-1 Naphthobenzothiophenes	201	212
C-2 Naphthobenzothiophenes	229	241
C-3 Naphthobenzothiophenes	0	0
Benzo (a) Anthracene	66.3	69.8
Chrysene	30	33
C1- Chrysenes	143	150
C2- Chrysenes	162	170
C3- Chrysenes	2	2
C4- Chrysenes	ND	ND
Benzo (b) Fluoranthene	ND	ND
Benzo (k) Fluoranthene	ND	ND
Benzo (e) Pyrene	2	3
Benzo (a) Pyrene	3.41	3
Perylene	2.13	2
Indeno (1,2,3 - cd) Pyrene	ND	ND
Dibenzo (a,h) anthracene	ND	ND
Benzo (g,h,i) perylene	ND	ND
Total Aromatics	**6379**	**6829**
% Surrogate Recovery		
5 Alpha Androstane	0.84	1.00
Phenanthrene-d-10	0.95	1.00

NUTRIENT STATISTICS FOR SURROGATE CORRECTED DATA Testing Dates: Day 28						
	Replicate #1 Conc (ng/mL)	Replicate #2 Conc (ng/mL)	Replicate #3 Conc (ng/mL)	Average	Stdev	%RSD
Alkane Analyte:						
nC-10 Decane	ND	ND	ND	ND	ND	ND
nC-11 Undecane	ND	ND	ND	ND	ND	ND
nC-12 Dodecane	ND	ND	ND	ND	ND	ND
nC-13 Tridecane	ND	ND	ND	ND	ND	ND
nC-14 Tetradecane	6	9	8	8	0	6.4
nC-15 Pentadecane	57	69	57	64	7	11
nC-16 Hexadecane	586	483	413	489	48	10
nC-17 Heptadecane	1018	882	823	841	104	11
Pristane	627	679	518	574	55	9.6
nC-18 Octadecane	1321	1379	1241	1314	69	5.9
Phytane	869	771	824	821	81	10
nC-19 Nonadecane	1494	1713	1524	1581	117	7.4
nC-20 Eicosane	1601	1901	1802	1835	58	3.1
nC-21 Heneicosane	1871	1821	1851	1851	27	1.4
nC-22 Docosane	1987	2084	2047	2039	49	2.4
nC-23 Tricosane	1942	1949	2027	1973	47	2.4
nC-24 Tetracosane	1838	1856	2200	1965	201	10
nC-25 Pentacosane	1637	1785	1779	1767	71	4.0
nC-26 Hexacosane	1563	1919	2116	1867	281	15
nC-27 Heptacosane	1341	1341	1389	1357	24	1.8
nC-28 Octacosane	1324	1418	1365	1369	47	3.5
nC-29 Nonacosane	1265	1182	1238	1228	58	4.6
nC-30 Triacontane	1093	1074	951	1039	74	7.1
nC-31 Hentriacontane	1008	1055	1212	1092	81	7.7
nC-32 Dotriacontane	716	838	633	729	103	14
nC-33 Tritriacontane	84	89	91	88	4	4.1
nC-34 Tetratriacontane	354	376	295	343	40.19	12
nC-35 Pentatriacontane	231	271	227	243	24	9.7
Total Alkanes	**26202**	**26756**	**26563**	**26507**	**281**	**1.1**
Aromatic Analyte:	Conc (ng/mL)	Conc (ng/mL)	Conc (ng/mL)	Average	Stdev	%RSD
Naphthalene	ND	ND	ND	ND	ND	ND
C1-Naphthalenes	ND	ND	ND	ND	ND	ND
C2-Naphthalenes	57	70	87	71	15	21
C3-Naphthalenes	142	124	169	145	23	16
C4-Naphthalenes	267	255	341	288	46	16
Fluorene	15	14	12	14	1	9
C1-Fluorenes	136	169	121	142	24	17
C2-Fluorenes	299	367	294	317	43	14
C3- Fluorenes	351	439	390	393	69	18
Dibenzothiophene	165	171	121	152	26	17
C1-Dibenzothiophenes	306	442	442	397	78	20
C2-Dibenzothiophenes	661	1050	899	870	197	23
C3- Dibenzothiophenes	792	792	771	785	12	1.5
Phenanthrene	208	195	227	210	16	7.6
C1-Phenanthrenes	705	869	768	781	57	7.3
C2-Phenanthrenes	1136	1372	1153	1220	139	11
C3-Phenanthrenes	855	1025	1057	979	88.6	9.0
C4-Phenanthrenes	393	362	397	384	19	5.0
Anthracene	365.44	388.65	320.84	358.70	34.69	10
Fluoranthene	5.0	5.3	6.2	5.5	0.64	12
Pyrene	4.6	4.6	6.7	5.3	1.2	23
C1- Pyrenes	197	126	110	144	36.2	25
C2- Pyrenes	241	230	180	217	33	15
C3- Pyrenes	3	3	4	3	0.3	8.6
C4- Pyrenes	3	3	3	3	0.2	6.1
Naphthobenzothiophene	84	61	69	71	12	17
C-1 Naphthobenzothiophenes	167	151	212	177	33	19
C-2 Naphthobenzothiophenes	204	261	241	235	29	12
C-3 Naphthobenzothiophenes	0	0	0	0	0	0
Benzo (a) Anthracene	84.9	69.8	69.8	81.5	10.70	13
Chrysene	41	36	33	36	4.6	13
C1- Chrysenes	145	152	150	153	7.04	4.6
C2- Chrysenes	153	161	170	161	9	5.4
C3- Chrysenes	2	2	2	2	0.1	6.1
C4- Chrysenes	ND	ND	ND	ND	ND	ND
Benzo (b) Fluoranthene	ND	ND	ND	ND	ND	ND
Benzo (k) Fluoranthene	ND	ND	ND	ND	ND	ND
Benzo (e) Pyrene	3	3	3	3	0.29	11.0
Benzo (a) Pyrene	2.5	2.5	2.5	2.5	0.15	7.7
Perylene	2.5	2.2	2.5	2.4	0.22	12
Indeno (1,2,3 - cd) Pyrene	ND	ND	ND	ND	ND	ND
Dibenzo (a,h) anthracene	ND	ND	ND	ND	ND	ND
Benzo (g,h,i) perylene	ND	ND	ND	ND	ND	ND
Total Aromatics	**8962**	**8434**	**8829**	**8742**	**639**	**7.3**

PRODUCT, REPLICATE 1 Testing Date: Day 28 Initial Oil Weight: 610 mg Final Extracted Volume: 10 mL			PRODUCT, REPLICATE 2 Testing Date: Day 28 Initial Oil Weight: 619 mg Final Extracted Volume: 10 mL			PRODUCT, REPLICATE 3 Testing Date: Day 28 Initial Oil Weight: 610 mg Final Extracted Volume: 10 mL			PRODUCT STATISTICS FOR SURROGATE CORRECTED DATA Testing Date: Day 28					
	Concentration (ng/mL)	Surrogate Corrected (ng/mL)		Concentration (ng/mL)	Surrogate Corrected (ng/mL)		Concentration (ng/mL)	Surrogate Corrected (ng/mL)		Replicate #1 Conc (ng/mL)	Replicate #2 Conc (ng/mL)	Replicate #3 Conc (ng/mL)	Average	Stdev	%RSD
Alkane Analyte:			Alkane Analyte:			Alkane Analyte:			Alkane Analyte:						
nC-10 Decane	ND	ND	nC-10 Decane	ND	ND	nC-10 Decane	ND	ND	nC-10 Decane	ND	ND	ND	ND	ND	ND
nC-11 Undecane	ND	ND	nC-11 Undecane	ND	ND	nC-11 Undecane	ND	ND	nC-11 Undecane	ND	ND	ND	ND	ND	ND
nC-12 Dodecane	ND	ND	nC-12 Dodecane	ND	ND	nC-12 Dodecane	ND	ND	nC-12 Dodecane	ND	ND	ND	ND	ND	ND
nC-13 Tridecane	ND	ND	nC-13 Tridecane	ND	ND	nC-13 Tridecane	ND	ND	nC-13 Tridecane	ND	ND	ND	ND	ND	ND
nC-14 Tetradecane	0.29	0.3	nC-14 Tetradecane	0.2	0.2	nC-14 Tetradecane	0.2	0.2	nC-14 Tetradecane	0	0	0	0	0	14
nC-15 Pentadecane	4	4	nC-15 Pentadecane	5	5	nC-15 Pentadecane	5	5	nC-15 Pentadecane	4	5	5	5	1	15
nC-16 Hexadecane	36	45	nC-16 Hexadecane	42	45	nC-16 Hexadecane	47	52	nC-16 Hexadecane	45	45	52	48	4	10
nC-17 Heptadecane	124	138	nC-17 Heptadecane	134	146	nC-17 Heptadecane	160	178	nC-17 Heptadecane	138	146	178	154	21	14
Pristane	109	112	Pristane	101	116	Pristane	106	120	Pristane	112	119	120	114	5	5
nC-18 Octadecane	184	204	nC-18 Octadecane	207	205	nC-18 Octadecane	239	265	nC-18 Octadecane	204	205	265	224	35	15
Phytane	151	168	Phytane	135	148	Phytane	148	164	Phytane	168	148	164	160	11	6.6
nC-19 Nonadecane	238	251	nC-19 Nonadecane	250	272	nC-19 Nonadecane	270	301	nC-19 Nonadecane	251	272	301	275	26	9.1
nC-20 Eicosane	288	312	nC-20 Eicosane	286	322	nC-20 Eicosane	322	358	nC-20 Eicosane	312	322	358	330	24	7.3
nC-21 Heneicosane	283	314	nC-21 Heneicosane	315	344	nC-21 Heneicosane	339	359	nC-21 Heneicosane	314	344	359	339	27	8.0
nC-22 Docosane	314	348	nC-22 Docosane	335	365	nC-22 Docosane	351	403	nC-22 Docosane	348	365	403	371	27	7.3
nC-23 Tricosane	317	352	nC-23 Tricosane	340	376	nC-23 Tricosane	361	390	nC-23 Tricosane	352	376	390	373	19	5.2
nC-24 Tetracosane	299	332	nC-24 Tetracosane	352	352	nC-24 Tetracosane	369	409	nC-24 Tetracosane	332	352	409	371	45	12
nC-25 Pentacosane	189	210	nC-25 Pentacosane	249	231	nC-25 Pentacosane	264	294	nC-25 Pentacosane	210	231	294	269	41	16
nC-26 Hexacosane	182	202	nC-26 Hexacosane	268	252	nC-26 Hexacosane	254	285	nC-26 Hexacosane	202	252	285	257	28	11
nC-27 Heptacosane	204	227	nC-27 Heptacosane	210	228	nC-27 Heptacosane	220	245	nC-27 Heptacosane	227	228	245	233	11	5.0
nC-28 Octacosane	165	184	nC-28 Octacosane	184	200	nC-28 Octacosane	170	194	nC-28 Octacosane	184	200	194	193	8	4.2
nC-29 Nonacosane	172	192	nC-29 Nonacosane	159	142	nC-29 Nonacosane	142	158	nC-29 Nonacosane	192	142	158	164	11	7.0
nC-30 Triacontane	145	162	nC-30 Triacontane	92	100	nC-30 Triacontane	93	104	nC-30 Triacontane	162	100	104	122	19	15
nC-31 Hentriacontane	107	119	nC-31 Hentriacontane	123	144	nC-31 Hentriacontane	97	108	nC-31 Hentriacontane	119	144	108	124	18	14
nC-32 Dotriacontane	113	126	nC-32 Dotriacontane	73	79	nC-32 Dotriacontane	64	71	nC-32 Dotriacontane	126	79	71	92	29	32
nC-33 Tritriacontane	68	76	nC-33 Tritriacontane	40	45	nC-33 Tritriacontane	35	39	nC-33 Tritriacontane	76	45	39	53	19	36
nC-34 Tetratriacontane	49	55	nC-34 Tetratriacontane	35	39	nC-34 Tetratriacontane	27	30	nC-34 Tetratriacontane	55	39	30	41	13	32
nC-35 Pentatriacontane	29	33	nC-35 Pentatriacontane	19	21	nC-35 Pentatriacontane	15	17	nC-35 Pentatriacontane	33	21	17	24	8	35
nC-36 Pentatriacontane	28	31													
Total Alkanes	*3481*	*3879*	*Total Alkanes*	*3954*	*4298*	*Total Alkanes*	*4099*	*4543*	*Total Alkanes*	*3879*	*4298*	*4543*	*4273*	*293*	*6.8*
Aromatic Analyte:			Aromatic Analyte:			Aromatic Analyte:			Aromatic Analyte:	Conc (ng/mL)	Conc (ng/mL)	Conc (ng/mL)	Average	Stdev	%RSD
Naphthalene	ND	ND	Naphthalene	ND	ND	Naphthalene	ND	ND	Naphthalene	ND	ND	ND	ND	ND	ND
C1-Naphthalenes	ND	ND	C1-Naphthalenes	ND	ND	C1-Naphthalenes	ND	ND	C1-Naphthalenes	ND	ND	ND	ND	ND	ND
C2-Naphthalenes	ND	ND	C2-Naphthalenes	ND	ND	C2-Naphthalenes	ND	ND	C2-Naphthalenes	10	8	10	9	1	15
C3-Naphthalenes	7	10	C3-Naphthalenes	6	8	C3-Naphthalenes	7	10	C3-Naphthalenes	20	28	27	25	2	8.6
C4-Naphthalenes	21	26	C4-Naphthalenes	20	28	C4-Naphthalenes	19	27	C4-Naphthalenes	26	28	27	27	1	3.2
Fluorene	1	1.7	Fluorene	1.2	1.7	Fluorene	1.2	1.6	Fluorene	1	2	2	2	0.1	5.1
C1-Fluorenes	12	17	C1-Fluorenes	13	17	C1-Fluorenes	13	16	C1-Fluorenes	17	17	16	17	0.9	5.1
C2-Fluorenes	34	34	C2-Fluorenes	34	47	C2-Fluorenes	30	42	C2-Fluorenes	54	47	42	47	6	12.4
C3- Fluorenes	41	57	C3- Fluorenes	36	52	C3- Fluorenes	41	58	C3- Fluorenes	57	52	58	55	3	5.3
Dibenzothiophene	22	32	Dibenzothiophene	21	30	Dibenzothiophene	20	28	Dibenzothiophene	32	28	28	30	2	7.7
C1-Dibenzothiophenes	67	66	C1-Dibenzothiophenes	64	88	C1-Dibenzothiophenes	68	96	C1-Dibenzothiophenes	66	88	96	83	4	4.8
C2-Dibenzothiophenes	113	157	C2-Dibenzothiophenes	96	132	C2-Dibenzothiophenes	90	126	C2-Dibenzothiophenes	157	132	126	138	13	9.3
C3- Dibenzothiophenes	65	90	C3- Dibenzothiophenes	63	84	C3- Dibenzothiophenes	65	91	C3- Dibenzothiophenes	90	84	91	88	3	3.6
Phenanthrene	51	72	Phenanthrene	64	88	Phenanthrene	47	66	Phenanthrene	72	88	66	75	11	14
C1-Phenanthrenes	115	159	C1-Phenanthrenes	105	144	C1-Phenanthrenes	114	161	C1-Phenanthrenes	159	144	161	155	9.0	5.8
C2-Phenanthrenes	127	178	C2-Phenanthrenes	106	145	C2-Phenanthrenes	117	165	C2-Phenanthrenes	178	145	165	163	17	10
C3-Phenanthrenes	104	143	C3-Phenanthrenes	84	115	C3-Phenanthrenes	81	114	C3-Phenanthrenes	143	115	114	124	16	13
C4-Phenanthrenes	25	35	C4-Phenanthrenes	24	33	C4-Phenanthrenes	24	34	C4-Phenanthrenes	35	33	34	34	1.1	3.4
Anthracene	41	57.44	Anthracene	41.93	56	Anthracene	40.55	57	Anthracene	57.44	56.21	57.11	56.92	0.64	1.1
Fluoranthene	1	1.5	Fluoranthene	0.8	1.2	Fluoranthene	1.0	1.5	Fluoranthene	1.5	1.2	1.5	1.4	0.17	12
Pyrene	1	1.1	Pyrene	1.0	1.4	Pyrene	0.8	1.1	Pyrene	1	1	1	1	0.15	12
C1- Pyrenes	7	10	C1- Pyrenes	6	10	C1- Pyrenes	6	10	C1- Pyrenes	10	10	10	10	0.6	5
C2- Pyrenes	10	14	C2- Pyrenes	14	19	C2- Pyrenes	13	18	C2- Pyrenes	14	19	18	17	2.65	17
C3- Pyrenes	1	1	C3- Pyrenes	0	1	C3- Pyrenes	1	1	C3- Pyrenes	1	1	1	1	0.1	12
C4- Pyrenes	0	1	C4- Pyrenes	0	0	C4- Pyrenes	0	1	C4- Pyrenes	1	0	1	1	0.1	12
Naphthobenzothiophene	9	13	Naphthobenzothiophene	9	13	Naphthobenzothiophene	10	13	Naphthobenzothiophene	13	13	13	0.49	3.9	
C-1 Naphthobenzothiophene	29	43	C-1 Naphthobenzothiophene	22	30	C-1 Naphthobenzothiophene	22	31	C-1 Naphthobenzothiophene	42	30	31	34	6	18
C-2 Naphthobenzothiophene	1	1	C-2 Naphthobenzothiophene	1	1	C-2 Naphthobenzothiophene	1	1	C-2 Naphthobenzothiophene	1	1	1	1	0	13
C-3 Naphthobenzothiophene	0	0	C-3 Naphthobenzothiophene	0	0	C-3 Naphthobenzothiophene	0	1	C-3 Naphthobenzothiophene	0	0	1	0	0.1	15
Benzo (a) Anthracene	6	9.6	Benzo (a) Anthracene	4.9	6.7	Benzo (a) Anthracene	6.5	9.1	Benzo (a) Anthracene	6.49	6.87	9.09	8.06	1.29	16
Chrysene	4	6	Chrysene	3	4	Chrysene	5	6	Chrysene	6	4	6	5	0.87	17
C1- Chrysenes	15	22	C1- Chrysenes	15	22	C1- Chrysenes	14	20	C1- Chrysenes	22	22	20	21	1.2	5.9
C2- Chrysenes	12	17	C2- Chrysenes	15	20	C2- Chrysenes	14	18	C2- Chrysenes	17	20	18	18	1.6	8.6
C3- Chrysenes	ND	ND	C3- Chrysenes	ND	ND	C3- Chrysenes	ND	ND	C3- Chrysenes	ND	ND	ND	ND	ND	ND
C4- Chrysenes	ND	ND	C4- Chrysenes	ND	ND	C4- Chrysenes	ND	ND	C4- Chrysenes	ND	ND	ND	ND	ND	ND
Benzo (b) Fluoranthene	2.6	3.7	Benzo (b) Fluoranthene	2.3	3.1	Benzo (b) Fluoranthene	1.9	2.7	Benzo (b) Fluoranthene	3.7	3.1	2.7	3.2	0.52	16
Benzo (j) Fluoranthene	ND	ND	Benzo (j) Fluoranthene	ND	ND	Benzo (j) Fluoranthene	ND	ND	Benzo (j) Fluoranthene	ND	ND	ND	ND	ND	ND
Benzo (k) Fluoranthene	ND	ND	Benzo (k) Fluoranthene	ND	ND	Benzo (k) Fluoranthene	ND	ND	Benzo (k) Fluoranthene	ND	ND	ND	ND	ND	ND
Benzo (e) Pyrene	ND	ND	Benzo (e) Pyrene	ND	ND	Benzo (e) Pyrene	ND	ND	Benzo (e) Pyrene	ND	ND	ND	ND	ND	ND
Benzo (a) Pyrene	ND	ND	Benzo (a) Pyrene	ND	ND	Benzo (a) Pyrene	ND	ND	Benzo (a) Pyrene	ND	ND	ND	ND	ND	ND
Perylene	ND	ND	Perylene	ND	ND	Perylene	ND	ND	Perylene	ND	ND	ND	ND	ND	ND
Indeno (1,2,3 - cd) Pyrene	ND	ND	Indeno (1,2,3 - cd) Pyrene	ND	ND	Indeno (1,2,3 - cd) Pyrene	ND	ND	Indeno (1,2,3 - cd) Pyrene	ND	ND	ND	ND	ND	ND
Dibenzo (a,h) anthracene	ND	ND	Dibenzo (a,h) anthracene	ND	ND	Dibenzo (a,h) anthracene	ND	ND	Dibenzo (a,h) anthracene	ND	ND	ND	ND	ND	ND
Benzo (g,h,i) perylene	ND	ND	Benzo (g,h,i) perylene	ND	ND	Benzo (g,h,i) perylene	ND	ND	Benzo (g,h,i) perylene	ND	ND	ND	ND	ND	ND
Total Aromatics	*360*	*1352*	*Total Aromatics*	*876*	*1200*	*Total Aromatics*	*888*	*1250*	*Total Aromatics*	*1352*	*1200*	*1250*	*1268*	*76*	*6.1*
% Surrogate Recovery			% Surrogate Recovery			% Surrogate Recovery									
5 Alpha Androstane	0.90	1.00	5 Alpha Androstane	0.82	1.00	5 Alpha Androstane	0.90	1.00							
Phenanthrene d-10	0.71	1.00	Phenanthrene d-10	0.75	1.00	Phenanthrene d-10	0.71	1.00							

	Day 0 (g)	Day 7 (g)	Day 28 (g)	% Reduction	Avg % Red.
Ctrl. #1	0.097	0.093	0.082	15.5	
Ctrl. #2	0.099	0.093	0.084	15.2	16.5
Ctrl. #3	0.100	0.094	0.081	19.0	
Mean	0.099	0.093	0.082		
Nutrient #1	0.097	0.081	0.044	54.6	
Nutrient #2	0.101	0.077	0.049	51.5	52.0
Nutrient #3	0.104	0.079	0.052	50.0	
Mean	0.101	0.079	0.048		
Product #1	0.099	0.077	0.018	81.8	
Product #2	0.101	0.078	0.014	86.1	85.4
Product #3	0.101	0.075	0.012	88.1	
Mean	0.100	0.077	0.015		

	Vial wt. (g)	Vial + DCM + Oil (g)	Vial + Oil (g)	Oil (g)
D0-C-1	13.473	26.985	13.570	0.097
D0-C-2	14.015	27.530	14.114	0.099
D0-C-3	13.865	26.751	13.965	0.100
D0-N-1	14.249	27.189	14.346	0.097
D0-N-2	13.785	27.087	13.886	0.101
D0-N-3	13.591	27.025	13.695	0.104
D0-P-1	13.687	27.176	13.786	0.099
D0-P-2	13.798	27.115	13.899	0.101
D0-P-3	13.981	27.125	14.082	0.101
D7-C-1	13.976	27.043	14.069	0.093
D7-C-2	14.151	27.148	14.244	0.093
D7-C-3	13.591	26.887	13.689	0.098
D7-N-1	13.687	26.964	13.768	0.081
D7-N-2	13.798	27.195	13.875	0.077
D7-N-3	13.981	27.045	14.060	0.079
D7-P-1	14.211	27.193	14.288	0.077
D7-P-2	14.323	27.187	14.401	0.078
D7-P-3	14.063	27.131	14.138	0.075
D28-C-1	13.976	26.864	14.058	0.082
D28-C-2	14.151	27.112	14.235	0.084
D28-C-3	13.591	27.058	13.672	0.081
D28-N-1	13.687	27.283	13.731	0.044
D28-N-2	14.111	27.217	14.160	0.049
D28-N-3	13.981	27.156	14.033	0.052
D28-P-1	14.211	26.947	14.229	0.018
D28-P-2	14.323	26.852	14.337	0.014
D28-P-3	14.063	27.099	14.075	0.012

	Day 0 (MPN, per ml)	Day 7 (MPN, per ml)	Day 28 (MPN, per ml)
Ctrl. #1	7,968	8,406	9,843
Ctrl. #2	8,179	8,072	10,136
Ctrl. #3	7,647	8,724	9,549
Nutrient #1	8,493	1,832,536	7,274,655
Nutrient #2	7,647	2,015,665	7,967,738
Nutrient #3	7,852	2,115,255	7,646,602
Product #1	8,724	7,274,655	182,054,230
Product #2	8,406	7,967,738	175,038,856
Product #3	8,972	7,646,602	197,910,169

APPENDIX II

General Linear Model: ALKANES versus DAY, TREATMENT

```
Factor     Type Levels Values
DAY        fixed      3  0  7 28
TREATMEN   fixed      3 Control  Nutrient OSI

Analysis of Variance for ALKANES, using Adjusted SS for Tests

Source          DF     Seq SS     Adj SS     Adj MS       F      P
DAY              2 1746813937 1746813937  873406968  697.73  0.000
TREATMEN         2 1082517417 1082517417  541258708  432.39  0.000
DAY*TREATMEN     4  761225884  761225884  190306471  152.03  0.000
Error           18   22531957   22531957    1251775
Total           26 3613089194

Dunnett Simultaneous Tests
Response Variable ALKANES
Comparisons with Control Level
DAY       = 0
TREATMEN = Control subtracted from:

Level              Difference       SE of                 Adjusted
DAY*TREATMEN        of Means  Difference   T-Value    P-Value
 0  Nutrient           -2600       913.5     -2.85     0.0597
 0  OSI                -1439       913.5     -1.58     0.5103
 7  Control            -3920       913.5     -4.29     0.0029
 7  Nutrient           -8354       913.5     -9.15     0.0000
 7  OSI               -16854       913.5    -18.45     0.0000
28  Control            -7373       913.5     -8.07     0.0000
28  Nutrient          -16663       913.5    -18.24     0.0000
28  OSI               -38896       913.5    -42.58     0.0000
```

General Linear Model: ALKANES versus DAY, TREATMENT

```
Factor     Type Levels Values
DAY        fixed      3  0  7 28
TREATMEN   fixed      3 Control  Nutrient OSI

Analysis of Variance for ALKANES, using Adjusted SS for Tests

Source          DF     Seq SS     Adj SS     Adj MS       F      P
DAY              2 1746813937 1746813937  873406968  697.73  0.000
TREATMEN         2 1082517417 1082517417  541258708  432.39  0.000
DAY*TREATMEN     4  761225884  761225884  190306471  152.03  0.000
Error           18   22531957   22531957    1251775
Total           26 3613089194

Dunnett Simultaneous Tests
Response Variable ALKANES
Comparisons with Control Level
DAY       = 7
TREATMEN = Control subtracted from:

Level              Difference       SE of                 Adjusted
DAY*TREATMEN        of Means  Difference   T-Value    P-Value
 0  Control            3920       913.5      4.29     0.0029
 0  Nutrient           1319       913.5      1.44     0.5977
 0  OSI                2480       913.5      2.72     0.0772
 7  Nutrient          -4435       913.5     -4.85     0.0009
 7  OSI               -12934       913.5    -14.16     0.0000
28  Control            -3453       913.5     -3.78     0.0086
28  Nutrient          -12743       913.5    -13.95     0.0000
28  OSI               -34977       913.5    -38.29     0.0000
```

General Linear Model: ALKANES versus DAY, TREATMENT

```
Factor     Type Levels Values
DAY        fixed      3  0  7 28
TREATMEN   fixed      3 Control  Nutrient OSI

Analysis of Variance for ALKANES, using Adjusted SS for Tests

Source          DF      Seq SS      Adj SS      Adj MS       F      P
DAY              2 1746813937 1746813937   873406968  697.73  0.000
TREATMEN         2 1082517417 1082517417   541258708  432.39  0.000
DAY*TREATMEN     4  761225884  761225884   190306471  152.03  0.000
Error           18   22531957   22531957     1251775
Total           26 3613089194

Dunnett Simultaneous Tests
Response Variable ALKANES
Comparisons with Control Level
DAY       = 28
TREATMEN = Control subtracted from:

Level             Difference       SE of              Adjusted
DAY*TREATMEN       of Means  Difference   T-Value     P-Value
 0  Control           7373       913.5      8.07      1.0000
 0  Nutrient          4773       913.5      5.22      1.0000
 0  OSI               5934       913.5      6.50      1.0000
 7  Control           3453       913.5      3.78      1.0000
 7  Nutrient          -981       913.5     -1.07      0.4720
 7  OSI              -9481       913.5    -10.38      0.0000
28  Nutrient         -9290       913.5    -10.17      0.0000
28  OSI             -31523       913.5    -34.51      0.0000
```

General Linear Model: RANK_ALKANES versus DAY, TREATMENT

```
Factor     Type Levels Values
DAY        fixed      3  0  7 28
TREATMEN   fixed      3 Control  Nutrient OSI

Analysis of Variance for RANK_ALK, using Adjusted SS for Tests

Source          DF     Seq SS     Adj SS     Adj MS       F      P
DAY              2    1178.00    1178.00     589.00  182.79  0.000
TREATMEN         2     298.67     298.67     149.33   46.34  0.000
DAY*TREATMEN     4     103.33     103.33      25.83    8.02  0.001
Error           18      58.00      58.00       3.22
Total           26    1638.00

Dunnett Simultaneous Tests
Response Variable RANK_ALK
Comparisons with Control Level
DAY       = 0
TREATMEN = Control subtracted from:

Level             Difference       SE of              Adjusted
DAY*TREATMEN       of Means  Difference   T-Value     P-Value
 0  Nutrient          -5.00      1.466     -3.41      0.0094
 0  OSI               -2.00      1.466     -1.36      0.3439
 7  Control           -8.33      1.466     -5.69      0.0001
 7  Nutrient         -13.33      1.466     -9.10      0.0000
 7  OSI              -19.33      1.466    -13.19      0.0000
28  Control          -12.33      1.466     -8.41      0.0000
28  Nutrient         -18.33      1.466    -12.51      0.0000
28  OSI              -23.33      1.466    -15.92      0.0000
```

General Linear Model: RANK_ALKANES versus DAY, TREATMENT

```
Factor     Type Levels Values
DAY        fixed      3  0  7 28
TREATMEN   fixed      3 Control  Nutrient OSI

Analysis of Variance for RANK_ALK, using Adjusted SS for Tests

Source          DF     Seq SS     Adj SS     Adj MS       F      P
DAY              2    1178.00    1178.00     589.00  182.79  0.000
TREATMEN         2     298.67     298.67     149.33   46.34  0.000
DAY*TREATMEN     4     103.33     103.33      25.83    8.02  0.001
Error           18      58.00      58.00       3.22
Total           26    1638.00

Dunnett Simultaneous Tests
Response Variable RANK_ALK
Comparisons with Control Level
DAY      = 7
TREATMEN = Control subtracted from:

Level              Difference       SE of              Adjusted
DAY*TREATMEN        of Means  Difference   T-Value     P-Value
 0  Control            8.33       1.466      5.69      1.0000
 0  Nutrient           3.33       1.466      2.27      0.9999
 0  OSI                6.33       1.466      4.32      1.0000
 7  Nutrient          -5.00       1.466     -3.41      0.0094
 7  OSI              -11.00       1.466     -7.51      0.0000
28  Control           -4.00       1.466     -2.73      0.0376
28  Nutrient         -10.00       1.466     -6.82      0.0000
28  OSI              -15.00       1.466    -10.23      0.0000
```

General Linear Model: RANK_ALKANES versus DAY, TREATMENT

```
Factor     Type Levels Values
DAY        fixed      3  0  7 28
TREATMEN   fixed      3 Control  Nutrient OSI

Analysis of Variance for RANK_ALK, using Adjusted SS for Tests

Source          DF     Seq SS     Adj SS     Adj MS       F      P
DAY              2    1178.00    1178.00     589.00  182.79  0.000
TREATMEN         2     298.67     298.67     149.33   46.34  0.000
DAY*TREATMEN     4     103.33     103.33      25.83    8.02  0.001
Error           18      58.00      58.00       3.22
Total           26    1638.00

Dunnett Simultaneous Tests
Response Variable RANK_ALK
Comparisons with Control Level
DAY      = 28
TREATMEN = Control subtracted from:

Level              Difference       SE of              Adjusted
DAY*TREATMEN        of Means  Difference   T-Value     P-Value
 0  Control           12.33       1.466      8.415     1.0000
 0  Nutrient           7.33       1.466      5.003     1.0000
 0  OSI               10.33       1.466      7.050     1.0000
 7  Control            4.00       1.466      2.729     1.0000
 7  Nutrient          -1.00       1.466     -0.682     0.6528
 7  OSI               -7.00       1.466     -4.776     0.0005
28  Nutrient          -6.00       1.466     -4.094     0.0022
28  OSI              -11.00       1.466     -7.505     0.0000
```

General Linear Model: AROMATICS versus DAY, TREATMENT

```
Factor     Type Levels Values
DAY       fixed      3  0  7 28
TREATMEN  fixed      3 Control  Nutrient OSI

Analysis of Variance for AROMATIC, using Adjusted SS for Tests

Source          DF     Seq SS     Adj SS     Adj MS       F      P
DAY              2  122630081  122630081   61315041  142.02  0.000
TREATMEN         2   60150172   60150172   30075086   69.66  0.000
DAY*TREATMEN     4   76909629   76909629   19227407   44.54  0.000
Error           18    7770989    7770989     431722
Total           26  267460872

Dunnett Simultaneous Tests
Response Variable AROMATIC
Comparisons with Control Level
DAY       = 0
TREATMEN = Control subtracted from:

Level              Difference       SE of              Adjusted
DAY*TREATMEN        of Means  Difference   T-Value     P-Value
 0  Nutrient            350       536.5      0.65      0.9772
 0  OSI                 719       536.5      1.34      0.9971
 7  Control           -1080       536.5     -2.01      0.1364
 7  Nutrient          -1537       536.5     -2.87      0.0288
 7  OSI               -3364       536.5     -6.27      0.0000
28  Control           -1902       536.5     -3.54      0.0071
28  Nutrient          -2497       536.5     -4.66      0.0007
28  OSI              -10168       536.5    -18.95      0.0000
```

General Linear Model: AROMATICS versus DAY, TREATMENT

```
Factor     Type Levels Values
DAY       fixed      3  0  7 28
TREATMEN  fixed      3 Control  Nutrient OSI

Analysis of Variance for AROMATIC, using Adjusted SS for Tests

Source          DF     Seq SS     Adj SS     Adj MS       F      P
DAY              2  122630081  122630081   61315041  142.02  0.000
TREATMEN         2   60150172   60150172   30075086   69.66  0.000
DAY*TREATMEN     4   76909629   76909629   19227407   44.54  0.000
Error           18    7770989    7770989     431722
Total           26  267460872

Dunnett Simultaneous Tests
Response Variable AROMATIC
Comparisons with Control Level
DAY       = 7
TREATMEN = Control subtracted from:

Level              Difference       SE of              Adjusted
DAY*TREATMEN        of Means  Difference   T-Value     P-Value
 0  Control             1080       536.5      2.01      0.9997
 0  Nutrient            1430       536.5      2.67      1.0000
 0  OSI                 1799       536.5      3.35      1.0000
 7  Nutrient            -457       536.5     -0.85      0.5756
 7  OSI               -2283        536.5     -4.26      0.0016
28  Control            -821        536.5     -1.53      0.2788
28  Nutrient          -1417        536.5     -2.64      0.0445
28  OSI               -9088        536.5    -16.94      0.0000
```

General Linear Model: AROMATICS versus DAY, TREATMENT

```
Factor     Type Levels Values
DAY        fixed      3  0  7 28
TREATMEN   fixed      3 Control  Nutrient OSI

Analysis of Variance for AROMATIC, using Adjusted SS for Tests

Source          DF      Seq SS      Adj SS      Adj MS       F      P
DAY              2   122630081   122630081    61315041  142.02  0.000
TREATMEN         2    60150172    60150172    30075086   69.66  0.000
DAY*TREATMEN     4    76909629    76909629    19227407   44.54  0.000
Error           18     7770989     7770989      431722
Total           26   267460872

Dunnett Simultaneous Tests
Response Variable AROMATIC
Comparisons with Control Level
DAY       = 28
TREATMEN = Control subtracted from:

Level                Difference       SE of                 Adjusted
DAY*TREATMEN          of Means  Difference   T-Value    P-Value
 0  Control              1902       536.5      3.54     1.0000
 0  Nutrient             2251       536.5      4.20     1.0000
 0  OSI                  2621       536.5      4.88     1.0000
 7  Control               821       536.5      1.53     0.9985
 7  Nutrient              364       536.5      0.68     0.9788
 7  OSI                 -1462       536.5     -2.73     0.0379
28  Nutrient             -596       536.5     -1.11     0.4554
28  OSI                 -8266       536.5    -15.41     0.0000
```

General Linear Model: Rank_aromatics versus DAY, TREATMENT

```
Factor     Type Levels Values
DAY        fixed      3  0  7 28
TREATMEN   fixed      3 Control  Nutrient OSI

Analysis of Variance for Rank_aro, using Adjusted SS for Tests

Source          DF     Seq SS     Adj SS     Adj MS      F      P
DAY              2    1102.89    1102.89     551.44  67.37  0.000
TREATMEN         2     194.00     194.00      97.00  11.85  0.001
DAY*TREATMEN     4     193.78     193.78      48.44   5.92  0.003
Error           18     147.33     147.33       8.19
Total           26    1638.00

Dunnett Simultaneous Tests
Response Variable Rank_aro
Comparisons with Control Level
DAY       = 0
TREATMEN = Control subtracted from:

Level                Difference       SE of                 Adjusted
DAY*TREATMEN          of Means  Difference   T-Value    P-Value
 0  Nutrient             1.33      2.336     0.571     0.9716
 0  OSI                  2.67      2.336     1.142     0.9946
 7  Control             -4.33      2.336    -1.855     0.1753
 7  Nutrient            -7.67      2.336    -3.282     0.0123
 7  OSI                -16.33      2.336    -6.992     0.0000
28  Control             -9.67      2.336    -4.138     0.0020
28  Nutrient           -12.67      2.336    -5.422     0.0001
28  OSI                -19.33      2.336    -8.276     0.0000
```

General Linear Model: Rank_aromatics versus DAY, TREATMENT

```
Factor     Type Levels Values
DAY        fixed      3  0  7 28
TREATMEN   fixed      3 Control  Nutrient OSI

Analysis of Variance for Rank_aro, using Adjusted SS for Tests

Source          DF     Seq SS     Adj SS     Adj MS       F      P
DAY              2    1102.89    1102.89     551.44   67.37  0.000
TREATMEN         2     194.00     194.00      97.00   11.85  0.001
DAY*TREATMEN     4     193.78     193.78      48.44    5.92  0.003
Error           18     147.33     147.33       8.19
Total           26    1638.00

Dunnett Simultaneous Tests
Response Variable Rank_aro
Comparisons with Control Level
DAY       = 7
TREATMEN = Control subtracted from:

Level              Difference       SE of                 Adjusted
DAY*TREATMEN        of Means  Difference   T-Value    P-Value
 0  Control            4.33       2.336     1.855     0.9995
 0  Nutrient           5.67       2.336     2.426     0.9999
 0  OSI                7.00       2.336     2.997     1.0000
 7  Nutrient          -3.33       2.336    -1.427     0.3186
 7  OSI              -12.00       2.336    -5.137     0.0002
28  Control           -5.33       2.336    -2.283     0.0862
28  Nutrient          -8.33       2.336    -3.567     0.0068
28  OSI              -15.00       2.336    -6.421     0.0000
```

General Linear Model: Rank_aromatics versus DAY, TREATMENT

```
Factor     Type Levels Values
DAY        fixed      3  0  7 28
TREATMEN   fixed      3 Control  Nutrient OSI

Analysis of Variance for Rank_aro, using Adjusted SS for Tests

Source          DF     Seq SS     Adj SS     Adj MS       F      P
DAY              2    1102.89    1102.89     551.44   67.37  0.000
TREATMEN         2     194.00     194.00      97.00   11.85  0.001
DAY*TREATMEN     4     193.78     193.78      48.44    5.92  0.003
Error           18     147.33     147.33       8.19
Total           26    1638.00

Dunnett Simultaneous Tests
Response Variable Rank_aro
Comparisons with Control Level
DAY       = 28
TREATMEN = Control subtracted from:

Level              Difference       SE of                 Adjusted
DAY*TREATMEN        of Means  Difference   T-Value    P-Value
 0  Control           9.667       2.336     4.138     1.0000
 0  Nutrient         11.000       2.336     4.709     1.0000
 0  OSI              12.333       2.336     5.280     1.0000
 7  Control           5.333       2.336     2.283     0.9999
 7  Nutrient          2.000       2.336     0.856     0.9872
 7  OSI              -6.667       2.336    -2.854     0.0294
28  Nutrient         -3.000       2.336    -1.284     0.3778
28  OSI              -9.667       2.336    -4.138     0.0020
```

General Linear Model: ALKANES versus DAY, TREATMENT

```
Factor     Type Levels Values
DAY        fixed      3  0  7 28
TREATMEN   fixed      3 Control  Nutrient OSI

Analysis of Variance for ALKANES, using Adjusted SS for Tests

Source          DF      Seq SS      Adj SS      Adj MS       F      P
DAY              2 1746813937 1746813937   873406968  697.73  0.000
TREATMEN         2 1082517417 1082517417   541258708  432.39  0.000
DAY*TREATMEN     4  761225884  761225884   190306471  152.03  0.000
Error           18   22531957   22531957     1251775
Total           26 3613089194

Tukey Simultaneous Tests
Response Variable ALKANES
All Pairwise Comparisons among Levels of DAY*TREATMEN

DAY      = 0
TREATMEN = Control subtracted from:

Level              Difference       SE of              Adjusted
DAY*TREATMEN        of Means  Difference   T-Value     P-Value
 0  Nutrient           -2600       913.5     -2.85      0.1683
 0  OSI                -1439       913.5     -1.58      0.8056
 7  Control            -3920       913.5     -4.29      0.0102
 7  Nutrient           -8354       913.5     -9.15      0.0000
 7  OSI               -16854       913.5    -18.45      0.0000
28  Control            -7373       913.5     -8.07      0.0000
28  Nutrient          -16663       913.5    -18.24      0.0000
28  OSI               -38896       913.5    -42.58      0.0000

DAY      = 0
TREATMEN = Nutrient subtracted from:

Level              Difference       SE of              Adjusted
DAY*TREATMEN        of Means  Difference   T-Value     P-Value
 0  OSI                 1161       913.5      1.27      0.9275
 7  Control            -1319       913.5     -1.44      0.8661
 7  Nutrient           -5754       913.5     -6.30      0.0002
 7  OSI               -14254       913.5    -15.60      0.0000
28  Control            -4773       913.5     -5.22      0.0015
28  Nutrient          -14062       913.5    -15.39      0.0000
28  OSI               -36296       913.5    -39.73      0.0000

DAY      = 0
TREATMEN = OSI subtracted from:

Level              Difference       SE of              Adjusted
DAY*TREATMEN        of Means  Difference   T-Value     P-Value
 7  Control            -2480       913.5     -2.72      0.2097
 7  Nutrient           -6915       913.5     -7.57      0.0000
 7  OSI               -15415       913.5    -16.87      0.0000
28  Control            -5934       913.5     -6.50      0.0001
28  Nutrient          -15223       913.5    -16.66      0.0000
28  OSI               -37457       913.5    -41.00      0.0000

DAY      = 7
TREATMEN = Control subtracted from:

Level              Difference       SE of              Adjusted
DAY*TREATMEN        of Means  Difference   T-Value     P-Value
 7  Nutrient           -4435       913.5     -4.85      0.0032
 7  OSI               -12934       913.5    -14.16      0.0000
```

28	Control	-3453	913.5	-3.78	0.0289
28	Nutrient	-12743	913.5	-13.95	0.0000
28	OSI	-34977	913.5	-38.29	0.0000

DAY = 7
TREATMEN = Nutrient subtracted from:

Level DAY*TREATMEN		Difference of Means	SE of Difference	T-Value	Adjusted P-Value
7	OSI	-8500	913.5	-9.30	0.0000
28	Control	981	913.5	1.07	0.9710
28	Nutrient	-8308	913.5	-9.09	0.0000
28	OSI	-30542	913.5	-33.43	0.0000

DAY = 7
TREATMEN = OSI subtracted from:

Level DAY*TREATMEN		Difference of Means	SE of Difference	T-Value	Adjusted P-Value
28	Control	9481	913.5	10.38	0.0000
28	Nutrient	191	913.5	0.21	1.0000
28	OSI	-22042	913.5	-24.13	0.0000

DAY = 28
TREATMEN = Control subtracted from:

Level DAY*TREATMEN		Difference of Means	SE of Difference	T-Value	Adjusted P-Value
28	Nutrient	-9290	913.5	-10.17	0.0000
28	OSI	-31523	913.5	-34.51	0.0000

DAY = 28
TREATMEN = Nutrient subtracted from:

Level DAY*TREATMEN		Difference of Means	SE of Difference	T-Value	Adjusted P-Value
28	OSI	-22234	913.5	-24.34	0.0000

General Linear Model: AROMATICS versus DAY, TREATMENT

Factor	Type	Levels	Values
DAY	fixed	3	0 7 28
TREATMEN	fixed	3	Control Nutrient OSI

Analysis of Variance for AROMATIC, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DAY	2	122630081	122630081	61315041	142.02	0.000
TREATMEN	2	60150172	60150172	30075086	69.66	0.000
DAY*TREATMEN	4	76909629	76909629	19227407	44.54	0.000
Error	18	7770989	7770989	431722		
Total	26	267460872				

Tukey Simultaneous Tests
Response Variable AROMATIC
All Pairwise Comparisons among Levels of DAY*TREATMEN

DAY = 0
TREATMEN = Control subtracted from:

Level	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0 Nutrient	350	536.5	0.65	0.9989
0 OSI	719	536.5	1.34	0.9056
7 Control	-1080	536.5	-2.01	0.5535
7 Nutrient	-1537	536.5	-2.87	0.1629
7 OSI	-3364	536.5	-6.27	0.0002
28 Control	-1902	536.5	-3.54	0.0462
28 Nutrient	-2497	536.5	-4.66	0.0048
28 OSI	-10168	536.5	-18.95	0.0000

DAY = 0
TREATMEN = Nutrient subtracted from:

Level	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0 OSI	369	536.5	0.69	0.9984
7 Control	-1430	536.5	-2.67	0.2273
7 Nutrient	-1887	536.5	-3.52	0.0487
7 OSI	-3713	536.5	-6.92	0.0001
28 Control	-2251	536.5	-4.20	0.0124
28 Nutrient	-2847	536.5	-5.31	0.0013
28 OSI	-10518	536.5	-19.60	0.0000

DAY = 0
TREATMEN = OSI subtracted from:

Level	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
7 Control	-1799	536.5	-3.35	0.0668
7 Nutrient	-2256	536.5	-4.21	0.0122
7 OSI	-4083	536.5	-7.61	0.0000
28 Control	-2621	536.5	-4.88	0.0030
28 Nutrient	-3216	536.5	-6.00	0.0003
28 OSI	-10887	536.5	-20.29	0.0000

DAY = 7
TREATMEN = Control subtracted from:

Level	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
7 Nutrient	-457	536.5	-0.85	0.9931
7 OSI	-2283	536.5	-4.26	0.0110

28	Control	-821	536.5	-1.53	0.8273
28	Nutrient	-1417	536.5	-2.64	0.2362
28	OSI	-9088	536.5	-16.94	0.0000

DAY = 7
TREATMEN = Nutrient subtracted from:

Level DAY*TREATMEN		Difference of Means	SE of Difference	T-Value	Adjusted P-Value
7	OSI	-1826	536.5	-3.40	0.0607
28	Control	-364	536.5	-0.68	0.9985
28	Nutrient	-960	536.5	-1.79	0.6881
28	OSI	-8631	536.5	-16.09	0.0000

DAY = 7
TREATMEN = OSI subtracted from:

Level DAY*TREATMEN		Difference of Means	SE of Difference	T-Value	Adjusted P-Value
28	Control	1462	536.5	2.73	0.2063
28	Nutrient	866	536.5	1.61	0.7855
28	OSI	-6804	536.5	-12.68	0.0000

DAY = 28
TREATMEN = Control subtracted from:

Level DAY*TREATMEN		Difference of Means	SE of Difference	T-Value	Adjusted P-Value
28	Nutrient	-596	536.5	-1.11	0.9650
28	OSI	-8266	536.5	-15.41	0.0000

DAY = 28
TREATMEN = Nutrient subtracted from:

Level DAY*TREATMEN		Difference of Means	SE of Difference	T-Value	Adjusted P-Value
28	OSI	-7671	536.5	-14.30	0.0000

APPENDIX III

Two-Sample T-Test and CI: Con_0, OSEI_0

```
Two-sample T for Con_0 vs OSEI_0

         N      Mean     StDev   SE Mean
Con_0    3   0.10067   0.00379    0.0022
OSEI_0   3   0.10833   0.00289    0.0017

Difference = mu Con_0 - mu OSEI_0
Estimate for difference:  -0.00767
95% lower bound for difference: -0.01414
T-Test of difference = 0 (vs >): T-Value = -2.79  P-Value = 0.966  DF = 3
```

Two-Sample T-Test and CI: Con_7, OSEI_7

```
Two-sample T for Con_7 vs OSEI_7

         N      Mean     StDev   SE Mean
Con_7    3   0.09800   0.00200    0.0012
OSEI_7   3   0.09600   0.00265    0.0015

Difference = mu Con_7 - mu OSEI_7
Estimate for difference:  0.00200
95% lower bound for difference: -0.00251
T-Test of difference = 0 (vs >): T-Value = 1.04  P-Value = 0.187  DF = 3
```

Two-Sample T-Test and CI: Con_28, OSEI_28

```
Two-sample T for Con_28 vs OSEI_28

          N      Mean     StDev   SE Mean
Con_28    3   0.09533   0.00321    0.0019
OSEI_28   3  0.015667  0.000577   0.00033

Difference = mu Con_28 - mu OSEI_28
Estimate for difference:  0.07967
95% lower bound for difference: 0.07416
T-Test of difference = 0 (vs >): T-Value = 42.25  P-Value = 0.000  DF = 2
```

Two-Sample T-Test and CI: Con_0, Nutr_0

```
Two-sample T for Con_0 vs Nutr_0

         N      Mean     StDev   SE Mean
Con_0    3   0.10067   0.00379    0.0022
Nutr_0   3  0.097667  0.000577   0.00033

Difference = mu Con_0 - mu Nutr_0
Estimate for difference:  0.00300
95% lower bound for difference: -0.00346
T-Test of difference = 0 (vs >): T-Value = 1.36  P-Value = 0.154  DF = 2
```

Two-Sample T-Test and CI: Con_7, Nutr_7

```
Two-sample T for Con_7 vs Nutr_7

         N      Mean     StDev   SE Mean
Con_7    3   0.09800   0.00200    0.0012
Nutr_7   3   0.08500   0.00100   0.00058

Difference = mu Con_7 - mu Nutr_7
Estimate for difference:  0.01300
95% lower bound for difference: 0.00923
T-Test of difference = 0 (vs >): T-Value = 10.07  P-Value = 0.005  DF = 2
```

Two-Sample T-Test and CI: Con_28, Nutr_28

```
Two-sample T for Con_28 vs Nutr_28

          N      Mean     StDev   SE Mean
Con_28    3   0.09533   0.00321    0.0019
Nutr_28   3   0.02400   0.00173    0.0010

Difference = mu Con_28 - mu Nutr_28
Estimate for difference:  0.07133
95% lower bound for difference: 0.06637
T-Test of difference = 0 (vs >): T-Value = 33.84  P-Value = 0.000  DF = 3
```

APPENDIX B
SECTION 2.6

EVALUATION OF TOXICITY TESTING BY THE OSEI CORPS. FOR SOUTH KOREAN GOVERNMENT

P.O. Box 515429
Dallas, Texas 75251
Ph: (972) 669-3390
Email oseicorp@msn.com
Web www.osei.us

Date June 30, 2008

Fresh Water Marine Toxicity Test Summary
South Korea (Minnows)

The OSEI Corporation performed a toxicity test for the Korean Government approval process involving minnows (Pimephales promelas). The toxicity test was a 24 hour acute toxicity test. The LC50 value for this test was 707.11 mg/l at a 20% concentration, which is the concentration the Korean government test required. If you extrapolate the test value, had the test been performed at the OSE II application concentration of 2% instead of 20%, then the LC50 would have been over 1337.11 mg/l which proves OSE II to be virtually non toxic. There are several government agencies around the world that try to force specific tests to be performed at a single concentration without allowing for the application rate of a product. So while they come up with a value at a certain concentration it may, or may not be applicable to every product, which is why we point out the extrapolation calculation for OSE II at the recommended application rate.

Steven Pedigo
Chairman/CEO OSEI Corporation

OIL SPILL EATER II (2%)
ACUTE PRODUCT TEST

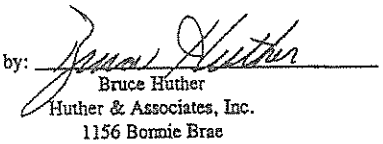
June 2008

24-Hour Acute Toxicity Test Results

Pimephales promelas

Prepared for:

Kwang Keun, Kim
Korea Institute of Construction anticorrosive Technology
95-6 Munjung-dong, Songpa-Ku
Seoul, Korea 138-869
Tel: 02-3401-8388
kicatkim@hanmail.net

Prepared by: 

Bruce Huther
Huther & Associates, Inc.
1156 Bonnie Brae
Denton, Texas 76201
(940) 387-1025 Fax: (940) 387-1036
huther@flash.net

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Huther and Associates, Inc.
environmental toxicologists, biologists, consultants

ACUTE LC50 PRODUCT REPORT

Client . . . . . OSEI, Corporation
Sample . . . . . .Oil Spill Eater II
Project No. OS457
Test Date . . . . . June 2008

Results:

24-hr. *P. Promelas* LC50:	5,856.34 mg/L
95% Upper Confidence Limits:	6,265.67 mg/L
95% Lower Confidence Limits:	5,473.76 mg/L

INTRODUCTION

A product identified as Oil Spill Eater II, Concentrate was delivered to Huther and Associates, Inc. on June 26, 2008. One acute toxicity test was conducted: a static acute 24-hour definitive toxicity test using *Pimephales promelas* (fathead minnow). Test procedures followed recommended methods contained in "*Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition*", EPA-821-R-02-012, October 2004.

P. promelas are a freshwater aquatic indicator organism frequently used to evaluate the potential toxicity of a compound or an effluent. The acute toxicity of a compound or effluent is generally measured using a multi-concentration, or definitive test, consisting of a control water and a minimum of five increasing concentrations of product added to control water. The test is designed to provide dose-response information, expressed as the concentration that is lethal to 50% of the test organisms (LC50).

SAMPLE PREPARATION

Oil Spill Eater II was initially prepared for definitive testing by adding the product to distilled, deionized water at a ratio of 50 parts water to 1 part product (2% concentration; stock solution). Seven test concentrations of stock solution were prepared in distilled, deionized water reconstituted to 104 mg/L as $CaCO_3$. The seven concentrations were 250, 500, 1000, 2000, 4000, 8000 and 16,000 mg/L. Dissolved oxygen, pH and conductivity were measured in each concentration prior to test initiation and at 24-hours. The test was conducted at 25^{0}C in a photoperiod of 16 hours light and 8 hours dark.

TEST DESIGN
Pimephales promelas

The definitive *Pimephales promelas* test was conducted in 300 mL beakers containing 250 mL of test solution. The test was initiated June 28, 2008. Ten *P. promelas* larvae were added to each of two replicate beakers per concentration. Larvae originated from laboratory cultures and were 48-hours old at test initiation. Larvae were fed *Artemia* nauplii prior to test initiation.

A control of two replicate beakers containing ten *P. promelas* larvae each in laboratory water was conducted concurrently with the test. Survival data were statistically analyzed using the Trimmed Spearman-Karber point estimate test to determine the LC50.

RESULTS

Pimephales promelas

The following LC50 value was determined for Oil Spill Eater II (2%):

24-Hour Definitive Test

Conc. (mg/L)	# exposed	# alive	#dead	% survival
Control	20	20	0	100.0
250	20	20	0	100.0
500	20	20	0	100.0
1000	20	20	0	100.0
2000	20	20	0	100.0
4000	20	20	0	100.0
8000	20	1	19	5.0
16000	20	0	20	0.0

Percent Spearman-Karber Trim:	0.00%
Estimated LC50 (mg/L):	**5,856.34**
95% Lower C.L. (mg/L):	5,473.76
95% Upper C.L. (mg/L):	6,265.67

The pH in all solutions was within the organism's tolerance range.

DISCUSSION AND CONCLUSIONS

One LC50 determination was made for Oil Spill Eater II tested at a 2% concentration: 24-hour *Pimephales promelas* LC50: 5,856.34 mg/L. The acute test was conducted from June 28, 2008 to June 29, 2008.

Huther and Associates, Inc.
environmental toxicologists, biologists, consultants

24-HOUR *PIMEPHALES PROMELAS* SURVIVAL

CLIENT: OSE - 2%

PROJECT #: 05457

CONC.	NUMBER ORGANISMS, 0 HRS A	NUMBER ORGANISMS, 0 HRS B	NUMBER ORGANISMS, 24 HRS A	NUMBER ORGANISMS, 24 HRS B
Ctrl	10	10	10	10
250 mg/L	10	10	10	10
500	10	10	10	10
1000	10	10	10	10
2000	10	10	10	10
4000	10	10	10	10
8000	10	10	1/9	0/10
16,000	10	10	0/0	0/10
DATE/TIME	mm		mm	
TECHNICIAN	6/28/08 1430		6/29/08 1430	

1156 North Bonnie Brae Denton, Texas 76201 Ph: (940) 387-1026 Fax: (940) 387-1036

· OIL SPILL EATER

Test @ 2%

CHEMISTRY MEASUREMENTS

Date	Client	Sample #	ST pH 24hr	ST DO 24hr	Hardness	Alkalinity	Cond.	Ammonia	Salinity	Analyst
6/28/05										
			ST | 24hr	ST | 24hr				pH @ 96hr	D.O @ 96hr	
	LAB CONTROL		7.84 | 8.60	8.23 | 7.43			390	7.91	7.92	
	250 mg/L		7.84 | 8.59	8.22 | 7.38			389	7.94	8.14	
	500		7.85 | 8.52	8.20 | 7.44			387	7.95	8.27	
	1000		7.85 | 8.48	8.15 | 7.38			387	7.94	8.26	
	2000		7.86 | 8.42	8.10 | 7.53			388	7.93	8.20	
	4000		7.86 | 8.42	8.15 | 7.28			390	7.92	8.21	
	8000		7.85 | 8.31	8.05 | 7.31			393	7.92	805	
	16,000		7.81 | 8.14	7.87 | 7.12			399	7.94	8.03	
	LAB WATER CONTROL		7.84		104	64					

TRIMMED SPEARMAN-KARBER METHOD. VERSION 1.5

DATE: JUNE 200 TEST NUMBER: 1 DURATION: 24 H
TOXICANT : OSE II
SPECIES: P. PROMELAS

RAW DATA: Concentration (MG/L)	Number Exposed	Mortalities
.00	20	0
1000.00	20	0
2000.00	20	0
4000.00	20	0
8000.00	20	19
*******	20	20

16000.00 ZK

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 5856.34
95% LOWER CONFIDENCE: 5473.76
95% UPPER CONFIDENCE: 6265.67

P.O. Box 515429
Dallas, Texas 75251
Ph: (972) 669-3390
Email oseicorp@msn.com
Web www.osei.us

Date June 30, 2008

Toxicity Test Summary for a Ceridaphnia Dubia
Fresh Water Flea

The OSEI Corporation performed a toxicity test for a land, water, and airborn based species a Ceriodaphnia Dubia (water flea). The estimated LC 50 for this species even at a higher concentration 20%, than OSE II is applied was 2199.62 which shows that OSE II is also virtually non toxic to bugs as well. The extrapolated value for the LC 50 at OSE II normal application rate of 2% would have been over 4000 mg/l, which shows OSE II is virtually non toxic to water fleas.

Steven Pedigo
Chairman/ CEO OSEI Corporation

OIL SPILL EATER II (2%)
ACUTE PRODUCT TEST

June 2008

24-Hour Acute Toxicity Test Results

Ceriodaphnia dubia

Prepared for:

Oil Spill Eater International, Corporation
13127 Chandler Drive
Dallas, Texas 75243
Tel: 972-669-3390

Prepared by: _____

Bruce Huther
Huther & Associates, Inc.
1156 Bonnie Brae
Denton, Texas 76201
(940) 387-1025 Fax: (940) 387-1036
huther@flash.net

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Huther and Associates, Inc.
environmental toxicologists, biologists, consultants

ACUTE LC50 PRODUCT REPORT

Client . . . . . OSEI, Corporation
Sample . . . . . 2% Oil Spill Eater II
Project No. OS457
Test Date . . . . . June 2008

Results:

24-hr. *C. dubia* LC50: **>16,000.00 mg/L**
95% Upper Confidence Limits: N/A
95% Lower Confidence Limits: N/A

INTRODUCTION

A product identified as Oil Spill Eater II, Concentrate was delivered to Huther and Associates, Inc. on June 26, 2008. One acute toxicity test was conducted: a static acute 24-hour definitive toxicity test using *Ceriodaphnia dubia* (water flea). Test procedures followed recommended methods contained in "*Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition*", EPA-821-R-02-012, October 2004.

C. dubia are a freshwater aquatic indicator organism frequently used to evaluate the potential toxicity of a compound or an effluent. The acute toxicity of a compound or effluent is generally measured using a multi-concentration, or definitive test, consisting of a control water and a minimum of five increasing concentrations of product added to control water. The test is designed to provide dose-response information, expressed as the concentration that is lethal to 50% of the test organisms (LC50).

SAMPLE PREPARATION

Oil Spill Eater II was initially prepared for definitive testing by adding the product to distilled, deionized water at a ratio of 50 parts water to 1 part product (2% concentration; stock solution). Seven test concentrations of stock solution were prepared in distilled, deionized water reconstituted to 104 mg/L as $CaCO_3$. The seven concentrations were 250, 500, 1000, 2000, 4000, 8000 and 16,000 mg/L. Dissolved oxygen, pH and conductivity were measured in each concentration prior to test initiation and at 24-hours. The test was conducted at 25°C in a photoperiod of 16 hours light and 8 hours dark.

TEST DESIGN
Ceriodaphnia dubia

The definitive *Ceriodaphnia dubia* test was conducted in 25 mL beakers containing 15 mL of test solution. The test was initiated June 28, 2008. Five C. *dubia* neonates were added to each of four replicate beakers per concentration. Neonates originated from laboratory cultures and were 24-hours old at test initiation. Neonates were fed *Selenastrum capricornutum* prior to test initiation.

A control of four replicate beakers containing five *C. dubia* each in laboratory water was conducted concurrently with the test. Survival data were statistically analyzed using the Trimmed Spearman-Karber point estimate test to determine the LC50.

RESULTS
Ceriodaphnia dubia

The following LC50 value was determined for Oil Spill Eater II (2%):

24-Hour Definitive Test

Conc. (mg/L)	# exposed	# alive	#dead	% survival
Control	20	20	0	100.0
250	20	20	0	100.0
500	20	20	0	100.0
1000	20	20	0	100.0
2000	20	20	0	100.0
4000	20	19	1	95.0
8000	20	20	0	100.0
16000	20	17	3	85.0

Percent Spearman-Karber Trim:	0.00%
Estimated LC50 (mg/L):	**>16,000.00**
95% Lower C.L. (mg/L):	N/A
95% Upper C.L. (mg/L):	N/A

The pH in all solutions was within the organism's tolerance range.

DISCUSSION AND CONCLUSIONS

One LC50 determination was made for Oil Spill Eater II tested at a 2% concentration: 24-hour *Ceriodaphnia dubia* LC50: >16,000.00 mg/L. The acute test was conducted from June 28, 2008 to June 29, 2008.

Luther and Associates, Inc.
environmental toxicologists, biologists, consultants

24-HOUR *CERIODAPHNIA DUBIA* SURVIVAL

CLIENT: OSE 2%

PROJECT #: 05457

CONC.	NUMBER ORGANISMS, 0 HRS				NUMBER ORGANISMS, 24 HRS			
	A	B	C	D	A	B	C	D
CON	5	5	5	5	5	5	5	5
250 mg/L	5	5	5	5	5	5	5	5
500	5	5	5	5	5	5	5	5
1000	5	5	5	5	6	5	5	5
2000	5	5	5	5	5	5	5	5
4000	5	5	5	5	5	5	5	4,
8000	5	5	5	5	5	5	5	5
16,000	5	5	5	5	4,	4,	5	4,
DATE/TIME	6/28/08 1245				6/29/08 1245			
TECHNICIAN	mm				mm			

1445 MacArthur Blvd. Ste. 216 Carrollton, Texas 75007 (972) 242-6844

OIL SPILL EATER

Test @ 2%

CHEMISTRY MEASUREMENTS

Date	Client	Sample #	ST pH 24hr	ST DO 24hr	Hardness	Alkalinity	Cond	Ammonia	Salinity	Analyst
6/28/05										
			ST | 24hr	ST | 24hr				pH @ 96hr	D.O @ 96hr	
	LAB CONTROL		7.87 | 8.60	8.23 | 7.43			390	7.91	7.92	
	250 mg/L		7.84 | 8.59	8.22 | 7.38			389	7.94	8.14	
	500		7.85 | 8.52	8.20 | 7.44			387	7.95	8.27	
	1000		7.85 | 8.48	8.15 | 7.38			387	7.94	8.26	
	2000		7.86 | 8.47	8.10 | 7.53			388	7.93	8.20	
	4000		7.86 | 8.42	8.15 | 7.28			390	7.92	8.21	
	8000		7.85 | 8.31	8.05 | 7.31			393	7.92	8.05	
	16,000		7.81 | 8.14	7.87 | 7.12			399	7.94	8.03	
	LAB WATER CONTROL		7.84		104	64				

ACUTE REFERENCE TOXICANT TEST RESULTS

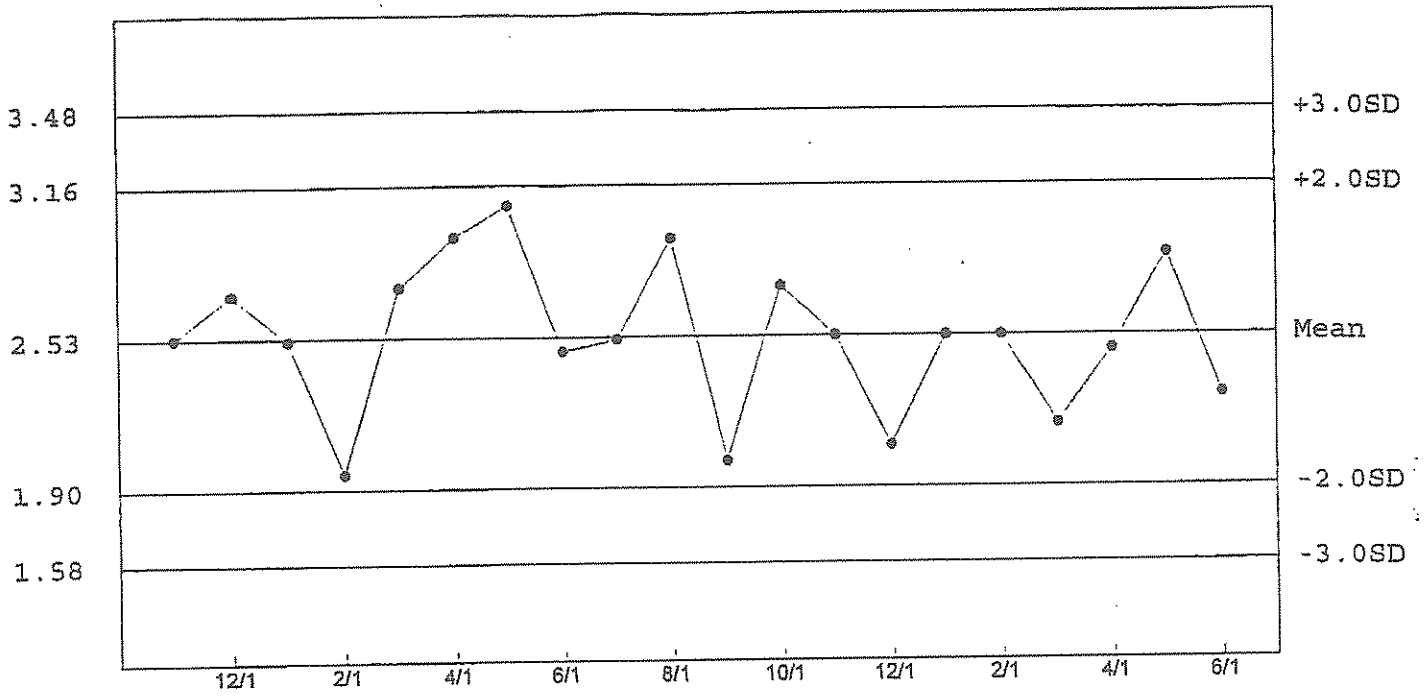
SPECIES:	*Ceriodaphnia dubia*
CHEMICAL:	Sodium Chloride
DURATION:	48-Hours
TEST NUMBER:	6
TEST DATE:	June 2008
STATISTICAL METHOD:	Spearman-Karber

CONCENTRATION (g/L)	NUMBER EXPOSED	NUMBER DEAD
1.0	10	0
1.5	10	0
2.0	10	0
2.5	10	9
3.0	10	10
4.0	10	10

LC50	95% LOWER CONFIDENCE LIMITS	95% UPPER CONFIDENCE LIMITS
2.28 g/L	2.20 g/L	2.37 g/L

Ref. Toxicant Sodium chloride g/L

Ceriodaphnia dubia LC50



n= 20 Mean= 2.53 SD= 0.32 CV= 12.49% Min= 1.96 Max= 3.08

APPENDIX B
SECTION 2.7

EVALUATION OF EPA AND NETAC EFFICACY TESTING REPORT

P.O. Box 515429
Dallas, Texas 75075
Ph: (972) 669-3390
Fax: (469) 241-0896
Email: oseicorp@msn.com
Web: http://www.osei.us

SUMMARY

U.S EPA and NETAC EFFICACY TESTING

The United States Environmental Protection Agency spent one and one-half years testing and evaluating protocols using OIL SPILL EATER II.

Mr. Tom Merski (August 18, 1993) explained the control (oil and seawater only) showed such an insignificant change (no reduction in TPH) that the control results were not even released.

NOTE - that OIL SPILL EATER II Biodegraded Alaskan Crude Oil 98% in 21 days in NETAC's Tier II Test. This test specifically shows the reduction of Polynuclear Aromatic Hydrocarbons that are the Hydrocarbons that are more persistent and difficult to Bioremediate!

This test proves that using OIL SPILL EATER II is beneficial over doing nothing, and that 98% of a spill can be mitigated as opposed to mechanical cleanups, which after 30 days or more can only blot up 20% of a spill. Using OIL SPILL EATER II can reduce the impact to marine organisms and ECO systems faster and more efficiently than mechanical cleanups. This means huge savings on the cleanup costs and environmental damage assessment fees.

By: Steven R. Pedigo
Chairman
OSEI, CORP.

SRP/AJL

National Environmental Technology Applications Center
UNIVERSITY OF PITTSBURGH APPLIED RESEARCH CENTER
615 William Pitt Way · Pittsburgh, PA 15238
Facsimile (412) 826-5552
(412) 826-5511
NETAC

July 22, 1993

Mr. George Lively
President
OSEI Corporation
Oil Spill Eater International
Suite 1116, 5545 Harvest Hill
Dallas, TX 75230

New address as of Oct. 1999
13127 Chandler Drive
Dallas, TX 75243

Dear Mr. Lively:

Subject: ***Oil Spill Eater II Methods Validation Data***

Per your request, enclosed is the efficacy data generated with "Oil Spill Eater II" from the development and validation of our oil spill response bioremediation evaluation methods. The Toxicity data from this process will be provided as soon as it is released from the EPA Office of Research and Development laboratories. We have included information on the experimental methods and objectives intended to assist you in understanding the meaning of the numbers generated for this report.

On behalf of NETAC and all the members of our Oil Spill Product Protocol Development Panel, we wish to express our appreciation for the contribution of your bioremediation agent for use in validating these methods and for your availability to answer questions about how this agent was intended to be used. Your patience and cooperation over the past two years has been commendable.

As you are aware, these experiments were conducted by the NETAC and EPA Office of Research and Development laboratories in Cincinnati, OH and in Gulf Breeze, FL. These data give you a general idea of how your product may behave in an open environment. Note that these data were obtained during the development of our methods. Numerous refinements have been made to increase the sensitivity of these tests; therefore, your product may provide different results in future tests due to this increased sensitivity as well as from the natural variability of the product and the constituent(s) used in the test sequence.

Please bear in mind that, although the Tier II methods have been finalized, we anticipate that all of the methods will be refined and updated periodically as we learn more about these systems. This means that data which was incidentally obtained for your product during the development of the protocols as it currently stands may change as the protocol is further refined. We must emphasize the research nature of the data we are providing to you today!

These data are provided to give you an indication of how your product behaved in this particular phase of the research. Different results may occur with the newly refined methods. We recommend that you evaluate this information as another set of intermediate data. We strongly suggest that you initiate additional testing applying the final Tier II method to develop a product performance baseline.

We also wish to emphasize that the participation of any bioremediation agent in the development of validation of the protocol does not constitute endorsement, approval or recommendation on the part of either NETAC or the EPA Office of Research and Development.

Enclosed for your convenience are the tabulated results of the Day 21 Shaker flask experiment for efficacy testing, and a Statistical Method Summary used to generate data about your product. This statistical method can be found in the July 1993 issue of the *Evaluation Methods Manual for Oil Spill Response Bioremediation Agents*. This document is currently being printed and a copy of the manual will be sent to you as soon as possible.

If you have questions about the data which we have provided, its potential use or application, or development of the protocol please call me at (412) 826-5511.

Sincerely,

A. Thomas Merski
Vice-Chairman,
Treatability Protocol Development Subcommittee
Bioremediation Action Committee

ATM\MRM:tmw
H:\public\bpec\OSEI-2.ltr
310-2015-141

cc: W.M. Griffin

NETAC

RESULTS:

TIER II EFFICACY DATA

PERCENT REDUCTION

	OIL SPILL EATER II (DAY 21)
ANALYTE	LAB A (n = 3) (%)
PRISTANE	88
C18	66
PHYTANE	82
C30	83
TOTAL n-PARAFFINS	77
FLUORENE	92
PHENANTHRENE	97
CHRYSENE	165
TOTAL AROMATICS	98

APPENDIX B
SECTION 2.8

EVALUATION OF SECOND US EPA AND NETAC BIOREMEDIATION TEST REPORT

OIL SPILL EATER INTERNATIONAL, CORP.

P.O. Box 515429
Dallas, Texas 75075
Ph: (972) 669-3390
Fax: (469) 241-0896
Email: oseicorp@msn.com
Web: http://www.osei.us

SUMMARY

SECOND U.S. EPA/NETAC (Bioremediation Test)
Using OIL SPILL EATER II
February 28, 2001

The second U.S. EPA/NETAC Test was more thorough with different days for testing the amount of bioremediation occurring. EPA/NETAC wanted to determine if there was a statistical difference between the control (doing nothing at all), the nutrient control (EPA – Dr. Venosa's nutrients) and the test product, **OIL SPILL EATER II.**

Table 2 shows the raw data where on day 0 the control, nutrient control and OSE II started at approximately 8,000 ppm (parts per million). In seven (7) days, OSE II had remediated the oil to an average of 6,529 ppm. The control and nutrient control were still around 8,000 ppm. On day twenty eight (28), OSE II had remediated the oil to 3,658 ppm. While the control was where it started and the nutrient control showed only minimal reduction of the oil.

In fact, OSE II remediated more of the oil in seven (7) days than the nutrient or nutrient control remediated in twenty eight (28) days.

EPA/NETAC through scientifically valid testing wanted to determine through an Anova Table if there was significant statistical difference between the nutrient, nutrient control, and the test product, OSE II.

In this very limited closed system, OSE II reduced the oil over 50%, while very little reduction occurred in the control or nutrient control. In fact, on Page 3, in the last paragraph, EPA/NETAC explains that for OSE II (Group 3) "at day 7 and day 28 are significantly different from (Group 1) and (Group 2)."

This test is reproduced as the example in the U.S. Code of Federal Regulations under Bioremediation Efficacy Test.

EPA/NETAC conclude, "Therefore in terms of total aromatic degradation, the test indicates the desired statistically significant difference between the mean of the product (OSE II) and the mean of the non-nutrient control.

EPA/NETAC's scientifically valid Bioremediation Test proves that OSE II is a very significant oil spill cleanup product.

By: Steven R. Pedigo
Chairman

SRP/AJL

NETAC

National Environmental Technology Applications Center
UNIVERSITY OF PITTSBURGH APPLIED RESEARCH CENTER
615 William Pitt Way · Pittsburgh, PA 15238
Facsimile (412) 826-5552
(412) 826-5511

OIL SPILL RESPONSE BIOREMEDIATION AGENTS EVALUATION METHODS VALIDATION TESTING DISCUSSION OF RESULTS

The following data are provided for the oil spill response bioremediation agent producer as a means to begin to assess how this bioremediation agent may behave in response to an oil spill in the environment. The data we are providing are limited to the gas chromatographic/mass spectrometer (GC/MS) results.

Note that a total of 69 analytes (components naturally occurring in oil) were measured in these experiments. These analytes constitute a small but highly representative fraction of the toxic and biodegradable portion of oil. We are providing you with a summary of the ultimate results and a summary of the most germane analytes to facilitate our reporting of this information and to reduce confusion in reporting caused by the modification of the selected test results.

The following table of GC/MS results indicate the percent reduction of analyte(s) versus the same analyte(s) present in the control (i.e., product results/control results x 100). For example, if 100 percent of an analyte is present at Day 21 after mixing oil, seawater and product as compared to the control (oil and seawater only) then the product did not stimulate the decomposition of hydrocarbons in oil. Note, that the greater the number of analytes with a low percentage the more capable the product of enhancing the biodegradation of oil.

From this experiment, the results indicated that there was sufficient comparability of the data between the laboratories conducting this experiment. The resultant data presented for this bioremediation agent and the comparative nutrient treatment did not show a significant statistical difference between the product mean and the control mean at the $p \leq 0.05$ level of significance. That is, biodegradation was occurring but not significantly faster than the control. We note that even though these treatments did not produce statistical significant degradation of the test oil, several of the products in this research did achieve this standard.

An analysis of the total aromatic data (in ppm) was conducted for the following three groups:

GROUP 1: Non-nutrient Control
GROUP 2: Nutrient Control
GROUP 3: Test Product – OSE II

The raw data is shown in Table 2 below. Note the three replications for each group-time combination.

TABLE 2

PRODUCT TEST DATA
TOTAL AROMATICS (PPM)

	GROUP 1	GROUP 2	GROUP 3
DAY 0	8153	7912	7711
	8299	8309	8311
	8088	8111	8200
DAY 7	8100	7950	6900
	8078	8200	6702
	7999	8019	5987
DAY 28	8259	8102	4000
	8111	7754	3875
	8344	7659	3100

Table 3 gives the summary statistics (number of observations, means, and standard deviations) for each group-time combination.

TABLE 3

SUMMARY STATISTICS FOR PRODUCT TEST DATA
TOTAL AROMATICS (PPM)

	GROUP 1	GROUP 2	GROUP 3
DAY 0	8153	7912	7711
	8299	8309	8311
	8088	8111	8200
DAY 7	8100	7950	6900
	8078	8200	6702
	7999	8019	5987
DAY 28	8259	8102	4000
	8111	7754	3875
	8344	7659	3100

Table 4 shows the results of the two-way ANOVA.

TABLE 4

TWO-WAY ANOVA TABLE

Source	df	Sum of Squares	Mean Square	F-Statistic	p-Value
GROUP	2	23944857.41	11972428.70	151.94	0.0001
TIME	2	10954731.19	5477365.59	69.51	0.0001
INTERACTION	4	19347589.04	4836897.26	61.39	0.0001
ERROR	18	1418303.33	78794.63		
TOTAL	26	55665480.96			

From the ANOVA table, we see that the F-statistic for INTERACTION is significant (F=61.39, p=0.0001). This indicates that group differences exist for one or more days. Protected LSD mean separations were then conducted for each day to determine which group differences exist. The results are summarized in Table 5. Note that means with the same letter (T grouping) are not significantly different.

TABLE 5

PAIRWISE PROTECTED LSD MEAN SEPARATION

T Grouping	Mean	n	Interaction
A	8238.0	3	Group 1, Day 28
A	8180.0	3	Group 1, Day 0
A	8110.7	3	Group 2, Day 0
A	8074.0	3	Group 3, Day 0
A	8059.0	3	Group 1, Day 7
A	8056.3	3	Group 2, Day 7
A	7838.3	3	Group 2, Day 28
B	6529.7	3	Group 3, Day 7
C	3658.3	3	Group 3, Day 28

Significance Level = 0.05
Degrees of Freedom = 18
Mean Square Error = 78794.63
Critical Value = 2.10
Least Significant Difference = 481.52

The grouping letters indicate that the product mean values (group 3) at day 7 and day 28 are significantly different from those of both the nutrient control (group 2) and the non-nutrient control (group 1) for those days. No other significant differences are shown. Therefore, in terms of total aromatic degradation, the test indicates the desired statistically significant difference between the mean of the product and the mean of the non-nutrient control.

EXPERIMENTAL DESIGN

The shaker flask evaluation conducted in Tier II is an experiment designed to determine the product's ability to degrade crude oil components at a rate or extent greater than a natural seawater microbial population. The experimental design includes a control, nutrient treatment, and the product treatment. The resultant data are compared and tested statistically using a two-way analysis of variance to determine data trends. The experimental design for Tier II testing is known as a factorial experiment with two factors. The first factor is product/control group; the second factor is time (as measured in days). For example, if two groups (product A and a non-nutrient control) are tested at each of three points in time (day 0, 7, and 28), the experiment is called a 2x3 factorial experiment. There were three replications (replicated shaker flasks) of each group-time combination.

DATA ANALYSIS METHODS

For each analyte and each product used in Tier II, a product is deemed a success by the demonstration of a statistically significant difference between the mean analyte degradation by the product and the mean analyte degradation by the non-nutrient control. Such a determination will be made by performing a two-way analysis of variance (ANOVA) on the sample data. The technical aspects of this procedure are outlined in Snedecor and Cochran (1980). Most statistical software packages support the use of two-way ANOVA. However, the format required for the input data differs among the various commercial packages. Whichever package is used, the following ANOVA table will be provided as part of the output.

TABLE 1
TWO WAY ANOVA TABLE

Source	df	Sum of Squares	Mean Square	F-statistic	p-value
Group	p-1	SSG	MSG = MSG/MSE	MSG/MSE	*
Time	t-1	SST	MST = MST/MSE	MST/MSE	*
Interaction	(p-1)(t-1)	SSI	MSI = MSI/MSE	MSI/MSE	*
Error	pt(n-1)	SSE	MSE = SSE		
TOTAL	npt-1	SSTOT			

* To be determined from the value of the F-statistic

In the degrees of freedom column (df) of Table 1, p denotes the number of product/ control groups, t denotes the number of days at which each group is analyzed and n denotes the number of replications. For the example of the 2x3 factorial experiment discussed in the previous section, p=2, t=3, and n=3. The significance of the F-statistics (as indicated by their corresponding p-value) are used to interpret the analysis.

INTERPRETATION

If the F-statistic for the INTERACTION is significant at the 0.05 level (i.e. the p-value is less than 0.05), the data indicate that the mean response of at least two groups being tested differ for at least one point in time. In order to find out which groups and at which points in time the difference occurs, pairwise comparisons between the group means should be conducted for all time points. These comparisons can be made using protected least squared difference (LSD) or Dunnett mean separation techniques. The protected LSD procedure is detailed in Snedecor and Cochran (1980); the Dunnett procedure is outlined in Montgomery (1991). For both methods, the mean square error (MSE) from the two-way ANOVA table should be used to compute the separation values.

If the F-statistic for the INTERACTION is not significant at the 0.05 level (i.e. the p-value is not less than 0.05), but the F-statistic for the GROUP is significant (i.e. the p-value is less than 0.05), but data indicate that any differences which exist among the group means are consistent across time. To find out which group means differ, a pairwise comparison of the group means should be carried out by pooling data across all points in time. Again, the mean square error (MSE) from the two-way ANOVA table should be used to compute the separation values.

If the F-statistic corresponding to both INTERACTION and GROUP are not significant at the 0.05 level, the data indicate no difference between the group means at any point in time. In this case, no further analysis is necessary.

Finally, Snedecor and Cochran (1980) caution about the use of multiple comparisons. If many such comparisons are being conducted, then about 5 percent of the tested differences will erroneously be concluded as significant. The researcher must guard against such differences causing undue attention.

REQUIRED DOCUMENTATION

The following documents should be included to summarize findings from a product test.

- Data listings for each analyte that was analyzed. These should show all raw data.
- A table of summary statistics for each analyte. The table should include the mean, standard deviation and sample size for each group at each day.
- An ANOVA table for each analyte. The table should be of the same format as Table 1.
- A clear summary of the mean separations (if mean separations were necessary). The mean separation methods (LSD or Dunnett), the significance level, the minimum significant difference value and the significant differences should be clearly marked on each output page.
- All computer outputs should be included. No programming alterations are necessary. The specific computer package used to analyze the data should be included in the report.

APPENDIX B
SECTION 2.9

EVALUATION OF TEXAS A & M UNIVERSITY REPORT

P.O. Box 515429
Dallas, Texas 75075
Ph: (972) 669-3390
Fax: (469) 241-0896
Email: oseicorp@msn.com
Web: www.osei.us

OSEI CORPORATION'S SUMMARY
of
Texas A&M's
Microbial Petroleum Degradation Enhancement
By Oil Spill Bioremediation Products

The General Land Office for the state of Texas (USA) asked the University of Texas A&M to perform a study on 13 bioremediation products listed in the EPA National Contingency Plan for oil spills.

The efficacy tests were to be performed using the EPA / NETAC guidelines in their test protocol for bioremediation agents.

The test covered the total oil and grease (O&G), the aliphatic fraction of oil, the aromatic fraction of oil, and the plate counts on the numbers of hydrocarbon degraders grown or colonized during this test.

OIL SPILL EATER II IS PRODUCT 10.

Oil Spill Eater II was one of the best products at reducing the oil and grease. *Oil Spill Eater II* was the most effective product at reducing the aliphatic fraction of the oil.

Oil Spill Eater II was the most effective product at reducing the Polar-aromatic (PAH, more toxic) fraction of the oil.

Oil Spill Eater II grew the most hydrocarbon degraders, an acceptable product grew 10^5 numbers of hydrocarbon degraders while *OSE II* outperformed them all at enhancing hydrocarbon degraders at $10^{7.5}$.

Oil Spill Eater II proved it was the most efficient product at biodegrading Alaskan North Slope crude oil out of the 13 EPA / NCP Listed products tested.

Steven R. Pedigo
Chairman

Microbial Petroleum Degradation Enhancement By Oil Spill Bioremediation Products

A Report Submitted to the Texas General Land Office

October 12, 1995

Principal Investigators:

James S. Bonner

Robin L. Autenrieth

Contributing Students:

Salvador Aldrett

Marc A. Mills

Frank Stephens

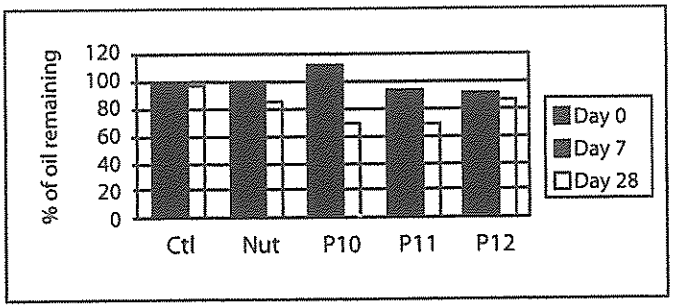
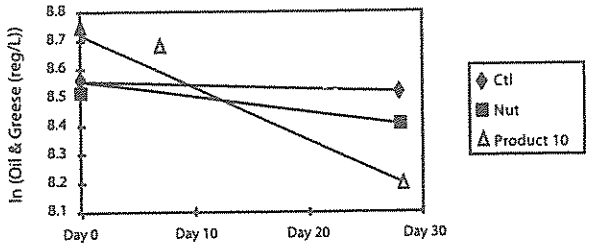


Figure 4 – Oil and Grease results (Batch D)
P10 is OSE II

High O&G numbers can be a result of a high production of extractable materials such as biomass or metabolites. As shown in Batch D, Product 10 is causing an increase in the O&G values at day 0 and 7, with an average value of 11% more of the initial weight. However, microbial counts indicate a high aliphatic degrader population through this period, as will be shown later Figure 16. After 28 days the oil was degraded more extensively by Product 10 than by the nutrient control. This suggests that the polar fraction is possibly being increased by the product's contents, on days 0 and 7, but does not imply that the oil is remaining undegraded. Microbial degradation of Product 10 could be producing metabolites that are being completely oxidized between day 7 and day 28.



Treatment	Slope	R square
Control	-0.0013	0.9505
Nutrient	-0.00563	0.8041
Product 10	-0.01859	0.9228

Figure 10 – Ln concentration change with time for product 10 (P10) as compared with the nutrient and non-nutrient control

Figure 10 suggests a lag phase for Product 10 between day 0 and 7, after this period the microbial population shows a high degradation rate, achieving a final degradation extent higher than that of the nutrient and non-nutrient control.

The rate of oil removal is an important factor to consider when comparing the performance of each product. Table 7 presents a summary with the different rates of oil removal as well as the average.

	Product	Rate	Non-nutrient control	Nutrient control
	Product	0.007	0.00013	0.004
	Product	0.012	0.00013	0.004
	Product	0.014	0.002	0.005
	Product	0.017	0.0003	0.014
OSE II→	Product	0.018	0.00013	0.005
	Product	0.011	0.00013	0.005
	Average	0.013	0.0005	0.005

Table 7 - Rates of oil removal for the products passing the O7G criteria (mg of oil/L-Day)

OSE II had the highest rate of oil removal of the 13 EPA NCP Listed Products tested.

According to these results the average half-life of the petroleum mixture for this specific experiment is approximately 40 days. Prior studies suggest a half-life for petroleum mixtures of approximately 2 months (Stewart et. al., 1993).

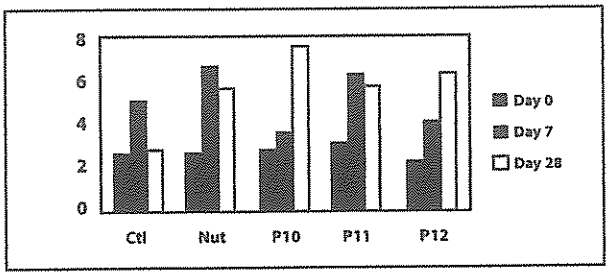


Figure 16 - MSIv aliphatic degraders results (Batch D)

OSE II grew the highest number of oil degrading bacteria at $10^{7.5}$.

Products with a significant extent of oil removal show microbial counts in the order of 10^5 for the aliphatic degraders as presented in Figure 14, Figure 15, Figure 16, and Figure 17. Treatments with higher microbial populations, but similar degradation extents a compared with the control suggest the addition of an alternative carbon source other than the petroleum hydrocarbons.

Figures 32-34 show the composition of aliphatics, aromatics, and polars for batch D. As presented earlier for batches A and B, the aliphatic fraction is being degraded more severely than the aromatic fraction. The same results are found in the next two figures. Microbial counts for aliphatic degraders (Figure 16) show a higher number for Product 10, with a value of 4.06 E7 at day 28, as compared with the rest of the treatments in this batch, with values in the order of 10^6 at the most. This is reflected as a decrease in the aliphatic fraction composition from a 100% to 46% after 28 days.

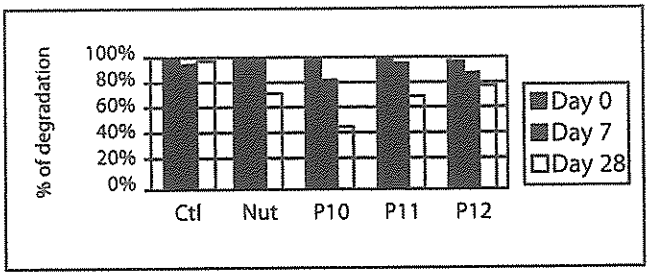


Figure 32 – Aliphatic fraction composition through time (% of degradation (Batch 1))

OSE II had the highest rate of degradation.

Products 10, 11, and 12 are decreasing in aliphatic and aromatic composition up to 50% for the aliphatic fraction and 25% for the aromatic. It is clear from these results that the oil is being degraded, and therefore, changing its composition. However, the aliphatic fraction is being degraded at a greater extent than the aromatic fraction, as mentioned before. Product 10 is showing a significant extent of hydrocarbons removal as presented in Figure 33 and Figure 34 for Product 10.

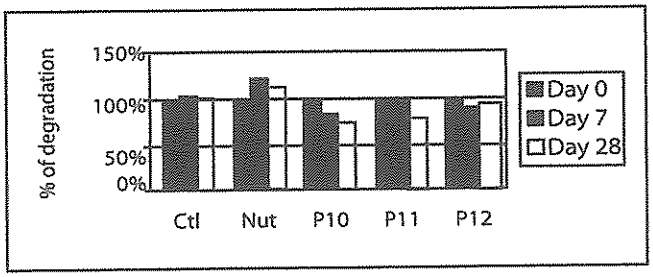


Figure 33 – Aromatic fraction composition through time (% of degradation (Batch D))

OSE II had the most (highest rate of) degradation of the aromatic fraction of the oil.

As presented in Figures 23 and 33 show the average of aliphatic fraction biodegraded was 34% (54% decrease for OSE II), while only 21% of the aromatic fraction showed to be biodegraded. The most degradation was by OSE II.

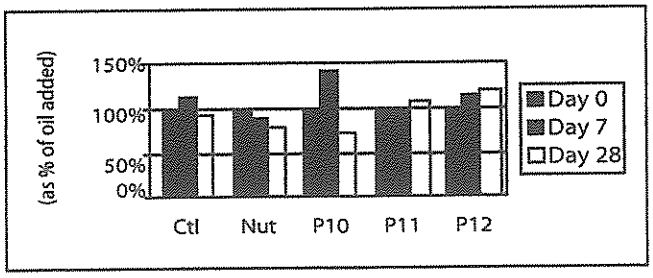


Figure 34 – Polar fraction composition through time as a percentage of the amount initially present (Batch D)

OSE II had the most or highest rate of (Polar) aromatic hydrocarbon degradation.

APPENDIX B
SECTION 2.10

EVALUATION OF RECIPROCITY- TEST REPORT

P.O. Box 515429
Dallas, Texas 75075
Ph: (972) 669-3390
Fax: (469) 241-0896
Email: oseicorp@msn.com
Web: http://www.osei.us

OIL SPILL EATER II
EPA TEST – MARCH 1993
OIL SPILL EATER II – RESPIROCITY TEST - SUMMARY

This Respirocity Test was developed by NETAC and the Environmental Protection Agency to verify if a product could actually mitigate hydrocarbons to an end point of CO2 and water. The test was designed to measure the amount of oxygen-enhanced bacteria used. This would confirm the bacteria are in fact breaking the hydrocarbons down to CO2 and water.

At 100 parts Alaskan Gulf Seawater to 1 part OIL SPILL EATER II – applied at a 1 to 1 ratio to 1,000 parts per million Alaskan Prudhoe Bay Crude, the oxygen uptake is dramatic. This dramatic oxygen uptake proves a large amount of bacterial growth and decomposition of Prudhoe Bay Crude. The Chart on Page 2 shows an 86% decrease in heavy-end hydrocarbons and a 50% decrease in the aromatics. The test was stopped at 30 days; the test time prescribed by the EPA.

Our Standard Application Instructions for crude oil are 50 parts water to 1 part OIL SPILL EATER II applied at a 1 to 1 ratio to crude oil. The test results may be extrapolated to determine that with a 50 to 1 dilution, a 98% decrease in heavy-ends would occur in 24 days while an 85% decrease in aromatics would occur in 30 days. OIL SPILL EATER II can very effectively mitigate an oil spill.

After reviewing copies of the EPA Test on 10 other products, a comparison was initiated on the 2 products EPA claimed out-performed the other 9 products they tested. One product reduced the TPH approximately 158 parts per million and the other product reduced to 157 ppm of TPH. OIL SPILL EATER II reduced the TPH to 870 PPM. We feel this is a significant difference in efficacy.

March 1993
Respirocity Test

The Prudhoe Crude was supplied by the EPA, and was supposed to be the same crude used on the other two products. The crude sent to us for testing had a higher TPH (1,000 PPM) compared to the bacteria products tested by the EPA which only had a TPH of 168 ppm. Additionally, this crude did not have aromatics which the crude oil OSE II was tested on, did. The aromatics were reduced 50%.

It is our opinion that if you apply bacteria directly to a hydrocarbon with aromatics, that the toxicity of the aromatics will kill the bacteria. OIL SPILL EATER II first breaks the hydrocarbon walls, then grows bacteria so the toxicity is reduced first.

The accumulate oxygen uptake was also tested which shows bacterial activity. One of the products the EPA tested, they claim, performed well, had an uptake of 280 mg/L in 10 days and 460 mg/L in 30 days. The other product the EPA tested had 40 mg/L at 10 days and 440 mg/L at 30 days. OIL SPILL EATER II had an uptake of 520 mg/L at 10 days and 810 mg/L at 30 days. OSE II had more oxygen uptake at 10 days than the best bacterial products had at 30 days; on the 30 day comparison, OSE II had almost double the oxygen uptake any other product.

The EPA screened 31 products and tested 10. This test shows OIL SPILL EATER II reduced dramatically more TPH than these other products. OSE II produces more microbial activity than products with bacteria, and additionally, OSE II reduces aromatics. This test should help prove why we feel OSE II is the better product.

NOTE: In the summer of 2000 – Dr. Al Venosa (one of the EPA's top scientists at the time, on oil spills) reviewed this test. Dr. Venosa concluded that OSE II did, in fact biodegrade alkanes and aromatics. Dr. Venosa went on to explain that OSE II may be effective in degrading oil.

By: Steven R. Pedigo
Chairman
OSEI, Corp.

SRP/AJL

CHEMICAL ANALYSIS, INC.

Chemical * Polymer * Design

Research and Development
Consultation
Legal and Expert Witness

July 3, 1990

Failure Analysis
Formula Analysis
Engineering Design

Mr. Steve Pedigo
Sky Blue Chems
13355 Noel Road
1 Galleria Tower, Suite 500
Dallas, Texas 75240

NEW ADDRESS AS OF 10/96

OSEI, CORP.
13127Chandler Drive
Dallas, TX 75243

Subject: Oil Spill Eater Respirocity Evaluation
CAI Lab. No. 3265

Dear Mr. Pedigo:

Chemical Analysis, Inc. being an independent third party laboratory was employed to evaluate an oil spill additive for respirocity efficacy. The oil spill additive submitted to the laboratory was a product identified as Oil Spill Eater batch No. 124-E. The additive was evaluated at two different concentrations which included 1/100 and 1/500, additive parts to solution parts, respectively.

The concentration of the oil was 1000 parts per million (ppm). The oil and seawater was submitted to the laboratory to be similar to field material.

The results of our evaluation are attached to the report. Observing the results, it can be seen that the additive has a meaningful and significant effect on decreasing the oil concentration and increasing the oxygen take up.

The effect on decreasing the aliphatic content of the oil was in the range of 80 percent and the decrease of the aromatic content was in the range of 40 percent. An additive concentration of 1/500 appears to be effective. The concentration of the additive may have an adequate effect at even a lower concentration than 1/500.

The inherent effect of oxygen takeup was observed to be 178 mg/L for the additive (1/500), 12 for the seawater, and 8 for the oil. The net effect of the additive was 512 mg/L.

If there are any questions or if we may be of further assistance, please advise.

Sincerely yours,
CHEMICAL ANALYSIS, INC.

Galen Bartman
Laboratory Director
GWH:es

Oil Spill Eater (OSE) Respirocity Results

Sample	Oil	Additive	Seawater	Accumulated Oxygen Uptake 0 mg/L	10 mg/L	20 mg/L	30 days mg/L	Aliphatic Content 0 ppm	10 ppm	20 ppm	30 days ppm	Aromatic Content 0 ppm	30 days ppm	Percent Aliphatic Decrease	Percent Aromatic Decrease
1	+	1/500	+	16	380	620	690	712	570	233	151	246	133	79	46
2	+	1/500	+	18	410	660	730	693	542	274	138	240	149	80	38
3	-	1/500	+	5	152	174	186	-	-	-	-	-	-	-	-
4	-	1/500	+	5	141	168	194	-	-	-	-	-	-	-	-
5	-	-	+	0	5	8	12	-	-	-	-	-	-	-	-
6	-	-	+	0	6	8	11	-	-	-	-	-	-	-	-
7	+	-	+	2	12	18	22	705	710	695	682	251	248	3	1
8	+	-	+	3	13	16	19	684	680	681	675	238	237	1	0
9	+	1/100	+	26	460	680	770	690	512	210	105	245	115	85	53
10	+	1/100	+	33	520	740	810	695	486	260	89	250	127	87	49
11	Spill Eater Batch No. 124-E														

APPENDIX B
SECTION 2.11

EVALUATION OF UNIVERSITY OF ALASKA, FAIRBANKS, ALASKA REPORT

OIL SPILL EATER INTERNATIONAL, CORP.

P.O. Box 515429
Dallas, Texas 75075
Ph: (972) 669-3390
Fax: (469) 241-0896
Email: oseicorp@msn.com
Web: http://www.osei.us

March 23, 1990

OIL SPILL EATER II
BIODEGRADATION TESTS
CONCLUSIONS

These tests were conducted by the University of Alaska in Fairbanks, AK. The first test was on a heavy-end hydrocarbon (Hexadecane), which is left over once the light-ends volatize off. The mineral nutrients in nature refers to the use of Alaskan Sea Water used to perform the test. At 50 to 1, it shows good reduction and if the test would have continued another 48 hours, the results would have been substantially increased. The OIL SPILL EATER II has a good food source for bacteria and there was more food source than sea water ratio to grow a large colony quickly; therefore, the bacteria engulfed the food sources in the OSE II and slowly converted to hydrocarbons. Once all the OSE II food source runs out, then the only food source left are the hydrocarbons—so they switch over to stay alive. At 1 to 500 and 1 to 1000 absolute biodegradation was proven, the bacteria colonized quickly and ran out of food source because they started with less food source. The bacteria switched over quickly and a dramatic reduction in hexadecane was accomplished.

The second test was run on Naphthalene using minerals and nutrients (Alaskan Sea Water). Naphthalene is a polynuclear aeromatic hydrocarbon and are harder to break down than heavy-end hydrocarbons and they are the most toxic. These tests also show that OIL SPILL EATER II is a very effective means of mitigating naphthalene, a PAH which EPA's Dr. Al Venosa deems the hardest target compounds to Bioremediate!

By: Steven R. Pedigo
Chairman

P.O. Box 515429
Dallas, Texas 75075
Ph: (972) 669-3390
Fax: (469) 241-0896
Email: oseicorp@msn.com
Web: http://www.osei.us

OIL SPILL EATER II

A PROTEIN POWER PACKAGE

The lack of knowledge about biological treatment of hydrocarbons has led to slow acceptance of proven methods of Bioremediation, particularly with respect to oil spills. However, following the EXXON VALDEZ incident, the U.S. Environmental Protection Agency undertook the first major governmental effort to use biological methods for site remediation. Although the early results are mixed, EPA is to be commended for its efforts which included application of a French Product (Inipol EPA 22) to enhance microbial degrading of weathered crude oil from beaches. Inipol has been described as "Popeye's Spinach" supplement to enhance the rate and extent of hydrocarbon degradation by naturally occurring microbial populations. The Inipol formulation probably does enhance the growth of hydrocarbon degradation bacteria (although this has not been clearly shown in the field portion of the EPA Study), but suffers in that it contains the potentially toxic solvent, 2-butoxyethanol.

There are many other agents which have potential to stimulate hydrocarbon removal from contaminated environments. These range from the solvent based cleaners and dispersants to simple water soluble inorganic fertilizers. One such product that has shown great potential for enhancing hydrocarbon biodegradation in standardized laboratory tests at the University of Alaska Fairbanks is OIL SPILL EATER II. If Inipol is a "Popeye's Spinach" formulation for hydrocarbon degrading micro-organisms, OIL SPILL EATER II is a "Protein Power Package" of mineral nutrients, enzymes and a carbon source concentrated in a non-toxic oleophilic surfactant. The surfactant base dissolves into hydrocarbon matrices with the aid of protease and amylase enzymes that act as micro-surface cleaners. The mineral nutrients enhance growth of natural hydrocarbon degrading micro-organisms with the pulse of easily metabolized carbon to quickly increase bio-mass. The high bio-mass, then begins to degrade hydrocarbon substrates and to product biosurfactants until the hydrocarbon substrate is depleted.

OIL SPILL EATER II
A PROTEIN POWER PACKAGE

In the aftermath of the EXXON VALDEZ Oil Spill, researchers from the University of Alaska evaluated the potential for naturally occurring micro-organisms to biodegrade oil contaminated beaches. Their studies showed that while natural micro-organisms have the potential to biodegrade both linear alkanes and aromatic hydrocarbons, their numbers and related metabolic activities can be substantially increased. In standard laboratory tests, these researchers showed that the marine formulation of OIL SPILL EATER II diluted into artificial seawater containing a consortium of micro-organisms and hydrocarbons from Prince William Sound, Alaska will degrade Hexadecane—300% faster than the same consortium amended with mineral nutrients and hydrocarbons without OIL SPILL EATER II.

By: Dr. Ed Brown
University of Alaska

DEB/AJL

OIL SPILL EATER CONCENTRATE
MINERALIZATION OF HEXADECANE BY A MICROBIAL CONSORTIUS FROM PRINCE WILLIAM SOUND, ALASKA (1)

Sample	Mineral Nutrients in nature HO OSE	Mineral Nutrients in nature 1/50 Dilution of Oil Spill Eater II	Mineral Nutrients in nature 1/500 Dilution of Oil Spill Eater II	Mineral Nutrients in nature 1/1000 Dilution of Oil Spill Eater II	Mineral Nutrients in nature 1/10 Dilution of Oil Spill
Hexadecane Transformation (I transformed to CO2) Mean of 3 trials	16	19.3	50	43.7	0

Need more time so bacteria can use up molasses & convert to Hydrocarbon

300 increase

proven efficacy

Should totally eliminate Hydrocarbons

1. Consortius was incubated for 70 hours with 100 mg of labeled hexadecane per sample.

Test Conducted at University of Alaska-Fairbanks

OIL SPILL EATER II CONCENTRATE
Mineralization of Naphthalene by a Microbial Consortius From
Prince William Sound, Alaska (1)
Alaskan Seawater

Sample	MINERAL Nutrients in nature No OSE	MINERAL Nutrients in nature 1/50 Dilution of Oil Spill Eater II	MINERAL Nutrients in nature 1/500 Dilution of Oil Spill Eater II	MINERAL Nutrients in nature 1/1000 Dilution of Oil Spill Eater II
NAPHTHALENE Transformation (% transformed To CO_2 Mean of 3 trials	3	29	46	27
		More time would have been allowed for the bacteria to completely use up the molasses and completely convert to hydrocarbon for its food source	1533% increase proven efficacy should totally eliminate raphthalene hydrocarbons	

1. Consortium (Alaska Sea Water) was incubated for 51 hours with 100 mg of labeled Naphthalene per 10 ML sample.

Test conducted at the University of Alaska
1/9/90

APPENDIX B
SECTION 2.12

EVALUATION OF SOUTHWEST RESEARCH INSTITUTE REPORT

OIL SPILL EATER INTERNATIONAL, CORP.

P.O. Box 515429
Dallas, Texas 75075
Ph: (972) 669-3390
Fax: (469) 241-0896
Email: oseicorp@msn.com
Web: http://www.osei.us

August 13, 1990

MEGA BORG BIODEGRADATION TEST

Southwest Research Institute – one of the United States largest and most respected labs performed TPH reduction tests and residual weight tests using OIL SPILL EATER. This product, OSE, was applied to South African Crude Oil – spilled from the Mega Borg Tanker off the coast of Galveston, Texas. The sample of crude was supplied by the U.S. Coast Guard – Sky Blue Chems sent the sea water from Galveston to the Lab.

The initial TPH was 100,070 ppm; in 216 hours the TPH was reduced to 529 for a 99.5% reduction. This is a dramatic decrease and it proves Oil Spill Eater is a very viable Bioremediation product. This dramatic decrease shows how effective Oil Spill Eater is in reducing the chemical (toxic) constituent of the crude oil. The TPH was reduced approximately 90% in 48 hours rendering the crude oil virtually harmless quickly.

The physical reduction of the crude oil was also determined. In 216 hours, 94.7 of the residual weight of the South African Crude was remediated.

These tests prove "OIL SPILL EATER" is an extremely effective Bioremediation product that decreases not only the chemical components of crude oil, but it also Biodegrades the physical components as well.

Steven R. Pedigo
Chairman

SRP/AJL

SOUTHWEST RESEARCH INSTITUTE
6220 CULEBRA ROAD • SAN ANTONIO, TX 78238-5100 • (210) 684-5111

August 3, 1990

CHEMISTRY AND CHEMICAL ENGINEERING DIVISION
DEPARTMENT OF ENVIRONMENTAL SCIENCE

Attention: Mr. Steven R. Pedigo

Subject: Second Test for Sky Blue Chemical 01-3108-092

A sample of Megaborg oil and seawater was analyzed as per your instructions. The results of this initial test were inconclusive and a second test was requested. The second test was more extensive and included more time points. Samples were taken at 48,72, and 96 hours for the sample and control. The sample consisted of 600 ml seawater, 6 ml Megaborg oil, and 6 ml of the oil-eater provided. The control consisted of 600 ml seawater, and 6 ml Megaborg oil. The sample and control were stirred constantly at a very low speed. Sampling procedure: Vigorously stir the solution and remove 100 ml. Extract for TRPH analysis. After 90 hours the client requested addition of more seawater to improve the efficiency of the oil-eater, this was performed. A final analysis for TRPH was performed at 216 hours and was a complete sample extraction. In order to better compare the control and oil-eater results, results are shown in % Recoverable Oil, assuming that 1 gram of oil is equal to 1 ml of oil (since oil density is unknown). The percent recoverable oil is calculated as follows:

equation not clear

$$\frac{\text{TRPH g/ml} \times \frac{100 \text{ ml}}{1000 \text{ g/ml}}}{\text{theoretical amount of oil extracted in each aliquot} = 1 \text{ g}} \times \frac{100}{1000 \text{ mg/g}} = \%$$

TRPH and % Recoverable Oil for each time are shown for the sample and control in tables 1 and 2, respectively. Megaborg oil itself was found to have a TRPH of 1,070,000 mg/l.

Sincerely,

Mary Riddle

Mary Riddle
Research Scientist

Approved:

Donald E Johnson

Donald E. Johnson, Ph.D.
Director

SAN ANTONIO TEXAS

Table 1

01-3108-092
Sample With Oil-Eater II

Time Elapsed	TRPH (mg/10)	% Recoverable Oil
48 hours	7520	75.2
72 hours	6910	69.1
96 hours	5990	59.9
*216 hours	529	5.3

95% Reduction of TPH in 216 hours. Chemical reduction of TPH.

94.7% residual weight reduction in 216 hours. Physical reduction of oil.

* Total sample analyzed

APPENDIX B
SECTION 2.13

EVALUATION OF SOUTHWEST RESEARCH INSTITUTE REPORT

P.O. Box 515429
Dallas, Texas 75075
Ph: (972) 669-3390
Fax: (469) 241-0896
Email: oseicorp@msn.com
Web: http://www.osei.us

SUMMARY OF BETX TEST

The objective was to have a third party testing laboratory show how OSE II (OIL SPILL EATER II Concentrate) worked well even on Benzene, Ethyl Benezene, Toulene and Xylene. The final composition – after all dilutions were performed, was 2,000 parts water to one (1) part OSE II Concentrate.

Even at this low level, the total BETX was reduced 32%. The correlation of strengths should prove that at 1,000 to one (1) reduction would have been 64%, a 500 to one (1) reduction would have been 80%; a 100 to one (1) reduction would have been 98%, almost completely Biodegraded.

At 2,000 to one (1) OSE II is a cost-effective product for Ballast Water Treatment.

The reduction correlation's with the increasing ratios also show that OSE II is an effective product for gasoline and diesel spills. OSE II would reduce gasoline or diesel spills on the surface and around leaking Underground Storage Tanks. OSE II would also be a good product to clean up any oil sheen on water surfaces and concrete surfaces.

Steven R. Pedigo
Chairman

CHEMICAL ANALYSIS, INC.
Chemical ❋ Polymer ❋ Design

Research and Development
Consultation
Legal and Expert Witness

Failure Analysis
Formula Analysis
Engineering Design

March 14, 1990

Mr. Steve Pedigo

Subject: BETX Analysis
CAI Lab. No. 3229

Dear Mr. Pedigo:

Chemical Analysis, Inc. being a third party independent laboratory was employed to evaluate a product identified as Oil Spill Eater and its affect on BETX solution. The procedural method was provided to our laboratory which outlined the preparation of several solutions.

Solution I: BETX

COMPONENTS	% BY VOLUME
Benzene	5.0
Ethylbenzene	5.0
Toluene	5.0
Xylene	5.0
Florida Sea Water	80.0
TOTAL	100.0%

Solution II: OSE-Florida Sea Water

COMPONENTS	% BY VOLUME
Oil Spill Eater	0.20
Florida Sea Water	99.80
TOTAL	100.0%

The percentage ratio of these two components represents a 1 to 500 mix ratio respectively.

3001 Skyway Circle North, Suite 100. Las Colinas Irving, Texas 75038 (214) 255-4100

Solution III: BETX/OSE-Florida Sea Water

COMPONENTS	% BY VOLUME
Solution I	50.00
Solution II	50.00
TOTAL	100.0%

Solution IV: BETX/OSE-Florida Sea Water Solution

COMPONENTS	% BY VOLUME
Solution III	50.00
Florida Sea Water	50.00
TOTAL	100.0%

Final Solution Composition:

COMPONENTS	% BY VOLUME
Aromatics	5.00
OSE Additive	0.05 (1:2000 weight ratio)
Florida Sea Water	94.95
TOTAL	100.0%

The final solution identifies the composition of the final mixture when the various solutions are prepared and mixed together based on the procedural instructions. The resultant final solution was allowed to stir for a period of (96) hours and the volume of BETX aromatic content was evaluated. The initial percent volume of aromatic discontinuous phase in the final solution represented five percent after the test. As a result of the evaluation, it was observed that 1.6% of the BETX solution had decreased from the discontinuous aromatic phase; this represented a 32% volume reduction in the aromatic content. Turbidity was observed to have increased in the water phase which indicated that incompatable components were incorporated into the water phase.

The 1:2000 weight ratio concentration of OSE in the final solution is based on the assumption that the OSE additive is 100% active; if the OSE is less than 100% active then one needs to proportionate the concentration accordingly.

If there are any questions or if we can be of further assistance, please advise.

Sincerely yours,
CHEMICAL ANALYSIS, INC.

Galen W. Hartman
Laboratory Director

GWH/cmc

مركز المساعدة المتبادلة للطوارئ البحرية
Marine Emergency Mutual Aid Centre (MEMAC)
REGIONAL ORGANIZATION FOR THE PROTECTION OF THE MARINE ENVIRONMENT
المنظمة الاقليمية لحماية البيئة البحرية

OSEI Corporation
P.O. Box 515429,
Dallas, Texas 75251,
USA

Ref : 337/12-RHD
Date : 12th August 2012

Subject : OSEI – II

Dear Sirs,

MEMAC would like to advise that a revision has been made for the Bioremediation product known as OSE II, which is non-toxic and can be used within our Region.

The OSE II Bioremediation product is enlisted in the list of MEMAC Oil Spill Combating Products used within the ROPME Region.

For MEMAC (Marine Emergency Mutual Aid Centre)

ص.ب:١٠١١٢ المنامة – مملكة البحرين – هاتف: ١٧٢٧٤٥٥٤ – فاكس: ١٧٢٧٤٥٥١
P.O. Box. : 10112, MANAMA, KINGDOM OF BAHRAIN. TEL: 17274554 - FAX: 17274551
E-mail: memac@batelco.com.bh
Web site: http://www.memac-rsa.org

جامعة الملك فهد للبترول و المعادن
معهد البحوث
مركز البيئة والمياه

التقرير النهائي

تقرير حول تقييم خصائص المعالج الحيوي للتسربات النفطية (OSE-II)

مقدم إلى

شركة رواد المجرة للمقاولات العامة (RMC)

الخبر – المملكة العربية السعودية

صفر ١٤٣٦هـ

ديسمبر ٢٠١٤م

دراسة رقم CEW4906-14015

التقرير النهائي

تقرير حول تقييم خصائص المعالج الحيوي للتسربات النفطية (OSE-II)

مقدم إلى

شركة رواد المجرة للمقاولات العامة (RMC)

الخبر - المملكة العربية السعودية

إعداد

مركز البيئة والمياه

صفر ١٤٣٦هـ

ديسمبر ٢٠١٤م

جامعة الملك فهد للبترول والمعادن

معهد البحوث

الظهران - المملكة العربية السعودية

الخلاصة

طلبت شركة رواد المجرة للمقاولات العامة (RMC) بالخبر من معهد البحوث بجامعة الملك فهد للبترول والمعادن في الظهران تقييم الخصائص الفنية والتحليلية لمنتج خاص بالمعالجة الحيوية للتسرب النفطي يسمى آكل التسربات٢ (OSE-II). وقد تم تطوير هذا المنتج عام ١٩٨٩ بواسطة شركة سكاي بلو كيمز (Sky Blue Chems Company) بالولايات المتحدة المملوكة الآن لمؤسسة أو إس إي ١، الواقعة في مدينة دالاس بولاية تكساس الأمريكية.

وكجزء من المهمة قام معهد البحوث بجامعة الملك فهد للبترول والمعادن بتقييم التقارير الفنية والتحليلية لاستعمال مادة آكل التسربات٢ (OSE-II) لمعالجة التسرب النفطي في الأنهار والبحار، وذلك بناء على الجوانب النظرية والعملية والفنية بالإضافة إلى إخضاع المنتج لاختبارات كيمائية ذات علاقة بتجارب تسربات مركبة. كما تم الأخذ بالاعتبار استعمال هذا المنتج في أجزاء أخرى من العالم وتم استخلاص استنتاجات عن ملاءمة منتج المعالجة الحيوية للتسرب النفطي وإمكانية تطبيق استعمالاته على التسربات النفطية في المملكة العربية السعودية.

ويحتوي المنتج على أنزيمات وإضافات غذائية ضرورية لنمو البكتيريا. وعند الاستعمال يتم تخفيفه بالماء ٥٠ مرة حجم/حجم، ثم يتم رشه على المناطق الملوثة بالتسربات النفطية. وتتكون خطوات العملية من تخفيف المنتج بالماء ثم رشه على التسربات النفطية التي تتحول إلى معلقات ثم تنحل إلى جزئيات وغازات. وخلال هذه العملية تقوم الأنزيمات بتفكيك المركبات ذات الأوزان الجزيئية العالية مثل الهيدروكربونات البترولية بينما تقوم الميكروبات المتوفرة بالبيئة بإحداث المزيد من تفكيك النفط. وفي تلك الأثناء فإن البكتيريا ذاتية التغذية (chemolithoautotrophs) تحصل على المغذيات من المواد المكملة والماء والطاقة الناتجة من عملية تفكيك النفط.

واستنادا على تقييمنا فإنه يمكن اعتبار مادة آكل التسربات٢ (OSE-II) إضافة مبتكرة للمعالجة الحيوية للتسرب النفطي. وهذا المنتج يوفر حلا اقتصاديا للتسربات النفطية بمختلف مصادرها حيث يتميز بانخفاض تكلفة التشغيل والكفاءة العالية للمعالجة، وهو فعال جدا في معالجة أنواع مختلفة من التسربات النفطية. ويمكن أن يستعمل هذا المنتج محليا لمعالجة التسربات النفطية في مختلف البيئات من مياه الأنهار والبحار أو التربة الملوثة.