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**OSEI Corporation Summary of  
Testing Done on OSE II by Dr. Tsao of British Petroleum  
at LSU Laboratories Regarding the Effectiveness of OSE II on  
Remediating Oil from the Deepwater Horizon, Macando  
Oil Blow Out in The Gulf of Mexico  
2010/2011**

The major oil company British Petroleum tested the OSEI Corporation's product called Oil Spill Eater II (OSE II) at Louisiana State University from November 2010 through approximately January 2011. Relevant sections of BP's BCST (Bio Chem Strike Team) test results and summary "interim report" are attached.

OSE II was tested at our normal mixing and application rate as laid out in the protocols we provided. OSE II was applied to Louisiana sweet crude from Bay Jimmy, Louisiana. This oil had the additional components of Exxon's chemical dispersant product called "Corexit", as this dispersant had been applied to it in the field as a part of the BP spill response actions.

British Petroleum formed a group named the Bio Chem Strike Team (BCST). Under the direction of Dr. Tsao, BCST was established in response to the Deepwater Horizon incident by the Alternative Response Technology (ART) program. The BCST consisted of experts from BP, LSU, LDEQ (Louisiana Department of Environmental Quality), USCG (U.S. Coast Guard), OSPR (California), SCAT, and highly experienced oil spill response consultants. Furthermore, BCST operated in conjunction with advice from EPA and NOAA. The overall stated objectives of the BCST were to evaluate the thousands of alternative approaches that were submitted as potential solutions to the oil cleanup. Through the ART system, those biological and chemical technologies were to be determined as to which ones best met the needs of Unified Command on oil spill cleanup. In order to accomplish this, the team reviewed and subsequently determined which technologies would undergo "desktop evaluations" (literature review), laboratory scale testing (at the aquatic toxicology laboratory at Louisiana State University (LSU), and/or field testing. Ultimately, the results of this

work by the BCST would provide recommendations for best available technologies for use by the Unified Command.

The OSEI Corporation submitted information about our product, OSE II, and, as a result, OSE II has been put through an exhaustive review by the BCST group from June 2010 until the OSEI Corporation received an email from Dr. TSAO stating OSE II had made it through their tier reviewed process of four tiers. BP had stated earlier that only a few products would be capable of making it through their review process.

OSE II was then slated for testing and the tests were started in November of 2010, and concluded in January of 2011. The tests were very thorough and measured several pertinent aspects in regards to remediating hydrocarbons/oil. The tests were conducted with Gas Chromatograph Mass Spectrometry EPA test procedures. Bacteria counts, as well as dissolved oxygen, nitrogen, and phosphorous levels were measured, and PAH and Alkane degradation was quantified.

The results from the tests of OSE II were excellent and demonstrated the statements we have made to BP regarding the effectiveness of the product as being factual. The first week's test results tracked exactly with the thousands of tests that have been performed on OSE II by universities around the world, as well as the many legal "closure" letters we have received which are required to verify the completion of a cleanup project in the U.S.

The week-two test showed that additional PAH's had been unexplainably added to the test; however, the reduction of PAH's 2 weeks later showed that, despite the unexplained addition of PAH's in the second week, even greater remediation of the PAH's had occurred. The inexplicable addition of PAH's to the OSE II test vials, however, may have slowed the final results somewhat.

The test measured Alkanes which is a component of oil that has limited toxicity to the environment and aquatic life. PAH's are called poly cyclic aromatic hydrocarbons and have been denoted by the US EPA as the most persistent toxic component of oil, and are the hydrocarbons they deem to be the ones most in need of remediation.

OSE II showed a great ability in the closed laboratory test to be able to remediate PAH's, as well as the Alkanes. In fact, by the conclusion of the testing time frame, OSE II had remediated 80% of both components of the oil released by BP which ended up in Bay Jimmy, Louisiana. It is very important to note that laboratory tests actually hinder OSE II's ability to remediate oil, due to the fact that, in a closed laboratory setting, there is less available indigenous bacteria to enhance, less wave action, and less oxygen. Therefore, one can easily predict that the percentage of results seen in the same time frame in the Gulf of Mexico waters and environment

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will be far greater, as has already been shown in the many real field tests and clean-up projects we have done.

One aspect of the BP spill in the Gulf of Mexico is the fact that the water column is being depleted of oxygen because of the dispersants sinking oil into the water column. The BCST test verified OSE II has minimum negative impact upon the oxygen level.

This test by a major oil company is the second major testing of OSE II on two of the largest spills on water in the history of planet Earth caused by Man. Exxon tested OSE II in 1989 at Florham Park, New Jersey and discovered OSE II was the most effective product in the world by a factor of better than 90% on the North Slope Alaskan Crude oil from the Valdez spill. This was verified by Dr. Brown of the University of Alaska and Steve Hinton, a chemical engineer for Exxon. Upon the completion of that test, both of these men called the OSEI Corporation alerting Steven Pedigo of the success of its results, which were like none they had ever seen before.

BP has now successfully tested OSE II on their spill in the Gulf of Mexico which is estimated, at this time, to be over 6,000,000 gallons of oil spilled.

Dr. Tsao wrote in his report **“After nearly one year since the Deepwater Horizon spill, residual weathered oil remains in many locations. The need for a field trial to establish operational criteria for final bioremediation work plans should be initiated before early Spring 2011.”**

The OSEI Corporation has alerted BP that, after over 16,000 spill clean ups in the past 21½ years, the logistics in regard to the successful application of OSE II were worked out some time ago. We have also let BP know that OSE II has been demonstrated several times on their Gulf of Mexico blow out on sandy beaches and marsh grass. They can see the video on our Website under “News videos, WLOX TV in Mississippi” where OSE II was demonstrated on Waveland beach for MS State Senator Gollot and the RRT IV team.

The remediation of the PAH’s also verifies that OSE II is an extremely effective first response bioremediation product, and has among its many benefits:

- 1) it causes the oil to float which limits the negative toxic impact to the water column or ocean floor of the oil and dispersant,
- 2) it causes the reduction of the adhesion properties so the oil cannot stick to birds, grass, rock or sand on shorelines,
- 3) it causes the elimination of fire hazard,
- 4) it has been proven to be non-toxic by the numerous formal toxicity tests, the fact that you can safely wash your hands with it, and the TV news program in which Retired Rear Admiral Lively drank some of it.

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- 5) Boom deployment actually works and can help when OSE II is used because OSE II causes the oil to float,
- 6) Although OSE II causes the oil to float, because of the method in which it goes to work on the oil, it is still very difficult to see,
- 7) it has a defined end point of turning the oil into water and CO2.

All of the above clearly demonstrate that it is the best and only needed oil spill response and that it will, even at this late date, remediate both fresh and weathered oil and dispersant currently in the Gulf.

Steven Pedigo  
Chairman/CEO OSEI Corporation

## **Laboratory Screening of Commercial Bioremediation Agents for the Deepwater Horizon Spill Response**

### **Report**

#### **Submitted to:**

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**Remediation Engineering and Technology Specialist**  
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#### **Submitted by:**

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**March 3, 2011**

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*This report provides a brief summary of findings to date for OSE II from the National Oil and Hazardous Substances Pollution Contingency Plan (NCP) Product Schedule.*

## **1.0 Introduction and Summary of Methods**

The BioChem Strike Team (BCST) was established in response to the Deepwater Horizon incident by the Alternative Response Technology (ART) program. The BCST consisted of experts from BP, LSU, LDEQ, USCG, OSPR (California), SCAT, and highly experienced oil spill response consultants. Furthermore, the BCST operated in conjunction with advice from EPA and NOAA. The overall objectives of the BCST were to evaluate among the thousands of submissions of alternative approaches through the ART system, those biological and chemical technologies that best meet the needs of Unified Command on oil spill cleanup. In order to accomplish this, the team reviewed and subsequently determined which technologies would undergo desktop evaluations (literature review), laboratory scale testing (at the aquatic toxicology laboratory at Louisiana State University, LSU), and/or field testing. Ultimately the results of this work by the BCST will be to provide recommendations for best available technologies for use by the Unified Command.

This interim report summarizes Oil Spill Eater II (OSE II) evaluated at the laboratory scale. Specifically, the BCST determined that OSE II a product listed on the National Oil and Hazardous Substances Pollution Contingency Plan (NCP) Product Schedule warranted further testing to determine effectiveness in degrading oil under the specific environmental, climate, and ecological conditions generated by the 2010 Gulf oil spill. Using pre-defined test protocols, OSE II was evaluated and compared to natural (inherent) biodegradation occurring through indigenous microflora and micronutrients present in Gulf waters. The selected product was analyzed in a controlled flask-study to determine the remediation potential on weathered crude oil recovered from south Louisiana marshes.

It should be noted this shows only OSE II, as established by OSEI Corporation to eliminate un-needed data to reduce confusion.

## **2.0 Materials and Methods**

The experimental design protocol specified a flask study, incubated at room temperature on a consistently rotating, 200 rpm, orbital shaker. The samples were sacrificed over 5 separate sampling events including Time 0, 1, 2, 4 and 12 Weeks. The original protocol called for the last sampling event to occur at 8 weeks. However, the final sampling event was changed to 12 weeks while the backlog of analytical characterization for the earlier

samples was being worked through. Each flask was analyzed for total nitrates (NO<sub>3</sub><sup>-</sup>), total phosphates (PO<sub>4</sub><sup>3-</sup>), total organic carbon (TOC), total alkanes, total polyaromatic hydrocarbons (PAHs) and the physical parameters, pH, dissolved oxygen (DO) and temperature. Specific aromatic fractions were also analyzed, but the full dataset is not reported here.

## **2.1 Chemical Analyses**

### **2.1.1 GC/MS Methods**

Extraction of PAHs and alkanes in water-amended with oil follows methods outlined in EPA Method 8270 series. Approximately 100 ml of water is poured into a 250-ml separatory funnel and adjusted to a pH of 7. A 30-ml aliquot of dichloromethane is added to the separatory funnel and spiked with a known amount of standard surrogate. The funnel is capped and shaken for approximately 3 minutes, venting occasionally to remove solvent pressure. The solvent and water are allowed to separate and the solvent is drained through an anhydrous sodium sulfate funnel into a 250-ml flat-bottom flask. The solvent addition and draining step are repeated 2 more times. The sodium sulfate funnel is rinsed with dichloromethane and allowed to drain completely. The flat-bottom flask is then placed on a rotary evaporation system and concentrated to a volume of 5-10 ml dichloromethane and placed in a calibrated extraction thimble. If concentrating is necessary, the extract volume is placed under a nitrogen blow down concentrator and reduced to a volume of 1.0 ml. The dichloromethane extract is exchanged to hexane using approximately 4-5 ml of hexane. A micro distillation column is added to the extraction thimble and placed in a hot water bath. The dichloromethane is evaporated off and the remaining hexane extract is reduced to a volume of 1-2 ml. The hexane extract is placed beneath a nitrogen blow down device and reduced to a final volume of 1.0 ml hexane.

### **2.1.2 GC/MS Instrumental analyses**

After addition of internal standards, samples were analyzed using an Agilent 7890A GC fitted with a 0.25 mm i.d. °—30 m HP-5MS column and an Agilent 7683B autosampler. The injector was set to 250°C and the detector to 280°C. Detection of analytes involves the utilization of a HP 5975C Inert XL Series Mass Selective Detector operating in the Selected Ion Monitoring mode. The column was held at 60°C for 1 min and then ramped at 25°C/min to 160°C followed by 3°C/min to 268°C and 12°C/min to 300°C, where it was held for 8 min. Concentrations of parent PAHs were based on calibrations using a five-point curve which were checked for each batch of samples analyzed. Concentrations were reported on a dry weight basis. Approximate alkylated PAH concentrations were calculated assuming the same response factors for each parent and corresponding alkylated analogues. For alkylated phenanthrene/anthracenes, the results were reported as pairs to incorporate the uncertainty of the measurements and quantification based on the average response factor of the individual parent PAHs.

## **2.2 Other analytical approaches**

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A. Water quality analysis

- DO, pH, temperature and salinity were measured using standard field equipment, (YSI 85-10 meter) appropriately calibrated.
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- B. Microbial analysis
  - Microbial activity was measured by epifluorescence direct cell count (EDCC) for Most Probable Number (MPN).
- C. Nutrients
  - Total phosphates (PO<sub>4</sub><sup>3-</sup>) using EPA 365.4, total nitrates (NO<sub>3</sub><sup>-</sup>) using standard method 4500-NO<sub>3</sub> F modified and total organic (TOC) using US EPA Method 9060.

### **3.0 Screening Protocol**

#### **3.1 Preparation of Oiled Flasks**

The crude oil and Gulf water used in the study were recovered in Bay Jimmy (coordinates: 29°27'238" N, 89°53'510" W) on August 20, 2010. A half (0.5) g of weathered crude oil were weighed out and deposited in the bottom of a sterile 250 ml Erlenmeyer flask. Before the oil was added to the flasks, each flask was rinsed with de-ionized water and autoclaved to ensure sterility. Ten (10) ml of the solvent Dichloromethane (DCM) was added to the flasks and the flasks were placed on the shaker table for approximately 10 minutes until the oil had completely dissolved in the DCM. The flasks were then left uncovered under a ventilation hood to allow the DCM to flash off, leaving a ring of crude oil on the bottom of each 250 ml test flask.

Each of the 180 test flasks, including the 30 control flasks and 150 product flasks, were prepared in this exact manner.

#### **3.2 Preparation of Controls**

controls were prepared in triplicate for each of the five sampling events.

- Negative Control treatments consisted of 100 ml of sterile Gulf water and 0.5 ml of weathered crude oil per test flask. As in all other test flasks, 0.5 ml of oil were dissolved in 10 ml of DCM, creating a coating of weathered oil in the bottom of each flask. 100 ml of autoclaved Gulf water was then added to each flask. No nutrients were added.

- Positive Control 1 treatments consisted of 100 ml of Gulf water and 0.5 g dissolved oil per flask. No nutrients were added. As in all other test flasks, 0.5 ml of oil were dissolved in 10 ml of DCM, creating a coating of weathered oil in the bottom of each flask.

- Positive Control 3 treatments required a solution of 0.09g of hexadecane and 0.01g of chrysene per flask containing 100 ml of Gulf water. Based on the difficulty P8

of accurately weighing 0.01 and 0.09 grams of each component, a stock solution of hexadecane

and chrysene in Dichloromethane (DCM) was prepared. The solution could then be accurately pipetted into each test flask. The calculations to produce 30 ml of solution are as follows:

### **3.3 Stock solutions**

30 ml of DCM containing 0.01 g chrysene per ml DCM and 0.12 ml hexadecane per ml DCM

30 ml DCM contained 0.3 g chrysene and 3.6 ml hexadecane 0.3 g of chrysene were added to 30 ml of DCM and allowed to dissolve. Once dissolved, 3.6 ml of hexadecane was added to the chrysene/DCM solution. One (1) ml of the composite solution was then added to each Positive Control 3 test flask. Based on the passive volatilization of DCM as compared to hexadecane and chrysene, the DCM was flashed off under a vented hood in order to leave the desired amount of chrysene and hexadecane in the bottom of the test flask. Nutrients were also added to each flask.

The final Positive Control 3 flasks consisted of 100 ml of Gulf water, 0.5 g crude oil, 0.25 g KH<sub>2</sub>PO<sub>4</sub> and 0.5 g NH<sub>4</sub>NO<sub>3</sub> and 1 ml of the solution of hexane and chrysene described above.

### **3.4 Preparation of Products**

*The following products were added to triplicate flasks using formulations and approaches provided by product representatives to LSU.*

#### **Oil Spill Eater (OSEII)**

Nutrients Added: No

Based on the manufactures ratio describing the application of the product to dispersed oil, 0.5 ml of OSE II was added to each test flask.



#### 4.0 Findings

*OSE II, tested in the laboratory screening study is listed in the U.S. Environmental Protection Agency's (USEPA) Office of Emergency Management Regulatory and Policy Division's National Oil and Hazardous Substances Pollution Contingency Plan (NCP) Product Schedule.*

OSE II demonstrated the ability to biodegrade and/or reduce total concentrations of the weathered oil (including alkanes, PAHs) recovered from Bay Jimmy. Additionally, the flask study has verified that the remaining dispersed and weathered oil in coastal environments along the Louisiana and northern Gulf of Mexico will continue to biodegrade. **(The prior statement has been refuted by the Woods Hole Oceanographic Institute.)**

This is not a new finding and has been the opinion of many scientists as a reasonable outcome for any oil spill affecting the coastlines of Gulf States. However, the study does demonstrate the need for accelerated biodegradation strategies so as to minimize the toxicological legacy of the spill over time.

Data sets are included in Appendix A of the report. Representative chromatograms for the first four weeks of the study are in Appendix B.

Microbial cell counts using epifluorescence direct cell count (e.g. MPN) revealed that all samples contained natural and/or supplemented microbial populations above 10<sup>6</sup> viable cells per ml.

*Specific findings for control and commercial products are as follows:*

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**Negative Control:** The negative control flasks consisted of weathered oil added to sterile Gulf water. The flasks indicated minimal reductions in alkanes and PAHs over the 12 week period. Total alkanes from time 0 to week 12 were reduced 14.2% and total PAHs were reduced 14.2% over the same time period.

**Positive Control 1:** The series of control flasks consisted of weathered oil added to non-sterile Gulf water with no additional nutrients. Data sets demonstrated an 11.9% increase in total

alkanes over the 12 week period. Based on the variability of 0.5 gram oil measurements within each flask, this slight increase is an acceptable result for the Control 1 data series. Additionally, visual observation over the 12 weeks indicated minimal degradation of oil. However over the 12 week study, the total PAH concentration was reduced only 28.6%. The Positive Control 1 data series suggested microbial activity produced modest reductions in weathered oil. This is consistent with earlier USEPA studies indicating the need for nutrient amendment so as to maintain steady biodegradation/mineralization. **This was not the case for OSE II.**

Positive Control 3: Positive control 3 consisted of site water with indigenous microflora and the chrysene/hexadecane additive as primary carbon sources. No weathered oil was added to the flask series. A 32.6% reduction in chrysene was demonstrated over the 12 week test period.

**Oil Spill Eater (OSE II):** OSE II is a biological enzyme additive and the product was added to non-sterile site water and weathered oil. By the end of the 12 week test period, 80.1% of the alkane constituents and 79.2% of the PAH constituents were degraded. The product degraded more of the PAH components than Positive Control 2 and about the same percent of alkanes.

NOTE: if you include the 2<sup>nd</sup> week spike then the total degradation was approximately over 4 times any controls degradation.

**OSE II showed the ability to degrade both components of weathered crude oil equally well.**[emphasis mine]

## 5.0 General Discussion

As a general trend, the PAH groups including C1-C3 Phenanthrenes, C3 and C4 Pyrenes as well as C2 and C3 Fluorenes were left intact by the end of 12 weeks. The PAHs of toxicological concern including the Benzo constituents were degraded in every treatment flask. As expected, the shorter-chain alkanes including nC10 to nC14 were most often thoroughly degraded by the end of 12 weeks, while the heavier chains were left in greater concentrations. Importantly to the time frame of the field trials, the greatest reduction in PAHs by OSE II occurred over 4 weeks. The PAH concentration then returned to elevated levels in the weeks between sampling event four (week 4) and five (week 12), for the controls and other products, however this did not occur for OSE II, as the test data shows, OSE II showed continued remediation of the PAH's throughout the duration of the test.

The current laboratory study showed that OSE II an NCP product can promote the conversion, or biodegradation, of oil to CO<sub>2</sub>, biomass and water. The study has also demonstrated that nitrogen and phosphorous amendments also work to enhance in the degradation of oil under controlled closed systems. (while the fertilizer in this closed study showed some ability to reduce the alkanes the least toxic component of oil, however the concentration of fertilizer used would preclude its use in aquatic eco systems due to the fact that at these high concentrations of nitrogen and phosphorous used for this test, you would cause P12

the water to become toxic to aquatic life) Data sets from earlier EPA research into remediation of spilled oil argued that the limiting factor for biodegradation/mineralization is dependent upon the availability of nitrogen and phosphorus. Other factors such as temperature, salinity and dissolved oxygen may affect not only nutrient availability but also acclimated biomass performance. Field demonstration trials are needed to document the efficacy of bioremediation products on weathered oil and to determine their net contribution to biodegradation/mineralization.

**After nearly one year since the Deepwater Horizon spill, residual weathered oil remains in many locations. The need for a field trial to establish operational criteria for final bioremediation workplans should be initiated before early Spring 2011.**[emphasis mine]

**The following 3 paragraphs were written by Steven Pedigo of the OSEI Corporation.**

**The EPA was cited above as arguing that the limiting factor in remediation of oil is mineral nutrients (fertilizer). The EPA has tried unsuccessfully to utilize fertilizer/mineral nutrients in aquatic spill situations in fast release, and slow release versions. They have failed numerous times with the application of fertilizers. Dr. Al Venosa of the EPA Cincinnati Research and Development lab attempted to utilize fertilizer in the Delaware River on an oil spill several years ago. He tried a starting concentration of fertilizer and could not produce any results since in the field you have to deal with dilution in aquatic scenarios. Dr. Venosa became frustrated and loaded up a high concentration and applied it to the spill, he caused the water to become toxic with too much nitrogen which created a large fish kill.**

**The EPA also tried to clean up a spill on the Osage Indian reservation in 2003/2004 with fertilizer on the shores of a creek. Oil had coated the creek for several miles from a pipeline break. After fertilizer failed to produce any results the EPA utilized OSE II in cold temperatures and in 45 days the oil had been cleaned up to the State of Oklahoma DEQ's acceptable levels.**

**The problem with fertilizers is the question of how much do you use. If you use too much, it kills the aquatic life. There is no reason to risk the adverse affects to aquatic life when you can use OSE II, a product whose application ratios do not create a toxicological problem for aquatic life.**

**(End of the interjection by Steven Pedigo. )**

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#### **Appendix A. Data sets from shaker flask studies**

Data sets for the first 8 products with controls are presented for the 12 week screening period. Data sets are incomplete on two additional products added later in the study. Screening studies for these two products were initiated in December 2010. They will be included in the final report.

**Time = 0 11/10/2010**

**Flask Series      NO3-N mg/L   PO4 3- mg/L    TOC mg/L    Alkanes mg/kg    PAHs mg/kg  
pH   DO mg/L   Temp °C**

Negative Control								
A			2.20	0.00	7.29	27400	394	
7.96	9.5	25.0						
B			1.90	0.00	8.20	28200	415	7.96
9.5	25.0							
C			2.30	14.60	7.56	28200	415	7.96
9.5	25.0							
Positive Control 1								
A			1.40	21.80	6.60	21300	452	7.96
9.5	25.0							
B			3.50	7.40	7.56	19900	437	7.96
9.5	25.0							
C			1.60	0.00	7.00	22400	423	7.96
9.5	25.0							
A			1.70	0.00	8.77	0	12100	7.96
9.5	25.0							
B			1.00	0.00	7.96	0	12500	7.96
9.5	25.0							
C			1.70	0.00	8.54	0	11100	7.96
9.5	25.0							
Oil Spill Eater II								
A			1.00	2.90	12.46	17000	486	7.96
9.5	25.0							
B			0.80	2.00	11.61	18600	533	7.96
9.5	25.0							
C			1.40	1.20	12.58	20100	500	7.96
9.5	25.0							

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Time = 1 Week 11/17/2010

Flask Series NO3-N mg/L PO4 mg/L TOC Alkanes mg/kg PAHs mg/kg pH  
DO mg/L Temp °C

Negative Control								
A			1.10	0.00	9.64	20500	390	8.01
5.75	23.4							
B			1.10	0.00	9.95	21800	414	8.06
5.10	23.4							
C			1.20	0.80	10.51	28000	551	8.06
4.88	23.4							
Positive Control 1								

A		1.10	0.00	7.56	19200	368	7.88
4.38	24.0						
B		0.80	0.00	7.50	19900	376	7.93
4.44	24.3						
C		0.90	0.00	7.50	17700	301	7.96
4.39	24.0						
Positive Control 3							
A		0.50	0.00	5.63	0	17700	7.95
4.32	24.0						
B		1.60	0.00	4.91	0	17600	7.97
4.61	24.0						
C		0.90	0.10	7.29	0	14100	7.98
4.61	24.0						
Oil Spill Eater II							
A		0.60	0.00	28.56	7570	306	7.49
3.23	24.4						
B		1.30	0.00	28.97	8940	328	7.42
3.17	24.2						
C		1.10	0.00	32.83	9790	387	7.40
3.16	24.2						

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Time = 2 Weeks 11/24/2010

Flask Series	NO3-N mg/L	PO43- mg/L	TOC	Alkanes mg/kg	PAHs mg/kg	pH
DO mg/L	Temp °C					
Negative Control						
A	1.00	0.0	9.30	10400	521	7.82
4.80	23.5					
B	0.60	0.2	9.87	10500	499	7.89
4.74	24.1					

C	1.10	0.0	9	.85	9110	390	7.92
4.72	24.2						
Positive Control 1							
A	1.20	0.0		9.29	10500	487	7.90
4.53	24.3						
B	1.10	0.0		6.41	4990	215	7.95
4.37	24.3						
C	0.60	1.0		8.15	9630	572	7.96
4.57	24.4						
Positive Control 3							
A	0.90	0.6		10.54	0	16800	7.91
4.56	24.5						
B	1.60	0.0		9.23	0	18700	7.96
4.20	24.5						
C	1.20	0.0		10.54	0	17000	7.96
4.49	24.8						
Oil Spill Eater II							
A	1.00	0.0		32.49	4050	914	7.70
3.84	25.8						
B	1.60	0.5		33.57	3190	981	7.70
3.70	25.7						
C	1.40	0.0		30.81	4280	940	7.70
3.73	25.9						

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**Time = 4 Weeks 12/8/2010**

Flask Series	NO3-N mg/L	PO4 mg/L	TOC	Alkanes mg/kg	PAHs mg/kg	pH	DO
Negative Control							
A	0.6	0.0	12.80	13900	267	7.87	4.61
24.2							
B	0.6	0.1	13.49	14200	254	7.93	3.99
24.1							
C	0.9	0.0	11.72	14300	269	7.97	4.57
24.4							
Positive Control 1							
A	0.8	0.0	9.95	11500	67.7	7.93	4.47
25.1							

B		1.1	0.0	13.04	1330	99.5	7.97	4.56
25.2								
C		0.8	1.0	11.61	11800	73.1	7.98	4.01
25.4								
Positive Control 3								
A		0.6	0.4	10.68	0	19100	7.86	
4.28	25.4							
B		0.9	0.0	10.45	0	18800	7.90	
4.15	25.7							
C		0.8	0.0	10.47	0	19500	7.92	
4.22	DNR							
Oil Spill Eater II								
A		1.1	0.0	36.43	3230	219	7.68	
3.37	25.2							
B		1.1	0.4	39.11	4070	308	7.66	
3.94	25.4							
C		0.9	0.0	26.02	4490	310	7.73	
4.30	25.3							

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**Time = 12 Weeks 2/3/2011**

Flask Series	NO3-N mg/L	PO4 mg/L	TOC	Alkanes mg/kg	PAHs mg/kg	pH	DO
mg/L	Temp °C						
Negative Control							
A		0.7		25100	401	7.81	5.58
22.1							
B		0.6		23400	309	7.89	4.81
22.1							
C		0.9		23400	341	7.96	5.02
22.3							
Positive Control 1							
A		0.5		25100	341	7.77	
4.90	21.9						
B		0.7		23000	291	7.78	
4.61	21.9						
C		0.4		24100	303	7.91	
5.03	22.4						



Positive Control 3						
A		0.3		0	12500	7.88
4.70	22.1					
B		0.5		0	13800	7.93
4.72	22.1					
C		0.4		0	12100	7.96
4.64	22.3					
Oil Spill Eater II						
A		0.6		4050	47.6	7.71
4.90	21.1					
B		0.6		5560	143	7.74
4.70	21.2					
C0.9			1450	124	7.82	4.58 21.4

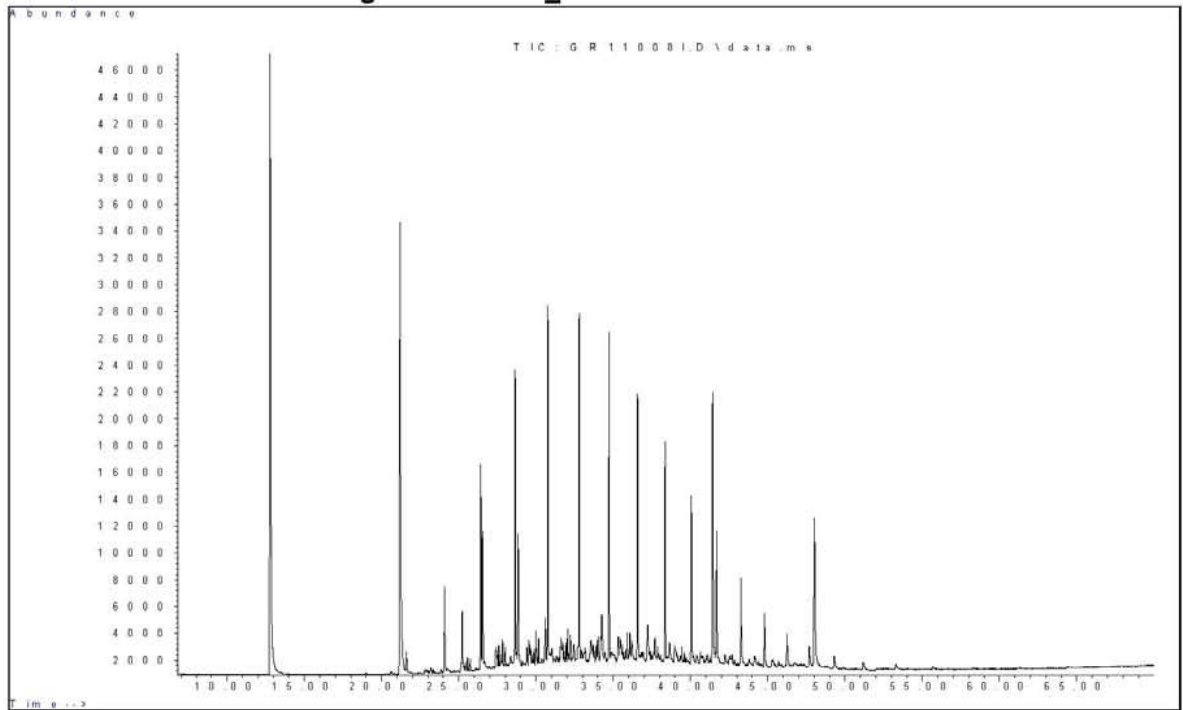
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### Appendix B. Chromatographs of Extracted Flasks Over Time

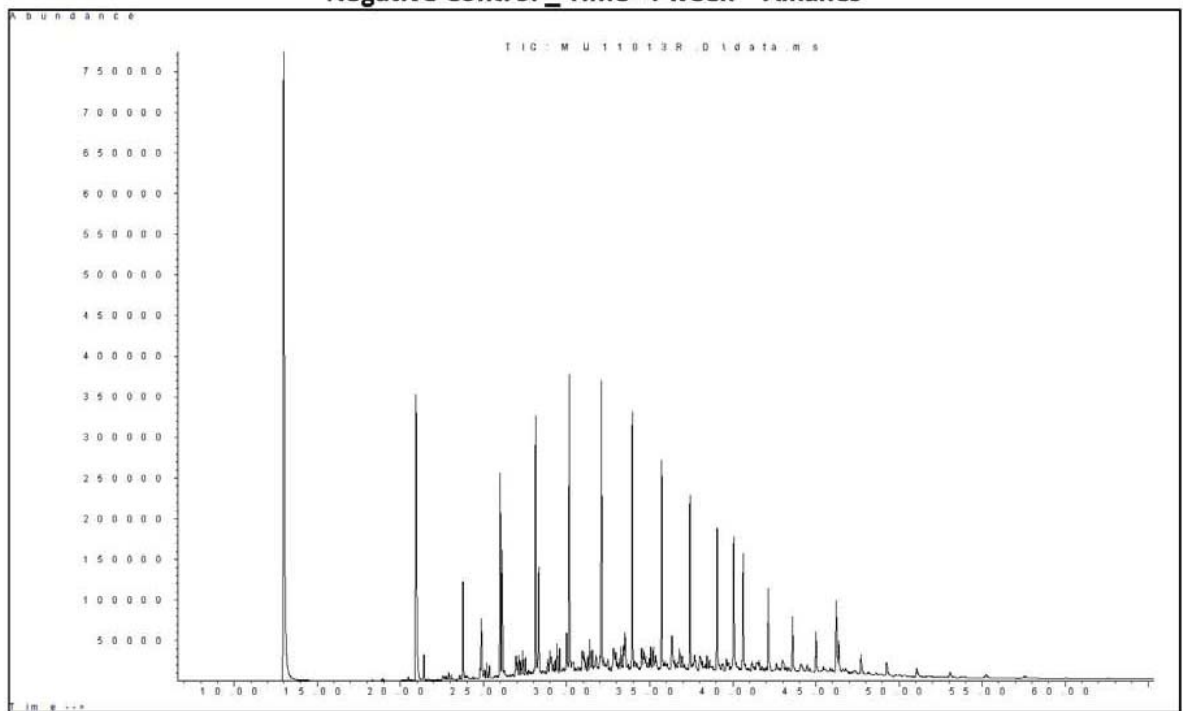
Data sets presented are for total alkanes from Weeks 1 through 4 of the study. Chromatographs from Week 12 showed minimal changes as compared to Week 4. They will be included in the final report.



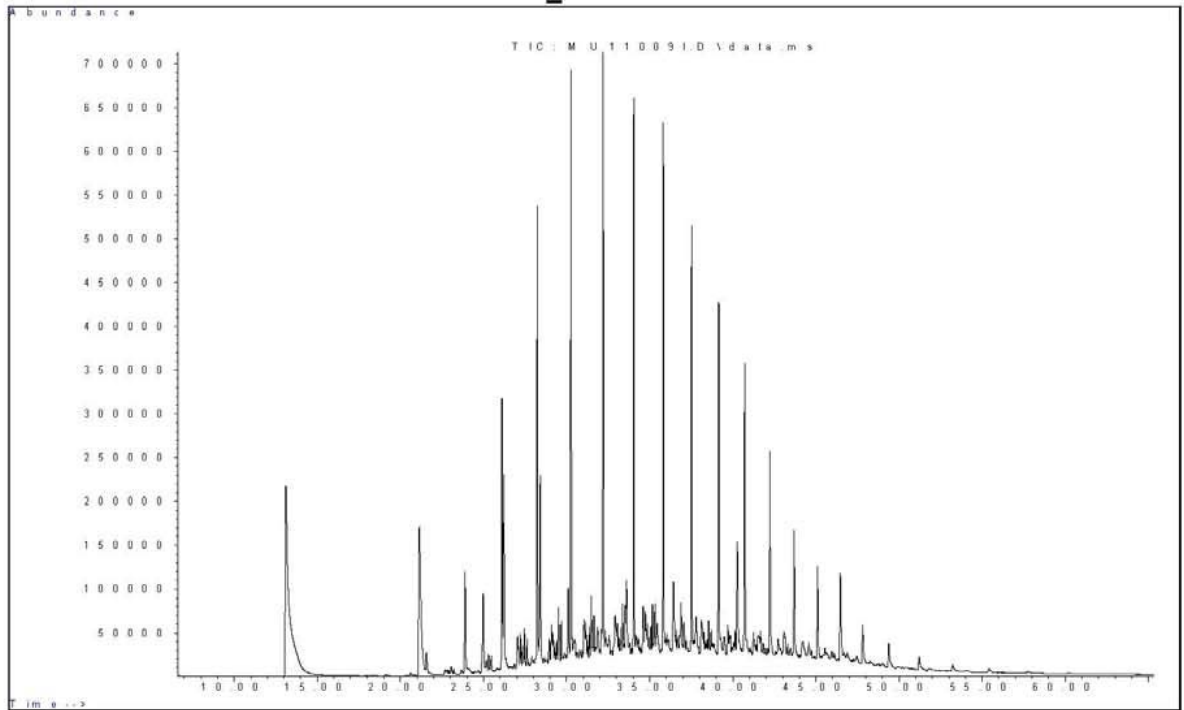
### Negative Control \_ Time=2 week – Alkanes



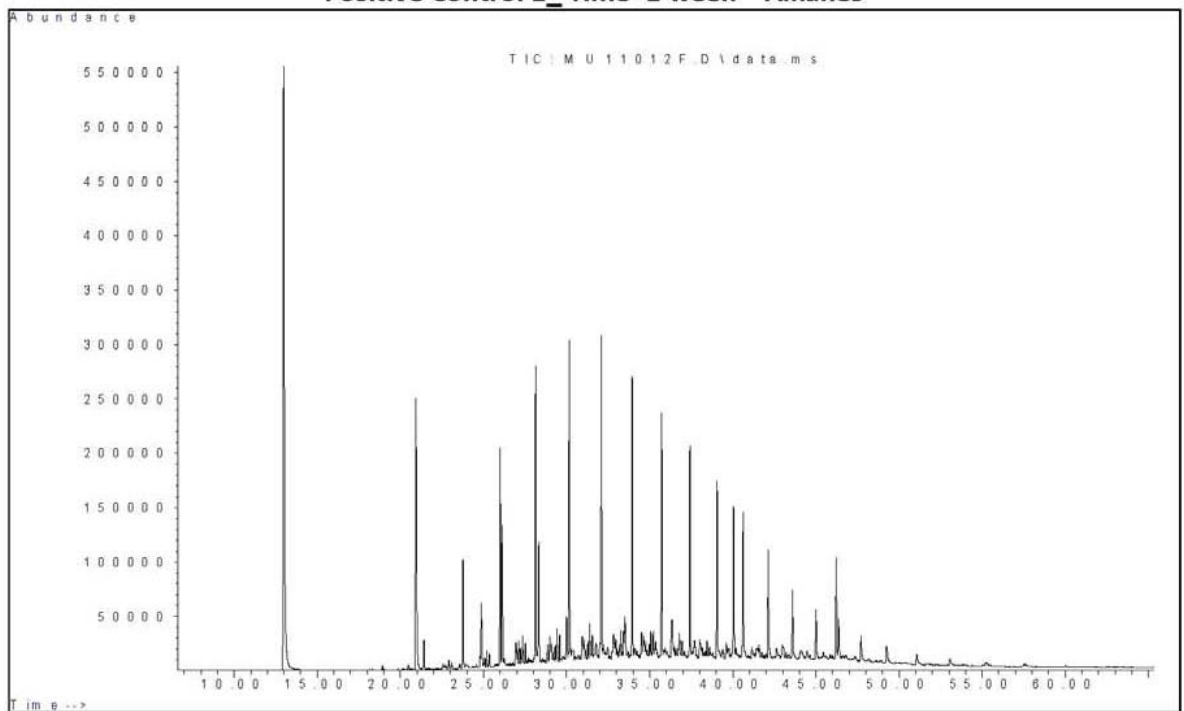
### Negative Control \_ Time=4 week – Alkanes



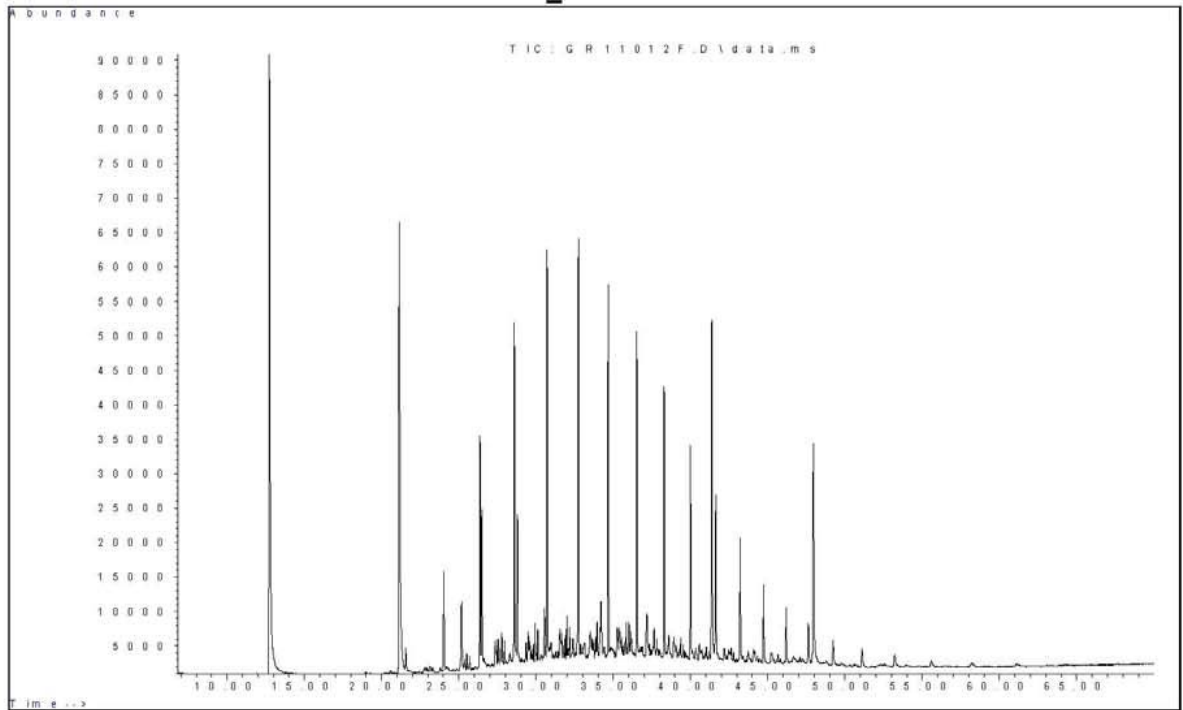
Positive Control 1\_Time=0 week – Alkanes



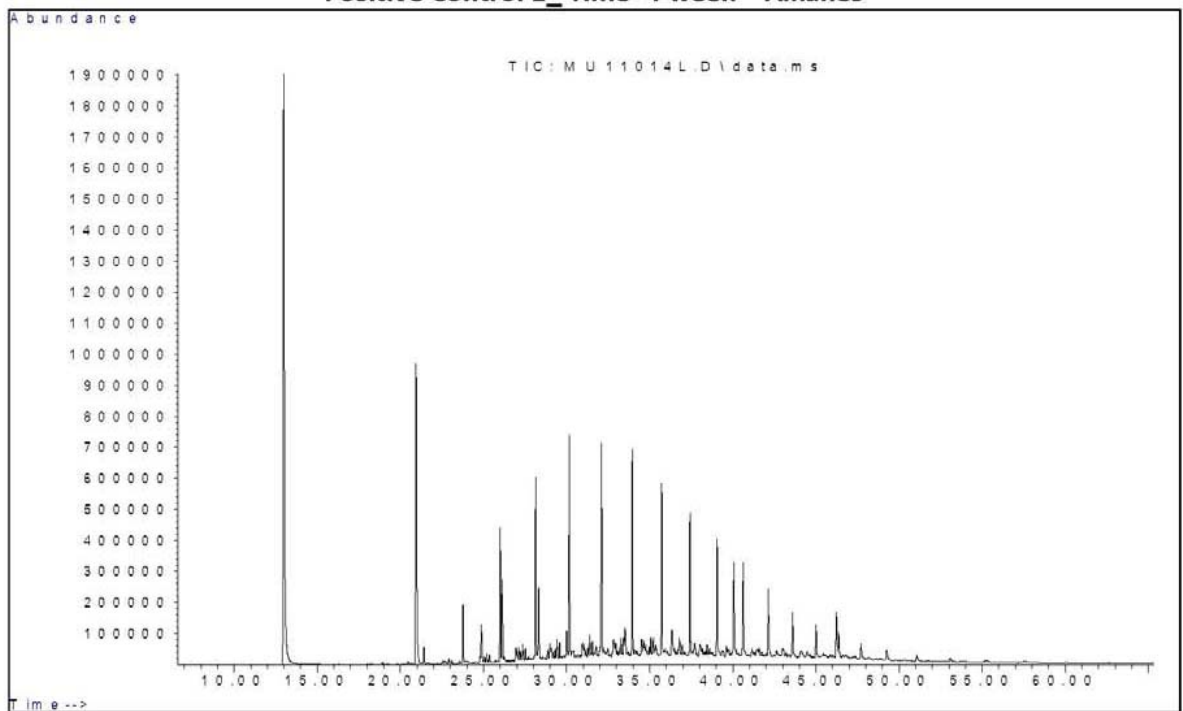
Positive Control 1\_Time=1 week – Alkanes



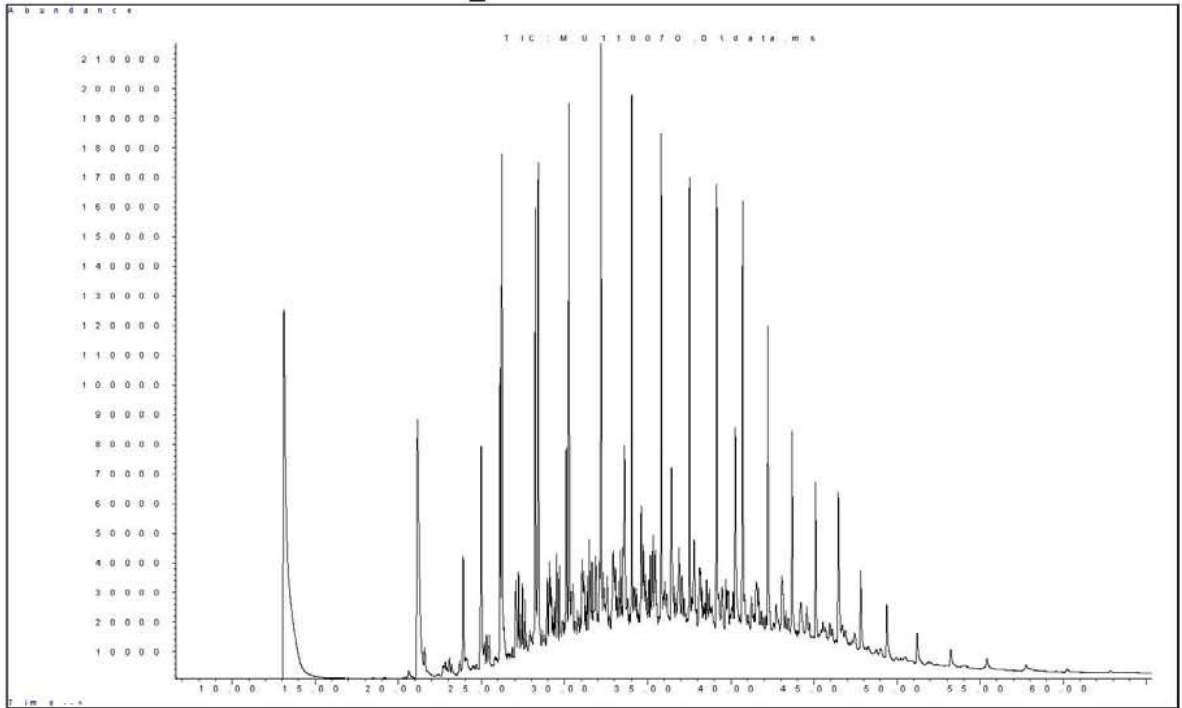
### Positive Control 1\_Time=2 week – Alkanes



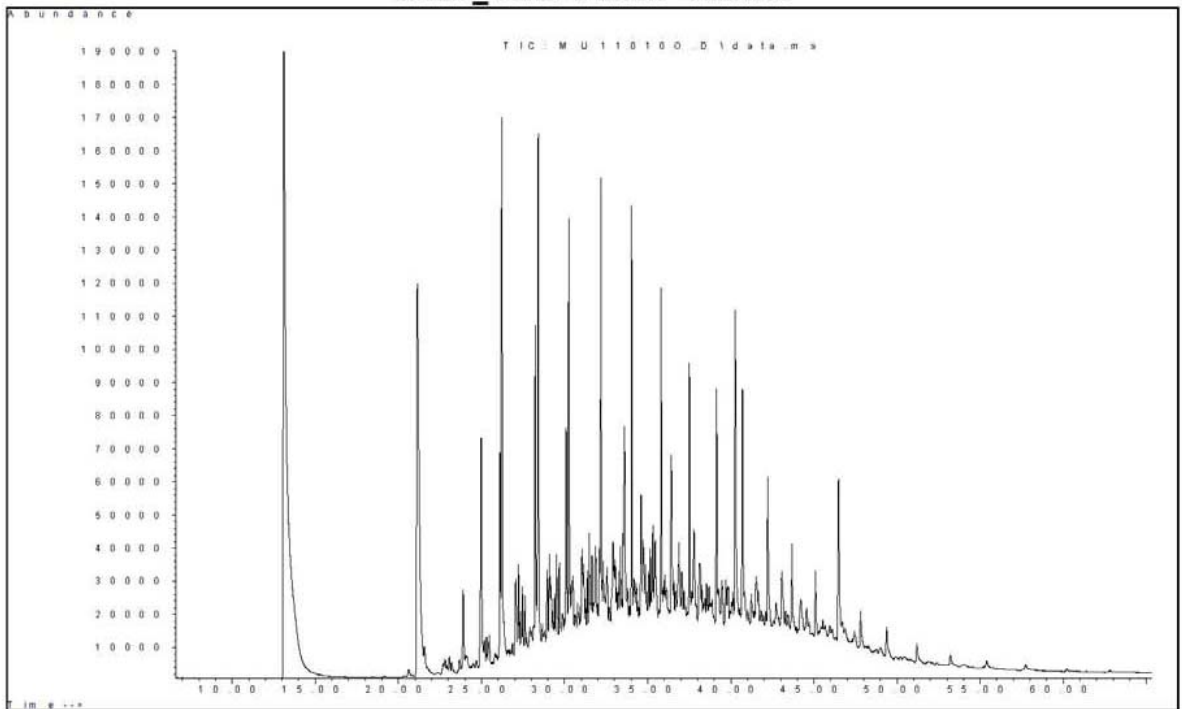
### Positive Control 1\_Time=4 week – Alkanes



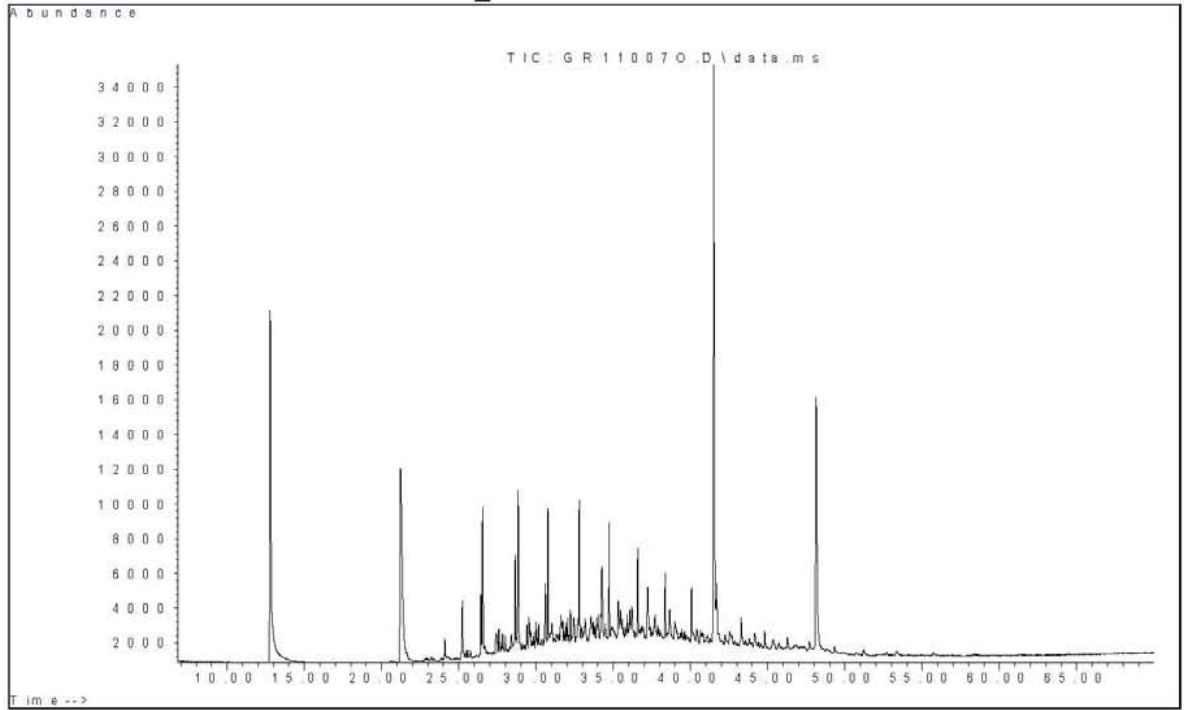
OSE II \_Time=0 week – Alkanes



OSE II \_Time=1 week – Alkanes



OSE II \_Time=2 week – Alkanes



OSE II \_Time=4 week – Alkanes

