Executive Summary

At the request of the Federal On-Scene-Coordinator (FOSC) U.S. EPA Region 5, a bench scale activity screening level oil biodegradation study was conducted on residual oil from the Enbridge Oil Spill, Kalamazoo River, MI. The primary objective of the study was to determine if residual oil in the Kalamazoo River, from the Enbridge Oil Spill, could undergo biodegradation beyond the weathering and in-situ degradation which has occurred since the spill event.

The study approach selected was to utilize both Kalamazoo River sediment and a soil known to contain organisms capable of degrading oil as the inoculum, applied to a nutrient broth to which recovered Enbridge Spill oil was added. The tests were run for a total of 28 days, with samples tested at Days 0, 14 and 28. Degradation was evaluated through the use of a combination of gravimetric evaluation, total petroleum hydrocarbon (TPH) analyses and GC/MS oil fingerprinting analyses. Additional interpretation of the biodegradation potential of the residual oil was made from the evaluation of GC/MS oil fingerprinting analyses on samples of oil recovered during the oil spill response, oil recovered from sediment samples from the Kalamazoo River which contained oil from the spill, and from literature available on the oil spill crude oil source.

The conclusions of the testing conducted include: 1) currently the TPH content of river sediments from the Kalamazoo River impacted by the oil spill can be dominated by compounds which have origins other that the Enbridge Oil Spill, both naturally generated compounds and contamination sources other than the Enbridge Oil Spill; 2) 35-40% of the mass of the oil which remains from the Enbridge Oil Spill is not quantifiable using GC or GC/MS techniques 3) it is estimated that approximately 25% of the TPH, which originated from the Enbridge Oil Spill, was degraded in the bench scale studies; 4) the majority of the biodegradation of Enbridge oil in the bench scale studies was degraded by day 14; 5) while there is evidence that biodegradation of residual Enbridge oil continued after day 14, the amount of oil degraded declined dramatically.

Overall it may be concluded that the residual oil within the Kalamazoo River from the Enbridge Oil Spill has the potential to undergo further degradation. However, the absolute amount of oil which may be removed via degradation is limited to roughly 25% of the current residual oil mass. Additional degradation may occur but would be expected to occur over an extended time period. Field conditions where the residual oil exists will impact the rate and extent of residual oil degradation. While nutrient levels may not be limiting to in-situ biodegradation, low oxygen conditions, which typically exist in subsurface sediments, will limit the rate of biodegradation. Lastly, the physical nature of the residual oil will affect the degradation of residual oil, it has been noted that the residual oil in Kalamazoo river sediments exists in discrete masses or "globules"; this physical behavior limits the surface area upon which oil biodegrading organisms can access the oil, which appears to limit the extent of Enbridge oil biodegradation within the river.

Introduction

On July 26, 2010 a rupture of Line 6B was reported within the drainage of the Kalamazoo River in Marshall MI. It was reported that overt 20,000 barrels of crude oil were released from line 6B during this event. The Crude oil released was reported to be a mixture of Cold Lake and Western Canadian Special crude oil and contained a condensate to facilitate transport in the pipeline. During the initial response, spilled oil was recovered from the water surface, however, after a little more than a week, it was observed that the oil sank to the bottom of the river. Efforts to recover this submerged oil has been conducted since that time.

Biodegradation of residual oil has been a demonstrated technology for oil removal. However, this technology has been limited to aerobic systems and typically this involves the use of land farming techniques or batch treatment cells. During the biodegradation process, rapid degradation of oil components is typically measured and observed on the straight chain hydrocarbons (n-alkanes) and "lighter end" compounds which dominate refined oil products. Typically, most crude oils contain a significant percentage of the easily degradable "light ends" and straight chain hydrocarbons. However, the chemical composition of the spilled crude oil mixture was atypical and contained large amounts of branched and "heavy" hydrocarbons as well as asphaltenes which are more resistant to biodegradation.

The first step in any biodegradation assessment is to determine if the material is fundamentally degradable at a rate or percentage which could make biodegradation a viable treatment technology. To this end the Region 5 FOSC tasked the Environmental Response Team (ERT) to determine if the residual oil from the Line 6B spill was biodegradable. The ERT through its support contract (SERAS – Lockheed Martin) conducted a screening level biodegradation study to determine the biodegradation potential of oil released from Line 6B under idealized conditions.

Materials and Methods

Residual Oils Obtained

The first task for this evaluation was to obtain residual oil released from line 6B. The desired material to be evaluated was oil that is representative of the residual oil that currently exists within the sediments of the Kalamazoo River. The residual oil within the Kalamazoo River has undergone mechanical weathering including the loss of the condensate and an undetermined degree of biodegradation.

Two 5 gal buckets of *oil contaminated* sediment were collected by Region 5 START from location 10.75 and shipped to ERT in Edison NJ. The sediments were collected from a river side channel which is depositional. At this location mechanical oil recovery had occurred, however, the location was "re-oiled" presumably from river transport and was anticipated to contain representative oil. The sediments from both buckets were extracted and analyzed for TPH and oil fingerprint analysis using GC/MS. The sediment extracts from the two buckets are labeled SERAS-017-0001 and SERAS-017-0002. The oil (TPH) concentrations found in the sediments were considered insufficient to conduct meaningful biodegradation assessments. The results of the TPH analysis are presented in ***Table 01***. In addition to the low TPH concentration, a significant portion of the TPH concentration was attributed to the normal "background" organics. The GC/MS TPH and fingerprint analysis conducted by the ERT laboratory showed that a greater percentage of the TPH was attributed to the normal background in the sediments from both buckets. ***Exhibit EX07*** shows the TPH fingerprints for the oil extracted from the two bucket samples, compared a sample of oil skimmed from the surface water inside one of the buckets (SERAS-017-0000) and a sample of spilled oil collected from the bank of the river (SERAS-017-0004).

In an effort to understand the composition of the residual oil within the sediments, the sediments within one of the buckets of sediment from 10.75 was disturbed and oil globules which migrated to the water surface were collected and analyzed by the oil fingerprinting techniques. This oil sample collected by ERT is referred to as SERAS-017-0000.

During the initial response, released oil was recovered and sent to a processing facility in Indiana; at that facility, a portion of the recovered oil had been stored. A request was submitted to obtain a sample of this oil recovered from the initial oil spill response. This recovered oil is suspected to be "fresh crude" that had not undergone significant mechanical weathering as evidenced by the observed low viscosity and other physical characteristics. A sample of this oil was sent to SERAS and is referred to as SERAS-017-0003.

After some discussion, it was determined that ERT could conduct a more effective biodegradation study using a sample of oil that exhibits a "degraded and weathered" state similar to the oil that currently remains in the river, rather than a "fresh crude" source. An alternative Line 6B residual oil sample was found and obtained during a bank excavation at MP 13.40 in a subsurface void. This residual oil was highly viscous and believed to be mechanically weathered within the Kalamazoo River. A sample of this material was requested and sent to ERT for potential use in the biodegradation assessment. This sample is referred to as SERAS-017-0004.

Two additional samples of oil from Line 6B were also sent to ERT. These samples were taken from the pipeline directly rather than released oil which was recovered. These samples are referred to as SERAS-017-0005 and SERAS-017-0006

Attachment 01 lists the sediment samples and oil samples supplied to ERT along with the identification numbers and observational information. All the samples were analyzed by GC/MS for oil fingerprinting and only the sediment samples (0001, 0002) were analyzed by GC/MS TPH analysis.

Initial Oil Testing

Both qualitative (oil fingerprinting) and quantitative (TPH concentration) information on residual oils is necessary in order to evaluate the biodegradation potential of an oil. The samples listed in **Appendix A, *Attachment 01*** were analyzed by GC/MS utilizing oil fingerprinting techniques (SERAS *draft* GC/MS Method 1803). TPH analyses were performed using SERAS draft GC/MS Method 1841. ***Attachment 02*** contains copies of the chain of custody records and work order requests for the samples listed in ***Attachment 01***.

The TPH analysis of the river sediment samples SERAS-017-0001 and SERAS-017-0002, which were collected from mile marker 10.75, are reported in ***Table 1.0.***

Quantification of oil within the sediment matrix from the Kalamazoo River is problematic in that there is a high degree of natural background hydrocarbons and organics that *are not related* to or linked to the Enbridge spill.

Biodegradation Studies

Selection of oil samples for biodegradation assessment
Based upon the analyses conducted, including the interpretation of the relationship between the oil samples 0001 through 0006, two samples were selected for use in the biodegradation assessments, 0003 and 0004. Sample 0003 represents the best example of the mixed crude during the release, and sample 0004 represents a moderately weathered version of the mixed crude that is most similar to the "skimmed oil", or oil globules that have been recently recovered from the bottom of the river.

Oil Sample Processing & Preparation for Biodegradation Study

Typically, oil samples are sterilized for use in biodegradation assessments; this is often accomplished through filtration. Given the highly viscous nature of oil 0004, filtration was deemed infeasible. It was therefore decided that oil sterilization would not be intentionally accomplished or maintained.

Recovered oil from spills normally will contain material which has been entrained within the oil as it moved through the environment, this includes water. Normal procedures for the removal of water and other material proved ineffective specifically for oil 0004. Sample 0004 was centrifuged at 3000RPM for 30 minutes to separate the oil from the entrained material including water, and the processed product then autoclaved at 125 degrees centigrade for 30 minutes to achieve sterilization. Fingerprinting analyses were conducted on the oil prior to, and after autoclaving ***(Exhibit EX08A)*** in order to determine if the autoclaving process qualitatively altered oil 0004. It was concluded that the autoclaving process did not substantively alter oil 0004*;* therefore oil 0004 was prepared for the biodegradation assessment by initially centrifuging the viscous oil to remove extraneous material from the oil, then autoclaving the cleaned up product. ***Exhibit EX08B*** shows the effect of autoclaving on the "fresh crude" of sample 0003 compared to sample 0004. The TPH fingerprint of this fresh crude oil is not typical and illustrates that most of the "mass" of the crude consists of the "unresolved complex mixture" of hydrocarbons that elute between 15 to 50 minutes. When the crude oil was baked, only a small fraction of "light ends" (compounds eluting between 5 to 15 minutes) was lost due to evaporation, and after baking, the fingerprint of what was left of the fresh "crude" sample 0003, was a very close match to the viscous sample 0004 collected from the river bank.

Biodegradation Assessment Process

Attachment 01 summarizes the biodegradation studies that were performed between 03/01/12 through 04/18/12 at the SERAS laboratory.

The following is a synopsis of the procedures followed:

Approximately 200 mg of oil was gravimetrically placed into 250 ml flasks to which 50 ml of Bushell Haas mineral salt medium amended with 0.05% Tween 80was added. Oil 0003 and 0004 samples were inoculated with prepared sediments from mile marker 10.75. A separate set of 0004 samples were inoculated with prepared soil from an ongoing biodegradation study being conducted simultaneously. This alternate inoculation was conducted as it was known to contain an active oil biodegradation culture, and there was not opportunity during the assessment process to determine the existence and viability of the oil degrading microorganism community which existed in the sediments from mile marker 10.75.

Exposures were run in duplicate with an additional MS/MSD sample. With the exception of time 0 samples, flasks were place on an orbital shaker and incubated at 30 degrees C for 14 and 28 days. At 14 days duplicate samples of each exposure were removed from the shaker, "killed" and extracted. Extracts were prepared for fingerprinting and quantitative analyses. Residual extracts were retained for potential future analyses. This process was repeated at day 28.

Tables 2.0, 3.0, and 4.0 contain the results of the GC/MS TPH analysis of the Day 0, Day 14 and Day 28 biodegradation studies. The TPH results are reported as "total mg" sample.

Due to questions regarding recovery and the subsequent degradation loss calculation; aliquots of select samples were evaporated to remove the sample solvent and the residual was measured gravimetrically. Those results are reported in ***Table 03a.*** The gravimetric results correlate with literature sources ***(Attachment 04)*** concerning the asphaltene content and how it affects the actual "measured" TPH concentration compared to the gravimetric TPH concentration.

Data Interpretation & Conclusions

Oil Fingerprint Analysis:

- Based upon the nearly all the observed fingerprints, sample 0003 and 0004 are from the same source. It was determined that sample 0004 is a mechanically weathered version of sample 0003. The mechanical weathering includes, but is not limited to loss of many of the light hydrocarbons due to simple evaporative weathering. The fingerprints EX03 and EX08a,b illustrate this point.
- The oil extracted from the sediments collected from location 10.75 exhibited fingerprints that matched the line 6B oil, but the majority of hydrocarbons that are typically calculated as TPH are actually from naturally occurring background organic material. The hopane fingerprints of EX01 and EX02 can be used as evidence that the line 6B oil is present in the sediment. There is a "partial match" of the sediment hopanes when compared to the spilled oil because of naturally occurring hopanes produced from organic plant debris that can not be separated out.
- It was determined that the "oil" extracted from the sediment consists of mostly hydrocarbons from decaying organic material, possibly oil from "other" unknown sources, and pyrogenic PAH's from an unknown source – probably creosote. It can be accurately estimated that more than half of the measured TPH is ***not*** attributed to the spilled oil.
- Oil sample 0003 matches the two line 6B samples of 0005 and 0006.
- Mechanical and or evaporative weathering of sample 0003 will produce sample 0004. This was also demonstrated at the SERAS lab when sample 0003 was baked at 125 degrees centigrade during a biodegradation study.
- Biological weathering (biodegradation) will produce the fingerprint of the "oil globules" collected from the river. The fingerprints shown in EX05 show sample 0003, 0004 and 0000 which illustrate the fresh crude, mechanically weathered crude, and mechanical & biodegraded crude oil in succession. There is no attempt to approximate the "time frame" that lead to the mechanical and biodegraded sample 0000 because there are many variables that have to be considered.

Fingerprint Conclusion: Samples 0003 and 0006 are exact matches of each other and exhibit very little weathering. Sample 0005 is a very close match to 0003 and 0006, and shows a slight variation in the C17/pristane and C18/phytane ratios when compared to 0003 and 0006. This change is due to the loss of some of the n-alkanes which are the first compounds to be affected by biodegradation. Sample 0004 has a similar C17/pristane and C18/phytane ratio that matches 0005 and has been determined to be a mechanically weathered version of sample 0005, and 0005 a slightly weathered version of samples 0003 & 0006. Sample 0000 retains just the pristane/phytane peaks and ratio that is observed in sample 0005. Several distinct biomarker compounds that are present samples 0000 and 0004 are observed in the TPH that was extracted from the sediment samples 0001 and 0002.

It was determined that the sediments from location 10.75 contained <200mg/Kg of TPH. Based upon the fingerprints, probably more than half of the TPH concentration is due to naturally occurring background organics that are *not* petroleum related and *not* from the spilled oil.

Biodegradation Study:

The TPH fingerprint of 0004 is the closest match to the TPH fingerprint of sample 0000, the oil globule. Oil obtained from sample 0004 was purified, processed, used to calibrate the GC/MS system to measure TPH concentration, and spiked into the biodegradation study media. The results of the biodegradation studies are reported as total mg oil extracted for the Day 0, 14 and 28 day biodegradation studies. The TPH results reported in ***Tables 2.0, 3.0 & 4.0*** were *not* corrected for the effects of the high asphaltenes

and other solvent extracted constituents that contribute to the TPH weight, and response factor for analytical calculations. Based on the literature and the gravimetric analysis performed at SERAS, approximately 35-40% of the TPH is not quantifiable using GC or GC/MS techniques. It is estimated that approximately 25% of the TPH was degraded by measuring the total mg of spiked oil against the results of the day 14 and 28 day studies.

The fingerprints ***Exhibit EX09*** show the TPH fingerprints of sample 0000 (globule) against the day 14 and day 28 TPH fingerprints of the biodegradation study samples. Within the sample 0000 TPH "UCM" hump, some isoprenoids (I), cycloalkanes (.), and some of the biomarker hopane peaks (T3, T4, h) are observed. The effects of the day 14 and day 28 biodegradation studies indicate that a significant amount of the more degradable ion 85 alkanes, ion 83 alkenes/napthenes, and ion 113 isoprenoids/alkanes have been removed . What remains are the highly resistant biomarker compounds such as bicyclic sesquiterpanes, sterane, hopanes, and multi-ring terpanes. The biodegradation experiment removed much of the degradable alkanes and alkenes from the TPH "UCM" and once removed, exposed the recalcitrant biomarker compounds. Although small, there wasn't a significant difference change in the TPH or observed fingerprints between day 14 and day 28. The identified biomarker compounds that remained after day 28 are considered to be the most resistant compounds to biodegradation, and it is beyond the scope of this report to comment on the degradability of what is left of the oil after the day 28 study.

Appendix A contains ***Attachment 01,*** which lists all the samples received and evaluated through ERT, and biodegradation work performed at SERAS. ***Attachment 02*** contains the chain of custodies, and work order documentation for all work received and performed. **Appendix B** contains ***Attachment 03,*** the oil fingerprint report followed by the TPH results tables. **Appendix C** contains ***Attachment 04,*** a document titled "**Chemical Fingerprints of Alberta Oil Sands and Related Petroleum Products**", which is used to support the oil fingerprint assessment. **Appendix D** contains the experimental design for the activity screening.

J. Sylo ver. 10/18/2012

APPENDIX A

Attachment 01

Attachment 01
Summary of Enbridge Oil Samples Recieved: 02/01/12 - 03/01/12

COC#	Sample ID	Date Received	Analysis Requested	Date Analyzed	Matrix / Sample Description
SERAS-017-02/01/12-0001	SERAS-017-0001	2/1/2012	TPH	2/2/2012	This was one of two 5 gallon buckets recieved that contained water and rever sediment. The bucket was 3/4 full and the aqueous/sediment layer was approximately equal. The dried sediment was extracted and analyzed on 2/2/12 for TPH.
	SERAS-017-0000	*This sample is referred to as the "skimmed" oil. I took a one liter beaker and grabbed a subsample of this bucket which resulted in ~300mL of sediment and bottome debris topped with ~500mL water. I skimmed a small quantity of the visible product that flated to the surface and placed it in 1.0mL of DCM/hexane and analyzed this for fingerprint analysis. I did not obtain a weight for the "skimmed" product. This sample is referred to as either the "skimmed" product or sample 00X in some of the chromatograms since it was not officially logged in on a COC.*				
SERAS-017-02/08/12-0002	SERAS-017-0002	2/1/2012	TPH	2/15/2012	The sediment of this sample was extracted on 2/14/12 and analyzed for TPH on 2/15/12. The fingerprints of this sample and the TPH were compared to SERAS-017-0001
SERAS-017-02/21/12-0003	SERAS-017-0003	2/21/2012	TPH* fingerprint	2/27/2012	Pure product: Called "product of recovered oil in tank". Mobile black liquid with characteristic odor of "crude" oil. Was not analyzed for TPH since it was product. Analyzed 2/27/12 for fingerprinting.
SERAS-017-02/23/12-0004	SERAS-017-0004**	2/23/2012	TPH* fingerprint	2/27/2012	The matrix indicates "pure product" but the sample was an emulsion of thick black product and fine silt. When the product was separated from the water/silt, it was a thick-black-viscous product. It was analyzed on 2/27 for fingerprinting, but not TPH since the TPH/silt was not the issue. Also referred to as "tar from excavation"
072223 Conestoga-Rovers & Associates	SERAS-017-0005 SERAS-017-0006	3/1/2012	fingerprint	3/1/2012	These two samples were recieved as duplicates in 40mL VOA vials on the Conestoga-Rovers chain of custody. The SERAS numbers were assigned to the sample numbers listed on the COC and information is provided below:
	SERAS-017-0005 SERAS-017-0006	is Sample No. WCS-6B-072223-092910-JPS-KA-002-20. two bottles recieved with custody seal # 701795. This is a mobile-black crude oil sample. is Sample No. CL-6B-072223-092710-JPS-KA-001-33. two bottles recieved with custody seal # 701688. This is a mobile-black crude oil sample. Both of these samples were analyzed on 3/1/12 for fingerprint analysis.			

* Chain of custody indicates TPH analysis. These were samples contained pure product and fingerprint analysis was performed.

** This oil from this sample was processed and used to perform the biodegradation studies.

Summary of Additional Analytical Work Performed: Biodegredatation Studies : 03/01/12 - 04/18/12

COC#	# of Samples	Date Received	Analysis Requested	Date Analyzed	Comments
On 03/07/2012 a portion of sample SERAS-017-0004 was processed to remove all silt, debris, water, and baked at 125 for 1 hour degrees to simulate autoclaving. Approxximately 6.5 grams was delivered to the SERAS microbiology lab to perform degradation studies. A GC/MS TPH calibration range was performed on 03/22/2012 using the processed oil, and TPH results calculated using the "site" oil.					
SERAS-017-03/15/12-0005	7	3/19/2012	TPH ***	3/23/2012	**Day 0 Samples** of biodegradation study. Results in **Table 02.**
SERAS-017-03/22/12-0006	6	3/22/2012	TPH ***	3/23/2012	**Day 14 Samples** of biodegradation study. Results in **Table 03.**
*After reviewing the Day 0 and Day14 TPH results, gravimetric analysis was perfomed on 2 select Day 0 and Day 14 samples to obtain information concerning the asphaltene content. **Table 3a** shows the TPH as calculated using the GC/MS system and TPH calculated by gravimetric analysis of the TPH extract for the two selected samples.*					
SERAS-017-04-06/12-007	7	4/12/2012	TPH ***	4/18/2012	**Day 28 Samples** of biodegradation study. Results in **Table 04.**

*** TPH analysis was performed using SERAS GC/MS TPH Method #1841 and additional fingerprint chromatograms were submitted to supply visual evidence of degradation.

Attachment 02

EPA/ERT
SERAS, Edison, NJ
EPA Contract Number: EP-W-09-031

CHAIN OF CUSTODY RECORD
Site #: SERAS-017
Contact Name: T. Ferrell Miller
Contact Phone: [redacted]

No: SERAS-017-02/01/12-0001

Lab: SERAS Laboratory
Lab Phone: 732-321-4212

WO# R202001

Lab #	Sample #	Location	Analyses	Matrix	Collected	Numb Cont	Container	Preservative	MS/MSD
01	SERAS-017-0001	275-44-21	TPH-DRO	River Sediment	1/31/2012	1	68-oz HDPE	None	Y

TFM
2/1/12

RECEIVED
FEB 2 2012

Special Instructions:

SAMPLES TRANSFERRED FROM CHAIN OF CUSTODY #

Items/Reason	Relinquished by	Date	Received by	Date	Time	Items/Reason	Relinquished By	Date	Received by	Date	Time
Analysis	T. F. Miller	2/1/12	[signature]	2/1/12	11:00	All Analysis	[signature]	2/2/12	[signature]	2/2/12	10:45

E

WORK ORDER

Printed: 6/1/2012 4:37:43PM

R202001

ERT/SERAS Laboratory

Bucket SED

Client: US EPA/ERT (Edison)
Project: Oil Spill Response Support

Project Manager: Vinod Kansal
Project Number: SERAS-017

Report To:
US EPA/ERT (Edison)
Harry Allen
2890 Woodbridge Avenue
Edison, NJ 08837
Phone: (732) 321-6747
Fax: (732) 321-6724

Invoice To:
US EPA/ERT (Edison)
Alan Humphrey
2890 Woodbridge Avenue
Edison, NJ 08837
Phone :(732) 321-6748
Fax: (732) 321-6724

TPH + Fing

Date Due:	02/22/12 17:00 (15 day TAT)		
Received By:	Lawrence Martin	Date Received:	02/01/12 12:35
Logged In By:	Lawrence Martin	Date Logged In:	02/01/12 12:35

Samples Received at: 23°C

Custody Seals	No	Received On Ice	No
Containers Intact	Yes		
COC/Labels Agree	Yes		
Preservation Confir	No		

Analysis	Due	TAT	Expires	Comments
R202001-01 SERAS-017-0001 [Soil] Sampled 01/31/12 00:00 Eastern				**275-44-21**
Fingerprint	02/01/12 16:00	8	02/07/12 00:00	

Reviewed By

Date

EPA/ERT
SERAS, Edison, NJ
EPA Contract Number: EP-W-09-031

CHAIN OF CUSTODY RECORD
Site #: SERAS-017
Contact Name: T. Ferrell Miller
Contact Phone: [redacted]

No: SERAS-017-02/08/12-0002

Lab: SERAS Laboratory
Lab Phone: 732-321-4212

WO# R202008

Lab #	Sample #	Location	Analyses	Matrix	Collected	Numb Cont	Container	Preservative	MS/MSD
01	SERAS-017-0002	275-45-34	TPH-DRO	River Sediment	2/8/2012	1	68-oz HDPE	None	Y

TFm 2/8/12

RECEIVED
FEB 14 2012

Special Instructions:	SAMPLES TRANSFERRED FROM CHAIN OF CUSTODY #

Items/Reason	Relinquished by	Date	Received by	Date	Time	Items/Reason	Relinquished By	Date	Received by	Date	Time
1/analysis	T.F. Miller	2/8/12	[signature]	2/8/12	15:00	All/Analysis	[signature]	2/13/12	[signature]	2/13/12	14:15

E

WORK ORDER

Printed: 6/1/2012 4:40:03PM

R202008

ERT/SERAS Laboratory

Bucket Sed

Client: US EPA/ERT (Edison)
Project: Oil Spill Response Support

Project Manager: Vinod Kansal
Project Number: SERAS-017

Report To:
US EPA/ERT (Edison)
Harry Allen
2890 Woodbridge Avenue
Edison, NJ 08837
Phone: (732) 321-6747
Fax: (732) 321-6724

Invoice To:
US EPA/ERT (Edison)
Alan Humphrey
2890 Woodbridge Avenue
Edison, NJ 08837
Phone :(732) 321-6748
Fax: (732) 321-6724

Fingerprint + TPH

Date Due:	02/29/12 17:00 (15 day TAT)		
Received By:	Lawrence Martin	Date Received:	02/08/12 15:43
Logged In By:	Lawrence Martin	Date Logged In:	02/08/12 15:43

Samples Received at: **23°C**

Custody Seals	No	Received On Ice	No
Containers Intact	Yes		
COC/Labels Agree	Yes		
Preservation Confir	No		

Analysis	Due	TAT	Expires	Comments
R202008-01 SERAS-017-0002 [Soil] Sampled 02/08/12 00:00 Eastern				**275-45-34**
Fingerprint	02/08/12 16:00	8	02/15/12 00:00	

Reviewed By

Date

EPA/ERT
SERAS, Edison, NJ
EPA Contract Number: EP-W-09-031

CHAIN OF CUSTODY RECORD
Site #: SERAS-017
Contact Name: T. Ferrell Miller
Contact Phone: [redacted]

No: SERAS-017-02/21/12-0003
Lab: SERAS Laboratory
Lab Phone: 732-321-4212

WO# R202016

Lab #	Sample #	Location	Analyses	Matrix	Collected	Numb Cont	Container	Preservative	MS/MSD
01	SERAS-017-0003	275-55-05	TPH-DRO	Pure Oil Source	2/21/2012	1	2 oz amber	4 C	Y

RECEIVED
FEB 22 2012

TFM
2/21/12

Special Instructions: TPH and fingerprint analysis are requested.

SAMPLES TRANSFERRED FROM CHAIN OF CUSTODY #

Items/Reason	Relinquished by	Date	Received by	Date	Time	Items/Reason	Relinquished By	Date	Received by	Date	Time
1/analysis	T. F. Miller	2/21/12	[signature]	2/21/12	14:30	All Analysis	[signature]	2/22/12	[signature]	2/22/12	0905

E

EPA/ERT
SERAS, Edison, NJ
EPA Contract Number: EP-W-09-031

CHAIN OF CUSTODY RECORD
Site #: SERAS-017
Contact Name: T. Ferrell Miller
Contact Phone: [redacted]

No: SERAS-017-02/23/12-0004

Lab: SERAS Laboratory
Lab Phone: 732-321-4212

WO# R202016

Lab #	Sample #	Location	Analyses	Matrix	Collected	Numb Cont	Container	Preservative	MS/MSD
02	SERAS-017-0004	275-56-24	TPH-DRO	Pure Oil Source	2/23/2012	1	2 oz amber	4 C	Y

T. F. Miller 2/23/12

RECEIVED
FEB 23 2012

Special Instructions: Please analyze the sample for TPH content and fingerprint analysis.

SAMPLES TRANSFERRED FROM CHAIN OF CUSTODY #

Received Room Temp Jay 2-123/12

Items/Reason	Relinquished by	Date	Received by	Date	Time	Items/Reason	Relinquished By	Date	Received by	Date	Time
1/analysis	T. F. Miller	2/23/12	[signature]	2/23/12	16:00	All/Analysis	[signature]	2/25/12	[signature]	2/23/12	16:06

E

WORK ORDER

Printed: 6/1/2012 4:37:41PM

R202016

ERT/SERAS Laboratory

Client: US EPA/ERT (Edison)
Project: Oil Spill Response Support

Project Manager: Vinod Kansal
Project Number: SERAS-017

Report To:
US EPA/ERT (Edison)
Harry Allen
2890 Woodbridge Avenue
Edison, NJ 08837
Phone: (732) 321-6747
Fax: (732) 321-6724

Invoice To:
US EPA/ERT (Edison)
Alan Humphrey
2890 Woodbridge Avenue
Edison, NJ 08837
Phone :(732) 321-6748
Fax: (732) 321-6724

Date Due: 03/13/12 17:00 (15 day TAT)

Received By: Lawrence Martin
Date Received: 02/21/12 16:07

Logged In By: Lawrence Martin
Date Logged In: 02/21/12 16:07

Samples Received at: 23°C
Custody Seals No Received On Ice No
Containers Intact Yes
COC/Labels Agree Yes
Preservation Confir No

Analysis	Due	TAT	Expires	Comments
R202016-01 SERAS-017-0003 [Oil] Sampled 02/21/12 00:00 Eastern				**275-55-05**
Oil Fingerprint	02/22/12 16:00	8	02/28/12 00:00	
R202016-02 SERAS-017-0004 [Oil] Sampled 02/23/12 00:00 Eastern				**275-56-24**
Oil Fingerprint	02/22/12 16:00	8	03/01/12 00:00	

Reviewed By

Date

CHAIN OF CUSTODY RECORD

CONESTOGA-ROVERS & ASSOCIATES
2055 Niagara Falls Blvd
Niagara Falls, NY 14304

SHIPPED TO (Laboratory Name):
Raymond Siegener, PhD
Alpha Analytical
320 Forbes Blvd
Mansfield, MA 02048

REFERENCE NUMBER:
Line 6B Sampling
072223

SAMPLER'S SIGNATURE: Christa Nunn PRINTED NAME: Christa Nunn

SEQ. No.	DATE	TIME	SAMPLE No.	SAMPLE TYPE	No. of Containers	PARAMETERS	REMARKS
3	2/22/12	1600	WCS-LB3-072223-092910-JPS-KA-002-20	CO	1	5	Bottle Custody Seal # 701795
4	1	1	CL-LB3-072223-092710-JPS-KA-001-33	1	1	6	7016898

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MAR 1 2012

ENBRIDGE Oil

5 = SERAS-017-0005
6 = SERAS-017-0206

*Sample Petroleum Crude Oil, 3, UN1267, PGII

TOTAL NUMBER OF CONTAINERS: 2

HEALTH/CHEMICAL HAZARDS:

RELINQUISHED BY: ① Christa Nunn	DATE: 2/22/12 TIME: 1615	RECEIVED BY: ① FedEx	DATE: TIME:
RELINQUISHED BY: ② FedEx	DATE: 2/24/12 TIME: 1050	RECEIVED BY: ②	DATE: 2/24/12 TIME: 1050
RELINQUISHED BY: ③	DATE: 2/27/12 TIME: 1135	RECEIVED BY: ③ UPS	DATE: TIME:

METHOD OF SHIPMENT: FedEx Ground WAY BILL No. 391687133007 6591

White —Fully Executed Copy
Yellow —Receiving Laboratory Copy
Pink —Shipper Copy
Goldenrod —Sampler Copy

SAMPLE TEAM: Christa Nunn

RECEIVED FOR LABORATORY BY:
DATE: 3/01/2012 TIME: 15:50

Nº CRA 25320

1001 (D) APR 28/97(NF) REV. 0 (F-15)

WORK ORDER

Printed: 6/1/2012 4:37:40PM

R203003

ERT/SERAS Laboratory

Client: US EPA/ERT (Edison)
Project: Enbridge Oil Spill

Project Manager: Vinod Kansal
Project Number: SERAS-017

Report To:
US EPA/ERT (Edison)
Alan Humphrey
2890 Woodbridge Avenue
Edison, NJ 08837
Phone: (732) 321-6748
Fax: (732) 321-6724

Invoice To:
US EPA/ERT (Edison)
Alan Humphrey
2890 Woodbridge Avenue
Edison, NJ 08837
Phone :(732) 321-6748
Fax: (732) 321-6724

Date Due: 03/22/12 17:00 (15 day TAT)

Received By: Lawrence Martin
Logged In By: Lawrence Martin

Date Received: 03/01/12 14:21
Date Logged In: 03/06/12 14:21

Samples Received at: 23°C
Custody Seals No
Containers Intact No
COC/Labels Agree No
Preservation Confir No
Received On Ice No

Analysis	Due	TAT	Expires	Comments
R203003-01 Does Not Exist [Other] Sampled 03/05/12 00:00 Eastern				
R203003-02 Does Not Exist [Other] Sampled 03/05/12 00:00 Eastern				
R203003-03 SERAS-017-0005 [Other] Sampled 02/22/12 00:00 Eastern				**WCS-6B-072223-092910-JPS-KA-002-20**
Oil Fingerprint	03/01/12 16:00	8	02/29/12 00:00	
R203003-04 SERAS-017-0006 [Other] Sampled 02/22/12 00:00 Eastern				**CL-6B-072223-092710-JPS-KA-001-33**
Oil Fingerprint	03/01/12 16:00	8	02/29/12 00:00	

Reviewed By

Date

E1/0

EPA/ERT
SERAS, Edison, NJ
EPA Contract Number: EP-W-09-031

CHAIN OF CUSTODY RECORD
Site #: SERAS-017
Contact Name: T. Ferrell Miller
Contact Phone: [redacted]

No: SERAS-017-03/15/12-0005
Lab: SERAS Laboratory
Lab Phone: 732-321-4212

WO# R203011

Lab #	Sample #	Location	Analyses	Matrix	Collected	Numb Cont	Container	Preservative	MS/MSD
01	SERAS-017-0007	275-78-10	TPH-DRO	Nutrient Medium	3/6/2012	1	4-oz. amber	formaldehyde	N
02	SERAS-017-0008	275-78-11	TPH-DRO	Nutrient Medium	3/6/2012	1	4-oz. amber	formaldehyde	N
03	SERAS-017-0009	275-78-12	TPH-DRO	Nutrient Medium	3/8/2012	1	4-oz. amber	formaldehyde	N
04	SERAS-017-0010	275-78-13	TPH-DRO	Nutrient Medium	3/8/2012	1	4-oz. amber	formaldehyde	N
05	SERAS-017-0011	275-78-14	TPH-DRO	Nutrient Medium	3/8/2012	1	4-oz. amber	formaldehyde	N
06	SERAS-017-0012	275-78-15	TPH-DRO	Nutrient Medium	3/8/2012	1	4-oz. amber	formaldehyde	N
07	SERAS-017-0013	275-78-16	TPH-DRO	Nutrient Medium	3/14/2012	3	4-oz. amber	formaldehyde	Y

T7M
3/15/12

Special Instructions: Each sample contains 65 mL (nutrient medium),10 mL methylene chloride, 6.5 mL 37% v/v formaldehyde and 200 mg TPH. Extract the entire sample. Please use sample SERAS-017-0013 for matrix, matrix spike and matrix spike duplicate analyses.

SAMPLES TRANSFERRED FROM
CHAIN OF CUSTODY #

Items/Reason	Relinquished by	Date	Received by	Date	Time	Items/Reason	Relinquished By	Date	Received by	Date	Time
9/analyses	T. F. Miller	3/15/12	[signature]	3/16/12	8:00	All Analysis	[signature]	3/19/12	[signature]	3/19/12	11:20

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MAR 21 2012

WORK ORDER

Printed: 6/1/2012 4:37:40PM

R203011

ERT/SERAS Laboratory

Client: US EPA/ERT (Edison)
Project: Bioremediation Support for Oil

Project Manager: Vinod Kansal
Project Number: 0-017

Report To:
US EPA/ERT (Edison)
Harry Allen
2890 Woodbridge Avenue
Edison, NJ 08837
Phone: (732) 321-6747
Fax: (732) 321-6724

Invoice To:
US EPA/ERT (Edison)
Harry Allen
2890 Woodbridge Avenue
Edison, NJ 08837
Phone :(732) 321-6747
Fax: (732) 321-6724

Date Due: 04/06/12 17:00 (15 day TAT)

Received By: Lawrence Martin
Logged In By: Lawrence Martin

Date Received: 03/16/12 09:49
Date Logged In: 03/19/12 09:49

Samples Received at: 23°C
Custody Seals No
Received On Ice No
Containers Intact Yes
COC/Labels Agree Yes
Preservation Confir No

Analysis	Due	TAT	Expires	Comments
R203011-01 SERAS-017-0007 [Water] Sampled 03/06/12 00:00 Eastern				**275-78-10**
Oil Fingerprint	03/16/12 16:00	8	03/13/12 00:00	
R203011-02 SERAS-017-0008 [Water] Sampled 03/06/12 00:00 Eastern				**275-78-11**
Oil Fingerprint	03/16/12 16:00	8	03/13/12 00:00	
R203011-03 SERAS-017-0009 [Water] Sampled 03/08/12 00:00 Eastern				**275-78-12**
Oil Fingerprint	03/16/12 16:00	8	03/15/12 00:00	
R203011-04 SERAS-017-0010 [Water] Sampled 03/08/12 00:00 Eastern				**275-78-13**
Oil Fingerprint	03/16/12 16:00	8	03/15/12 00:00	
R203011-05 SERAS-017-0011 [Water] Sampled 03/08/12 00:00 Eastern				**275-78-14**
Oil Fingerprint	03/16/12 16:00	8	03/15/12 00:00	
R203011-06 SERAS-017-0012 [Water] Sampled 03/08/12 00:00 Eastern				**275-78-15**
Oil Fingerprint	03/16/12 16:00	8	03/15/12 00:00	
R203011-07 SERAS-017-0013 [Water] Sampled 03/08/12 00:00 Eastern				**275-78-16**
Oil Fingerprint	03/16/12 16:00	8	03/15/12 00:00	

Reviewed By

Date

END

EPA/ERT
SERAS, Edison, NJ
EPA Contract Number: EP-W-09-031

CHAIN OF CUSTODY RECORD
Site #: SERAS-017
Contact Name: T. Ferrell Miller
Contact Phone: [redacted]

No: SERAS-017-03/22/12-0006
Lab: SERAS Laboratory
Lab Phone: 732-321-4212

WO# R203015

Lab #	Sample #	Location	Analyses	Matrix	Collected	Numb Cont	Container	Preservative	MS/MSD
01	SERAS-017-0014	275-82-07	TPH-DRO	Nutrient Medium	3/20/2012	1	4-oz. amber	formaldehyde	N
02	SERAS-017-0015	275-82-08	TPH-DRO	Nutrient Medium	3/20/2012	1	4-oz. amber	formaldehyde	N
03	SERAS-017-0016	275-82-09	TPH-DRO	Nutrient Medium	3/22/2012	1	4-oz. amber	formaldehyde	N
04	SERAS-017-0017	275-82-10	TPH-DRO	Nutrient Medium	3/22/2012	1	4-oz. amber	formaldehyde	N
05	SERAS-017-0018	275-82-11	TPH-DRO	Nutrient Medium	3/22/2012	1	4-oz. amber	formaldehyde	N
06	SERAS-017-0019	275-82-12	TPH-DRO	Nutrient Medium	3/22/2012	1	4-oz. amber	formaldehyde	N

TFM
3/22/12

Special Instructions: Each sample consists of 65 mL water (nutrient medium), 10 mL methylene chloride, 6.5 mL 37% v/v formaldehyde and 200 mg TPH. Samples SERAS-017-0018 and SERAS-017-0019 contain 17.5 mL methylene chloride rather than 10 ml.

SAMPLES TRANSFERRED FROM
CHAIN OF CUSTODY #

Items/Reason	Relinquished by	Date	Received by	Date	Time	Items/Reason	Relinquished By	Date	Received by	Date	Time
6/analysis	T.F. Miller	3/22/12	[signature]	3/22/12	15:00	All/Analysis	[signature]	3/22/12	[signature]	3/22/12	15:30

MAR 22 2012

WORK ORDER

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R203015

ERT/SERAS Laboratory

Client: US EPA/ERT (Edison)
Project: Bioremediation Support for Oil

Project Manager: Vinod Kansal
Project Number: 0-017

Report To:
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Harry Allen
2890 Woodbridge Avenue
Edison, NJ 08837
Phone: (732) 321-6747
Fax: (732) 321-6724

Invoice To:
US EPA/ERT (Edison)
Harry Allen
2890 Woodbridge Avenue
Edison, NJ 08837
Phone :(732) 321-6747
Fax: (732) 321-6724

Date Due: 04/12/12 17:00 (15 day TAT)

Received By: Lawrence Martin
Logged In By: Lawrence Martin

Date Received: 03/22/12 15:28
Date Logged In: 03/22/12 15:28

Samples Received at: 23°C
Custody Seals No
Containers Intact Yes
COC/Labels Agree Yes
Preservation Confir No
Received On Ice No

Analysis	Due	TAT	Expires	Comments
R203015-01 SERAS-017-0014 [Water] Sampled 03/20/12 00:00 Eastern				**275-82-07**
Oil Fingerprint	03/22/12 16:00	8	03/27/12 00:00	
R203015-02 SERAS-017-0015 [Water] Sampled 03/20/12 00:00 Eastern				**275-82-08**
Oil Fingerprint	03/22/12 16:00	8	03/27/12 00:00	
R203015-03 SERAS-017-0016 [Water] Sampled 03/22/12 00:00 Eastern				**275-82-09**
Oil Fingerprint	03/22/12 16:00	8	03/29/12 00:00	
R203015-04 SERAS-017-0017 [Water] Sampled 03/22/12 00:00 Eastern				**275-82-10**
Oil Fingerprint	03/22/12 16:00	8	03/29/12 00:00	
R203015-05 SERAS-017-0018 [Water] Sampled 03/22/12 00:00 Eastern				**275-82-11**
Oil Fingerprint	03/22/12 16:00	8	03/29/12 00:00	
R203015-06 SERAS-017-0019 [Water] Sampled 03/22/12 00:00 Eastern				**275-82-12**
Oil Fingerprint	03/22/12 16:00	8	03/29/12 00:00	

Reviewed By

Date

EPA/ERT
SERAS, Edison, NJ
EPA Contract Number: EP-W-09-031

CHAIN OF CUSTODY RECORD
Site #: SERAS-017
Contact Name: T. Ferrell Miller
Contact Phone: [redacted]

No: SERAS-017-04/06/12-0007
Lab: SERAS Laboratory
Lab Phone: 732-321-4212

WO# R204002

Lab #	Sample #	Location	Analyses	Matrix	Collected	Numb Cont	Container	Preservative	MS/MSD
01	SERAS-017-0020	275-89-06	TPH-DRO	Nutrient Medium	4/3/2012	1	4-oz. amber	formaldehyde	N
02	SERAS-017-0021	275-89-07	TPH-DRO	Nutrient Medium	4/3/2012	1	4-oz. amber	formaldehyde	N
03	SERAS-017-0022	275-89-08	TPH-DRO	Nutrient Medium	4/5/2012	1	4-oz. amber	formaldehyde	N
04	SERAS-017-0023	275-89-09	TPH-DRO	Nutrient Medium	4/5/2012	1	4-oz. amber	formaldehyde	N
05	SERAS-017-0024	275-89-10	TPH-DRO	Nutrient Medium	4/5/2012	1	4-oz. amber	formaldehyde	N
06	SERAS-017-0025	275-89-11	TPH-DRO	Nutrient Medium	4/5/2012	1	4-oz. amber	formaldehyde	N

TJM
4/6/12

Special Instructions: Each sample contains 65 mL water (nutrient medium), 10 mL methylene chloride, 6.5 mL 37% v/v formaldehyde and 200 mg TPH. SERAS-017-0025 contains 15 mL methylene chloride instead of 10 mL.

SAMPLES TRANSFERRED FROM
CHAIN OF CUSTODY #

Items/Reason	Relinquished by	Date	Received by	Date	Time
6/analysis	T. F. Miller	4/6/12	[signature]	4/6/12	12:00

Items/Reason	Relinquished By	Date	Received by	Date	Time

RECEIVED
APR 18 2012

[signature]

WORK ORDER

Printed: 6/1/2012 4:37:38PM

R204002

ERT/SERAS Laboratory

Client: US EPA/ERT (Edison)
Project: Bioremediation Support for Oil

Project Manager: Vinod Kansal
Project Number: 0-017

Report To:
US EPA/ERT (Edison)
Harry Allen
2890 Woodbridge Avenue
Edison, NJ 08837
Phone: (732) 321-6747
Fax: (732) 321-6724

Invoice To:
US EPA/ERT (Edison)
Harry Allen
2890 Woodbridge Avenue
Edison, NJ 08837
Phone :(732) 321-6747
Fax: (732) 321-6724

Date Due: 04/27/12 17:00 (15 day TAT)

Received By: Lawrence Martin
Logged In By: Lawrence Martin

Date Received: 04/06/12 12:22
Date Logged In: 04/06/12 12:22

Samples Received at: 23°C
Custody Seals No
Containers Intact Yes
COC/Labels Agree Yes
Preservation Confir No
Received On Ice No

Analysis	Due	TAT	Expires	Comments
R204002-01 SERAS-017-0020 [Water] Sampled 04/03/12 00:00 Eastern				**275-89-06**
Oil Fingerprint	04/06/12 16:00	8	04/10/12 00:00	
R204002-02 SERAS-017-0021 [Water] Sampled 04/03/12 00:00 Eastern				**275-89-07**
Oil Fingerprint	04/06/12 16:00	8	04/10/12 00:00	
R204002-03 SERAS-017-0022 [Water] Sampled 04/05/12 00:00 Eastern				**275-89-08**
Oil Fingerprint	04/06/12 16:00	8	04/12/12 00:00	
R204002-04 SERAS-017-0023 [Water] Sampled 04/05/12 00:00 Eastern				**275-89-09**
Oil Fingerprint	04/06/12 16:00	8	04/12/12 00:00	
R204002-05 SERAS-017-0024 [Water] Sampled 04/05/12 00:00 Eastern				**275-89-10**
Oil Fingerprint	04/06/12 16:00	8	04/12/12 00:00	
R204002-06 SERAS-017-0025 [Water] Sampled 04/05/12 00:00 Eastern				**275-89-11**
Oil Fingerprint	04/06/12 16:00	8	04/12/12 00:00	

Reviewed By

Date

APPENDIX B

Attachment 03

Attachment 03

Results of the GC/MS Oil Fingerprint Analysis on Enbridge Oil Sediments, Product and Biodegradation Studies

The samples and fingerprints were evaluated by direct visual comparison of selected fingerprints and backed with information provided in the following document: **Chemical Fingerprints of Alberta Oil Sands and Related Petroleum Products**, which will be referred to as CFA.

Based on the evaluation of the fingerprints the following conclusions can be made from examining the fingerprints:

- **Exhibit EX01** shows the hopane fingerprints for samples 000, 003, 004, 005 and 006. Using the ion 191 hopane fingerprints, all the product samples originate from a common source.
- **Exhibit EX02** shows the hopane fingerprint for sample 000 compared to the fingerprints from sediment samples 001 and 002. This confirms the presence of 000 in the extracted sediment. The anomalous peaks in the two sediment fingerprints are due to naturally occurring hopanes in the sediment.
- **Exhibit EX03** shows the total TPH fingerprint for the non-sediment samples. Samples 003, and 006 are exact matches to each other, while 005 shows a slight difference in the C17/pristane and C18/phytane ratios. Sample 004 is a mechanically/evaporative weathered version of 003 to 006, and sample 000 is a match of 004 that has undergone biodegradation.
- **Exhibits EX04:** Shows the total methyl- and dimethyl-chrysene fingerprints for sample 004 and sediment sample 001. The sediments were contaminated with a second source of PAH's that masked *all* of the PAH and alkyl-PAH fingerprints from the spilled oil and could not be used.
- **Exhibit EX05** shows the total TPH of sample 003, 004 and 000 which illustrate the fresh unweathered crude, mechanically weathered crude and the mechanically and biodegraded form of the crude oil.
- Sediment samples 001 and 002 contain a mixture of sulfur and TPH that is identified as naturally occurring background TPH. The hopane fingerprint of **EX02** supplies the only conclusive evidence that oil from samples 0000, or any of the crude oil is present.

I was asked to supply evidence of the "degradability" of the Enbridge Oil sample 0000. Based on my experience with degradation studies of similar oils I've provided fingerprints that show day 0 and day 84 of an oil mixture that is similar in composition to sample 0000. **Exhibits EX06A** show how a similar oil was degraded in an 84 day biodegradation study. **Exhibit EX06B** shows sample 0000. The shape and overall appearance of the UCM changed from day 0 to day 84 and we would expect the same results and degradation effects on the chromatograms of **EX06B.**

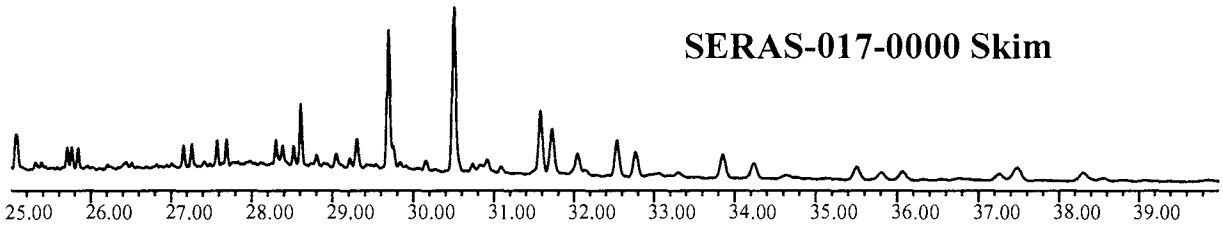
- **Exhibit EX07** show the TPH fingerprints for the two sediment extracts and source oil samples 017-0000 and 017-0004. It is difficult to determine whether either of the source oils are present using just the TPH fingerprint and that was determined by examining trace biomarkers fingerprints within the source oils and finding those fingerprints within the oil extracted from the river sediments. This set of fingerprints was used to illustrate that the TPH fingerprint of the oil extracted from the sediments indicate that this "oil" is composed mostly of naturally occurring background hydrocarbons derived from plants and other organic material.
- **Exhibits EX08a & EX08b** show the fingerprints of sample 0004 before and after processing, and that baking (autoclaving) samples 0003 and 0004 had little effect on the actual TPH fingerprint.
- **Exhibit EX09** shows the TPH fingerprint of sample 0000 and the effects of the 14 and 28 day biodegradation studies performed on the processed oil from sample 0004 , The biodegradation effectively "cleaned up" the oil to expose and highlight just the highly resistant biomarker compounds.

Fingerprint Conclusion: Samples 0003 and 0006 are exact matches of each other and exhibit very little weathering. Sample 0005 is a very close match to 0003 and 0006, and shows a slight variation in the C17/pristane and C18/phytane ratios when compared to 0003 and 0006. This change is due to the loss of some of the n-alkanes which are the first compounds to be affected by biodegradation. Sample 0004 has a similar C17/pristane and C18/phytane ratio that matches 0005 and has been determined to be a mechanically weathered version of sample 0005, and 0005 a slightly weathered version of samples 0003 & 0006. Sample 0000 retains just the pristane/phytane peaks and ratio that is observed in sample 0005. Several distinct biomarker compounds that are present samples 0000 and 0004 are observed in the TPH that was extracted from the sediment samples 0001 and 0002.

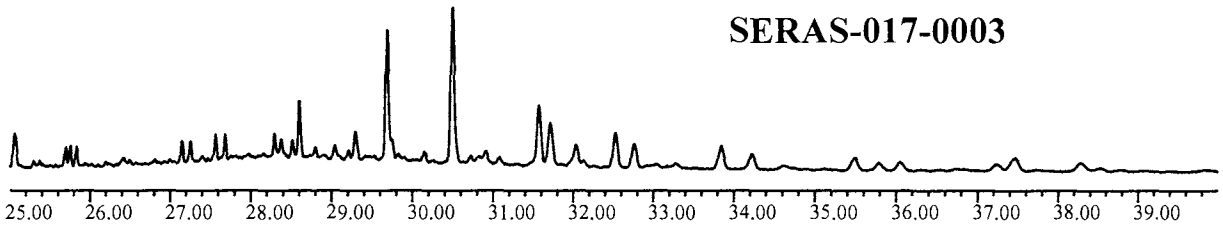
Fingerprint Exhibits

EX01: Ion 191 Hopane Fingerprints

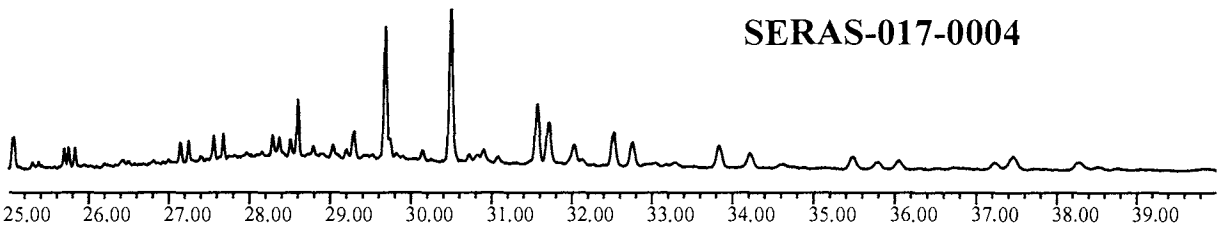
Ion 191.00 (190.70 to 191.70): SL02878.D\data.ms (*)



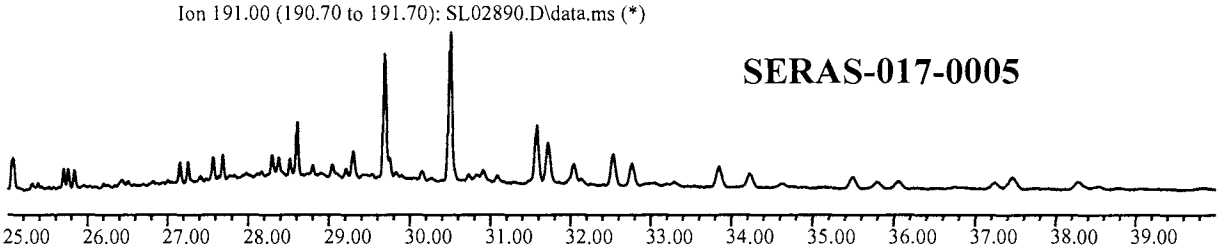
Ion 191.00 (190.70 to 191.70): SL02875.D\data.ms (*)



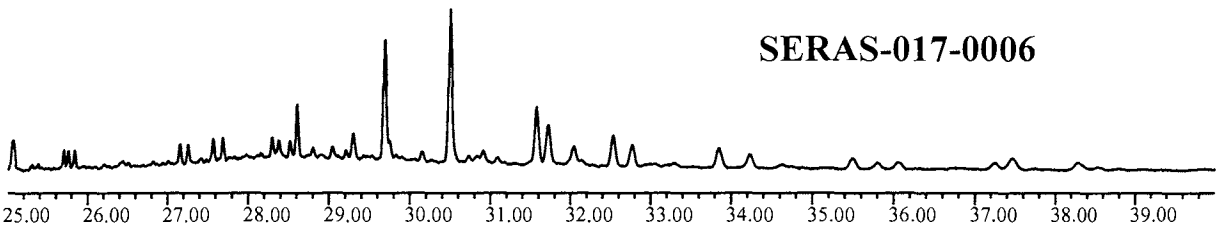
Ion 191.00 (190.70 to 191.70): SL02877.D\data.ms (*)



Ion 191.00 (190.70 to 191.70): SL02890.D\data.ms (*)



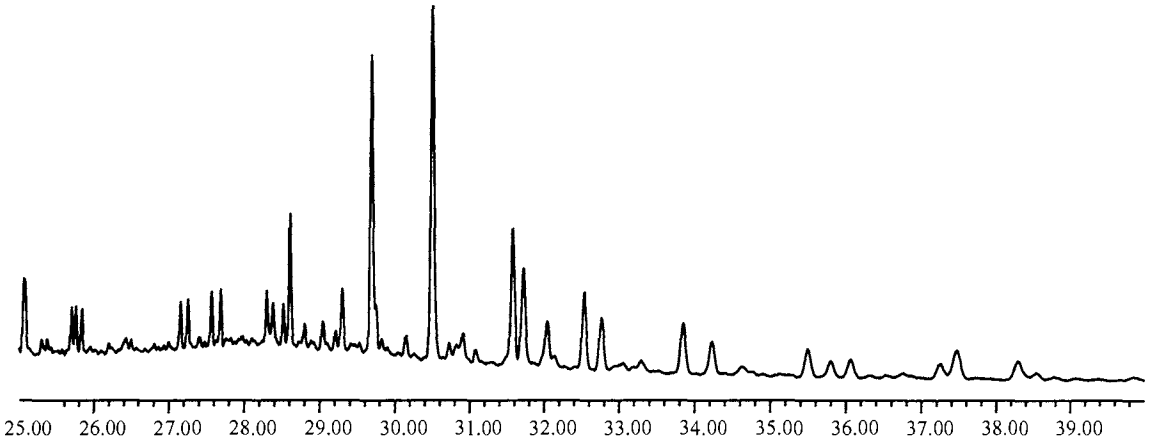
Ion 191.00 (190.70 to 191.70): SL02892.D\data.ms (*)



EX02: Ion 191 Hopane Fingerprints

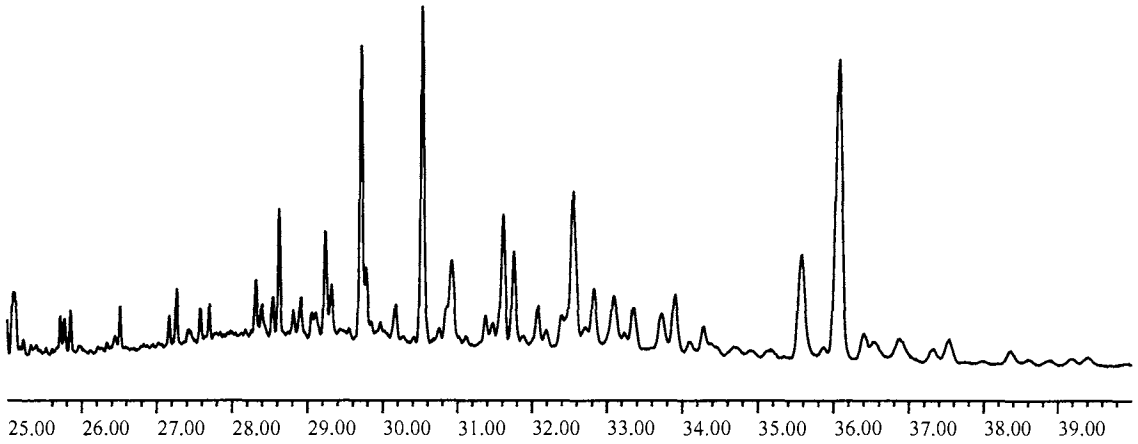
SERAS-017-0000 Skim

Ion 191.00 (190.70 to 191.70): SL02878.D\data.ms (*)



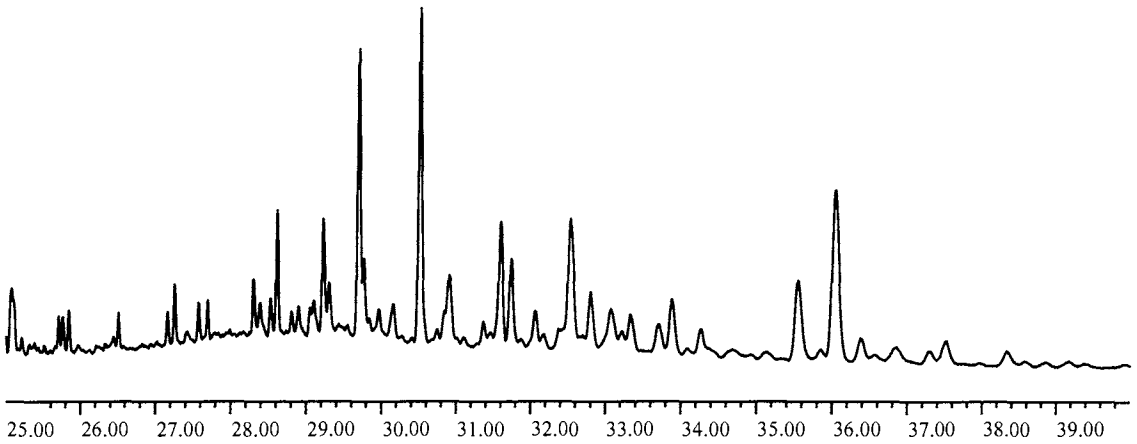
SERAS-017-0001 Sediment

Ion 191.00 (190.70 to 191.70): SL02674.D\data.ms (*)

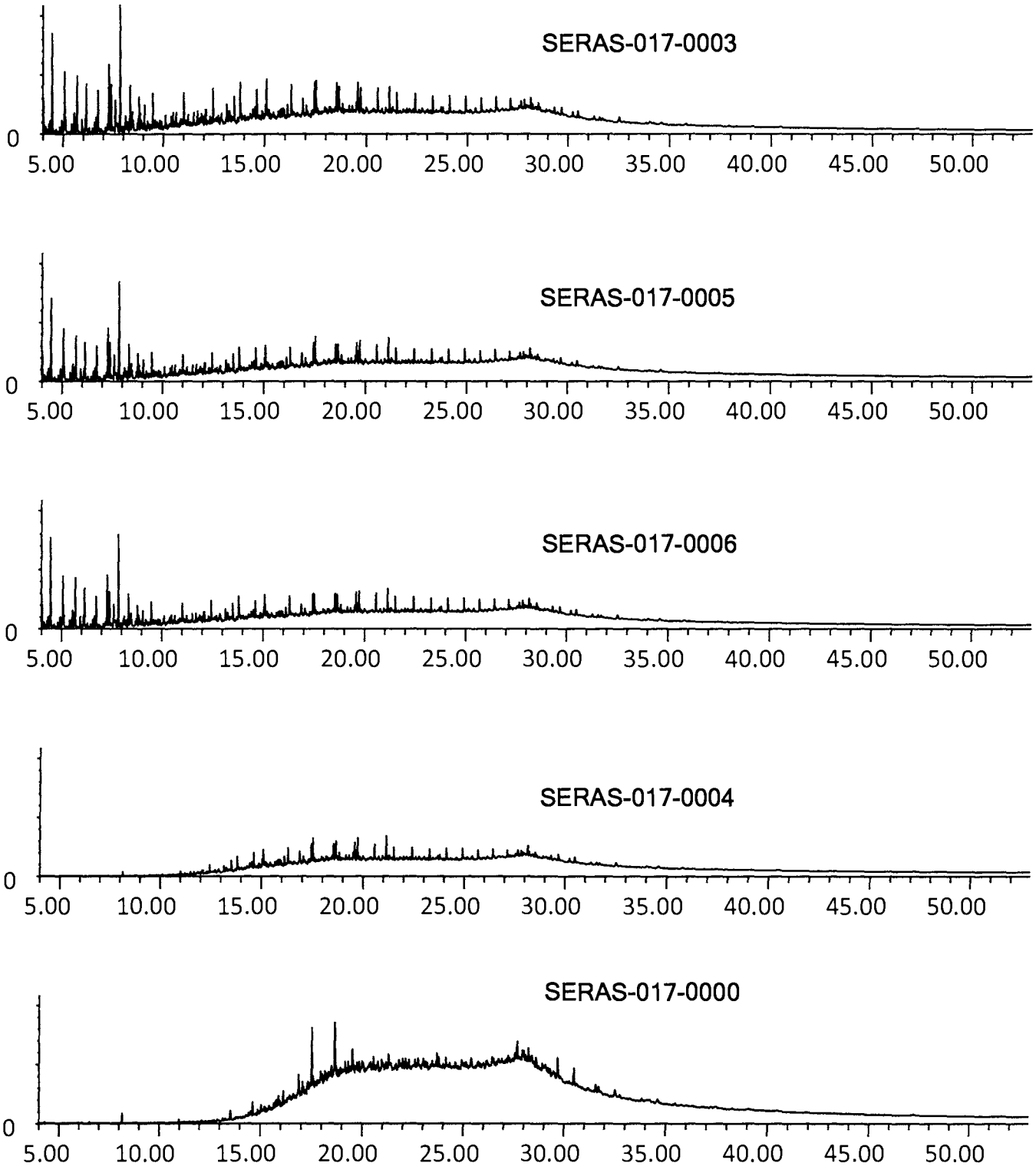


SERAS-017-0002 Sediment

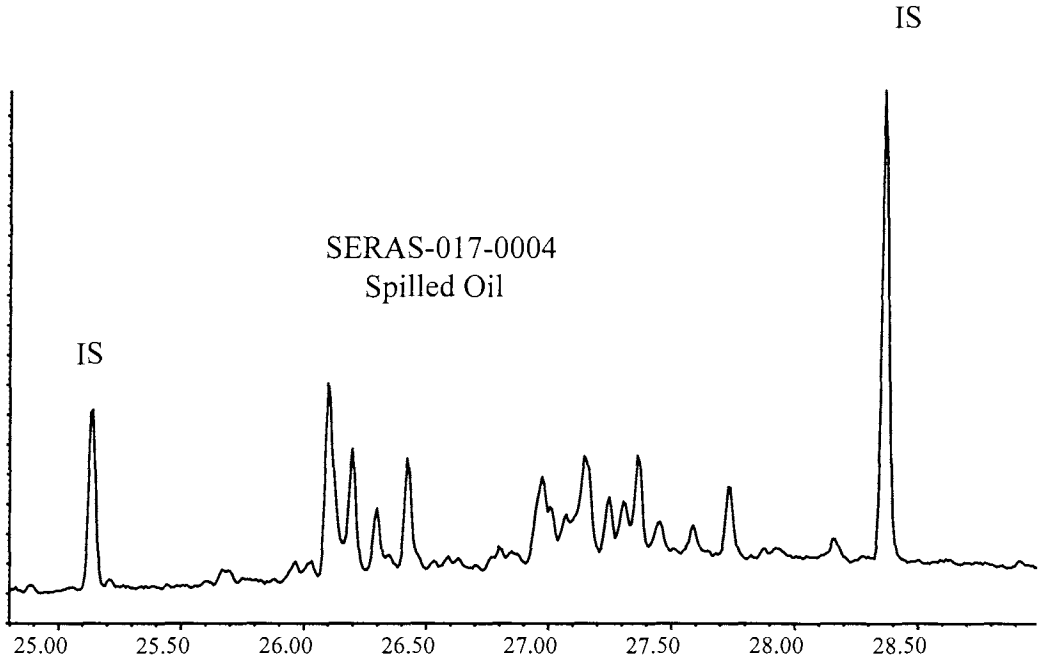
Ion 191.00 (190.70 to 191.70): SL02829.D\data.ms (*)

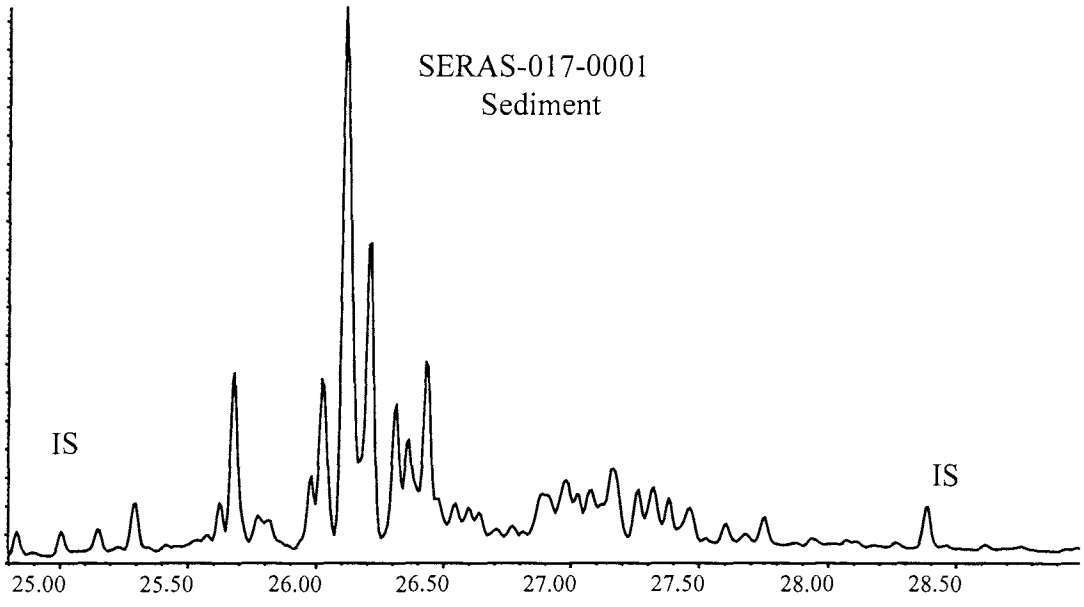


EX03: TPH Fingerprints

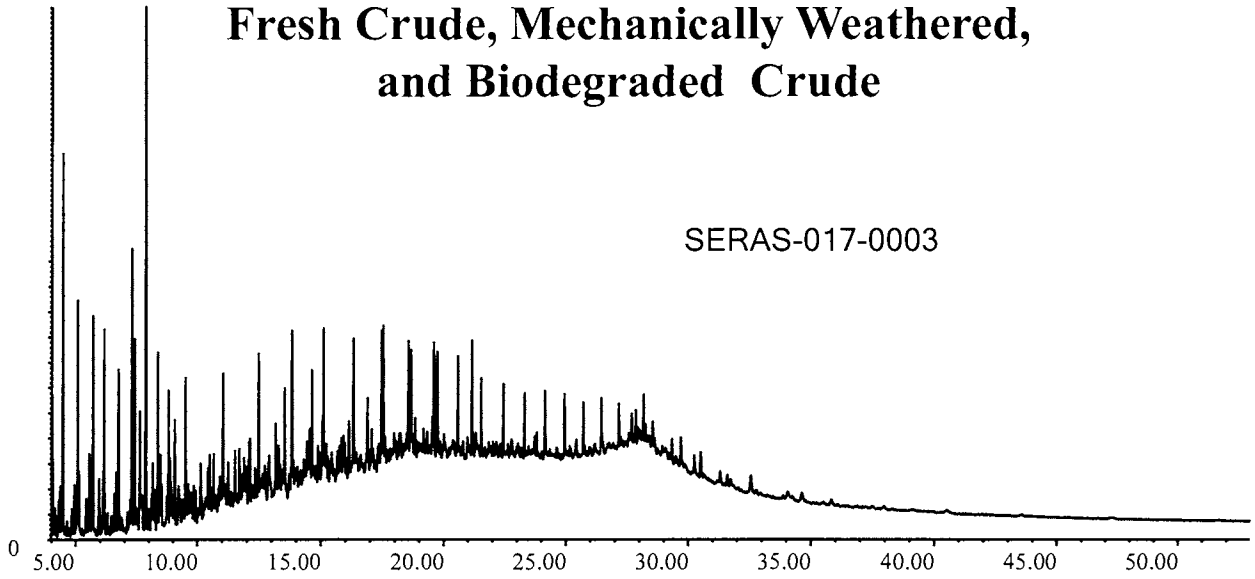


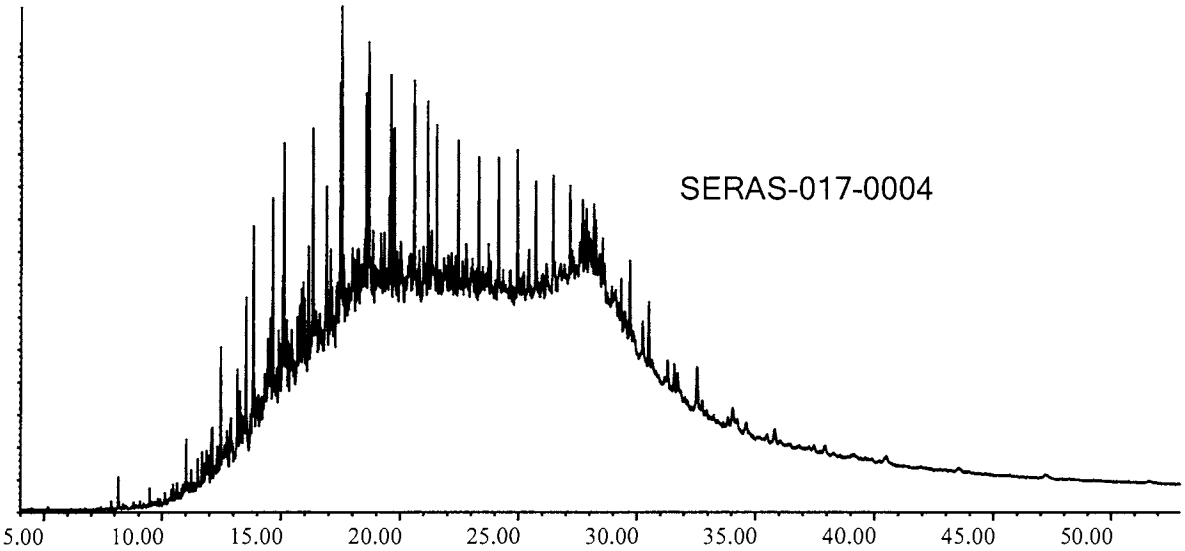
EX04: Total methyl- & dimethyl-Chrysene Fingerprints



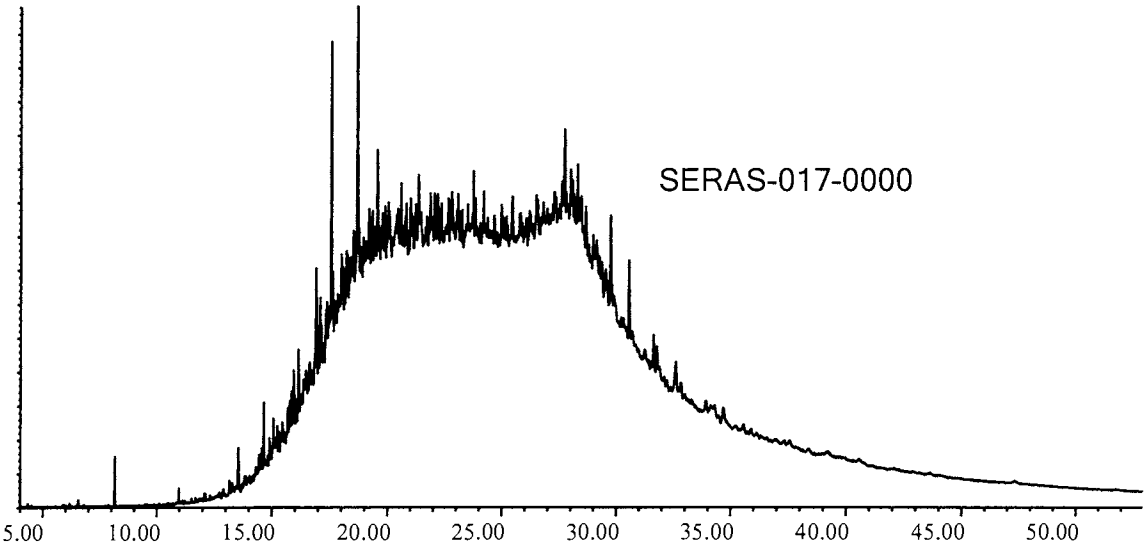


EX05: TPH Fingerprints Illustrating Fresh Crude, Mechanically Weathered, and Biodegraded Crude





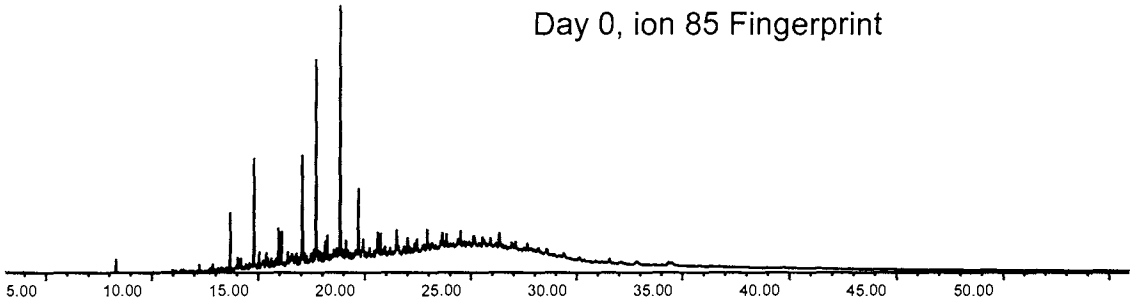
Time-->



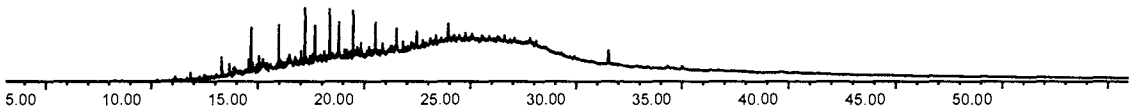
Time-->

EX06A: Biodegradation of Ion 85 & ion 83 Compounds During an 84 Day Degradation Study

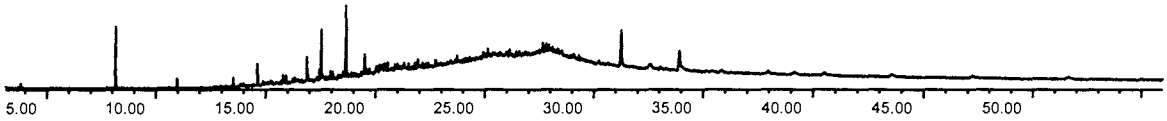
Day 0, ion 85 Fingerprint



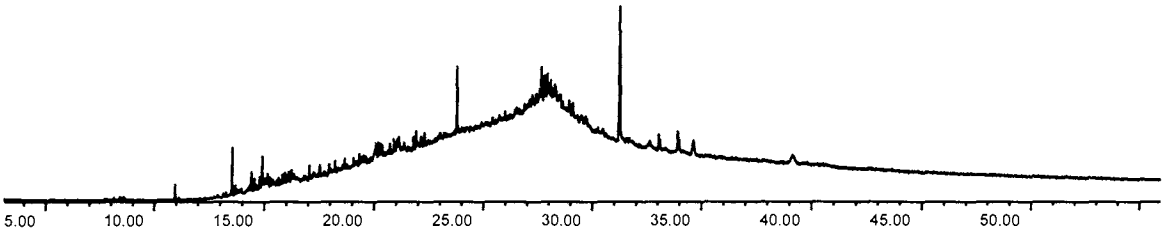
Day 0, ion 83 Fingerprint



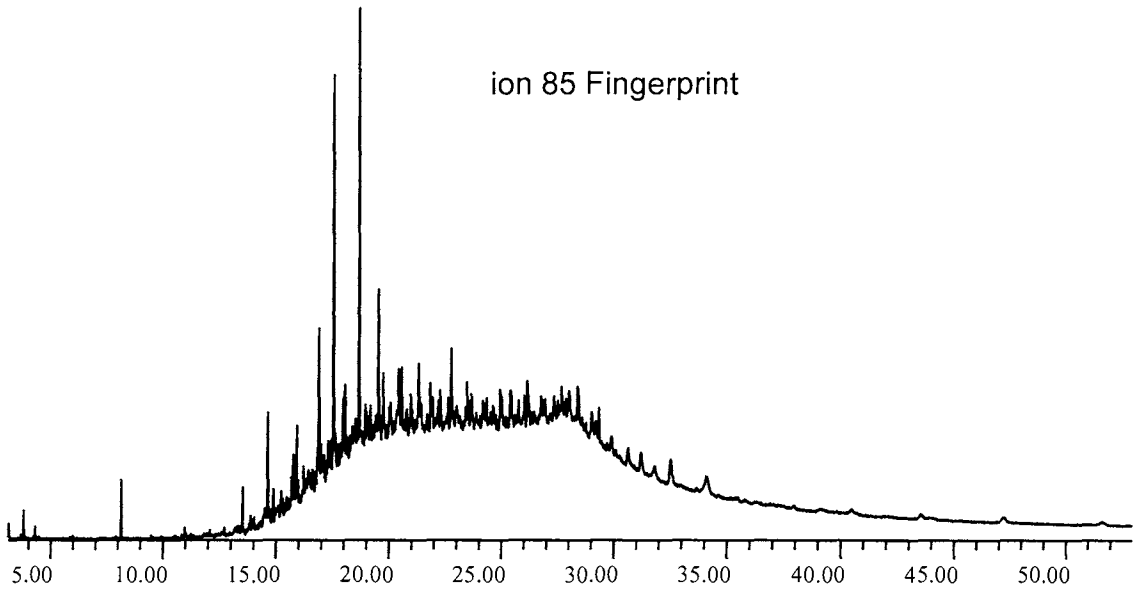
Day 84, ion 85 Fingerprint

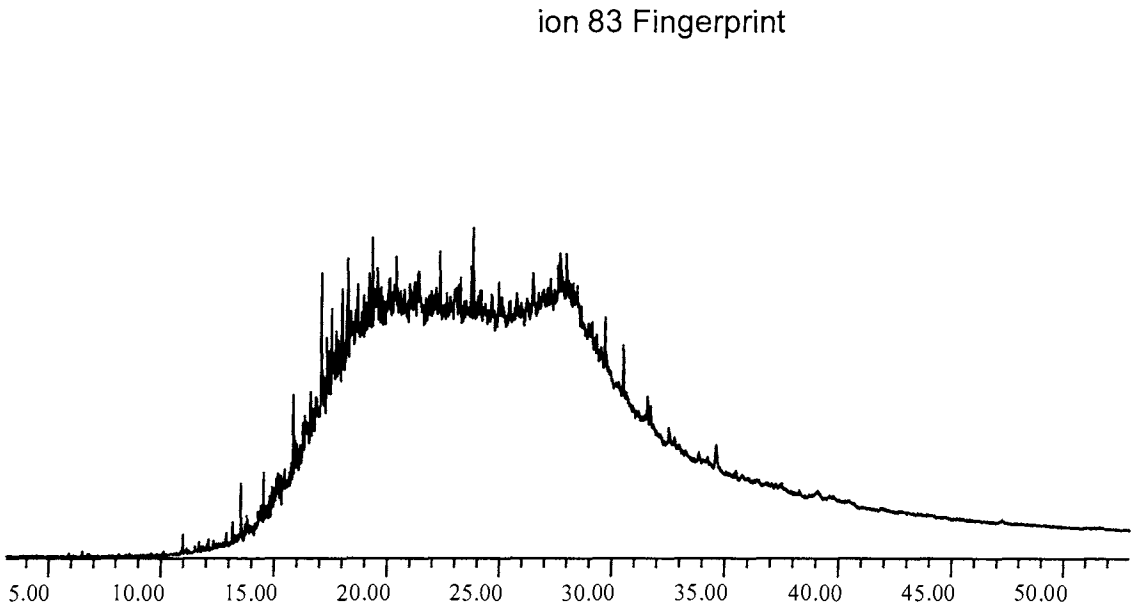


Day 84, ion 83 Fingerprint

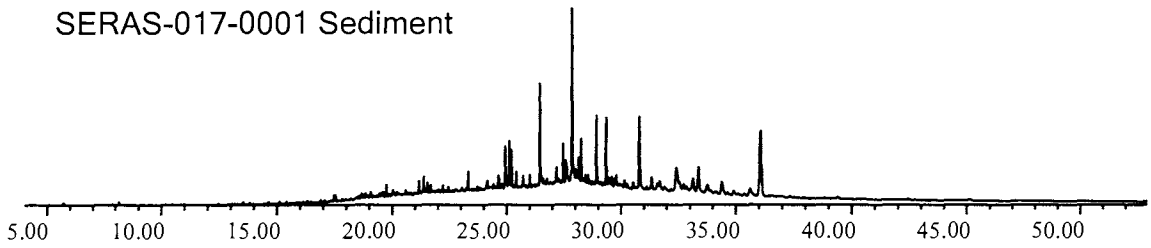


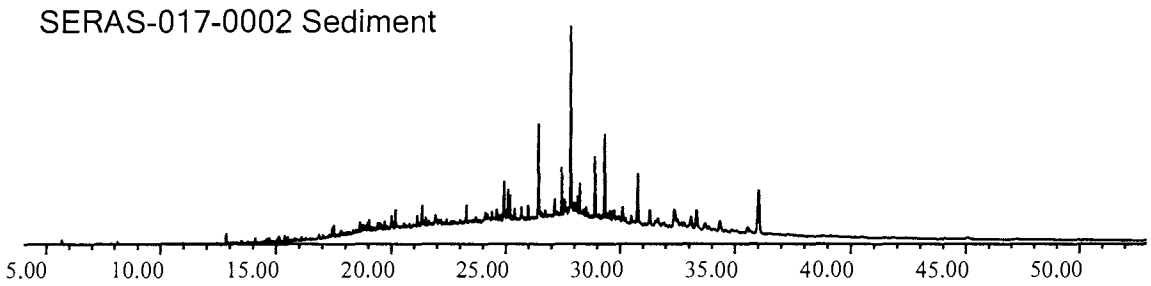
EX06B: Sample 017-0000 Ions 85 & 83 Fingerprints

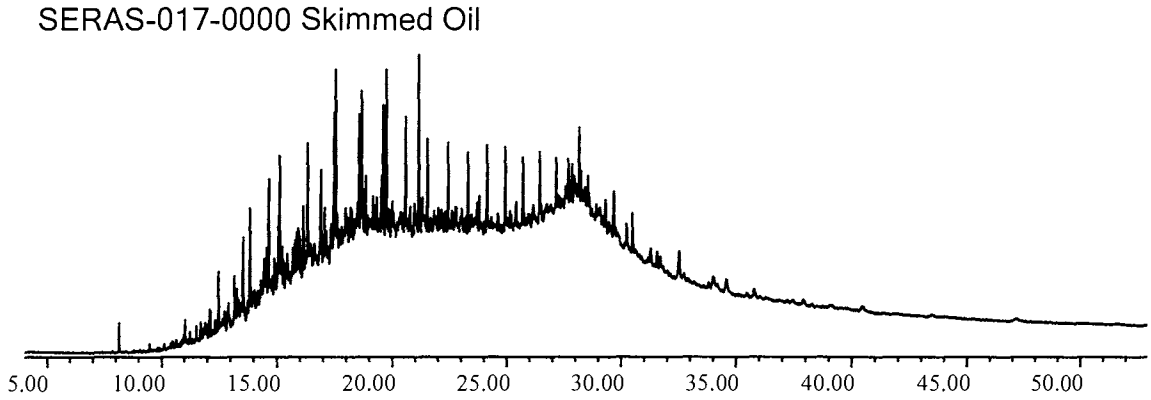


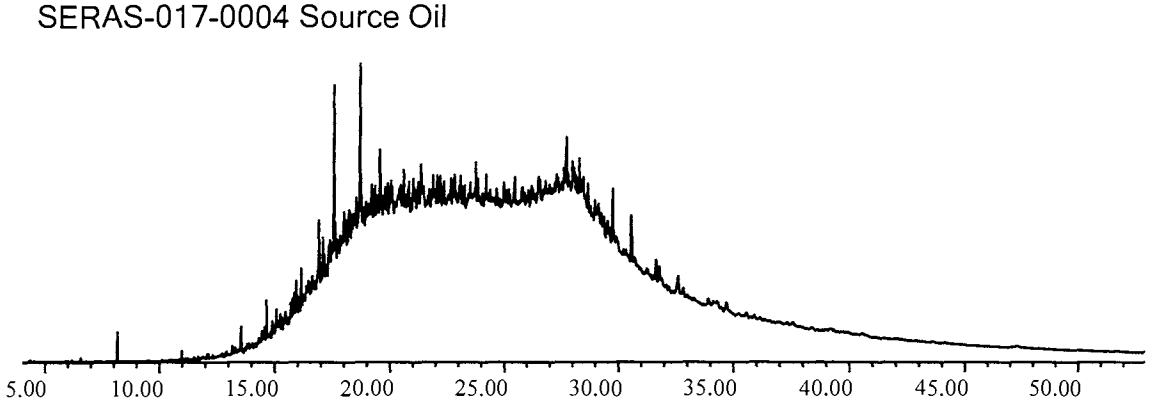


EX07: TPH Fingerprints of the two Sediments Samples Compared to Spilled Oil Samples Illustrating Natural or Introduced "Background Organics"



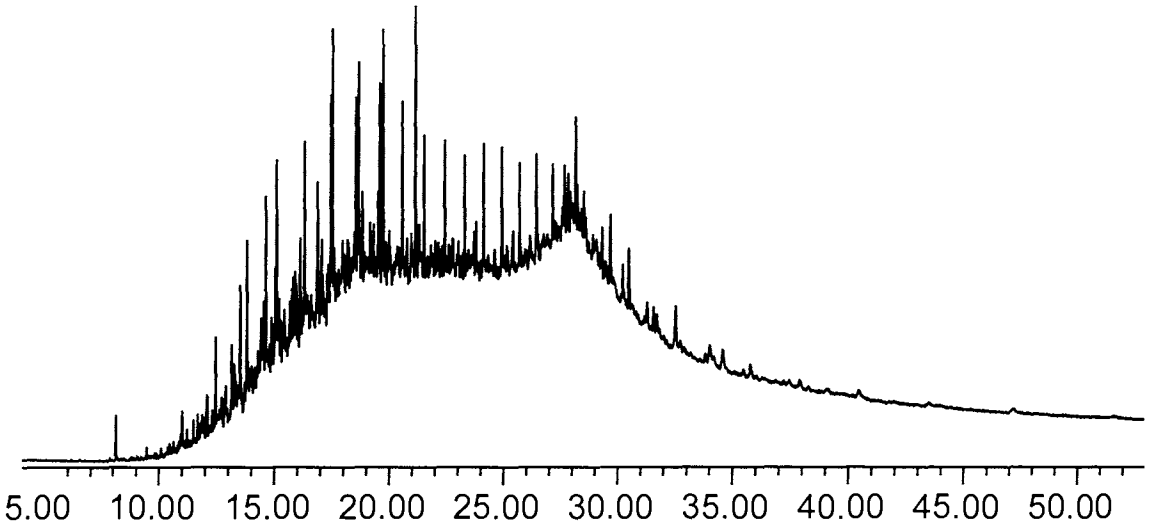




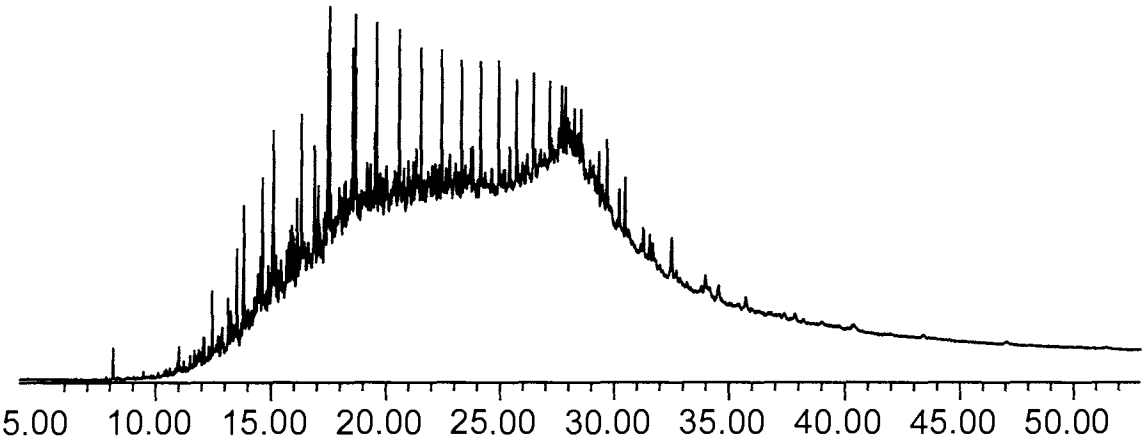


EX08A: Illustrates Fingerprint of Unprocessed Spilled Oil and Fingerprint of Processed and Autoclaved Oil: Sample 017-0004

Sample 017-0004; Unprocessed Sample

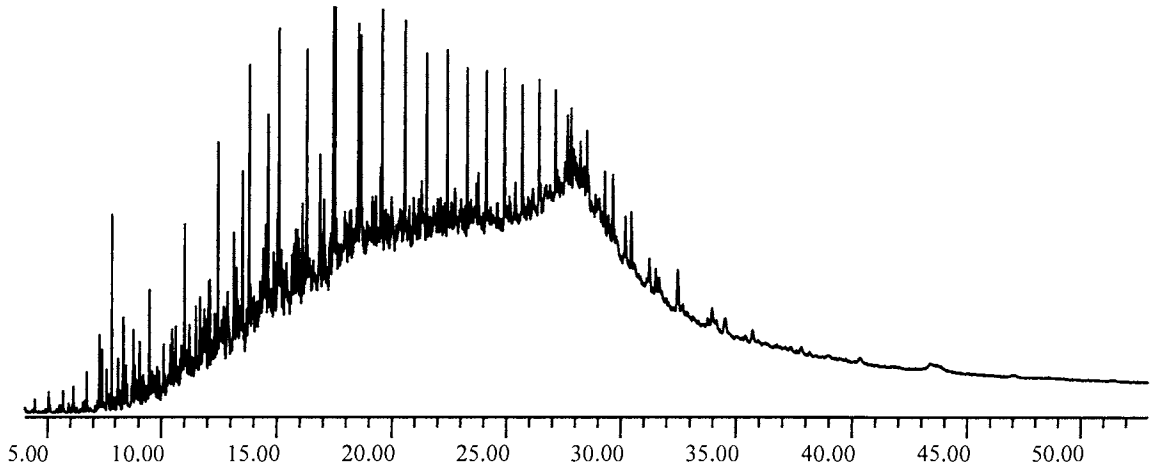


Sample 017-0004; Processed and Autoclaved

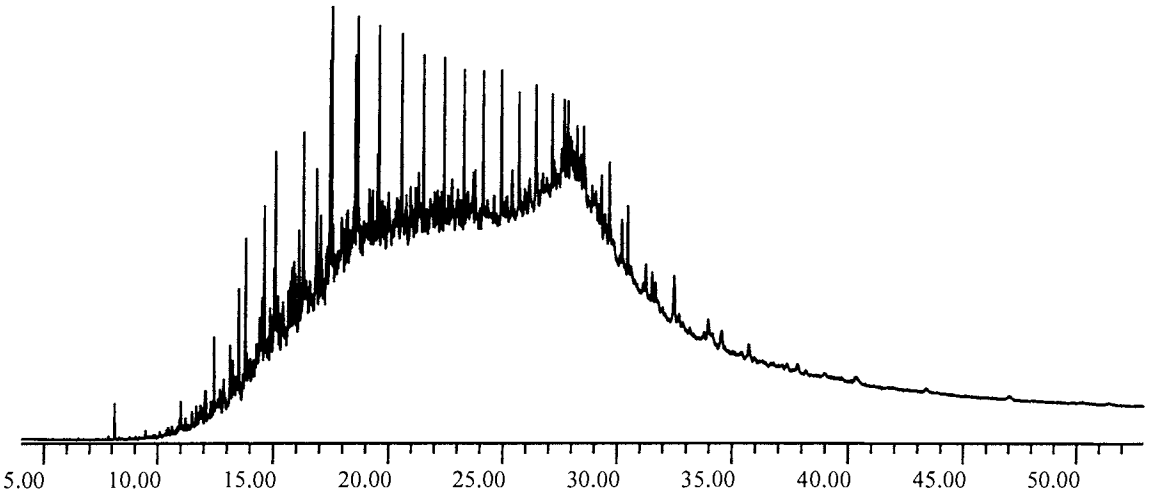


EX08B: Illustrates Fingerprint of Unprocessed Fresh Crude Oil and Fingerprint of Processed and Autoclaved Oil: Sample 017-0003

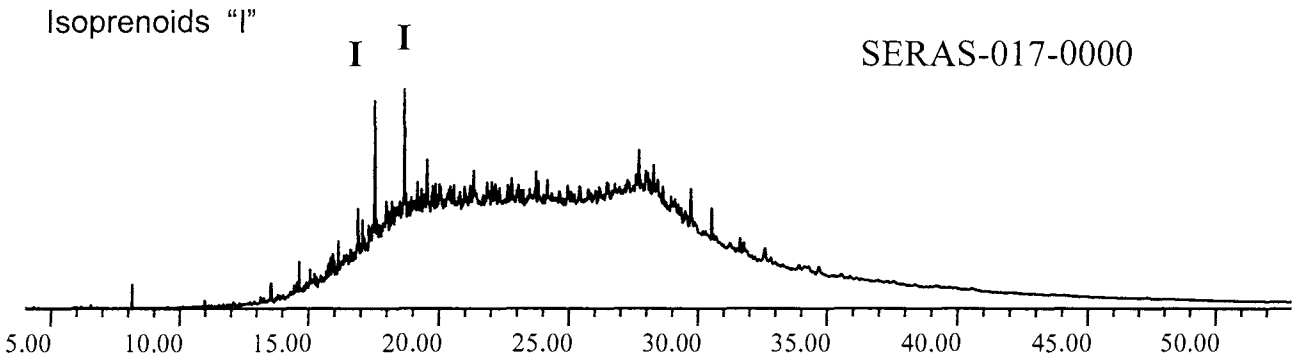
Sample 017-0003: Unprocessed Sample

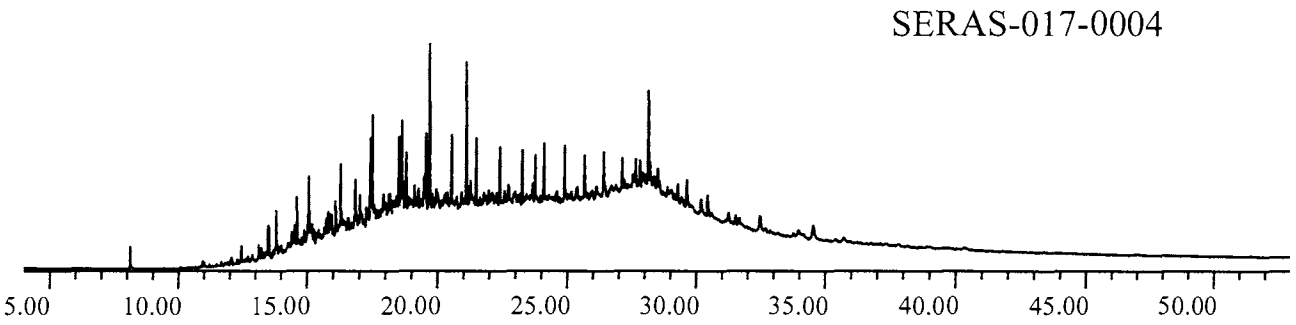


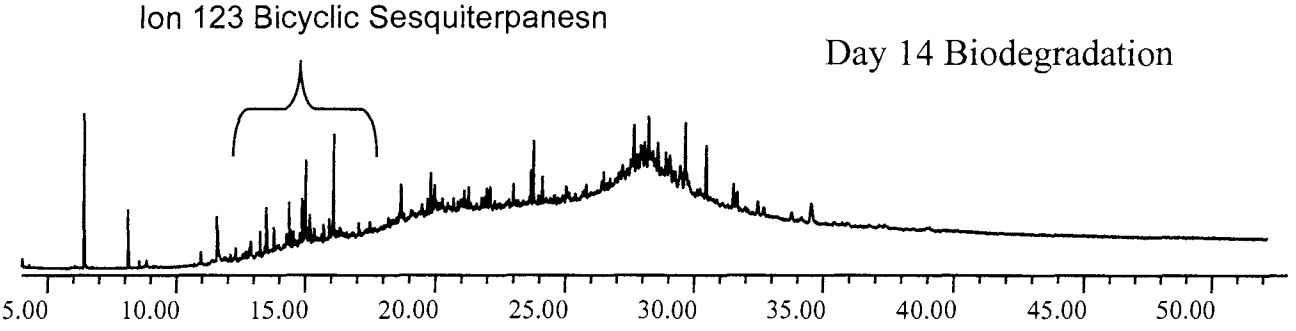
Sample 017-0003: Processed & Autoclaved

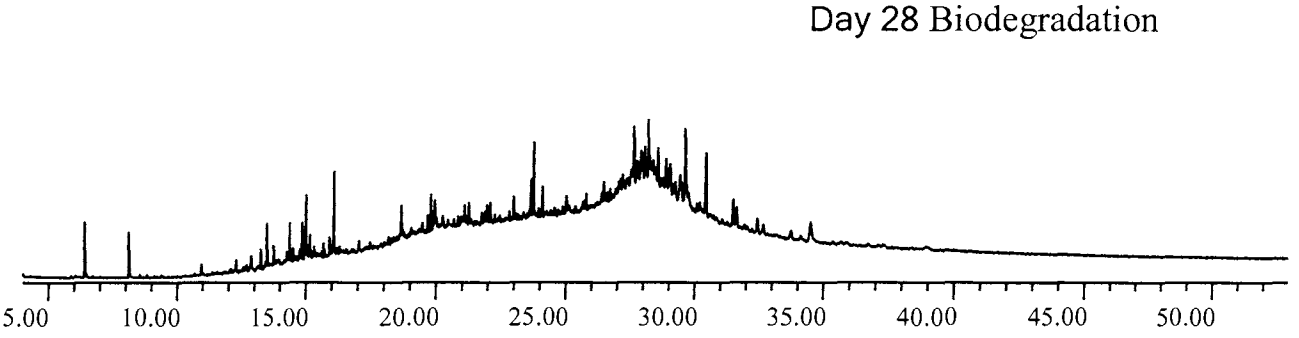


EX09: TPH Fingerprints



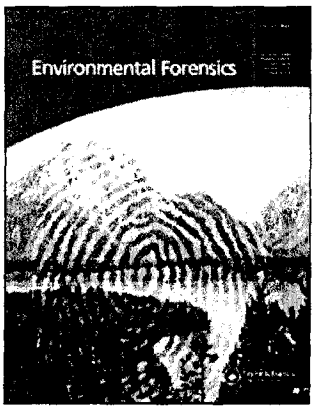






APPENDIX C

Attachment 04



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Chemical Fingerprints of Alberta Oil Sands and Related Petroleum Products

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Chemical Fingerprints of Alberta Oil Sands and Related Petroleum Products

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Emergencies Science and Technology Section, Environment Canada, Ottawa, ON, Canada

Alberta oil sands are known to contain the world's largest reserves of bitumen. The rapid growth in their production could result in a significant environmental impact. Fingerprinting bitumen and petroleum products from the Alberta oil sands is essential in order to better understand the chemical compositions of oil sands, prepare for potential oil spills, and address the associated environmental problems. This study presents an integrated quantitative chemical characterization of Alberta oil sands bitumen and other related Alberta oils using gas chromatography-flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS). The characterized target hydrocarbons include *n*-alkanes, unsubstituted polycyclic aromatic hydrocarbons (PAHs) and their alkylated homologues (APAHs), biomarker terpanes and steranes, bicyclic sesquiterpanes, and diamondoids. The chemical features of bitumen in oil sands are clearly distinguishable from those of most other conventional crude oils. The chemical fingerprints of diluted oil sands bitumen and Albian Heavy Synthetic crude were significantly altered by either the diluent blended with the former or the upgrading processing of crude bitumen in the latter. A chromatographic hump of unresolved complex mixtures (UCMs) eluting between n-C_{10} to n-C_{40} is pronounced and *n*-alkanes are nearly absent in bitumen extracted from oil sands. Alkylated naphthalenes account for only a small proportion of the total APAHs in Alberta oil sands extracts. The PAH compounds in oil sands extracts and diluted bitumen are dominated by alkylated homologues with the relative distribution of $C_0- < C_1- < C_2- < C_3-$ for all five APAH series. Biomarker terpanes and cage-like adamantanes were determined in almost identical abundance and distribution profile in oil sands extracts and diluted crude bitumen, while biomarker steranes and bicyclic sesquiterpanes were removed to varying degrees by physical weathering or biodegradation.

Keywords: Alberta oil sands, bitumen, petroleum, fingerprinting, polycyclic aromatic hydrocarbons (PAHs), biomarkers, gas chromatography-mass spectrometry (GC-MS)

Although sustainable and renewable technologies to generate more power for the future are being explored, petroleum is expected to remain a dominant fuel for many decades. As conventional crude oil reserves on land are rapidly being depleted, heavy oils such as oil sands are attracting increasing investment interest. The Alberta oil sands are known as the world's largest reserves of bitumen. According to the Alberta Energy Resources Conservation Board, in 2009 the province of Alberta (mainly in the Peace River, Athabasca, and Cold Lake) remains 169.9 billion barrels of recoverable bitumen as estimated using current technologies (Energy Resources Conservation Board [ERCB], 2010). Oil sands production, including raw bitumen production and upgraded synthetic crude oil, currently make up approximately 50% of Canada's total crude oil production. This figure is expected to grow from more than 1.2 million barrels per day (b/d) in 2008 to approximately 2.2 million b/d in 2015 and 3.3 million b/d in 2025 following current trends (Canadian Association of Petroleum Producers [CAPP], 2009).

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Oil sands are a mixture of 4% to 6% water, 83 to 85% host sediment (sands and other mineral material), and 10% to 12% bitumen. Bitumen from the oil sands is a viscous, heavy oil with an API gravity typically of <10 (Speight, 2006). As the bitumen in oil sands has lost most of the low molecular weight paraffins and naphthenes, it generally contains a high percentage of complex molecules such as asphaltenes and resins. At room temperature, the crude bitumen is in an almost solid state and must be converted to upgraded crude using processes such as coking, distillation, catalytic conversion, and hydrotreating in order to be further refined into more valuable and pipeline-transportable products.

The rapid growth in production of the Alberta oil sands has inevitably resulted in unprecedented environmental impact. The mining, extraction, and production of oil sands have left significant footprints in Alberta such as a huge consumption of water resources, vast emissions of greenhouse gases, and a large number of tailing ponds. Oil sands mining and processing have been contributing to the pollution of the Athabasca River for more than a decade (Kelly et al., 2009). If water from the tailing ponds escaped to nearby rivers carrying a heavy load of toxic waste, the environmental damage would be very severe and could last for decades. Accidental spills in the transportation of oil sands and

relevant oil products within Canada and to United States, and to other countries could potentially cause significant impacts on the environment.

The ability to characterize Alberta oil sands and their oil products is essential in order to assess the potential adverse effects on human health and the surrounding ecosystem, better prepare for potential spills, and address the environmental concerns associated with oil sands products. There are a few studies on the organic geochemistry of Alberta oil sands bitumen (Dimmler et al., 1984; Fowler and Brooks, 1987; Brooks et al., 1988; Strausz et al., 2010). However, to date, detailed quantitative fingerprints are still not available in the literature. This study presents a quantitative chemical characterization on the concentration and distribution profiles of target compounds in Alberta oil sands and several other related Alberta oils. The samples studied include raw Alberta oil sands, diluted crude oil sands bitumen, and upgraded heavy synthetic crude. Alberta Sweet Mixed Blend (ASMB), a mixture of conventional Alberta oils, is used for comparison purposes. Among these oils, diluted crude oil sands bitumen and heavy synthetic crude are commonly transported in Canada and to United States through pipelines.

Experimental

Reagents and Materials

Normal alkane standards from n-C_9 to n-C_{36} used to determine individual and total petroleum hydrocarbons (TPHs) and polycyclic aromatic hydrocarbons (PAHs) calibration mixture were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Restek (Bellefonte, PA, USA). The biomarker standards of hopanes and steranes were obtained from Chiron Laboratory (Trondheim, Norway). Calibration standards for the determination of bicyclic sesquiterpanes, including cis-decahydronaphthalene (cis-decalin, $C_{10}H_{18}$), d_{18}-decahydronaphthalene (d_{18}-decalin, $C_{10}D_{18}$), and 1-methyldecalin ($C_{11}H_{20}$), were purchased from Sigma-Aldrich (St Louis, MO, USA) and Chiron Laboratory (Trondheim, Norway), respectively. Diamondoid calibration standards were obtained from Chiron Laboratory except adamantane and 1,3-dimethyladamantane (1,3-DMA), which were obtained from Sigma-Aldrich. Silica gel (100 to 200 mesh, 150 Å, pore 1.2 cm^2/g, active surface 320 m^2/g) was obtained from Sigma-Aldrich (Milwaukee, WI, USA). All solvents were of the highest purity available without further purification.

Sample Preparation

Six Alberta oil samples were collected from various sources by the Emergencies Science and Technology Section (ESTS) of Environment Canada. These samples are three raw Alberta oil sands (AOS), a diluted crude oil sands bitumen (DOB, diluted with diluents), Albian Heavy Synthetic (AHS), and Alberta Sweet Mixed Blend (ASMB).

Approximately 1.0 g of Alberta oil sands was extracted with dichloromethane (DCM) and made up to 10 mL of bitumen solution. In this study, oil sands extract was used to represent the materials extracted by DCM in order to differentiate it from the bitumen produced from oil sands plants. 1.0 mL of the extracted bitumen solution was taken and blown down to dryness to determine the total solvent extractable materials (TSEMs). For subsequent fingerprinting analysis, 1.0 mL of the above extracted bitumen solution was exchanged into n-hexane. For the other three oil samples, about 0.8 g of oil was dissolved in hexane and made up to the final volume of 10 mL. An aliquot of 1.0 mL of oil sands bitumen solution (in n-hexane) or 200 μL of other oil solutions containing approximately 16 mg of oil was spiked with appropriate surrogates (100 μL of 200 ppm of o-terphenyl and 100 μL of a mixture of deuterated naphthalene, acenaphthene, phenanthrene, benz[a]anthracene, and perylene, 10 ppm each) and then quantitatively transferred into the preconditioned 3.0 g of silica gel column (inner diameter 11 mm) for sample cleanup and fractionation. 12 mL of hexane and 15 mL of DCM-hexane (1:1, v/v) were used to elute the saturated and aromatic hydrocarbons, respectively. For each sample, the hexane fraction (labeled F1) was concentrated under a gentle stream of nitrogen to appropriate volumes, spiked with appropriate internal standards (IS) including 5-α-androstane, 17β(H),21β(H)-hopane and decalin-d_{18} and adamantane-d_{16} used for analysis of total saturated hydrocarbons (TSHs) and n-alkanes, biomarker terpane and sterane, bicyclic sesquiterpanes and diamondoids, respectively. The hexane/DCM fraction (labeled F2) was concentrated and spiked with 5-α-androstane and terphenyl-d_{14} as internal standards for analysis of total aromatic hydrocarbons (TAHs), alkylated homologous PAHs, and other unsubstituted United States Environmental Protection Agency (US EPA) priority PAHs, respectively.

Analytical Methods

Analyses for total GC-detectable petroleum hydrocarbons (TPHs) and n-alkane distribution (n-C_8 through n-C_{40}, plus pristane and phytane) were carried out on an Agilent 6890 gas chromatograph equipped with a flame ionization detector (gas chromatography-flame ionization detection [GC-FID]) system and an Agilent 7683 autosampler. A DB-5HT fused silica column (30 m × 0.25-mm I.D., 0.10 μm film thickness) was used. The carrier gas was hydrogen at 2.0 mL/min. The injector and detector temperatures were set at 290°C and 300°C, respectively. The temperature program used for TPH determination was the following: 40°C for 2 min, increased to 340°C at 20°C/min, and hold for 17 min.

Analyses of target PAH compounds (including five alkylated PAH homologous groups and 15 unsubstituted PAHs), biomarker terpanes and steranes, and bicyclic sesquiterpanes and diamondoids were performed on an Agilent 6890 GC system equipped with an Agilent 5973 mass-selective detector. The GC separation was achieved using an HP-5MS capillary column (30 m × 0.25-mm I.D., 0.25 μm film thickness) with

Table 1. Hydrocarbon group analysis results

Samples	AOS #1	AOS #2	AOS #3	DOB	AHS	ASMB
TPH analysis						
TSEM (mg/g sample)	143	123	140			
TPH (mg/g TSEM or oil)*	327	352	308	413	426	474
TSH (mg/g TSEM or oil)	175	180	153	247	270	338
TAH (mg/g TSEM or oil)	151	171	155	166	155	135
TSH/TPH (%)	53.7	51.3	49.7	59.8	63.5	71.3
TAH/TPH (%)	46.3	48.7	50.3	40.2	36.5	28.5
Resolved peaks/TPH (%)	2.1	3.0	2.0	8.2	13.0	36.7
UCM/TPH (%)	97.9	97.0	98.0	91.8	87.0	73.3
TPH allocation						
$<n$-C_{10} (%)	0.1	0.2	0.1	3.3	4.7	8.8
n-C_{10}~n-C_{16} (%)	10.0	13.6	8.0	15.3	12.1	22.3
n-C_{16}~n-C_{34} (%)	65.5	64.5	67.0	62.6	55.1	54.8
$>n$-C_{34} (%)	24.4	21.8	24.9	18.7	28.1	14.1
n-Alkane analysis						
$\sum n$-$C_{9\sim44}$ (mg/g oil)	ND**	ND	ND	7.41	14.5	58.0

*The unit for TPH, TSH, and TAH is mg g^{-1} of TSEM for Alberta oil sands and mg g^{-1} of oil for other samples.
**ND: not detectable.

different temperature programs for specific target compounds. Samples were injected in splitless mode (injector temperature at 280°C) with 1.0 mL/min of helium as carrier gas. The MS was performed in the selected ion monitoring (SIM) mode. System control and data acquisition were achieved with the Agilent Enhanced MSD ChemStation.

The quantitation methodologies of individual compounds and analysis quality control are defined in the literature (Wang et al., 1999a; 2005; Yang et al., 2006; 2009). The identification of individual target compounds was based on internal standards and comparison with reference chromatograms of the well characterized ESTS reference oil (Prudhoe Bay crude oil, 13.1% weathered, prepared from Prudhoe Bay crude by rotary evaporation in this lab) (Wang et al., 2004). The peak area of target compounds was acquired from reliable instrumental analysis and by careful manual integration. Diagnostic ratios were calculated based on the concentration of target compounds.

Results and Discussion

Determination of Hydrocarbon Groups and n-Alkane Distributions

The prevailing theory about the origins of Alberta oil sands deposits is that the oil sand bitumens of northeast Alberta were formed by the biodegradation and water washing of conventional pooled oils during the Lower Cretaceous period (Brooks et al., 1988; Rubinstein et al., 1977; Bachu, 1995). Normal alkanes along with some light hydrocarbons were nearly completely depleted by the natural weathering by evaporation and bacterial degradation through permeable porous rocks and sediments during the formation of bitumen from light crude oil in British Columbia and southern Alberta that migrated eastward and upward (Brooks et al., 1988; Bachu, 1995).

The Alberta oil sands bitumen content in deposits varies from 1 to 18% (typically 10–12%) depending on the deposit residence and the proportion of bitumen in the sands, which generally increases with depth of the formation. Using DCM as solvent, the total solvent extractable material (TSEM) was determined to be 143, 123, and 140 mg/g by weight in three raw Alberta oil sands (Table 1), at the equivalent range of bitumen extracted by industrial processing using hot water. The chemical features of hydrocarbon groups and n-alkanes in the Alberta oil sands samples studied were examined by GC-FID. The GC-FID chromatograms of the oil sands extracts, diluted crude bitumen, and Albian Heavy Synthetic compared to conventional ASMB are shown in Figure 1. Bitumen is recognized as the heaviest form of petroleum. The gross chemical composition of the bitumens varies greatly, not only from deposit to deposit but also among samples from a single deposit (Brooks et al., 1988). A large chromatographic hump of unresolved complex mixtures (UCMs) eluting between n-C_{10} to n-C_{40} is pronounced in all oil sands extracts and bitumen samples, indicating a significant biodegradation of their original crudes. A small UCM hump eluting between n-C_{27} to n-C_{31} is seen on the shoulder of the main UCM hump. This shoulder UCM hump likely contains a complex mixture of branched cyclic and aromatic hydrocarbons that are relatively highly resistant to biodegradation. Albian Heavy Synthetic is a blend of sweet Premium Albian Synthetic (PAS, API ~ 34) upgraded from oil sands bitumen with the ebullated-bed hydrocracking residua (Brierley et al., 2006). It is understandable that the abundance and distribution of TPHs in Albian Heavy Synthetic are significantly different from those in bitumen extracts. Since Premium Albian Synthetic consists mostly of light hydrocarbons and is absent of vacuum residue, all UCM contents is contributed by the heavier portion of hydrocracking residues.

The hydrocarbon group analysis results of Alberta oil samples are given in Table 1. In addition to the GC-TPH values

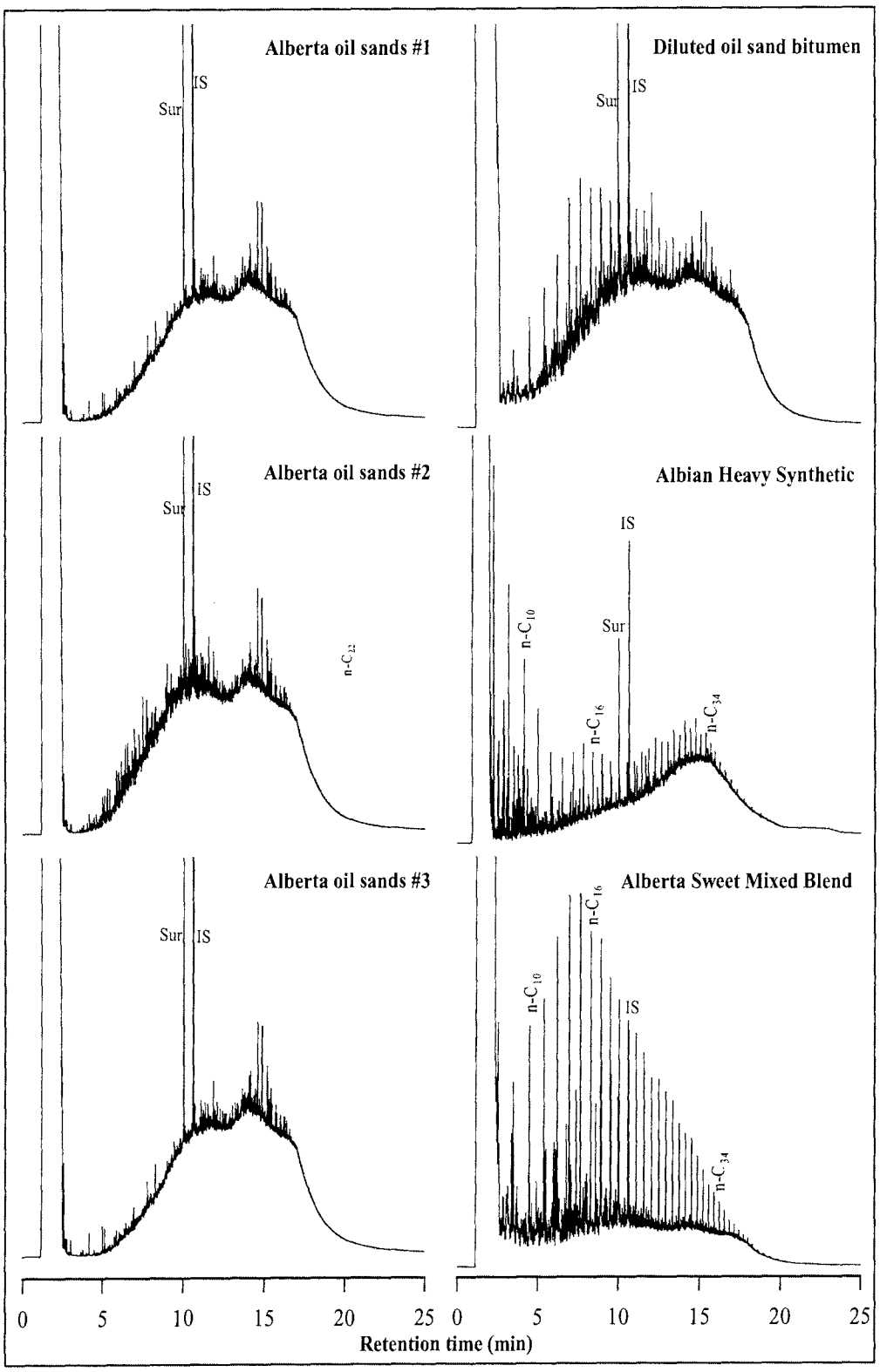

Figure 1. Gas chromatography/flame ionization detection (GC-FID) chromatograms of Alberta oil sands samples.

and percentages of saturates and aromatics in the TPH, the ratios of resolved peaks/TPH, UCM/TPH, and TPH distribution are also listed. The GC-TPHs account for only 46.8, 43.3, and 43.1 mg/g of raw oil sands studied. In order to compare with other oils on the same basis, therefore, the values are calculated and expressed as 327, 352, and 308 mg/g of TSEM, respectively. Higher GC-TPH values were determined to be 413 and 426 mg/g for oil sands bitumen and Albian Heavy Synthetic and 474 mg/g for ASMB crude, respectively. It should be noted that the GC-detectable TPH represents only a portion of these heavy oils, which excludes the components of asphaltenes and resins and those high molecular weight hydrocarbons that were removed by silica-gel column cleanup, or retained on the GC injector port and GC column. The total aromatic compounds in Alberta oil sands extracts account for 46 to 50% of TPHs, which is significantly higher than in other samples. The ratios of resolved peaks to the total GC areas were determined to be only 2.0 to 3.0% for three oil sands extracts compared to 36.7% for the lighter ASMB crude oil. The resolved peaks in DOB increase to 8.2% because this bitumen is blended with light petroleum products. The 13.0% of resolved components in AHS are largely from the portion of sweet Premium Albian Synthetic, which mainly consists of smaller molecule from upgrading of oil sands bitumen. As seen in Table 1, the hydrocarbon distributions of the four fractions in all four heavy oils are similar; they all contain little of the lightest fraction ($<n$-C_{10}) and are dominated by heavier hydrocarbons eluted between n-C_{16} to n-C_{34}. Compared to the oil sands products, the ASMB crude oil obviously contains relatively more lighter content, in which $<n$-C_{16} hydrocarbons account for about 30% of TPH.

Normal alkanes as well as pristane and phytane, which are more resistant to biodegradation, were not detected in the Alberta oil sands studied. The diluted sands bitumen (DOB) was determined to contain 7.4 mg/g of n-alkanes, primarily in the carbon range of n-C_9 to n-C_{30} with most at approximately n-C_{14} to n-C_{17}. These resolved n-alkanes probably did not originate from the diluted crude bitumen itself, but rather from the diluents. Normal alkanes in Albian Heavy Synthetic are obviously at a low concentration of 14.5 mg/g compared to typical conventional crude oils and these alkanes are largely contributed by its blended oil. The concentration of n-alkanes in ASMB is 58.0 mg/g of oil (Table 1), which is similar to other conventional crudes (Wang et al., 2004).

Distribution of Target Alkylated PAH Homologues and US EPA Priority PAHs

Quantitation results of five target petroleum-characteristic alkylated PAH homologues and other US EPA priority PAHs in six Alberta samples are summarized in Table 2. The distribution of the target PAHs in these samples is depicted in Figure 2. Crude oils generally contain significant amounts of PAHs, in particular the alkylated homologues of naphthalene, phenanthrene, dibenzothiophene, fluorene, and chrysene (Wang et al., 1999a). Compared to fresh conventional crudes, bitumens from Alberta oil sands present distinguishable PAH features.

The sum of the five alkylated PAHs was determined to be 1884 and 1055 μg/g of TSEM for AOS #1 and AOS #3 and 4933, 4467, and 5131 μg/g for AOS #2, DOB, and AHS, respectively. The highest value is 9857 μg/g for ASMB with a predominance of alkylated naphthalenes. The results suggest varying degrees of biodegradation of bitumens in these samples and the bitumens in raw oil sands #1 and #3 were more biodegraded than those in raw oil sands #2 and the bitumen products. The PAH distribution patterns are similar for Alberta oil sands extracts and diluted crude bitumen but significantly different than those for AHS and ASMB. The C_1- to C_3-naphthalene homologues are often the most abundant in many fresh crude oils, but distinctively, they were readily removed in Alberta oil sands with a very low concentration. C_0- to C_3-naphthalene isomers are all detected in appreciable concentrations in the diluted crude bitumen, and these naphthalenes are mostly contributed by the diluent. The abundance and distribution profiles of PAHs in AHS are altered by the upgrading process. Compared to the conventional crude ASMB, AHS has lower alkylated dibenzothiophenes. Another characteristic feature of AHS is its high content of alkylated chrysenes, at about six times that in the other five samples. Nevertheless, the PAH distribution of AHS still somewhat reflects its origin from bitumen. The five target alkylated PAH series in ASMB largely demonstrate the typical "bell-shape" distribution profiles. The biodegradation of alkylated PAHs varies with the number of aromatic rings of these compounds. From the results given in Table 2, among the five target alkylated PAH homologues, the 2-ring alkylated naphthalene homologues were the most susceptible to biodegradation, while the alkyl homologues of 4-ring chrysene were the most resistant to biodegradation. This is consistent with previous findings that the rate of degradation of PAHs is inversely proportional to the number of rings in the PAH molecule (Wang et al., 1998). It is apparent in Figure 2 that five oil-characteristic alkylated PAH series in all oil sands extracts have a distribution profile of C_0- < C_1- < C_2- < C_3-homologues. This can be explained by the fact that the susceptibility to microbial degradation decreases as the alkylation level increases in each alkylated PAH family.

The unsubstituted individual PAHs in oil sands extracts range from 36.9 to 50.2 μg/g of TSEM. The PAHs determined in heavily biodegraded bitumen AOS #1 and AOS #3 are composed of relatively higher 3- to 5-ring compounds than conventional crudes, particularly light crude oil, which is generally dominated by 2- or 3-ring PAH compounds (Wang et al., 2004). Blended with hydrocracking residues, Albian Heavy Synthetic was found to contain an extremely high content of unsubstituted PAHs, with a total concentration of 624 μg/g. Among these PAHs, pyrene and benzo(ghi)perylene are as high as 206 and 109 μg/g. The pyrogenic index, which is defined as the sum of 15 unsubstituted PAHs divided by the sum of the five target alkylated PAH homologues (Wang et al., 1999b), is as high as 0.12, significantly greater than the corresponding value of 0.01~0.03 for other oil sands samples and for conventional

Table 2. Quantitation results for target polycyclic aromatic hydrocarbon (PAHs)

Compounds	AOS #1 (μg/g TSEM)	AOS #2 (μg/g TSEM)	AOS #3 (μg/g TSEM)	DOB (μg/g oil)	AHS (μg/g oil)	ASMB (μg/g oil)
Alkylated PAHs						
C_0-naphthalene	ND	ND	ND	24.5	30.5	367
C_1-naphthalenes	ND	ND	ND	112	155	1328
C_2-naphthalenes	ND	19.4	ND	376	333	2101
C_3-naphthalenes	14.2	350	7.30	682	406	1891
C_4-naphthalenes	103	741	49.3	741	354	1075
Sum of naphthalenes	117	1111	56.6	1935	1278	6763
C_0-phenanthrene	3.53	8.65	1.88	30.9	55.1	111
C_1-phenanthrenes	69.4	173	14.0	101	195	310
C_2-phenanthrenes	184	407	63.1	166	321	412
C_3-phenanthrenes	296	456	163	200	374	384
C_4-phenanthrenes	218	294	173	146	357	281
Sum of phenanthrenes	771	1339	414	643	1302	1499
C_0-dibenzothiophene	ND	ND	ND	47.2	36.9	128
C_1-dibenzothiophenes	21.4	167	12.0	188	147	301
C_2-dibenzothiophenes	179	662	97.6	406	302	437
C_3-dibenzothiophenes	351	813	194	509	390	339
Sum of dibenzothiophenes	552	1642	303	1150	877	1205
C_0-fluorene	ND	2.71	ND	20.4	14.1	57.4
C_1-fluorenes	10.6	66.0	4.01	70.3	53.9	161
C_2-fluorenes	59.9	231	20.4	171	124	301
C_3-fluorenes	141	303	60.4	251	188	348
Sum of fluorenes	212	602	84.8	513	380	867
C_0-chrysene	15.4	9.18	14.2	7.54	53.2	7.44
C_1-chrysenes	40.7	49.4	35.4	47.6	359	52.5
C_2-chrysenes	88.8	93.7	68.2	88.7	502	91.8
C_3-chrysenes	88.1	86.5	78.5	82.9	379	94.0
Sum of chrysenes	233	239	196	227	1294	246
Total alkylated PAH_S	1884	4933	1055	4467	5131	10580
US EPA priority PAHs						
Biphenyl (Bph)	ND*	ND	ND	9.39	13.0	53.3
Acenaphthylene (Acl)	ND	ND	ND	2.84	1.94	7.99
Acenaphthene (Ace)	2.17	8.54	0.77	7.01	4.75	9.33
Anthracene (An)	ND	ND	ND	9.64	6.22	4.18
Fluoranthene (Fl)	3.79	3.74	3.00	5.65	19.2	2.45
Pyrene (Py)	11.7	12.7	10.1	18.3	206	13.2
Benz(a)anthracene (BaA)	2.37	3.06	0.98	4.02	37.8	2.19
Benzo(b)fluoranthene (BbF)	4.40	4.24	4.12	5.09	25.0	2.67
Benzo(k)fluoranthene (BkF)	0.38	0.59	0.45	0.73	5.66	ND
Benzo(e)pyrene (BeP)	6.10	5.72	5.81	7.00	75.3	7.35
Benzo(a)pyrene (BaP)	2.38	1.93	1.95	3.94	49.6	1.32
Perylene (Pe)	5.29	4.79	4.56	8.60	28.1	7.24
Indeno(1,2,3-cd)pyrene (IP)	1.26	1.08	1.18	2.03	20.5	ND
Dibenzo(ah)anthracene (DA)	1.77	1.50	1.58	1.62	22.2	0.92
Benzo(ghi)perylene (BP)	2.46	2.32	2.35	4.82	109	1.86
Total EPA priority PAHs	44.1	50.2	36.9	90.7	624	114
Diagnostic ratios						
2-m-N:1-m-N	NA**	NA	NA	1.56	1.62	1.75
(3-+2-)/(4-/9-+1-m-phen)	0.64	0.44	0.31	0.81	1.57	0.98
$\sum$(2–3 ring PAHs)/$\sum$(4–6 ring PAHs)	0.05	0.33	0.02	0.47	0.04	1.91
Pyrogenic Index	0.02	0.01	0.03	0.02	0.12	0.01

*ND: not detectable; **NA: not applicable.

crude oils. These unsubstituted PAHs are obviously generated in the upgrading process due to coking of bitumen at temperature around 500°C (Oil Sands Discovery Centre, 2010).

The gas chromatography-mass spectrometry (GC-MS) chromatograms of three clusters of aromatic hydrocarbons, including methylphenanthrenes (m/z 192), methyl-pyrenes/fluoranthrenes (m/z 216), and triaromatic steranes (m/z 231), are shown in Figure 3. The information from these compounds is important to forensic oil analysis, particularly when the oils involved are heavily weathered. The positions of the alkyl substituents can strongly affect the rate of biodegradation. It can be seen that biodegradation of methylphenanthrenes is in a decreasing order of 3-MP or 2-MP>1-MP>>9-/4-MP. This observation is similar to the results reported by Wang et al. (1998). Since

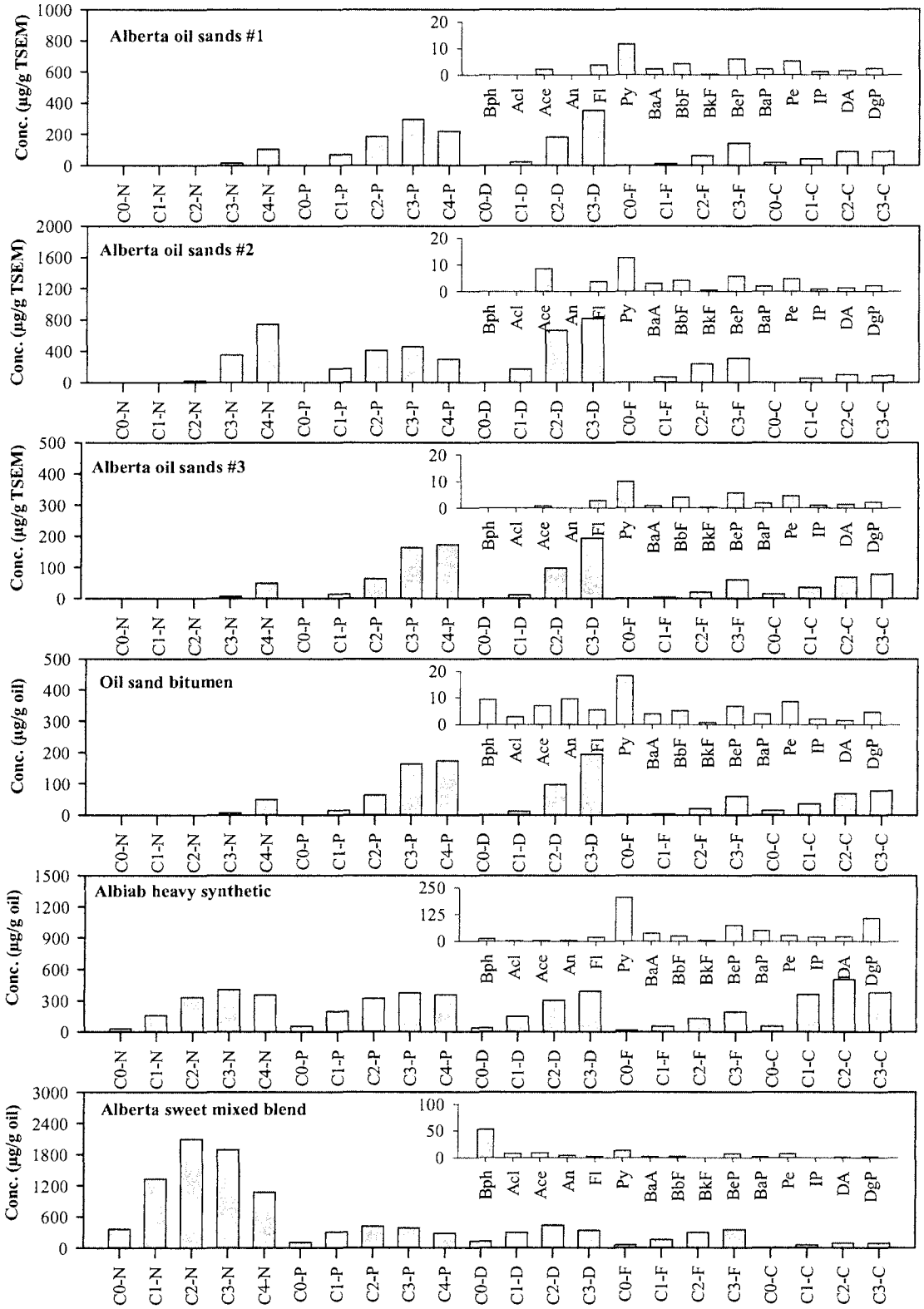

Figure 2. Target polycyclic aromatic hydrocarbon (PAH) fingerprints of Alberta oil sands samples.

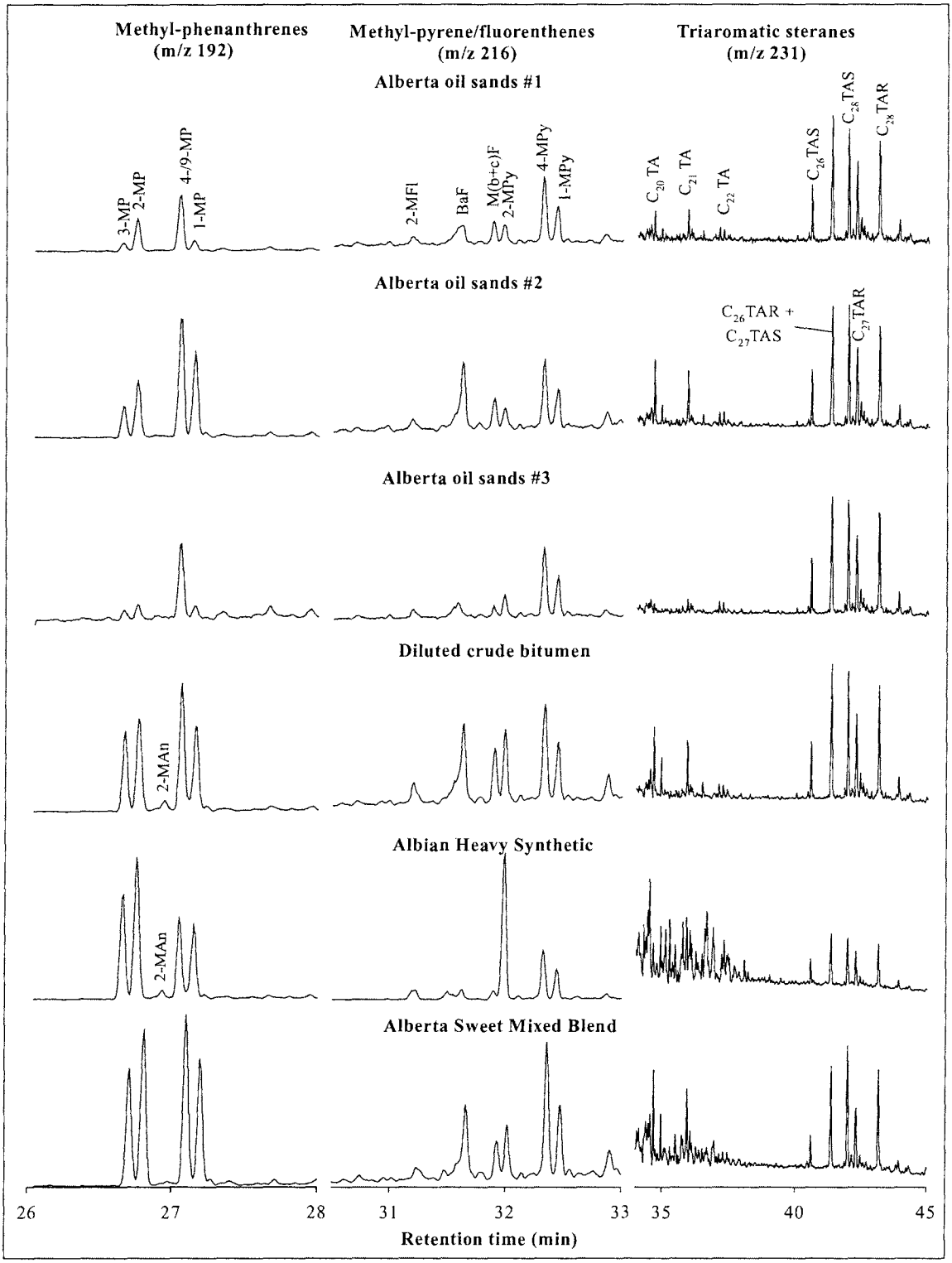

Figure 3. Gas chromatography/mass spectrometry (GC-MS) chromatograms of methylphenanthrenes (m/z 192), methyl-pyrenes/fluoranthrenes (m/z 216), and triaromatic steranes (m/z 231) in Alberta oil sands samples.

methylanthracenes are generally absent in most conventional crude oils or are present only in low concentrations relative to methylphenanthrenes (Uhler et al., 2007), the relatively high presence of 2-methyl-anthracene (2-MAn) in AHS might be a result of the evidence of cracking process. The most refractory isomers in the m/z 216 cluster appear to be 4-methyl-pyrene (4-MPy) and 1-methyl-pyrene (1-MPy), while other isomers were degraded in different degrees. The C_{26-28} isomers of triaromatic steranes (TASs) are very resistant to biodegradation and only degraded under extreme conditions (Volkman et al., 1984). These steroids remain unaffected in their abundance and distribution pattern in the m/z 231 fragmentogram (Figure 3). Preferential depletion of C_{20} to C_{22} triaromatic sterane isomers is obviously found for the severely biodegraded AOS #1 and AOS #3.

Characterization of Petroleum Biomarkers

Petroleum biomarkers are compounds that are similar in structure to their original biological molecules. Petroleum biomarkers are often used to investigate the depositional environment of crude oil and to track such processes as genesis, maturation, migration, and biodegradation (Brooks et al., 1988; Peters and Moldowan, 1991; Zumberge, 1987). The typical biomarker compounds such as polycyclic terpanes and steranes have been frequently applied in source identification and differentiation of forensic oil spill analysis (Wang et al., 1999a; Daling et al., 2002).

Different classes of petroleum hydrocarbons have different susceptibilities to biodegradation. There is much discussion in the literature about the biodegradation of biomarker terpanes and steranes. The degradation of aliphatic hydrocarbons generally follows an order of n-alkanes > isoalkanes plus anteisoalkanes > cyclohexylalkanes and/or methylcyclopentylalkanes > acyclic isoprenoids > C_{27-29} steranes > C_{30-35} hopanes > diasteranes > C_{27-29} hopanes > C_{21-22} steranes > tricyclic terpanes (Brooks et al., 1988; Connan, 1984; Wang et al., 2001). The quantitation results for the target biomarker terpanes and three pairs of $\alpha\beta\beta$-steranes (C_{27} to C_{29}) are summarized in Table 3. The total target biomarkers were determined to be 848 and

Table 3. *Quantitation results for biomarkers*

Compounds	AOS #1 (μg/g TSEM)	AOS #2 (μg/g TSEM)	AOS #3 (μg/g TSEM)	DOB (μg/g oil)	AHS (μg/g oil)	ASMB (μg/g oil)
Biomarker terpanes						
C_{21}	39.5	37.5	39.7	30.9	15.9	16.0
C_{22}	18.7	18.2	18.6	14.1	678	6.29
C_{23}	120	113	120	92.3	46.9	41.3
C_{24}	62.0	58.9	62.8	47.8	24.2	21.2
Ts	29.3	27.7	29.3	25.4	13.1	31.1
Tm	102	95.0	101	85.9	54.4	33.2
C_{29}	239	230	237	200	97.1	86.8
C_{30}	288	268	283	240	114	97.2
C_{31}(S)	126	120	125	101	46.3	37.2
C_{31}(R)	91.6	88.4	91.8	75.5	35.7	25.9
Gammacerane	55.3	50.0	52.0	42.6	21.2	12.8
C_{32}(S)	79.5	75.3	78.5	65.4	30.4	26.9
C_{32}(R)	58.4	55.1	57.8	47.6	23.6	19.9
C_{33}(S)	59.8	58.0	58.5	47.1	24.2	18.3
C_{33}(R)	39.1	37.2	38.0	31.7	16.1	12.1
C_{34}(S)	39.4	36.9	39.7	31.4	17.8	14.5
C_{34}(R)	25.2	23.9	24.6	19.6	10.8	9.03
C_{35}(S)	41.6	39.9	42.2	32.0	19.0	11.8
C_{35}(R)	27.4	26.3	28.2	21.3	13.6	8.11
Biomarker steranes						
C_{27} $\alpha\beta\beta$	69.8	80.2	75.2	166	76.5	106
C_{28} $\alpha\beta\beta$	48.7	54.6	51.8	124	56.5	62.0
C_{29} $\alpha\beta\beta$	60.0	70.2	68.1	206	84.9	122
Total biomarkers	1720	1664	1723	1748	848	820
Diagnostic ratios						
C_{23}/C_{24}	1.93	1.92	1.91	1.93	1.94	1.95
C_{23}/C_{30}	0.41	0.42	0.43	0.39	0.41	0.42
C_{24}/C_{30}	0.22	0.22	0.22	0.20	0.21	0.22
C_{29}/C_{30}	0.83	0.86	0.84	0.84	0.85	0.89
C_{31}(S)/C_{31}(R)	1.38	1.36	1.36	1.34	1.30	1.43
C_{32}(S)/C_{32}(R)	1.36	1.37	1.36	1.37	1.29	1.35
C_{34}(S)/C_{35}(S)	0.95	0.93	0.94	0.98	0.94	1.23
C_{34}(R)/C_{35}(R)	0.92	0.91	0.87	0.92	0.79	1.11
Ts/Tm	0.29	0.29	0.29	0.30	0.24	0.94
$C_{30}/\sum C_{31\sim35}$	0.45	0.44	0.44	0.51	0.48	0.53
C_{27} $\alpha\beta\beta$/ C_{29} $\alpha\beta\beta$	1.16	1.14	1.10	0.80	0.90	0.87
$\sum$steranes/$\sum$terpanes	0.11	0.14	0.12	0.39	0.35	0.56

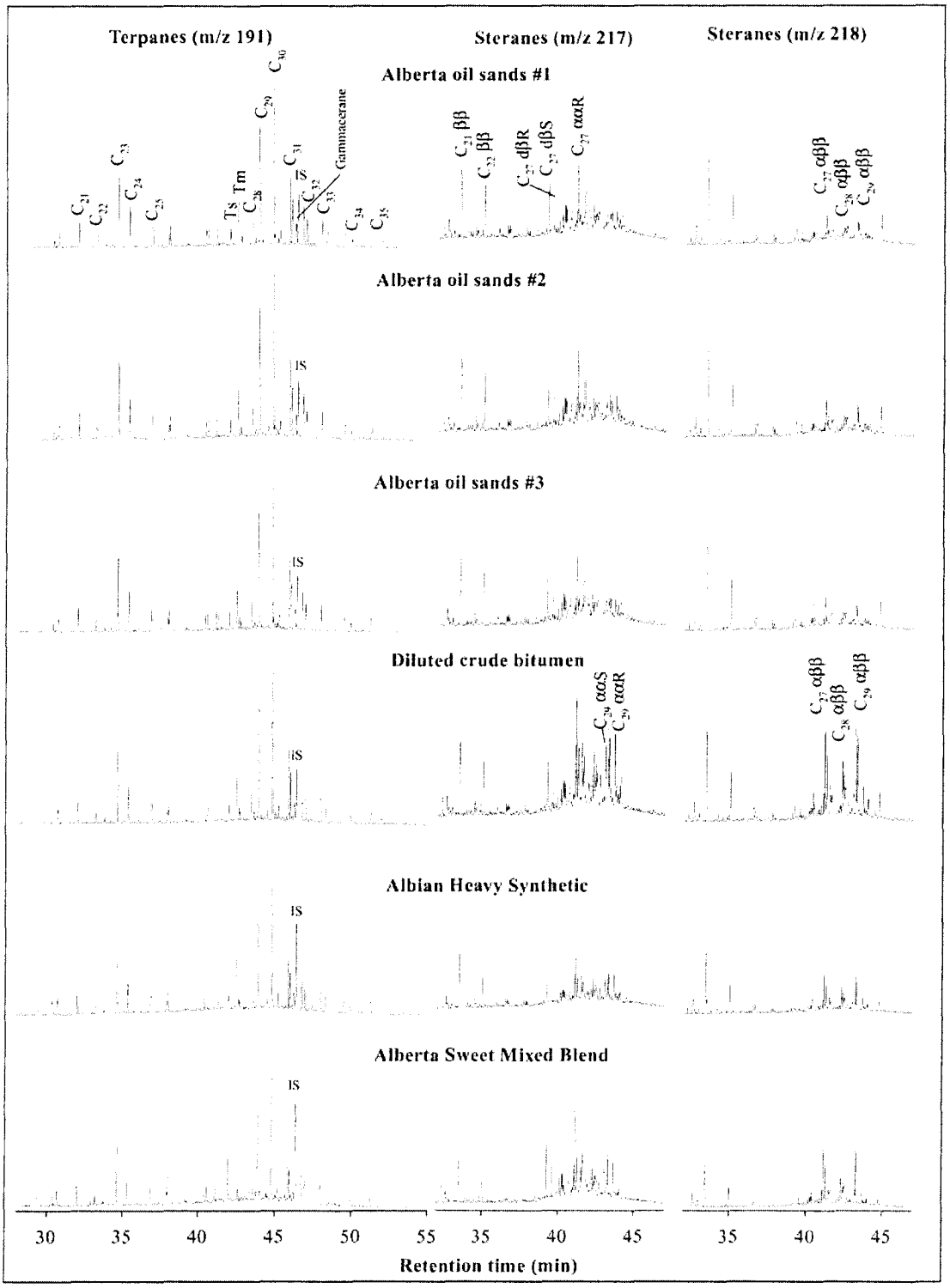

Figure 4. Gas chromatography/mass spectrometry (GC-MS) chromatograms of biomarker terpanes (m/z 191) and steranes (m/z 217 and 218) in Alberta oil sands samples.

820 μg/g for AHS and ASMB, respectively. The biomarkers in AHS are mostly contributed by the heavier portion of residues of upgrading process. The concentrations of biomarker terpanes and steranes in three oil sands extracts and diluted oil sands bitumen are all about 1700 μg/g. The GC-MS distribution profiles of the highly degradation-resistant biomarker terpane and sterane compounds are shown in Figure 4. It is noted that the profiles of biomarker terpanes (m/z 191) in oil sands extract and crude bitumen are quite similar, while at the same time, all molecular diagnostic ratios in Table 3 are also nearly identical, which indicates that they are from the same geologic source rock sequence. Furthermore, both the biomarker quantity and chromatographic features of three oil sands samples are almost identical at ion m/z 191, m/z 217, and m/z 218. This reflects similar biodegradation levels for these samples in terms of biomarkers. A wide range of terpanes, from C_{21} to C_{35} is present in these samples. Among these biomarkers, C_{21} to C_{25} tricyclic terpanes are in significant concentration with 17α(H),21β(H)-30-norhopane (C_{29}) and 17α(H),21β(H)-hopane (C_{30}) being the most abundant. All samples display a distribution pattern of $C_{29} < C_{30}$ and a regular decreasing profile in the abundance of the extended C_{31} to C_{35} homohopanes.

As shown in Figure 4, the presence of 28,30-bisnorhopane (C_{28}) and gammacerane is evident in the oil sands extracts, diluted crude bitumen, and Albian Heavy Synthetic, which is consistent with results from others (Fowler and Brooks, 1987; Brooks et al., 1988; Strausz et al., 2010). The occurrence of gammacerane may suggest a hypersaline depositional environment of the original oil in the Alberta oil sands. One other specific feature of C_{34} (S) < C_{35} (S) and C_{34} (R) < C_{35} (R) is noticed for homohopanes in all samples studied, with the exception of ASMB, which suggests that these oils were derived from source rocks deposited under anoxic conditions (Peters and Moldowan, 1991). The molecular ratios of Ts/Tm are close, ranging from 0.24 to 0.32 for the four oil sands products, but as high as 0.94 for ASMB, indicating a different origin for this oil. C_{21} $\beta\beta$- and C_{22} $\beta\beta$- pregnanes are strongly present in the m/z 217 fragmentogram because pregnanes are highly resistant to biodegradation. As seen in Table 4 and Figure 4, C_{27} $\alpha\beta\beta$, C_{28} $\alpha\beta\beta$, and C_{29} $\alpha\beta\beta$ steranes in Alberta oil sands consistently have a relative abundance in the "V" shape distribution pattern. This distribution occurs in Albian rocks and has been documented in core extracts (Creaney and Allan, 1992). In addition, the ratio of biomarker steranes to terpanes for oil sands is relatively lower than conventional crude ASMB.

Characterization of Bicyclic Sesquiterpanes

Bicyclic sesquiterpanes are commonly found in crude oils, intermediate petroleum distillates, and finished petroleum products. Their relative concentrations vary considerably from oil to oil (Wang et al., 2005; Yang et al., 2009; Philp et al., 1981). These bicyclic terpenoids probably have a microbiological source and are produced from the biodegradation of larger terpanes or are formed directly from bicyclic compounds of the same carbon framework (Dimmler et al., 1984; Alexander et al., 1984). These drimane skeleton compounds are resistant to slight to medium weathering, particularly from biodegradation (Wang et al., 2005; Yang et al., 2009; Peters et al., 2005). However, if evaporation has affected a fuel beyond *n*-C_{13}, then a relative depletion of the lower boiling $C_{14}H_{26}$ sesquiterpanes might be expected (Stout et al., 2005). Recently, sesquiterpane analysis was applied in oil spill correlation and identification (Williams et al., 1986; Wang

Table 4. *Quantitation results for bicyclic sesquiterpanes*

Compounds	AOS #1 (μg/g TSEM)	AOS #2 (μg/g TSEM)	AOS #3 (μg/g TSEM)	DOB (μg/g oil)	AHS (μg/g oil)	ASMB (μg/g oil)
C_{14} sesquiterpanes						
BS1	221	304	164	314	168	264
BS2	ND	78.0	ND	88.5	19.0	142
C_{15} sesquiterpanes						
BS3	63.3	281	ND	264	122	425
BS4	36.1	212	ND	198	87.0	313
BS5	639	667	509	644	364	1213
BS6	ND	223	ND	170	76.1	365
C_{16} sesquiterpanes						
BS7	48.8	93.8	ND	85.4	44.6	138
BS8	58.8	58.8	45.3	97.2	41.5	144
BS9	ND	ND	ND	46.7	14.2	75.8
BS10	556	696	620	668	365	844
Total sesquiterpanes	1763	2652	1339	2577	1302	3923
Diagnostic ratios						
BS1/BS5	0.35	0.46	0.32	0.49	0.46	0.22
BS3/BS5	0.10	0.42	NA	0.41	0.34	0.35
BS3/BS4	1.75	1.33	NA	1.33	1.40	1.36
BS4/BS5	0.06	0.32	NA	0.31	0.24	0.26
BS6/BS5	NA	0.33	NA	0.26	0.21	0.30
BS8/BS10	0.11	0.08	0.07	0.15	0.11	0.17
BS3/BS10	0.11	0.40	NA	0.40	0.33	0.50
BS5/BS10	1.15	0.96	0.82	0.96	1.00	1.44

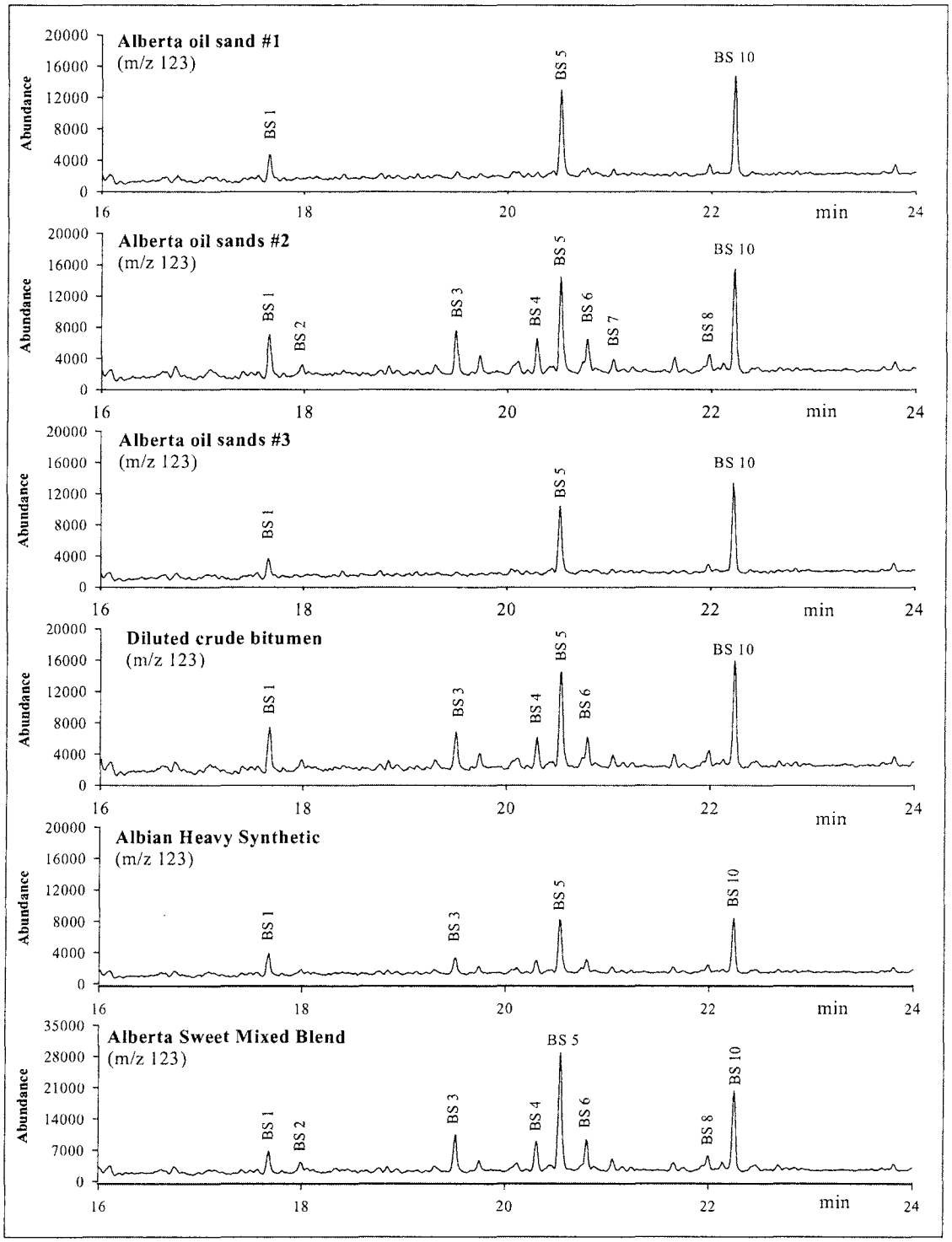

Figure 5. Gas chromatography/mass spectrometry (GC-MS) chromatograms of bicyclic sesquiterpanes (m/z 123) in Alberta oil sands samples.

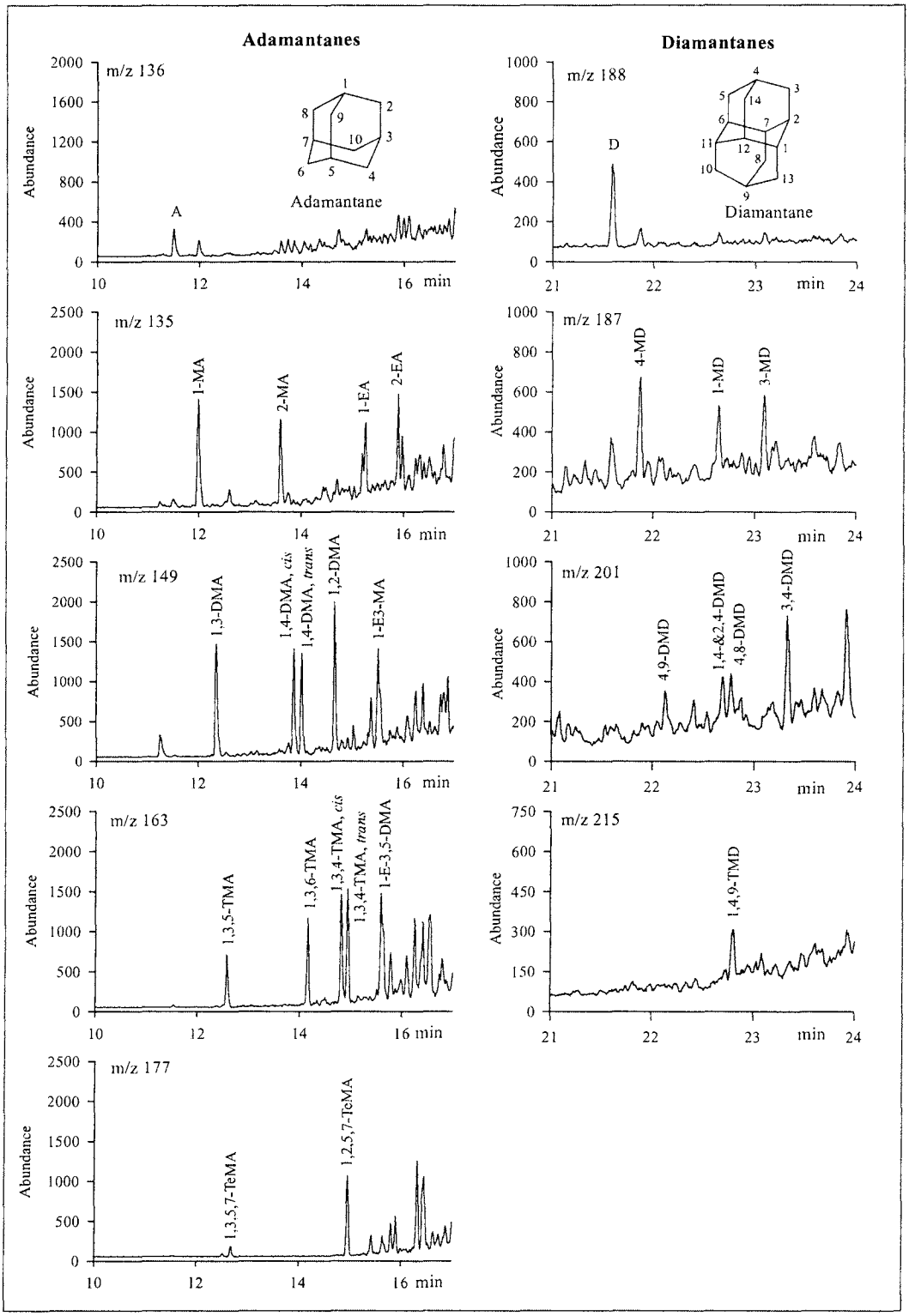

Figure 6. Gas chromatography/mass spectrometry (GC-MS) chromatograms of adamantanes (m/z 136, 135, 149, 163, and 177) and diamantanes (m/z 188, 187, 201, and 215) in Alberta oil sands #3.

Table 5. Quantitation results for diamondoid compounds

Compounds	AOS #1 (μg/g TSEM)	AOS #2 (μg/g TSEM)	AOS #3 (μg/g TSEM)	DOB (μg/g oil)	AHS (μg/g oil)	ASMB (μg/g oil)
Adamantanes						
A	3.79	3.45	3.18	9.15	5.91	7.69
1-MA	9.13	7.70	7.89	18.8	11.5	15.7
1,3-DMA	7.32	6.32	6.68	12.7	7.84	10.8
1,3,5-TMA	3.04	2.75	2.95	4.82	2.77	3.99
1,3,5,7-TeMA	0.65	0.55	0.60	0.93	0.56	0.85
2-MA	11.0	10.2	10.1	15.8	9.50	14.6
1,4-DMA, *cis*	5.70	5.16	5.54	8.26	4.86	7.39
1,4-DMA, *trans*	5.61	5.06	5.56	7.72	4.51	7.17
1,3,6-TMA	4.53	4.14	4.27	5.98	3.81	5.41
1,2-DMA	8.31	7.28	7.87	9.96	6.08	8.68
1,3,4-TMA, *cis*	5.72	5.42	5.96	6.95	5.09	6.96
1,3,4-TMA, *trans*	5.34	4.90	5.54	6.39	3.70	5.55
1,2,5,7-TeMA	3.83	3.64	3.91	4.63	2.87	3.89
1-EA	3.85	3.63	4.13	4.65	2.76	3.95
1-E-3-MA	8.18	7.58	8.20	5.73	5.95	8.98
1-E-3,5-DMA	8.92	8.49	9.48	10.6	7.21	7.41
2-EA	4.97	4.59	4.96	5.81	3.50	5.22
∑adamantanes	100	90.8	96.9	139	88.4	124
Diamantanes						
D	2.13	2.54	1.74	2.71	3.96	3.11
4-MD	1.53	1.36	1.39	1.48	1.98	1.99
4,9-DMD	0.61	0.64	0.62	0.44	ND	0.71
1-MD	0.57	0.55	0.85	0.94	ND	1.84
1,4- & 2,4-DMD	0.53	0.43	0.56	0.52	ND	0.59
4,8-DMD	0.56	0.45	0.55	0.55	ND	0.65
TMD	0.55	0.44	0.56	0.47	ND	0.69
3-MD	1.37	1.40	1.19	1.34	ND	1.91
3,4-DMD	2.06	1.82	1.71	1.57	ND	1.69
∑diamantanes	9.90	9.64	9.18	10.0	5.93	13.2
Total diamondoids	110	101	106	149	94.3	138
Diagnostic ratios						
1-MA/(1-MA+2-MA)	0.45	0.43	0.44	0.54	0.55	0.52
2-EA/(1-EA+2-EA)	0.56	0.56	0.55	0.56	0.56	0.57
1,4-DMA, cis/(cis+trans)	0.50	0.51	0.50	0.52	0.52	0.51
1,3,4-TMA cis/(cis+trans)	0.52	0.53	0.52	0.51	0.58	0.56
1-MA/(1-MA+4-MD)	0.86	0.85	0.85	0.85	0.85	0.89

et al., 2005). Analysis of bicyclic sesquiterpanes will provide another criterion to fingerprint oil sands in addition to PAHs and biomarkers.

A series of bicyclic terpenoids were determined in the Athabasca oil sand bitumen saturates (Dimmler et al., 1984; Strausz et al., 2010). GC-MS chromatograms (m/z 123) in Figure 5 clearly demonstrate the presence of bicyclic sesquiterpanes in all representative Alberta oils studied in this work despite the depletion of n-alkanes by biodegradation in oil sands and bitumen. These compounds are relatively low-boiling saturated hydrocarbons, usually eluting between *n*-C_{12} and *n*-C_{16}. The concentrations of 10 commonly recognized C_{14} to C_{16} bicyclic sesquiterpanes in Alberta oils are given in Table 4. The concentrations of 10 target sesquiterpanes in oil sands bitumen and Albian Heavy Synthetic range from 1000 to 2600 μg/g, which is at the same level as many other crude oils (Yang et al., 2009). Total bicyclic sesquiterpanes in conventional ASMB crude can reach as high as 3923 μg/g and are significantly higher than in bitumen. The selected ion chromatograms at m/z 123 show distribution patterns of BS10>BS5>BS1 for oil sands extract and Alberta oil sands bitumen, BS10 · BS5>BS1 for Albian Heavy Synthetic, and BS5>BS10>BS1 for ASMB, respectively. Based on Figure 5 and data presented in Table 4, the principal dominant bicyclic sesquiterpanes are BS10 (8β(H)-homodrimane, C_{16}), BS5 (8β(H)-drimane, C_{15}), BS1 (nordrimane, C_{14}), and BS3 (rearranged drimane, C_{15}), which together account for over 70% of all detected bicyclic sesquiterpanes. It is evident that bicyclic sesquiterpanes have been partially biodegraded in these heavy oil samples. 8β(H)-homodrimane is likely the most abundant homologue in oil sands extracts and the bitumen sample, which suggests that this bicyclic sesquiterpane has the least degradability among all 10 sesquiterpanes. This finding contrasts with the observation of Williams *et al.* (1986) that 8β(H)-homodrimane is more susceptible than 8β(H)-drimane in South Texas Eocene Jackson oils. This could possibly be due to the different geological conditions.

Characterization of Diamondoids

Diamondoids are a class of saturated hydrocarbons that consist of three-dimensionally fused cyclohexane rings, which result in

a diamond-like structure. The simplest diamondoid compound is adamantane ($C_{10}H_{16}$), followed by its pseudo-homologous series including diamantane ($C_{14}H_{20}$), triamantane, tetramantane, pentamantane, higher polymantanes, and their alkylated homologues. Diamondoid compounds occur widely in crude oils, mid-range distillate fuels, and finished petroleum products and their relative concentrations vary considerably from oil to oil (Yang et al., 2006; Wei et al., 2007). Crude oils from different sources have different signatures for both the absolute concentrations and relative distribution patterns of diamondoids (Yang et al., 2006).

Williams et al. (1986) reported that diamondoids demonstrated resistance to biodegradation in a severely degraded Canadian oil, in which pentacyclic triterpanes were almost completely demethylated. They thought that the degradation resistance of adamantanes is at least as strong as that of tricyclic terpanes and that the adamantane series should therefore be useful for correlating severely biodegraded oils. Wei et al. (2007) reported that the concentration of total diamondoids tends to decrease as the biodegradation rank of oil deposits increases. Adamantanes behave similarly with respect to their abundance because diamondoids are generally dominated by adamantanes. With increasing biodegradation, the concentration of diamantanes decreases only slightly.

GC-MS chromatograms of adamantanes and diamantanes in oil sands extract are shown in Figure 6. These diamondoids generally represent most of the lighter resolved peaks in the GC-FID chromatograms of the saturated hydrocarbon fraction in heavily biodegraded oils, for example, oil sands extracts in Figure 1. The concentration of adamantanes, diamantanes, and their homologues in Alberta oil sands samples studied are shown in Table 5. Both adamantanes and diamantanes occur in considerable quantities in all of the samples studied. Overall, the one-cage adamantanes in the heavy oil samples are in the concentration of 100 μg/g range, approximately ten times more abundant than two-cage diamantanes. Among the detected adamantanes, either 1-MA (bridgehead-substituted) or 2-MA is the most abundant homologue in all oil samples. Adamantanes in Table 5 and Figure 6 are likely unaffected by biodegradation in all oil samples although these oils were biodegraded in various extent. It was also found that all diagnostic ratios of target diamondoids except the methyl adamantane index [MAI, 1-MA/(1-MA+2-MA)] are almost identical for all six Alberta samples, which is possibly a result of blended diluent and/or the upgrading process of bitumen. Diamantane isomers generally occur in crude oils only in relatively low abundance, which could result in high measurement uncertainty in oil analysis. Diagnostic ratios associated with diamantanes should therefore be used cautiously as criteria.

Conclusions

This paper presents a study on the chemical fingerprints of raw Alberta oil sands, diluted crude bitumen, and Albian Heavy Synthetic derived from crude bitumen. The quantitatively characterized hydrocarbons include n-alkanes, target alkylated PAHs and other US EPA priority PAHs, biomarker terpanes and steranes, and bicyclic sesquiterpanes. Based on the results described in this paper, the following conclusions can be derived.

1. All three oil sands extracts have a similar gross composition. The chemical characteristics of diluted crude bitumen and Albian Heavy Synthetic are altered significantly due to blending with diluents or upgrading processing. One significant chromatographic feature of the Alberta oil sands and crude bitumen is the predominance of unresolved complex mixtures (UCMs).
2. All of the Alberta oil sands samples analyzed in this study show varying degrees of biodegradation. Normal alkanes and isoprenoid alkanes have been almost completely depleted by biodegradation and/or water washing.
3. Alkylated naphthalenes occur in relatively lower concentration in the oil sands studied. Albian Heavy Synthetic contains a high content of unsubstituted PAHs. A series of five alkylated PAHs present an increasing distribution profile of C_0- < C_1- < C_2- < C_3- in oil sands extracts and bitumen due to preferential biodegradation.
4. The oil sands extracts, oil sands bitumen, and Albian Heavy Synthetic show many similar chemical characteristics of biomarker terpanes. The terpanes are likely unaffected by biodegradation. These distinctive biomarker compositions and ratios indicate that they were generated from the same or very similar sources.
5. Bicyclic sesquiterpanes are present in all Alberta oil samples. 8β(H)-homodrimane and 8β(H)-drimane are the dominant bicyclic sesquiterpanes due to their high resistance to biodegradation.
6. Cage-like diamondoids were determined in significant abundancee in samples of Alberta oil sands. The distribution profiles of adamantanes likely remain unchanged in crude bitumen and Albian Heavy Synthetic. These small biomarkers are less susceptible to biodegradation and may be applicable in oil-oil correlation of severely biodegraded oils.

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APPENDIX D

EXPERIMENT EOS-1
Evaluation of TPH-Degrading Activity in Enbridge Oil Spill Site Sediments

1. Soil Samples

Three soil samples were tested in activity screening studies to assess TPH-degrading activity. These samples are summarized in Table 1.

Sample SERAS-017-0001 (Bucket #2 soil)

This site soil sample was obtained from Bucket #2 that was sent to SERAS. The bucket contents were originally collected from the bottom of a stream at the Enbridge Oil site. A sample of sediment was collected from the bucket, air-dried in a fume hood and sieved through a #10 SS sieve. The natural moisture content (NMC) of the composite sample was determined by drying the sample to constant weight at 95-105°C. These results are shown in Table 2.

Sample SERAS-017-0002 (Bucket #1 soil)

This site soil sample was obtained from Bucket #1 that was sent to SERAS. The bucket contents were originally collected from the bottom of a stream at the Enbridge Oil site. A sample of sediment was collected from the bucket, air-dried in a fume hood and sieved through a #10 SS sieve. The NMC of the composite sample was determined by drying the sample to constant weight at 95-105°C. These results are shown in Table 2.

SERAS-017-0001/0002 Composite Soil

A composite soil sample was prepared from samples SERAS-017-0001 (-0001) and SERAS-017-0002 (-0002). A 10-gram (g) (dry weight) aliquot of -0001 was mixed with a 10-g (dry weight) aliquot of -0002 in a stainless steel mixing bowl and stored in an amber glass bottle at room temperature until used. The NMC of the soil composite was mathematically determined. The sample inoculum was referred to as EN-SED

CUC-PP Soil Composite (Work Assignment SERAS-135)

This sample was obtained from the original batch of a soil composite used in a bench-scale solid-phase study. The composite sample consisted of samples CUC-01, CUC-02, CUC-03, CUC-06 and CUC-08. The composite was prepared on 6/21/11. The sample inoculum was referred to as CUC-SOIL. Bench-scale solid-phase studies showed that this soil exhibited potent TPH-degrading activity.

The NMC of the composite sample was determined by drying the sample to constant weight at 95-105°C. These results are shown in Table 2.

Determination of Soil Weight to Inoculate Test Flasks

Determine the soil inoculum weight (wet weight) for flask inoculation. Enter results in Table 3.

2. Screening Medium

Prepare the mineral salts medium shown in Table 4. This medium is a modified Bushnell Haas mineral salts (BH-MS) medium that was amended with 0.05% Tween 80 (BH-MS-T medium).

Dispense medium in 500-milliliter (mL) aliquots into 1,000-mL solution bottles.

Sterilize the medium at 121°C for 15 minutes.

Cool medium to room temperature and add 0.50 mL 0.5% $FeCl_3 \cdot 6H_2O$ stock solution to each bottle (filter-sterilize stock solution). Mix.

Set volume to 500 mL with sterile deionized water. Mix.

3. Oil Source

Two sources of oil were used: (1) SERAS-017-0003 (referred to as -0003) and SERAS-017-0004 (referred to as -0004). The samples were sent to SERAS from the Enbridge Oil site.

-0003 oil was used "as is" and was not sterilized before use. -0004 oil was sedimented in a centrifuge to remove particulates prior to use. Like -0003 oil, it was not sterilized.

4. Oil Addition to Sterile Flasks

Eighteen 250-mL flasks were plugged with foam plugs and sterilized at 121°C for 15 minutes and dried in a hot air oven (100°C for 2 hours).

Using disposable sterile 10-µL inoculating loops, a 0.20 g aliquot of oil was transferred ("dabbed") onto the bottom surface of each flask. The oil weight was confirmed by weighing the unamended flask and "dabbing"oil into the flask until the desired weight had been achieved. One-µL inoculating loops were occasionally used to remove small quantities of oil to achieve the desired oil weight. The oil concentration in each flask was 4,000 ppm.

-0003 oil was added to 6 labeled flasks while -0004 soil was added to 12 labeled flasks.

5. Setup of Screening Flasks for Inoculation

After the addition of the designated TPH to labeled flasks, each flask was then amended with 50 mL of BH-MS-T medium.

The flasks were labeled in three sets of 6 flasks. Each set was labeled in duplicate with the source of oil tested (-0003 or -0004 oil), the soil inocula used (EN-SED or CUC-SOIL) and the culture age at harvest (Days 0A/0B, Days 14A/14B and Days 28A/28B). Flask contents were collected on the date of harvest and samples prepared. The organization of flasks is summarized in Table 5.

6. Inoculation of Test Flasks/Preparation of Matrix (M)/Matrix Spike (MS)/Matrix Spike Duplicate (MSD) flasks

BH-MS-T-0003 Oil Medium Inoculation

Weigh 1.00 g (dry weight) EN-SED soil and add to 6 respective flasks containing BH-MS-T-0003 oil.

BH-MS-T-0004 Oil Medium Inoculation

Weigh 1.00 g (dry weight) EN-SED soil and add to 6 respective flasks containing BH-MS-T-0004 oil medium.

Weigh 1.00 g (dry weight) CUC-SOIL soil and add to 6 respective flasks containing BH-MS-T-0004 oil medium.

Preparation of M/MS/MSD Flasks

Prepare 3 respective flasks containing BH-MS-T-0004 oil medium. Label respective flasks as M, MS and MSD.

7. Soil Screening Study

Harvest the Day 0 samples according to instructions in Section 8.

Place the Days 14 and 28 flask sets on the gyratory shaker set at 30°C and 200 rpm and incubate for 28 days with samples collected at Days 14 and 28.

Monitor the flasks daily for microbial growth (turbidity) and for effects on suspended or caked oil removal at the medium interface in the flask.

Using a plugged 250-mL flask containing 50-mL deionized water, determine the rate of evaporation in the flask after 3 days **(3 mL)** and 7 days **(5mL)** incubation.

Add 5 mL sterile deionized water to test flasks on a weekly basis until harvested.

Evaporation was also monitored by weighing the flasks at 7-day intervals. The flask tare weight plus 0.2 g of oil is a known value. Addition of 50 mL (assume 50 g) of medium provides a starting weight when the flasks are placed on the shaker. The volume loss of approximately 5 mL/week was confirmed by this manner. It was also confirmed that the 50-mL volume from a 50-mL disposable pipet can be off by as much as 5% (2 to 2.5 mL).

8. Preparation of Samples for Oil Analysis

- Decant the medium from the flask into a labeled 4-oz. amber glass bottle fitted with a Teflon-lined cap.
- Rinse/flush the flask surfaces with four 2.5 mL methylene chloride rinses using a 5-mL disposable glass pipet. Transfer each solvent rinsate to the sample bottle. Use additional solvent rinses if necessary. Terminate the rinsing procedure when the rinsate is clear.
- Wash flask surfaces with three 5-mL deionized water rinses. Use the same 5-mL glass pipet (used in solvent rinses) to thoroughly wash flask surfaces. Transfer each aqueous rinsate to the sample bottle. Collect as much of the heavy sediment particles as possible without plugging the pipet.
- Add 6.5 mL of 37% volume/volume (v/v) formaldehyde to the sample bottle. Mix.
- Prepare the remainder of the Day 0 samples in a similar fashion.
- Prepare samples from the Day 14 and Day 28 flask sets in a similar fashion.
- Store the samples at 4°C until submitted for TPH analysis. The Chain of Custody numbers are recorded in Table 6.

9. TPH Degradation

- Submit the 18 samples for Total Petroleum Hydrocarbon (TPH) analysis and score the results as mg TPH/flask.
- Average the duplicate samples and determine the extent of TPH removal by the following equation.

$$\text{Degradation (\%)} = \frac{\text{Avg. Day 0 TPH conc.} - \text{Avg. (Day 14 or Day 28) TPH conc.}}{\text{Avg. Day 0 TPH conc.}} \times 100$$

- Enter results in Table 7 and determine the susceptibility of -0003 and -0004 oil to biodegradation. Determine the potential of EN-SED and CUC-SOIL microbial populations to degrade -0003 and -0004 oil sources.

TABLE 1
Samples Tested in the TPH-Degrading Activity Screening Study
Evaluation of TPH-Degrading Activity in Enbridge Oil Spill Site Sediments
Enbridge Oil Spill Site
Marshal, Michigan
March 2012

Soil Sample	Sample #	Sample Inoculum	Site Location
River Sediment	SERAS-017-0001	-0001	Enbridge Oil Site - Bucket #2 Sediment
River Sediment	SERAS-017-0002	-0002	Enbridge Oil Site - Bucket #1 Sediment
River Sediment Composite	SERAS-017-0001/-0002	EN-SED	Enbridge Oil Site - Composite of Sediment Samples SERAS-017-0001 and SERAS-017-0002
Bench-Scale Solid-Phase (BSSP) Soil Composite	SERAS-135-BSSP Composite	CUC-SOIL	CUC-PP Bioremediation Site

TABLE 2
Calculation of Natural Moisture Content in Test Soil
Evaluation of TPH-Degrading Activity in Enbridge Oil Spill Site Sediments
Enbridge Oil Spill Site
Marshal, Michigan
March 2012

Vial #	Sample #	Location	Vial Wt. (g)[1]	Vial Wt. + Wet Soil (g)	Soil Wet Wt. (g)	Pan Wt. + Dry Soil (g)	Soil Dry Wt. (g)	Water Wt. (g)	NMC (%)[2]	Avg. NMC (%)	Solids (%)	Avg. Solids (%)
1A	-0001	Bucket #2	24.56	29.56	5.00	29.33	4.77	0.23	4.6	4.2	95.4	95.8
1B			24.48	29.48	5.00	29.29	4.81	0.19	3.8		96.2	
2A	-0002	Bucket #1	24.60	29.60	5.00	28.89	4.29	0.71	14.2	13.9	85.8	86.1
2B			24.31	29.31	5.00	28.63	4.32	0.68	13.6		86.4	
3A	EN-SED	(-0001:-0002) (1:1) (wt.:wt.)	-	-	22.04	-	20.00	-	-	-	-	90.7
3B			-	-		-		-	-		-	
4A	CUC-SOIL	BSSP Soil Composite	24.58	26.58	2.00	26.44	1.86	0.14	7.0	7.0	93.0	93.0
4B			24.55	26.55	2.00	26.41	1.86	0.14	7.0		93.0	

[1]g - gram
[2]% - percent

TABLE 3
Calculation of the Soil Inoculum Weight for Test Flasks
Evaluation of TPH-Degrading Activity in Enbridge Oil Spill Site Sediments
Enbridge Oil Spill Site
Marshal, Michigan
March 2012

Sample Inoculum	Soil Inoculum Dry Wt. (g)[1]	Solids (%)[2]	Soil Inoculum Wet Wt. (g)
EN-SED	1.00	90.7	1.10
CUC-SOIL	1.00	93.0	1.08

[1] g - gram
[2] % - percent

TABLE 4
Nutrient Medium Used in Screening TPH-Degrading Activity in Enbridge Oil Spill Site Sediments
Evaluation of TPH-Degrading Activity in Enbridge Oil Spill Site Sediments
Enbridge Oil Spill Site
Marshal, Michigan
March 2012

Ingredient	Conc. (g/L)[1]	Conc. (mL/L)[2]	Conc. (g/50 mL)	Stock Solution (%)
-0003 or -0004 Oil[3]	-	-	0.20	-
Tween 80	-	0.50	-	-
NH_4NO_3	1.0	10.0	-	10.0
KH_2PO_4	1.0	10.0	-	10.0
K_2HPO_4	1.0	10.0	-	10.0
$CaCl_2 \cdot 2H_2O$	0.020	10.0	-	0.20
$MgSO_4 \cdot 7 H_2O$	0.20	10.0	-	2.0
$FeCl_3 \cdot 6 H_2O$	0.005	1.0	-	0.50

[1] g/L - grams per liter
[2] mL/L - milliliters per liter
[3] The oil sources were used "as is" and were not sterilized.

INSTRUCTIONS

1. Add nutrients based on a 1,000-mL volume to 900 mL of deionized water
2. Add 0.50 mL Tween 80; flush out pipet until all of the detergent is removed.
3. Adjust pH to 7.1 with 1.0 N sodium hydroxide solution.
4. Set volume at 1,000 mL with deionized water. Mix.
5. Dispense in 500-mL aliquots in 1,000-mL solution bottles. Sterilize at 121°C for 15 minutes. Cool.
6. Weigh 0.10 g $FeCl_3 \cdot 6H_2O$ in a 25-mL beaker and dissolve in 15 mL deionized water.
7. Transfer solution to 50-mL graduated cylinder, set volume at 20 mL. Mix. Syringe-filter sterilize the solution into a sterile pyrex test tube.
8. Add 0.50 mL of the Fe stock solution to each bottle and set volume to 500 mL with sterile deionized water.
9. Sterilize (18) 250-mL flasks at 121°C for 15 minutes. Oven-dry flasks for 2 hours at 100°C.
10. Dab 0.20 grams -0003 or -0004 oil into each of 18 flasks.
11. Aseptically dispense medium in 50 mL aliquots into 250-mL flasks (x18).
12. Label flasks in sets of 6 based on soil inoculum (EN-SED/CUC-SOIL), oil source (-0003/-0004) and age (Days 0, 14, and 28).
13. Seal the flasks with parafilm and place in plastic resealable bags until used.

TABLE 5
Organization of Screening Flasks
Evaluation of TPH-Degrading Activity in Enbridge Oil Spill Site Sediments
Enbridge Oil Spill Site
Marshal, Michigan
March 2012

Flask #	Oil Source	Soil Inocula	Sample Description
1	-0003	EN-SED	Day 0A
2	-0003	EN-SED	Day 0B
3	-0003	EN-SED	Day 14A
4	-0003	EN-SED	Day 14B
5	-0003	EN-SED	Day 28A
6	-0003	EN-SED	Day 28B
7	-0004	EN-SED	Day 0A
8	-0004	EN-SED	Day 0B
9	-0004	EN-SED	Day 14A
10	-0004	EN-SED	Day 14B
11	-0004	EN-SED	Day 28A
12	-0004	EN-SED	Day 28B
13	-0004	CUC-SOIL	Day 0A
14	-0004	CUC-SOIL	Day 0B
15	-0004	CUC-SOIL	Day 14A
16	-0004	CUC-SOIL	Day 14B
17	-0004	CUC-SOIL	Day 28A
18	-0004	CUC-SOIL	Day 28B

TABLE 6
Chain of Custody Identification Sheet
Evaluation of TPH-Degrading Activity in Enbridge Oil Spill Site Sediments
Enbridge Oil Spill Site
Marshal, Michigan
March 2012

Chain of Custody #	Sample Location	Oil	Soil Inocula	Age (Days)
SERAS-017-0007	275-78-10	-0003	EN-SED	Day 0A
SERAS-017-0008	275-78-11	-0003	EN-SED	Day 0B
SERAS-017-0009	275-78-12	-0004	EN-SED	Day 0A
SERAS-017-0010	275-78-13	-0004	EN-SED	Day 0B
SERAS-017-0011	275-78-14	-0004	CUC-SOIL	Day 0A
SERAS-017-0012	275-78-15	-0004	CUC-SOIL	Day 0B
SERAS-017-0013	275-78-16	-0004	M/MS/MSD	Day 0A/0B/0C
SERAS-017-0014	275-82-07	-0003	EN-SED	Day 14A
SERAS-017-0015	275-82-08	-0003	EN-SED	Day 14B
SERAS-017-0016	275-82-09	-0004	EN-SED	Day 14A
SERAS-017-0017	275-82-10	-0004	EN-SED	Day 14B
SERAS-017-0018	275-82-11	-0004	CUC-SOIL	Day 14A
SERAS-017-0019	275-82-12	-0004	CUC-SOIL	Day 14B
SERAS-017-0020	275-89-06	-0003	EN-SED	Day 28A
SERAS-017-0021	275-89-07	-0003	EN-SED	Day 28B
SERAS-017-0022	275-89-08	-0004	EN-SED	Day 28A
SERAS-017-0023	275-89-09	-0004	EN-SED	Day 28B
SERAS-017-0024	275-89-10	-0004	CUC-SOIL	Day 28A
SERAS-017-0025	275-89-11	-0004	CUC-SOIL	Day 28B

TABLE 7
Evaluation of TPH-Degrading Activity in EN-SED and CUC-SOIL Composite Samples
Evaluation of TPH-Degrading Activity in Enbridge Oil Spill Site Sediments
Enbridge Oil Spill Site
Marshal, Michigan
March 2012

Soil Inoculum	Flask	Oil Source	TPH[1] Concentration (mg/flask)[2] with Time (Days)								
			Day 0			Day 14			Day 28		
			TPH Conc. (mg/flask)	Avg. TPH Conc. (mg/flask)	Biodeg.[3] (%)[4]	TPH Conc. (mg/flask)	Avg. TPH Conc. (mg/flask)	Biodeg. (%)	TPH Conc. (mg/flask)	Avg. TPH Conc. (mg/flask)	Biodeg. (%)
EN-SED	A	-0003	192	215	0.0	73.1	75	65.1	65.9	64	70.2
	B		237			77.0			62.6		
EN-SED	A	-0004	175	186	0.0	72.7	78	58.1	65.5	66	64.5
	B		196			84.2			66.3		
CUC-SOIL	A	-0004	172	175	0.0	77.7	75	57.1	68.3	68	61.1
	B		178			71.8			67.5		

[1]TPH - Total Petroleum Hydrocarbons
[2]mg/flask - mg TPH per flask
[3]Biodeg. - biodegradation
[4]% - percent

TABLES

Table 1.0: Results of theTPH in Soil Analysis by GC/MS
Enbridge Oil: WA# 0-017
TPH as DRO+ORO (Total TPH} and Based on Dry Weight in Sediment

Method: SERAS SOP 1841

Sample No.	Sampling Location	GC/MS File	Conc. (mg/Kg)	RL (mg/Kg)
Soil Blank	1200012-BLK1	SL02672	U	1.67
SERAS-017-0001	275-44-21	SL02674	129	1.67
SERAS-017-0002	275-45-24	SL02829	194	1.67

Samples from COC#s: SERAS-017-02/01/12-0001
SERAS-017-02/08/12-0002

Table 2.0: Results of the TPH in Water Analysis by GC/MS
Day 0 Biodegradation Study Samples: Enbridge Oil: WA# 0-017
TPH Calculated from Site Specific Oil and Reported as Total Miligrams (mg) Extracted

Method: SERAS GC/MS SOP 1841

Sample No.	Sampling Location	GC/MS File	Conc. *Total mg*	RL *Total mg*
03/22/12 Sequence				
Water Blank	1200039-BLK1	SL02967	U	1.00
SERAS-017-0007	275-78-10	SL02969	192	20.00
SERAS-017-0008	275-78-11	SL02970	237	20.00
SERAS-017-0009	275-78-12	SL02971	175	20.00
SERAS-017-0010	275-78-13	SL02972	196	20.00
SERAS-017-0011	275-78-14	SL02973	172	20.00
SERAS-017-0012	275-78-15	SL02974	178	20.00
SERAS-017-0013	275-78-16	SL02975	188	20.00

Samples from COC#: SERAS-017-03/15/12-0005

Table 3.0: Results of the TPH in Water Analysis by GC/MS
Day 14 Biodegradation Study Samples: Enbridge Oil: WA# 0-017
TPH Calculated from Site Specific Oil and Reported as Total Miligrams (mg) Extracted

Method: SERAS GC/MS SOP 1841

Sample No.	Sampling Location	GC/MS File	Conc. *Total mg*	RL *Total mg*
03/23/12 Sequence				
Water Blank	1200040-BLK1	SL02981	U	1.00
SERAS-017-0014	275-82-07	SL02982	73.1	20.00
SERAS-017-0015	275-82-08	SL02983	77.0	20.00
SERAS-017-0016	275-82-09	SL02984	72.7	20.00
SERAS-017-0017	275-82-10	SL02985	84.2	20.00
SERAS-017-0018	275-82-11	SL02986	77.7	20.00
SERAS-017-0019	275-82-12	SL02987	71.8	20.00

Samples from COC#: SERAS-017-03/22/12-0006

Table 3a: Results of the TPH Analysis by GC/MS & Gravimetric Analysis
Select Day 0 & 14 Samples: Enbridge Oil: WA# 0-017
TPH Calculated from Site Specific Oil & Determined Gravimetrically
and Reported as Total Miligrams (mg) Extracted

Method: SERAS GC/MS SOP 1841		GC/MS Calculated Conc.	Gravmetric Calculated Conc.	RL
Sample No.		*Total mg*	*Total mg*	*Total mg*
SERAS-017-0007	Day 0	192	183	20.00
SERAS-017-0014	Day 14	73.1	136	20.00
SERAS-017-0013	Day 0	188	182	20.00
SERAS-017-0019	Day 14	71.8	148	20.00

Table 4.0: Results of the TPH in Water Analysis by GC/MS
Day 28 Biodegradation Study Samples: Enbridge Oil: WA# 0-017
TPH Calculated from Site Specific Oil and Reported as Total Miligrams (mg) Extracted

Method: SERAS GC/MS SOP 1841

Sample No.	Sampling Location	GC/MS File	Conc. *Total mg*	RL *Total mg*
04/18/12 Sequence				
Water Blank	1200055-BLK1	SL03053	U	1.00
SERAS-017-0020	275-89-06	SL03056	65.9	20.00
SERAS-017-0021	275-89-07	SL03057	62.6	20.00
SERAS-017-0022	275-89-08	SL03058	65.5	20.00
SERAS-017-0023	275-89-09	SL03059	66.3	20.00
SERAS-017-0024	275-89-10	SL03060	68.3	20.00
SERAS-017-0025	275-89-11	SL03061	67.5	20.00

Samples from COC#: SERAS-017-04/06/12-0007