Application of Compound Specific Isotope Analysis (CSIA) for Evaluating Chlorinated Solvent Degradation

Paul B. Hatzinger, PhD
APTIM

ESTCP ER18-5022
Some questions CSIA may help to answer - CVOCs?

- Is my chlorinated solvent degrading?
- Are daughter products also degrading?
- What is the dominant degradation mechanism?
- What are the approximate rate(s) of degradation?
- Is this actually my solvent anyway?

- Isotope chemists often do not work on a consultants timescale
- There are no EPA certified methods or SOPs
Some fundamentals – What are isotopes?

Isotopes of an element have the same number of protons and electrons but a different number of neutrons.

Environmentally relevant stable isotopes

<table>
<thead>
<tr>
<th>Element</th>
<th>Isotopes</th>
<th>Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen</td>
<td>$^1\text{H}$, $^2\text{H}$</td>
<td>99.99 %</td>
</tr>
<tr>
<td>Oxygen</td>
<td>$^{16}\text{O}$, $^{17}\text{O}$, $^{18}\text{O}$</td>
<td>99.76 %</td>
</tr>
<tr>
<td>Carbon</td>
<td>$^{12}\text{C}$, $^{13}\text{C}$</td>
<td>98.89 %</td>
</tr>
<tr>
<td>Chlorine</td>
<td>$^{35}\text{Cl}$, $^{37}\text{Cl}$</td>
<td>75.78 %</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>$^{14}\text{N}$, $^{15}\text{N}$</td>
<td>99.63 %</td>
</tr>
<tr>
<td>Sulfur</td>
<td>$^{32}\text{S}$, $^{34}\text{S}$</td>
<td>95.01 %</td>
</tr>
</tbody>
</table>

Isotopes of Carbon

- $^{12}\text{C}$
  - 6 protons
  - 6 neutrons
  - “light” stable

- $^{13}\text{C}$
  - 6 protons
  - 7 neutrons
  - “heavy” stable

- $^{14}\text{C}$
  - 6 protons
  - 8 neutrons
  - Radioactive

Source: Pace Analytical, Inc and ITRC.
Why are stable isotopes useful for evaluating contaminant behavior?

- Compounds can have different ratios of stable isotopes (e.g., $^{13}\text{C}/^{12}\text{C}$) depending on how they were formed and whether they have been degraded since environmental release.

Source: figure modified from ITRC
Some fundamentals – How are isotope ratios measured?

- Stable isotope ratios are typically measured using Isotope Ratio Mass Spectrometers (IRMS).
- If chemicals are analyzed individually (e.g., separated first by GC) then the process is termed “Compound Specific Isotope Analysis (CSIA)”
Some fundamentals – How are isotope ratios reported?

- Isotopic ratios of light elements are generally reported relative to a known standard as “delta” (δ) values and measured in parts-per-thousand (denoted “‰” = per mil)

\[
\delta^{13}C \text{ in } \% = \frac{(^{13}C/^{12}C_{\text{sample}} - ^{13}C/^{12}C_{\text{standard}}) \times 1000}{^{13}C/^{12}C_{\text{standard}}}
\]

Example: \(\delta^{13}C = +30 \%\)

30 parts-per-thousand (3 %) higher ratio of \(^{13}C/^{12}C\) in sample relative to a known isotopic standard
Contaminant degradation – Isotope fractionation makes CSIA useful!

- Slight differences in bond strength between heavy and light isotopes in an element can lead to *isotope fractionation* during reaction.

- “Kinetic Isotope Effect” (KIE)

- Many abiotic and biological reactions
Contaminant degradation – Isotope fractionation makes CSIA useful?

Kinetic isotope effect (KIE)

\[
\frac{1^3 k}{12 k} \quad \text{TCE} \rightarrow \text{product}
\]

Fractionation factor

\[
\alpha = \frac{1^3 k}{12 k}
\]

Enrichment factor

\[
\varepsilon = 1000 \times (\alpha - 1)
\]

Rayleigh Fractionation

\[
\delta = \delta_0 + \varepsilon \times \ln f
\]

(2) Hirschorn et al., 2004 Pathway dependent isotopic fractionation during aerobic biodegradation of 1,2-dichloroethane ES&T 38:4775-4871.

FIGURE 5. $\delta^{13}C$ values for 1,2-DCA during biodegradation by *X. autotrophicus* GJ10, *A. aquaticus* AD20, and *Pseudomonas* sp. Strain DCA1. Closed circles represent replicates of *X. autotrophicus* GJ10.
Enrichment factors (ɛ)?

- ɛ values are best determined in a laboratory setting (pure cultures; enzymes and/or chemical reactants) – not in microcosms or field studies.

- ɛ values often vary among different degradative mechanisms but should be reasonably consistent for the same mechanism.

- To measure degradation in a field setting isotopic fractionation must occur to with an ɛ of at least ~ 2 - 3 ‰.

- In a field setting, ɛ is often influenced and generally reduced by environmental factors – poor mixing in environmental matrices.

- More isotopes are usually better (C and Cl).
Is my chlorinated solvent degrading in groundwater - CSIA?

<table>
<thead>
<tr>
<th>compound</th>
<th>condition</th>
<th>mechanism</th>
<th>bacteria</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>cDCE</td>
<td>13C/12C</td>
<td>anaerobic</td>
<td>beta-Proteobacterium strain JS666</td>
<td>Abe et al. 2009</td>
</tr>
<tr>
<td>cDCE</td>
<td>-8.5</td>
<td>reductive dehalogenation</td>
<td>Dehalococcoides</td>
<td>Abe et al. 2009</td>
</tr>
<tr>
<td>cDCE</td>
<td>-18.5</td>
<td>oxidation (persulfate)</td>
<td></td>
<td>Marchesi et al. 2012</td>
</tr>
<tr>
<td>cDCE</td>
<td>-7.6</td>
<td>abiotic (Zero-valent iron)</td>
<td></td>
<td>Audi-Miró et al. 2012</td>
</tr>
<tr>
<td>cDCE</td>
<td>-20.5</td>
<td>abiotic (Zero-valent iron)</td>
<td></td>
<td>Elsner et al. 2008</td>
</tr>
<tr>
<td>cDCE</td>
<td>-21.7</td>
<td>aerobic (toluene 2,3-dioxygenase)</td>
<td>Pseudomonas putida F1</td>
<td>Clingenpeel et al. 2012</td>
</tr>
<tr>
<td>cDCE</td>
<td>-1.1</td>
<td>aerobic (toluene 2,3-dioxygenase)</td>
<td>Pseudomonas fluorescens CFS215</td>
<td>Clingenpeel et al. 2012</td>
</tr>
<tr>
<td>cDCE</td>
<td>-0.9</td>
<td>aerobic (toluene 3-monoxygenase)</td>
<td>Pseudomonas mendocina KR1</td>
<td>Clingenpeel et al. 2012</td>
</tr>
<tr>
<td>cDCE</td>
<td>-19.9</td>
<td>aerobic biodegradation</td>
<td></td>
<td>Jennings et al. 2009</td>
</tr>
<tr>
<td>cDCE</td>
<td>-15.2</td>
<td>aerobic biodegradation</td>
<td></td>
<td>Schmidt et al. 2010</td>
</tr>
<tr>
<td>cDCE</td>
<td>-14.9</td>
<td>reductive dehalogenation</td>
<td>Dhc sp. strain BAV1</td>
<td>Fletcher et al. 2011</td>
</tr>
<tr>
<td>cDCE</td>
<td>-18.4</td>
<td>reductive dehalogenation</td>
<td>Dhc strains BAV1, FL2, GT, and VS</td>
<td>Fletcher et al. 2011</td>
</tr>
<tr>
<td>cDCE</td>
<td>-25.3</td>
<td>reductive dehalogenation</td>
<td>Dhc-containing culture (BDI)</td>
<td>Fletcher et al. 2011</td>
</tr>
<tr>
<td>cDCE</td>
<td>-26.8</td>
<td>reductive dehalogenation</td>
<td>Dhc mixed culture</td>
<td>Kuder et al. 2013</td>
</tr>
<tr>
<td>cDCE</td>
<td>n.e.</td>
<td>Oxic, cometabolic</td>
<td>Methylosinus trichosporium OB3b</td>
<td>Chu et al., 2004</td>
</tr>
<tr>
<td>cDCE</td>
<td>-14.1</td>
<td>Methanogenic, dehalogenating</td>
<td>Enrichment culture</td>
<td>Bloom et al., 2000</td>
</tr>
<tr>
<td>cDCE</td>
<td>-16.1</td>
<td>Methanogenic, dehalogenating</td>
<td>Enrichment culture</td>
<td>Bloom et al., 2000</td>
</tr>
<tr>
<td>cDCE</td>
<td>-19.9</td>
<td>Anoxic, dehalogenating</td>
<td>Microcosms</td>
<td>Hunkeler et al., 2002</td>
</tr>
<tr>
<td>cDCE</td>
<td>-20.4</td>
<td>Anoxic, dehalogenating</td>
<td>Consortium (MeOH)</td>
<td>Slater et al., 2001</td>
</tr>
<tr>
<td>cDCE</td>
<td>-21.1</td>
<td>Anoxic</td>
<td>Dehalococcoides ethenogenes strain</td>
<td>Lee et al., 2007</td>
</tr>
<tr>
<td>cDCE</td>
<td>-16.9</td>
<td>Anoxic</td>
<td>Dehalococcoides sp. strain BAV1</td>
<td>Lee et al., 2007</td>
</tr>
<tr>
<td>cDCE</td>
<td>-30.5</td>
<td>dehalogenation</td>
<td>Dhc BTF08</td>
<td>Schmidt et al. 2014</td>
</tr>
</tbody>
</table>
Is my chlorinated solvent degrading?

Clear evidence of degradation

Groundwater

MW-1
-35.0 ‰

MW-2
-25.0 ‰

MW-3
-20.4 ‰

MW-4
-19.7 ‰

MW-5
-5.4 ‰

MW-6
-10.2 ‰

MW-7
-2.7 ‰

δ¹³C
TCE

(˜ -35 to -23‰ typical in sources)

Various *Dehalococcoides* cultures

ε = - 15 to -30 ‰

Source: figure modified from Microseeps and ITRC
Is my chlorinated solvent degrading?

Evidence of Two Sources Mixing

Groundwater

MW-1 -35.0 ‰
MW-2 -25.0 ‰
MW-3 30.4 ‰
MW-4 -29.7 ‰
MW-5 -27.9 ‰
MW-6 -29.5 ‰
MW-7 -30.2 ‰

δ¹³C
TCE
Are daughter products degrading?

Biological Reductive Dechlorination

- PCE
- cis-DCE
- TCE
- VC
- Ethene
Are daughter products degrading?

Figure 3. Schematic of $\delta^{13}$C values versus time for microbial reductive dechlorination of TCE to ethene by KB-1 adapted from Slater et al.\textsuperscript{20}

Are daughter products degrading?

- For daughter products, fractionation alone does not prove daughter product degradation.
- If daughter product does not degrade, it still gets heavier as the parent gets heavier.
- The undegraded daughter product typically can get no heavier than the original (undegraded) parent product.
- In chlorinated solvent daughter products, a carbon isotopic ratio heavier than the parent is evidence that the daughter product is degrading.

Figure courtesy of Pace Analytical, Inc. and modified from Hunkeler et al., 1999. ES&T 33:2733-2738.
Vehicle Maintenance Area
- 3,000 ft plume to ~ 40 ft deep
- 100 µg/L TCE in 2007 after soil excavation in 1998, but high cis-DCE
- Two distinct sandy layers separated by clay/silt
- Low pH and mixed ORP
- 2009: Lower sand – lactate, potassium hydroxide, SDC-9 recirculation, final EVO
- 2009: Upper sand – EVO direct injection
- Overall very good results
- Sampled 6 wells – 2 upper sand, 2 lower sand, 2 downgradient - 5.5 years after treatment
- Microbial array; CSIA; PFM

Shallow Treatment Zone

Deep Treatment Zone
Raritan Arsenal - Long term biotreatment performance
ESTCP ER-201427

Starting cis-DCE (-30 %)

<table>
<thead>
<tr>
<th>Well</th>
<th>$\delta^{13}$C TCE (%)</th>
<th>TCE (µg/L)</th>
<th>$\delta^{13}$C DCE (%)</th>
<th>DCE (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>114</td>
<td>-20.1</td>
<td>8.1</td>
<td>-8.6</td>
<td>70</td>
</tr>
<tr>
<td>114A</td>
<td>-18.6</td>
<td>6.3</td>
<td>-8.2</td>
<td>1190</td>
</tr>
<tr>
<td>304D</td>
<td>-13.4</td>
<td>3.5</td>
<td>-18.6</td>
<td>56</td>
</tr>
<tr>
<td>306D</td>
<td>NA</td>
<td>&lt; 0.5</td>
<td>+22.7</td>
<td>4</td>
</tr>
<tr>
<td>302S</td>
<td>NA</td>
<td>&lt; 0.5</td>
<td>NA</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>303S</td>
<td>NA</td>
<td>&lt; 0.5</td>
<td>-8.5</td>
<td>4</td>
</tr>
</tbody>
</table>
Raritan Arsenal: What is the rate of contaminant degradation based on CSIA (back of envelope)

Assuming 1\textsuperscript{st} order degradation rates \((k)\), and constant seepage velocity \((V_x)\) and enrichment factor \((\epsilon)\), then:

\[
\delta^{13}C = \delta^{13}C_0 - \frac{k \cdot \epsilon}{V_x} \cdot x
\]

TCE: Assuming \(V_x \approx 2 \text{ ft/day}\) and \(\epsilon \approx -15 \text{‰}\) \(\Rightarrow k \approx 8 \times 10^{-3} \text{ d}^{-1}\) and \(t_{1/2} \approx 80 \text{ d}\) for TCE - slope = 0.0583

DCE: Assuming \(V_x \approx 2 \text{ ft/day}\) and \(\epsilon \approx -15 \text{‰}\) \(\Rightarrow k \approx 2 \times 10^{-2} \text{ d}^{-1}\) and \(t_{1/2} \approx 30 \text{ d}\) for DCE - slope = 0.139
What is the rate of contaminant degradation based on CSIA - modeling?

Integrated Stable Isotope – Reactive Transport Model Approach for Assessment of Chlorinated Solvent Degradation – ESTCP ER-201029

“Help site managers apply a reactive transport model approach for improved CSIA data interpretation and to use models to estimate more accurate attenuation processes for chlorinated solvents”


T. Kuder, P. Philip, B. van Breukelen, H. Thouement, M. Vandeford, C. Newell
Five historical bioremediation sites – TCE & cis-DCE CSIA compiled

- TCE generally shows less fractionation than expected at very low ratio to daughters
  - Re-partitioning from low permeability layers?
  - Threshold effect?

- Cis-DCE more consistent fractionation
  - Product - less re-partitioning from low permeability layers expected?
What is the dominant degradation mechanism in the field?

- Weight of evidence approach
- Geochemical & microbiological evidence critical
- Multiple isotopes are almost always necessary
- Multiple element isotope ratios can provide critical data
- Dual isotope analysis (2D)
What is the dominant degradation mechanism -1.2-DCA?

Scheme I. Aerobic and Anaerobic Biodegradation Pathways of 1,2-DCA in Aqueous Systems

a. Oxidation (Aerobic)

\[
\begin{align*}
\text{Cl} & + \text{R-O}^+ - \text{R-OH} \rightarrow \text{CO}_2 \\
\end{align*}
\]

b. Hydrolytic dehalogenation via S_n2 (Aerobic*)

\[
\begin{align*}
\text{Nu} & + \frac{\text{Cl}^-}{\text{Cl}} \rightarrow \frac{1}{2}\text{Cl}_2 \rightarrow \text{CO}_2 \\
\end{align*}
\]

c.d. Dihaloelimination (Anaerobic): c) concerted, d) stepwise

\[
\begin{align*}
\text{Cl} & + 2e^- - 2\text{Cl}^- \\
\text{Cl} & + e^- - \text{Cl}^- - \text{Cl}^- \\
\text{Cl} & + e^- - \text{Cl}^- - \text{Cl}^- - \text{Cl}^- \\
\end{align*}
\]

e. Hydrogenolysis (Anaerobic)

\[
\begin{align*}
\text{Cl} & + e^- - \text{Cl}^- + \text{H}^+ \\
\end{align*}
\]

f. Dehydrohalogenation (Anaerobic)

\[
\begin{align*}
\text{Cl} & + \text{HCl} \\
\end{align*}
\]

Is it my contaminant anyway – Vapor intrusion?
ESTCP ER-201025

Distinguishing “Indoor Source” from “Groundwater Source”

Are there still cVOCs in consumer products?

From Beckley et al., 2013. Final Report: Use of Compound-Specific Stable Isotope Analysis to Distinguish between Vapor Intrusion and Indoor Sources of VOCS. ESTCP Project ER-201025.(https://www.serdp-estcp.org/).
L. Beckley, T. McHugh, T. Kuder, P. Philip
Is it my contaminant anyway – Vapor intrusion?

Chlorinated Solvents

<table>
<thead>
<tr>
<th>Product</th>
<th>Price</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gun Cleaner</td>
<td>$19.95</td>
<td>&gt;90% TCE</td>
</tr>
<tr>
<td>Pepper Spray</td>
<td>$3.99</td>
<td>&gt;90% TCE</td>
</tr>
<tr>
<td>Hobby Glue</td>
<td>$4.95</td>
<td>&gt;90% PCE</td>
</tr>
<tr>
<td>Plastic Ornament</td>
<td>$4.95</td>
<td>1,2-DCA</td>
</tr>
</tbody>
</table>

Key point: Chlorinated VOCs are legal and are still used in a wide variety of consumer products currently available for purchase.
Is it my contaminant anyway – Vapor intrusion?

Figure 1: Conceptual Basis for Application of CSIA to Vapor Intrusion
Is it my contaminant anyway – Vapor intrusion?

Site Data: Raritan Building CP4, New Jersey

- **IA-1**: TCE, 1.3 µg/m³
- **IA-2**: TCE, 2.1 µg/m³
- **AA-1**: TCE, 0.057 µg/m³

- **CP4-SG-S**: TCE, 15 µg/m³
- **CP4-SG-3**: TCE, 93 µg/m³

**Indoor Air vs. Groundwater Isotope Signatures**

- Indoor Source Range
- Indoor Air
- Groundwater

TCE in groundwater: 7.6-120 µg/L
Summary and Questions

Benefits
- Provides direct evidence of biological degradation of cVOCs
- Can be useful for generating attenuation rates and mechanisms
- Provides information to identify multiple sources
- Commercially available

Limitations
- Need relevant laboratory fractionation factors for key reactions to calculate in situ attenuation rates
- Site CSIA data are rarely ideal – need interpretation
- CSIA results should always be evaluated with relevant geochemical, hydrogeological, microbiological data.