REMTECH 2019 CONFERENCE
BIOGENIC TOLUENE
A Laboratory's Perspective

AGENDA

- ➢ Biogenic Toluene Background

- ➢ Laboratory's Obligations

- ➢ Bureau Veritas' Approach

- ➢ What has the data indicated (so far)

- ➢ Conclusions and next steps

BACKGROUND

Low-Level Toluene Detections

- *Pristine Environments*

- *No history of industrial activity*

- *Typically associated with highly organic, moist, low-dissolved oxygen (anoxic) environments*

- *Peatland (Bogs/Fens)*

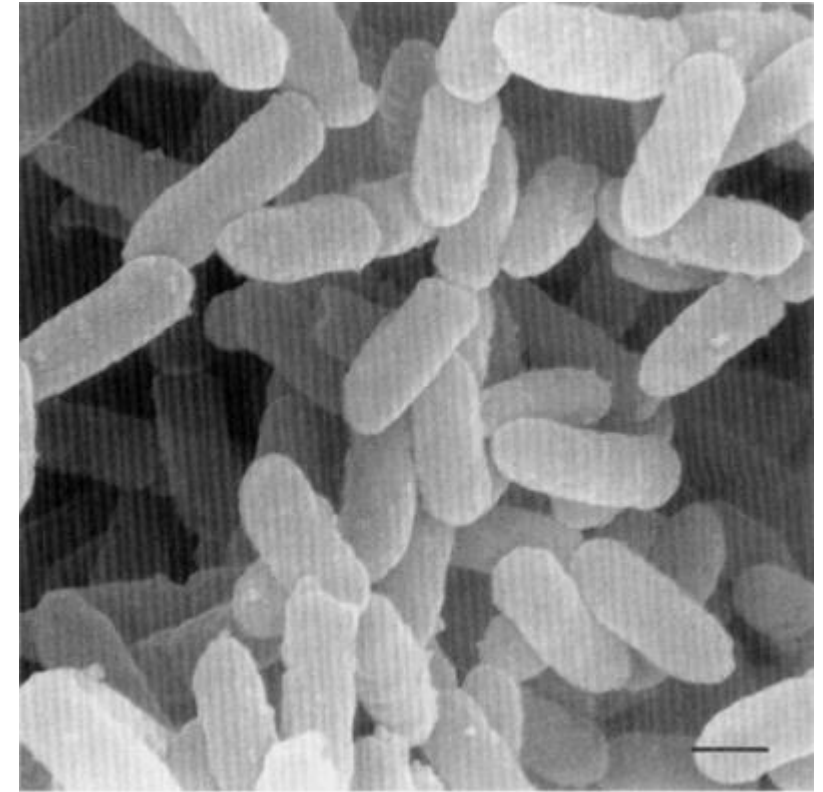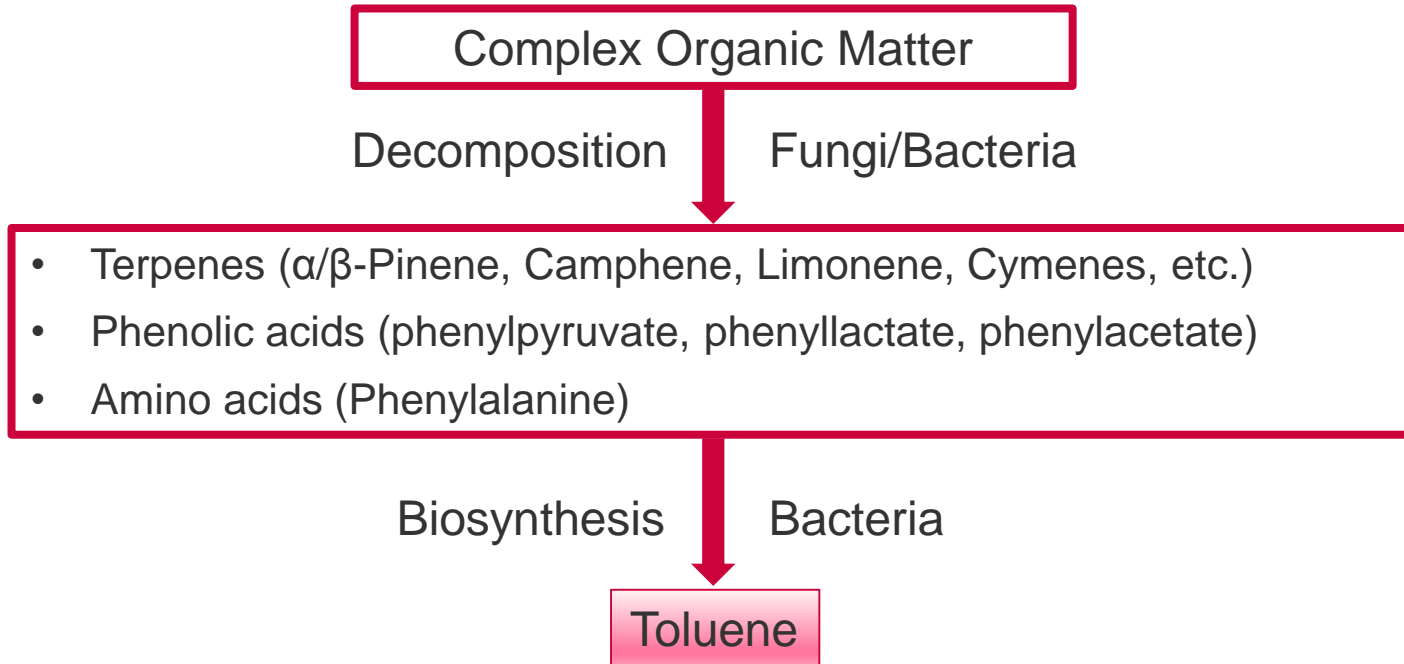- *Any anoxic, moist, high organic site (lake/marine sediments)*

BACKGROUND

FIG. 1. Scanning electron micrograph of strain TA 4ᵀ. Bar = 1 μm.

Tolumonas auensis
Sourced from freshwater lake sediment

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┌─────────────────────────────────────┐
│        Complex Organic Matter        │
└─────────────────────────────────────┘
                   │
Decomposition      ▼      Fungi/Bacteria

┌───────────────────────────────────────────────────────────┐
│  • Terpenes (α/β-Pinene, Camphene, Limonene, Cymenes, etc.)│
│  • Phenolic acids (phenylpyruvate, phenyllactate, phenylacetate)│
│  • Amino acids (Phenylalanine)                             │
└───────────────────────────────────────────────────────────┘
                   │
Biosynthesis       ▼      Bacteria

              ┌──────────┐
              │ Toluene  │
              └──────────┘
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Harry R. Beller, et al, 2018. Discovery of enzymes for toluene synthesis from anoxic microbial communities, Nature Chemical Biology

Zargar K, et al. 2016. In vitro characterization of phenylacetate decarboxylase, a novel enzyme catalyzing toluene biosynthesis in an anaerobic microbial community. Scientific Reports

Harder J, Probian C. 1995. Microbial degradation of monoterpenes in the absence of molecular oxygen. Appl Environ Microbiol

Fischer-Romero, C et al., Tolurnonas auensis gen. nov., sp. nov., a Toluene-Producing Bacterium from Anoxic Sediments of a Freshwater Lake, International Journal of Systematic Bacteriology, Jan. 1996, p. 183-188
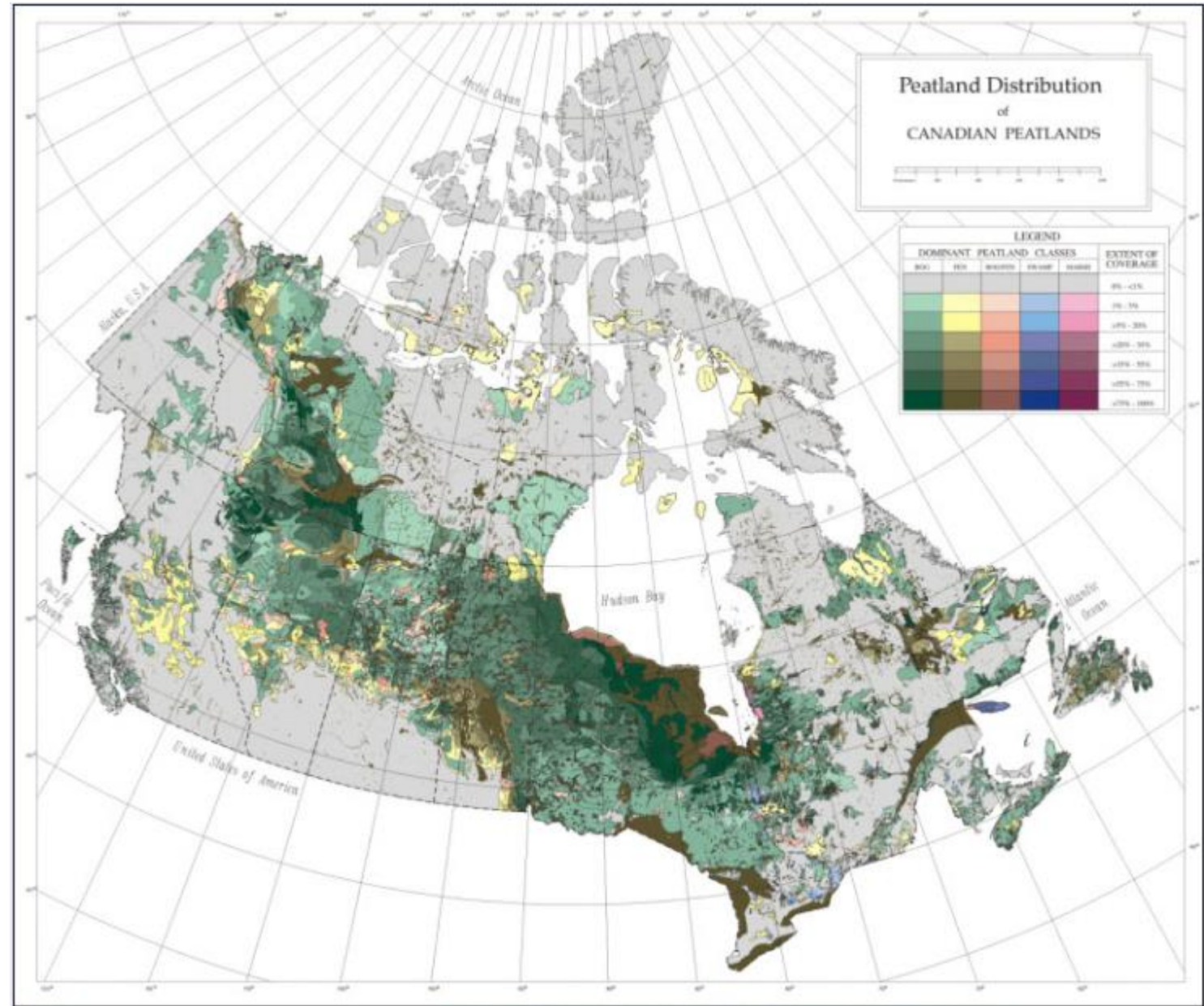
BACKGROUND

Potentially a <u>National</u> Issue

However…
Most inquiries are from Alberta as part of Oil & Gas Site Assessment and Remediation (SAR) activities.

Why?

BACKGROUND

Provincial Regulatory Limits are the primary driver.

Median Toluene concentration of Biogenic Toluene samples processed by Bureau Veritas:

Soil: 0.42 mg/kg
Water: 2.8 μg/L

Region	Lowest Soil/Sediment Limit (mg/kg)	Lowest Water Limit (μg/L)
Federal (CCME) [1]	0.08 (Fine)	2
BC (CSR) [2]	0.5 (Enviro.Prot.)	5 (F/W Aquatic)
Alberta (Tier 1 & 2) [3,4]	0.12 (Coarse)	0.5 (Aquatic Life)
Ontario (OMOECC) [5]	0.2	0.8
Quebec [6,1]	0.2	2
Atlantic (RBCA) [7]	0.35 (Coarse)	24

[1] CCME, Guidance Manual for Environmental Site Characterization in Support of Environmental and Human Risk Assessment, Vol. 4, 2016

[2] B.C. Reg. 375/96, Contaminated Sites Regulation, Schedule 3.1/3.2

[3] Alberta Tier 1 Soil and Groundwater Remediation Guidelines, January 10, 2019

[4] Alberta Tier 2 Soil and Groundwater Remediation Guidelines, January 10, 2019

[5] Reg.153 (ESA) Soil, Groundwater and Sediment Standards

[6] Guide d'intervention, Direction du Programme de réduction des rejets industriels et des Lieux contaminés Protection des sols et réhabilitation des terrains contaminés, Mars 2019

[7] Atlantic RBCA (Risk-Based Corrective Action) for Petroleum Impacted Sites in Atlantic Canada Version 3, January 2015

BACKGROUND

Why should I care…?

- Cost reduction

- Reduce environmental damage

- Establish local background concentrations

- Site liability management

LABORATORY'S OBLIGATIONS

➢ Procedure, interpretation and conclusions are based on **Sound Science**

➢ Process each sample in a standardized manner (SOP)

➢ Review and update the process as required to ensure it is meeting client/regulatory demands

➢ Keep technologically current (new processes/procedures)

BUREAU VERITAS' APPROACH

Analysis Objectives

➢ Base the process on **Sound Science**

➢ Make the process simple (internal/external)

➢ Standardize the process (nationally)

➢ Minimize communication/time

➢ Make it cost effective

BUREAU VERITAS' APPROACH

Staged Approach: *Most cost effective*

Stage 1: Initial Testing (completed already)

Stage 2: Supplemental Analysis
Secondary Data Review (of Stage 1)
(from a Biogenic Toluene perspective)

Stage 3: CSIA (<0.3%)

STAGE 1

BTEX,F1-F4
VPH,EPH

STAGE 2

Secondary
Data Review

+

Supplemental
Analysis

if necessary

STAGE 3

Compound
Specific Isotope
Analysis (CSIA)
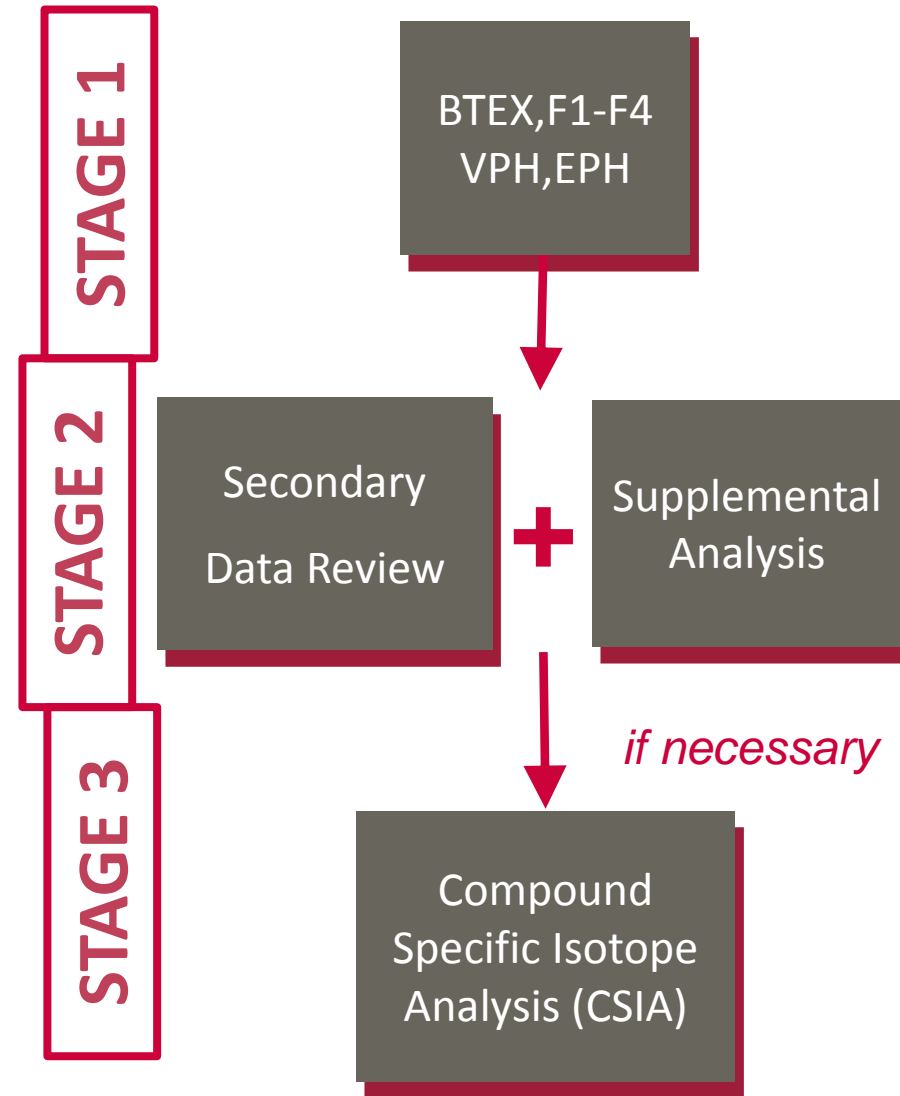
BUREAU VERITAS' APPROACH

STAGE 1: Data

Typical Biogenic Toluene Report

- *Elevated CCME F3*

- *High Moisture*

- *Toluene elevated*

AT1 BTEX AND F1-F4 IN SOIL (VIALS)

BV Labs ID		TX6875	
Sampling Date			
COC Number		M101651	
	UNITS		RDL
Ext. Pet. Hydrocarbon			
F2 (C10-C16 Hydrocarbons)	mg/kg	16	10
F3 (C16-C34 Hydrocarbons)	mg/kg	390	50
F4 (C34-C50 Hydrocarbons)	mg/kg	140	50
Reached Baseline at C50	mg/kg	Yes	N/A
Physical Properties			
Moisture	%	89	0.30
Volatiles			
Xylenes (Total)	mg/kg	<0.79	0.79
F1 (C6-C10) - BTEX	mg/kg	<180	180
Field Preserved Volatiles			
Benzene	mg/kg	<0.046	0.046
Toluene	mg/kg	0.27	0.12
Ethylbenzene	mg/kg	<0.073	0.073
m & p-Xylene	mg/kg	<0.71	0.71
o-Xylene	mg/kg	<0.35	0.35
F1 (C6-C10)	mg/kg	<180	180

BUREAU VERITAS' APPROACH

STAGE 2: Breakdown

Secondary Data Review

Percent Moisture
BTEX & Toluene Ratio
Chromatogram Review

+

Supplemental Analysis

Terpene Analysis

Cymene Ratio

BUREAU VERITAS' APPROACH

PERCENT MOISTURE

- Good indicator
- 85% biogenic samples >70%
- Most >80%
- 9% biogenic samples found <50%
- Not applicable to waters

- Biogenic
- Petrogenic
- Mixed Source (Biogenic and Petrogenic)

BUREAU VERITAS' APPROACH

TOLUENE RATIO

Toluene Ratio
$T_{ratio} = T / \Sigma(BTEX)$

- Good indicator
- 95% of biogenic samples >0.7
- 86% of biogenic samples $T_{ratio} = 1$

- Biogenic
- Petrogenic
- Mixed Source (Biogenic and Petrogenic)
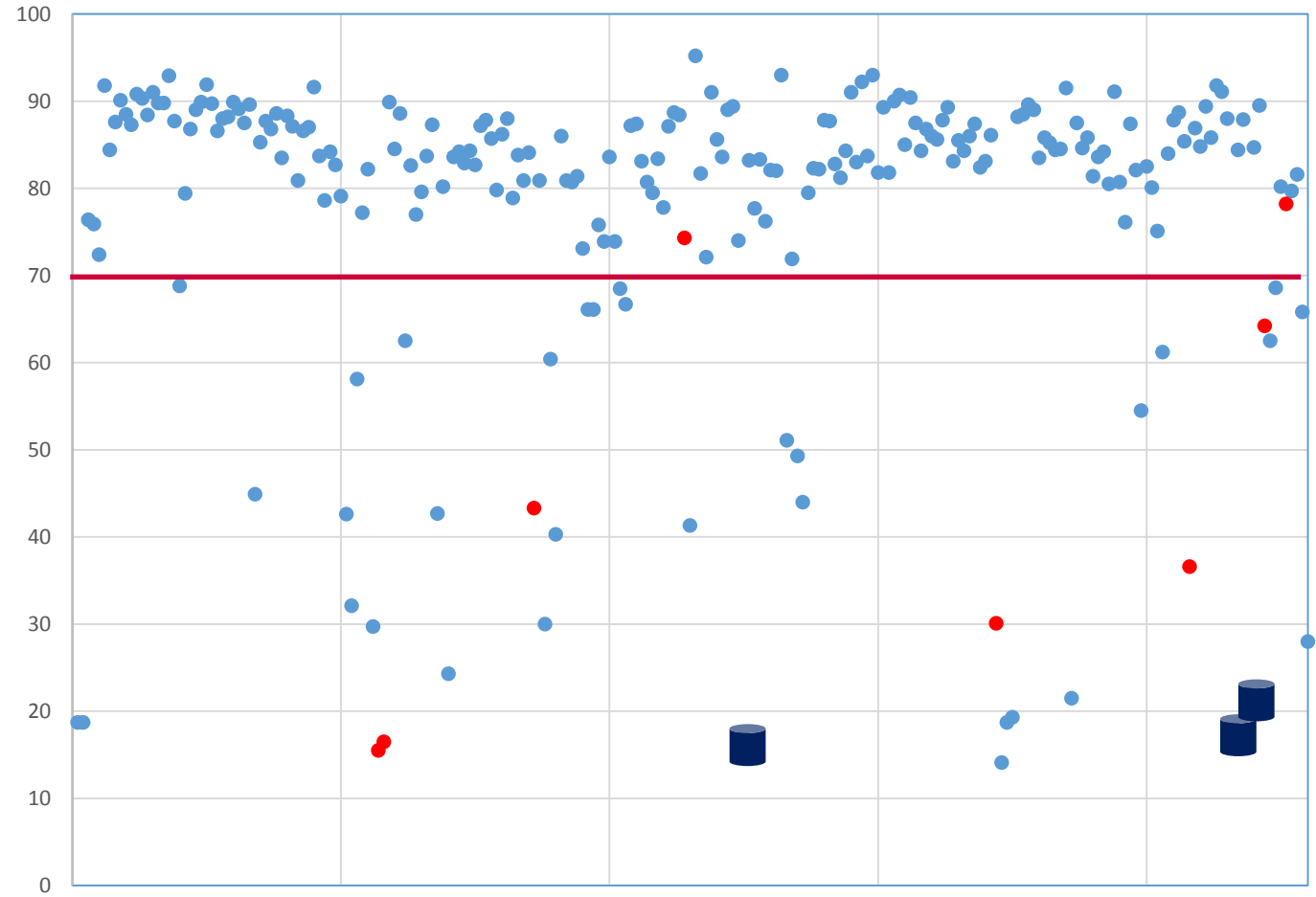
BUREAU VERITAS' APPROACH

BENZENE ETHYLBENZENE XYLENES

Observations:

- **14%** of Biogenic samples contained one/more other parameters (BEX)
- Xylene isomers are relatively rare
- Typically found at concentrations ≤1/5 the concentration of toluene

3%

5%

50%

42%

- Benzene
- Ethylbenzene
- o-Xylene
- m/p-Xylene
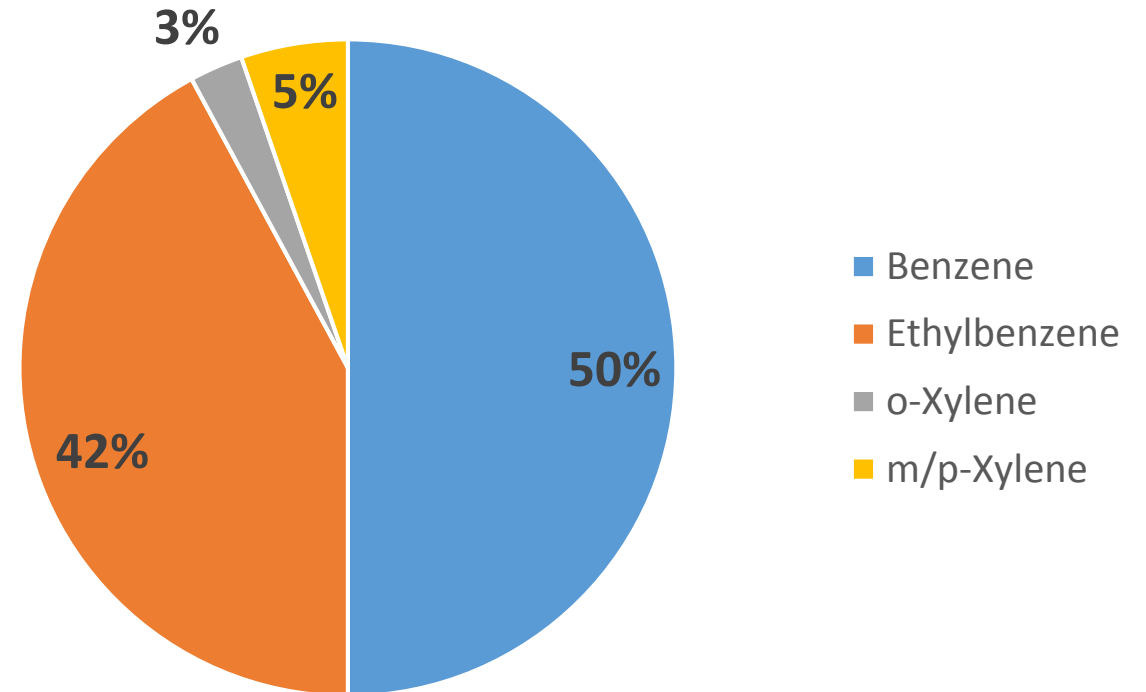
Juttner F. 1988. Benzene in the anoxic hypolimnion of a freshwater lake. Naturwissenschaften

BUREAU VERITAS' APPROACH

CHROMATOGRAM REVIEW

Biogenic cluster within nC30-34
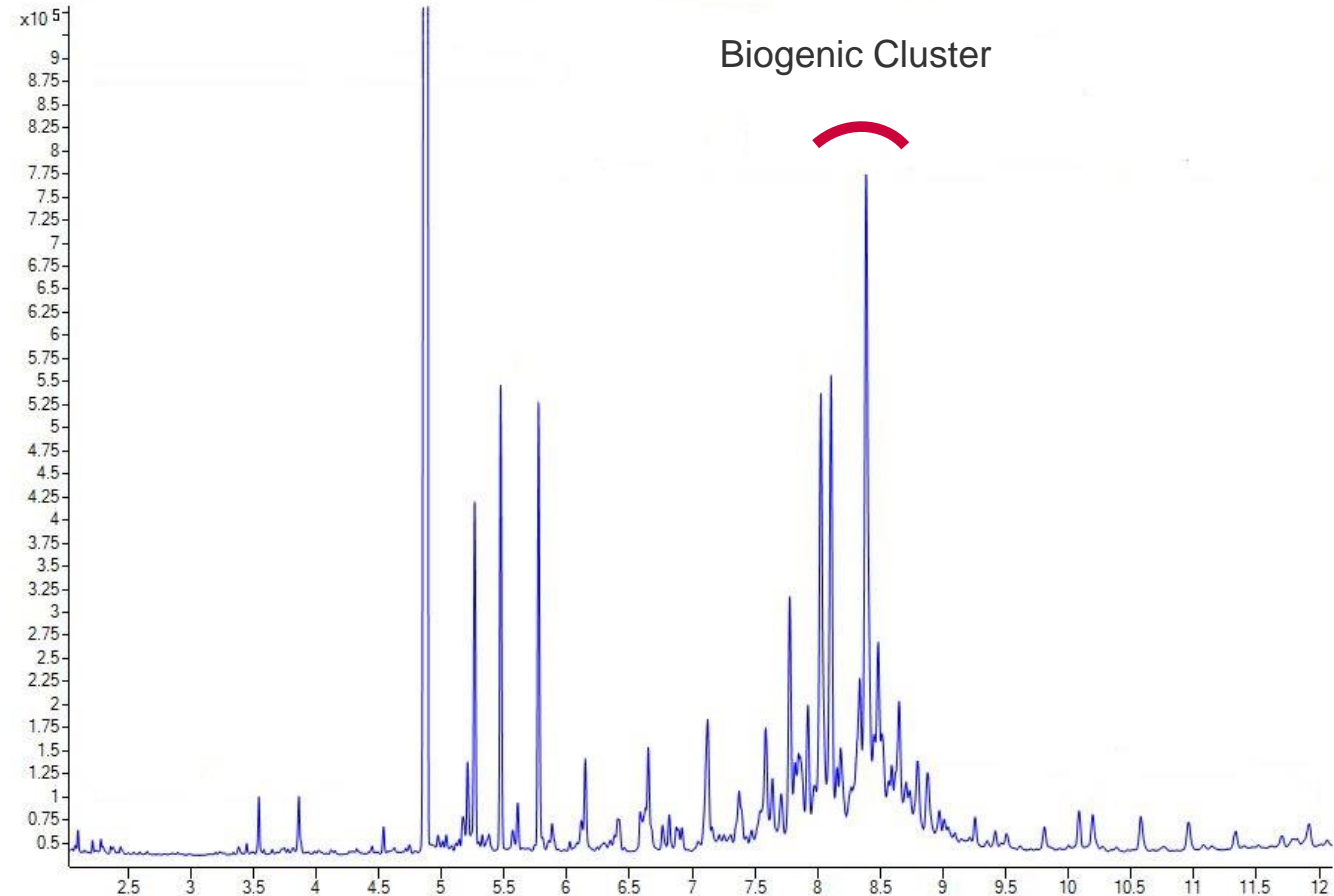
Biogenic Cluster

BUREAU VERITAS' APPROACH

CHROMATOGRAM REVIEW

Biogenic cluster within C30-34

Carbon Preference Index (CPI)
CPI = ∑odd nC/∑even nC

>1 likely Biogenic
~1 likely Petrogenic

■ Odd-Carbon n-Alkane
■ Even-Carbon n-Alkane
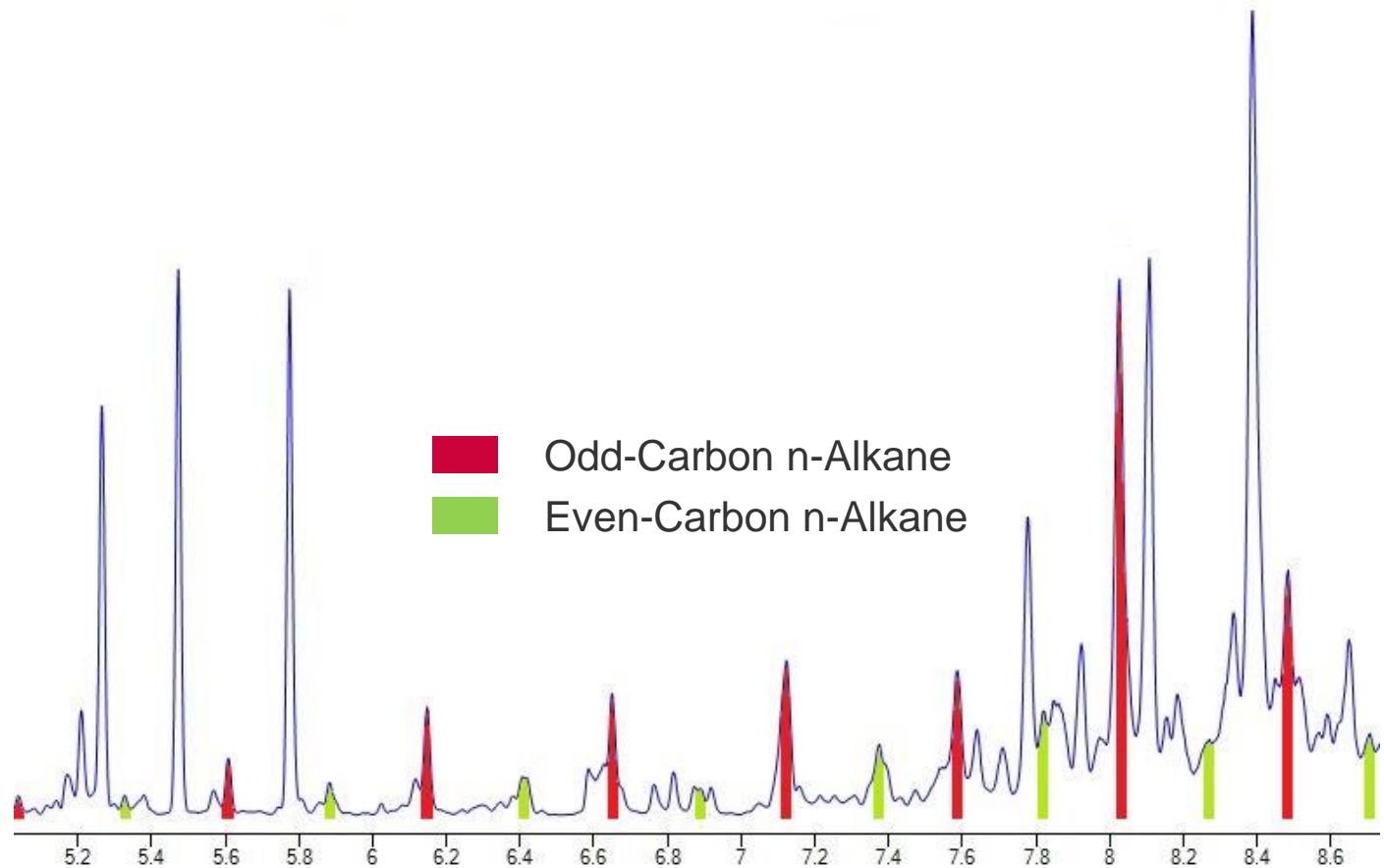
Peters, K.E., et al, The Biomarker Guide (Volume 2), Cambridge University Press, 2005.

BUREAU VERITAS' APPROACH

CHROMATOGRAM REVIEW

Biogenic cluster within C30-34

Carbon Preference Index (CPI)
CPI =∑odd nC/∑even nC

>1 likely Biogenic
~1 likely Petrogenic

Lack of Unresolved Complex Mixtures (UCM)

BUREAU VERITAS' APPROACH
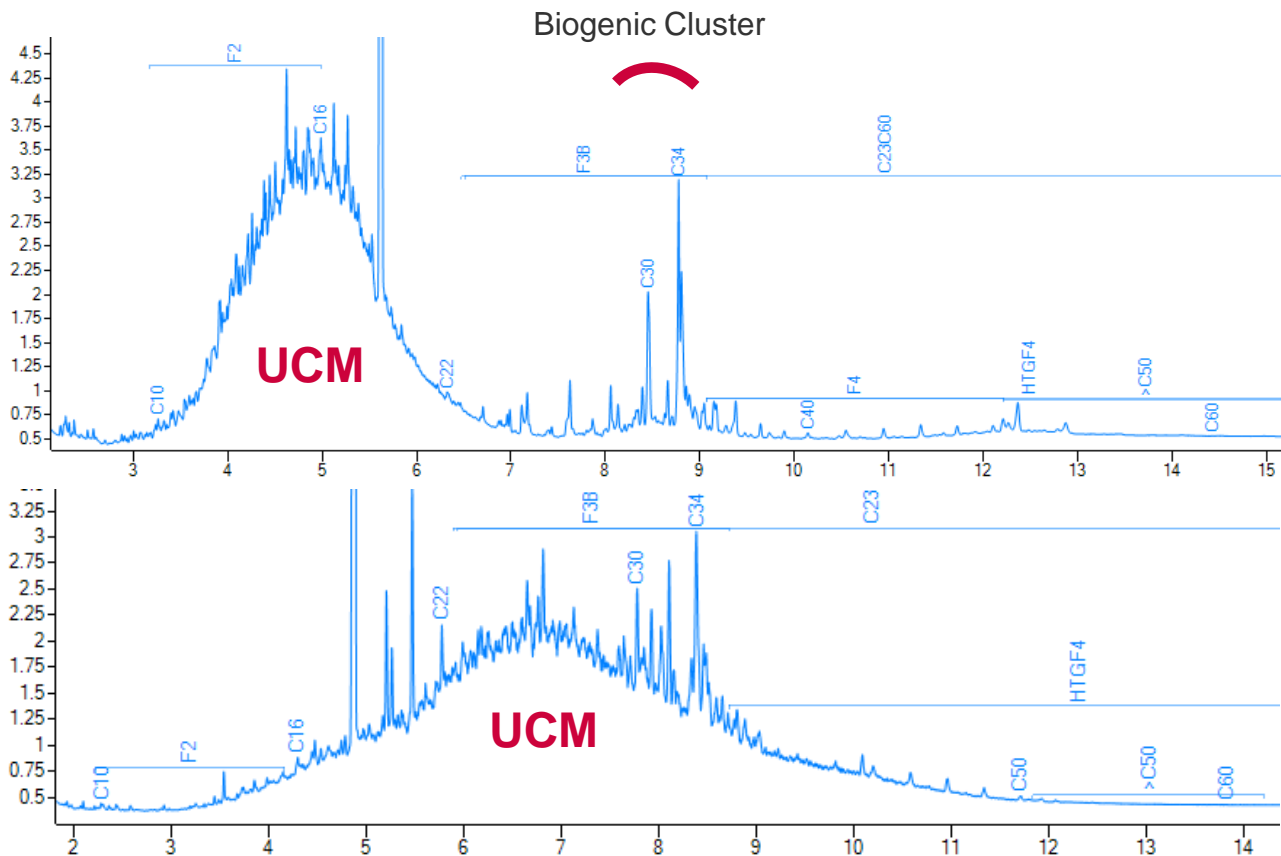
TERPENE ANALYSIS

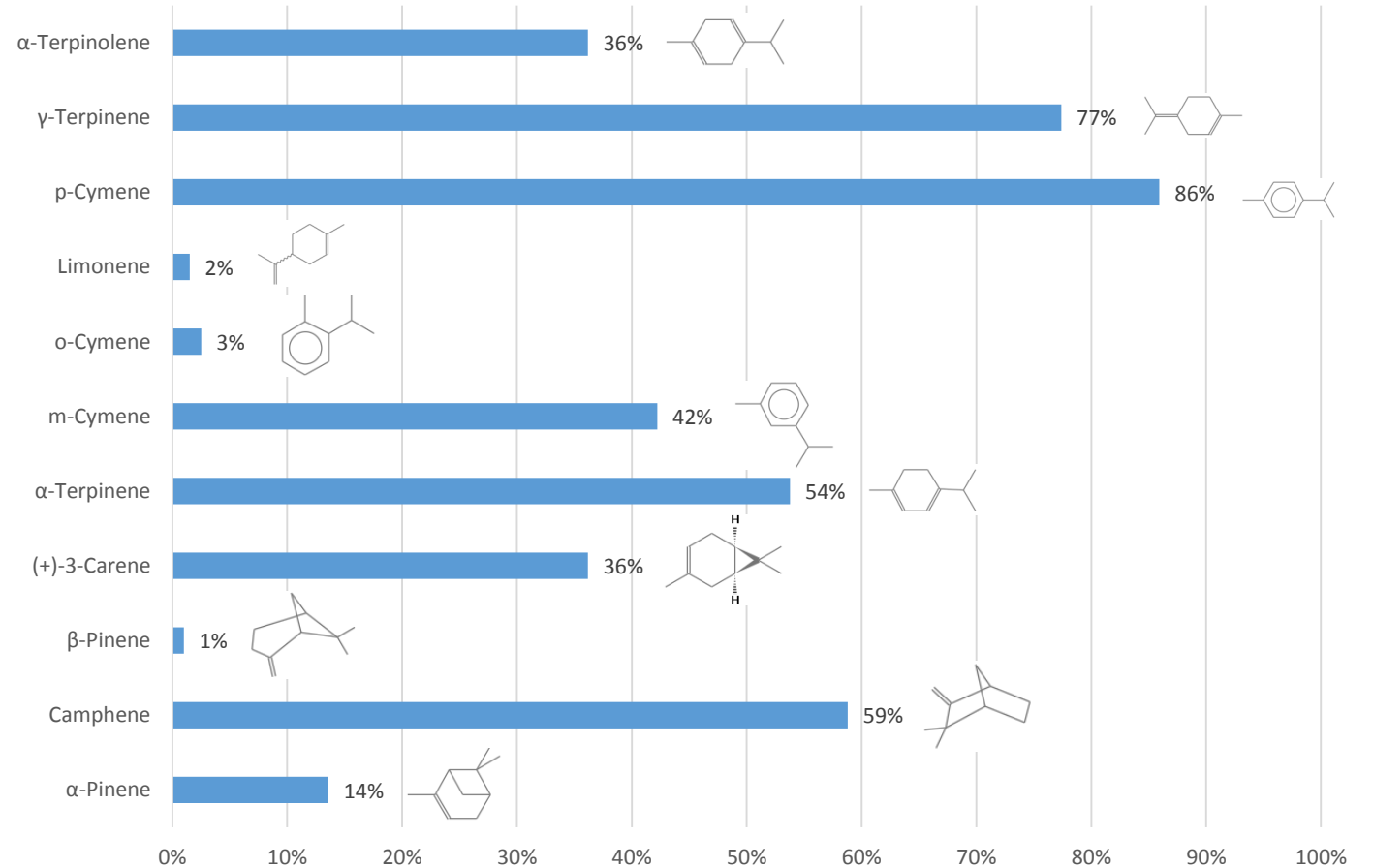Coupled with BTEX/F1 analysis
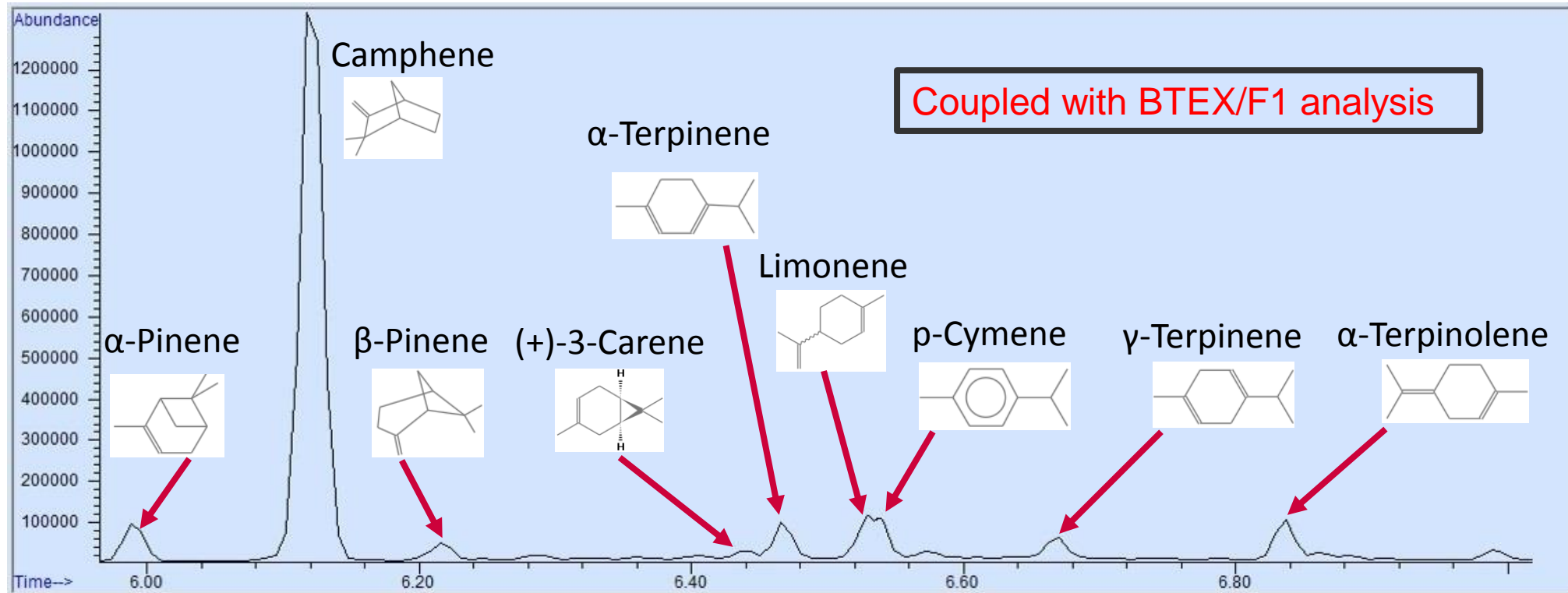
BUREAU VERITAS' APPROACH

TERPENE ANALYSIS

Prevalence within Biogenic Samples

Most Prevalent:
- p-Cymene
- γ-Terpinene
- Camphene

Typically 4-6 detected
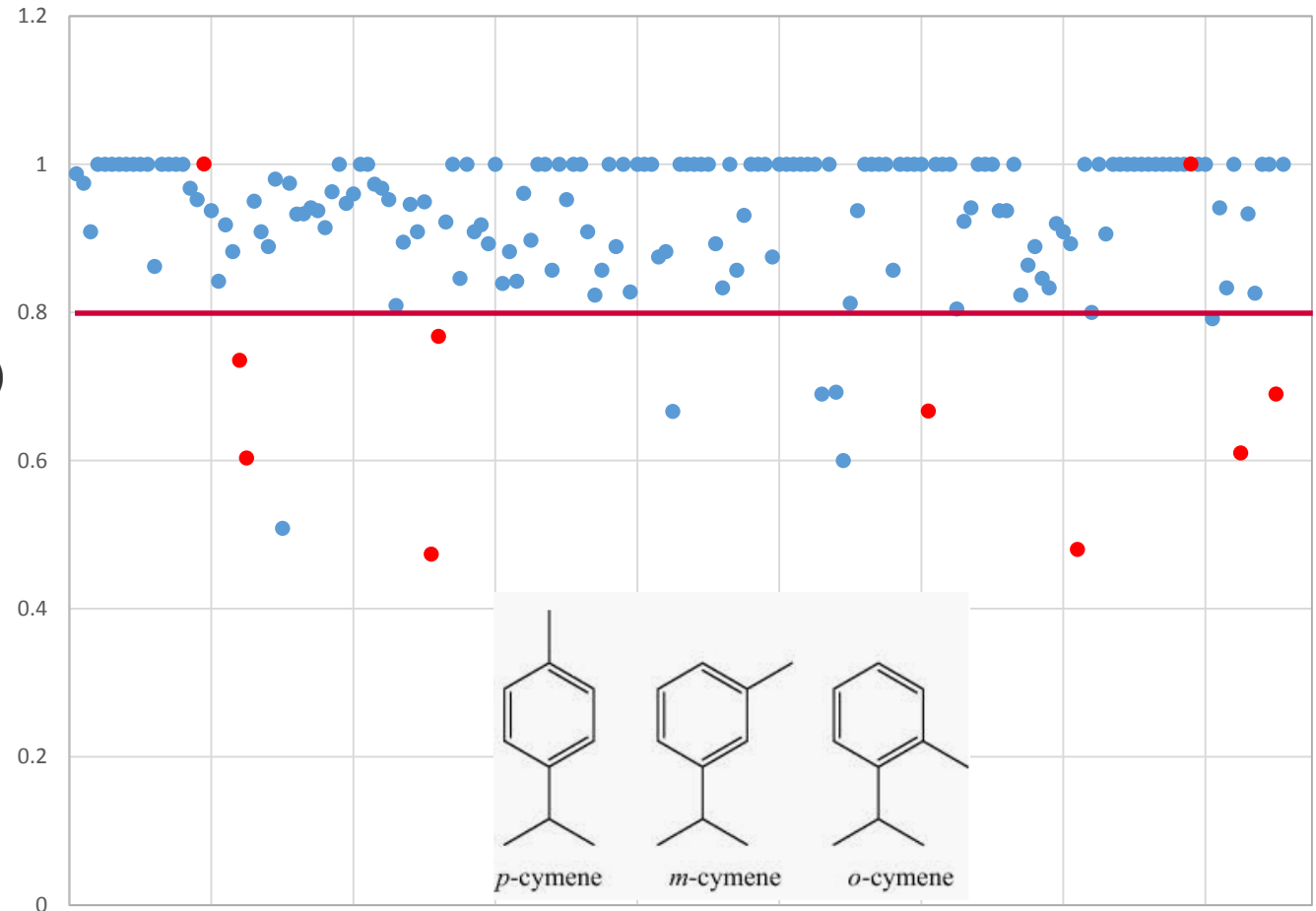
Rarely observe all together

BUREAU VERITAS' APPROACH

CYMENE RATIO

Cymene Ratio

Ratio = p-Cymene /Σ(Cymene isomers)

- Good Biogenic/Petrogenic Indicator
- 92% of biogenic samples >0.8
- Ratio calculated on 70% of samples

- Biogenic
- Mixed Source (Biogenic and Petrogenic)

BUREAU VERITAS' APPROACH

DOUBLE-RATIO PLOT

Observations:

- 85% of Biogenic samples within 0.8/0.8 (shaded area).
- Mixed Source tend to cluster outside
- Cymene ratio a slightly better predictor?

- Biogenic
- Mixed Source (Biogenic and Petrogenic)

WHAT THE DATA TELLS US (SO FAR)

Data Review Summary

Parameter	Biogenic	Petrogenic
Moisture	≥70% (typically >80%)	<50%
Unresolved Complex Mixtures (UCM)	Absent	Often Present
Presence of Benzene and Ethylbenzene	Absent or Trace	Present
Presence of Xylene Isomers	Rare	Present
Toluene Ratio	0.7 – 1.0	<0.7
Terpenes	Present	Absent (excl. Cymenes)
Cymene Ratio	≥0.8	<0.8

CONCLUSIONS AND NEXT STEPS

Biogenic Toluene Assessment Conclusions

➢ Biogenic: 79.5%

➢ Petrogenic: 1.0%

➢ Inconclusive (Evidence of both Biogenic and Petrogenic impacts): 4.0%

➢ Inconclusive (Absence of evidence for either): 15.5%

CONCLUSIONS AND NEXT STEPS

Biogenic Toluene Assessment Conclusions

- Biogenic   OK
- Petrogenic   ok…
- Inconclusive (Evidence of both Biogenic and Petrogenic Impacts)
- Inconclusive (Absence of evidence for either)

Now What?!

Inconclusive – Evidence of Both

Which brings up a commonly asked question…

Can you differentiate and quantify Petrogenic and Biogenic material separately within a mixed-source sample?
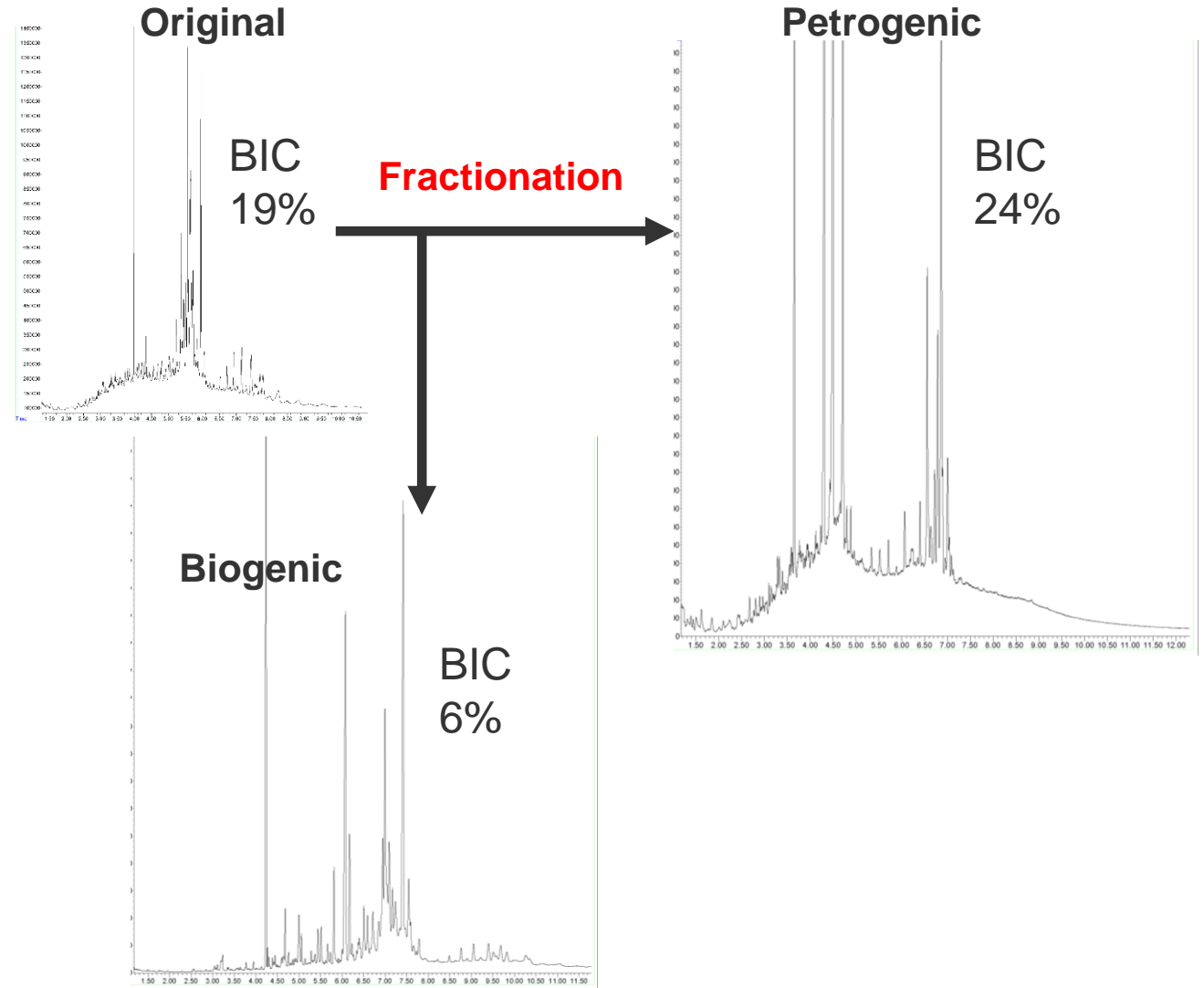
BIC: Biogenic Interference Calculation

Biogenic Cluster

BIC
19%

UCM
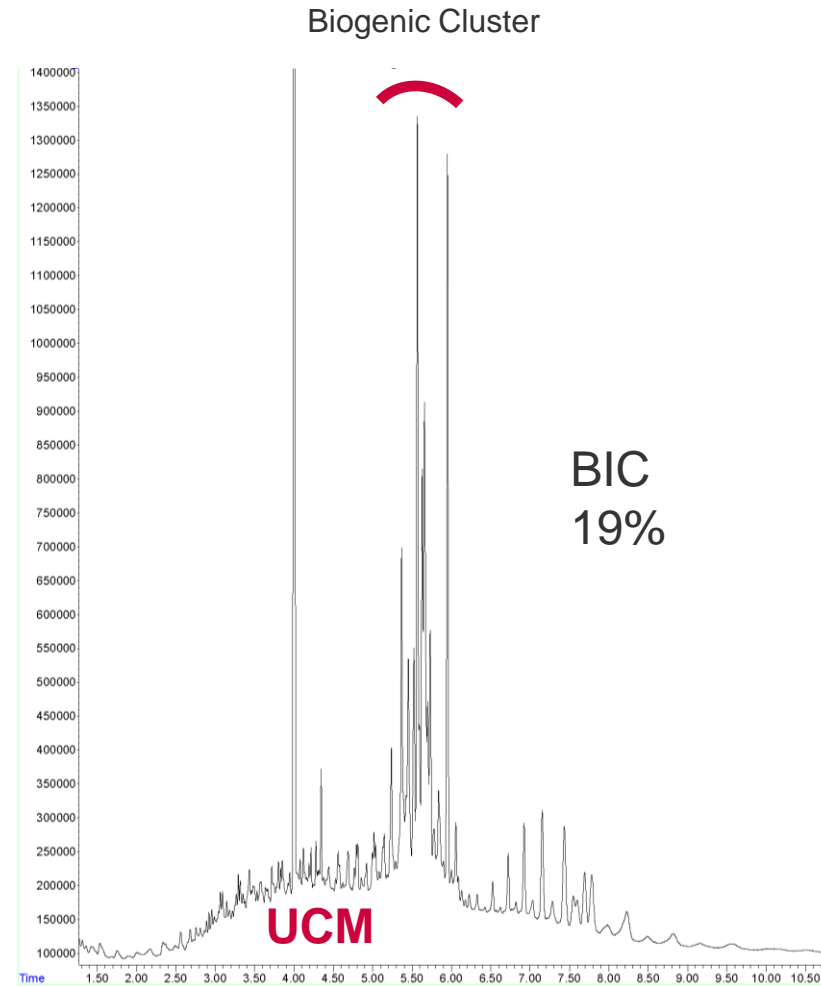
CONCLUSIONS AND NEXT STEPS

Inconclusive – Evidence of Both

For CCME F2-F4 (particularly F3) exceedances?

YES

BIC: Biogenic Interference Calculation

Original
BIC 19%

Fractionation

Petrogenic
BIC 24%

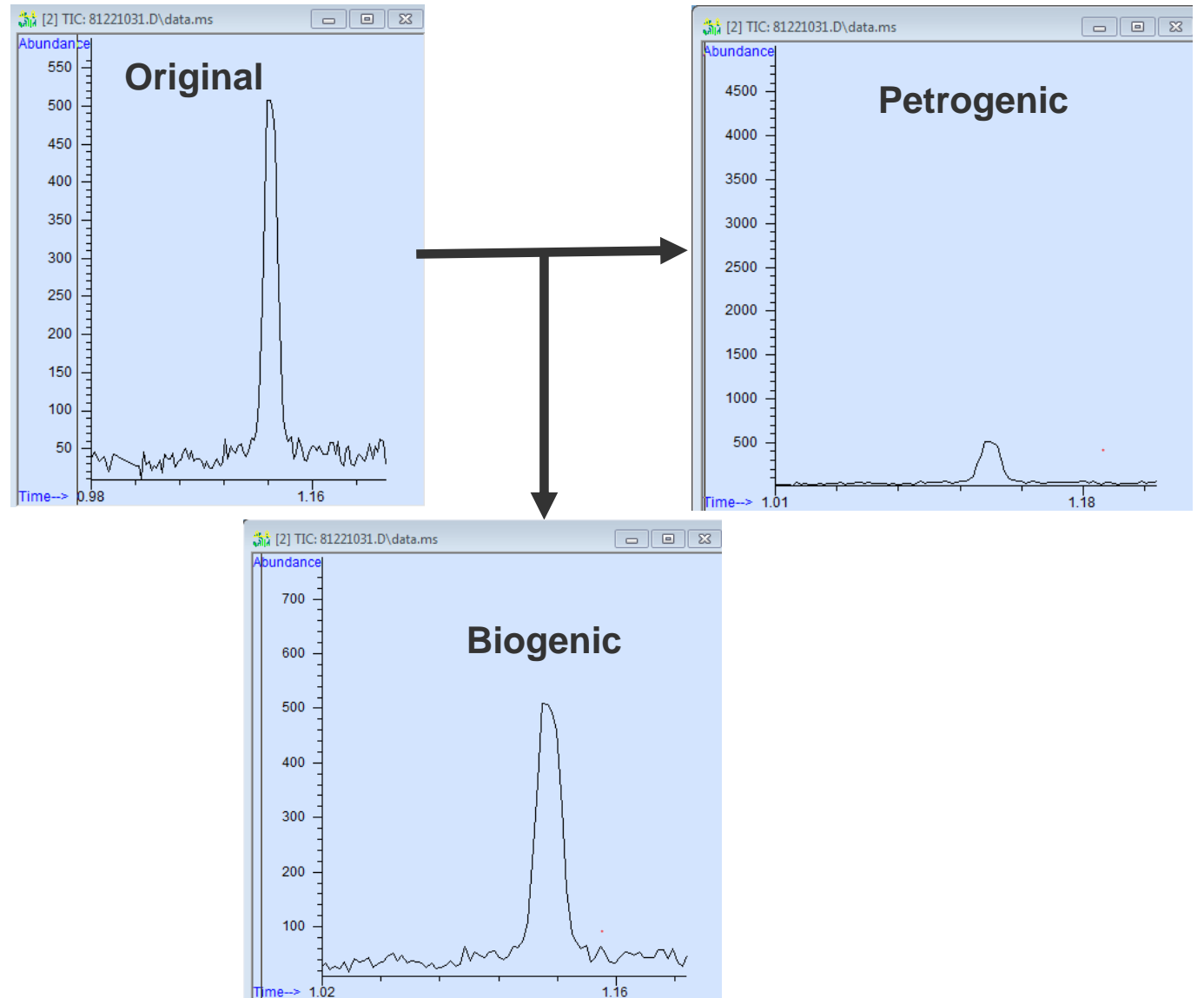**Biogenic**
BIC 6%

CONCLUSIONS AND NEXT STEPS

Inconclusive – Evidence of Both

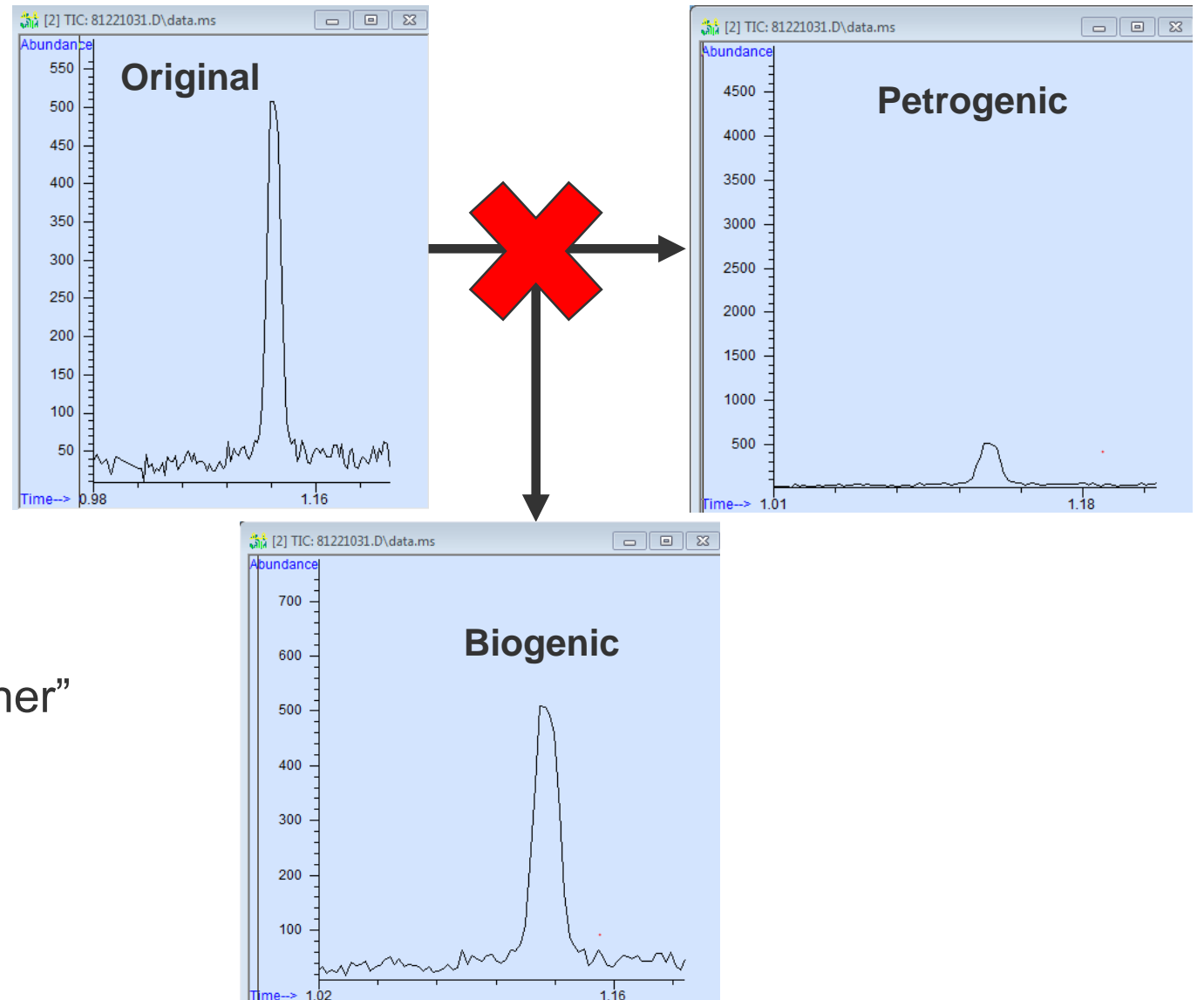What about **TOLUENE**?

CONCLUSIONS AND NEXT STEPS

Inconclusive – Evidence of Both

What about **TOLUENE**?

Unfortunately... NO

However,
For "Inconclusive – no evidence of either"

...Stage 3

CONCLUSIONS AND NEXT STEPS
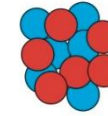
Compound Specific Isotope Analysis (STAGE 3)

- **Stable Isotopes:** *Carbon exists as 12C (most abundant, ~99%) & 13C (~1%)*
- **Compound Specific**: *Toluene only*
- Results indicative of source
- Provides an additional "Line of Evidence"
- **Not an absolute**; can also be inconclusive
- **Costly!**
- **Long TAT**: 6-8 weeks

$$\delta^{13}C\left(\%_{oo}\right) = \left[\frac{\left(^{13}C/^{12}C\right)_{sample} - \left(^{13}C/^{12}C\right)_{standard}}{\left(^{13}C/^{12}C\right)_{standard}}\right] \times 1000$$

$\delta^{13}C$ for **petrogenic toluene** → **-22 to -30‰**
$\delta^{13}C$ for **biogenic toluene** → **below -30‰.**

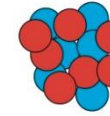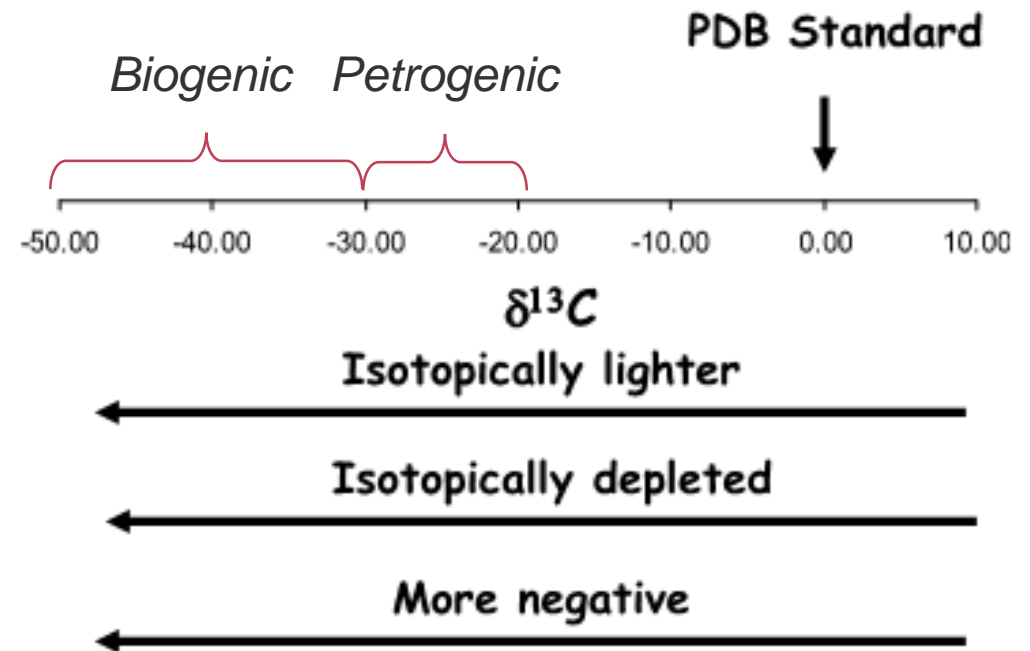carbon-12

¹²C
6 protons
6 neutrons
light

carbon-13

¹³C
6 protons
7 neutrons
heavy

PDB Standard

Biogenic   Petrogenic

-50.00   -40.00   -30.00   -20.00   -10.00   0.00   10.00

$\delta^{13}C$

Isotopically lighter

Isotopically depleted

More negative

BUREAU VERITAS' APPROACH

The "Take Home" Messages

➢ Toluene can be produced microbiologically

➢ Is associated with anoxic, highly organic environments (peat, sediments, etc.)

➢ Requires a multi-pronged assessment approach – multiple "Lines of Evidence"

➢ Completion of a "Stage 2" assessment assigned a probable source 80% of the time

BUREAU VERITAS' APPROACH

THANK YOU!