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Standardization of an Analytical Method to Differentiate Petrogenic and Biogenic Inputs in Contaminated and Background Soils

September 2012

Emergencies Science and Technology Section (ESTS)

Science and Technology Branch

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by

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This report presents a methodology to determine petroleum hydrocarbons (PHCs) in soil and sediment samples by gas chromatography-flame ionization detection (GC/FID) and also to investigate the identification and differentiation of the presence/absence of petrogenic and biogenic compounds by gas chromatography-mass spectrometry (GC/MS). This report also provides an interlaboratory method validation participated by six environmental analytical laboratories.

The findings and conclusions presented by the authors are their own and do not necessarily reflect the views of **Petroleum Technology Alliance Canada (PTAC)**. *Mention of trade names or commercial products does not constitute endorsement for use.*

The current studies are solely in the interest of advancing the analytical sciences for measurement of hydrocarbons in soils and sediments. The results of the present research efforts are of scientific interest and should not be taken as policy or recommendations of Environment Canada.

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PART I

METHOD

Determination of Total Petroleum Hydrocarbons and Differentiation of Petrogenic and Biogenic Inputs in Environmental Samples

Determination of Total Petroleum Hydrocarbons and Differentiation of Petrogenic and Biogenic Inputs in Environmental Samples

Oil Research Laboratory
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1 INTRODUCTION

"Total petroleum hydrocarbons" (TPHs) or "petroleum hydrocarbons" (PHCs) are one of the most widespread soil pollutants in Canada, North America, and worldwide. Clean-up of PHC-contaminated soils and sediments costs the Canadian economy hundreds of million of dollars annually. Much of this activity is driven by the need to meet regulated levels of PHC in soil.

In the environment, soil contamination generally originates from three main sources: biogenic, pyrogenic and petrogenic hydrocarbons. Biogenic substances are produced by organisms or generated from naturally occurring organic matter. These naturally occurring biogenic organic compounds (BOCs) are usually non-toxic and less hazardous than those from petrogenic and pyrogenic sources. BOCs present in soils and wet sediments can be easily misidentified and quantified as regulated PHCs during analysis using such methods for PHC determination. In some cases, biogenic interferences can exceed regulatory levels, resulting in unnecessary and costly remediation measures, while also wasting valuable landfill space. Therefore, it is critically important to characterize and differentiate PHCs and BOCs in contaminated sediments in PHC analysis.

This method describes a procedure for determining hydrocarbon constituents in soil or sediment samples by Soxhlet extraction or other suitable extraction techniques, silica-gel column cleanup, followed by gas chromatographic analysis. However, this method may be applied to liquid environmental samples, provided that the samples are extracted by appropriate techniques. Total petroleum hydrocarbons (PHCs) are quantitatively determined using gas chromatograph-flame ionization detection (GC/FID). In addition, a series of target hydrocarbons including *n*-alkanes, polycyclic aromatic hydrocarbons (PAHs), petroleum biomarkers including bicyclic sesquiterpanes (see list of analytes in Appendix Table 1) are investigated for identification and differentiation of the presence/absence of petrogenic and biogenic compounds in soil and sediment samples by gas chromatography-mass spectrometry (GC/MS). This method does not aim to quantitatively allocate the contribution of each source to PHCs in the sample.

The method reporting limits are <50 μg/g for total petroleum hydrocarbons (based upon extraction of 5.0 g soil in dry weight, or 10 g soil in wet weight, a final pre-injection extract volume of 1.0 mL, and a sample extract injection volume of 1.0 μL to GC).

This method is restricted to use by or under the supervision of analysts experienced in the use and interpretation of Gas Chromatography with Flame Ionization Detection (GC/FID) and GC coupled with mass spectrometry (GC/MS).

2 PRINCIPLE OF THE METHOD

Prior to extraction, the soil or sediment samples are spiked with appropriate surrogates. The sample is then extracted by Soxhlet extraction or other techniques with *n*-hexane/acetone (1:1, V:V) for 16 to 24 hours.

The extract is properly concentrated and is made up with *n*-hexane to an appropriate volume, i.e., 10 mL. 1.0 mL of the final concentrated extract is taken to determine the total solvent extractable materials (TSEM). Based on the TSEM values, an appropriate volume of aliquot of the concentrated extract (containing maximum 50 mg of TSEM; 20 mg is optimal) is quantitatively transferred to a 3 g silica gel clean-up column to remove polar components and other interferences. The column is eluted with 15 mL of mixture of *n*-hexane/dichloromethane (1:1, V:V).

The solution is concentrated to an appropriate volume, i.e., 2 mL. Internal standards are then added, and made up to the pre-injection volume, depending upon the concentrations of hydrocarbons in the solution. The solution is analyzed by GC/FID for total petroleum hydrocarbons. If the PHC value from GC/FID analysis exceeds a regulated PHC value, GC/MS analysis for *n*-alkanes, polycyclic aromatic hydrocarbons (PAHs), and petroleum biomarkers including bicyclic sesquiterpanes may be conducted to verify the presence or absence of petrogenic source hydrocarbons.

3 INTERFERENCES

Method interferences may be caused by contaminants in solvent reagents, on glassware, and on other sample processing hardware. All of these possible contamination sources must be routinely demonstrated to be free from interferences by performing laboratory method blanks.

Matrix interferences may be caused by contaminants co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the sample.

The silica gel clean-up procedure is useful to overcome many of these interferences. Some naturally occurring hydrocarbons may not be removed by the silica gel column cleanup and will be detected by this method. Acetone should be removed prior to silica-gel column cleanup in order to reduce the matrices effect and maintain the column efficiency.

Target compounds are detected by GC/MS monitoring selected ions. Other non-target compounds exhibiting the same ions may co-elute in the same window producing positive interferences.

4 SAFETY

All extraction and column clean-up operations should be carried out in fumehood.

Rinsing and cleaning of equipment must be done in a well-ventilated area with the operator using appropriate skin- and eye-protection.

This method does not address all safety issues associated with its use. Analysts should refer to the appropriate material safety data sheets, where available, for more information on health effects. If the toxicity or carcinogenicity of any reagent used in this method is not available, it must be treated as a potential health hazard. Exposure to these chemicals must be reduced to the lowest possible level by every means available.

The laboratory is responsible for maintaining a current awareness file of regulations regarding the safe handling of the chemicals specified in this method.

5 SAMPLE REQUIREMENTS

Recommended sample containers include clean glass bottles and jars with polytetrafluoroethylene (PTFE) lined caps. Soil or sediment samples are stored in a refrigerator at 5±3ºC. Shelf life of sample petroleum hydrocarbons depends on the molecular weights of individual hydrocarbons, and on matrix and biological activities associated with the samples. It is recommended to extract oily sediment and soil samples within 14 days. The samples could be stored in a freezer (at or lower than -20ºC) for up to 90 days before extraction.

6 EQUIPMENT, REAGENTS AND SUPPLIES

Equipments and materials used in this method may be replaced by equivalent products, and similar operation parameters are acceptable, as long as the method performance and validation meet all the criteria (see section 8 and section 9).

6.1 Solvents

All solvents, including acetone, and hexane and dichloromethane, should be free of interference.

6.2 Small Apparatus

All glassware should be proofed to be free of interference.

6.2.1 Soxhlet extractor kits:

Soxhlet extractors (250 to 1,000 mL capacity) and appropriate condensers, and disposable extraction thimbles. The extraction thimbles should be proven free of interference on a batch basis.

6.2.2 Evaporative Concentrators:

Rotary evaporator, dry-nitrogen “blow-down” device, or equivalent.

6.2.3 Analytical balance:

Must be capable of accurately weighing 0.0001 g. The balance must be properly calibrated and maintained.

6.2.4 Centrifuge tubes:

Calibrated, 15 mL, graduated, with a ground-glass stopper, or equivalent.

6.2.5 Graduated Cylinders:

10 mL, 50 mL and 100 mL.

6.2.6 Pipettes or equivalent:

Electronic or mechanical pipettes with capacities from 10 to 1000 µL. Pipettes must be calibrated and maintained according to relevant standard operating procedure (SOP).

6.3 Sample Clean-up Column

300 mm long × 10.5 mm ID plugged with Pyrex glass wool at the bottom and a PTFE stopcock. Fritted glass discs are not recommended because they are difficult to clean after highly concentrated extracts have been passed through.

6.4 Silica Gel, Sodium Sulphate and Glass Wool

6.4.1 Silica gel

6.4.1.1 Silica gel recommended is 100-200 mesh, pore size 150 Å, active surface 320 m^2/g. Prior to use, the silica gel should be cleaned by solvent rinsing.

6.4.1.2 Silica gel is poured into a pre-cleaned column and successively eluted with one column volume of acetone, *n*-hexane and dichloromethane. The solvents are discarded.

6.4.1.3 Then silica gel is air-dried completely in fumehood followed by proper activation, for example, for at least 20 hours at 160-180°C in a shallow glass tray, loosely covered with foil.

6.4.1.4 The silica gel is activated and stored in the oven at a temperature of 160-180 °C for use.

6.4.2 Sodium sulphate

6.4.2.1 Sodium sulphate should meet A.C.S. reagent [7757-82-6] specifications, and must be granular and anhydrous. Prior to use, the sodium sulphate should be cleaned by solvent rinsing to be free of interference.

6.4.2.2 Cleaned sodium sulphate is stored in the oven at a temperature of 180 ± 20°C for use.

6.4.3 Glass wool

6.4.3.1 Prior to use, the glass wool should be cleaned to be free of interference.

6.5 Gas Chromatograph with Flame Ionization Detector (GC/FID)

The complete GC system is composed of autosampler, split/splitless injector, capillary column and flame ionization detector. The signal output of GC is connected to a data acquisition and data analysis system.

The GC column in the GC/FID for PHC determination should be 100% polydimethylsiloxane or 5% phenyl-substituted phase. The recommended column is 30 m long × 0.25 mm ID, 0.1 μm film thickness capillary DB-5HT or equivalent.

6.6 Gas Chromatograph with Mass Spectrometer (GC/MS)

The mass spectrometer is operated in selected ion monitoring (SIM) mode using a 70 eV electron impact ionization mode and producing a qualified mass spectrum. A computer system, interfaced to the mass spectrometer, is used for system control as well as data acquisition, storage and data processing.

The GC column used in the GC/MS system for analyses of the target analytes should be at least 30 m long × 0.25 mm ID, 0.25 μm film thickness capillary, e.g. 5% phenyl containing phase HP-5MS or equivalent.

6.7 Standards and Reference Materials

Standard reference and calibration materials are to be purchased from established suppliers. The following standard materials are used in this method:

PHC Internal Standard (optional):	5α-androstane
PHC Calibration Standard:	RESTEK 31614, Connecticut ETPH Calibration Mixture, or equivalent standards containing at least *n*-C_{10}, *n*-C_{16} and *n*-C_{34}.
PHC Surrogate:	*ortho*-terphenyl
Oil Analysis Internal Standards (optional):	d_{14}-terphenyl
PAH Surrogates:	d_8-naphthalene, d_{10}-acenaphthene, d_{10}-phenanthrene, d_{12}-benz(a)anthracene, and d_{12}-perylene. Above deuterated PAHs are reommended, other appropriate compounds are acceptable.
Verification Standard:	ASTM 5442 (C_{12}-C_{60} analytical standards, or equivalent Prudhoe Bay 13.1% evaporated crude oil (*ESTS internal laboratory standard*), or other verification materials upon their validation.

6.7.1 Calibration standards for GC/FID PHC determinations

Calibration standards are prepared from the *n*-alkane stock standard solution by serial dilution. A typical series of calibration solutions is: 1.0, 10, 20, 50, and 100 μg/mL. Internal standard 5α-androstane and surrogate

ortho-terphenyl should be added at concentrations of 20 μg/mL to all calibration standards. All solutions are to be made up in *n*-hexane.

6.7.2 Calibration standards for GC/MS oil analysis

Calibration standards are prepared from purchased certified stock standard PAH solutions to cover, at a minimum, the concentration range of 0.05 to 20 μg/mL. Internal standard and surrogate compounds should be added at the same concentrations, i.e. 1 μg/mL, to all standard levels. All PAH calibration standards are to be made up in *n*-hexane.

6.7.3 Surrogate for PHC determinations

A stock solution of 200 μg/mL *ortho*-terphenyl is prepared from the pure solid or diluted from commercial standard solution.

6.7.4 Surrogates for PAH determinations

Stock solutions are prepared at 10 μg/mL each for the deuterated PAHs, such as d_8-naphthalene, d_{10}-acenaphthene, d_{10}-phenanthrene, d_{12}-benz(a)anthracene and d_{12}-perylene from the commercially-obtained standards.

6.7.5 Internal standard for PHC determinations

The recommended internal standards for PHC analysis is 5α-androstane. A stock solution of 200 μg/mL for 5α-androstane is prepared in *n*-hexane from the pure compound or diluted from commercial standard solution.

6.7.6 Internal Standards for Oil Fingerprinting Analysis

A stock solution of 10 μg/mL d_{14}-terphenyl is prepared in *n*-hexane from the commercially-obtained standards.

6.7.7 Verification standard for PHC determinations

A PHC verification standard of concentration 80 mg/mL is prepared from the reference oil.

6.7.8 Handling, storage and lifetime of standards and reference materials

Standards and other reference materials require special attention and care for use in the laboratory refer to relevant SOPs.

The expiration date of a standard reference material is that given by the certified supplier. If the certified supplier does not give an expiration date on the compound, then an expiration period of ten years from receipt of the compound is used.

For initial-dilution standard reference material stock solutions, an expiration period of ten years is used.

For multiple-dilution and daily-use standard and reference solutions, an expiration period of three years is used.

7 PROCEDURE

7.1 Soxhlet Extraction

Take 5 to 10 g of the sample (depending on the hydrocarbon content of the sample), record the weight to the nearest 0.01 g. Completely transfer the sample to a pre-cleaned Soxhlet thimble.

Spike the sample with the surrogate standards, 100 μL of 200 ppm *ortho*-terphenyl and 100 μL of the mixture containing five deuterated PAHs, 10 ppm each (see section 6.7.4), cover with a small amount of clean glasswool, and then transfer the thimble and contents to a clean Soxhlet body.

Extract the sample overnight (approximately 16 to 24 hours) with approximately 200 mL of *n*-hexane/acetone (1:1, V:V) (1 or 2 clean boiling chips in the extraction flask may be used to prevent extract bumping), a rate of 3 to 5 cycles per hour. Allow extract to cool when the extraction is complete.

The extract is washed twice by appropriate volume of interference-free water depending on the extract volume to remove acetone in the extract. Dry the extract with sufficient anhydrous sodium sulphate. Concentrate the dried extract to 3 to 5 mL by rotary evaporation at 30 to 50°C. Alternatively, acetone in the extract could be removed by exchanging solvent to *n*-hexane by adding ~30 mL of hexane each time (for two or three times), followed by rotary evaporation to a final volume of 3 to 5 mL.

Transfer the concentrated extract to appropriate volume, and make up to a final volume of 5 or 10 mL. If the extract will not be analyzed immediately, it should be transferred to a clean amber vial capped with a PTFE -lined screw-cap. Seal the vial with PTFE tape and mark the level of the sample on the vial. Ensure the vial is labelled properly. Store, refrigerated, in the dark for later analysis or cleanup.

Other extraction techniques are accepted in this method, provided they are validated.

7.2 Determination of Total Solvent-Extractable Materials (TSEM) by Gravimetric Method

An aliquot of the extract (0.5 to 1 mL) is taken and placed in a small accurately pre-weighed vial (using 0.0001 g accuracy analytical balance) and blown to dryness under gentle nitrogen flow. The residue is weighed until a constant weight is reached. The TSEM estimation is a necessary step to determine the amount of the extract needed for further analysis.

7.3 Sample Cleanup

Place 3 (±0.1) g of 100% activated silica gel into a 30-mm long, 10.5-mm ID chromatographic column. Gently tap the column to settle the silica gel. Add a 0.5 cm layer of anhydrous sodium sulphate on the top of silica gel.

Condition the column with hexane, discarding the eluate. When the solvent has drained to the top of the column bed, quantitatively transfer an appropriate aliquot of the final concentrated extract (1.0 to 2.0 mL, containing approximately 20 mg of

hydrocarbons or less, as estimated from the TSEM determination, section 7.2) onto the column using an additional 3 (±0.1) mL of hexane to complete the transfer. To avoid overloading the column, no more 50 mg of TSEM can be placed on the column.

Just prior to exposure of the silica gel to the air, elute the column with 15 mL of 1:1 (V:V) dichloromethane/hexane. Collect this eluate in a calibrated centrifuge tube, and gently concentrate the eluate to a small volume by evaporation under nitrogen in the "blow-down" apparatus. The concentrated eluate is spiked with 100 µL of the 200 ppm 5α-androstane stock and 100 µL of d_{14}-terphenyl as internal standard compounds. Make the solution up to the pre-injection volume (PIV) of 1.0 mL, or to a larger PIV if appropriate. The solutions are now ready for GC/FID and GC/MS analysis.

If immediate analyses are not performed, transfer the final solution to small vials capped with properly labelled PTFE -lined screw caps, and store refrigerated, in the dark, for later analysis (within 40 days).

7.4 Determination of Total Petroleum Hydrocarbons (PHC) by GC/FID

The analysis for total petroleum hydrocarbons, (approximately C_8 through C_{50}), is performed by high resolution capillary GC/FID. The following chromatographic conditions are recommended:

Instrument:	A gas chromatograph equipped with a flame ionization detector (i.e. Agilent 6890 equivalent or better)
Autosampler:	Agilent 7683 autosampler (equivalent or better)
Injection:	1µL volume, splitless injection, inlet held at 320°C.
Column:	30 m × 0.25 mm ID DB-5HT fused silica column or equivalent (0.1 µm film thickness).
Carrier Gas:	Helium at 1.0 mL/min, or hydrogen at 1.5 mL/min, constant flow.
Temperature program:	Initial hold at 40°C for 2 min, then 20°C/min to 340°C, hold 18 min. The total run time is 35 minutes.
Detector:	Flame ionization detector, at 340°C.
Make-up Gas:	Nitrogen, 30 mL/min
Detector Air:	400 mL/min
Detector Hydrogen:	30 mL/min

Prior to sample analysis, instrument stability is assured by a series of checks using the mid-range calibration standard, followed by a calibration check to establish the linearity and check sensitivity of the instrument. See section 7.6.

PHC analyses will follow an analysis sequence similar to the following order: a solvent blank, one mid-range calibration standard (i.e., 20 µg/mL), one low-range calibration standard, method blank, followed by 10 to 12 samples (matrix spike and matrix duplicate are treated as normal samples) and one sample is randomly

selected for a duplicate injection, and ending with one mid-range calibration standard and a solvent blank.

If either low-range or mid-range calibration standard exceeds a 20% difference from the most recent calibration, the instrument must be restabilized. Calibration and sensitivity are assured before the test can be repeated.

If the response for any analyte peak exceeds the linear range of the calibration, the extract of this sample should be diluted and reanalysed.

7.5 Determination of Petrogenic Hydrocarbons by GC/MS

If the PHC value from GC/FID analysis exceeds a regulated value, oil fingerprinting analysis of *n*-alkanes (*n*-C_{10} to *n*-C_{40}), alkylated polycyclic aromatic hydrocarbons (APAHs), bicyclic sesquiterpanes and petroleum biomarkers is conducted by GC/MS in the selected ion mode (SIM) to verify the presence or absence of petroleum source. Table 1 lists the selected characteristic ions used for analysis of these target hydrocarbons. The following chromatographic conditions are recommended.

Instrument:	A gas chromatograph equipped with a mass spectrometer (i.e. Agilent 6890 equivalent or better)
Autosampler:	Agilent 7683 autosampler (equivalent or better)
Injection:	1μL volume, splitless injection, inlet held at 280°C.
Column:	30 m × 0.25 mm ID HP-5MS or equivalent fused silica column (0.25 μm film thickness)
Carrier Gas:	Helium at 1.0 mL/min, or hydrogen at 1.5 mL/min, constant flow.
Temperature programs:	50°C for 2 min, then 6°C/min to 310°C, hold 20 min. The total run time is 65.33 minutes.
Detector:	Direct transfer (at 300°C) to a mass spectrometer (i.e. Agilent 5973 MSD equivalent or better).
MS mode:	Electron impact ionization at 70 eV, operated in the Selected Ion Monitoring (SIM) mode.
MS tune check:	The tune check may be performed as a separate analysis, or for routine MS tune verification. A standard Autotune and decafluorotriphenylphosphine (DFTPP) are recommended.
Solvent delay:	4.50 min
Mass group:	Group 1, 4.50 to 25 min, dwell time: 30 msec.
	Target ions: 85, 123, 128, 136, 142, 152, 153, 154, 156, 164, 166, 170, 179, 184, 193, 207
	Group 2, 25 to 35 min, dwell time: 30 msec.
	Target ions: 85, 178, 184, 188, 191, 192, 198, 206, 212, 220, 226, 234, 244

Group 3, 35 to 65.33 min, dwell time: 30 msec.

Target ions: 85, 191, 217, 218, 228, 234, 240, 242, 252, 256, 264, 270, 276, 278.

Above ion groups and time windows may be adjusted to allow for the detection of all target ions.

Prior to sample analysis, instrument stability is assured by a series of checks using the mid-range calibration standard, followed by a calibration check to establish the linearity and assure the sensitivity of the instrument.

The analyses will follow an analysis sequence similar to the following order: a solvent blank, one mid-range calibration standard (i.e., 1.0 µg/mL), one low-range calibration standard, method blank, followed by 10 – 12 samples and one sample is randomly selected for a duplicate injection, one mid-range calibration standard and a solvent blank.

Quantitative analysis of each of the target compounds or homolog groups is not required in this method. The selection criteria and reporting of each target compound and homolog group were based primarily on the presence of selected ions in the correct retention time window and by comparison with the peaks in the reference oils (refer to Appendixes).

The target ions used for biomarker analysis are *m/z* 191 for tri- to penta-cyclic terpanes and *m/z* 217 and 218 for steranes. Bicyclic sesquiterpanes are determined at *m/z* 123 and confirmed by ions of *m/z* 179, 193 and 207. Alkylated PAH series are determined by GC/MS with a suite of parent molecular ions, for examples, *m/z* 128, 142, 156, 170 and 184 for C_0- to C_4- naphthalenes, *m/z* 178, 192, 206, 220 and 234 for C_0- to C_4-phenenanthrenes, *m/z* 184, 198, 212 and 226 for C_0- to C_3-dibenzothiphenes, and *m/z* 228, 242, 256 and 270 for C_0- to C_3-chrysenes.

If the response for any ion exceeds the working range of the GC/MS system, dilute the extract and reanalyze.

7.6 Calibration

7.6.1 Linearity

The GC/FID linear dynamic range is established by a calibration check of five samples such as 1.0, 10, 20, 50 and 100 µg/mL of *n*-alkane standard solutions. Relative response factors (RRFs) are calculated for each individual analyte in the calibration mixtures at each of the concentration levels. The relative standard deviation (RSD) of an analyte's response factor must be 20% or smaller,

Calibration should be checked at the beginning of each project. For especially large projects, those lasting more than several months of continuous instrument run-time, mid-project calibration checks are required.

7.6.2 Calibration Checks and Instrument Performance

Mid-level concentration calibration standards are to be run with each batch of samples. These standards are typically 20 ppm for each analyte for the

PHC determinations and 1 ppm for each analyte for the petroleum hydrocarbon determinations. A minimum of two standards are to be run with each sample batch, one prior to the samples, and one after (see sections 7.4 and 7.5 for details). If more than 10 to 12 samples are run in a single batch then one additional mid-level calibration standard (duplicate injections) is to be run after each 10 to 12 samples.

The mid-level standard relative response factors are checked against the calibration curve current at the time of measurement. The RRFs should not vary by more than 30% from the calibration curve, with no more than four analytes outside of this limit. Up to 10% of the analytes may exceed the quoted limits by up to 10% absolute. For example, if the acceptance criterion is ± 30% and a deviation of 35% for one compound is acceptable but a deviation of 45% is unacceptable. Corrective action would be required.

The calibration standards should show good chromatography for all analytes. Problems such as excessive peak tailing, split peaks, asymmetric peaks and/or poor baselines must be corrected prior to sample analysis.

For PHC determinations, the *n*-C_{20} and 5α-androstane peaks must be fully resolved to baseline and the RRF for the high molecular-weight *n*-alkane measured (*i.e.*, *n*-C_{34} and *n*-C_{36} in RESTEK 31614) must be above 70% of the average RRF for all the *n*-alkanes. The response factor of *n*-C_{50} in ASTM 5442 verification standards or equivalent must be no less than 30% of the average response of the *n*-C_{10}, *n*-C_{16} and *n*-C_{34} alkanes.

7.6.3 Standard Accuracy

The mid-level concentration calibration standards must have been verified against independent reference solutions within six months prior to use. A diluted alternate reference material is run as a sample. Calculated concentrations for the alternate reference material must agree with the supplier's specifications to within the measurement uncertainty, with no more than 20% of the analytes falling outside of this range.

7.7. Determination of Percent Moisture

Transfer 5 to 10 g of sample into a tared (± 0.01 g or better) aluminum pan or a glass beaker and determine "wet weight". Evaporate the sample in fumehood if the sample apparently contains high content of volatile hydrocarbons. Dry this 5 to 10 g sample in an oven overnight at 101~110°C or obtain a constant weight. Allow the container to cool in a dessicator and reweigh (± 0.01 g or better).

Calculate the percent moisture of the sample using the Equation 1:

$$Moisture\ (\%) = \frac{W_{wet} - W_{dry}}{W_{wet}} \times 100 \qquad (1)$$

where:

W_{wet}= Wet weight of sample used for moisture analysis (g),

W_{dry}= Dry weight of sample used for moisture analysis (g).

Calculate dry weight for PHC result using the following Equation 2:

$$W_{dry,S}\ (g) = (1 - \frac{\%Moisture}{100}) \times W_{wet,S} \quad (2)$$

where:

$W_{wet,S}$= Wet weight of sample used for PHC analysis (g),

W_{dry}= Dry weight of sample used for PHC analysis (g).

Soil and sediment results must be reported on a dry-weight basis. The loss of weight in moisture determination includes water plus loss of volatile hydrocarbons.

8 CALCULATION OF RESULTS AND REPORTING CRITERIA

8.1 Relative Response Factors

Calculate the relative response factors (RRFs) for each analyte (including the surrogates) in the mid-level PHC calibration standards relative to the internal standards (5α-androstane for PHC determination and d_{14}-terphenyl for oil fingerprinting analysis) using Equation 3. The relative response factors are dimensionless. Average of relative response factors of all of the analytes in the mid-level calibration standard is used for the quantitation of Total Petroleum Hydrocarbons (PHC).

$$RRF = \frac{A_S C_{IS}}{A_{IS} C_S} \quad (3)$$

where:

A_S = Response for the target analyte to be measured,

A_{IS}= Response for the internal standard,

C_{IS}= Concentration of the internal standard (µg/mL), and

C_S = Concentration of the target analyte (µg/mL).

8.2 Total Petroleum Hydrocarbons (PHC)

To calculate the concentration of total PHC in the sample, the area response attributed to the petroleum hydrocarbons must be determined. This area includes all of the resolved peaks and the "unresolved complex mixture" (UCM). This total area must be adjusted to remove the area response of the solvent, internal standards, surrogates and GC column bleed.

Column bleed is defined as the reproducible baseline shift that occurs during temperature programming of the GC. To determine this area, a solvent-blank injection should be analyzed at the beginning of the sample sequence and after every 10 samples to determine the baseline response. This baseline is then set at a stable reproducible point just before the solvent peak. The GC/FID chromatogram

can be divided into four fractions according to their retention times. Reference oil, ASTM 5442 or equivalent is used to identify and define the retention time window of each fraction.

Fraction 1 hydrocarbons (PHC_{F1}), <*n*-C_{10}, are determined by integration of all areas before *n*-C_{10} peak apex, excluding the interference from solvent peaks (optional) (refer to CCME method to for the analysis of CCME-F1 of light hydrocarbons).

Fraction 2 hydrocarbons (PHC_{F2}), *n*-C_{10} to *n*-C_{16}, are determined by integration of all areas between *n*-C_{10} peak apex and *n*-C_{16} peak apex.

Fraction 3 hydrocarbons (PHC_{F3}), *n*-C_{16} to *n*-C_{34}, are determined by integration of all areas between *n*-C_{16} peak apex and *n*-C_{34} peak apex.

Fraction 4 hydrocarbons (PHC_{F4}), *n*-C_{34} to *n*-C_{50}, are determined by integration of all areas between *n*-C_{34} peak apex and *n*-C_{50} peak apex.

Calculate the petroleum hydrocarbon of each fraction using Equation 4:

$$PHC_{F_i}(\mu g / g) = \frac{A_{TPH,F_i} W_{IS} D}{A_{IS} RRF_{TPH} W_S} \quad (4)$$

where:

$A_{PHC,\ Fi}$ = The corrected total area of the sample chromatogram for each fraction

RRF_{PHC} = Average of relative response factors of all of the analytes in the mid-level calibration standard from Equation 3

W_{IS} = Amount (µg) of internal standard added to the sample

W_S = Weight of dry sample (g), and

D = Dilution factor. The dilution factor is dimensionless.

Calculate the total petroleum hydrocarbon using Equation 5:

$$TPH(\mu g / g) = TPH_{F2} + TPH_{F3} + TPH_{F4} \quad (5)$$

8.3 Surrogate Recoveries

Prior to extraction, each sample is spiked with a small amount of surrogate(s). For PHC determinations, *ortho*-terphenyl is used (see section 7.1). The recovery of the surrogate is monitored in each sample using the relative response to the internal standard, calculated using Equation 3. The percent recovery of the surrogate is calculated using Equation 6:

$$\text{Recovery}_S(\%) = \frac{A_S W_{IS} D}{A_{IS} W_S RRF_S} \quad (6)$$

where:

A_{IS} = Integrated area of the internal standard,

A_S = Integration area of the surrogate compound,

W_{IS} = Weight (μg) of the internal standard added to the sample,

W_S = Weight (μg) of the surrogate compound added to the sample.

RRF_S = Response factor of the surrogate relative to the internal standard, and

D = Dilution factor.

If the PHC result exceeds the regulated value, oil fingerprinting analysis using GC/MS is therefore performed. The recoveries of the PAH surrogates are monitored in each sample using the relative response to the internal standard of d_{14}-terphenyl, calculated using Equation 3.

8.4 Data Reporting

The following items should be included in each data report:

1) Total petroleum hydrocarbons (PHCs), PHC fractions of F1 (optional), F2, F3 and F4, reporting units are μg/g or mg/kg (dry weight)
2) Moisture percentage (%) of each sediment or soil sample
3) If oil fingerprinting analysis is performed, report the identification result of petroleum source – presence or absence and corresponding evidences including:
   i) GC/FID chromatogram of PHCs
   ii) GC/MS chromatogram of *n*-alkanes at m/z 85
   iii) GC/MS chromatogram of biomarker terpanes at m/z 191 and biomarker steranes at m/z 218
   iv) GC/MS chromatogram of bicyclic sesquiterpane at m/z 123
   v) Distribution of response of Alkylated PAHs: C_0-C_4 naphthalenes, C_0-C_4 phenanthrenes, C_0-C_3 dibenzothiophenes, and C_0-C_3 chrysenes.

   Positive identification of petrogenic source: presence of one or more typical petroleum chromatographic features including UCM, biomarkers, bicyclic sesquiterpanes and alkylated PAHs;

   Negative identification of petrogenic source: absence of any typical petroleum chromatographic features.

   The identification of target petroleum hydrocarbons should be conducted by an experienced chemist or an analyst with adequate training. The characterization and identification of oils are comprehensive and challenging due to the wide variability in petroleum products. Oil fingerprinting analysis becomes even more complicated once oil is released into environment and is subject to various weathering processes. This method does not describe how to conduct these works. It is recommended to refer to literatures cited for details.
4) Quality control report:

i) Recovery of the surrogate of *ortho*-terphenyl is reported for each sample analyzed (including laboratory method blank, and matrix spike analysis). Recoveries of the deuterated PAH surrogates are only required when GC/MS oil fingerprinting analysis is performed. Data should be flagged if the percent recovery is outside of the 50-140% range (40-140% for d_8-naphthalene). Reported values are not corrected for surrogate recovery

ii) Report the results of matrix duplicate analysis

iii) Report the results of matrix spike analysis (including percent recovery, %)

iv) Report the reporting limit if an analysis is reported as non-detectable.

9 QUALITY ASSURANCE

9.1 Calibration Accuracy

The calibration standard accuracy is assured by periodic checks against an independent standard reference material. A historical chart is kept of comparison of the two standards. All target compounds of interest must be evaluated using a 20% criterion (i.e., less than 20% difference or drift). Up to 10% of the analytes may exceed the quoted limits by up to 10% absolute.

9.2 Calibration Linearity

Instrument performance is assured by the establishment of a linear response for the range of measurement (see section 7.6.1).

9.3 Calibration Stability

Calibration stability is assured by duplicate injections of mid-level concentration calibration standards before and after each sample set, and after each 10 to 12 samples, if required (see sections 7.5 and 7.6). The relative response factors for each calibration analytes are checked in two ways.

The RRFs of mid-level calibration check must be within 30% of those of the most recent five-point calibration (see section 7.6.2).

9.4 Instrument Sensitivity

Instrument sensitivity should be monitored by injection of a low-level concentration standard at the beginning of each project as part of the calibration check. The chromatogram is compared with historical performance data to ensure that instrument sensitivity and an acceptable signal-to-noise ratio is maintained.

9.5 Instrument Stability

Solvent blanks are run before and after each sequence of samples to ensure chromatographic performance. Problems including dirty inlets or column heads, excessive column bleed or noisy detectors require instrument maintenance before any samples may be run.

9.6 Sample Recovery

Sample recovery efficiency is monitored by the surrogate spike recoveries from the samples. The acceptable surrogate recovery range is 50% to 140% range (40-140% for d_8-naphthalene). Samples with recoveries outside of this range should be reanalyzed, if possible.

9.7 Overall Method Performance Control

Laboratory method blank, matrix spike and matrix duplicate must be performed as part of the laboratory quality control at a minimum, for each analytical batch (up to 20 samples of similar matrix). Surrogate recoveries are calculated and considered the same as samples in section 9.6, above. Surrogate recoveries from the quality control analysis provide an additional check on both method accuracy and precision (bias and uncertainty) and assure positive method performance.

Laboratory method blank: a 5-g aliquot of blank soil (clean sand), spiked with surrogates is analyzed through all the sample manipulations and clean-up as the samples. Any measured result on a blank greater than the reporting limits, or greater than the 95% confidence limit of the historical average of method blanks requires that corrective action, usually instrument maintenance, be taken.

Matrix spike: a 5 g aliquot of blank soil (clean sand), spiked with medium range of analytes (10 to 20 mg is recommended if the ESTS reference oil 13.1% weathered Prudhoe Bay crude oil is used) and surrogates, is processed as a normal sample. Matrix spike recovery is calculated, and the percentage recovery of PHCs should be between 50-140%. If the ESTS reference oil is applied, use ESTS's historic PHC results as the reference data.

Matrix duplicate: one of two sample aliquots from the same sample container and carried through the entire analytical process including all sample preparation steps. The relative percent difference of duplicate analysis should not be greater than 40%.

9.8 Glassware Proof

All glassware is proofed for cleanliness before use. See section 6.2.

9.9 Non-conformances

A non-conformance can be triggered either by a complaint from a client or by an error found by lab personnel.

10 METHOD VALIDATION AND DETECTION LIMITS

10.1 Instrument Detection Limit (IDL)

The instrument detection limit (IDL) is first estimated by half-$\log_{10}$ serial dilutions, *i.e*, 20, 10, 2, 1, 0.2, 0.1 ppm solutions, of the calibration standard until the standard response peaks in the chromatogram are seen to have heights three to five times greater than the width of the baseline noise.

When an appropriate concentration has been discovered, at least seven replicate injections are made at that concentration of the calibration standard. Average baseline noise and peak heights (not areas) are determined. When the peak heights of all analytes are within three to five time the average amplitude of the baseline noise, then the instrument detection limit is taken to be the solution concentration. If the average heights are above or below three to five times the baseline noise, the solution concentration must be adjusted and a further seven replicates injected.

Limited by the availability of standards, this method does not require the determination of the instrument detection limit (IDL) of petroleum biomarkers, bicyclic sesquiterpanes and alkylated PAHs. However, it is recommended to determine them if the standards of these compounds are available.

10.2 Method Detection Limit (MDL) and Limit of Quantitation (LOQ)

For the PHC limit of detection, an appropriate amount of proper reference material (*e.g.*, ESTS reference oil) is spiked into 5 g of blank soil at a level 1–10 times of the expected MDL. An initial estimate of the MDL can be taken to be ten times the IDL (see section 10.1). A minimum of eight replicates are performed through the entire analytical method.

Outliers identified using Grubb's test or Dixon's Q test may be rejected, but the method detection limit (MDL) is determined by a minimum of seven replicate analysis.

The standard deviations for each analyte, *s*, are calculated and multiplied by the double-tailed Student's-t factor at the 99% confidence level. This value represents the limit of detection (LOD). The limit of quantitation is determined by multiplying the LOD by a factor of 10/3. $\text{MDL} = t_{(\text{n-1, } \alpha=0.99)}\, s$ (7)

$$\text{LOQ} = 10/3\ \text{LOD} \tag{8}$$

The resultant calculated MDL must be with 1–10 times of the spike level. If these conditions are not met, the determination must be repeated until the calculated MDL concentration is 1–10 times the spike concentration.

10.3 Method Validation

The accuracy of the method is assessed by the determinations of replicates of synthetic samples prepared by spiking a mid-level amount of a check standard into a standard soil mix.

To validate the PHC determination, replicates of a 5-mg aliquot of reference oil are spiked into 5 g of soil and run as a sample.

The mean recovery, M_r, is calculated from the recoveries of replicate analysis. The accuracy of the method is reflected by the relative bias (100% - mean percent recovery). The relative bias in the method must not exceed 30% (positive or negative).

The precision (repeatability) of the measurement is determined from the relative standard deviation, RSD_r, of the recoveries. The relative standard deviation of the recoveries must not be more than 30% for the method.

11 METHOD UNCERTAINTY

The following briefly describes one method to estimate the analytical measurement uncertainty. The Method uncertainty can be determined using a combination of Type A and Type B uncertainties.

The path of the samples through the method is mimicked exactly by those of the validation samples (section 10.3) with one exception: the preparation of the spike materials and their addition to the base matrix. Therefore, the uncertainty in the recoveries of the validation samples includes both the sample uncertainty and the uncertainty in the spike preparation:

$$U_{Validation}^2 = U_{Sample}^2 + U_{Spike}^2 \tag{9}$$

The uncertainty in the spike preparation and addition can be estimated using a Type B approach, for example, for a two stage serial dilution and spike addition (for PAHs):

$$\frac{U_{Spike}}{V_{Spike}} = \sqrt{\left(\frac{U_{SRM}}{[SRM]}\right)^2 + \left(\frac{U_{Pipet}}{V_{Pipet}}\right)^2 + \left(\frac{U_{Flask}}{V_{Flask}}\right)^2 + \left(\frac{U_{Pipet}}{V_{Pipet}}\right)^2 + \left(\frac{U_{Flask}}{V_{Flask}}\right)^2 + \left(\frac{U_{Pipet}}{V_{Pipet}}\right)^2} \tag{10}$$

where:

[SRM], U_{SRM} are the concentration and uncertainty of the standard reference material, respectively, as described on the certificate of purity supplied by the manufacturer for the spike material,

V_{Pipet}, U_{Pipet} are the volumes and measured uncertainties of the pipettes used in the spike preparation and addition, and

V_{Flask}, U_{Flask} are the volumes and measured uncertainties (determined from manufacturers' certificates) of the volumetric flasks used in the spike dilution.

For PHC determinations the relative spike uncertainty, U_{Spike}/ V_{Spike}, is calculated to be less than 0.5%.

Relative recovery uncertainties typically fall in the range of 20% to 30%. Since this greatly exceeds three times the spike preparation uncertainty, the uncertainty in the validation spiked-matrix samples is concluded to be approximately equal to the expected uncertainty of a sample measurement. The uncertainty in the method is thus estimated using a Type A determination from the uncertainty in the validation standard recoveries.

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13 LIST OF REVISIONS

September 2011: New method is created by Chun Yang on the basis of the ESTS oil analysis method and Method 5.09/1.4M.

January 2012: Revised by Chun Yang according to the comments from internal and external reviewers.

March 2012: Revised by Chun Yang based on the discussion at the AUPRF Workshop on Feb. 27 – Mar. 02, 2012, Ottawa, Ontario.

14 CERTIFICATION FOR USE

This method is fit for its intended use.

Lead Reviewer: **Date:**

Title:

Approved by: **Date:**

PART II

METHOD VALIDATION

Determination of Total Petroleum Hydrocarbons and Differentiation of Petrogenic and Biogenic Inputs in Environmental Samples

1. AUPRF Method Validation Plan

In order to validate the PHC analysis method, the Oil Research Laboratory of the Emergencies Science and Technology Section (ESTS), Environment Canada, prepared this method validation plan. This plan was submitted by Environment Canada to the AUPRF Method working group in March 2012. This plan was discussed, revised and approved by AUPRF PHC Working Group members on March 02, 2012.

1.1 Specific Instruction

1.1.1 Use 5 g of Standard Ottawa sand (Anachemia Canada Co., Montreal) for each extraction.

1.1.2 0.1 mL of surrogates of 200 µg/mL of o-terphenyl and 10 µg/mL of deuterated PAHs surrogates (mixture of d_8-naphthalenes, d_{10}-acenaphthene, d_{10}-phenanthrene, d_{12}-benz(a)anthracene and d_{12}-perylene).

1.1.3 A final volume of 1.0 mL is used. It may need to be adjusted for each lab's specific practice).

1.2. Validation Plan

The validation process consists of the following steps:

1.2.1 Method blank

An investigation of specificity should be conducted during the validation. A method blank using 5~10 g of blank Ottawa sand is conducted (1~3 analyses).

1.2.2 Linearity

A linear relationship should be evaluated across the range of the analytical procedure. Three concentration spiking (0.50, 5.0 and 100 mg of reference oil, or 0.1, 1.0 and 20.0 mg reference oil per 1.0 g of blank soil, each concentration analysis is at least triplicate) will be conducted. This ESTS reference oil of the Prudhoe Bay crude oil (13.1% weathered

by laboratory evaporation) contains 590 µg/g of GC/FID-detectable petroleum hydrocarbons.

The low concentration analysis (0.50 mg spiked, or 0.1 mg/g) is at least 8 replicated. These analyses are also used for determination of the method detection limit (MDL).

At least middle range is analyzed by GC/MS.

1.2.3 Range

To be estimated by results from the linearity study.

1.2.4 Precision

Estimated by replicate analyses in linearity study; surrogate recoveries for MDLs studies. Mean results and relative standard deviation (%RSD) are to be reported.

1.2.5 Repeatability

To be evaluated by the results from whole validation study.

1.2.6 Accuracy

To be estimated by mean value of the results for repeatability study divided by ESTS's reference PHC data of the reference oil.

1.2.7 Detection Limit and Quantitation Limit

Approximately 5.0 g of Ottawa sand was fortified with 0.5 mL of 1.0 mg/mL of ESTS reference oil solution (0.50 mg of 13.1% weathered Prudhoe Bay crude oil), and 0.1 mL of surrogates of 200 µg/mL of o-terphenyl and 10 µg/mL of deuterated PAHs surrogates (mixture of d_8-naphthalenes, d_{10}-acenaphthene, d_{10}-phenanthrene, d_{12}-benz(a)anthracene and d12-perylene). Sample analysis should be replicated at least 8 times (minimum 8 analyses, conducted in Linearity study).

The standard deviations for each analyte, *s*, are calculated and multiplied by the double-tailed Student's-t factor at the 99% confidence level. This value represents the method detection limit (MDL). The limit of quantitation is determined by multiplying the LOD by a factor of 10/3.

$$MDL = t_{(n-1,\ \alpha=0.99)}\ s$$

$$LOQ = 10/3\ MDL$$

1.2.8 Robustness (from whole group)

The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters.

Examples of typical variations are:

- Stability of analytical solutions
- Extraction techniques and extraction time
- Different GC columns (different lots and/or suppliers)
- Temperature
- Flow rate.

This will be evaluated by the results form all participants following different lab's practice.

1.3 Validation Report

Each lab is to prepare a report. The report should include:

- Summary information: including sample extraction, sample pretreatment, and GC analysis conditions
- Results of all items required by the validation plan
- Findings and comments to improve this method.

1.4 Key Time

- ESTS will send the revised method and validation plan to the participant by March 10, 2012 for further comments.
- Comments on the method and validation plan must be received by March 19, 2012.
- ESTS will send revised the method and validation plan by March 23, 2012.
- ESTS will deliver the validation package (including standard materials, reference oils) to each participant by March 30, 2012
- Each participant will submit the validation report to ESTS by May 15th.
- In May 2012, ESTS will prepare the final validation report (anonymous) and send it to each lab for comments on the validation report and for opportunity to improve the method.
- ESTS will prepare final report in July 2012 and send it each participant for review.

2. Participants of the AUPRF Method Validation

Nine analytical laboratories were invited to participate in this method validation. Most of these laboratories expertize in environmental analysis and analyze a large number of soil samples every year. By the end of July 2012, six participants independently conducted the method validation and submitted their reports (Table 1).

Table 1 Laboratories participated in the method validation.

Lab Name	Location
Emergencies Science and Technology Section (ESTS), Environment Canada	Ottawa, Ontario
ALS Environmental Group	Waterloo, Ontario
Maxxam Analytics	Calgary, Alberta
Laboratory Services Branch, Ministry of the Environment	Toronto, Ontario
Pacific and Yukon Laboratory for Environmental Testing (PESC), Pacific Environmental Science Centre, Environment Canada	North Vancouver, British Columbia
Prairie and Northern Laboratory for Environmental Testing (PNLET), Environment Canada	Edmonton, Alberta

3. Validation Standard Materials

All participant labs used Ottawa sand as blank soil in the method validation. As mentioned in the method, the standards materials in the method are only recommended for use, each lab can use their own standards if valid. However, in order to easily compare the method validation results, the Oil Research Lab of Environment Canada prepared the calibration standards for all participants and reference oil solution. These standards and reference oil were delivered to each participant in April 2012. A list of

weight of each vials was also attached, this allows the participant to check the loss of standard solutions and their validation.

These standard solutions include:

- *Internal Standard*: 5α-androstane (for PHC analysis) and d_{14}-terphenyl (for oil analysis by GC/MS)
- *Surrogate Standard*: *ortho*-terphenyl (for PHC analysis by GC/FID) and d_8-naphthalene, d_{10}-acenaphthene, d_{10}-phenanthrene, d_{12}-benz(a)anthracene, and d12-perylene (for oil analysis by GC/MS).
- *PHC Calibration Standard*: RESTEK 31614, Connecticut ETPH Calibration Mixture (*n*-C_9, *n*-C_{10}, *n*-C_{16} and *n*-C_{34}).
- *Verification Standard:* ASTM 5442 (C_{12}-C_{60} analytical standards)

  Prudhoe Bay 13.1% evaporated crude oil (*ESTS internal laboratory standard*).
- *PAH Calibration Standard*: Mixture of d_{14}-terphenyl, PAH surrogates, and individual PAHs.

The following Tables 2 to 7 summarize the information about the standards material used in this validation study.

Table 2 Method validation standard solutions: internal standard solution.

Standard ID	**AUPRF Standard-1**
Standard Name	AUPRF Internal Standard
Solvent	n-Hexane
Components	**Concentration (µg/mL)**
5α-Androstane	200
d_{14}-Terphenyl (IS)	10.0

Instruction for AUPRF Standard-1:

- This standard solution is used to spike in the final extract (after silica-gel cleanup) as internal standard.
- Spike 100 µL for a final volume of 1.0 mL. The final concentration 5α-andrstane and d_{14}-terphenyl is 20 µg/mL and 1.0 µg/mL, respectively.

Table 3 Method validation standard solutions: surrogate solution.

Standard ID	**AUPRF Standard-2**
Standard Name	AUPRF Surrogates
Solvent	n-Hexane

Components	**Concentration (µg/mL)**	**Ion**
o-Terphenyl	200	
d_8-Naphthalene	10.0	136
d_{10}-Acenaphthene	10.0	164
d_{10}-Phenanthrene	10.0	188
d_{12}-Benz(a)anthracene	10.0	240
d_{12}-Perylene	10.0	264

Instruction for AUPRF Standard-2:

- This standard solution is used to spike in the sample prior to extraction in order to evaluate the quality of a sample analysis.
- Spike 100 µL for a final volume of 1.0 mL. The final concentration of o-terphenyl is 20 µg/mL, and the concentration of each PAH surrogate is 1.0 µg/mL.

Table 4 Method validation standard solutions: PHC calibration solution.

Standard ID	**AUPRF Standard-3**
Standard Name	AUPRF PHC Calculation Standard
Solvent	n-Hexane

Components	**Concentration (**μg/mL)
5α-Androstane (IS)	20.0
o-Terphenyl (Sur)	20.0
n-C_9	20.0
n-C_{10}	20.0
n-C_{12}	20.0
n-C_{14}	20.0
n-C_{16}	20.0
n-C_{18}	20.0
n-C_{20}	20.0
n-C_{22}	20.0
n-C_{24}	20.0
n-C_{26}	20.0
n-C_{28}	20.0
n-C_{30}	20.0
n-C_{32}	20.0
n-C_{34}	20.0
n-C_{36}	20.0

Instruction for AUPRF Standard-3:

This solution is used for PHC quantitative analysis by GC/FID.

Table 5 Method validation standard solutions: PHC verification solution.

Standard ID	**AUPRF Standard-4**
Standard Name	AUPRF PHC Verification Standard ASTM D5442
Solvent	n-Hexane

Components	**Concentration**
5α-Androstane (IS)	20.0
o-Terphenyl (Sur)	20.0
n-C_{12}	20.0
n-C_{14}	20.0
n-C_{16}	20.0
n-C_{18}	20.0
n-C_{20}	20.0
n-C_{22}	20.0
n-C_{24}	20.0
n-C_{26}	20.0
n-C_{28}	20.0
n-C_{30}	20.0
n-C_{32}	20.0
n-C_{36}	20.0
n-C_{40}	20.0
n-C_{44}	20.0
n-C_{50}	20.0
n-C_{60}	20.0

Instruction for AUPRF Standard-4:

- This solution is used as a verification standard for PHC quantitative analysis by GC/FID.

Table 6 AUPRF method validation standard solutions: PAH calibration solution.

Standard ID	**AUPRF Standard-5**
Standard Name	AUPRF PAH Calibration Standard
Solvent	n-Hexane

Components	**Concentration**	**Ion**
d_{14}-Terphenyl (IS)	1.00	244
d_8-Naphthalene (Sur)	1.00	136
d_{10}-Acenaphthene (Sur)	1.00	164
d_{10}-Phenanthrene (Sur)	1.00	188
d_{12}-Benz(a)anthracene (Sur)	1.00	240
d_{12}-Perylene (Sur)	1.00	264
Naphthalene	1.00	128
2-Methylnaphthalene	1.00	142
1-Methylnaphthalene	1.00	142
Biphenyl	1.00	154
2,6-Methylnaphthalene	0.50	156
Acenaphthylene	1.00	152
Acenaphthene	1.00	153
2,3,5-Trimethylnaphthalene	0.50	170
Fluorene	1.00	166
Dibenzothiophene	1.00	184
Phenanthrene	0.50	178
Anthracene	1.00	178
1-Methylphenanthrene	0.50	192
Fluoranthene	0.50	202
Pyrene	0.50	202
Benzo(a)anthracene	0.50	228
Chrysene	0.50	228
Benzo(b)fluoranthene	0.50	252
Benzo(k)fluoranthenene	0.50	252
Benzo(e)pyrene	0.50	252
Benzo(a)pyrene	0.50	252
Perylene	0.50	252
Indeno(1,2,3-cd)pyrene	0.50	276
Dibenz(ah)anthracene	0.50	278
Benzo(ghi)perylene	0.50	276

Instruction for AUPRF Standard-5:

- This solution is used for PAH quantitation by GC/MS (optional).

Table 7 AUPRF method validation standard solutions: reference oil.

Standard ID	**AUPRF Standard-6**
Standard Name	AUPRF Reference Oil
Solvent	n-Hexane
Components	**Concentration**
Prudhoe Bay crude oil, 13.1% weathered	400 mg/mL

Instruction for AUPRF Standard-6:

- This solution containing reference oil is used to spike in blank soil for method validation or a reference oil in the Round Robin study.
- Spiking level of 100 mg: spike 250 µL of 400 mg/mL reference oil solution.
- Spiking level of 5 mg: dilute 400 mg/mL reference oil properly to 20 mg/mL; spike 250 µL of 20 mg/mL reference oil solution.
- Spiking level of 0.5 mg: dilute 20 mg/mL reference oil properly to 2.0 mg/mL; spike 250 µL of 2.0 mg/mL reference oil solution.

4. Method Validation Experiments

Nine analytical laboratories were invited to participate in this method validation. Five participants performed the validation study and submitted their validation reports. Lab 06 conducted the validation experiments, but it was unable to submit their reports since their data processing software (Varian system) does not allow them to process the data files as Agilent ChemStation.

The PHC concentrations reported was all based on 5 g of dry-weight. PHC_{F1} (<n-C_{10}) was not evaluated in this method validation although some lab has reported relevant results.

4.1 Sample Preparation

The procedures of sample preparation used by six participants were summarized in Table 8. A code of Lab 01 to 06 was used to represent a participant, and the code is not necessarily in the same order as listed in Table 1. As shown in Table 8, the procedures of the sample preparation are all different. Various extraction techniques were applied in processing the soil samples. Two labs used classic Soxhlet extraction technique, and Lab 04 used the automated Soxhlet extraction system (Foss Soxtec extractor). The other three participants used a rotary tumbler, a mechanical shaker, and a sonicator, respectively.

All labs used the mixture of hexane : acetone (50:50, V:V) as extraction solvent, except that one lab used the mixture of dichloromethane (DCM) : acetonitrile (ACN) (50:50, V:V). The extract was either subject to DI water washing or solvent exchange to remove acetone prior to silica gel clean up. One lab directly analyzed the extract without column cleanup.

All the participants used the validation surrogate standards and internal standard in the method validation.

Table 8 Sample extraction techniques used in this method validation (to be continued).

Participants	Sample Preparation Procedures
Lab 01	**Extraction Apparatus:** Soxhlet extractor **Solvent:** Hexane : acetone (50:50, V:V) **Extraction Procedures:** Take 5 g of the sample (depending on the hydrocarbon content of the sample), record the weight to the nearest 0.01 g. Completely transfer the sample to a pre-cleaned Soxhlet thimble. Spike the sample with the surrogate standards, 100 μL of 200 ppm *ortho*-terphenyl and 100 μL of the mixture containing five deuterated PAHs, 10 ppm each, cover with a small amount of clean glasswool, and then transfer the thimble and contents to a clean Soxhlet body. Extract the sample overnight (approximately 16 to 24 hours) with approximately 200 mL of hexane/acetone (50:50, V:V) (1 or 2 clean boiling chips in the extraction flask may be used to prevent extract bumping), a rate of 3 to 5 cycles per hour. Acetone in the extract was removed by exchanging solvent to *n*-hexane by adding ~30 mL of hexane each time (for two or three times), followed by rotary evaporation to a final volume of 3 to 5 mL. Transfer the concentrated extract to appropriate volume, and make up to a final volume of 5 or 10 mL. If the extract will not be analyzed immediately, it should be transferred to a clean amber vial capped with a PTFE-lined screw-cap. Store, refrigerated, in the dark for later analysis or cleanup. **Sample Cleanup:** Used 3 g of activated silica gel to clean up the extract. Condition the column with hexane, discarding the eluate. Quantitatively transfer an appropriate aliquot of the final concentrated extract (1.0 to 2.0 mL, containing approximately 20 mg of TSEM or less, onto the column using an additional 3 mL of hexane to complete the transfer. Just prior to exposure of the silica gel to the air, elute the column with 15 mL of 1:1 (V:V) dichloromethane/hexane. Collect this eluate in a calibrated centrifuge tube, and gently concentrate the eluate to a small volume by evaporation under nitrogen in the "blow-down" apparatus. The concentrated eluate is spiked with 100 μL of the 200 ppm 5α-androstane stock and 100 μL of d_{14}-terphenyl as internal standard compounds. Make the solution up to the pre-injection volume (PIV) of 1.0 mL. The solutions are now ready for GC/FID and GC/MS analysis.

Table 8 Sample extraction techniques used in this method validation (continued).

Participants	Sample Preparation Procedures
Lab 02	**Extraction Apparatus:** rotary tumbler **Solvent:** Hexane : acetone (50:50, V:V) **Extraction Procedures:** Weigh 5 g of sample into a 125 mL PTFE bottle, use baked Ottawa sand for blanks and validation; added 200uL of surrogates (AUPRF Std #2); added 20 mL of acetone and 20 mL of hexane; tumble in rotary tumbler at 30 RPM for 2 hours; add DI water to get rid of the acetone; transfer as much hexane as possible to a 40 mL VOC vial and add DI water again; take 10 mL of hexane; use the left over hexane to check the TSEM, concentrate to 2 mL. **Sample Cleanup:** Transfer appropriate amount of extract to the 3 g silica column, rinse the vial with hexane. Elute with DCM : hexane (50:50, V:V) up to approximately 12 mL. Concentrate to just under 1mL and add 100 µL of internal standard (AUPRF std#1).
Lab 03	**Extraction Apparatus:** mechanical shaker **Solvent:** Hexane : acetone (50:50, V:V) **Extraction Procedures:** Approximately 5.0 g of Ottawa sand were weighted into a 40 mL screw top amber glass vial. The 400 mg/mL AUPRF Standard 6 Reference Oil was used to prepare a 2.0 mg/mL stock. From this stock, 250 mL was used to fortify all eight low level spikes. Then 10.0 mL of acetone/surrogate mixture (this mixture contains 200 mg/mL of o-terphenyl) are added. No PAH surrogates were added to the low level spikes. The samples were then vortexed until they were completely dispersed in the acetone. Then 10.0 mL of hexane is added. Samples were then placed on a mechanical shaker for 30 minutes. After removal from shaker, samples are centrifuged for a minimum of 2 minutes. Vials are then uncapped and approximately 15 mL of water is added. Vials are then vigorously hand shaken and centrifuged again. The upper solvent layer is removed and placed into a new 44 mL vial containing about 30 mL of water. The vial is again vigorously agitated and centrifuged to remove any remaining acetone. **Sample Cleanup:** Then 1.0 mL of the hexane extract was removed and run through a clean-up column containing 3.0 g of silica gel with the resulting elute.

Table 8 Sample extraction techniques used in this method validation (continued).

Participants	Sample Preparation Procedures
Lab 04	**Extraction Apparatus:** Foss Soxtec extraction **Solvent:** Hexane : acetone (50:50, V:V) **Extraction Procedures** 5 g of Ottawa sand was extracted with acetone : hexane using a Foss Soxtec extractor. The extraction conditions are: Hot plate temperature (max): 210°C Hot Plate temperature: 185°C Total boil time: 1 hour Total rinse time: 1 hour Transfer extract into a 1 L separatory funnel. Rinse sample container with 25 mL of extraction solvent and transfer rinse to the funnel. Add 250 mL of de-ionized water, let the solution stand to allow layers to separate, approximately 5-10 minutes. Drain the water layer and discard. Insert a glass fibre filter in a funnel placed in the original 125 mL flat bottom flask. Add approximately 5 g of anhydrous sodium sulphate to the filter paper. Rinse sodium sulphate with approximately 10 mL of hexane and discard rinse. Drain hexane layer from separatory funnel into 250 mL round bottom flask through filter. Rinse separatory funnel with approximately 25 mL of hexane and filter into the flask. Rinse filter with approximately 10 mL of hexane. Evaporate extract using a Rotary evaporator to approximately 2 to 3 mL. Transfer the evaporated sample extract through the clean-up procedure. **Sample Cleanup:** Rinse the column and reservoir with 50:50 DCM/hexane (20 - 25 mL). Collect into waste beaker. Add sufficient 50:50 DCM/hexane to cover silica gel and mix into slurry for column preparation by wet pack method. Add the silica gel slurry and pack to a height of approximately 19 cm by tapping gently and draining the solvent into a waste beaker. Transfer the evaporated sample extract to the top of the column. Rinse the round bottom flask with 1-2 mL of 50:50 DCM/hexane and transfer to the column just as the sample extract level reaches the top of the packing. Add approximately 25 mL of elution solvent and collect a total of 25 mL of eluate in a graduated tube. Evaporate the 25 mL of eluate in the tube to < 5.0 mL, using a Turbovap (temperature bath of 35°C with a 10~12 psi of gas pressure) blow down apparatus, or equivalent. Make final volume to 5.0 mL with hexane and cap. Samples may be diluted based on expected concentrations or visual observations.

Table 8 Sample extraction techniques used in this method validation (continued).

Participants	Sample Preparation Procedures
Lab 05	**Extraction Apparatus:** Soxhlet Extraction **Solvent:** Hexane : acetone (50:50, V:V) **Extraction Procedures:** 5 g of Ottawa sand (Fisher cat no. S23-3) was used for each extraction. Surrogates and/or spike solution was added as required. Glasswool was placed on top of the soil. Boiling chips were added to prevent bumping. Samples were extracted for 20 hours with a cycling time of between 4 to 6 cycles/hour. About 200 mL of 1:1 v/v acetone/hexane was used for each extraction. Extracts were quantitatively transferred through 30 g of sodium sulfate drying tube into 500 mL boiling flask. The original drying tube was rinsed with 10 mL x 3 aliquots of hexane. The extract was concentrated by rotary evaporation to less than 5 mL, then solvent exchanged into hexane by adding 30 mL hexane. The solvent exchange process was repeated two more times. The extract was concentrated to less than 2 mL, then quantitatively transferred to a 5 mL volumetric flask and made up to volume with hexane. To determine the TSEM, 0.5 mL of extract was added in a pre-weighed centrifuge tube. The extract was blown just dryness under a gentle stream of nitrogen. The tube was re-weighed. Above procedure was repeated till a constant weight was obtained. **Sample Cleanup:** Prepared (wet pack with hexane) a 3 g silica gel column (as per AUPRF method) and added 0.5 cm of sodium sulfate on the top. Conditioned column with hexane and discarded the eluant. Added an appropriate amount of the extract (as determined from previous step with TSEM, less than or equal to 20 mg) to the column. Added 3 mL of hexane to help complete the transfer. Used 15 mL of 1:1 v/v DCM/hexane to elute the column. Collected the eluent with pre-calibrated (to 1 mL) centrifuge tube. Concentrated the extract under a gentle stream of nitrogen to just under the mark, added internal standard, and made up to mark with hexane. Mixed the extract the transferred it to a GC vial.

Table 8 Sample extraction techniques used in this method validation (continued).

Participants	Sample Preparation Procedures
Lab 06	**Extraction Apparatus:** polypropylene centrifuge tube **Solvent:** Dichloromethane : acetonitrile (DCM/ACN, 50:50, V:V) **Extraction Procedures:** Aliquots of 5 g of homogeneous soil sample were weighed and placed into a 50mL polypropylene centrifuge tube with screw caps (Sarstedt, Numbrecht, Germany). Three g of Florisil were added to the sample and mixed well, followed by 200 µL of surrogate standard. The mixture was hand-shaken for 1 min. Afterwards, 15 mL of binary extraction solvent (DCM/ACN, 50/50) was added. The mixture was vortexed and then sonicated for 20 minutes. The sample was centrifuged at 5,000 rpm (4,696 g*)* for 5 minutes. The supernatant was decanted to another 50 mL polypropylene centrifuge tube. The remaining sample was re-extracted with 10 mL of the extraction solvent by first breaking up the "cake" with a spatula then vortexing. The slurry sample was centrifuged again at 5,000 rpm for 3 minutes. The supernatant from the second extraction was combined with the first one and made up volume to 25 mL. The sample was vortexed again and 10 mL were transferred to a 15 mL polypropylene centrifuge tube. The sample was centrifuged at 5,000 rpm for 3 min and 1 mL was transferred to a GC vial for GC/MS analysis. **Sample Cleanup:** Without cleanup.

4.2 Determination of Petrogenic Hydrocarbons by GC/FID and GC/MS

The instrument and chromatographic conditions used for petrogenic hydrocarbons analysis were summarized in the following Table 9. All participants used an Agilent 6890 or7890 GC/FID except one lab used a Varian 3600 CX for PHC analysis. These instruments were equipped with different capillary columns (different length, internal diameter and film thickness). The GC parameters used by these participants are also different. Three type of carrier gases including helium (mostly used), nitrogen and hydrogen were used.

Unlike GC/FID analysis, all participants used similar equipments for the characterization and identification of petroleum hydrocarbons. Agilent 6890/7890 GC coupled with a 5973 or 5975 MSD were used by all participants. HP-5ms or DB-5ms (30 m × 0.25 mm, 0.25 µm film thickness) fused capillary column were used for sample separation. The MS mode was all electron impact ionization at 70 eV, operated in the Selected Ion Monitoring (SIM) mode. The ions listed in method section 7.5 were used to determine the target analytes.

As requested, most of the participants analyzed the petrogenic hydrocarbons using GC-MS and submitted chromatograms of target compounds (not presented in this report).

Table 9 Chromatographic instruments used in this method validation (to be continued).

Participants	GC/FID Analysis		GC/MS Analysis	
	Instrument	**Column**	**Instrument**	**Column**
Lab 01	Agilent 6890 GC/FID	DB-5HT, 30 m × 0.25 mm (id) fused silica column (0.1 μm film thickness); Carrier gas: 1.5 mL/min of hydrogen; Temperature program: 40°C for 2 min, 20°C/min to 340°C, hold 18 min; Flame Ionization Detector at 340°C	Agilent 6890 GC; Agilent 5973 MSD	HP-5MS, 30 m × 0.25 mm (id) fused silica column (0.25 μm film thickness); inlet held at 280°C; Carrier gas: 1.0 mL/min of helium; Temperature program: 50°C for 2 min, then 6°C/min to 310°C, hold 20 min; MS ion source: 230ºC.
Lab 02	Agilent 6890 GC/FID	Agilent/J&W DB1-HT, 15 m × 0.32 mm (id) × 0.10 μm film; 1μL volume splitless injection; Carrier gas: H_2 at 1.5 mL/min; Temperature program: 40°C for 2 min, then 20°C/min to 320°C, hold 7 min; FID temperature: 340°C.	Agilent 6890 GC; Agilent 5973 MSD	HP-5MS, 30 m × 0.25 mm (id) fused silica column (0.25 μm film thickness); inlet held at 270°C; Carrier gas: 1.2 mL/min of helium MS ion source: 240ºC.
Lab 03	Agilent 6890 GC/FID	Agilent/J&W DB1-HT, 15 m × 0.32 mm (id) × 0.10 μm film; 2μL volume splitless injection; Carrier gas: N_2 at 1.6 mL/min; FID temperature: 370°C.	Agilent 7890 GC; Agilent 5975 MSD	Agilent/J&W DB5-MS UI, 30 m × 0.25 mm (id) × 0.25 μm film 0.5μL volume splitless injection; inlet held at 280°C; Carrier gas: 1.0 mL/min of helium MS ion source: 230ºC.

Table 9 Chromatographic instruments used in this method validation (continued).

	GC/FID Analysis		GC/MS Analysis	
Participants	**Instrument**	**Column**	**Instrument**	**Column**
Lab 04	Agilent 6890N GC/FID	DB-1HT, 30 m × 0.53 mm × 1.7 µm with a deactivated fused silica 5 m guard column on the front end of the capillary column; Carrier gas: 8.5 mL/min of helium; Temperature program: 35°C for 3 min, then 15°C/min to 360°C, hold 5 min; FID temperature: 350°C.	Agilent 6890 GC; Agilent 5973 MSD	HP-5MS, 30 m × 0.25 mm (id) fused silica column (0.25 µm film thickness); Carrier gas: 1.0 mL/min of helium; Temperature program: 50 ºC for 2 min, then 6ºC/min to 320 ºC, hold for 13 min; MS ion source: 230ºC.
Lab 05	Agilent 7890	DB-5HT, 30 m × 0.25 mm (id) fused silica column (0.1 µm film thickness); Carrier gas: 1.5 mL/min of helium Temperature program: 40°C for 2 min, then 20°C/min to 340°C, hold 18 min; FID temperature: 340°C.	Agilent 6890 GC; Agilent 5973 MSD	HP-5MS, 30 m × 0.25 mm (id) fused silica column (0.25 µm film thickness); Carrier gas: 1.0 mL/min of helium Temperature program: 50°C for 2 min, 6°C/min to 310°C, hold 20 min; MS ion source: 300°C.
Lab 06	Varian 3600 CX	J&W DB-5, 30 m × 0.32 mm (id) × 0.25µ column; Carrier gas: 2.7 mL/min of helium; Temperature program: 55°C for 2 min, then 8°C/min to 325°C, hold 24.25 min; Detector Temperature: 325ºC.	Agilent 7890 GC; Agilent 5975 MSD	HP-5MS, 30 m × 0.25 mm (id) fused silica column (0.25 µm film thickness), a guard column (10 m × 0.53 mm id, deactivated, Restek) was press-fitted to the analytical column; Carrier gas: 1.0 mL/min of helium; Temperature program: 50ºC for 2 min, then 6ºC/min to 310 ºC, hold for 20 min; MS ion source: 300°C.

5. Method Validation Results

5.1 Method Blank

An investigation of method blank was conducted during the validation by each participant. As described in the method, interferences may be caused by contaminants in solvent reagents, on glassware, and on other sample processing hardware. Matrix interferences may be caused by contaminants co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the sample.

The silica gel clean-up procedure is useful to overcome many of these interferences. Some naturally occurring hydrocarbons may not be removed by the silica gel column cleanup and will be detected by this method.

All laboratories used blank Ottawa Sand for the method blank study. The results were summarized in Table 10. Lab 01 conducted four method blank tests. The average concentrations of PHC_{F2}, PHC_{F3}, and PHC_{F4} are 9.25, 4.96 and 0.66 µg/g of soil (by dry weight). The total PHC analysis blanks (n-C_{10} to n-C_{50}) is 14.9 µg/g. Lab 02 also conducted four method blanks analysis, and it reported similar range of blank with Lab 01. As seen from Table 10, the method blank for the PHC analysis (n-C_{10} to n-C_{50}) is generally less than 25 µg/g of dry soil. It is noted that all labs reported low blank for PHC_{F4} (>n-C_{34}). However, lubricating oil contamination during sample preparation and capillary column bleeding at high temperature are common and could introduce high interference to GC/FID analysis of this fraction. One participant also reported that the increasing baseline at high oven temperature could be mistakenly measured as method blank.

Table 10 Results of method blanks analysis.

Test No.	Test 1	Test 2	Test 3	Test 4	Mean	RSD (%)
Lab 01						
Surrogate Recovery (%)	71.8	84.5	82.8	96.0	83.8	11.8
PHC_{F2} (µg/g)	11.8	8.03	5.48	11.7	9.25	33.2
PHC_{F3} (µg/g)	4.77	4.39	6.01	4.66	4.96	14.4
PHC_{F4} (µg/g)	0.22	0.77	0.53	1.14	0.66	58.4
Total (µg/g)	16.8	13.2	12.0	17.5	14.9	18.1
Lab 02						
Surrogate Recovery (%)	80.5	77.6	77.1	78.6	78.5	1.5
PHC_{F2} (µg/g)	<5	<5	<5	<5	<5	-
PHC_{F3} (µg/g)	<5	<5	<5	<5	<5	-
PHC_{F4} (µg/g)	<5	<5	<5	<5	<5	-
Total (µg/g)	<15	<15	<15	<15	<15	-
Lab 03						
Surrogate Recovery (%)	NA					
PHC_{F2} (µg/g)						
PHC_{F3} (µg/g)						
PHC_{F4} (µg/g)						
Total (µg/g)						
Lab 04						
Surrogate Recovery (%)	111	108			119	
PHC_{F2} (µg/g)	1.12	1.12			1.12	
PHC_{F3} (µg/g)	10.0	2.00			6.00	
PHC_{F4} (µg/g)	3.52	3.52			3.52	
Total (µg/g)	14.6	6.64			10.6	
Lab 05						
Surrogate Recovery (%)	77.5	75.8	88.1		80.5	8.3
PHC_{F2} (µg/g)	<5	<5	<5		<5	
PHC_{F3} (µg/g)	<10	<10	<10		<10	
PHC_{F4} (µg/g)	<5	<5	<5		<5	
Total (µg/g)	<20	<20	<20		<20	

5.2 Linearity and Range

Prior to the method validation, each participant is responsible to ensure its instrument to be calibrated in an appropriate PHC range and to establish a proper linearity of PHC analysis. The linear relationship of the PHC analysis method was evaluated by conducting three concentrations of PHC analysis. An aliquot of 0.50, 5.0 and 100 mg of reference oil, or 0.1, 1.0 and 20.0 mg reference oil per 1.0 g of blank soil, was spiked in 5 g of Ottawa sand. Each concentration analysis is at least triplicate.

Table 11 presents the results of linearity approaches from five participants. Five labs all reported the multiple analysis results of PHC_{F2}, PHC_{F2}, PHC_{F2} and total PHC, as well as the recovery of surrogate (o-terphenyl) for three levels of spiking.

The method linearity was evaluated by plotting the PHC concentrations or PHC response as a function of analyte concentration or content. As an example, Figure 1 illustrates the linear regression of PHC concentrations against the spiking oil mass (Lab-01), the regression equations and coefficients were also shown in this figure. All the results from five participants demonstrated good linear relationship.

Table 11 Results of method linearity studies.

Component Level	Level 1		Level 2		Level 3	
	Mean	**RSD (%)**	**Mean**	**RSD (%)**	**Mean**	**RSD (%)**
Lab 01						
N	8		3		3	
Surrogate Recovery (%)	73.4	9.7	77.0	5.5	-	-
PHC_{F2} (µg/g)	23.4	22.8	125	11.6	3,553	12.4
PHC_{F3} (µg/g)	40.5	13.5	310	12.3	8,383	12.7
PHC_{F4} (µg/g)	12.3	14.8	97.0	4.9	2,704	14.8
Total (µg/g)	76.2	14.5	532	10.4	14,641	13.0
Lab 02						
N	8		4		4	
Surrogate Recovery (%)	93.5	2.4	104	2.4	-	-
PHC_{F2} (µg/g)	15.9	9.8	141	7.3	3,090	11.1
PHC_{F3} (µg/g)	35.6	3.4	338	3.2	7,097	5.8
PHC_{F4} (µg/g)	16.1	10.4	143	1.4	3,417	8.7
Total (µg/g)	67.6	5.4	622	3.3	13,604	7.2
Lab 03						
N	8		3		3	
Surrogate Recovery (%)	90.1	5.8	87.5	4.7	70.1	18.4
PHC_{F2} (µg/g)	20.7	4.6	154	6.2	3,120	18.4
PHC_{F3} (µg/g)	41.8	3.9	311	2.8	6,273	19.2
PHC_{F4} (µg/g)	8.97	21.9	70.6	3.1	1,697	15.5
Total (µg/g)	71.5	5.3	545	3.8	11,690	18.4
Lab 04*						
N	9		3		3	
Surrogate Recovery (%)	100	3.2	108	2.5	109	3.6
PHC_{F2} (µg/g)	7.50	24.1	90.0	38.0	2,952	29.3
PHC_{F3} (µg/g)	55.5	6.6	346	13.5	8,476	8.3
PHC_{F4} (µg/g)	11.1	10.0	108	10.6	2,816	9.6
Total (µg/g)	74.2	7.5	545	14.1	14,245	12.8
Lab 05						
N	7		3		3	
Surrogate Recovery (%)	91.3	2.9	98.1	1.2	-	-
PHC_{F2} (µg/g)	15.0	9.8	102	11.4	2,730	3.8
PHC_{F3} (µg/g)	33.2	6.9	359	5.0	8,210	1.4
PHC_{F4} (µg/g)	10.1	13.2	122	2.3	2,440	4.1
Total (µg/g)	58.3	5.1	584	1.6	13,400	1.2

* Use 10 mg of oil for level 2.

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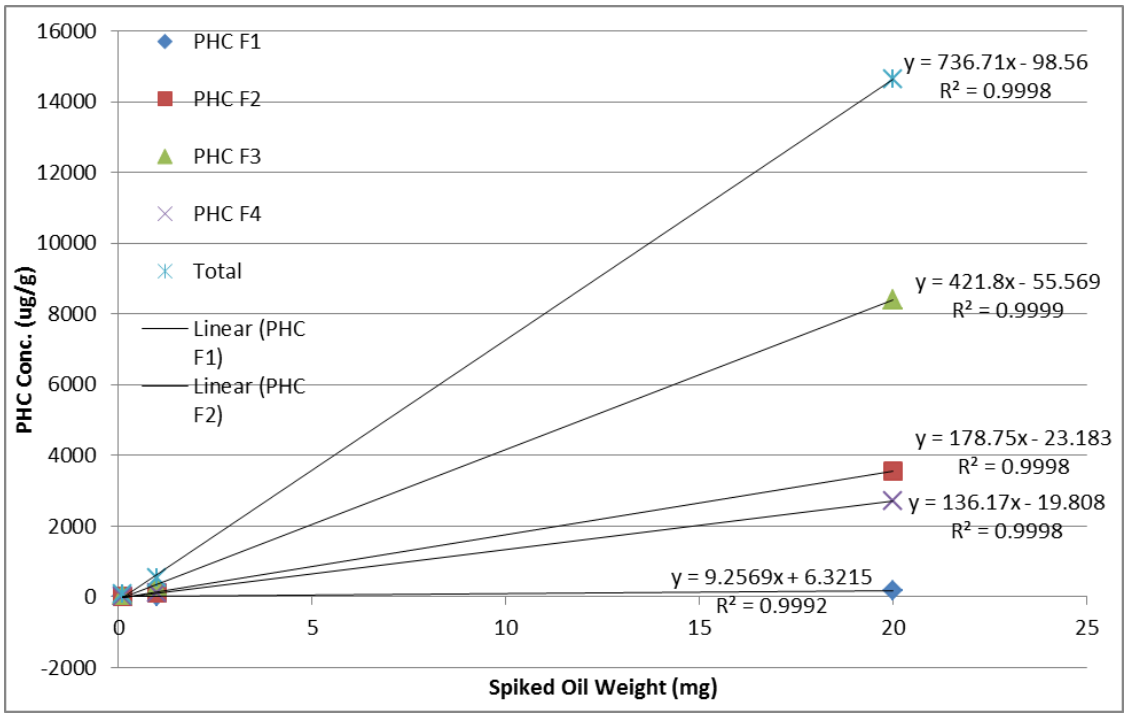


Figure 1 Linearity of PHC analysis (Lab-01).

5.3 Precision

To evaluate the method precision (repeatability and reproducibility, R&R), each lab conducts multiple measurements for each of three levels of PHCs. Every participant conducted 7 to 9 replicates for the lowest level, and triplicates for two other PHC levels. As observed from previous Table 11, the participants achieved the best repeatability for the middle level with an overall relative standard deviation of <10%. The RSD values for two other levels of PHC analysis are obviously higher than for the middle level, but largely less than <20%.

The reproducibility of this PHC analytical method was assessed by means of this interlaboratory validation. In Table 12, the mean value, standard deviation (*S*) and relative standard deviation of the surrogate, three PHC fractions and total PHC were calculated from the results from all five participants. Overall, the analysis of PHCs at low level has a lower reproducibility, with a RSD value

of 37.0%, 21.0%, 23.4% and 10.2% for PHC_{F2}, PHC_{F3}, PHC_{F4}, and total PHC, respectively.

Table 12 Reproducibility of results from five participants.

Component Level	Level 1	Level 2	Level 3
Surrogate Recovery			
Mean (%)	89.7	94.9	89.6
S (µg/g)	9.9	12.6	-
RSD (%)	11.0	13.3	-
PHC_{F2}			
Mean (µg/g)	16.5	122	3,089
S (µg/g)	6.10	26.5	302
RSD (%)	37.0	21.7	9.8
PHC_{F3}			
Mean (µg/g)	41.3	333	7,688
S (µg/g)	8.67	21.7	965
RSD (%)	21.0	6.5	12.6
PHC_{F4}			
Mean (µg/g)	11.7	108	2,615
S (µg/g)	2.74	27.1	626
RSD (%)	23.4	25.1	23.9
Total PHC			
Mean (µg/g)	69.6	566	13,516
S (µg/g)	7.1	37.1	1,135
RSD (%)	10.2	6.6	8.4

5.4 Accuracy

Accuracy is generally reported as percent recovery by the assay of known added amount of analytes in the sample. ESTS's reference oil Prudhoe Bay crude oil (13.1% weathered by laboratory rotary evaporation) was used in this study. The accuracy is estimated by mean value of the results for repeatability study divided by the PHC data of the reference oil. The Oil Research Lab of Environment Canada has been using this crude oil as a reference material for nearly ten years and generated a large dataset of this reference oil. Based on many analyses in last ten years, the reference oil contains 590 mg/g (S = 16.8) of PHC (oil sample was fractionated into saturated fraction and aromatic fraction, and determined by GC/FID). The accuracy of PHC analysis for 3 concentration levels was summarized in Table 13.

As shown in Table 13, participants obtained a recovery of about 70% to 130% for PHC analysis. The average PHC recoveries from five labs are 118%, 95.8% and 115%, with a standard deviation of 12.0%, 6.11%, and 9.62% for level 1, level 2 and level 3 spiking, respectively.

The recovery for surrogate (o-terphenyl) was calculated by dividing the measured concentration by initial spiked concentration. The average recoveries of surrogate of five labs are 89.7%, 94.9% and 89.6% for level 1, level 2 and level 3 spiking, respectively.

Table 13 Results of method accuracy studies.

Component Level	Level 1		Level 2		Level 3	
	Mean	***S***	**Mean**	***S***	**Mean**	***S***
Spiked Oil (mg)	0.50		5.0		100	
Converted PHC (mg/g)	59.0		590		11,800	
Lab 01						
Total PHC (µg/g)	76.2	11.0	532	55.3	14,641	1,903
PHC Recovery (%)	129	18.6	90.2	9.4	124	16.1
Surrogate Recovery (%)	73.4	7.12	77.0	4.24	-	-
Lab 02						
Total PHC (µg/g)	67.6	3.65	622	20.7	13,604	979
PHC Recovery (%)	115	6.19	105	3.5	115	8.3
Surrogate Recovery (%)	93.5	2.23	104	2.51	-	
Lab 03						
Total PHC (µg/g)	71.5	3.79	545	20.7	11,690	2,151
PHC Recovery (%)	121	6.4	92.4	3.5	99.1	18.2
Surrogate Recovery (%)	90.1	5.23	87.5	4.11	70.1	12.9
Lab 04*						
Total PHC (µg/g)	74.2	5.57	545	76.9	14,245	1,823
PHC Recovery (%)	126	9.5	92.4	13.0	121	15.5
Surrogate Recovery (%)	100	3.20	108	2.70	109	3.92
Lab 05						
Total PHC (µg/g)	58.3	2.97	584	9.34	13,400	161
PHC Recovery (%)	98.8	5.0	99.0	1.6	114	1.4
Surrogate Recovery (%)	91.3	2.65	98.1	1.18	-	-
Mean of PHC Recovery (%)	**118**		**95.8**		**115**	
Standard Deviation (%)	12.0		6.11		9.62	
Mean of Surrogate Recovery (%)	**89.7**		**94.9**		**89.6**	
Standard Deviation (%)	11.6		12.6		-	

5.5 Detection Limit and Quantitation Limit

To determine the method detection limit (MDL), 5 g of Ottawa sand was spiked with 0.50 mg of net reference oil or 0.1 mg/g of dry soil (equivalent to 295 µg net or 59 µg/g of GC/FID detectable PHCs in a range of n-C_{10} to n-C_{50}). This low level analysis was at least 8 replicated by every participant. All replicate analyses were processed through the entire analytical method used for the all samples in this method validation.

The standard deviations for each analyte (PHC fraction), *s*, are calculated from replicate measurement and multiplied by the double-tailed Student's-t factor at the 99% confidence level. This value represents the method detection limit (MDL). The student value for the 99% confidence level is 3.143, 2.998 and 2.896 for the degree of freedom (n-1) of 6, 7 and 8, respectively.

The limit of quantitation is determined by multiplying the MDL value by a factor of 10/3.

$$MDL = t_{(n-1,\ \alpha=0.99)}\ s$$

$$LOQ = 10/3\ MDL$$

The method detection limits for PHC analysis was presented in Table 14. The MDL reported by five participants is 2.85 to 16.0 µg/g for PHC_{F2}, 4.87 to 16.4 µg/g for PHC_{F3}, and 3.22 to 14.5 µg/g, respectively. The method detection limit of total PHC analysis (n-C_{10} to n-C_{50}) ranged from 11.4 to 38.2 µg/g with an average value of 23.0 µg/g. The MDLs of total PHCs all meet the criteria of: Calculated MDL < Spike Level < 10 × Calculated MDL.

Table 14 Method detection limits of the PHC analytical method.

Participants	**Lab 01**	**Lab 02**	**Lab 03**	**Lab 04**	**Lab 05**
N	8	8	8	9	7
Average Recovery (%)	73.6	93.5	90.1	100	91.4
PHC_{F2}					
Mean (µg/g)	23.4	15.9	20.7	7.50	15.0
S (µg/g)	5.33	1.56	0.95	1.81	1.46
MDL (µg/g)	16.0	4.69	2.85	5.24	4.60
LOQ (µg/g)	53.3	15.6	9.49	13.2	15.3
PHC_{F3}					
Mean (µg/g)	40.5	35.6	41.8	55.5	33.2
S (µg/g)	5.46	1.21	1.63	3.67	2.30
MDL (µg/g)	16.4	3.62	4.87	10.6	7.42
LOQ (µg/g)	54.5	12.1	16.2	34.5	24.1
PHC_{F4}					
Mean (µg/g)	12.3	16.1	8.97	11.1	10.1
S (µg/g)	1.83	1.68	3.81	1.11	1.33
MDL (µg/g)	5.48	5.03	11.4	3.22	4.19
LOQ (µg/g)	18.3	16.8	38.7	10.1	14.0
Total PHC					
Mean (µg/g)	76.2	67.6	71.5	74.2	58.3
S (µg/g)	11.1	3.65	3.81	5.53	2.95
MDL (µg/g)	33.2	11.0	11.4	16.0	16.0
LOQ (µg/g)	111	36.5	38.1	44.4	53.4

5.6 Robustness

The robustness of this analytical method has been proved by this interlaboratory method validation. A number of typical variations were involved through the sample extraction, extract cleanup, instrumental analysis and data processing, for example,

- Extraction technique and extraction time
- Equipment and analyst
- Different GC columns (different lots and/or suppliers)
- GC parameters
- Carrier gas and flow rate
- Random events, etc.

Despite of these variations, using this method, the participants obtained good accuracies, precision, and linearity for PHC analysis at three spiking levels.

6. Conclusions

This PHC analytical method has been successfully validated by six analytical laboratories. The method blank, linearity, accuracy, precision, and robustness were investigated. The method detection limit of total PHC (ranging from n-C_{10} to n-C_{50}) is determined to be <50 µg/g on the basis of 5 g dry weight.

During the method validation, the participants came across many challenges in sample preparation and instrumental analysis. To obtain satisfactory PHC analysis results, the analytical laboratory must properly adapt this method according to its own laboratory practice.

PART III

ROUND ROBIN

Determination and Differentiation of Petrogenic and Biogenic Inputs in Contaminated and Background Soils

1. Introduction

This Round Robin (RR) test was the first interlaboratory study on PHC analysis using the analytical method established in this AUPRF project. This RR test aimed to verify the validation and reliability of this new method. Six laboratories from Environment Canada, provincial and commercial labs from the National Capital Region (NCR) and three provinces participated in this test (page 39, Table 1 in Part II). The report evaluates the overall results from six participants.

2. Preparation of Soil Samples

In this Round Robin test, three soil samples were prepared by the Oil Research Lab of Environment Canada and were delivered to the participants in April 2012. Table 1 summarizes the information of three Round Robin 2012 soil samples. However, this information is confidential to the participants before they submitted their RR report. Sample 1 is a relatively clean soil collected in Ottawa suburb. Sample is an artificial petroleum-contaminated soil by spiking a reference oil in to blank soil 1. Sample3 is a petroleum contaminated soil collected in Alberta province. An instruction was also sent to the participants to use the AUPRF Round Robin 2012 Samples.

- Each sample is packaged in two vials. Each vial contains 5.0 g of soil (dry weight). Please use 5.0 g to calculate the PHC concentration.
- Open one vial and quantitatively transfer the **entire** contents of the vial to your extraction apparatus. Rinse the vial thoroughly with extraction solvent and transfer the solvent to the extraction apparatus.
- The other vial can be used for a backup or a duplicate analysis. Follow the AUPRF PHC Method for extraction and analysis of petroleum hydrocarbons in this sample.

Table 1 Round Robin soil sample Information.

Sample ID	RR Soil Sample 1	RR Soil Sample 2	RR Soil Sample 3
Sample Information	Blank soil, prepared from soil collected in Ottawa suburb in March, 2012	Prepared from blank RR Soil 1 by spiking reference oil	Oil contaminated soil collected in Alberta
PHC Concentration	NA	NA	NA
Dry Weight (g)	5.0	5.0	5.0
Water Content (%)	~30	~10	~8

3. PHC Analysis Results

The laboratories were requested to report the PHC concentrations, if the petrogenic hydrocarbons were detected in these soil samples, to provide the chromatographic evidences (selected ion chromatograms of target analytes) of the presence of petrogenic source. Three RR soil samples were processed by the same procedures and analyzed by the same chromatographic condition described in the method validation by each participant.

3.1 PHC Analysis Results of Round Robin Soil Samples.

The PHC Analysis Results of Round Robin Soil Samples was summarized in table 2. All of the PHC concentrations (μg/g) in this report are based on 5 g of dry weight of soil samples. All participants reported the surrogate (o-terphenyl) recoveries, PHC_{F2}, PHC_{F3}, PHC_{F4}, and total PHC in three RR samples. Lab 01 reported a duplicate analysis of RR soil sample 2, and Lab 03 report a duplicate analysis of RR soil sample 1. Figures 1A, 2A and 3A illustrated the GC/FID chromatogram of hydrocarbons in three Round Robin soil samples, respectively.

Six participants obtained a good surrogate recoveries, 78.8~103% for RR soil sample 1, 88.7~114% for RR soil sample 2, and 87.8~109% for RR soil sample

3. As seen in Table 2, the total PHC values were determined to be in the range of 21.1 to 100 µg/g for clean soil RR sample 1 with a mean value of 57.3 µg/g and standard deviation of 29.1 µg/g. Six participants reported more reproducible results for the total PHCs of petroleum-contaminated sample 2 and 3. The PHCs in RR soil 2 were determined to be 416~615 µg with a mean value of 509 µg/g and standard deviation of 68.6 µg/g. RR soil sample 3 contains the highest concentration of total PHCs, ranging from 2,667 to 3,473 µg/g with a mean value of 2,973 µg/g and a <10% of relative standard deviation. PHCs in all three RR soil samples are dominated by PHC_{F3} hydrocarbons (n-C_{16} to n-C_{34}), and this can be also noted in the GC/FID chromatograms in Figures 1A, 2A and 3A.

Table 2 PHC analysis results of the Round Robin soil samples.

Component	Lab 01	Lab 01 duplicate	Lab 02	Lab 03	Lab 03 duplicate	Lab 04	Lab 05	Lab 06	Mean	*S*
RR Soil 1										
Surrogate Recovery (%)	78.8		95.1	92.7	88.1	103	86.9		**90.8**	8.22
PHC_{F2} (µg/g)	9.62		<5.0	4.56	5.23	3.57	3.50	6.10	**5.43**	2.28
PHC_{F3} (µg/g)	19.3		15.8	32.7	26.5	60.9	53.4	23.1	**33.1**	17.4
PHC_{F4} (µg/g)	3.72		<5	10.4	9.33	35.8	24.5	48.2	**20.0**	17.4
Total (µg/g)	32.7		21.1	47.6	41.1	100	81.5	77.4	**57.3**	29.1
RR Soil 2										
Surrogate Recovery (%)	104	92.6	102	88.9		114	88.7		**98.4**	10.1
PHCF2 (µg/g)	18.1	21.5	28.1	36.4		31.2	23.9	77.6	**33.8**	20.2
PHCF3 (µg/g)	295	280	332	301		388	440	271	**330**	62.6
PHCF4 (µg/g)	161	165	124	78.1		154	151	232	**152**	46.4
Total (µg/g)	474	467	484	416		574	615	531	**509**	68.6
RR Soil 3										
Surrogate Recovery (%)	105		85.3	95.4		109	87.8		**96.5**	10.4
PHCF2 (µg/g)	321		284	469		306	339	443	**360**	76.7
PHCF3 (µg/g)	1,930		1,791	2,074		2,014	2,381	1,772	**1,994**	224
PHCF4 (µg/g)	601		592	546		620	752	603	**619**	69.8
Total (µg/g)	2,851		2,667	3,088		2,940	3,473	2,817	**2,973**	282

3.2 Characterization and Identification of Petrogenic Hydrocarbons.

All participants are able to characterize and identify petrogenic contamination by investigation the chromatographic features of samples. Table 3 summarizes the results of oil analysis for these three Round Robin samples. Most participants provided chromatographic evidences to support their conclusion. As examples, the appendices show GC/FID and GC/MS chromatograms of target analytes in three RR soil samples presented by Lab 01.

Table 3 Identification of petrogenic sources in Round Robin soil samples.

Lab Code	RR Soil 1	RR Soil 2	RR Soil 3
Lab 01	Trace	Positive	Positive
Lab 02	Negative	Positive	Positive
Lab 03	Negative	Positive	Positive
Lab 04	Negative	Positive	Positive
Lab 05	Negative	Positive	Positive
Lab 06	Negative	Positive	Positive

The clean RR soil sample 1 contains very low PHCs (averagely 57.3 µg/g). Figures 1B 2 shows the GC/MS chromatograms (at m/z 85) for n-alkane characterization in this soil sample. Distribution patterns of *n*-alkanes in RR sample 1 show a significant predominance of odd carbon number n-alkanes over the even number n-alkanes in the C_{21} to C_{35} range, with maxima at n-C_{29}, n-C_{31} and n-C_{33} alkanes, clearly indicating contribution of biogenic input from organic matter.

The presence of trace among of petrogenic contamination in RR sample 1 is also obvious, although some participants concluded a negative presence of petrogenic source. This can be supported by the APAH distribution and their chromatographic features.

As seen from these chromatograms, contribution of petrogenic hydrocarbons to PHCs determined in RR sample 2 and RR sample 3 are very clear. The selected ion chromatogram of biomarker terpanes (at m/z 191), biomarker steranes (m/z 218) and bicyclic sesquiterpanes (m/z 123) are shown in Figures 2C to 2E for RR soil sample 2 and Figures 2C to 2E for RR soil sample 3. Figures 3Cto 2J and 3F to 3J show chromatographic features of alkylated PAH homologous series (alkylated naphthalene, phenanthrene, dibenzothiophene, and chrysene series) and their distribution. All these chromatographic information provides sound evidence of the presence of petrogenic hydrocarbons in both RR soil samples 2 and 3.

Figure 2B illustrates the distribution patterns of *n*-alkanes in RR sample 2. A wide range of n-alkanes from n-C_{14} to n-C_{36} were detected in this soil, indicating petrogenic contamination (from spiked oil). The dominance of odd carbon number n-alkanes over the even number n-alkanes in the n-C_{27} to C_{33} range suggests a contribution of biogenic input (from blank soil) to total PHCs.

PART IV

WORKSHOP

Standardization of an Analytical Method to Differentiate Petrogenic and Biogenic Inputs in Contaminated and Background Soils

1. Workshop Program

Workshop on the *Standardization of an Analytical Method to Differentiate Petrogenic and Biogenic Inputs in Contaminated and Background Soils*

February 27 – March 2, 2012

Emergencies Science and Technology Section

Environment Canada

335 River Road, Ottawa, ON

"Total petroleum hydrocarbons" (TPHs) or "petroleum hydrocarbons" (PHCs) are one of the most widespread soil pollutants in Canada, North America, and worldwide. Clean-up of PHC-contaminated soils and sediments costs the Canadian economy hundreds of million of dollars annually. Much of this activity is driven by the need to meet regulated levels of PHC in soil.

In the environment, soil contamination generally originates from three main sources: biogenic, pyrogenic and petrogenic hydrocarbons. Biogenic substances are produced by organisms or generated from naturally occurring organic matter. These naturally occurring biogenic organic compounds (BOCs) are usually non-toxic and less hazardous than those from petrogenic and pyrogenic sources. BOCs present in soils and wet sediments can be easily misidentified and quantified as regulated PHCs during analysis using such methods for PHC determination. In some cases, biogenic interferences can exceed regulatory levels, resulting in unnecessary and costly remediation measures, while also wasting valuable landfill space. Therefore, it is critically important to characterize and differentiate PHCs and BOCs in contaminated sediments in PHC analysis.

Funded by Alberta Upstream Petroleum Research Fund (AUPRF), Environment Canada Oil Research Laboratory and other partner laboratories are conducting a projected "Standardization of an Analytical Method to Distinguish Petrogenic and Biogenic Inputs in Contaminated and Background Soils". The project is proposed to address above issues by adapting existing research on chemical forensic techniques to methods which can be used by analytical laboratories to prove the presence or absence of petroleum hydrocarbons in background soils in addition to the PHC analysis.

Workshop timing and organization:

A 5-day workshop will be held at 335 River Road Labs, Ottawa, Ontario, on February 27 to March 2, 2012. The purpose of the workshop is to discuss methodology for PHCs analysis and to develop a path forward for validation of analytical methods which would be used routinely for analysis in government and private laboratories. The meeting will be hosted by the Oil Research Laboratory, Emergencies Science and Technology Section (ESTS), Environment Canada.

Organizing Committee/Participants:

Chun Yang – ESTS, Environment Canada, Ottawa, ON

Zhendi Wang – ESTS, Environment Canada, Ottawa, ON

Bruce Hollebone – ESTS, Environment Canada, Ottawa, ON

Mike Landriault – ESTS, Environment Canada, Ottawa, ON

Dayue Shang – PESC, Environment Canada, North Vancouver, BC

Jim Sproull – PNLET, Environment Canada, Edmonton, AB

Chen Yang – PNLET, Environment Canada, Edmonton, AB

Ralph Ruffolo – Ministry of the Environment, Ontario, ON

Don Maxwell – Maxxam Analytics, Calgary, AB

Andrea Armstrong – ALS, Waterloo, ON

Marcus Kim – Agilent Technologies, Mississauga, ON.

(Participants from other labs and ESTS students are not listed above)

Workshop Program

Day 1: February 27, 2011

9:00 – 9:30 Arrival and Welcome (Zhendi Wang)
9:30 – 9:45 Workshop Objectives and Goals (Chun Yang)
9:45 – 10:15 Lab tour

10:15 – 10:30 Coffee Break

10:30 – 11:45 Introduction to AUPRF TPH Method (Chun Yang)

12:00 – 1:15 Lunch Break (on your own)

1:15 – 2:30 Sample Extraction (Mike Landriault)

2:30 – 2:45 Coffee Break

2:45 – 4:15 Oil Fingerprinting Analysis (Zhendi Wang)
4:15 – 4:45 Discussion (all)
4:45 – 4:55 Closing Comments to Day 1.

Day 2: February 28, 2012

9:00 – 9:15 Objectives and Goals
9:15 – 10:15 Sample pre-treatment (Mike Landriault)

10:15 – 10:30 Coffee Break

10:30 – 12:00 Oil Biomarker Chemistry (Zhendi Wang)

12:00 – 1:15 LUNCH (on your own)

1:15 – 2:15 GC×GC as a Potential Tool for Environmental Forensics (Ralph Ruffolo)

2:15 – 2:30 Coffee Break

2:30 – 4:15 Sample Pre-treatment and Instrumental Analysis (Mike Landriault)
4:15 – 4:30 Closing Comments to Day 2.

Day 3: February 29, 2012

9:00 – 9:15 Objectives and goals
9:15 – 10:15 Differentiation of Petrogenic and Biogenic Hydrocarbons in Environmental samples (Zhendi Wang/Chun Yang)

10:15 – 10:30 Coffee Break

10:30 – 11:15 Rapid Determination of Naphthenic Acids in Water Samples from Alberta

using LC/MS (Dayue Shang)
11:15 – 12:00 Case studies in Oil Weathering and Degradation (Bruce Hollebone).

12:00 – 1:15 LUNCH

1:15 – 2:15 Instrumental Analysis (Marcus Kim)

2:15 – 2:30 Coffee Break

2:30 – 3:30 Petroleum Hydrocarbons Analysis and Oil Fingerprinting/Matching at the Prairie and Northern Laboratory for Environmental Testing (Jim Sproull)
3:30 – 4:15 Discussion of Method (Chun Yang and all)

4:15 – 4:30 Closing Comments to Day 3.

Day 4: March 1, 2012

9:00 – 9:15 Objectives and Goals
9:15 – 10:15 Data Processing (Chun Yang and all)

10:15 – 10:30 Coffee Break

10:30 – 12:00 Discussion of AUPRF Method (to be continued)

12:00 – 1:15 LUNCH

1:15 – 1:45 Round Robin (Chun Yang)
1:45 – 2:15 (ALS, Maxxam)

2:15 – 2:30 Coffee Break

2:30 – 4:15 Discussion of AUPRF Method (continued)
4:15 – 4:30 Closing Comments to Day 4.

Day 5: March 2, 2012

9:00 – 9:15 Objectives and Goals
9:15 – 10:15 Discussion to finalize method text and instrumental method parameters (all).

10:15 – 10:30 Coffee Break

10:30 – 11:45 Discussion of the method validation plan, and Round Robin Plan (all).
11:45 – 12:00 Concluding Remarks and Wrap Up.

* All presentation and discussion will be in the ESTS training room (2nd floor). The experiment demo will be in the Oil Research Lab (room 345).

2. List of Workshop Attendees

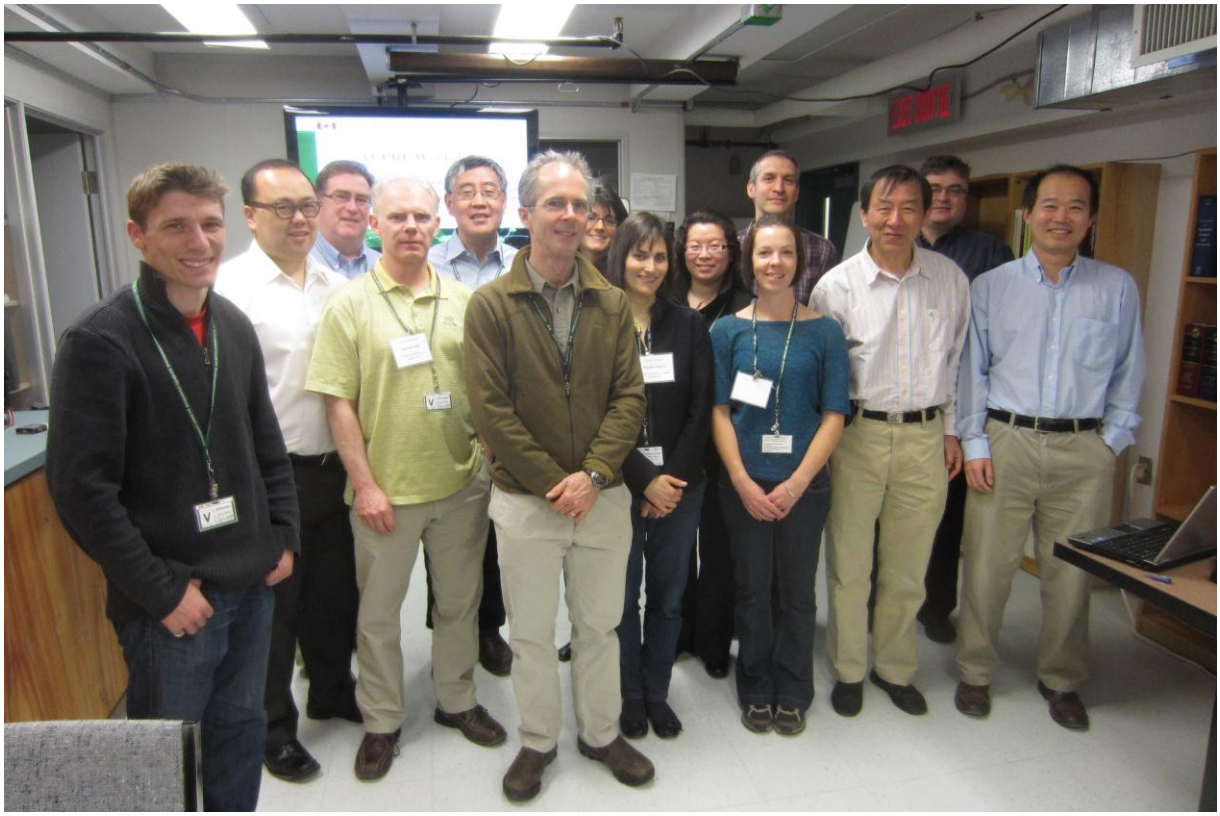
Workshop on *the Standardization of an Analytical Method to Differentiate Petrogenic and Biogenic Inputs in Contaminated and Background Soils*

February 27 – March 2, 2012
Emergencies Science and Technology Section
Environment Canada
335 River Road, Ottawa, ON

Name	Lab/Company
Ms. Andrea Armstrong	ALS, Waterloo, ON
Dr. Vladmir Blinov	ESTS, Environment Canada, Ottawa, ON
Mr. Jason Bornstein	Queen's University, Kingston, ON
Dr. Jennifer Gushue	Agilent Technologies, Montreal, QC
Dr. Bruce Hollebone	ESTS, Environment Canada, Ottawa, ON
Ms. Mireille Hugues	QLET, Environment Canada, Montreal, QC
Dr. Marcus Kim	Agilent Technologies, Mississauga, ON.
Dr. Wenxing Kuang	ESTS, Environment Canada, Ottawa, ON
Mr. Mike Landriault	ESTS, Environment Canada, Ottawa, ON
Mr. Ken Li	ESTS, Environment Canada, Ottawa, ON
Mr. James MacDonald	Maxxam Analytics, Bedford, NS
Mr. Don Maxwell	Maxxam Analytics, Calgary, AB
Dr. Ralph Ruffolo	Ministry of the Environment, Ontario, ON
Dr. Dayue Shang	PESC, Environment Canada, North Vancouver, BC
Mr. Keval Shah	ESTS, Environment Canada, Ottawa, ON
Mr. Jim Sproull	PNLET, Environment Canada, Edmonton, AB
Dr. Chunyan Wang	ESTS, Environment Canada, Ottawa, ON
Dr. Zhendi Wang	ESTS, Environment Canada, Ottawa, ON
Ms. Chen Yang	PNLET, Environment Canada, Edmonton, AB
Dr. Chun Yang	ESTS, Environment Canada, Ottawa, ON
Dr. Yuan (James) Yao	ESTS, Environment Canada, Ottawa, ON

3. Summary of the Workshop

The Oil Research Laboratory of the Emergencies Science and Technology Section (ESTS) hosted a successful workshop on the "*Standardization of an Analytical Method to Differentiate Petrogenic and Biogenic Inputs in Contaminated and Background Soils"* in Ottawa, from February 27 to March 2, 2012. Over twenty scientists from Environment Canada, provincial and commercial labs from the National Capital Region (NCR) and five provinces attended the workshop.



Participants in the photo (left to right): Jason Bornstein (Queen's University, Kingston, ON); Dr. Marcus Kim (Agilent Technologies, Mississauga, ON); James MacDonald (Maxxam Analytics, Halifax, NS); Don Maxwell (Maxxam Analytics, Calgary, AB); Dr. Dayue Shang (PESC, Environment Canada, North Vancouver, BC); Jim Sproull (PNLET, Environment Canada, Edmonton, AB); Dr. Jennifer Gushue (Agilent Technologies, Montreal, QC); Mireille Hugues (QLET, Environment Canada, Montreal, QC); Chen Yang (PNLET, Environment Canada, Edmonton, AB); Andrea Armstrong (ALS Environmental, Waterloo, ON); Dr. Ralph Ruffolo, (Ministry of the Environment, Ontario, ON); Dr. Zhendi Wang (ESTS, Environment Canada, Ottawa, ON); Dr. Bruce Hollebone (ESTS, Environment Canada, Ottawa, ON); and Dr. Chun Yang (ESTS, Environment Canada, Ottawa, ON).

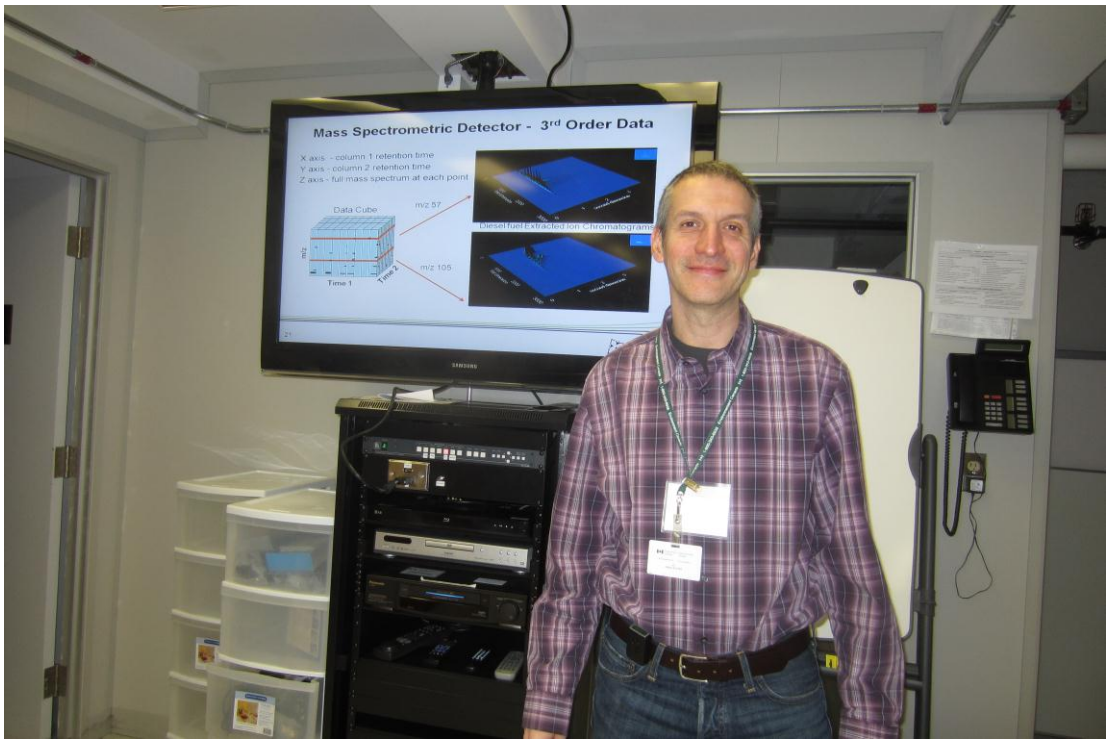
During the workshop, Dr. Zhendi Wang delivered a series of comprehensive lectures on petroleum chemistry, oil fingerprinting analysis, and identification and differentiation of petrogenic contamination from biogenic source hydrocarbons. Dr. Chun Yang chaired workshop and led the technical discussions with respect to the reliable determination of petroleum contamination in the environment.

The participants included; Dr. Ralph Ruffolo, Ontario Ministry of the Environment, Dr. Dayue Shang, Pacific and Yukon Laboratory for Environmental Testing (PYLET) and Jim Sproull, Prairie & Northern Laboratory for Environmental Testing (PNLET) who shared their knowledge and experience in the field of environmental analysis. Through this project and the associated workshop, Environment Canada has established a new PHC analysis methodology that was validated by the participating federal, provincial and commercial laboratories.

Dr. Zhendi Wang is delivering a lecture on the identification and differentiation of petrogenic contamination from biogenic source hydrocarbons.

Mr. Mike Landriault is demonstrating the sample preparation for the analysis of petroleum hydrocarbons in soil/sediment.

Dr. Chun Yang is introducing the new analytical method to differentiate petrogenic and biogenic inputs in contaminated and background soils.



Dr. Ralph Ruffolo (Ministry of the Environment, Ontario, ON) is sharing his knowledge and experience in environmental analysis.

Dr. Marcus Kim, Agilent Technologies Inc., is presenting “Analytical Solutions for Petrochemical Analysis”.