

## 5 Top Tips – Stable Isotope Probing (SIP)

**Is biodegradation occurring?** Stable isotope probing (SIP) can provide conclusive evidence of biodegradation of benzene and other petroleum hydrocarbons, fuel oxygenates, and even some emerging contaminants. Below are some of our top tips when using SIP to evaluate biodegradation:

- 1 Choose the contaminant of greatest concern for the SIP study.

The Bio-Trap® will be amended with a synthesized  $^{13}\text{C}$  form of the selected contaminant. For gasoline sites, SIP studies are most frequently performed with  $^{13}\text{C}$ -benzene although  $^{13}\text{C}$ -MTBE studies are also common. At diesel and MGP sites with PAHs, naphthalene is often chosen.

- 2 Use SIP to evaluate biodegradation of contaminants that microorganisms use as carbon and energy sources including BTEX, MTBE, chlorobenzene, and naphthalene.

In a SIP study, the  $^{13}\text{C}$  serves much like a tracer. If a contaminant is utilized by microbes as a carbon and energy source during the in-well deployment period, the  $^{13}\text{C}$  will be incorporated into biomass and/or mineralized to  $^{13}\text{CO}_2$  and then detected in post-deployment analyses.

- 3 Perform SIP in areas with highest contaminant concentrations – source zone and adjacent plume.

SIP studies are performed to evaluate biodegradation in impacted areas. Do not conduct SIP studies in background or other non-impacted monitoring wells.

- 4 Interpretation is straightforward. SIP study results are compared to well-established literature values for  $\delta^{13}\text{C}$  of natural PLFA and DIC.

Under natural conditions,  $\delta^{13}\text{C}$  values for DIC typically range from -25 to -10‰ while background  $\delta^{13}\text{C}$  values for PLFA are between -20 and -30‰ under natural conditions. During a SIP study however, biodegradation and incorporation of the  $^{13}\text{C}$  labeled contaminant into PLFA and/or DIC results in  $\delta^{13}\text{C}$  values greater (positive) than under background conditions.

➔ Thus, detection of  $^{13}\text{C}$  enriched PLFA and/or  $^{13}\text{C}$  enriched DIC is **conclusive evidence** of contaminant biodegradation.

- 5 SIP is routinely used as an additional line of evidence when evaluating monitored natural attenuation (MNA). However, SIP can also be performed with an **In Situ Microcosm (ISM)** study to simultaneously evaluate MNA and enhanced bioremediation options.

### Bonus Tip

**Stable Isotope Probing (SIP)** and **Compound Specific Isotope Analysis (CSIA)** sound similar but are fundamentally different. While SIP is appropriate for compounds used as carbon and energy sources, CSIA is commonly used to evaluate biodegradation of contaminants used as electron acceptors such as during reductive dechlorination of tetrachloroethene (PCE) and trichloroethene (TCE).

For more information, on SIP visit [www.microbe.com](http://www.microbe.com). You can also check out the recording of the MI EMD webinar “[Confirming in situ Benzene Biodegradation Under Anaerobic Conditions using SIP](#)” available on the Resources section of the MI website.

