Thank you for signing in early

The webinar will begin promptly at
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SERDP and ESTCP Webinar Series

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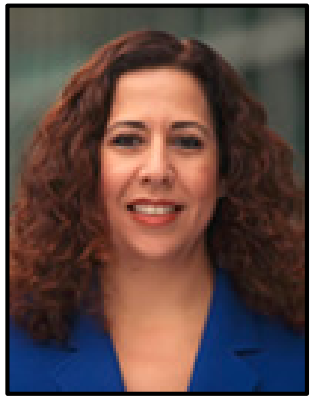
Applying Compound-Specific Isotope Analysis to Document Contaminant Degradation and Distinguish Sources

March 12, 2020

SERDP DOD • EPA • DOE | ESTCP

Welcome and Introductions



Rula A. Deeb, Ph.D.
Webinar Coordinator

SERDP DOD • EPA • DOE | ESTCP

Webinar Agenda

- **Webinar Logistics** (5 minutes)
  Dr. Rula Deeb, Geosyntec Consultants

- **Overview of SERDP and ESTCP** (5 minutes)
  Dr. Andrew Leeson, SERDP and ESTCP

- **Applying Compound-Specific Isotope Analysis to Document Contaminant Degradation and Distinguish Sources** (55 minutes + Q&A)
  Dr. Paul Hatzinger, APTIM

In Case of Technical Difficulties

- Use a compatible browser (Firefox, IE or Edge)
- If material is not showing on your screen or if screen freezes
  - Key in Ctrl + F5 to do a hard refresh of your browser
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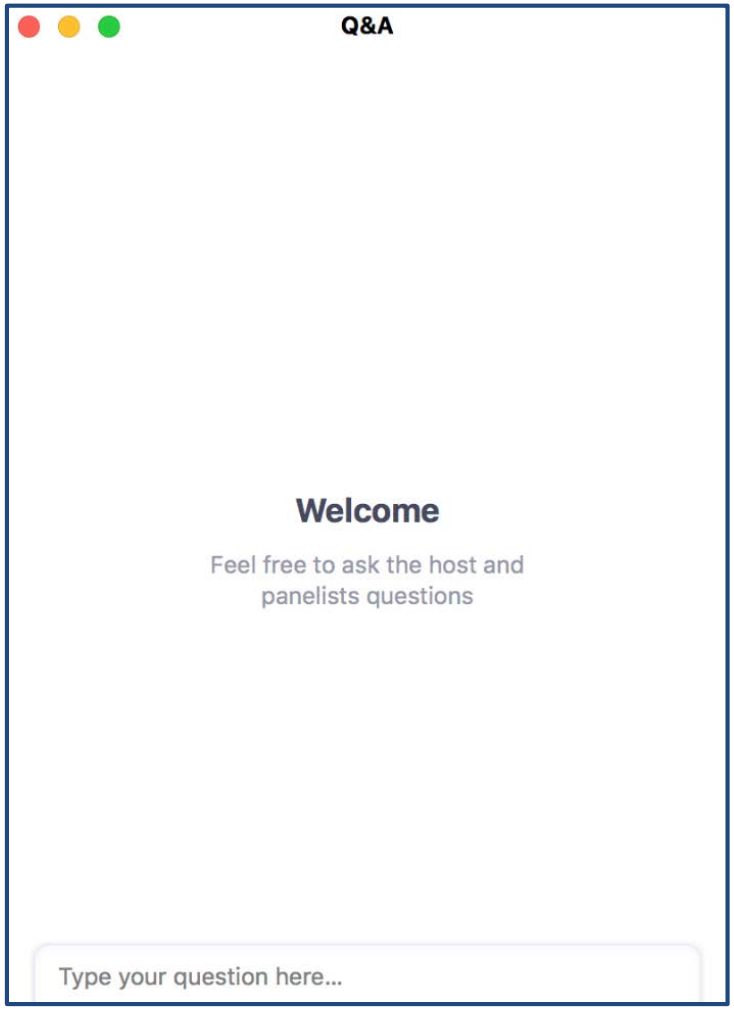
- Find the Q&A button on your control bar and type in your question(s)
- Make sure to add your organization name at the end of your question so that we can identify you during the Q&A sessions



SERDP and ESTCP Overview

Andrea Leeson, Ph.D.
SERDP and ESTCP

DoD's Environmental Technology Programs

Science and Technology

- Statutory program established 1991
- DoD, DOE, EPA partnership
  - Advanced technology development to address near-term needs
  - Fundamental research to impact real world environmental management

Demonstration and Validation

- Demonstrate innovative cost-effective environmental and energy technologies
  - Transition technology out of the lab
  - Establish cost and performance
  - Partner with end user and regulator
  - Technology transfer
    - Accelerate commercialization or broader adoption
    - Direct technology insertion

Environmental Drivers

Sustaining Ranges, Facilities and Operations

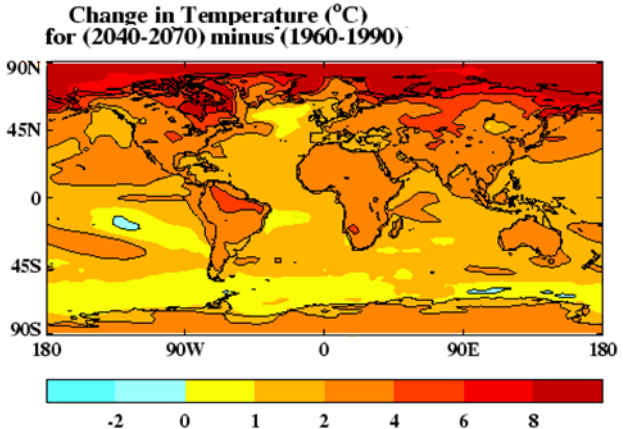
Maritime Sustainability
Threatened and Endangered Species

Toxic Air Emissions and Dust

UXO and Munitions Constituents

Noise

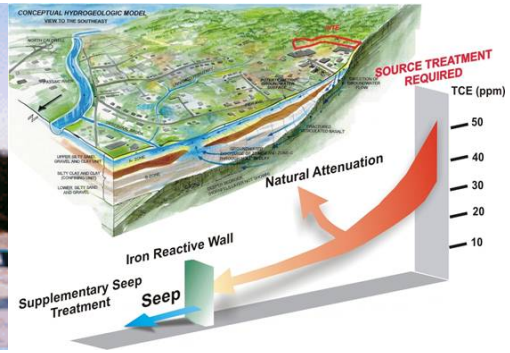
Sustainable FOB



Environmental Drivers

Reducing Current and Future Liability

Contamination from Past Practices



- Groundwater, soils and sediments
- Large UXO liability
- Emerging contaminants

Pollution Prevention to Control Life Cycle Costs

- Elimination of pollutants and hazardous materials in manufacturing, maintenance, and operations
- Achieve compliance through pollution prevention

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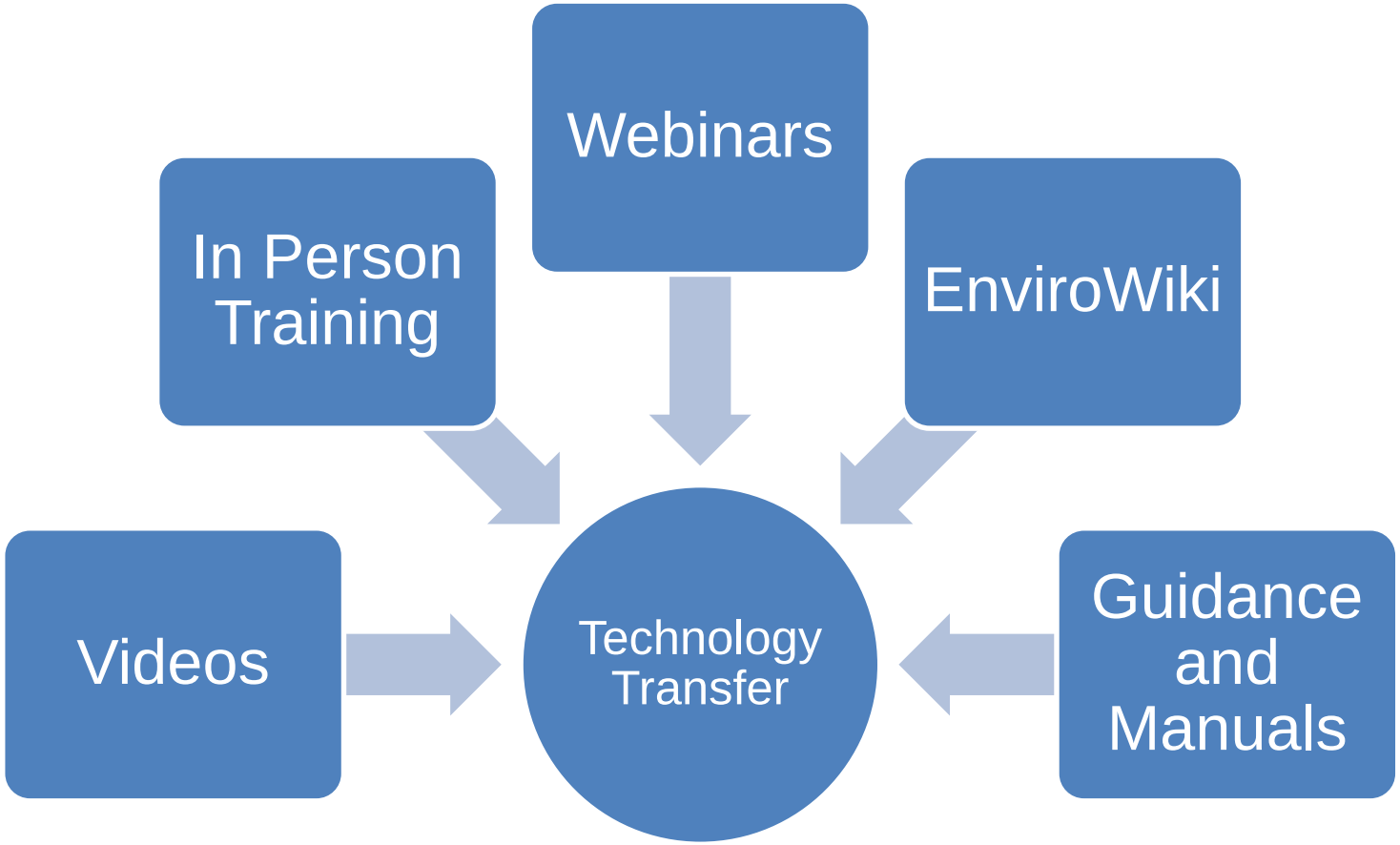

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Webinars

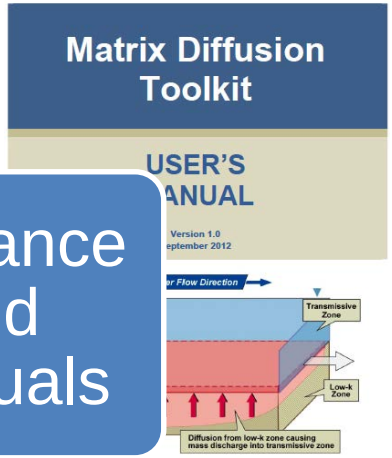
In Person Training

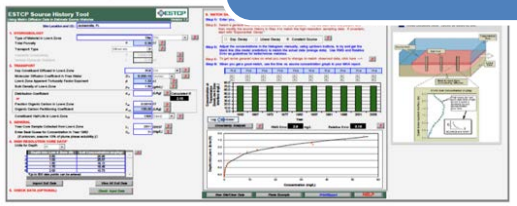
EnviroWiki

Videos

Technology Transfer

Guidance and Manuals





SERDP and ESTCP Webinar Series

Date	Topic
March 26, 2020	Long-Term Ecological Studies: Evaluating Responses to Ecosystem Restoration and Optimizing Recovery of Plant Communities
April 9, 2020	Ecological Risk Assessment Approaches at PFAS-Impacted Sites
April 23, 2020	Munitions Response Webinar: Project of the Year Award Winners
May 7, 2020	Innovative Technologies for PFAS Destruction in Investigation Derived Wastes
May 21, 2020	Analysis of Defense Related Ecosystem Services

For upcoming webinars, please visit

http://serdp-estcp.org/Tools-and-Training/Webinar-Series

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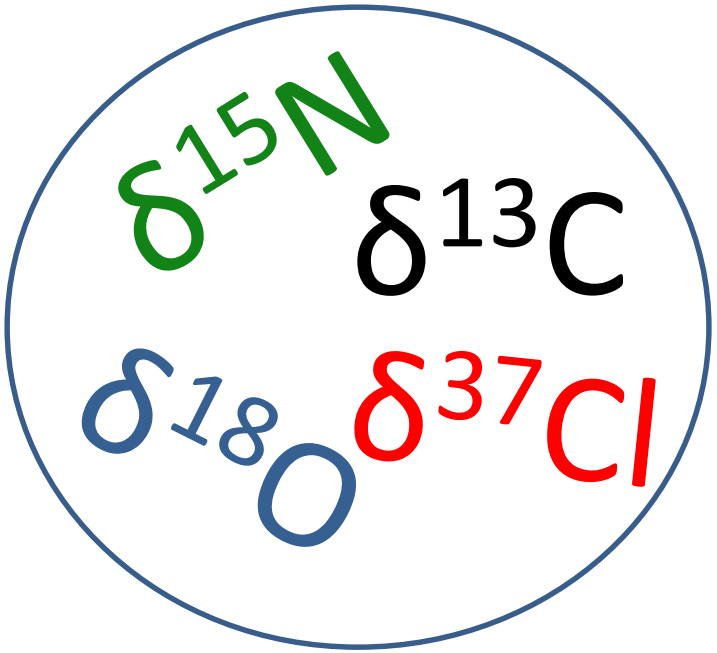
Applying Compound-Specific Isotope Analysis to Document Contaminant Degradation and Distinguish Sources

Paul B. Hatzinger, Ph.D.
APTIM

SERDP DOD • EPA • DOE | ESTCP

Webinar Overview

- Fundamentals of CSIA
- Applications
  - Documenting CVOC degradation
    - Parent CVOCs
    - Daughter products
  - Source discrimination – vapor intrusion
- Q&A
- Applications (Continued)
  - Other contaminants of concern
    - 1,4-Dioxane
    - 1,2-Dibromoethane
    - Perchlorate
- Summary
- Q&A



Some Questions CSIA Can Help to Answer

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

Some Fundamentals

What are Isotopes?

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

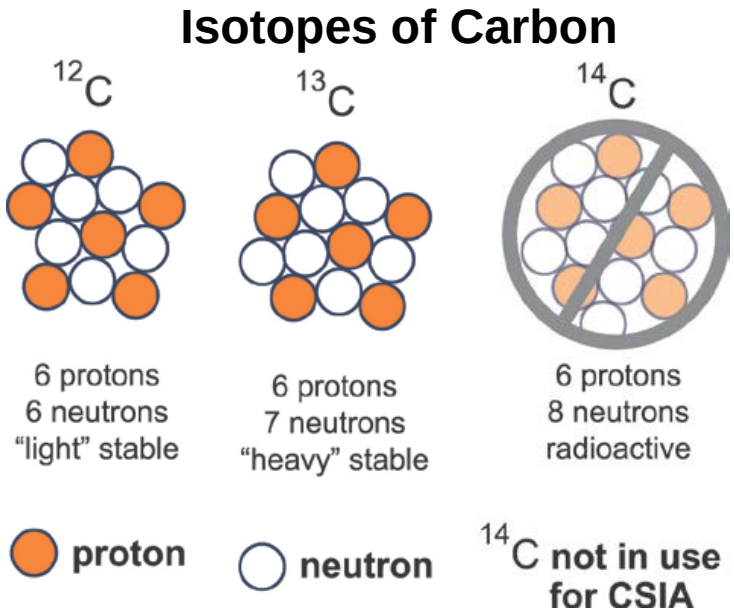


Figure Source: Ref 1

Some Fundamentals

What are Isotopes?

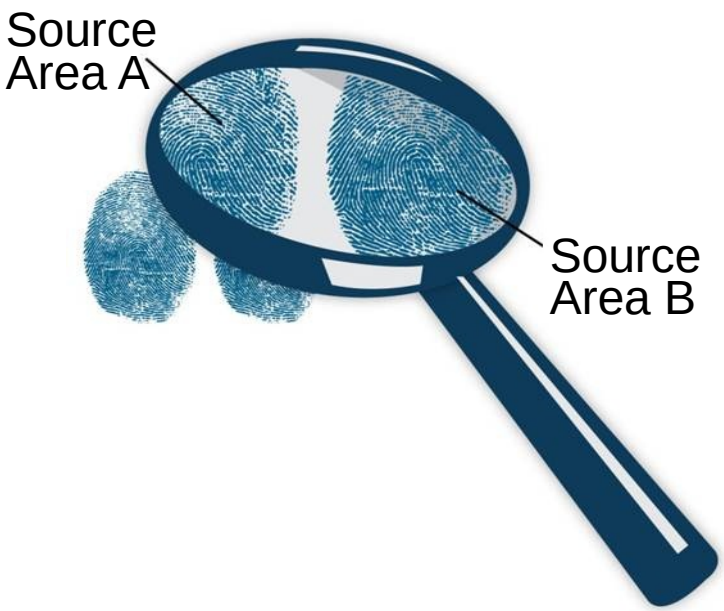
Some environmentally relevant stable isotopes

Element	Isotope	% Lighter Isotope in Nature
Hydrogen	^{1}H, ^{2}H	99.99 %
Oxygen	^{16}O, ^{17}O, ^{18}O	99.76 %
Carbon	^{12}C, ^{13}C	98.89 %
Chlorine	^{35}Cl, ^{37}Cl	75.78 %
Nitrogen	^{14}N, ^{15}N	99.63 %
Sulfur	^{32}S, ^{34}S	95.01 %

Why are Stable Isotopes Useful?

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

Forensics



Documenting degradation
(chemical or biological)

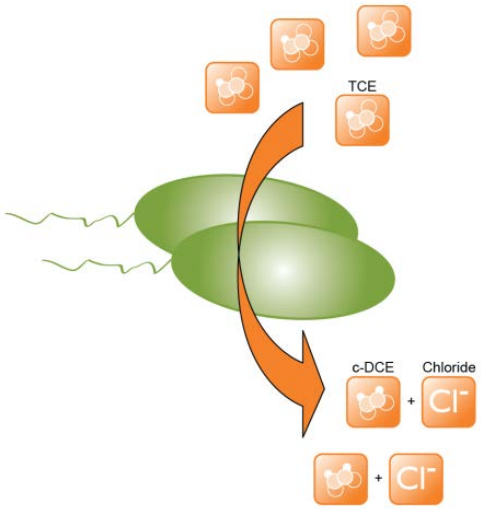


Figure Source: Ref 1

How are Stable Isotopes 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)"

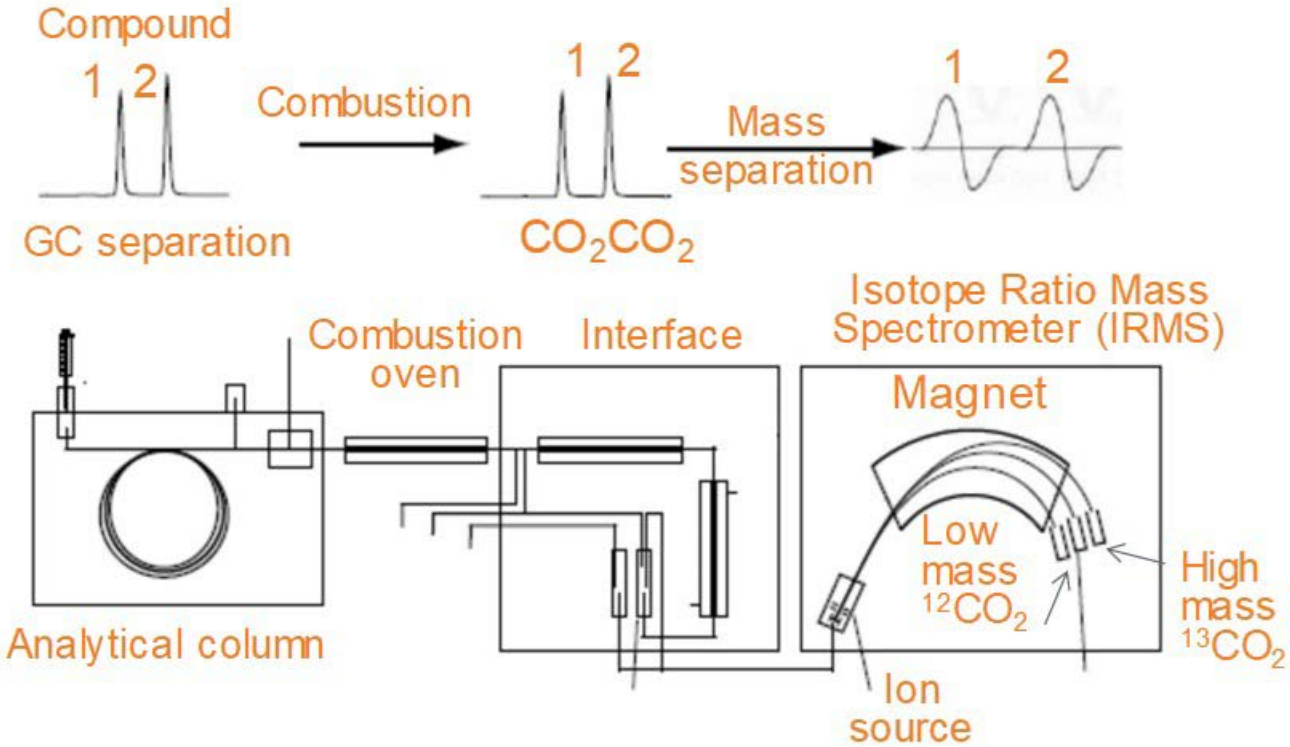


Figure Source: Ref 2

How are Stable Isotope Ratios Reported?

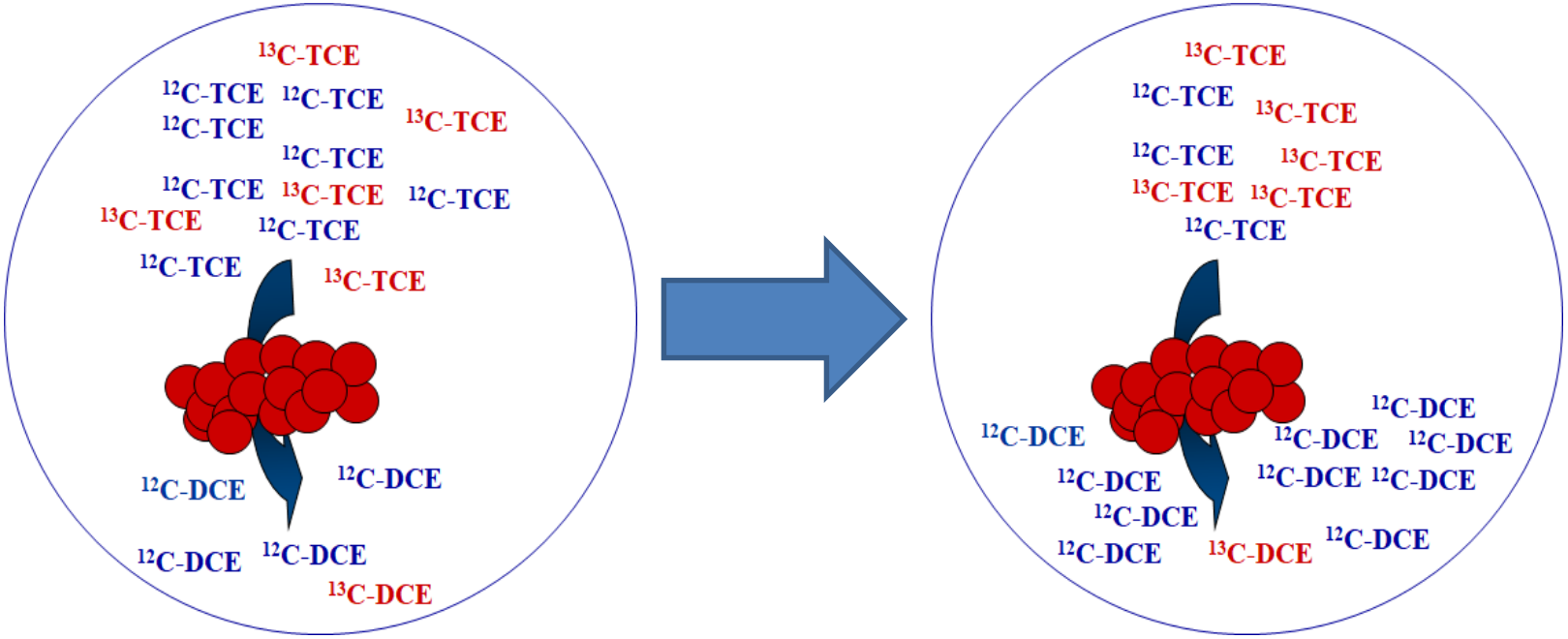
- Isotopic ratios of light elements are generally reported relative to a known standard as "delta" (δ) values and measured in parts-per-thousand (denoted "‰" = per mil)

$$\delta^{13}C \text{ in ‰} = \frac{\left({}^{13}C/{}^{12}C_{\text{sample}} - {}^{13}C/{}^{12}C_{\text{standard}}\right)}{{}^{13}C/{}^{12}C_{\text{standard}}} \times 1000$$

- 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

What is Isotope Fractionation?

- 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



Isotope Fractionation

Kinetic Isotope Effect

Kinetic isotope effect (KIE)

$$TCE \xrightarrow{{}^{13}k,\,{}^{12}k} product$$

Fractionation factor

$$\alpha = \frac{{}^{13}k}{{}^{12}k}$$

Enrichment factor

$$\varepsilon = 1000 * (\alpha - 1)$$

Rayleigh Fractionation

$$\delta = \delta_0 + \varepsilon * \ln f$$

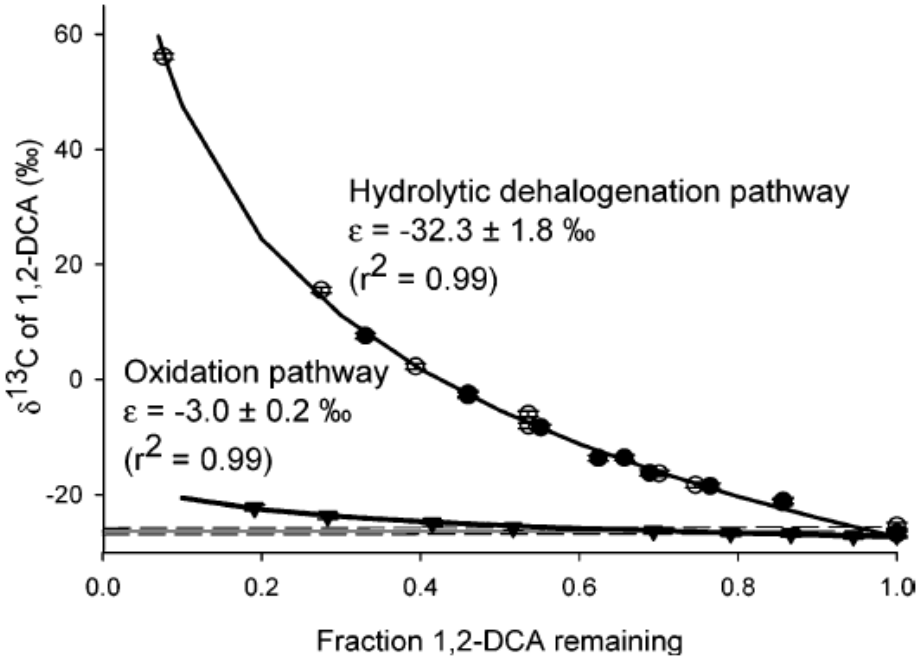


FIGURE 5. $\delta^{13}C$ values for 1,2-DCA during biodegradation by *X. autotrophicus* GJ10, *A. aquaticus* AD20, and *Pseudomonas* sp. Strain DCA1. Closed circles represent replicates of *X. autotrophicus* GJ10.

Figure Source: Ref 3

Enrichment Factors (ε)

- Best determined in a laboratory setting
- Often vary among degradative mechanisms but should be reasonably consistent for the same mechanism
- In a field setting isotopic fractionation must occur to with an ε of at least ~ - 2 to - 3 ‰
- ε values are often lower in environmental samples due to poor mixing and other factors
- More isotopes are usually better

Where Can I Find Relevant ε Values?

- USEPA. 2008. A Guide for Assessing Biodegradation and Source Identification of Organic Groundwater Contaminants Using Compound Specific Isotope Analysis (CSIA). U.S. EPA, Washington, D.C., EPA/600/R-08/148 https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NRMRL&dirEntryId=202171
- ISOFRAC Database on Isotope Enrichment Factors https://www.isodetect.de/en/applied-research/enrichment-factors/

compound	13C/12C	conditions, mechanism	bacteria	reference
cDCE	-20.5	abiotic (Zero-valent iron)		Audí-Miró et al. 2012
cDCE	-21.7	abiotic (Zero-valent iron)		Elsner et al. 2008
cDCE	-1.1	aerobic (toluene 2,3-dioxygenase)	Pseudomonas putida F1	Clingenpeel et al. 2012
cDCE	-1.2	aerobic (toluene 2,3-dioxygenase)	Pseudomonas fluorescens CFS215	Clingenpeel et al. 2012
cDCE	-0.9	aerobic (toluene 3-monooxygenase)	Pseudomonas mendocina KR1	Clingenpeel et al. 2012
cDCE	-19.9	aerobic biodegradation		Jennings et al. 2009
cDCE	-15.2	aerobic biodegradation		Schmidt et al. 2010
cDCE	-14.9	reductive dehalogenation	Dhc sp. strain BAV1	Fletcher et al. 2011
cDCE	-18.4	reductive dehalogenation	Dhc strains BAV1, FL2, GT, and VS	Fletcher et al. 2011
cDCE	-25.3	reductive dehalogenation	Dhc-containing culture (BDI)	Fletcher et al. 2011
cDCE	-26.8	reductive dehalogenation	Dhc mixed culture	Kuder et al. 2013

CSIA of CVOCs

- Sampling protocol
- Sampling strategy
- Isotopic evidence of parent CVOC degradation
- Isotopic evidence of daughter product degradation
- Case study
- Vapor intrusion of CVOCs – Source discrimination
- Q&A break

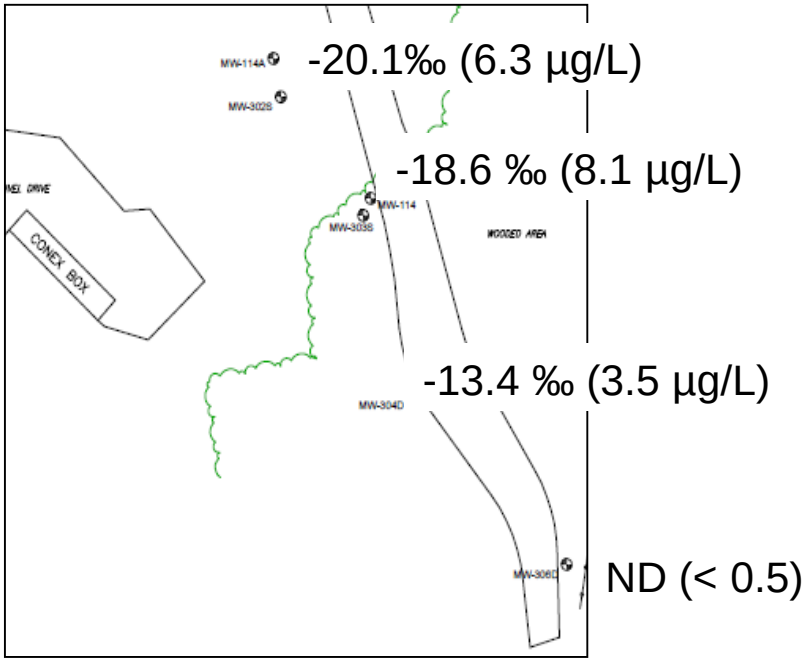
How Do I Sample CVOCs for Isotope Analysis?

- Typical low flow sampling
- Minimum concentration ~ 5 µg/L
- Typically collect 6 preserved VOA vials per well
- Also collect samples for quantitative CVOC analysis
- No specific hold time
- General cost estimate
  - \$ 500 for $\delta^{13}C$ first CVOC/ \$50 for $\delta^{13}C$ additional CVOCs
  - \$ 600 for $\delta^{37}Cl$ first CVOC/ \$200 for $\delta^{37}Cl$ additional CVOCs

Which Wells Do I Sample for CSIA?

- Objective of the work
  - MNA evaluation?
  - Active remedial technology?
- Sample along a flow path
- Sample wells over time
- Both strategies
- Good site model is critical if rates are of interest
  - Accurate groundwater flow rate
  - Accurate flow path

Trichloroethene (TCE)



Is My CVOC Degrading?

- Important to know the original range of isotopic ratios (e.g., $\delta^{13}C$ and $\delta^{37}Cl$)

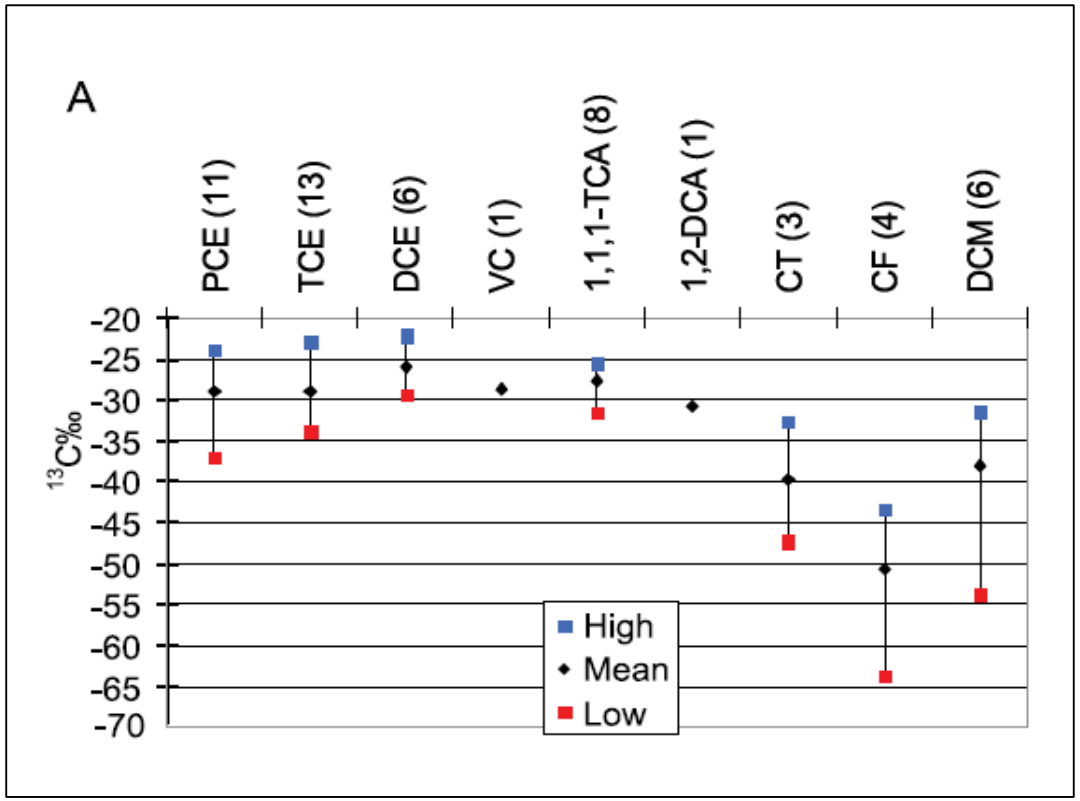
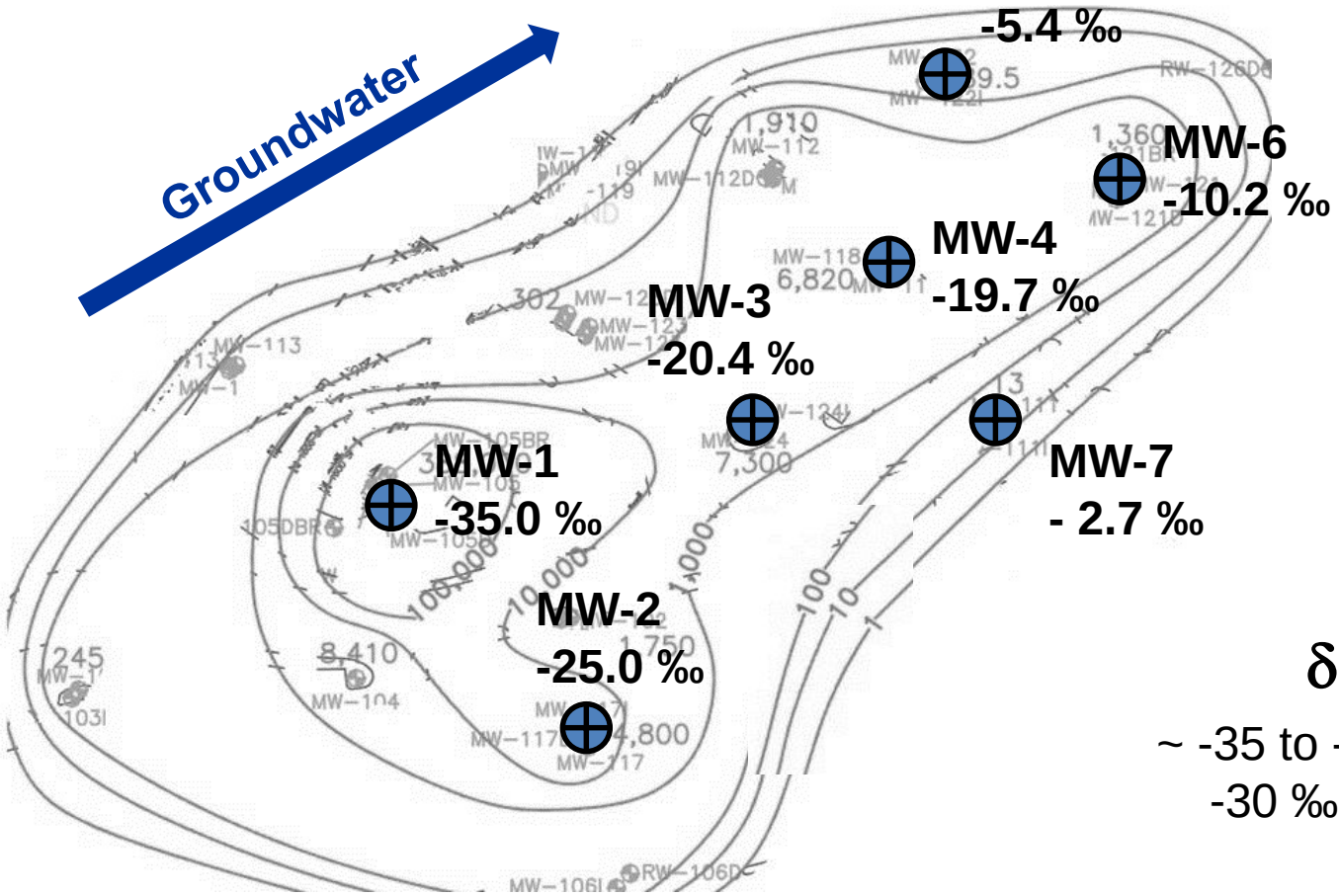


Figure Source: Ref 1

Is My CVOC Degrading? Example 1

Clear evidence of degradation



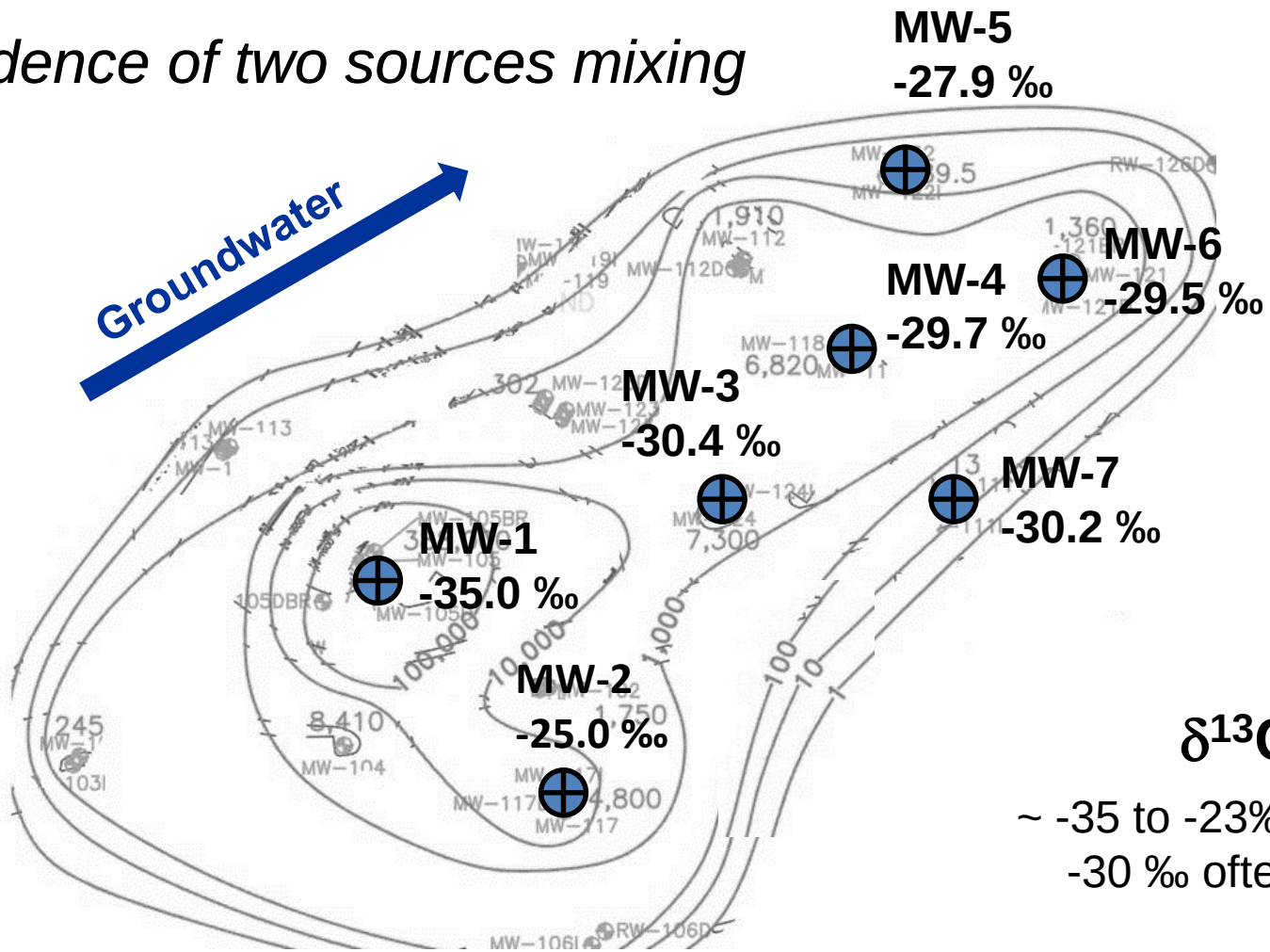
$\delta^{13}C$ - TCE

~ -35 to -23‰ typical in sources
-30 ‰ often used

***Note**: Isotope data for instructive purposes only (not real site data)*

Is My CVOC Degrading? Example 2

Evidence of two sources mixing



$\delta^{13}C$ - TCE

~ -35 to -23‰ typical in sources
-30 ‰ often used

***Note**: Isotope data for instructive purposes only (not real site data)*

Are CVOC Daughter Products Degrading?

Biological Reductive Dechlorination

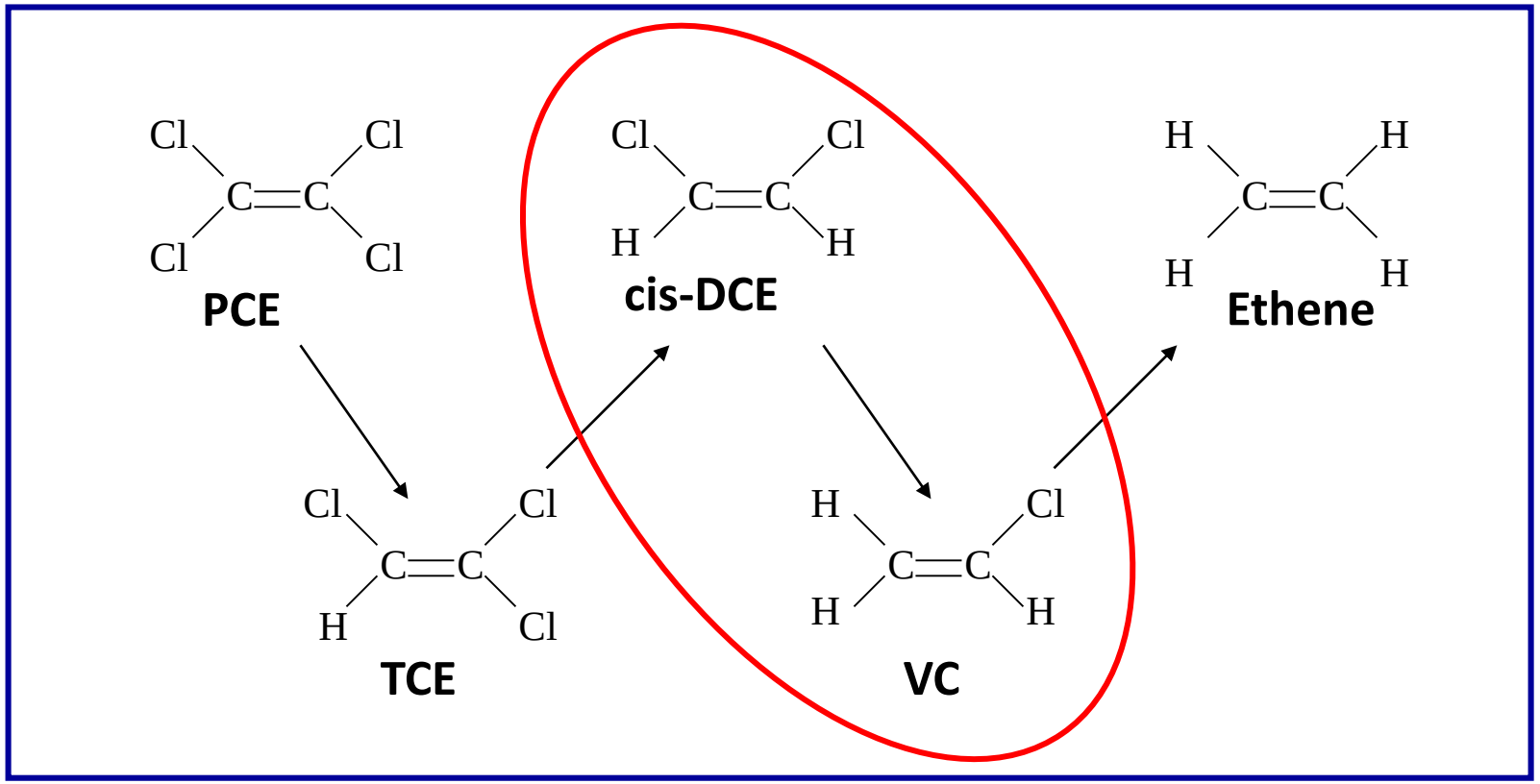


Figure Source: Ref 1

Are CVOC Daughter Products Degrading?

- For daughter products, fractionation alone does not prove degradation
- Even if the 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

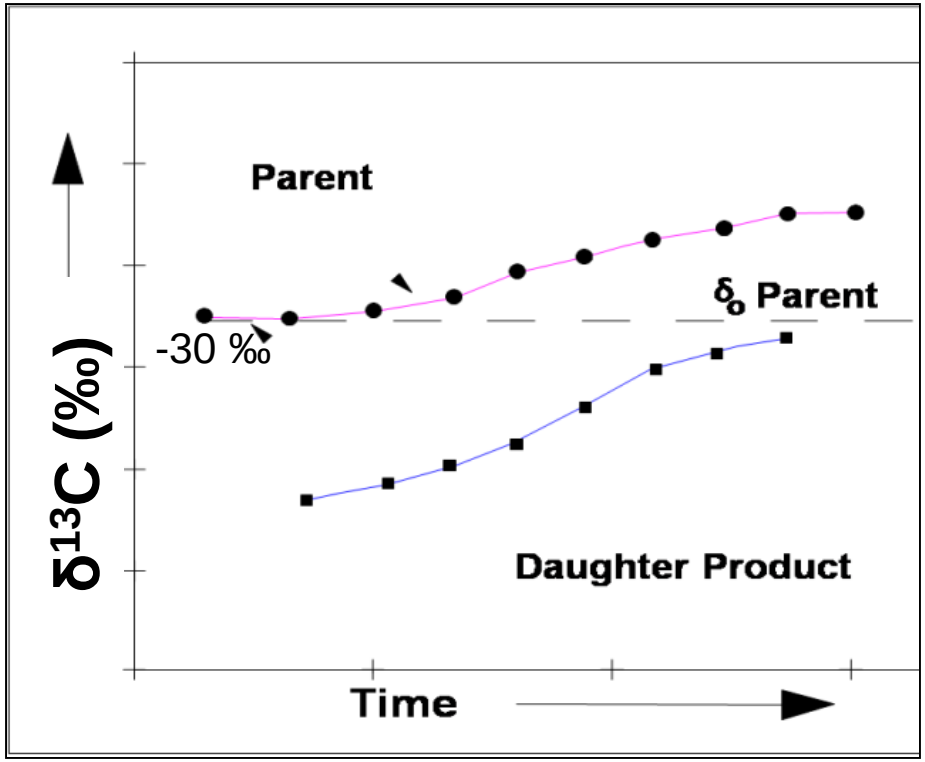


Figure Source: Pace Analytical, Inc.

Are CVOC Daughter Products Degrading?

- In chlorinated solvent daughter products, a carbon isotopic ratio heavier than the parent is evidence that the daughter product is degrading

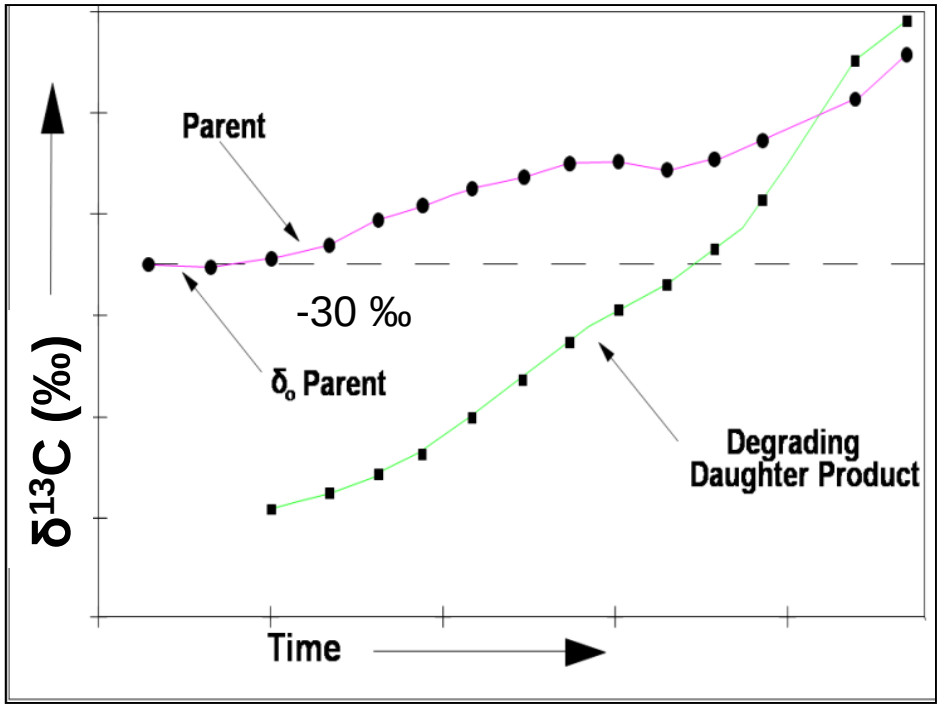


Figure Source: Pace Analytical, Inc.

CSIA Example of Degrading Parent and Daughter Product

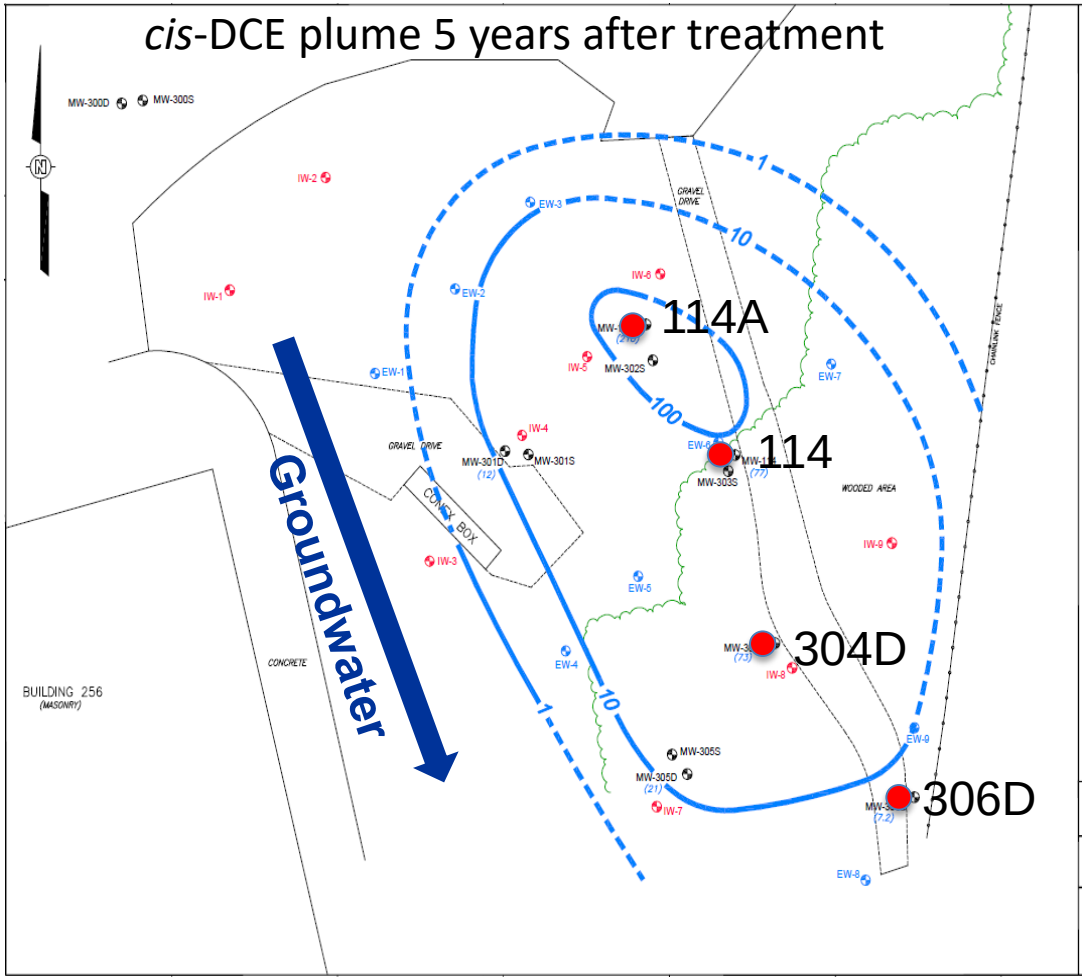
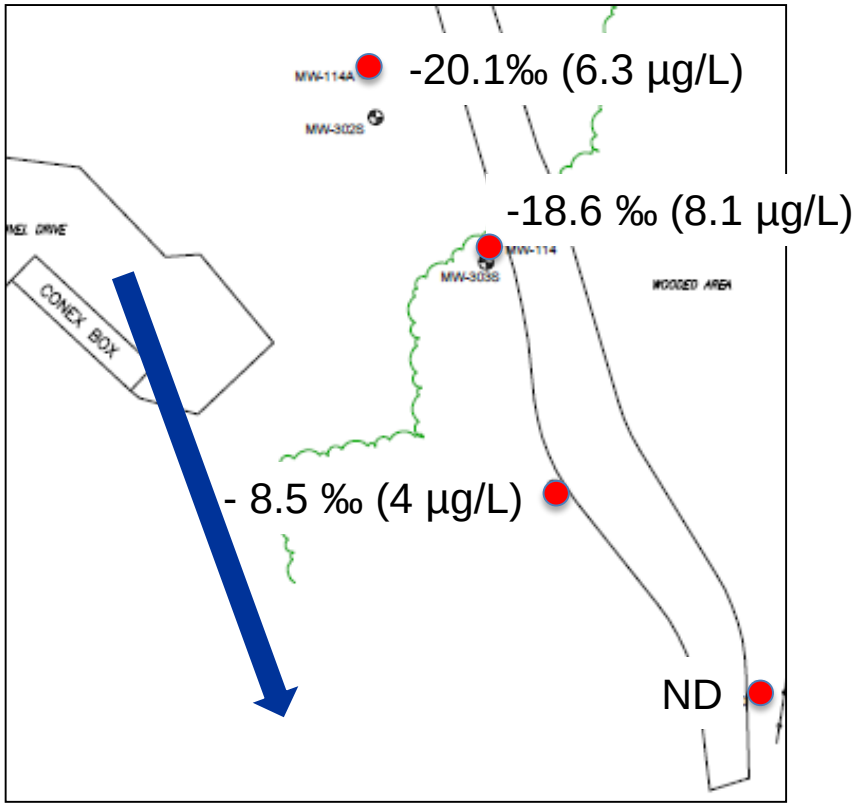


Figure Source: Ref 4

CSIA Example of Degrading Parent and Daughter Product

Starting TCE (- 30 ‰)



cis-DCE (Product of TCE**)**

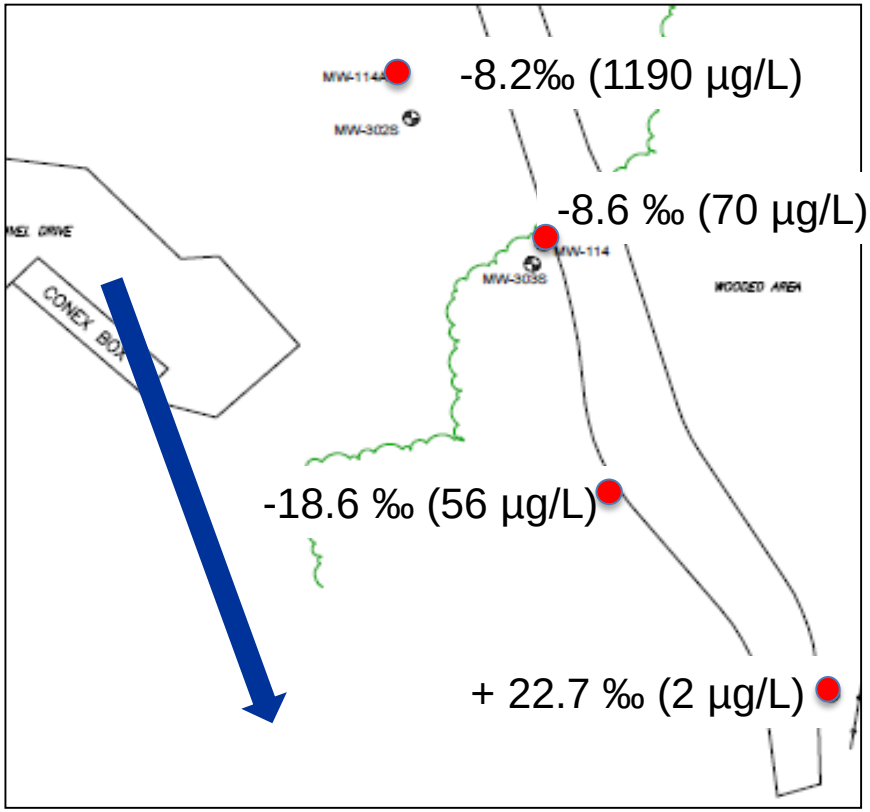


Figure Source: Ref 4

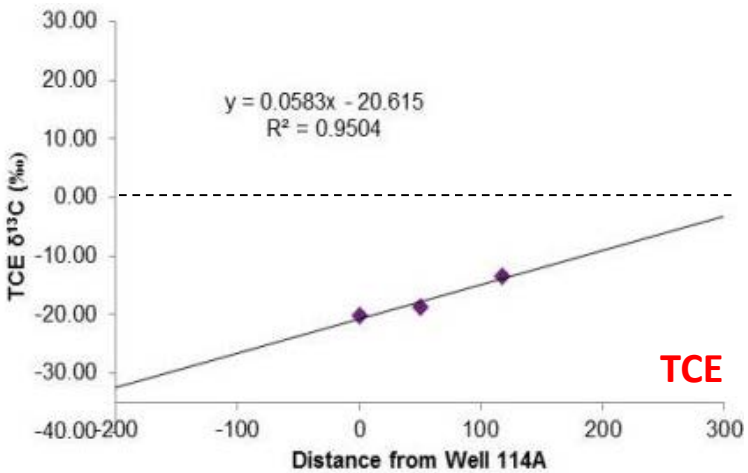
Rate of Contaminant Degradation

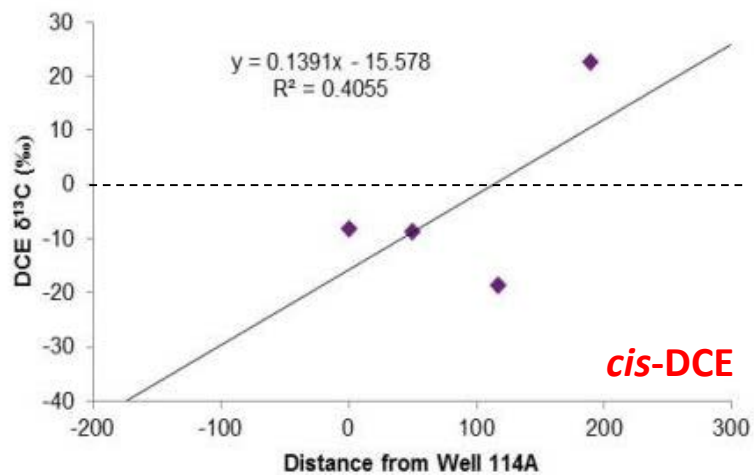
Assuming 1st order degradation rates (k), and constant seepage velocity (V_x) and enrichment factor (ε), then:

$$\delta^{13}C = \delta^{13}C_0 - \frac{k * \varepsilon}{V_x} * \mathrm{x}$$

Assuming $V_x \approx 2$ ft/day and $\varepsilon \approx -15$ ‰ →

TCE: $k \approx 8 \times 10^{-3}$ d^{-1} and $t_{1/2} \approx 80$ d - slope = 0.0583
DCE: $k \approx 2 \times 10^{-2}$ d^{-1} and $t_{1/2} \approx 30$ d - slope = 0.139

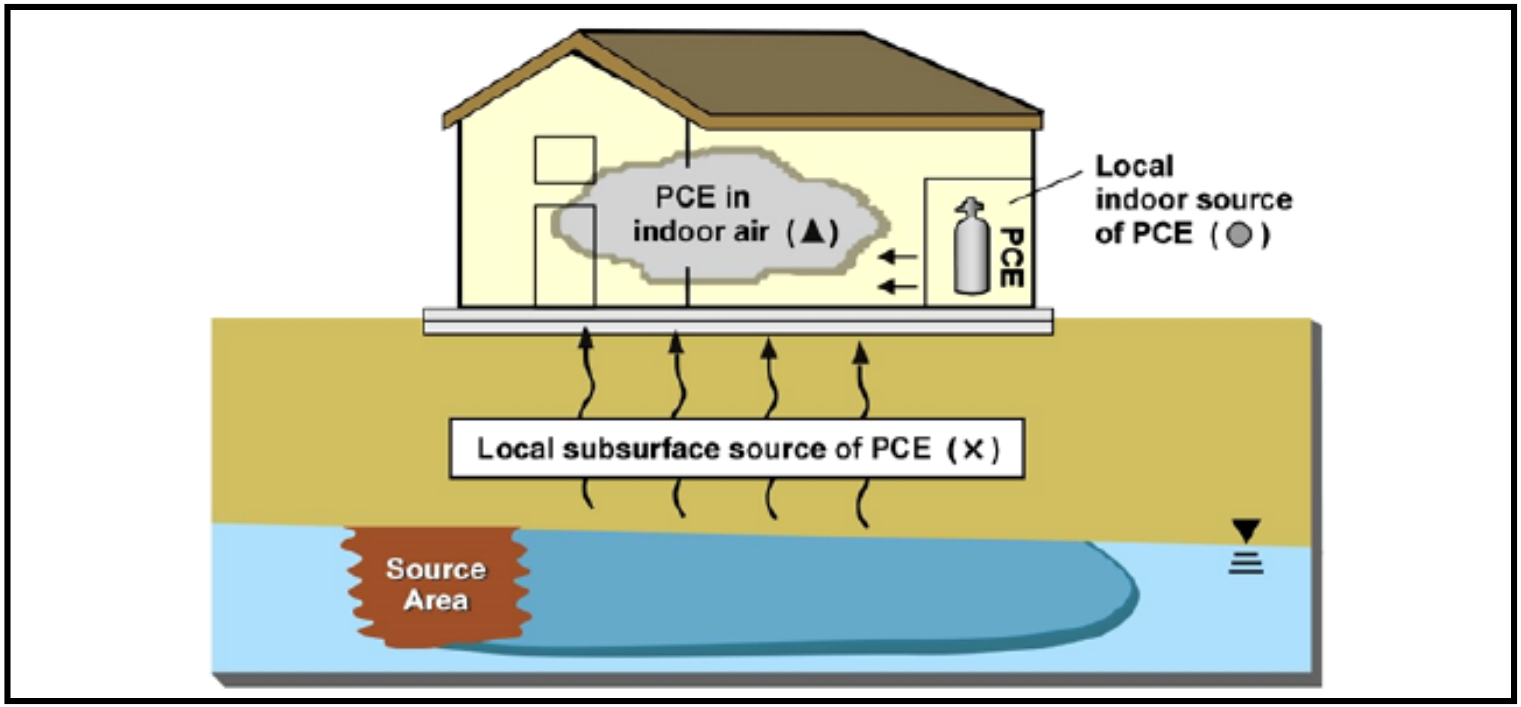




Is it My Contaminant Anyway?

Vapor Intrusion

Distinguishing "Indoor Source" from "Groundwater Source"



Are there still CVOCs in consumer products?

Is it my CVOC Anyway?

Vapor Intrusion

Gun Cleaner: $19.95
>90% TCE

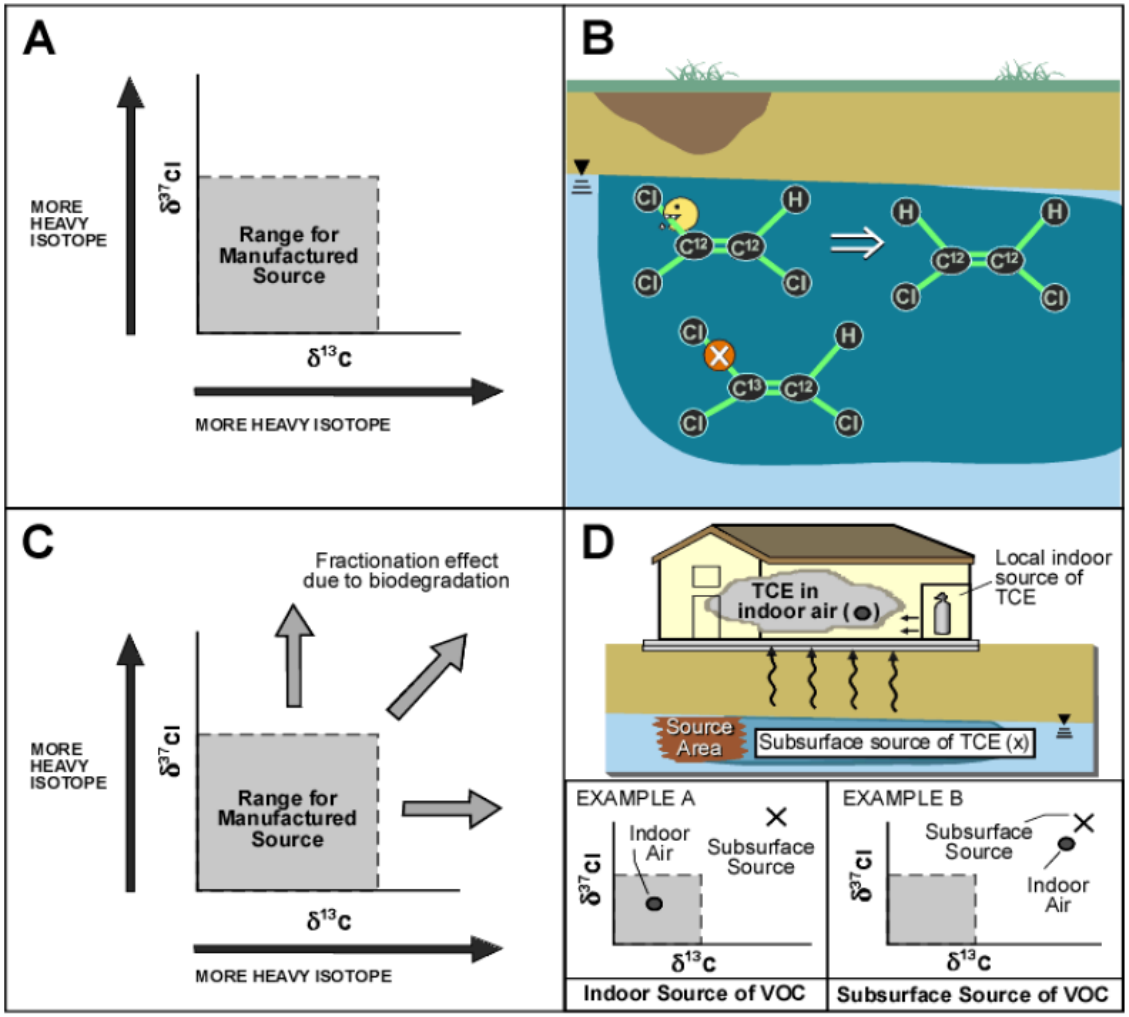
Pepper Spray: $3.99
>90% TCE

Hobby Glue: $4.95
>90% PCE

Plastic Ornament: $4.95
1,2-DCA

Key point: Chlorinated VOCs are legal and are still used in a wide variety of consumer products currently available for purchase

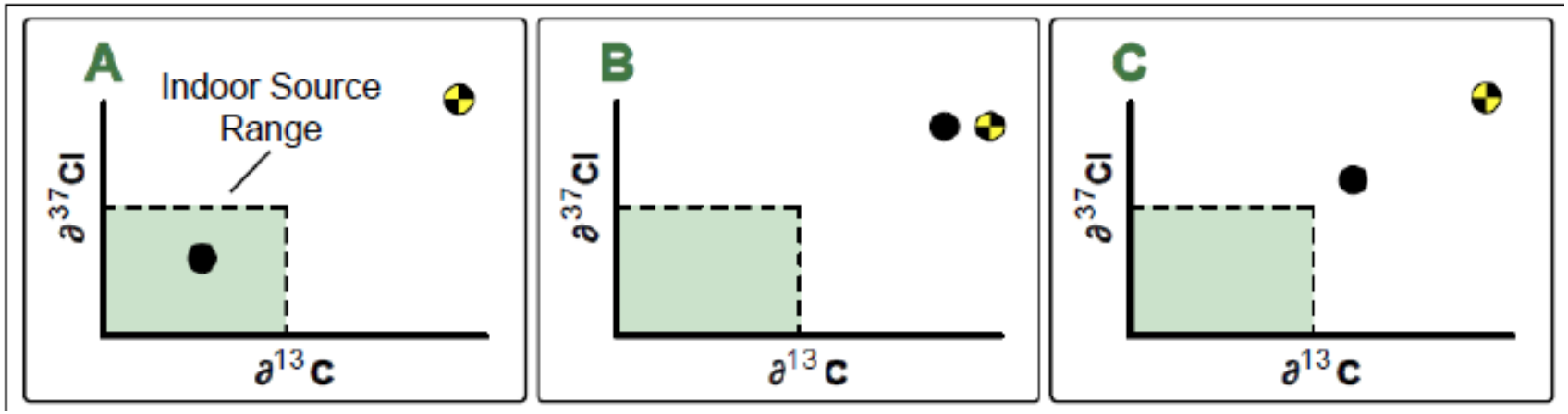
Conceptual Model: Vapor Intrusion vs. Indoor Source?



Conceptual Model: Vapor Intrusion vs. Indoor Source?

Scenarios:

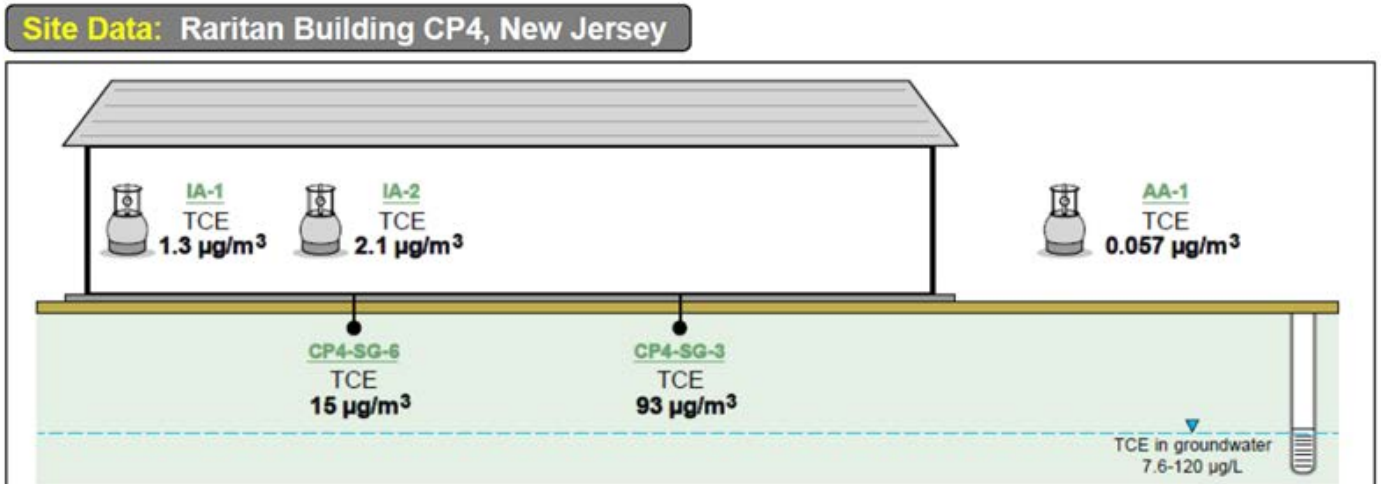
Indoor source | Vapor intrusion source | Mixed sources

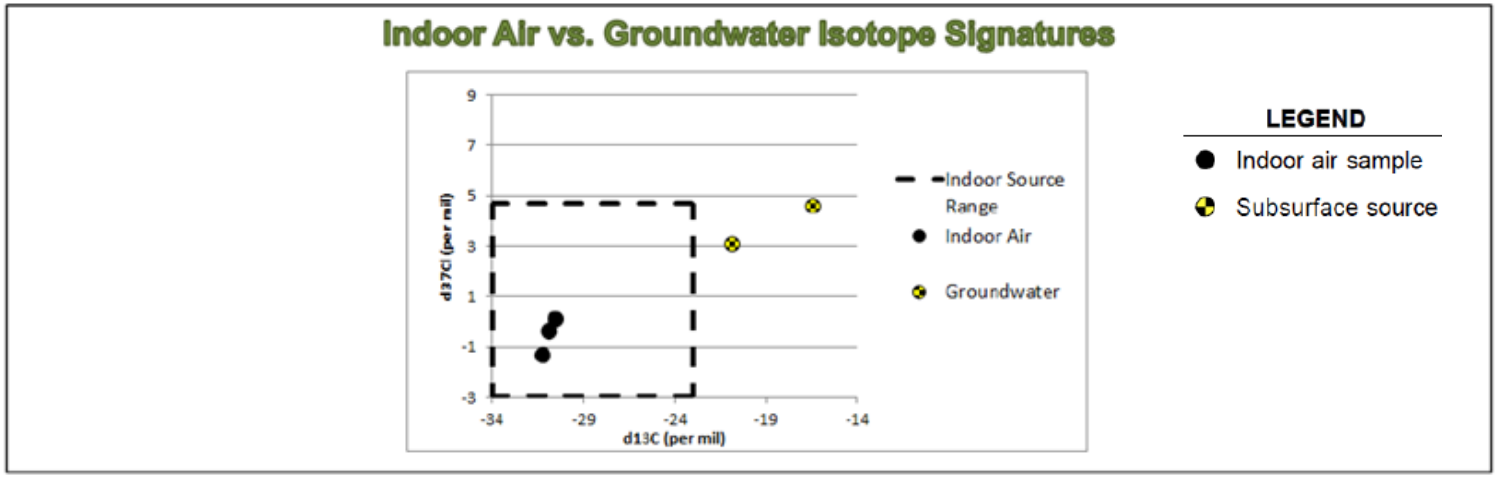


LEGEND

- Indoor air sample
- Subsurface source

Vapor Intrusion Forensics Example





Q&A Session 1

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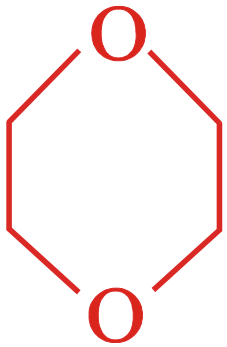
Other Contaminants of Concern

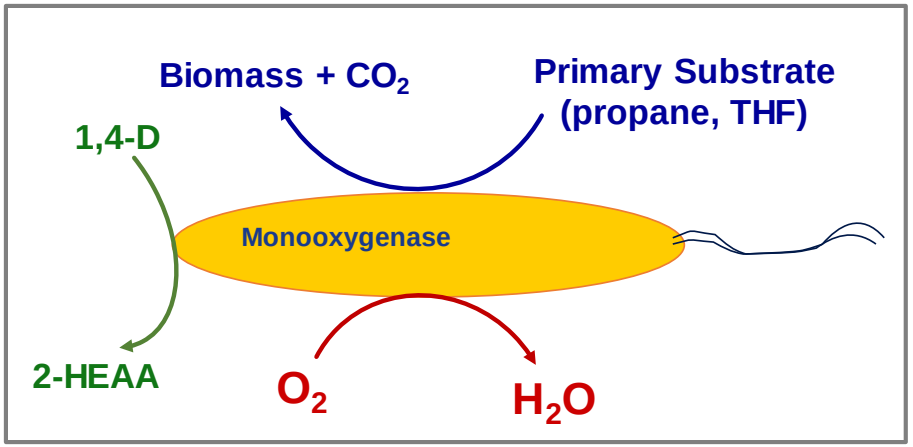
- CSIA applications
  - 1,4-Dioxane
  - 1,2-Dibromoethane (EDB)
  - Perchlorate
- Summary

1,4-Dioxane Background

- Characteristics
  - Cyclic ether; stabilizer in 1,1,1-TCA
  - High miscibility in water
  - Low Henry's Law coefficient
  - Low partitioning coefficient: K_{oc}= 1.23
  - ppb concentrations typical
  - Probable human carcinogen



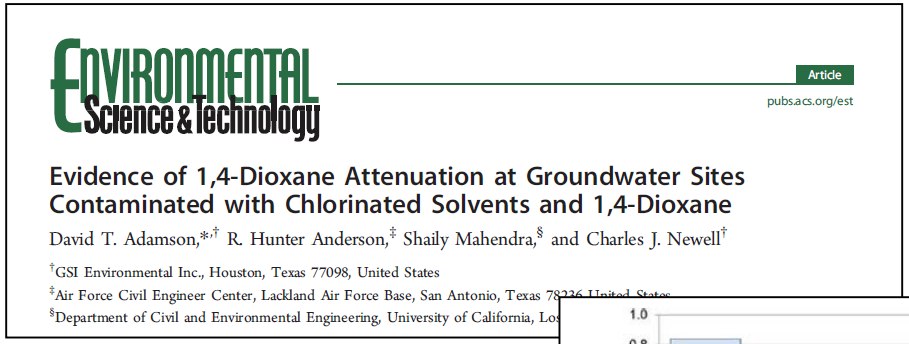
- Biodegradation
  - Growth-linked aerobic metabolism (e.g., strain CB1190)
  - Co-metabolic degradation with propane, ethane, tetrahydrofuran, others



1,4-Dioxane

Evidence for Natural Attenuation

- Is this truly degradation? How can I clearly document this?
- Cometabolic – linked to ethane, ethene or other cometabolic substrate?
- Is it growth-linked (e.g., CB1190 type organism)?



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Evidence of 1,4-Dioxane Attenuation at Groundwater Sites Contaminated with Chlorinated Solvents and 1,4-Dioxane

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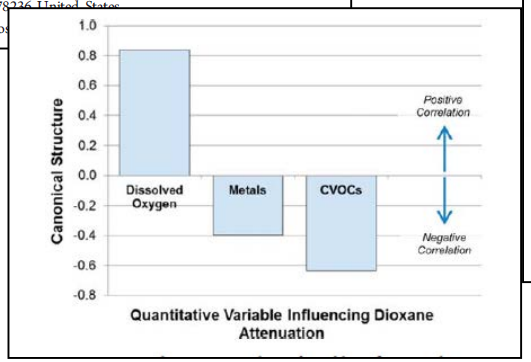


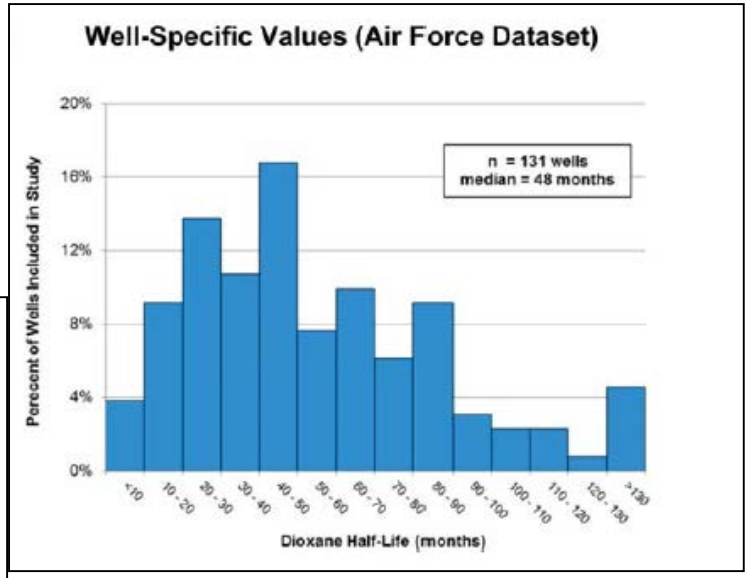


Figure Source: Ref 5

CSIA for 1,4-Dioxane

Dual Isotope Approach

- New method: Can be applied at low µg/L 1,4-dioxane
- Very limited fractionation data to date
  - Relatively small C fractionation (ε = - 1.8 to -5‰)
  - Large H fractionation - (ε = - 20 to - 150 ‰)
- Dual Isotope analysis should be useful for field evaluation
- Commercially available

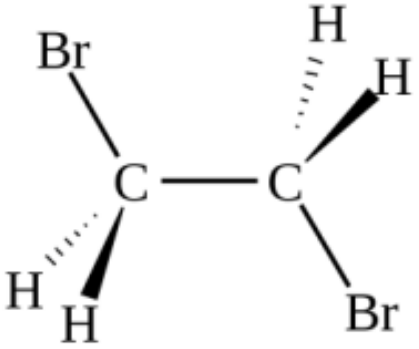
Table 1. Summary of Carbon and Hydrogen Isotopic Enrichment Factors and Apparent Kinetic Isotope Effects during 1,4-Dioxane Degradation in Microcosms

strain	growth substrate	ε_C (‰)	$AKIE_C$	ε_H (‰)	$AKIE_H$	$\Delta\delta^2H/\Delta\delta^{13}C$
R. rhodochrous ATCC 21198	propane	−2.7 ± 0.3	1.01	−21 ± 2	1.2	7.5 ± 1.1
R. rhodochrous ATCC 21198	isobutane	−2.5 ± 0.3	1.01	−28 ± 6	1.3	10.9 ± 2.2
P. tetrahydrofuranoxidans K1	THF	−4.7 ± 0.9	1.02	−147 ± 22	>10	37.2 ± 2.6

SERDP ER-2535 *Figure Source: Ref 6*

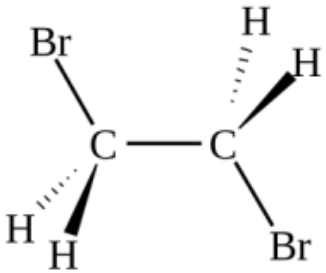
1,2-Dibromoethane (EDB) Background

- EDB is more “forgotten” than “emerging”
  - Fumigant (ended 1980s)
  - Lead scavenger in leaded fuels until 1980s (still in Avgas)
  - Suspected human carcinogen w/ USEPA MCL of 0.05 µg/L



1,2-Dibromoethane (EDB) Background

- EDB occurs widely in groundwater above MCL
  - Not detected to MCL by EPA 8260
- Biodegradable
  - Anaerobic (dehalogenation)
  - Aerobic (cometabolic)
- Abiotically degradable
  - Hydrolysis
  - Reduction with sulfide minerals



Occurrence of EDB in Groundwater (2004-2005)

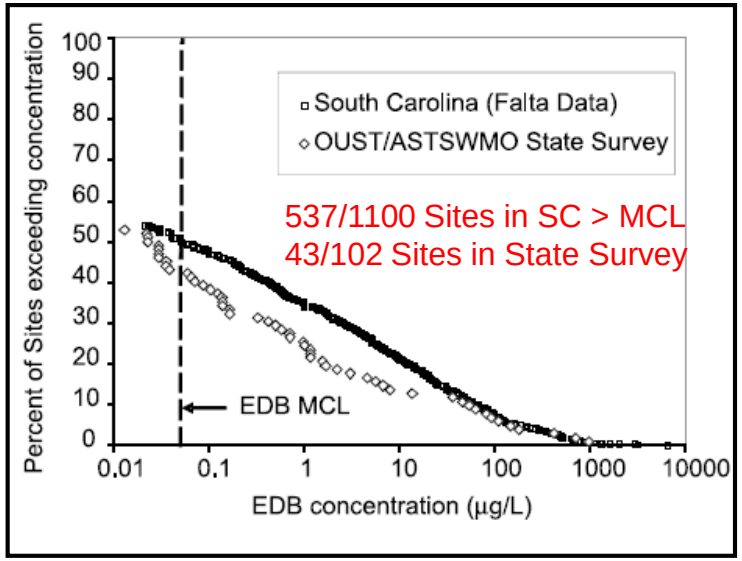
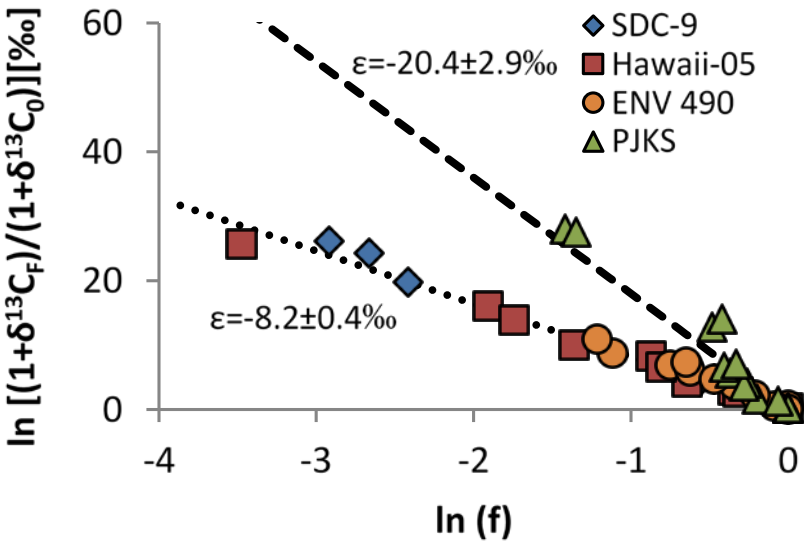


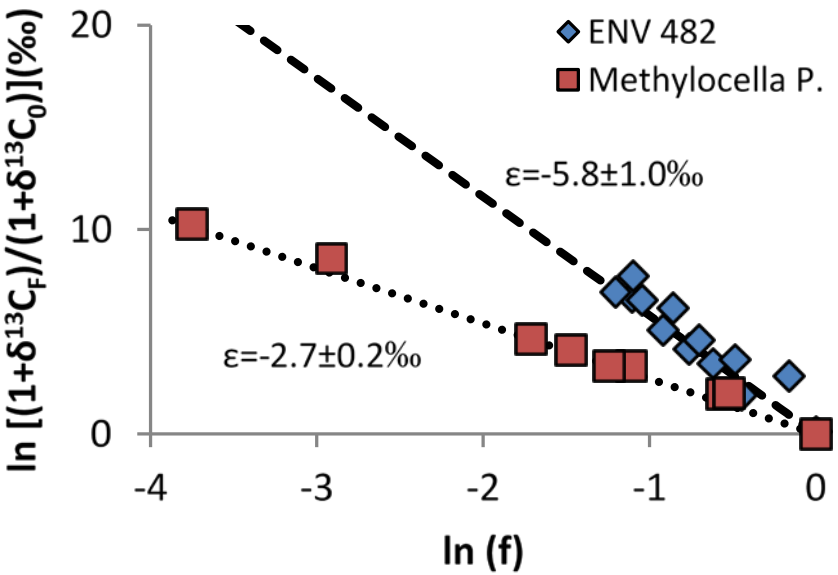
Figure Source: Ref 1

C Isotope Fractionation of EDB: Biodegradation

Anaerobic Biodegradation



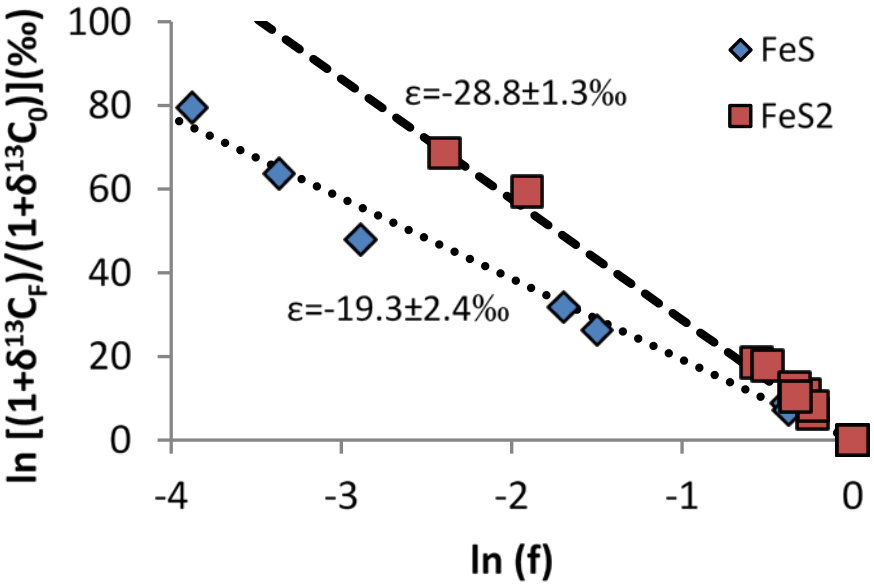
Aerobic Biodegradation



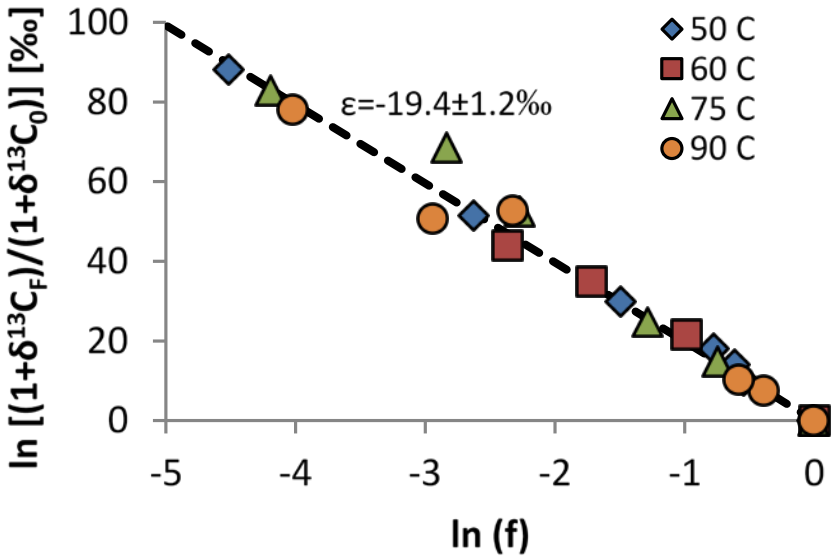
ESTCP ER-201331 *Figure Source: Ref 7*

C Isotope Fractionation of EDB: Abiotic Degradation

Iron Minerals



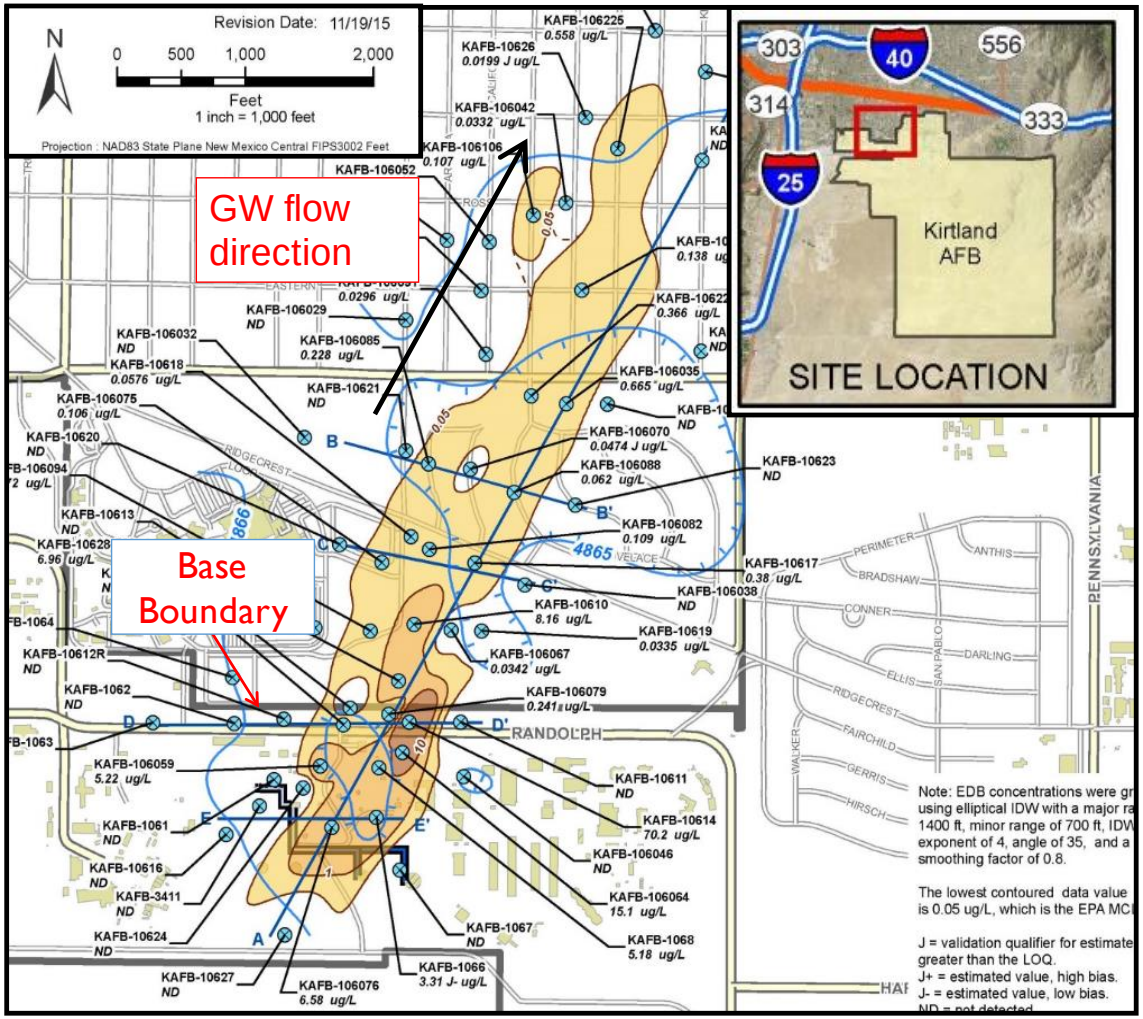
Hydrolysis



ESTCP ER-201331 *Figure Source: Ref 7*

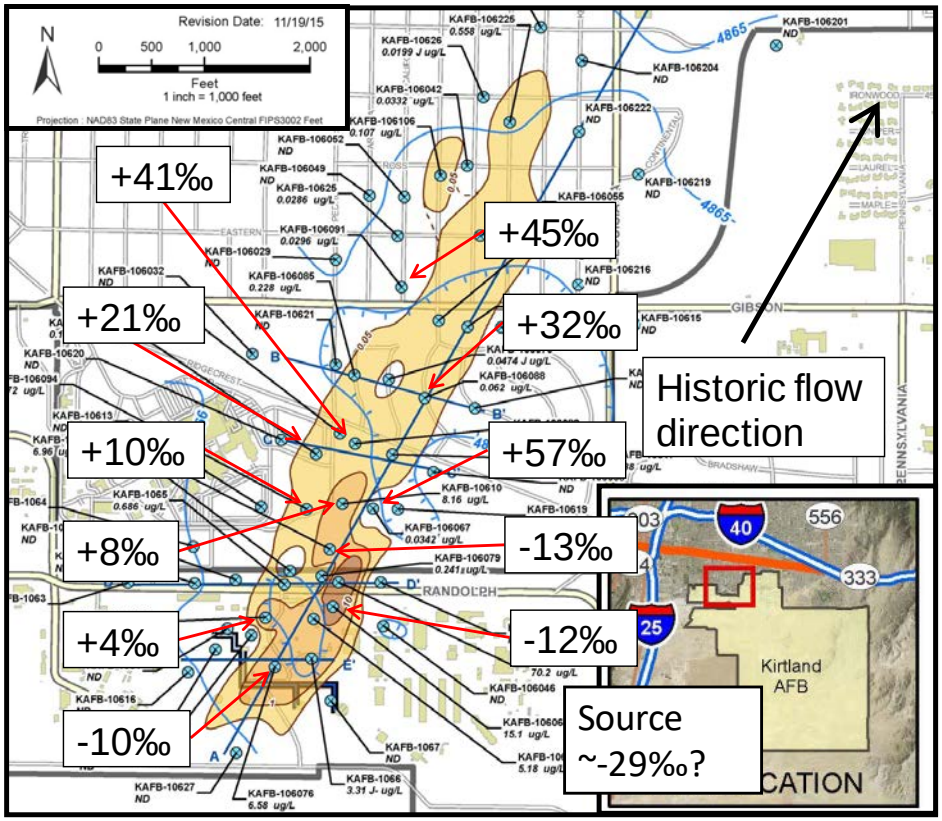
EDB at Former Kirtland AFB

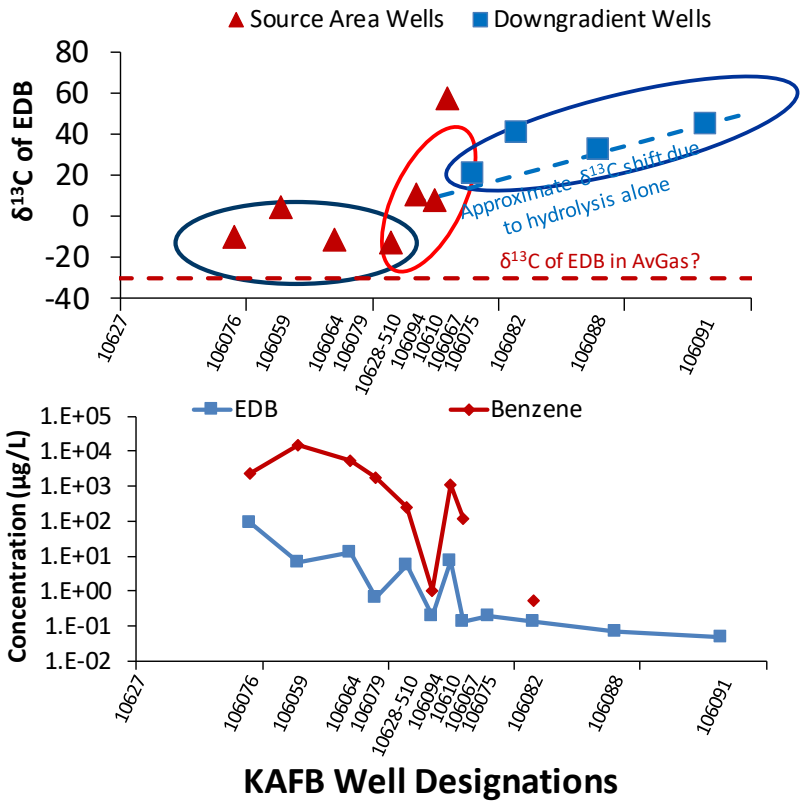
- Aviation gas/jet bulk fuel leak (1953 – 1999)
- AVGAS (EDB), JP-4, JP-8
- ~500 ft to water
- Pump and treat
- Amount of EDB attenuation?
- CSIA performed (ESTCP)



EDB at Former Kirtland AFB

- CSIA data clearly indicate attenuation of EDB at KAFB



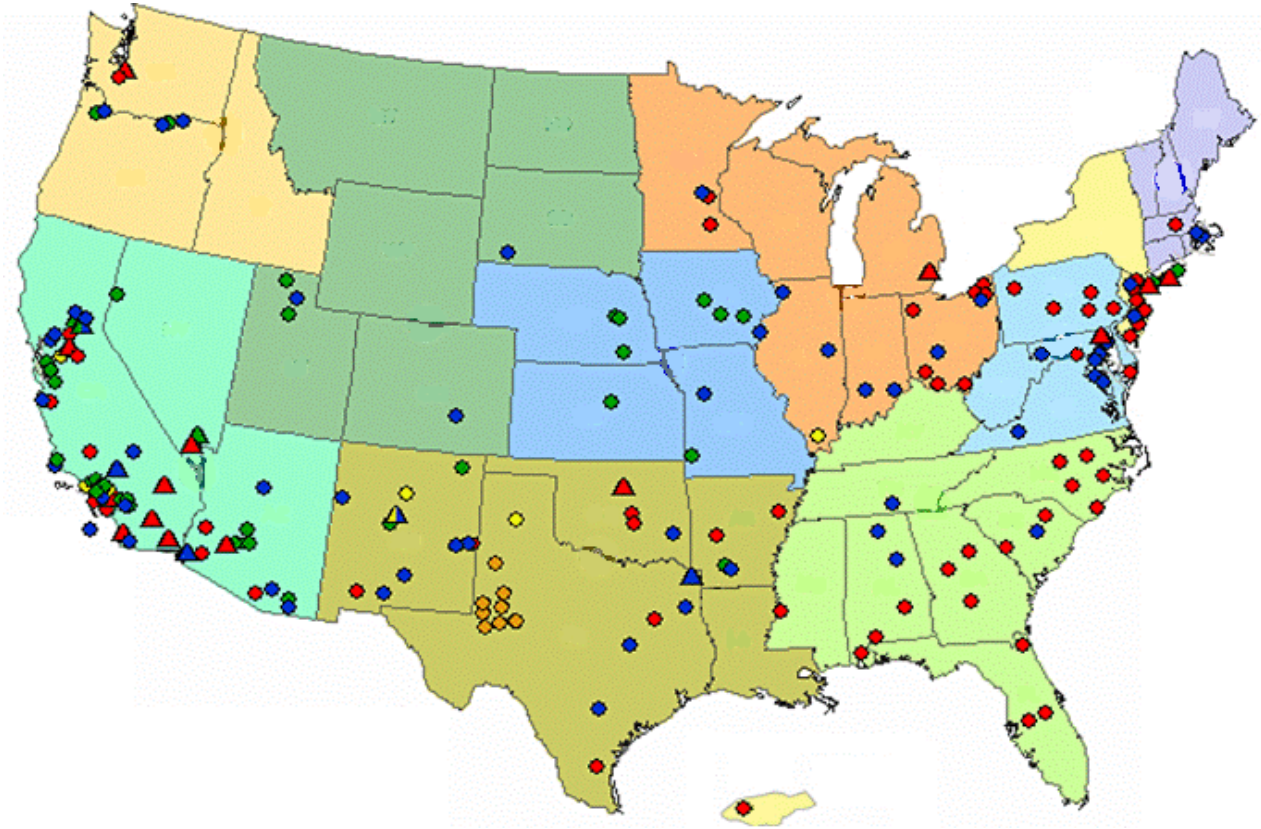


Perchlorate Historical Background

- Primary military and NASA application as a solid oxidant
- Expected limited sources of contamination
  - Rocket testing
  - Hog-out
  - Manufacturing
  - Training areas
  - OB/OD areas
  - Few commercial sites
- Readily biodegradable under anaerobic conditions

Photo Source: https://www.goodfreephotos.com

Perchlorate: USEPA Testing Beginning 2001



Puerto Rico

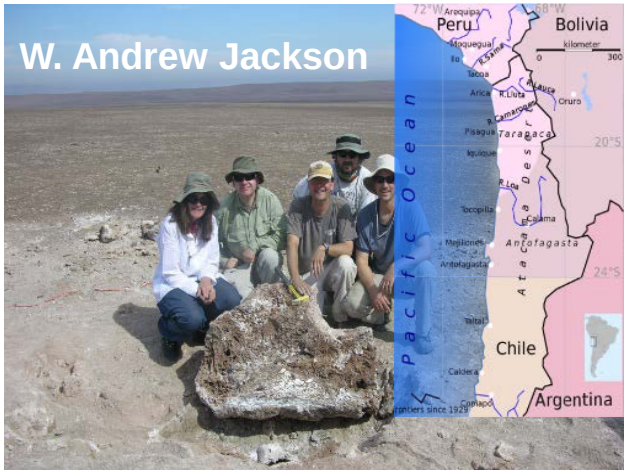
- Lower Detection Limits - 1998
- UCMR Testing - 2001

Legend

- One site
- Multiple sites
- DOD
- DOE, NASA, DOI
- Privately-owned
- UCMR detections
- University study detections

Other Sources of Perchlorate?

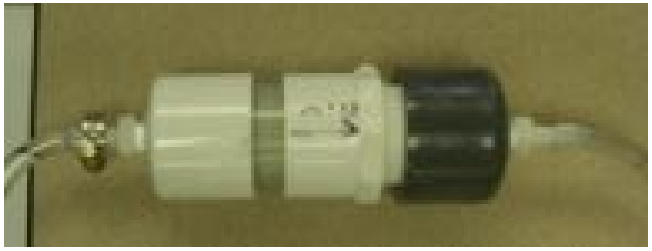
- Synthetic
  - Rockets, fireworks, flares, perchloric acid, chlorate, bleach 11,000 metric tons/yr of ClO_4^- from 1951 – 1997[1]
- Chilean (Atacama)
  - Once widely used as a N fertilizer
  - Conservatively, 0.2% ClO_4^- in processed Chilean nitrate[1,2]
  - 750 metric tons/yr of ClO_4^- from 1930 – 1993[1]
- Indigenous natural
  - Atmospheric formation
  - Accumulates in arid environments
  - Mobilized from vadose zone with irrigation
  - 130 metric tons/yr[1]



Source: Ref 8

Can CSIA Distinguish Natural from Synthetic Perchlorate?

- Methods have been developed to analyze for O and Cl isotopes in ClO_4^-
- Other lines of evidence
  - Groundwater dating
  - Co-contaminants
  - Other geochemical data



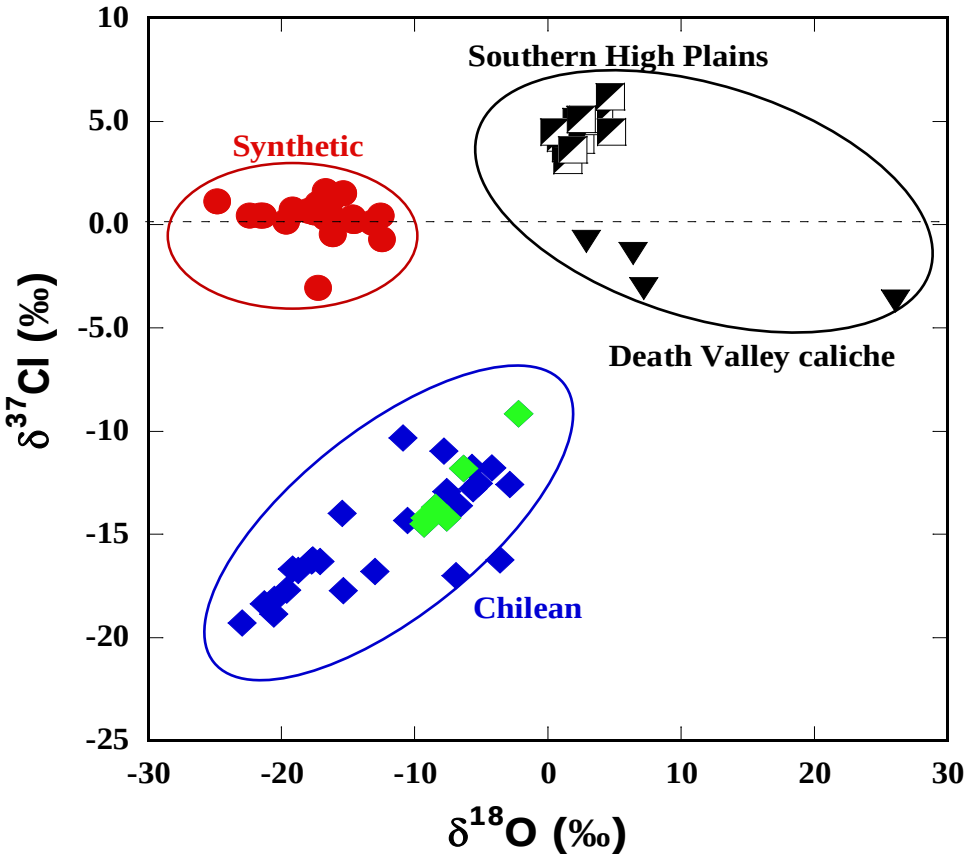
Stable Isotopes of O and Cl are measured in Perchlorate

Hydrogen	^{1}H, ^{2}H
Oxygen	^{16}O, ^{17}O, ^{18}O
Carbon	^{12}C, ^{13}C
Chlorine*	^{35}Cl, ^{37}Cl
Nitrogen	^{14}N, ^{15}N
Sulfur	^{32}S, ^{34}S

**Also measuring ^{36}Cl (radioactive)*

Perchlorate Source Isotope Characteristics: Dual Isotope Plots

Dual Isotope Plot Comparing $\delta^{37}Cl$ and $\delta^{18}O$ for Perchlorate Types.

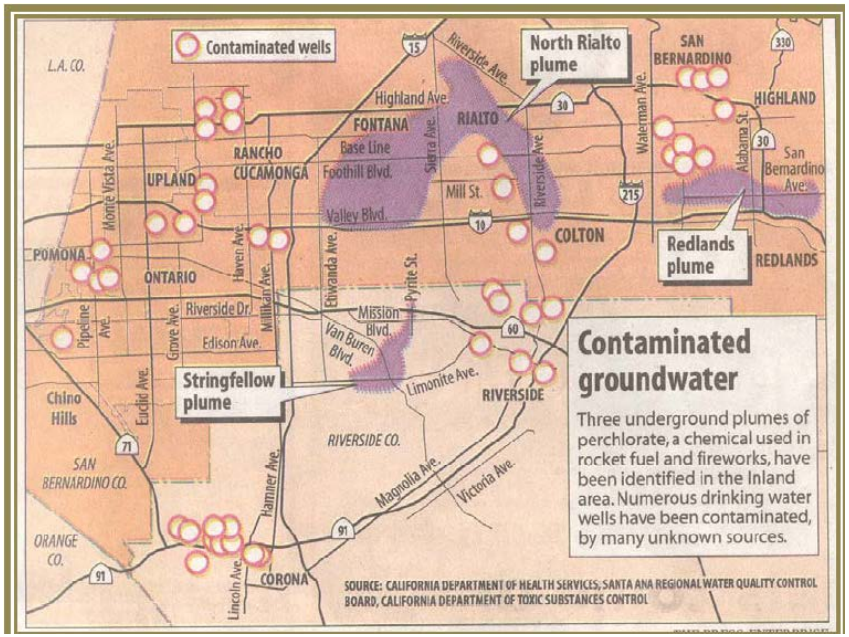


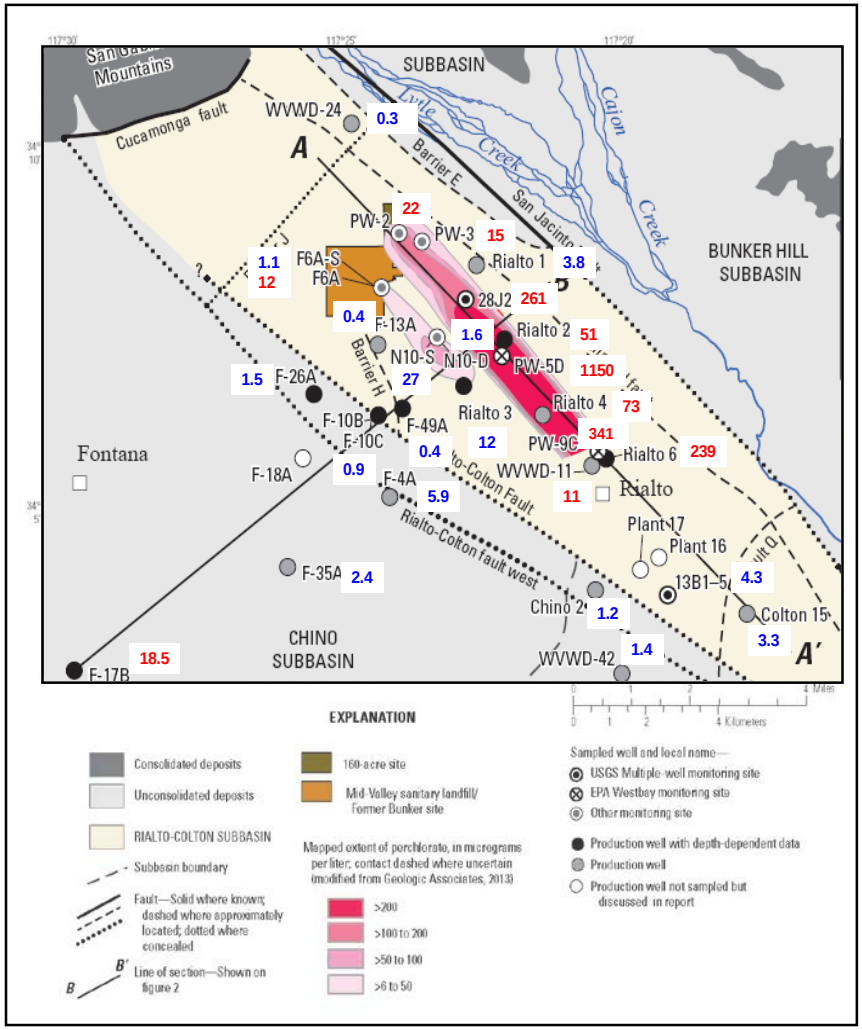
SERDP ER-1435, ESTCP ER-200509, ER-200942

Perchlorate Source Identification

Rialto, CA

- Two synthetic plumes
  - Mid-valley landfill
  - 160 acre site
- What are the main sources of perchlorate outside and downgradient of the mapped plumes?
- CSIA conducted throughout region



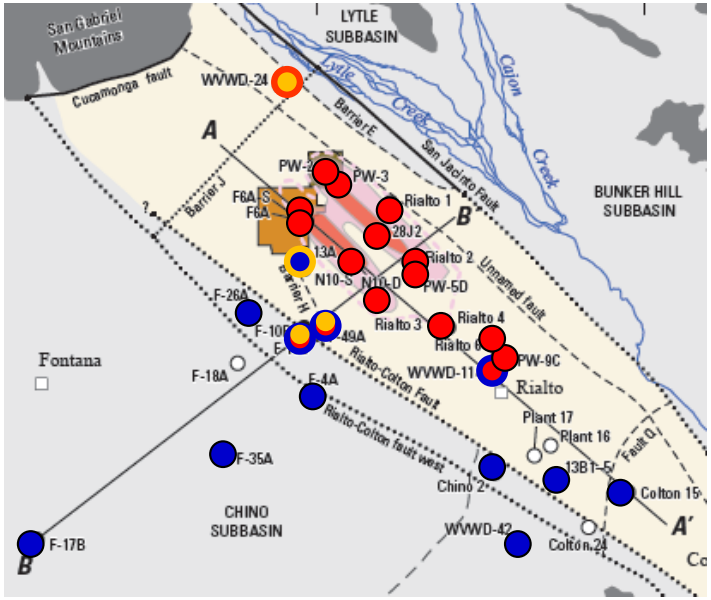


ESTCP ER-200942

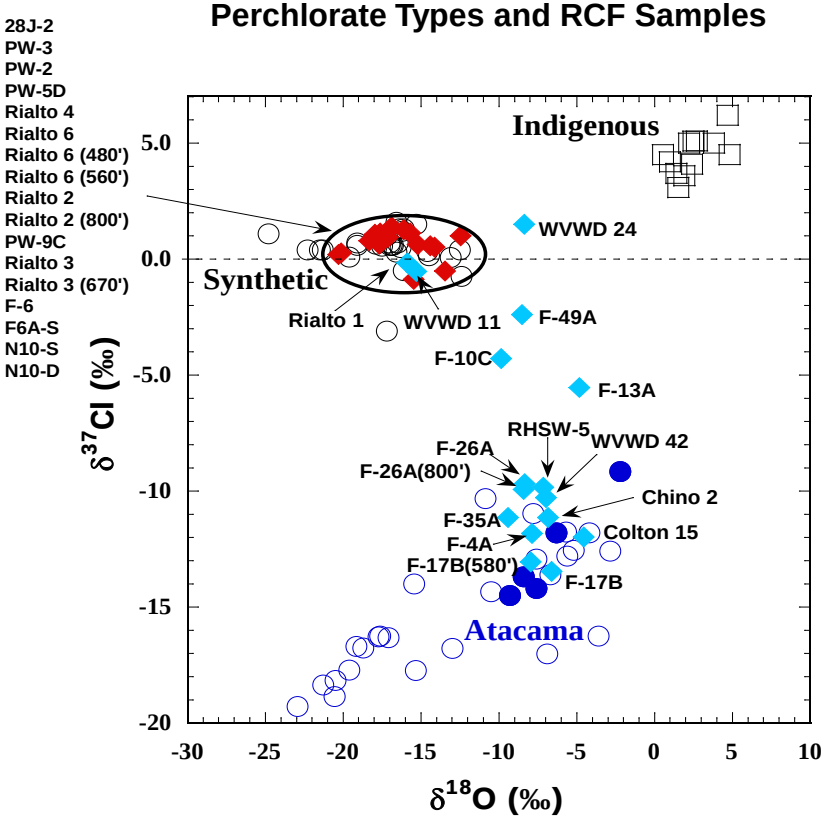
Rialto Stable Isotope Data

- Synthetic and Chilean natural perchlorate clearly distinguished by CSIA

Map of Well Locations with Interpretation of Dominant Perchlorate Source(s).



Dual Isotope Plot Comparing $\delta^{37}Cl$ and $\delta^{18}O$ for Perchlorate Types and RCF Samples



Summary: CSIA Benefits

- Provides direct evidence of biological degradation or abiotic attenuation
- Can be useful for generating attenuation rates and discerning mechanisms
- Provides information to identify sources of specific contaminants
- Commercially available for several compounds, including CVOCs and 1,4-dioxane

Summary: CSIA Limitations

- Need relevant laboratory fractionation factors and degrading 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

For additional information, please visit

https://www.serdp-estcp.org/Program-Areas/Environmental-Restoration/Contaminants-on-Ranges/Identifying-and-Evaluating-Sources/ER18-5022

Speaker Contact Information

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The next webinar is on March 26, 2020

Long-Term Ecological Studies: Evaluating Responses to Ecosystem Restoration and Optimizing Recovery of Plant Communities

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