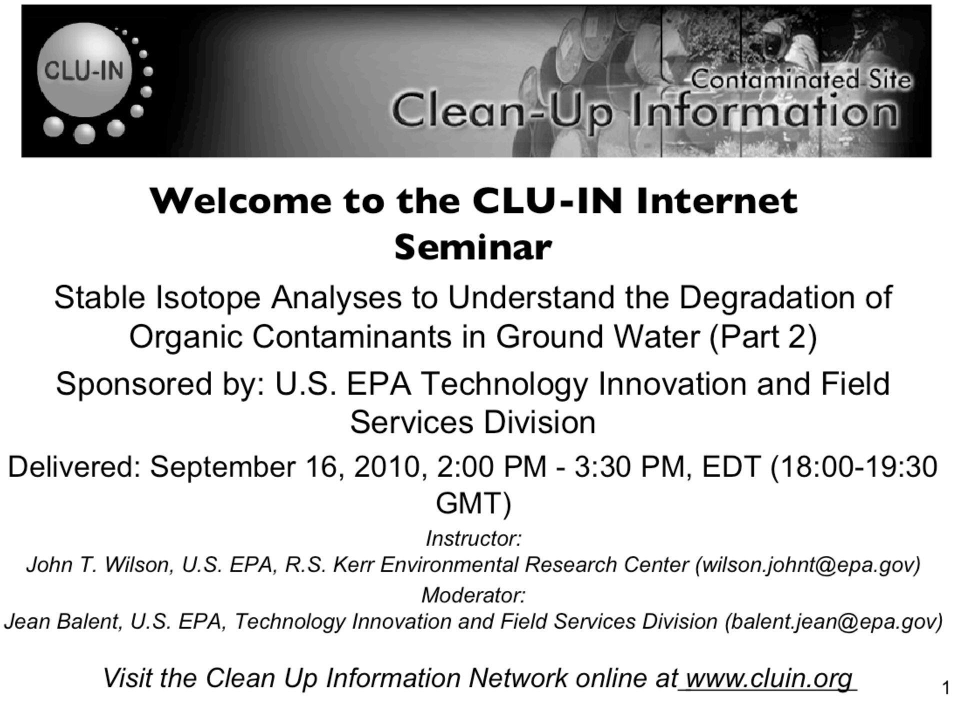

Welcome to the CLU-IN Internet Seminar

Stable Isotope Analyses to Understand the Degradation of Organic Contaminants in Ground Water (Part 2)

Sponsored by: U.S. EPA Technology Innovation and Field Services Division

Delivered: September 16, 2010, 2:00 PM - 3:30 PM, EDT (18:00-19:30 GMT)

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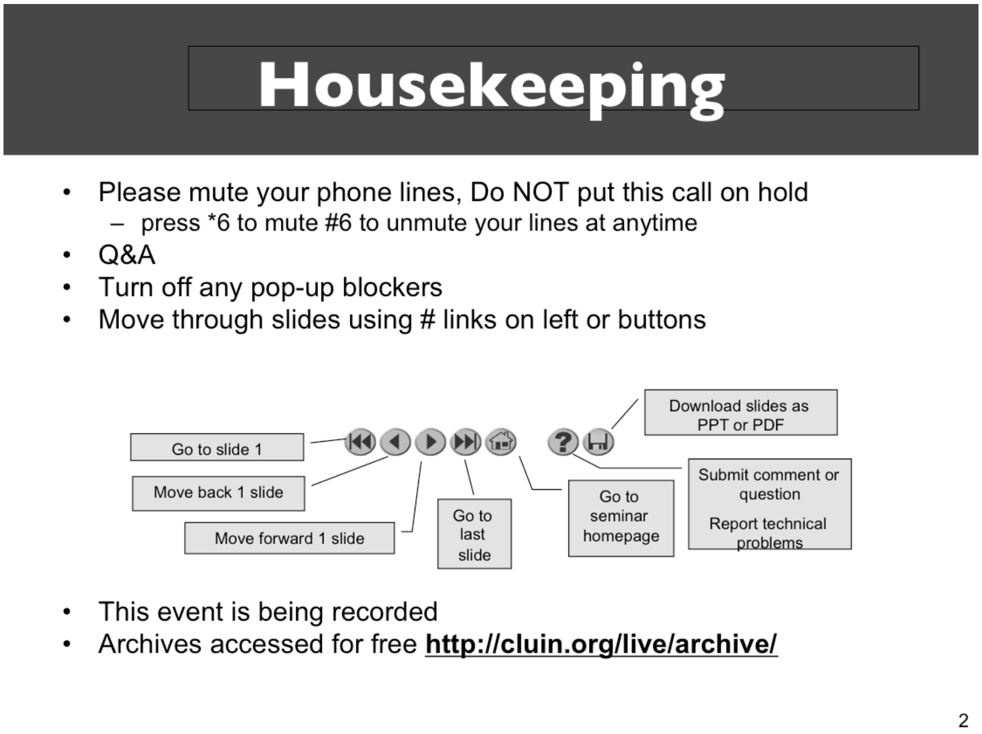

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Although I’m sure that some of you have these rules memorized from previous CLU-IN events, let’s run through them quickly for our new participants.

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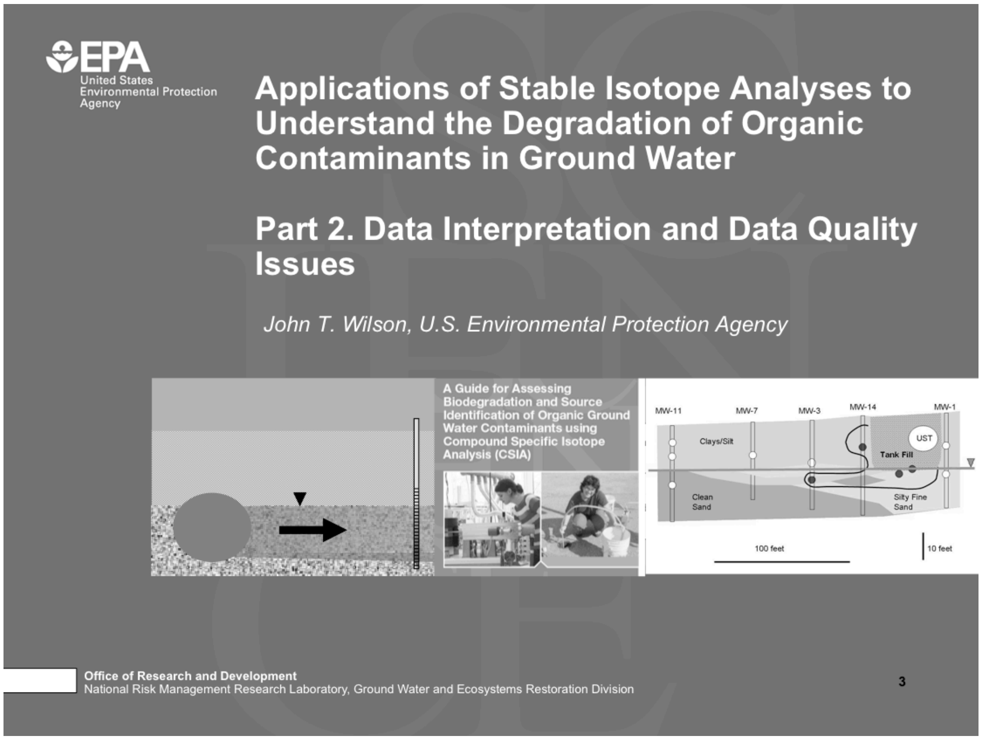
You should note that throughout the seminar, we will ask for your feedback. You do not need to wait for Q&A breaks to ask questions or provide comments. To submit comments/questions and report technical problems, please use the ? Icon at the top of your screen. You can move forward/backward in the slides by using the single arrow buttons (left moves back 1 slide, right moves advances 1 slide). The double arrowed buttons will take you to 1st and last slides respectively. You may also advance to any slide using the numbered links that appear on the left side of your screen. The button with a house icon will take you back to main seminar page which displays our agenda, speaker information, links to the slides and additional resources. Lastly, the button with a computer disc can be used to download and save today’s presentation materials.

With that, please move to slide 3.



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Applications of Stable Isotope Analyses to Understand the Degradation of Organic Contaminants in Ground Water

Part 2. Data Interpretation and Data Quality Issues

John T. Wilson, U.S. Environmental Protection Agency

A Guide for Assessing Biodegradation and Source Identification of Organic Ground Water Contaminants using Compound Specific Isotope Analysis (CSIA)

Office of Research and Development
National Risk Management Research Laboratory, Ground Water and Ecosystems Restoration Division

Documenting Quality for CSIR analyses

If you have questions, or want to request a copy of the Powerpoint file, send e-mail to wilson.johnt@epa.gov.

Element	Stable Isotopes	Relative Abundance
Hydrogen	^{1}H	0.99985
	^{2}H	0.00015
Carbon	^{12}C	0.9889
	^{13}C	0.0111
Chlorine	^{35}Cl	0.7577
	^{37}Cl	0.2423

As organic compounds degrade, the ratio of stable isotopes in the fraction remaining after degradation may change in a predictable way.

Compound specific stable isotope analyses (CSIA) can provide an unambiguous conservative boundary on the extent of degradation of organic compounds in ground water.

Analysis of Stable Carbon Isotope Ratios

The ratio of stable isotopes is determined with an Isotope Ratio Mass Spectrometer (IRMS).

The IRMS compares the ratio of ^{13}C to ^{12}C in the sample against the ratio of ^{13}C to ^{12}C in a reference standard.

$\delta^{13}C‰$

Delta C thirteen is the conventional unit for the stable carbon isotope ratio in the sample. It is a measure of how much it varies from the standard.

Notice that delta C thirteen is expressed in parts per thousand.

You will see this expressed as $^{o}/_{oo}$ or permil or per mill.

$$\delta^{13}C\text{‰} = \left[\frac{R}{R_s} - 1\right] \times 1000$$

Where R is the ratio of ^{13}C to ^{12}C in the sample and R_s is the ratio in the standard.

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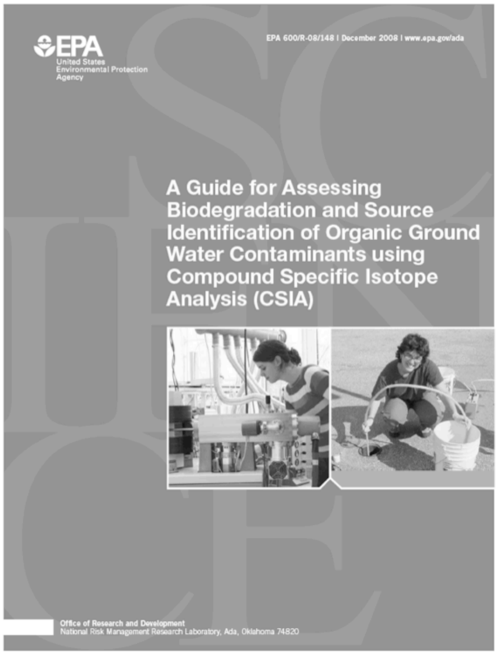
A *Guide for Assessing Biodegradation and Source Identification of Organic Ground Water Contaminants using Compound Specific Isotope Analysis (CSIA)*

EPA 600/R-08/148 |
December 2008 |
www.epa.gov/ada



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EPA 600/R-08/148 | December 2008 | www.epa.gov/ada

A Guide for Assessing Biodegradation and Source Identification of Organic Ground Water Contaminants using Compound Specific Isotope Analysis (CSIA)

Office of Research and Development
National Risk Management Research Laboratory, Ada, Oklahoma 74820

Recommendations for sample collection, sample preservation, and sample analysis.

Recommendations on QA/QC issues

Details on calculations.

Catalogue of values for $\delta^{13}C$, $\delta^{2}H$, and ε.

Sensitive QA/QC issues

1) Use of Standards to document accuracy and precision.
2) Recommendation for baseline separation of compounds during gas chromatography.

The following comments apply to analysis of stable isotopes of carbon.

They can be typically extrapolated as needed to analysis of stable isotopes of hydrogen and chlorine.

Standards for carbon isotope analyses

The isotope ratio mass spectrometer measures the isotope ratios of carbon dioxide produced from the combustion of the compound of interest. There are three standards:

The International Atomic Energy Agency standard (primary standard)

The laboratory's CO_2 working standard (secondary standard)

The laboratory's compound specific working standard for a particular VOA.

The International Atomic Energy Agency Standard

Samples provided by the IAEA that have a known ratio of carbon stable isotopes. In the United States the standard is provided by the National Bureau of Standards.

The IAEA Standard allows comparability of data from one lab to the other. Used for primary calibration of instruments.

The laboratory's CO_2 working standard

Calibrated against the IAEA international standard.

Used for tuning the instrument and day to day calibration checks of the instrument.

Provides the value against which all target peaks in a given run are standardized.

The laboratory's compound specific working standard

A sample of the compound being analyzed that has a known value for $\delta^{13}C$.

The standard is analyzed periodically in a sample set to document that the instrument is properly calibrated.

The compound specific working standard is also used to document the effective detection limit as discussed later.

At a minimum, the CO_2 working standard is analyzed twice at the beginning of each sample run. The automatic software routine uses one of these CO_2 peaks as the reference to calculate isotope ratios for the other peaks, including those of the second (or multiple) CO_2 peaks.

At least every fifth sample should be a sample replicate. At least every tenth sample should be the compound specific working standard.

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Require the vendor to provide the true value of $\delta^{13}C$ for each compound working standard.

Ask the vendor to report the values determined for their compound specific working standard during analysis of the samples.

The scope of work or the QAPP should specify an acceptable range of determined values from the true value for the compound working standard.

The determined values of the compound specific working standard should fall within the acceptable range from the true value.

Compounds in water are prepared for analysis by a variety of methods, including purge-and-trap and solid phase micro-extraction.

The method to prepare the sample for analysis will fractionate the sample.

The measured $\delta^{13}C$ should be corrected for the fractionation during preparation before it is reported.

If the analyst fails to correct for fractionation during sample preparation, the results will not be comparable from one laboratory to another.

To ensure the analysts has remembered to do this, you might ask the analyst to report the extent of fractionation during sample preparation.

How to determine the acceptable range of values to specify in the scope of work or the QAPP?

Ask the vendors to provide the acceptable range of values in their bid or quote.

Review and determine if the range in acceptable values meets the requirements of your project based on the comparisons you are going to make.

How much degradation can you detect at the proposed resolution?

$$F = C / Co = e^{((\delta\ ^{13}C_{MTBE in\ ground\ water} - \delta\ ^{13}C_{MTBE in\ gasoline}) / \varepsilon)}$$

Select a conservative value for the enrichment factor from the literature (a value with a low absolute value), and solve the equation for the fraction remaining (*F*), based on the difference in $\delta^{13}C$ that you can resolve based on the acceptable range of the compound specific working standard proposed by the vendor.

As a rule of thumb, you can resolve samples with good confidence when they differ by twice the acceptable range.

Values of ε are tabulated in Table 8.1 of the U.S. EPA Guide. Suppose you were interested anaerobic biodegradation of TCE.

The value of ε with the lowest absolute value is -2.56‰.

If the quoted acceptable range was 0.5‰, you can distinguish samples that differ by 1.0‰.

$$F = e^{1.0/-2.56} = 0.67$$

The extent of degradation that can be detected is-

$$1 - F = 1 - 0.67 = 0.33 \text{ or } 33\%$$

☐

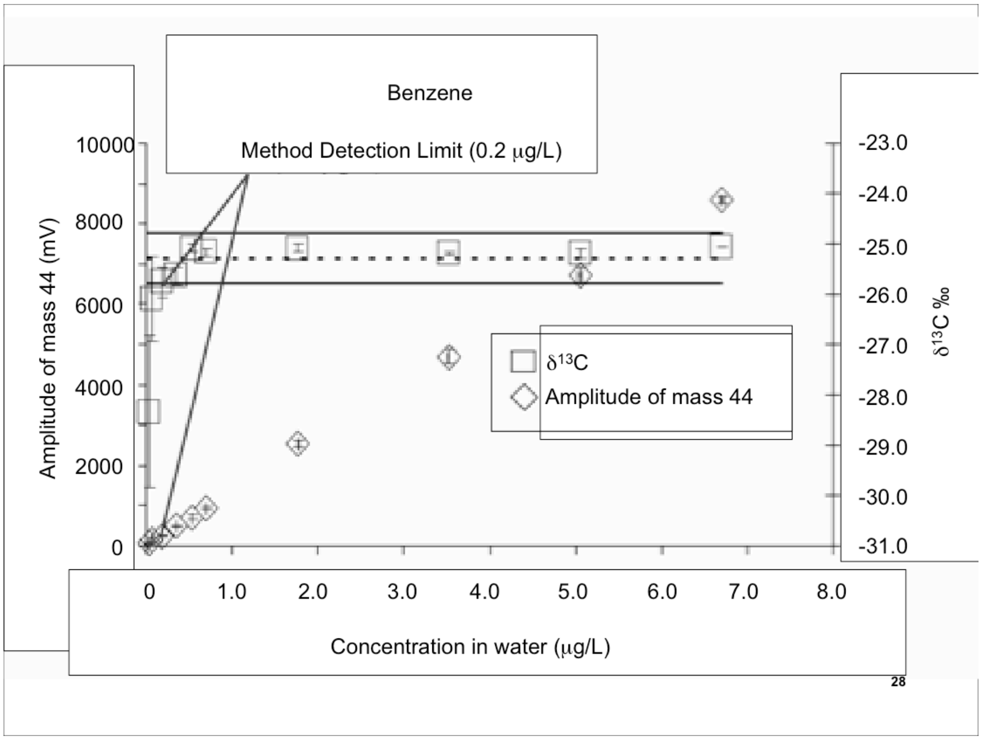
How to establish the effective detection limit for determination of $\delta^{13}C$ for a particular compound?

Customers tend to think of the detection limit as a concentration of the chemical being analyzed.

The effective detection limit for SCIR is the lowest concentration at which the variation in the determined values for $\delta^{13}C$ are acceptable.

The next slide is Figure 2.5 in A *Guide for Assessing Biodegradation and Source Identification of Organic Ground Water Contaminants using Compound Specific Isotope Analysis (CSIA).* The following is the figure legend.

Example of the evaluation of method detection limits (MDLs) in CSIA. The squares represent the δ^{13}C values in ‰ and the diamonds show the amplitude of mass 44 in mV. Error bars indicate the standard deviation of triplicate measurements. The horizontal broken line represents the iteratively calculated mean value after the methods of Jochmann et al. (2006) and Sherwood Lollar et al. (2007). The solid lines around the mean value represent the standard deviation on the mean of triplicate measurements. Figure modified after Jochmann et al. (2006).


Benzene
Method Detection Limit (0.2 μg/L)
Amplitude of mass 44 (mV)
10000
8000
6000
4000
2000
0
-23.0
-24.0
-25.0
-26.0
-27.0
-28.0
-29.0
-30.0
-31.0
δ13C ‰
δ13C
Amplitude of mass 44
0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
Concentration in water (μg/L)
28

Citations:

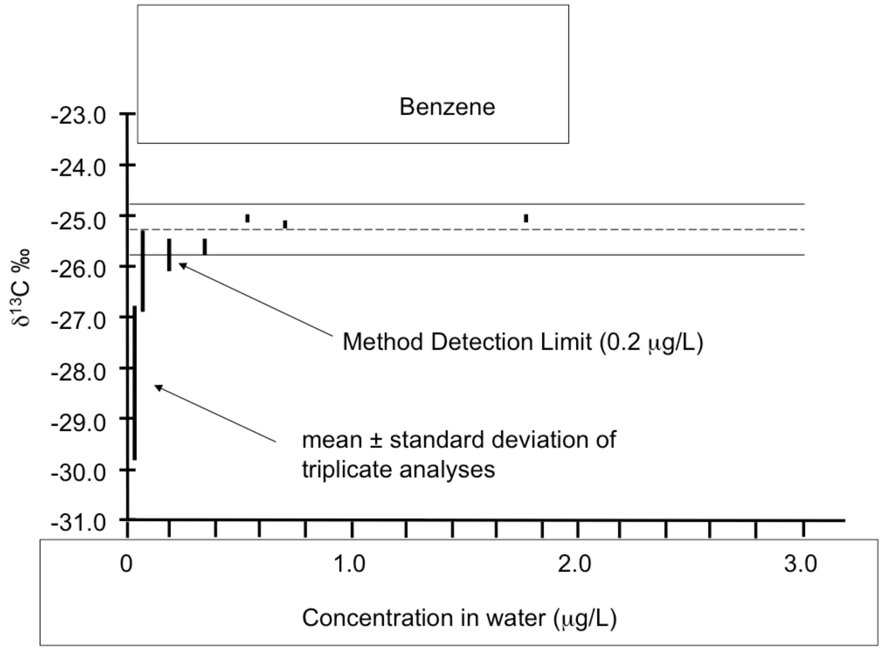
Jochmann, et al. A new approach to determine method detection limits for compound specific isotope analysis of volatile organic carbons. *Rapid Communications in Mass Spectrometry* 20: 3639-3648 (2006).

Sherwood Lollar,et al. An approach for assessing total instrumental uncertainty in compound-specific carbon isotope analysis: implications for environmental remediation studies. *Analytical Chemistry* 79: 3469-3475 (2007).

Jochmann et al. 2006 suggested an appropriate method detection limit (MDL) could be defined as *the signal size below which the standard deviation of the mean exceeds 0.5‰ and the* $\delta^{13}C$ *values are outside the 0.5‰ interval around the running mean.*

Sherwood Lollar et al. 2007 suggest that a more conservative approach might be to *define the method detection limit as the point at which the variance around the mean significantly increases (typically at signal size < 0.5 V).*

Benzene



Method Detection Limit (0.2 μg/L)

mean ± standard deviation of triplicate analyses

One vendor specifies a practical quantitation limit that is round number that is slightly larger than the method detection limit (MDL). This PQL allows for minor variations in the sensitivity of the instrument.

If the area of the mass 44 peak is less than the MDL, the vendor does not report an isotope ratio and flags the analysis as “U”.

If the peak area is between the MDL and PQL, the vendor reports the peak area and the isotope ratio and flags the isotope ratio with a “J”.

Provide guidance on QA in the scope of work or QA Project Plan.

Determine the difference in δ^{13}C or δ^{2}H that you need to resolve.

If you really don't know what difference you need to resolve, as a default, require that the standard deviation of the samples of triplicate samples of the compound working standard be equal to or less than +/-0.5‰ for δ^{13}C and equal to or less than +/-5‰ for δ^{2}H.

At this level of uncertainty, you can resolve a difference between samples when the difference in δ^{13}C >1‰ or the difference in δ^{2}H >10‰.

Detection limits should always be determined using the same chromatographic column and working conditions as the samples.

The compound working standards should be subjected to the entire analytical procedure including any extraction and concentration steps.

The compound working standards should be spiked into water, then extracted and prepared for gas chromatography following the same procedures as the samples.

Based on the requirements of your project, identify the lowest concentration of the VOA that you are interested in analyzing for isotope ratios.

The method detection limit, or practical quantitation limit, should correspond to a concentration that is lower than this lowest concentration you have identified for the project.

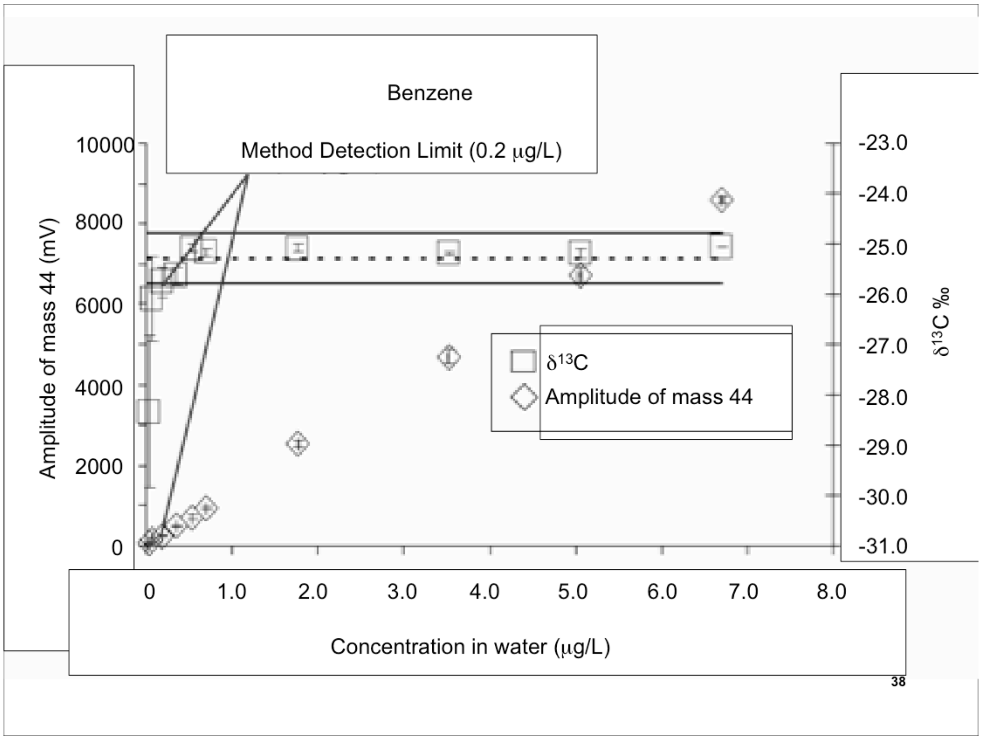
Ask the vendor to provide the MDLs or PQLs in their bid. Compare their capability to your requirement.

Require the vendor to report the values of replicate analyses (n=3) of the compound specific working standard at this lowest concentration.

As an estimate of precision in the determination of isotope ratio for the compound specific standard at this lowest concentration, calculate the mean and standard deviation on the samples.

The mean should not differ from the true value by more than the acceptable range. The sample standard deviation should not be greater than the acceptable range.

As an alternative, one vendor prefers to calculate the sample standard deviation of the range of the samples as the best indication of system performance.


Benzene
Method Detection Limit (0.2 μg/L)
Amplitude of mass 44 (mV)
10000
8000
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-24.0
-25.0
-26.0
-27.0
-28.0
-29.0
-30.0
-31.0
δ13C ‰
δ13C
Amplitude of mass 44
0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
Concentration in water (μg/L)
38

Notice that the amplitude of the mass 44 ion is roughly proportional to concentration of compound of interest.

There will be an amplitude of the mass 44 ions that is associated with the lowest concentration of compound that you are interested in.

An Alternative:

After you see the data, you may be very interested in certain samples that had low concentrations of the VOA. Those samples may have experienced the most biodegradation.

Require the vendor to flag $\delta^{13}C$ values that were performed on any samples where the amplitude of the mass 44 ion corresponded to concentrations that were below that lowest concentration of compound you identified to the vendor.

An Alternative:

Require the vendor to report the values of $\delta^{13}C$ of replicate analyses (n=3) of the compound specific working standard at a concentration that corresponds to the amplitude of the mass 44 ion in the flagged samples.

A replicate of the original sample should be included in the same sample run as the replicates of the diluted compound specific working standard.

An Alternative:

This is a very open-ended approach. It is impossible to determine before hand how much labor would be involved.

Expect to pay the vendor for the additional work necessary to determine the precision of the isotope ratio analysis on the flagged samples.

Detection limits should always be determined using the same chromatographic column and working conditions (including split ratios) as the samples.

The compound working standards should be subjected to the entire analytical procedure.

The compound working standards should be spiked into water, then extracted and prepared for gas chromatography following the same procedures as the samples.

An isotope ratio mass spectrometer can analyze samples over a fairly narrow range concentrations.

All samples should stay within the acceptable range and above the established threshold limit.

If a sample falls outside the acceptable range, the concentrations of the analytes should be adjusted, if possible, to bring the sample within the acceptable range, and the sample analyzed a second time.

This is one reason the vendors want so many replicate water samples. There may be several repeat analyses.

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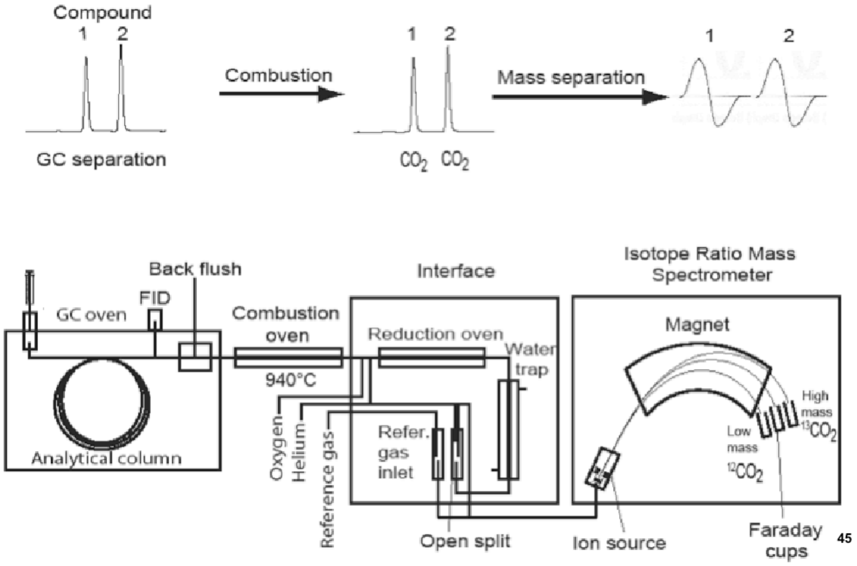
Figure 2.1 of U.S. EPA Guide.

GC IRMS requires clean separation of peaks of individual compounds.



In most plumes of chlorinated solvents, there are usually few VOA compounds in the water, and peaks of individual compound are clearly separated from each other.

In plumes that originate from spills of petroleum, conventional GC columns may not separate peaks for the compounds of interest from co-eluting compounds.

In many states, the concentrations of MTBE and benzene in ground water at UST sites are determined by Gas Chromatography with a Mass Spectrometer Detector (EPA Method 8260) instead of Gas Chromatography with a Flame Ionization Detector (EPA Method 8015).

The more expensive method (8260) is required because the GC column often can not separate the MTBE or benzene from other components of the fuel.

For some compounds baseline resolution is impossible:

1. isomers of chlorobenzene
2. higher molecular hydrocarbons in the gasoline or diesel range that elute on top of a rising baseline.
3. MTBE and 1,1-dichloroethane co-elute on some columns.

Determination of concentrations using GC Mass Spectrometry is fairly forgiving of overlapping peaks in the chromatograph. The ions characteristic to a specific compound can be used to recognize and quantify the compound of interest.

The Flame Ionization Detector works by burning the compounds. It can not distinguish between compounds in overlapping peaks.

Like a Flame Ionization Detector, the Isotope Ratio Mass Spectrometer is not forgiving. All of the compounds are oxidized to CO_2, and the mass ratio of the CO_2 that is derived from each compound is determined separately.

It is impossible to determine beforehand whether there will be overlap of peaks in the chromatograph from a particular sample.

As a result, it is difficult to protect against this source of error in a scope of work or QAPP.

However, there are things that can be done to recognize overlap of peaks and alternatives to improve the separation of peaks.

One way to detect problems with co-elution is to examine the chromatogram for shoulders on the front or back of each target peak, or more generally, any differences in peak shape as compared to the standard.

You might task the vendor to provide in the report one or a few of the most complex chromatographs. There are the chromatographs where there is a greater chance that peaks of other compounds will overlap the peaks for the compounds of interest.

You might require that “analyses only be performed when peaks for the compound of interest are clearly resolved from co-eluting peaks.” However, without some quantitative description of “clearly resolved”, the requirement is ambiguous.

Often, the Project Plan will require data on concentrations was well as isotopic ratios.

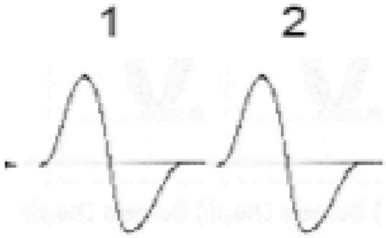
To minimize operator time on the IRMS, some vendors require that an analyses of concentrations of VOCs to be provided by the client.

Frequently, the samples will be analyzed for concentrations using Method 8260 or equivalent.

If the same chromatographic column and conditions are being used for Method 8260 and the CSIA, examine the full-scan mass spectra of the peak compared to the mass spectrum of a standard, and look for the presence of mass fragments in the sample spectrum that are unaccounted for in the spectrum of the standard.

Look at the non-background-subtracted spectra, to avoid subtracting out the contribution from a compound in the shoulder of the main peak. Anything that has an abundance greater than 10% of the base peak is suspect, and requires further consideration and evaluation.

Part of Figure 2.1 of U.S. EPA Guide.



The ratio of mass 45 to mass 44 ($^{13}C\text{-}CO_2$ to $^{12}C\text{-}CO_2$) is called the ratio trace of the peak. If there are co-eluting peaks, the shape of the trace will depart from a trace of the pure compound.

The error caused by co-eluting peaks will be the greatest when concentrations of the VOA are low, and the difference in isotopic ratios between samples is small.

When these circumstances apply, you might require the vendor to compare the ratio trace for each analysis against the trace of the compound specific standard. For particularly crucial analyses, you might require the vendor to provide copies of the ratio traces in the report.

Other factors can influence the shape of the ion trace, including the extent of isotopic fractionation of the sample compared to the compound specific standard.

Interpreting the trace is best left to an analytical chemist that is familiar with the instrument and the analytical protocol that was used to acquire the data.

As mentioned previously, the amplitude of area of the ion 44 peak is roughly proportional to the concentration of the VOA. Linear extrapolation can provide an estimate of the concentration of the VOA from the amplitude or area of the ion 44 peak.

If there is a concern with the symmetry of the ion ratio trace, you may task the analyst to compare the estimate of concentration from the IRMS to the concentration reported using GS/MS such as Method 8260. Extreme differences between the two estimates may indicate problems with co-elution or other matrix effects.

To perform CSIR of EDB in gasoline spills, Paul Philp’s lab at the University of Oklahoma had to use two dimensional chromatography to get good peak separation. In this case, “two dimensional” means they used two different GC columns in sequence to achieve adequate separation.

Natural Attenuation of the Lead Scavengers 1,2-Dibromoethane (EDB) and 1,2-Dichloroethane (1,2-DCA) at Motor Fuel Release Sites and Implications for Risk Management. EPA 600/R-08/107 | September 2008 | www.epa.gov/ada.

Analysis $\delta^{13}C$ or $\delta^{2}H$ of aromatic hydrocarbons or chlorinated VOCs should be performed on a conventional water sample in a 40 ml VOA vial preserved with HCl to pH <2.

Preserve samples of ethers such as MTBE with trisodium phosphate to pH>10.5.

As of this date, appropriate preservation of chlorinated VOCs for $\delta^{37}Cl$ has not been specifically evaluated but similar approaches to the above are likely to be required.

Require the vendor to specify the appropriate technique to preserve samples.

Under some circumstances, analyses for CSIA must wait for analyses on concentrations of VOAs in the samples. The clock is running on holding time while the vendor for the CSIA is waiting for the concentration data.

Data in the EPA Guide (Section 3.4) documents the capacity of hydrochloric acid and trisodium phosphate (as appropriate) to preserve samples for 28 days. Other studies have shown that samples can be held for even longer periods of time.

It is best to collect samples for analysis of concentrations and for CSIA at the same time.

The analyses can be performed on the same sample set, and the results are directly comparable.

Avoid collecting samples for concentrations and CSIA on different days.

Vendors may require as many as nine replicate samples from each well.

The vendor should specify the number of replicates in the bid.

You really can’t sample the same ground water twice. The cost of the vials is a tiny part of the cost of sampling. Collect more samples than you think you will need, and discard them if they are not needed.

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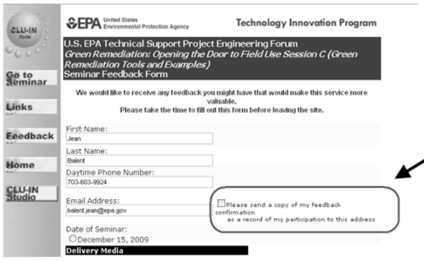

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Green Remediation: Opening the Door to Field Use Session C (Green Remediation Tools and Examples)
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