**Why perform CSIA?**

Compound specific isotope analysis (CSIA) is an analytical method that measures the ratio of stable isotopes (e.g. $^{13}$C/$^{12}$C, $^2$H/$^1$H, or $^{35}$Cl/$^{37}$Cl) of a contaminant.

For some compounds including chlorinated ethenes, the ratios of stable isotopes change in predictable ways as the compound is degraded (e.g. isotopic fractionation). Conversely, physical processes like volatilization, sorption and dilution usually do not appreciably impact isotopic ratios.

Therefore, CSIA can provide direct evidence of degradation of many common contaminants including chlorinated ethenes.

**Anaerobic Bioremediation (Reductive Dechlorination)**

Reductive dechlorination of PCE, TCE, DCE isomers and vinyl chloride results in significant carbon isotope fractionation making assessment of anaerobic biodegradation of chlorinated ethenes one of the most common applications for CSIA in environmental remediation.

<table>
<thead>
<tr>
<th>PCE</th>
<th>TCE</th>
<th>cDCE</th>
<th>VC</th>
<th>Ethene</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="PCE" /></td>
<td><img src="image2" alt="TCE" /></td>
<td><img src="image3" alt="cDCE" /></td>
<td><img src="image4" alt="VC" /></td>
<td><img src="image5" alt="Ethene" /></td>
</tr>
</tbody>
</table>

**Aerobic Bioremediation (Aerobic Cometabolism)**

Although enrichment factors ($\varepsilon$) differ, carbon isotope fractionation has been observed during aerobic biodegradation of chlorinated ethenes and CSIA has been used to assess aerobic biodegradation of TCE and DCE as a component of monitored natural attenuation (MNA).

**Abiotic Degradation**

Abiotic degradation of chlorinated ethenes mediated by zero valent iron (ZVI) and iron bearing minerals (e.g. FeS, pyrite) results in carbon isotope fractionation potentially providing conclusive evidence of abiotic degradation.

**Chemical Oxidation**

Significant carbon isotope fractionation has been reported during chemical oxidation of chlorinated ethenes by permanganate and a Fenton’s-like chemical oxidation product.

**Why does CSIA work?**

Chemical bonds with the light isotope ($^{12}$C) are slightly weaker than those formed with the heavier isotope ($^{13}$C) and react more quickly. Therefore…

- The parent compound (e.g. TCE) becomes enriched in the heavier isotope (increasing $\delta^{13}$C).
- The daughter product is initially depleted in the heavy isotope (lower or “more negative” $\delta^{13}$C).
- If the daughter product is degrading, it will also become increasingly $^{13}$C enriched.
Proof of Degradation

**Known Source:** CSIA provides conclusive evidence of contaminant degradation when the isotope signature ($\delta^{13}C$) of the compound in a sample is significantly greater (at least 2‰ greater) than the primary isotope signature of the unfraccionated compound released at the site.

$$\delta^{13}C_{\text{source (unfractionated))} + 2\%o = \delta^{13}C_{\text{threshold}}$$

**Unknown Source:** When the source is unknown, the highest (least negative) $\delta^{13}C$ value for the manufactured compound is frequently used as $\delta^{13}C_{\text{source}}$ as a conservative approach to evaluating biodegradation.

**Samples along the groundwater flow path:** If degradation is occurring, the $\delta^{13}C$ values of the compounds of interest in the downgradient wells will be enriched by at least 2‰.

**Example 1—CSIA along the Groundwater Flow Path**

Groundwater samples were collected along the groundwater flow path at TCE impacted site. If degradation is occurring, the $\delta^{13}C$ values of the compounds of interest in the downgradient wells will be enriched by at least 2‰.

**Has TCE degradation occurred? Yes.**

The $\delta^{13}C$ values for TCE detected in downgradient wells MW-6 and MW-7 were significantly greater than detected in upgradient wells MW-3 and MW-4 conclusively demonstrating TCE degradation along the groundwater flow path.

**Example 2—Unknown Source**

The study site is a former industrial facility where a shallow aquifer has been impacted by TCE. CSIA was performed to determine whether biodegradation of TCE and daughter products had under the prevailing site conditions.

Since the TCE source was unknown, the highest known $\delta^{13}C$ value of manufactured TCE (approximately -25‰) was used as $\delta^{13}C_{\text{source}}$ as a conservative approach for assessing degradation. Applying the safety factor of 2‰, the $\delta^{13}C_{\text{threshold}}$ for demonstrating biodegradation of TCE and daughter products was approximately -23‰.

**TCE Degradation? Yes.** At MW3, TCE (-21.7‰) was significantly $^{13}C$ enriched conclusively indicating that TCE and vinyl chloride degradation has occurred.

**DCE Degradation? Inconclusive.** The $\delta^{13}C$ value was within the $\delta^{13}C$ range for the parent compound TCE. Therefore, degradation of cis-DCE may or may not have occurred.

**Vinyl Chloride Degradation? Yes.** Vinyl chloride (-17.2‰) was significantly $\delta^{13}C$ enriched conclusively demonstrating that degradation has occurred.