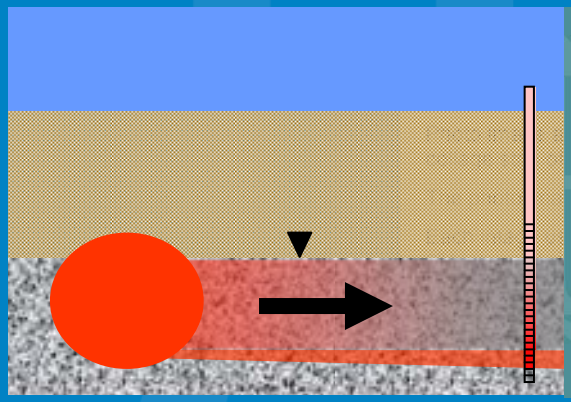
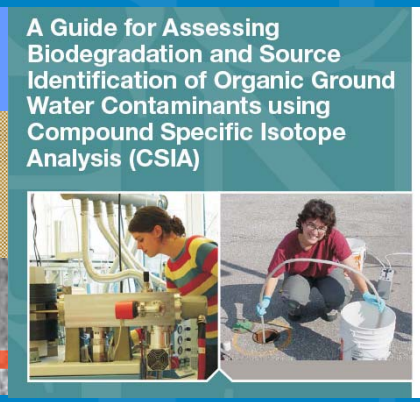
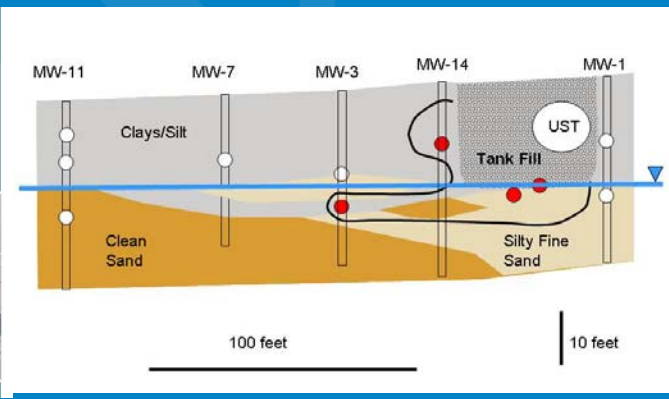
United States Environmental Protection Agency

What's New in the Site Characterization Tool Box: Molecular Biological Tools to Identify Microorganisms that Degrade Contaminants and Contaminant-Specific Isotope Analysis to Identify Sources and Document Degradation

John T. Wilson and Ann Keeley, EPA/ORD/Ada







May 5, 2011

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

EPA
United States
Environmental Protection
Agency

Biodegradation plays a Prominent Role in Fate and Transport of Contaminants

Although the potential for biodegradation has been well documented in the literature, there is a significant burden of proof and lag time associated with achieving the acceptance of natural and /or enhanced bioremediation by regulatory and public stakeholders.

United States Environmental Protection Agency

Molecular Biological Tools (MBTs)

Tools that target "biomarkers":

<u>Specific</u> nucleic acid sequences, peptides, proteins, or lipids

Outcome is to provide information about:

- **Types of microorganisms present**
- **Processes relevant to the assessment and/or remediation of natural or engineered systems**
- **microbial activity *in situ***

United States Environmental Protection Agency

Current State of Field Application of MBTs

Site Characterization Questions Prior to Selection

- **What is the potential for degradation based on the presence/absence of genes or microorganisms of interest?**
- **What is the link between the presence of target genes or microorganisms and the activity of interest?**
- **Is the spatial and temporal distribution of organisms appropriate to meet goals?**

General Description of Genomic & Molecular Tools

General Questions

Are the key microbes present?
Are their genes being expressed?
What other groups of microbes present?
What is the microbial density?

Tool Selection

Use DNA & RNA Based Tools for fingerprinting (Polymerase Chain Reaction [PCR])

General Description of Genomic & Molecular Tools

General Questions

How active is the microbial community?

Tool Selection

Use Protein Based Tools
(enzyme probes)

General Description of Genomic & Molecular Tools

General Questions

What groups of microbes are present?
What is the total biomass?

Tool Selection

Use Lipid Based Tools
(phospholipid fatty acids)

General Description of Genomic & Molecular Tools

General Questions

Can the contaminants be biologically degraded at the site under conditions that pertain in the groundwater?

Tool Selection

Use Stable Isotope Based Tools
(Stable Isotope Probing)

General Description of Genomic & Molecular Tools

General Questions

Are the contaminants actually being biodegraded?

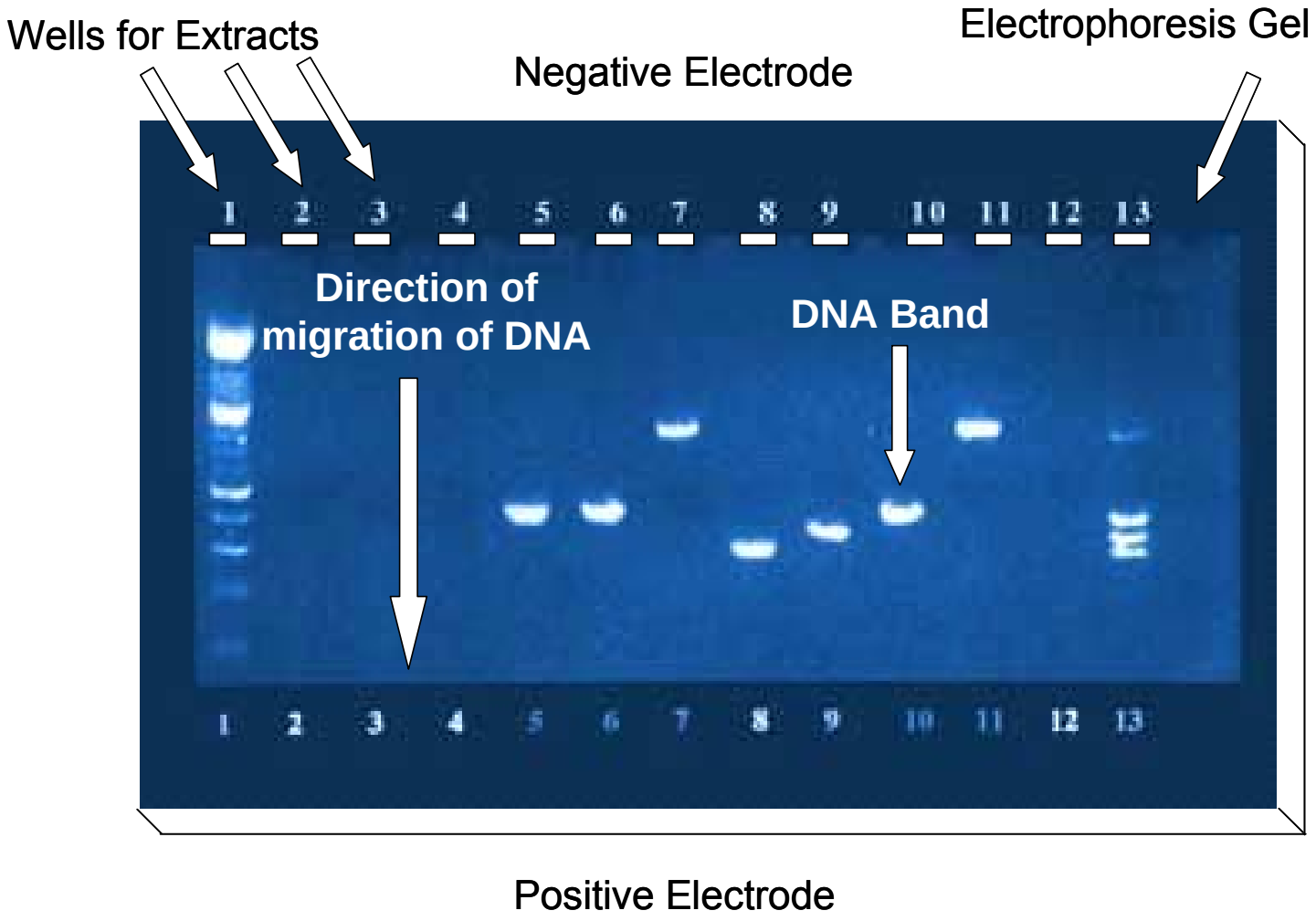
Tool Selection

Use Stable Isotope Based Tools
(Compound Specific Isotope Analysis)

EPA United States Environmental Protection Agency

PCR uses the polymerase chain reaction to copy DNA in a sample that binds with a short DNA primer that contains base sequences of the DNA being amplified. The copied DNA is copied, then the copies are copied, and so on, until there is enough DNA to measure.

The most common application copies the DNA for a component of the ribosome, the 16s rRNA gene.


Wells for Extracts
Negative Electrode
Electrophoresis Gel
1 2 3 4 5 6 7 8 9 10 11 12 13
Direction of migration of DNA
DNA Band
1 2 3 4 5 6 7 8 9 10 11 12 13
Positive Electrode

United States Environmental Protection Agency

Quantitative or Real time PCR uses primers with a fluorescent tag. As the primers are incorporated into DNA, this is detected in each PCR cycle by an increase in the fluorescence of the solution.

The original density of the organisms is related to the number of PCR cycles necessary to reach a predetermined fluorescence.

Quantitative RTm PCR (qPCR) is the Most Widely Used Genomic MBT in the Field

United States Environmental Protection Agency

Using DNA

- qPCR enables both detection and quantification of a specific sequence in a DNA sample (16S rRNA gene)

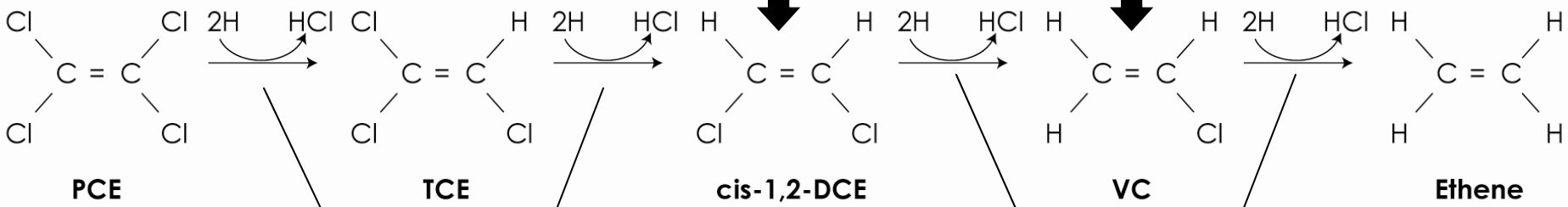
- It is offered as a commercial service by multiple laboratories to detect and quantify key genes of interest, especially for *Dehalococcoides spp*. (i.e., detection of “functional genes” such as reductive dehalogenase [(RDase) genes].

Using RNA

- The potential exists to detect an actual “activity” by using mRNA and expressed proteins from environmental sample.

Microbiology of Reductive Dechlorination of Chloroethenes

Can accumulate if requisite bacteria are not present



Dehalobacter
Dehalospirillum
Desulfitobacterium
Desulfuromonas
Dehalococcoides

some strains of
Dehalococcoides

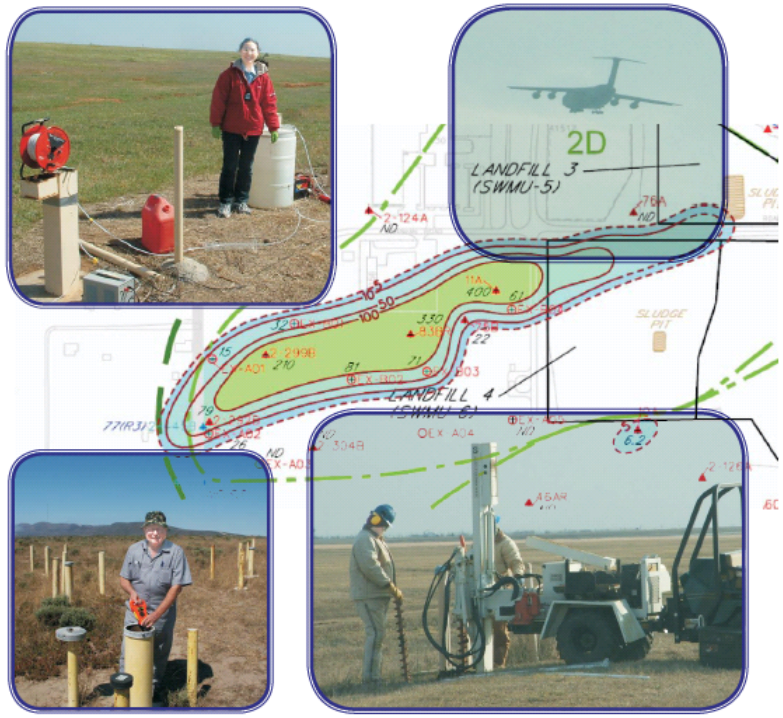
United States Environmental Protection Agency

qPCR: Key Organism Identification

- ***Dehalococcoides*** **is somewhat of an exception in bioremediation, where there is a strong link between the organism type (identification) and the activity (reductive dechlorination) because of the simple metabolic pathway.**

EPA United States Environmental Protection Agency

Evaluation of the Role of *Dehalococcoides* Organisms in the Natural Attenuation of Chlorinated Ethylenes in Ground Water

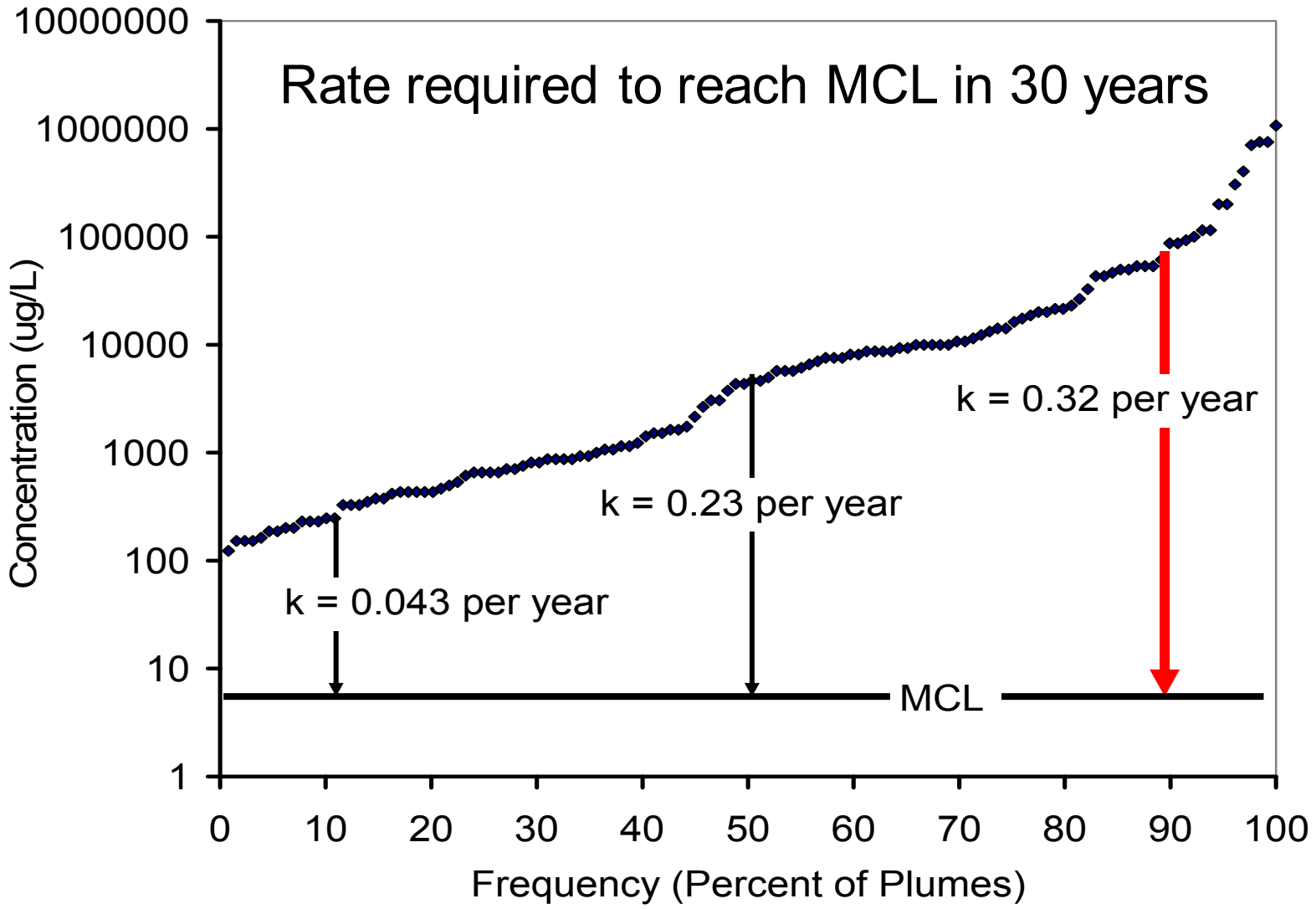


Evaluation of the Role of *Dehalococcoides* Organisms in the Natural Attenuation of Chlorinated Ethylenes in Ground Water. Xiaoxia Lu, Donald H. Kampbell and John T. Wilson. 2006.EPA/600/R-06/029

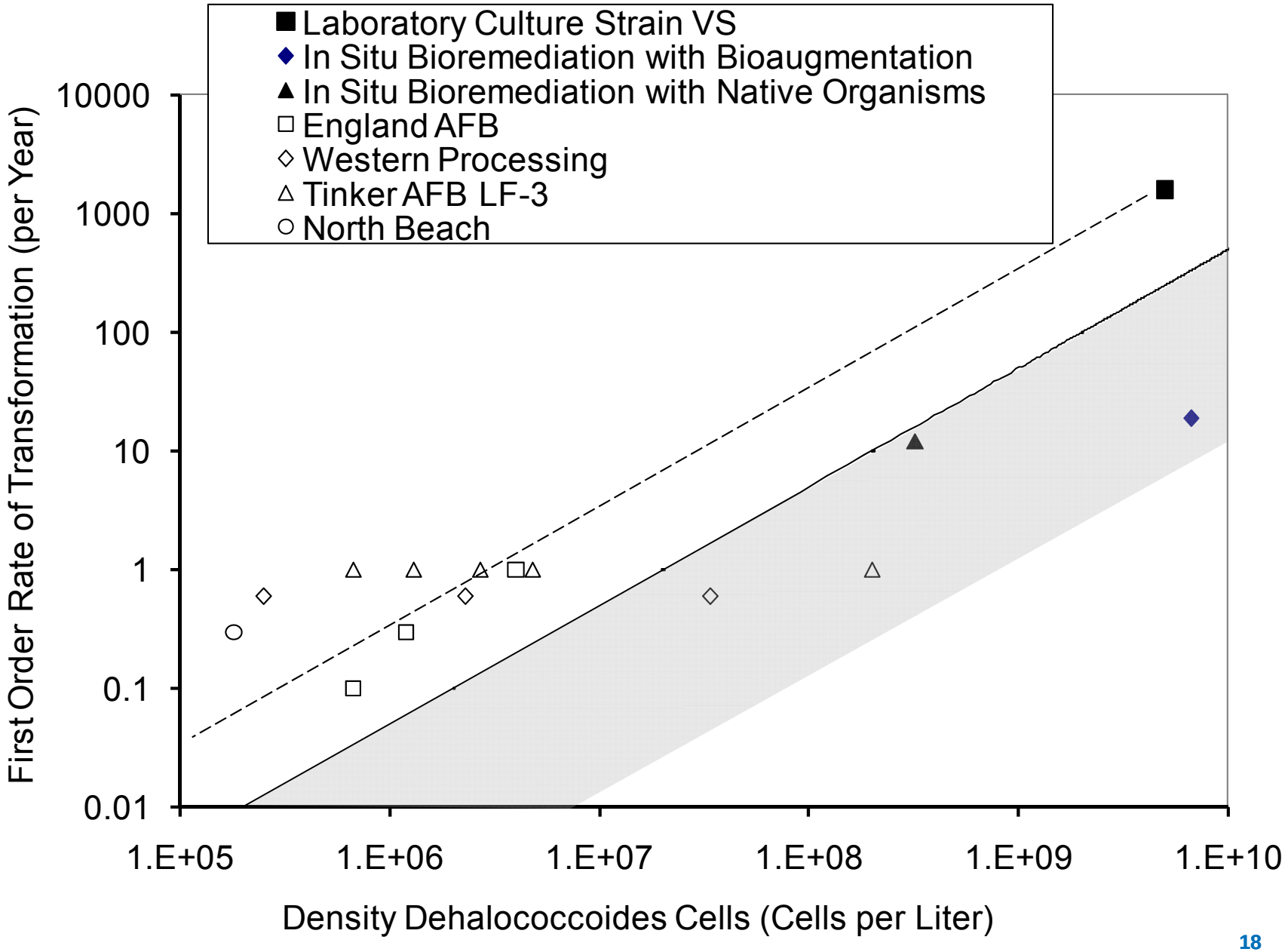
Relationship between *Dehalococcoides* DNA in Ground Water and Rates of Reductive Dechlorination at Field Scale. Xiaoxia Lu, John T. Wilson, and Donald H. Kampbell. 2006. *Water Research* 40(2006):3131-3140

United States Environmental Protection Agency

Compared the density of *Dehalococcides* cells in monitoring wells as determined by Direct PCR to the density as determined by Quantitative PCR, and to the rate of reductive dechlorination achieved at field scale.



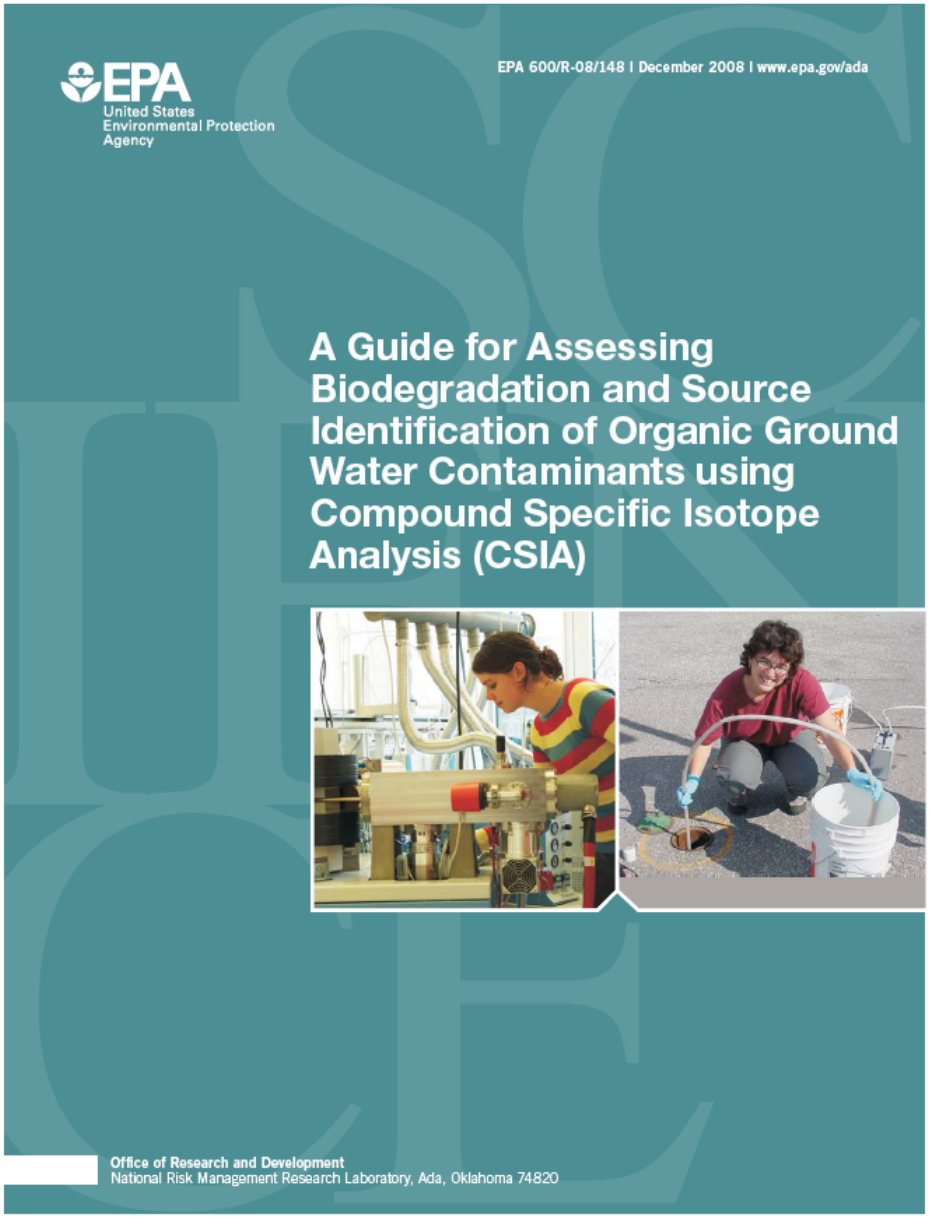
Evaluating chlorinated hydrocarbon plume behavior using historical case population analyses. *Bioremediation Journal* 4(4):311-335 (2000)


Laboratory Culture Strain VS
In Situ Bioremediation with Bioaugmentation
In Situ Bioremediation with Native Organisms
England AFB
Western Processing
Tinker AFB LF-3
North Beach
10000
1000
100
10
1
0.1
0.01
First Order Rate of Transformation (per Year)
1.E+05
1.E+06
1.E+07
1.E+08
1.E+09
1.E+10
Density Dehalococcoides Cells (Cells per Liter)

EPA
United States
Environmental Protection
Agency

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



United States Environmental Protection Agency

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

United States Environmental Protection Agency

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.

United States Environmental Protection Agency

$\delta^{13}C‰$

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.

EPA United States Environmental Protection Agency

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

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

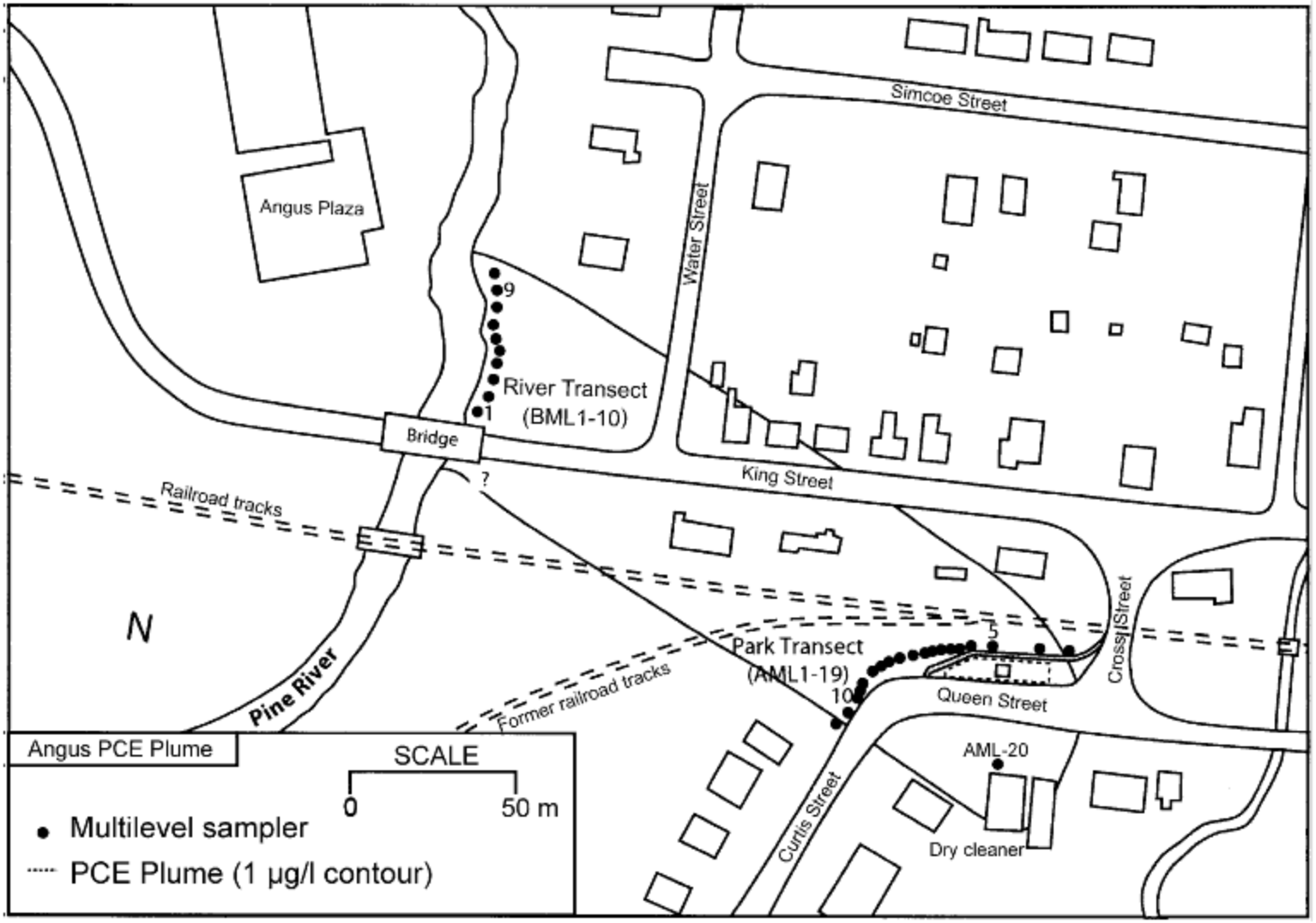
United States Environmental Protection Agency

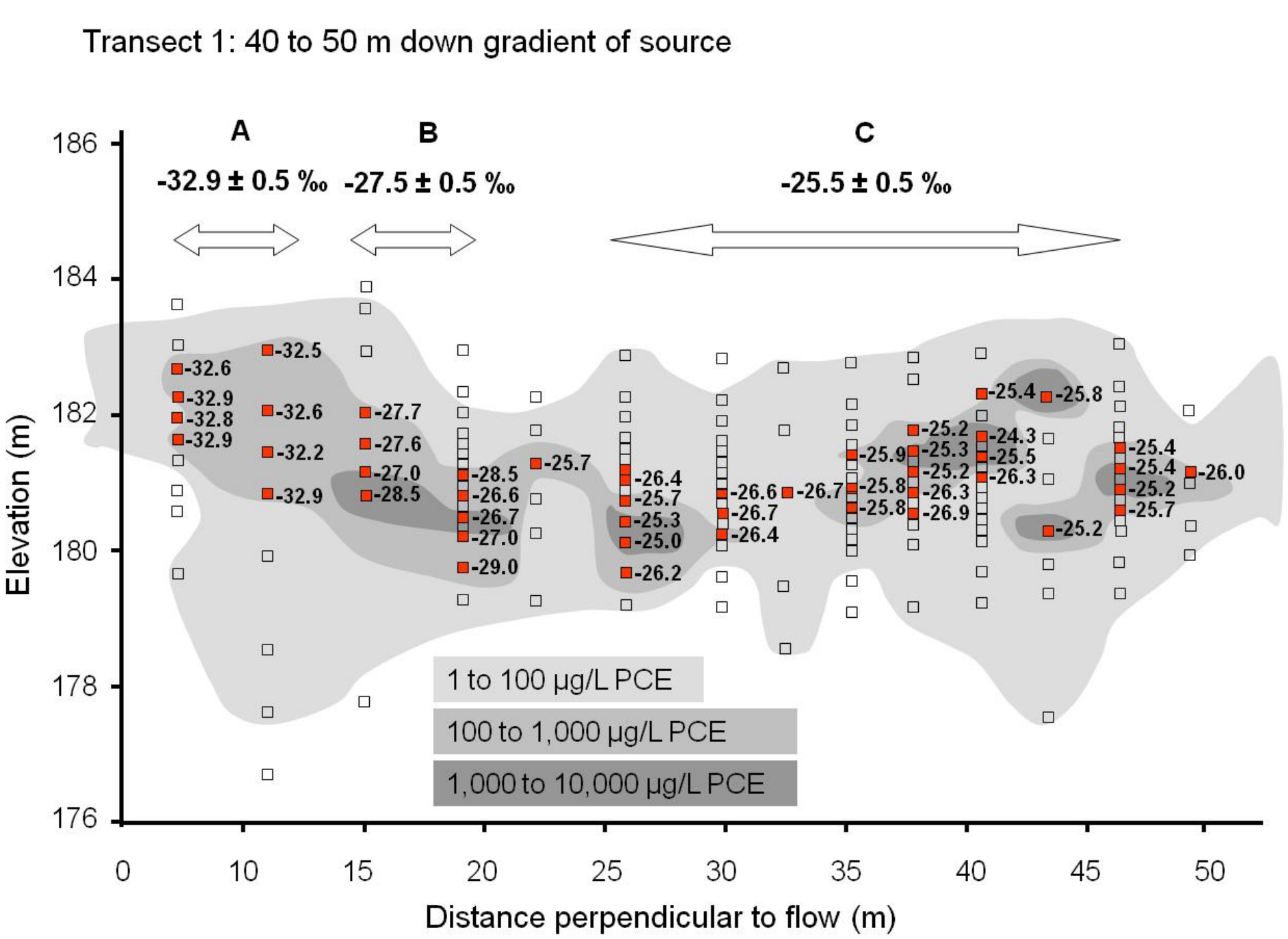
Can clearly resolve samples from each other if their $\delta^{13}C$ differ by more than 2‰.

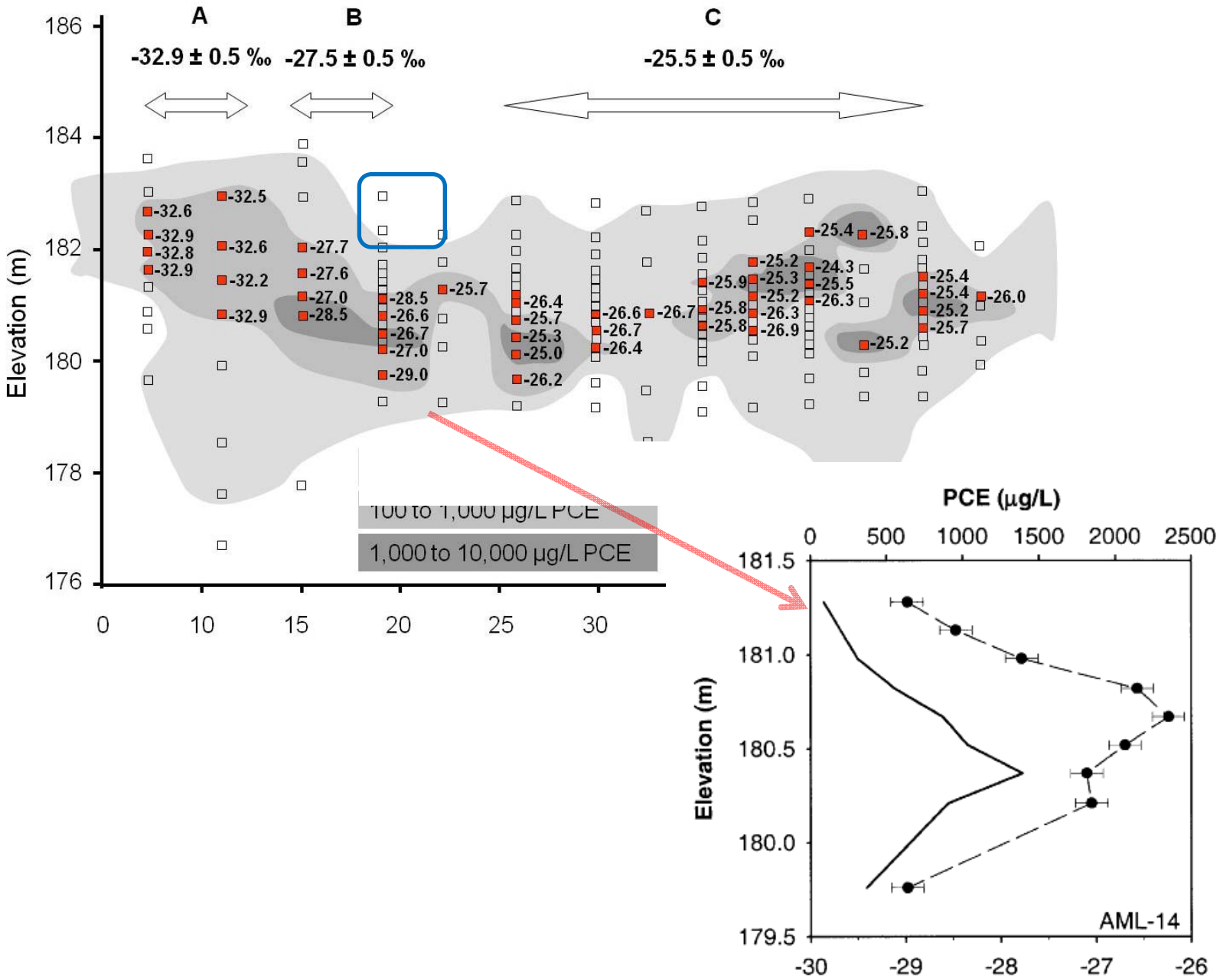
Can we use differences in isotopic ratios to track plumes, or to associate plumes with their sources?

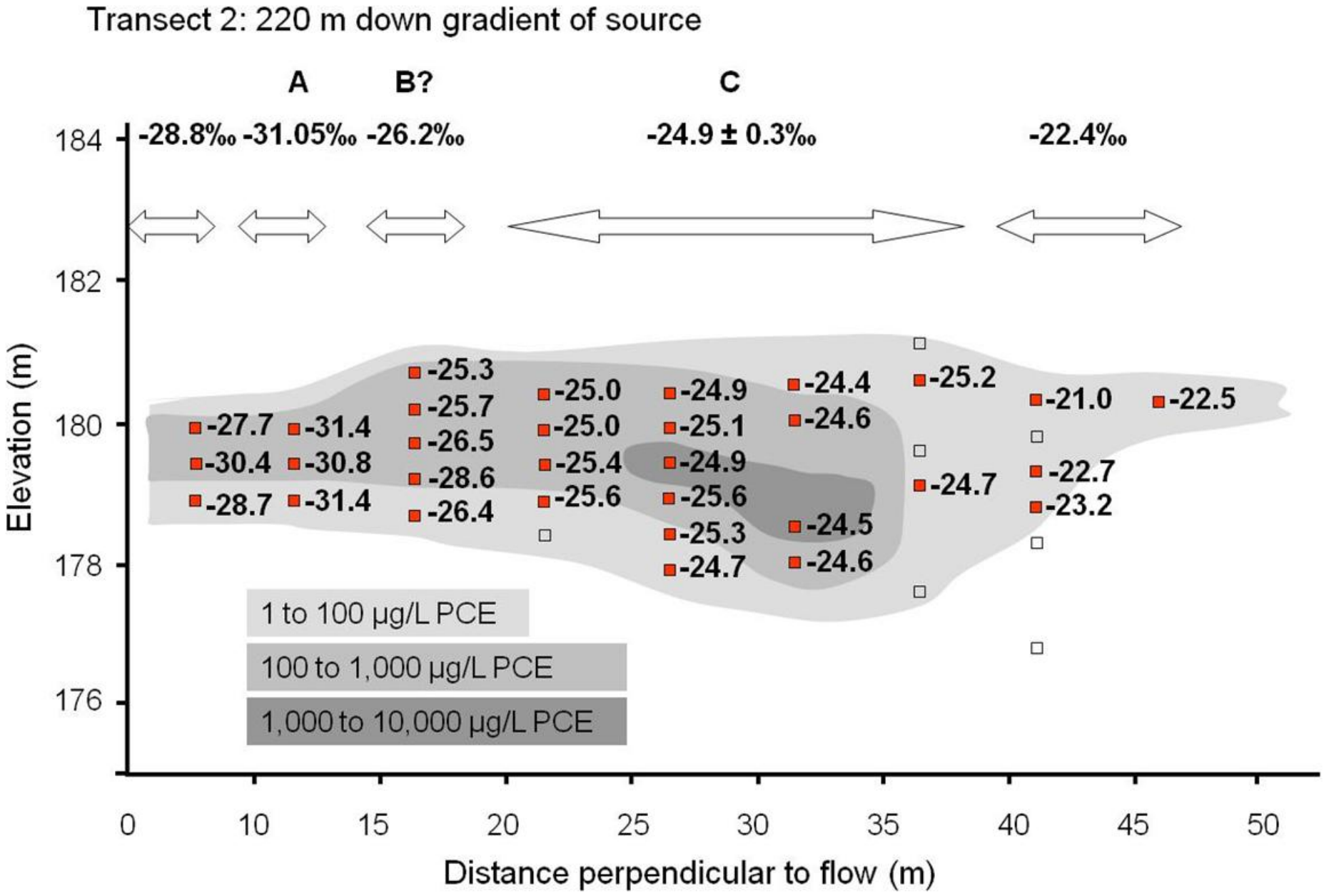
	PCE	TCE
Source	$\delta^{13}C$ (‰/PDB)	
Manufacturer A	-27.12±0.03	-31.53±0.01
Manufacturer B	-35.27±0.12	-27.90±0.08
Manufacturer C	-24.06±0.08	-29.93±0.18
Aldrich		-33.49±0.08
Dow	-23.19±0.10	-31.90±0.05
ICI	-37.20±0.03	-31.32±0.03
PPG	-33.84±0.03	-27.80±0.01
Vulcan	-24.1±0.04	
Range	**-23.19 to -37.20**	**-27.80 to -33.49**

Effect of source variability and transport processes on carbon isotope ratios of TCE and PCE in two sandy aquifers. *Journal of Contaminant Hydrology 74: 265-282 (2004)*



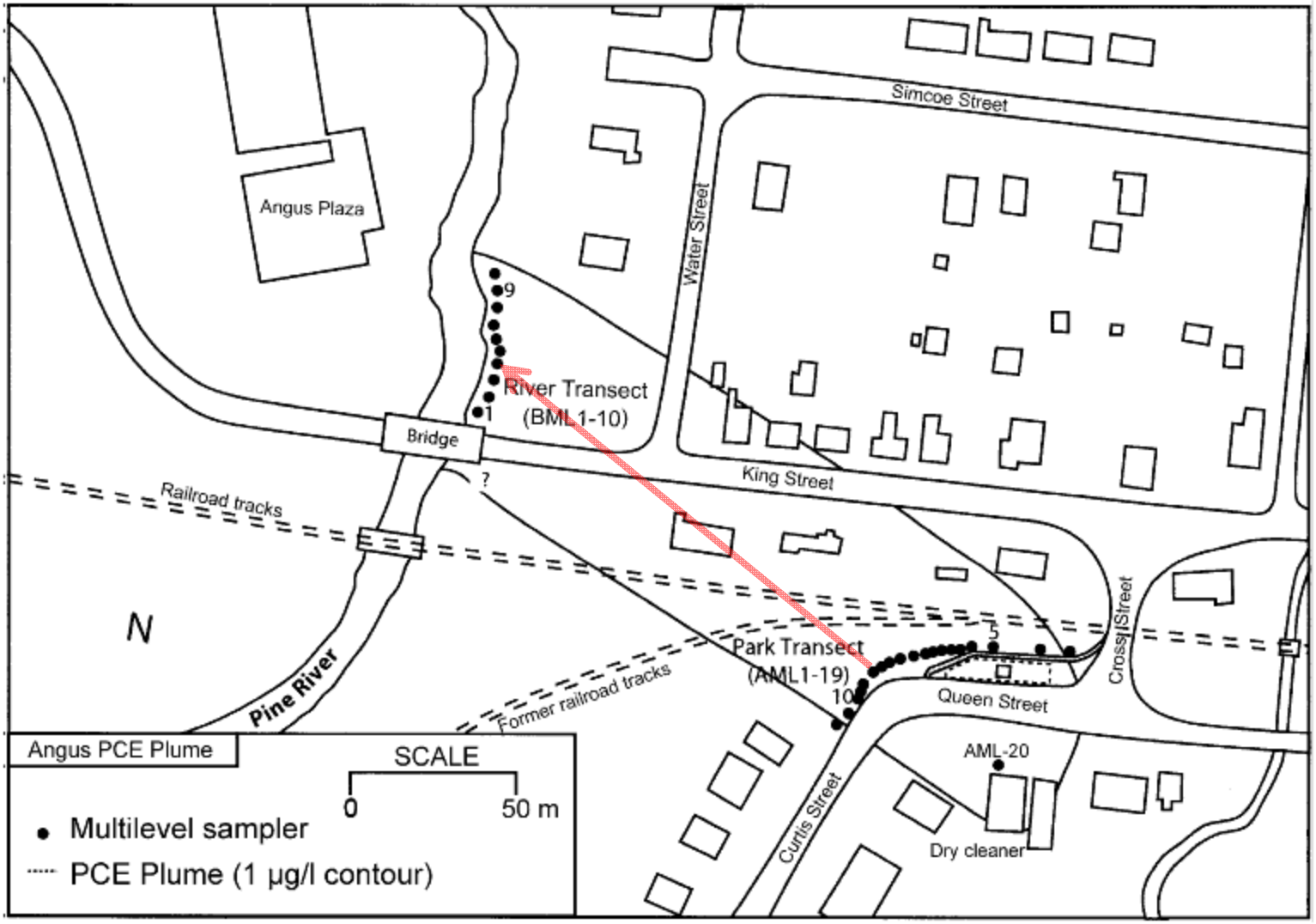

Transect 1: 40 to 50 m down gradient of source
A
B
C
-32.9 ± 0.5 ‰
-27.5 ± 0.5 ‰
-25.5 ± 0.5 ‰
-32.6
-32.9
-32.8
-32.9
-32.5
-32.6
-32.2
-32.9
-27.7
-27.6
-27.0
-28.5
-28.5
-26.6
-26.7
-27.0
-29.0
-25.7
-26.4
-25.7
-25.3
-25.0
-26.2
-26.6
-26.7
-26.4
-26.7
-25.9
-25.8
-25.8
-25.2
-25.3
-25.2
-26.3
-26.9
-25.4
-24.3
-25.5
-26.3
-25.8
-25.2
-25.4
-25.4
-25.2
-25.7
-26.0
1 to 100 µg/L PCE
100 to 1,000 µg/L PCE
1,000 to 10,000 µg/L PCE
Elevation (m)
186
184
182
180
178
176
0
10
15
20
25
30
35
40
45
50
Distance perpendicular to flow (m)

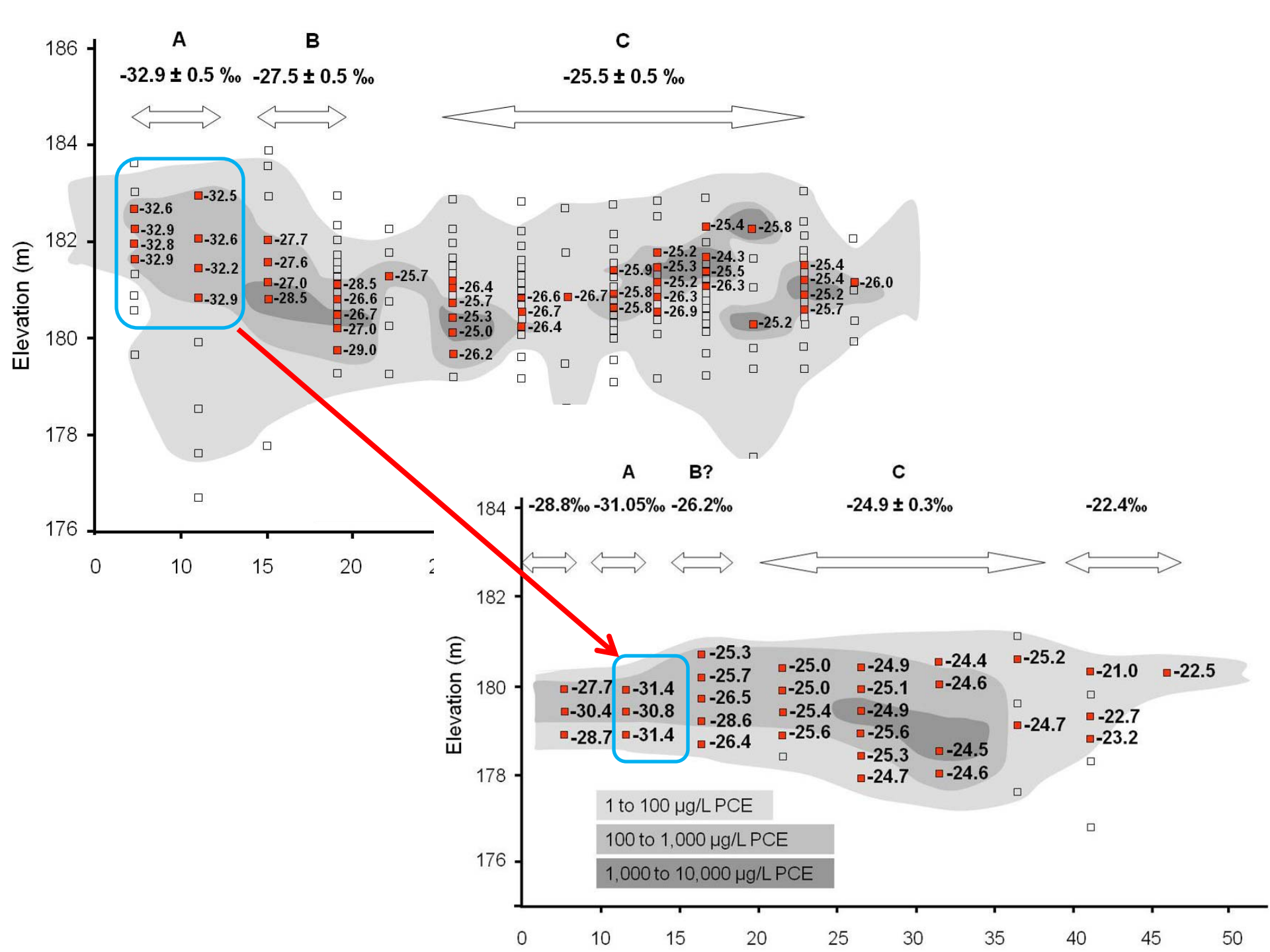

A
B
C
-32.9 ± 0.5 ‰
-27.5 ± 0.5 ‰
-25.5 ± 0.5 ‰
Elevation (m)
186
184
182
180
178
176
0
10
15
20
25
30
100 to 1,000 µg/L PCE
1,000 to 10,000 µg/L PCE
PCE (µg/L)
0
500
1000
1500
2000
2500
181.5
181.0
180.5
180.0
179.5
-30
-29
-28
-27
-26
AML-14


Transect 2: 220 m down gradient of source
A
B?
C
-28.8‰
-31.05‰
-26.2‰
-24.9 ± 0.3‰
-22.4‰
184
182
180
178
176
Elevation (m)
-27.7
-30.4
-28.7
-31.4
-30.8
-31.4
-25.3
-25.7
-26.5
-28.6
-26.4
-25.0
-25.0
-25.4
-25.6
-24.9
-25.1
-24.9
-25.6
-25.3
-24.7
-24.4
-24.6
-24.5
-24.6
-25.2
-24.7
-21.0
-22.7
-23.2
-22.5
1 to 100 µg/L PCE
100 to 1,000 µg/L PCE
1,000 to 10,000 µg/L PCE
0
10
15
20
25
30
35
40
45
50
Distance perpendicular to flow (m)

United States Environmental Protection Agency

Effect of source variability and transport processes on carbon isotope ratios of TCE and PCE in two sandy aquifers. *Journal of Contaminant Hydrology 74: 265-282 (2004)*




A
B
C
-32.9 ± 0.5 ‰
-27.5 ± 0.5 ‰
-25.5 ± 0.5 ‰
Elevation (m)
186
184
182
180
178
176
0
10
15
20
-32.6
-32.9
-32.8
-32.9
-32.5
-32.6
-32.2
-32.9
-27.7
-27.6
-27.0
-28.5
-28.5
-26.6
-26.7
-27.0
-29.0
-25.7
-26.4
-25.7
-25.3
-25.0
-26.2
-26.6
-26.7
-26.4
-26.7
-25.9
-25.8
-25.8
-25.2
-25.3
-25.2
-26.3
-26.9
-25.4
-25.8
-24.3
-25.5
-26.3
-25.2
-25.4
-25.4
-25.2
-25.7
-26.0
A
B?
C
-28.8‰
-31.05‰
-26.2‰
-24.9 ± 0.3‰
-22.4‰
Elevation (m)
184
182
180
178
176
0
10
15
20
25
30
35
40
45
50
-27.7
-30.4
-28.7
-31.4
-30.8
-31.4
-25.3
-25.7
-26.5
-28.6
-26.4
-25.0
-25.0
-25.4
-25.6
-24.9
-25.1
-24.9
-25.6
-25.3
-24.7
-24.4
-24.6
-24.5
-24.6
-25.2
-24.7
-21.0
-22.7
-23.2
-22.5
1 to 100 µg/L PCE
100 to 1,000 µg/L PCE
1,000 to 10,000 µg/L PCE

EPA United States Environmental Protection Agency

What does it take to make it work?

1) No appreciable biodegradation from source to impacted well.

2) An appreciable difference in $\delta^{13}C$ between plausible sources.

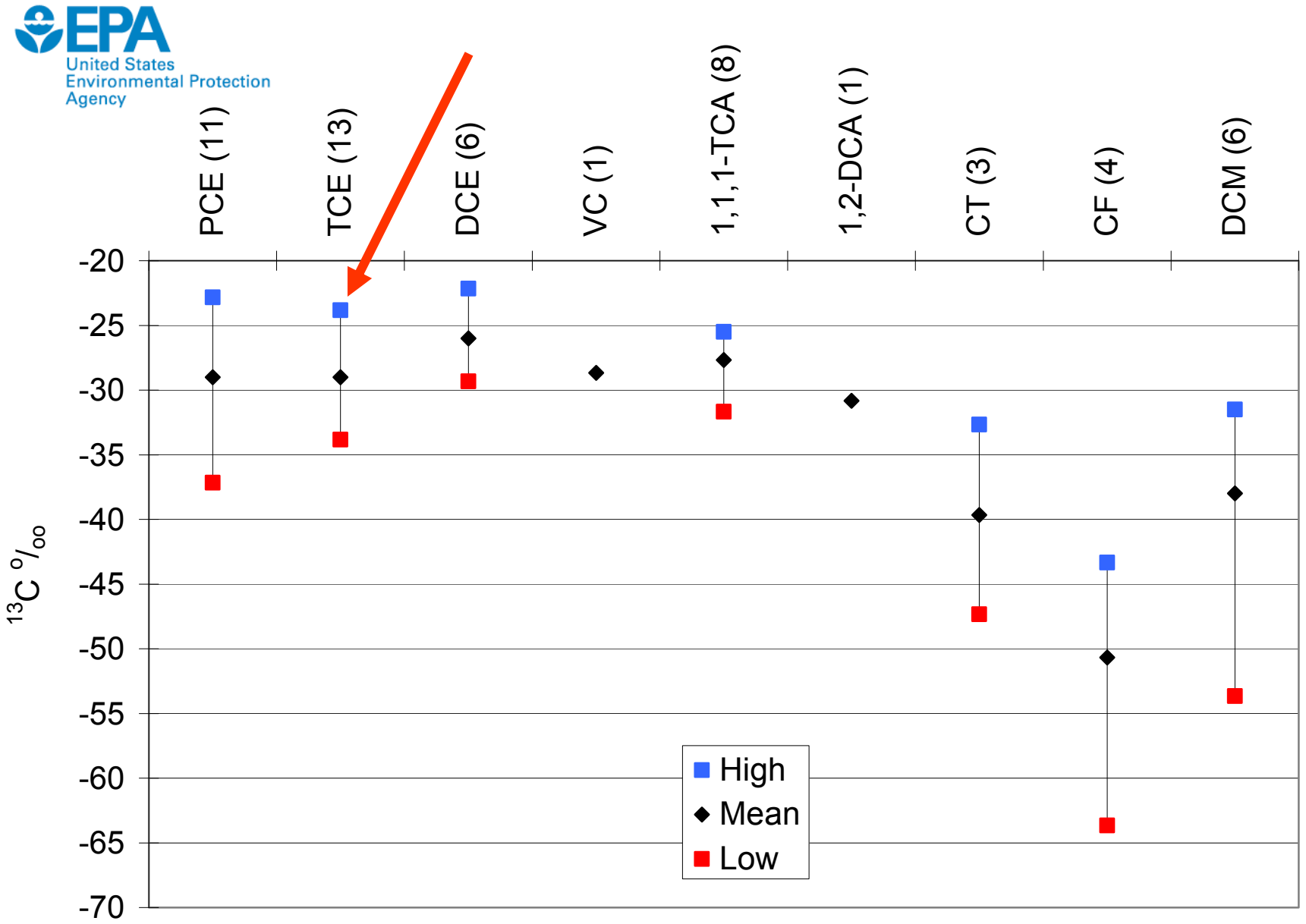
3) Samples from a transect perpendicular to ground water flow. Simple point to point comparisons may be misleading.

EPA United States Environmental Protection Agency

Application to a Superfund Site in Region 4. Can SCIR identify the source of a plume in fractured rock?

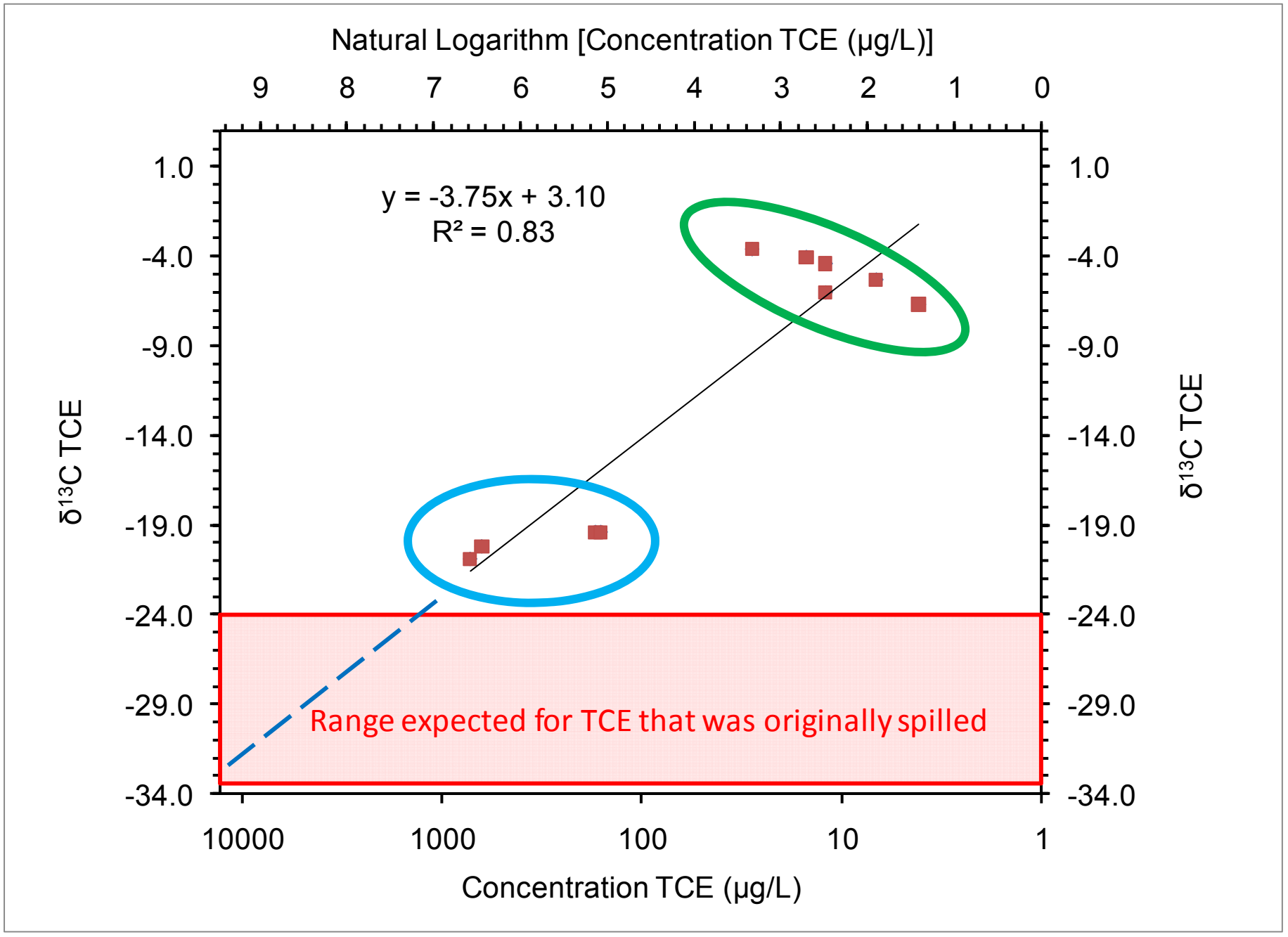
1)Sampled ground water in two impacted neighborhoods.

2)Compared the $\delta^{13}C$ in TCE to the range of $\delta^{13}C$ in commerce.



Range of $\delta^{13}C$ Reported for Un-fractionated Chlorinated Solvents

Figure 6.1 of EPA Guide


Natural Logarithm [Concentration TCE (µg/L)]
9
8
7
6
5
4
3
2
1
0
y = -3.75x + 3.10
R² = 0.83
1.0
-4.0
-9.0
-14.0
-19.0
-24.0
-29.0
-34.0
δ13C TCE
Range expected for TCE that was originally spilled
10000
1000
100
10
1
Concentration TCE (µg/L)

United States Environmental Protection Agency

Application to a Superfund Site in Region 4. Can SCIR identify the source of a plume in fractured rock?

1)Sampled ground water in two impacted neighborhoods.

2)Compared the $\delta^{13}C$ in TCE to the range of $\delta^{13}C$ in commerce.

3)If there is more than about 1% degradation products, probably not going to work.

EPA United States Environmental Protection Agency

What happens to $\delta^{13}C$ during biodegradation?

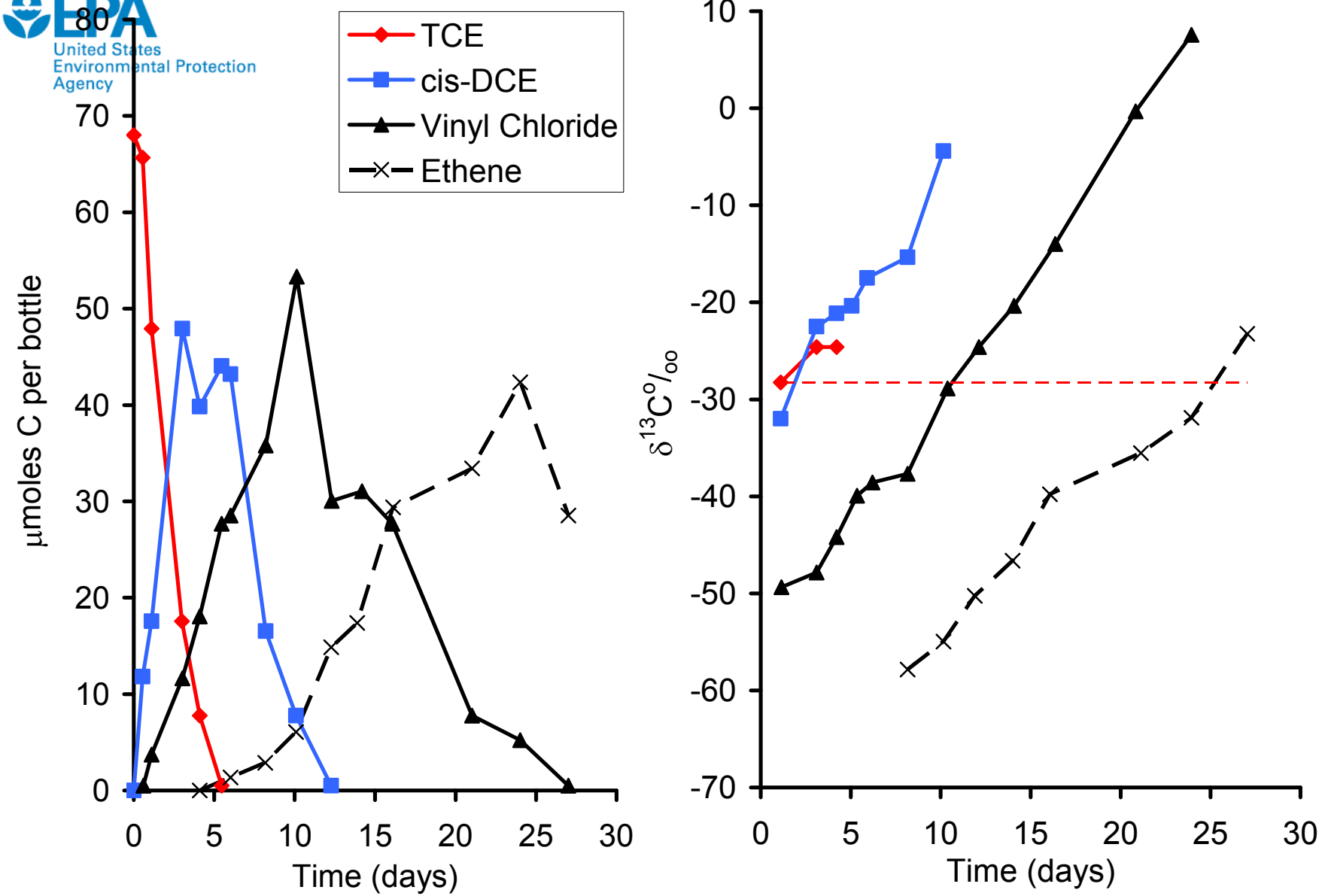
Can we use changes in $\delta^{13}C$ to understand biodegradation?

United States Environmental Protection Agency

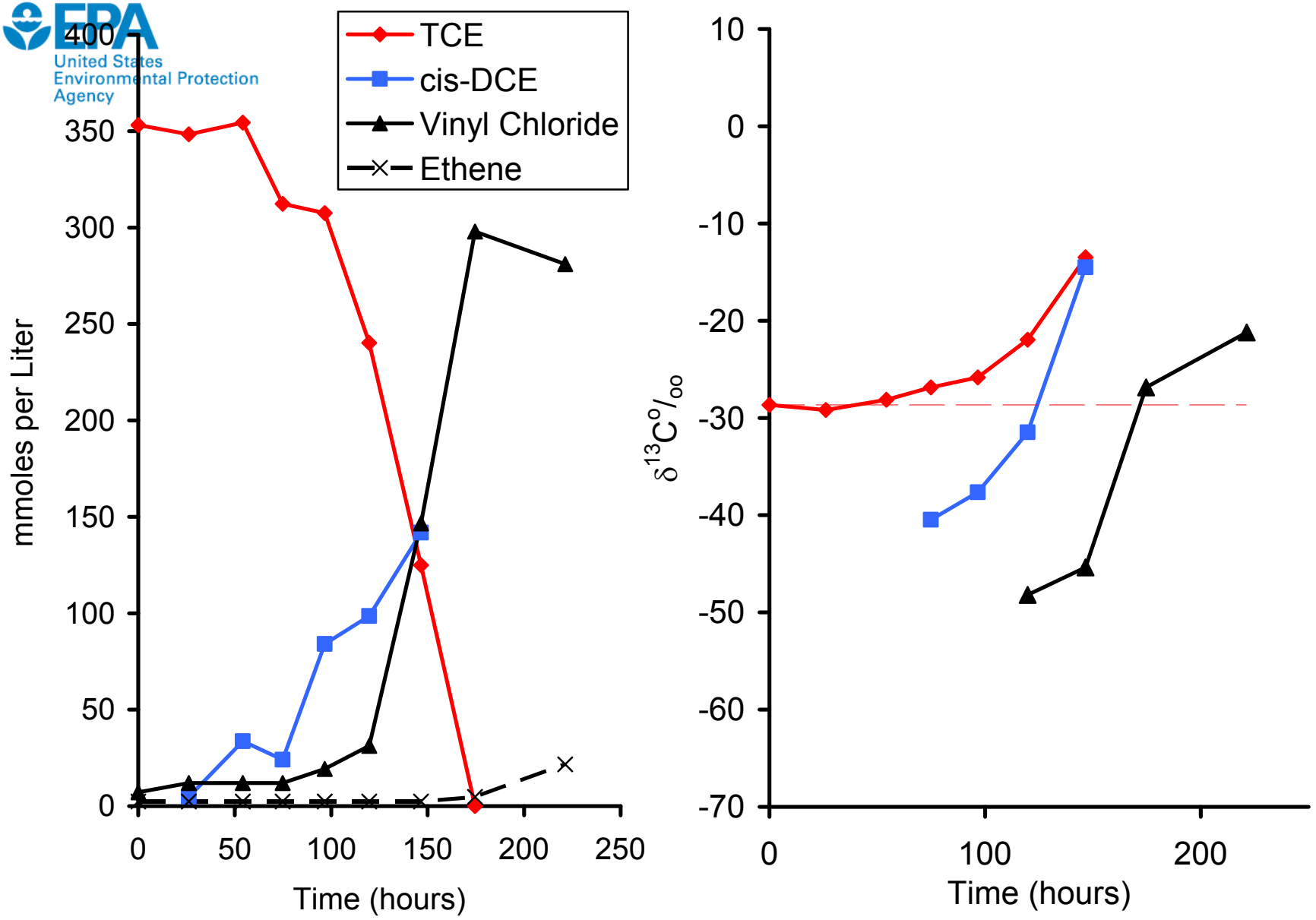
What happens to $\delta^{13}C$ during biodegradation?

Initially, the daughter product is lighter than the parent compound (the $\delta^{13}C$ is more negative).

As the daughter product degrades, it becomes heavier (the $\delta^{13}C$ becomes less negative).



Bloom et al. *ES&T* 34:2768-2772 (2002)



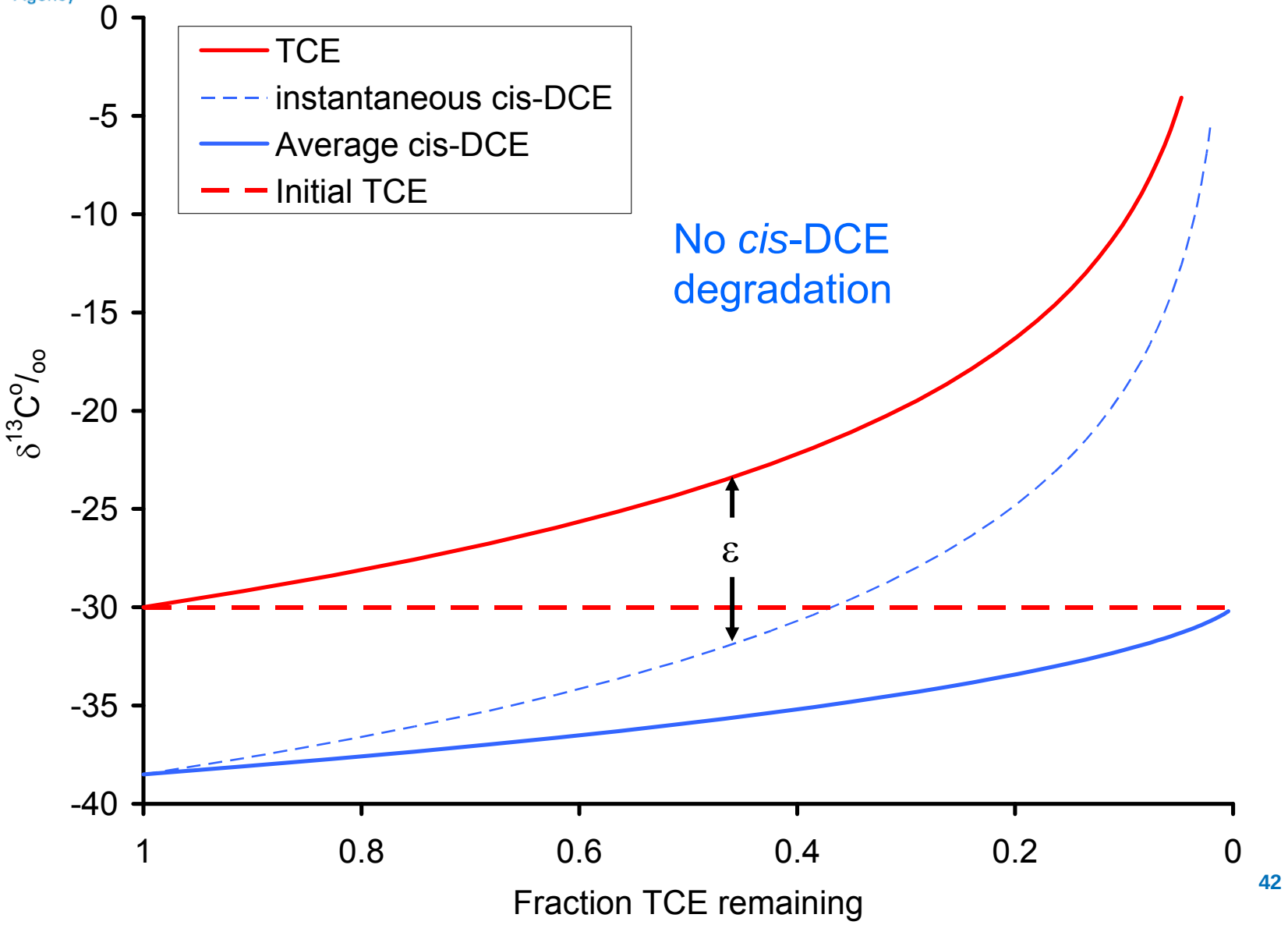
Cichocka et al. *Chemosphere* 639-648 (2008).

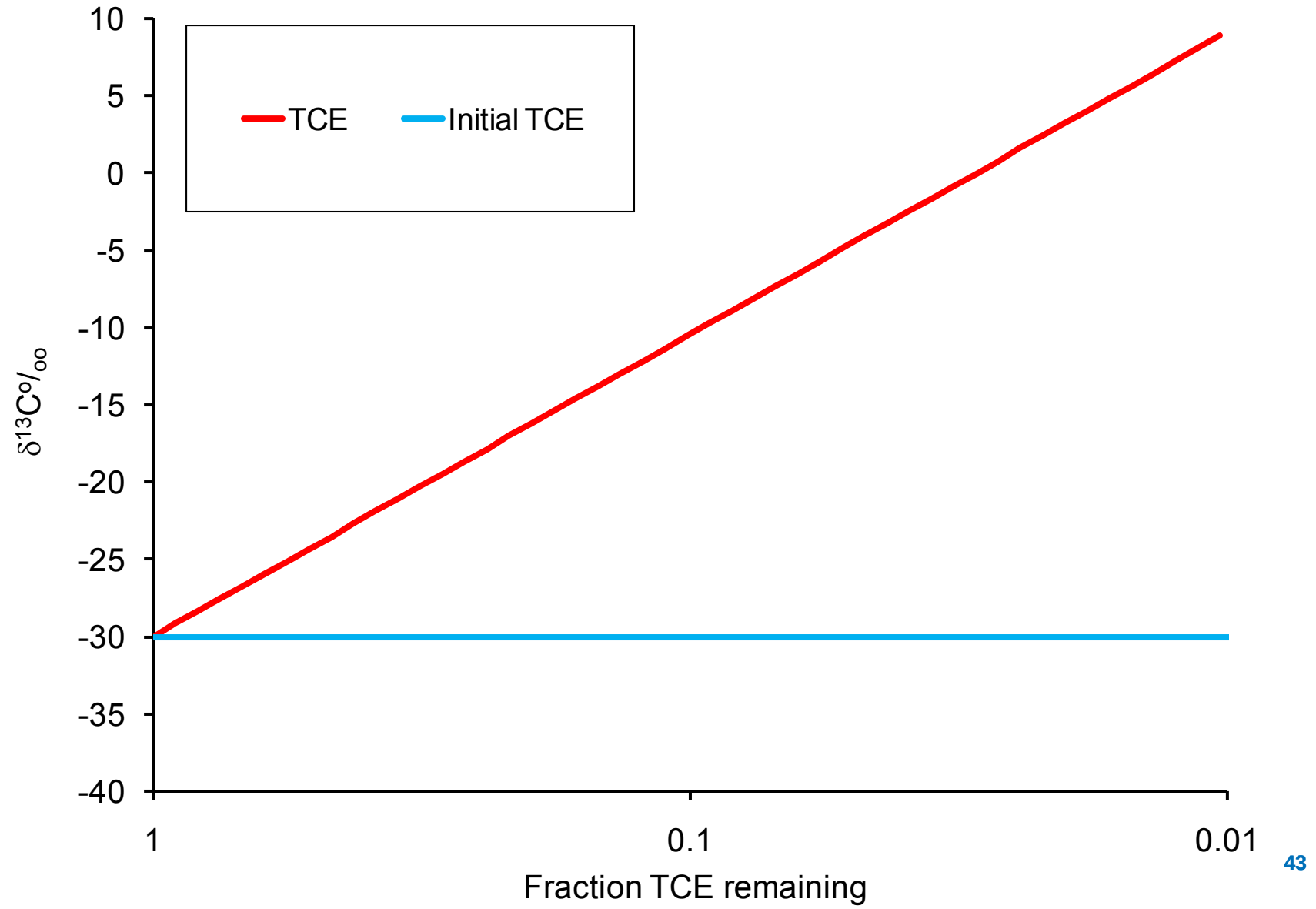
United States Environmental Protection Agency

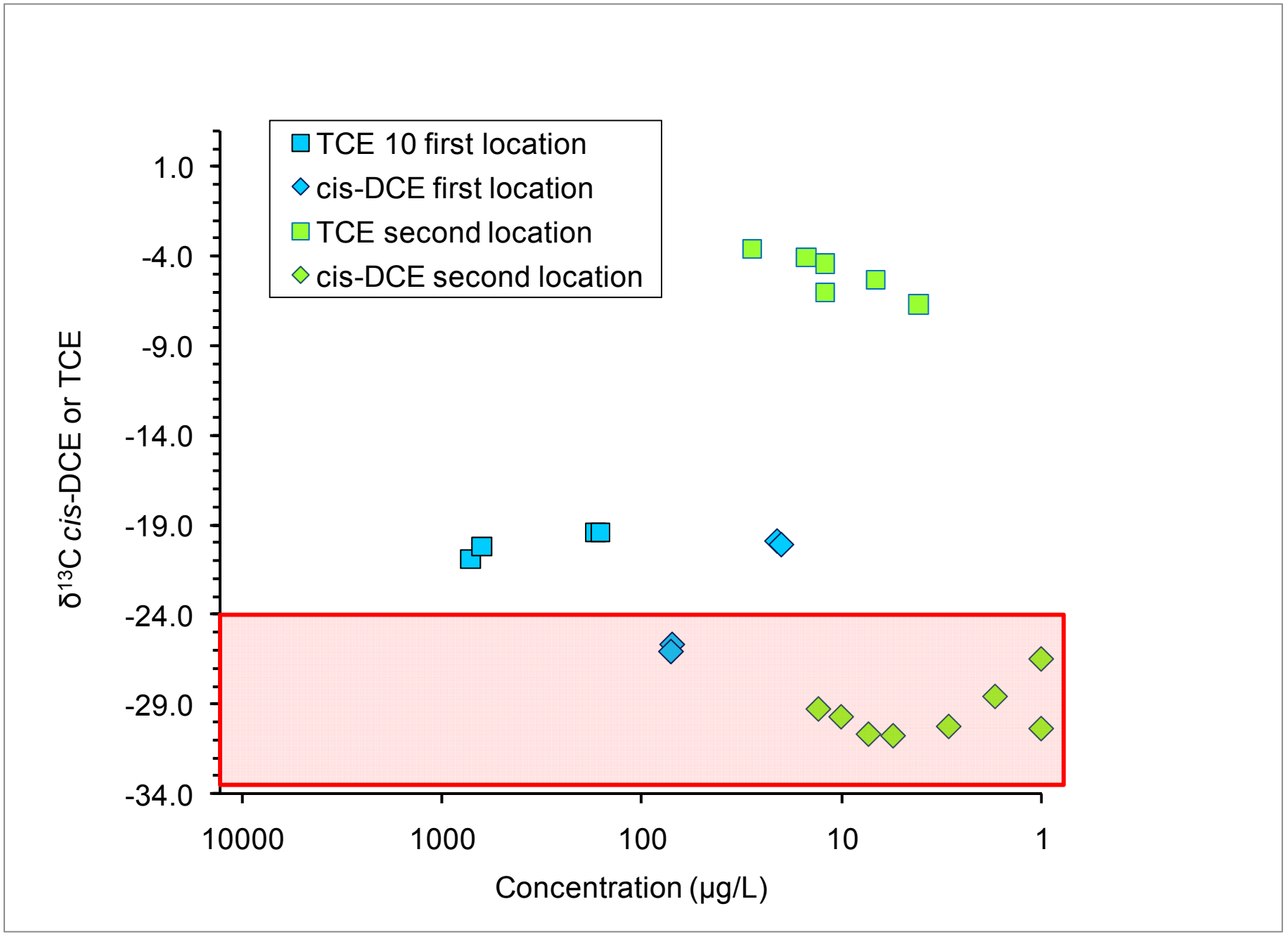
What happens to $\delta^{13}C$ during biodegradation?

If the daughter product is heavier (the $\delta^{13}C$ is less negative) than the plausible range for the parent, that is substantial evidence that the daughter product has also been degraded.

Figure 7.1 of EPA Guide




TCE
Initial TCE
10
5
0
-5
-10
-15
-20
-25
-30
-35
-40
δ13C‰
1
0.1
0.01
Fraction TCE remaining

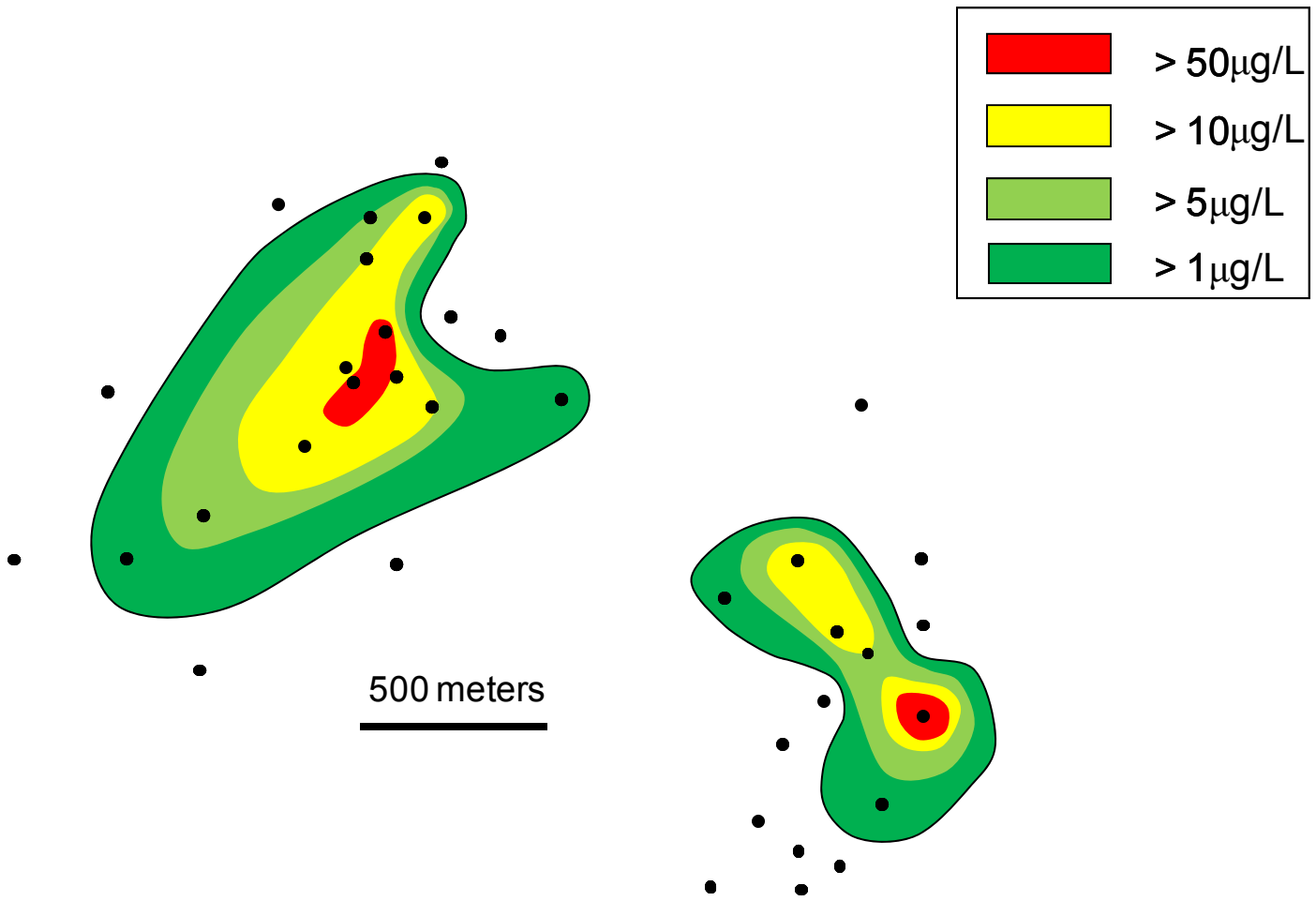

TCE 10 first location
cis-DCE first location
TCE second location
cis-DCE second location
1.0
-4.0
-9.0
-14.0
-19.0
-24.0
-29.0
-34.0
δ13C cis-DCE or TCE
10000
1000
100
10
1
Concentration (µg/L)

EPA United States Environmental Protection Agency

At locations along the flow path where the parent compound is entirely degraded, we can assume that the "original un-fractionated" $\delta^{13}C$ for the degradation product was the original un-fractionated $\delta^{13}C$ for the parent compound.

How can CSIA be used to determine whether a daughter product is degrading?

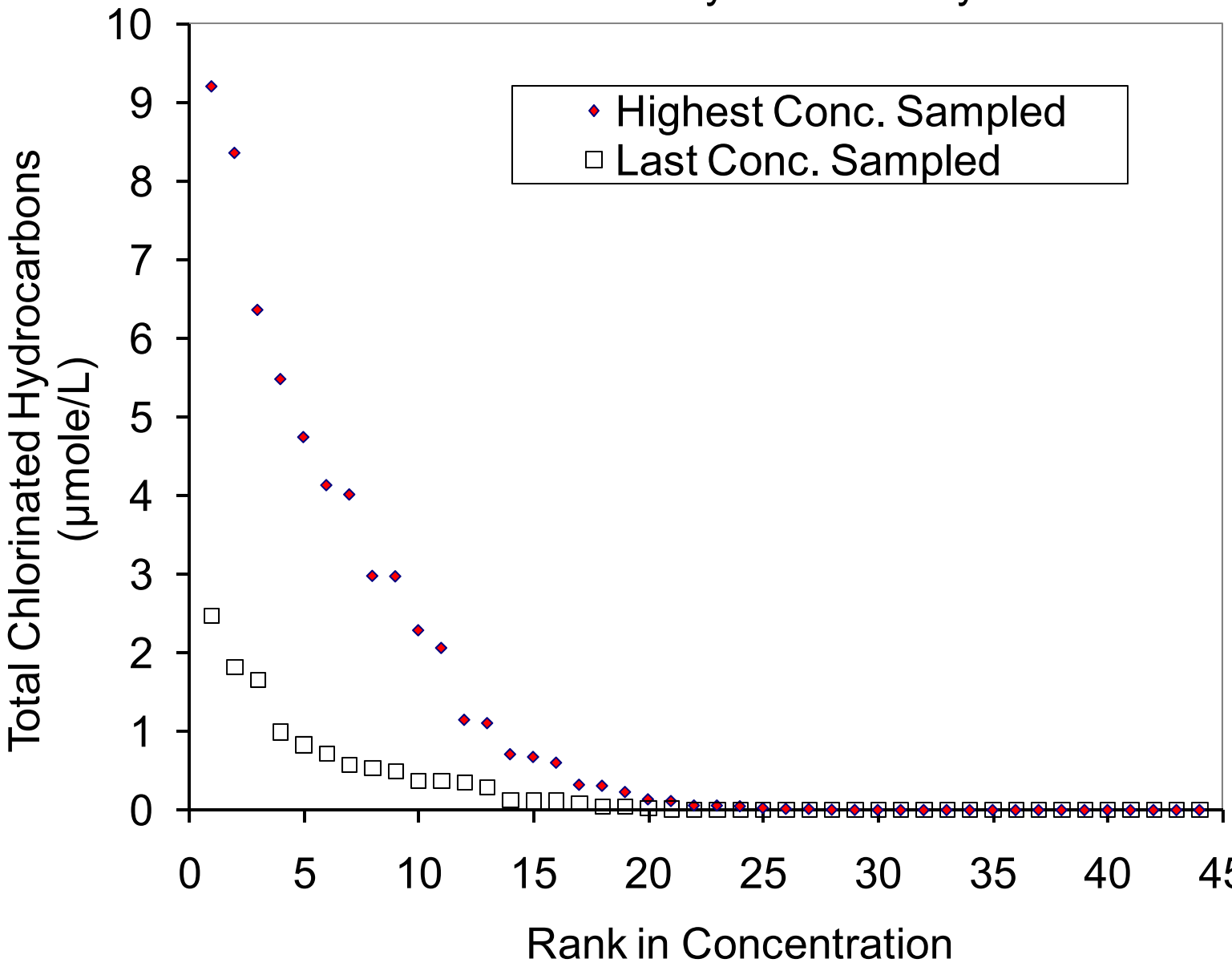
A site in Region 6. A “bull’s eye” plume. No predominant direction of ground water flow.

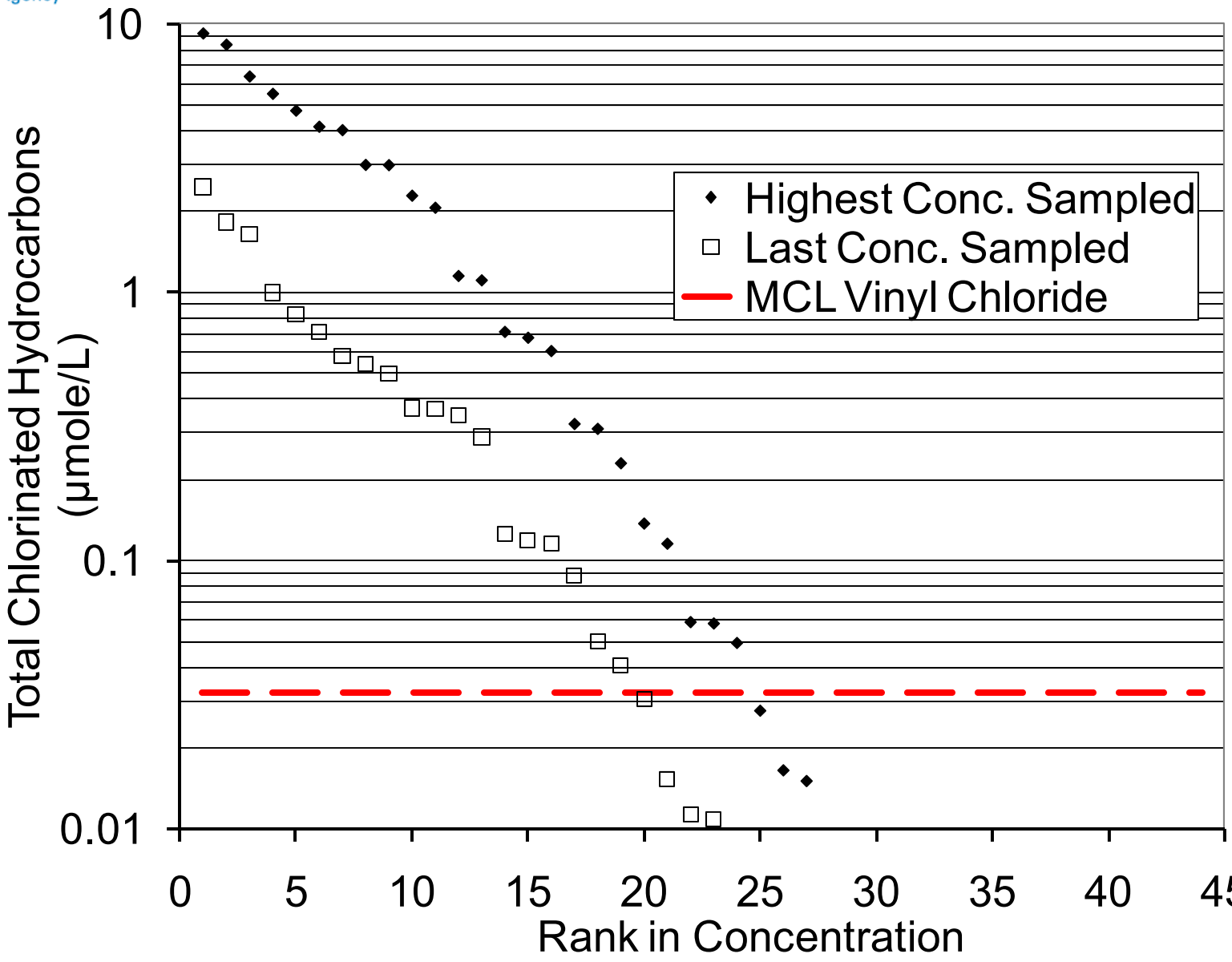


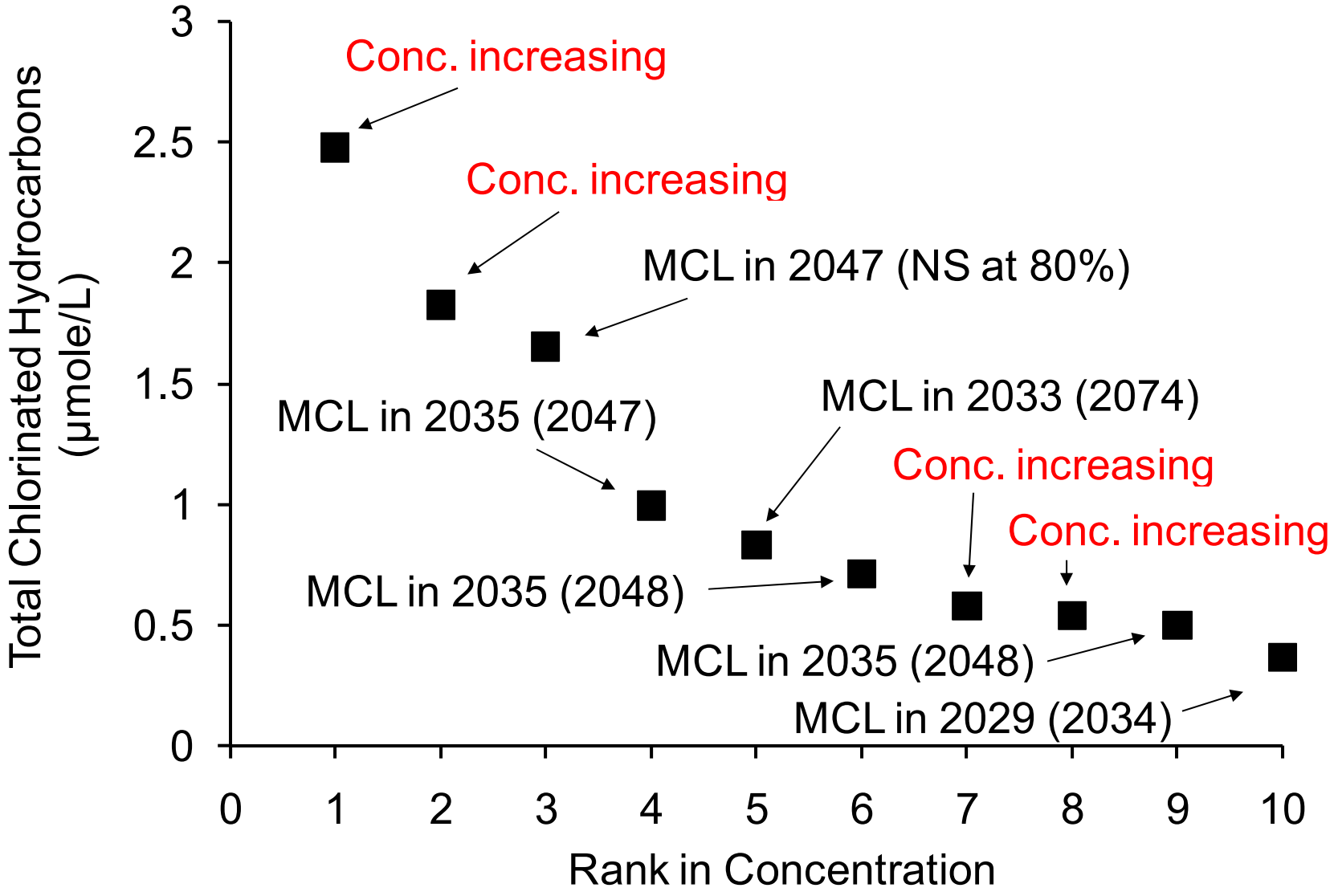
cis-DCE in the Intermediate Ground –Water Zone

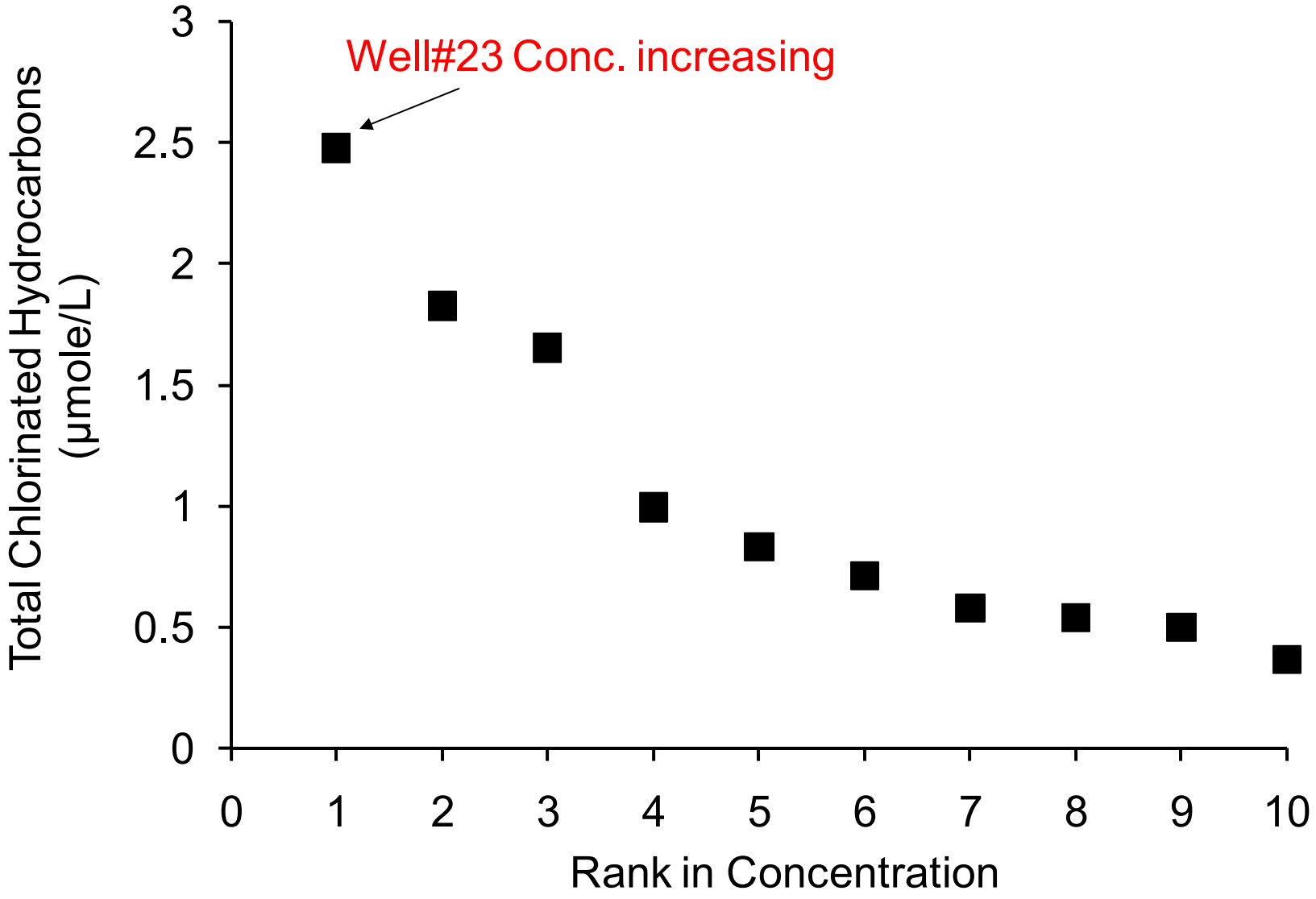
United States Environmental Protection Agency

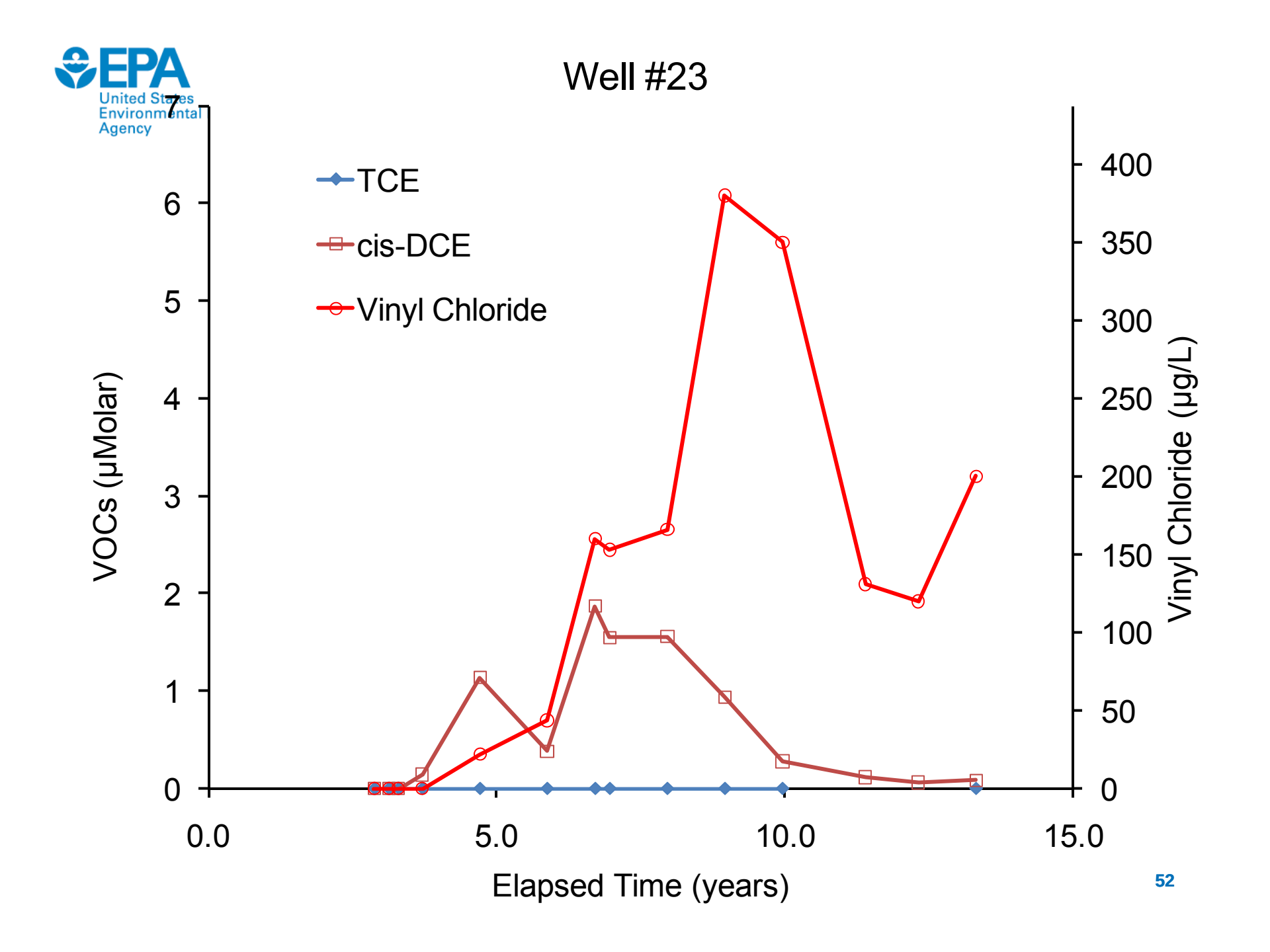
Distribution of concentrations of TCE and chlorinated degradation products at the end of the second five year review cycle.

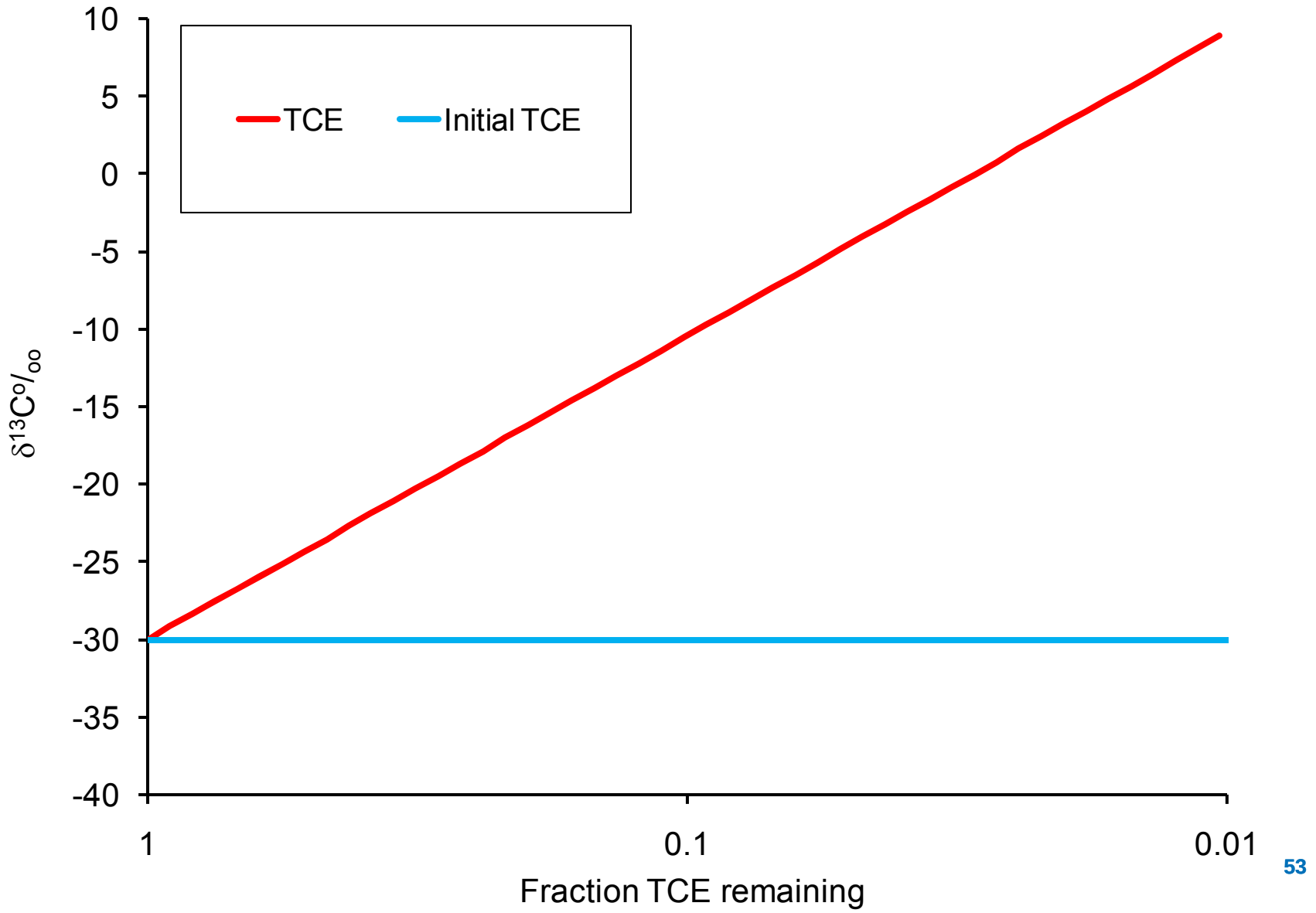


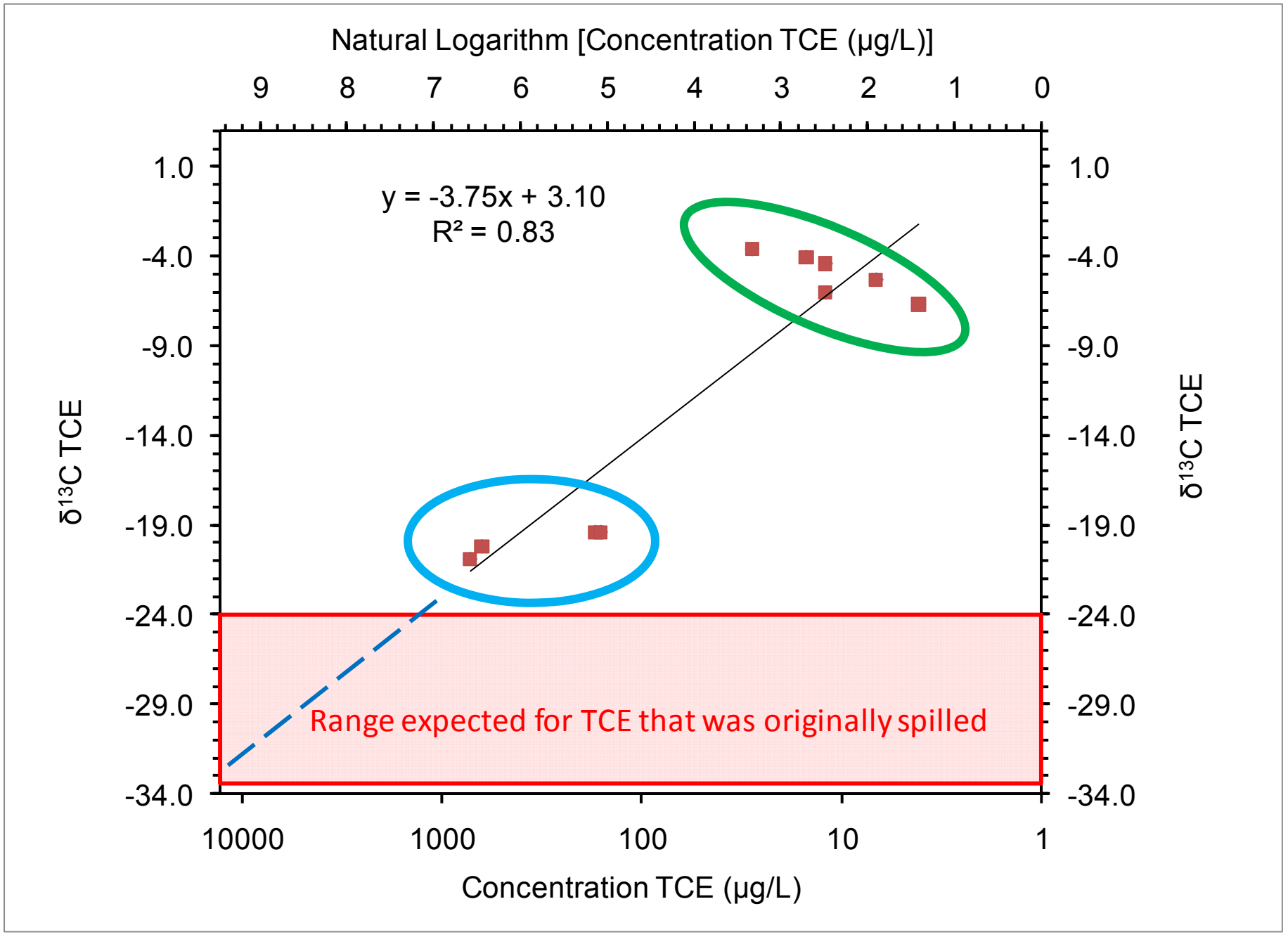

10
1
0.1
0.01
Total Chlorinated Hydrocarbons (µmole/L)
Highest Conc. Sampled
Last Conc. Sampled
MCL Vinyl Chloride
0
5
10
15
20
25
30
35
40
45
Rank in Concentration

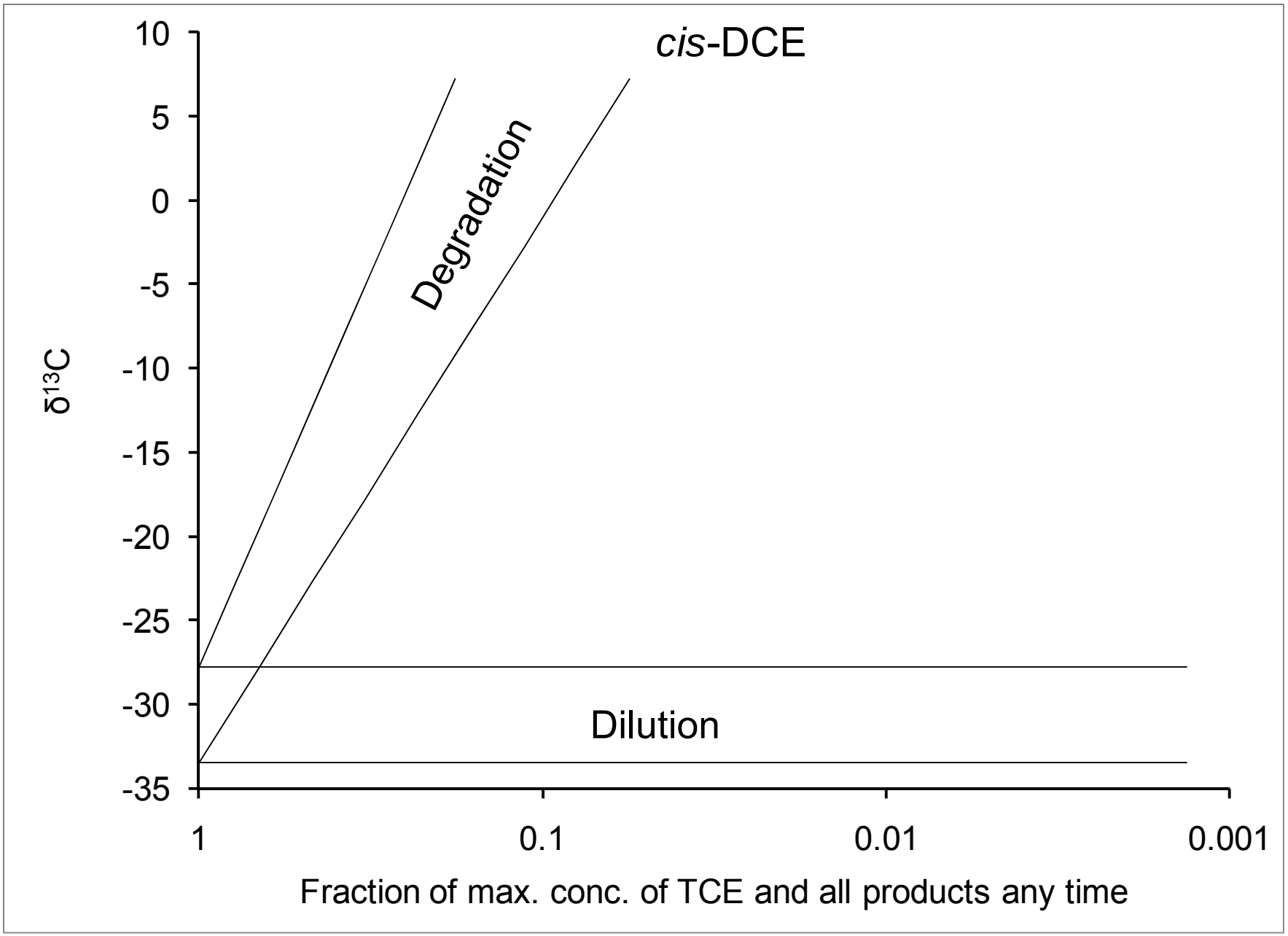

Total Chlorinated Hydrocarbons (µmole/L)
3
2.5
2
1.5
1
0.5
0
0
1
2
3
4
5
6
7
8
9
10
Rank in Concentration
Conc. increasing
Conc. increasing
MCL in 2047 (NS at 80%)
MCL in 2035 (2047)
MCL in 2033 (2074)
Conc. increasing
Conc. increasing
MCL in 2035 (2048)
MCL in 2035 (2048)
MCL in 2029 (2034)

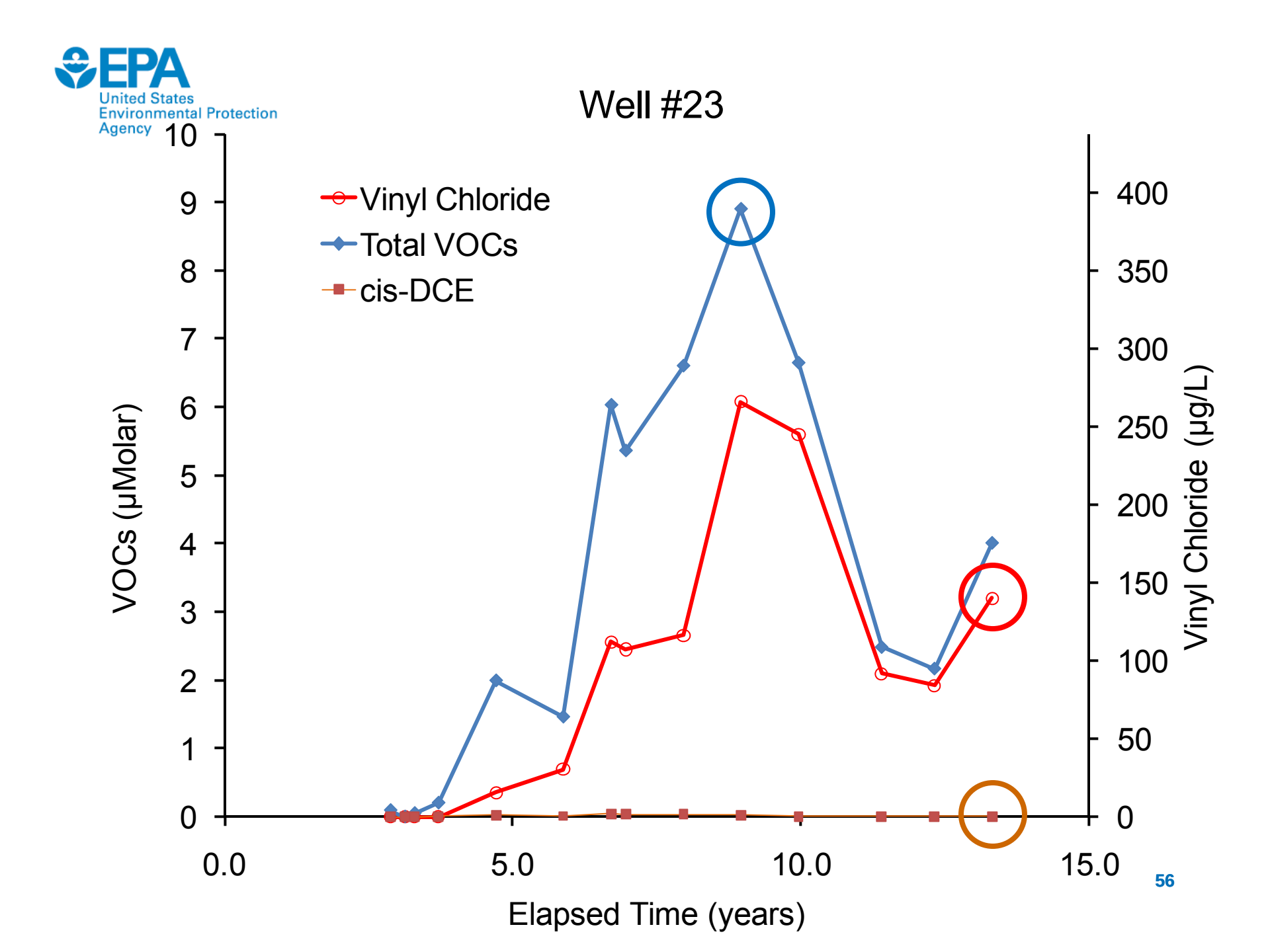

Well#23 Conc. increasing
Total Chlorinated Hydrocarbons (µmole/L)
3
2.5
2
1.5
1
0.5
0
0
1
2
3
4
5
6
7
8
9
10
Rank in Concentration

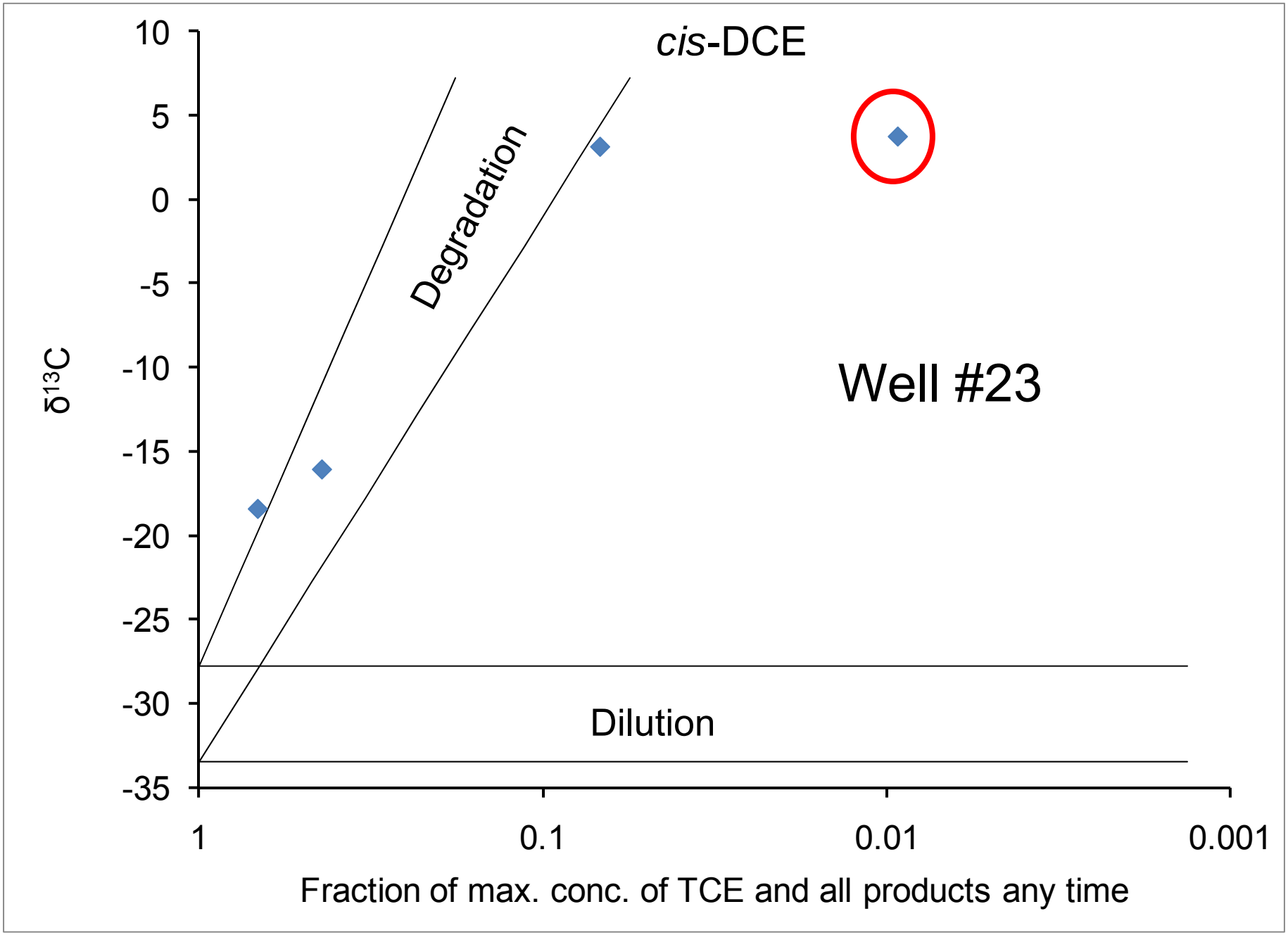

Well #23
TCE
cis-DCE
Vinyl Chloride
VOCs (µMolar)
Vinyl Chloride (µg/L)
Elapsed Time (years)
0
1
2
3
4
5
6
7
0
50
100
150
200
250
300
350
400
0.0
5.0
10.0
15.0

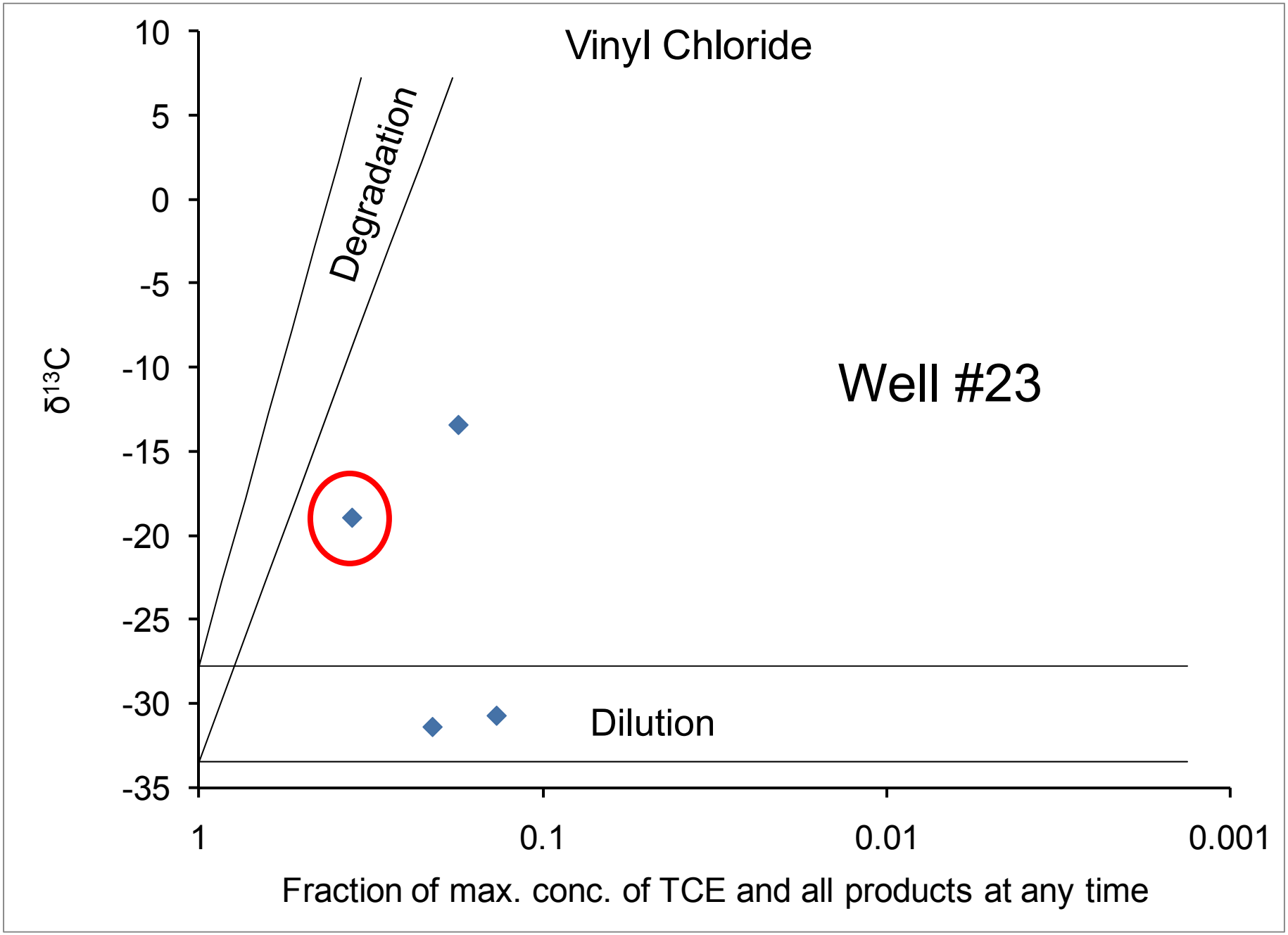

TCE
Initial TCE
10
5
0
-5
-10
-15
-20
-25
-30
-35
-40
δ13C‰
1
0.1
0.01
Fraction TCE remaining

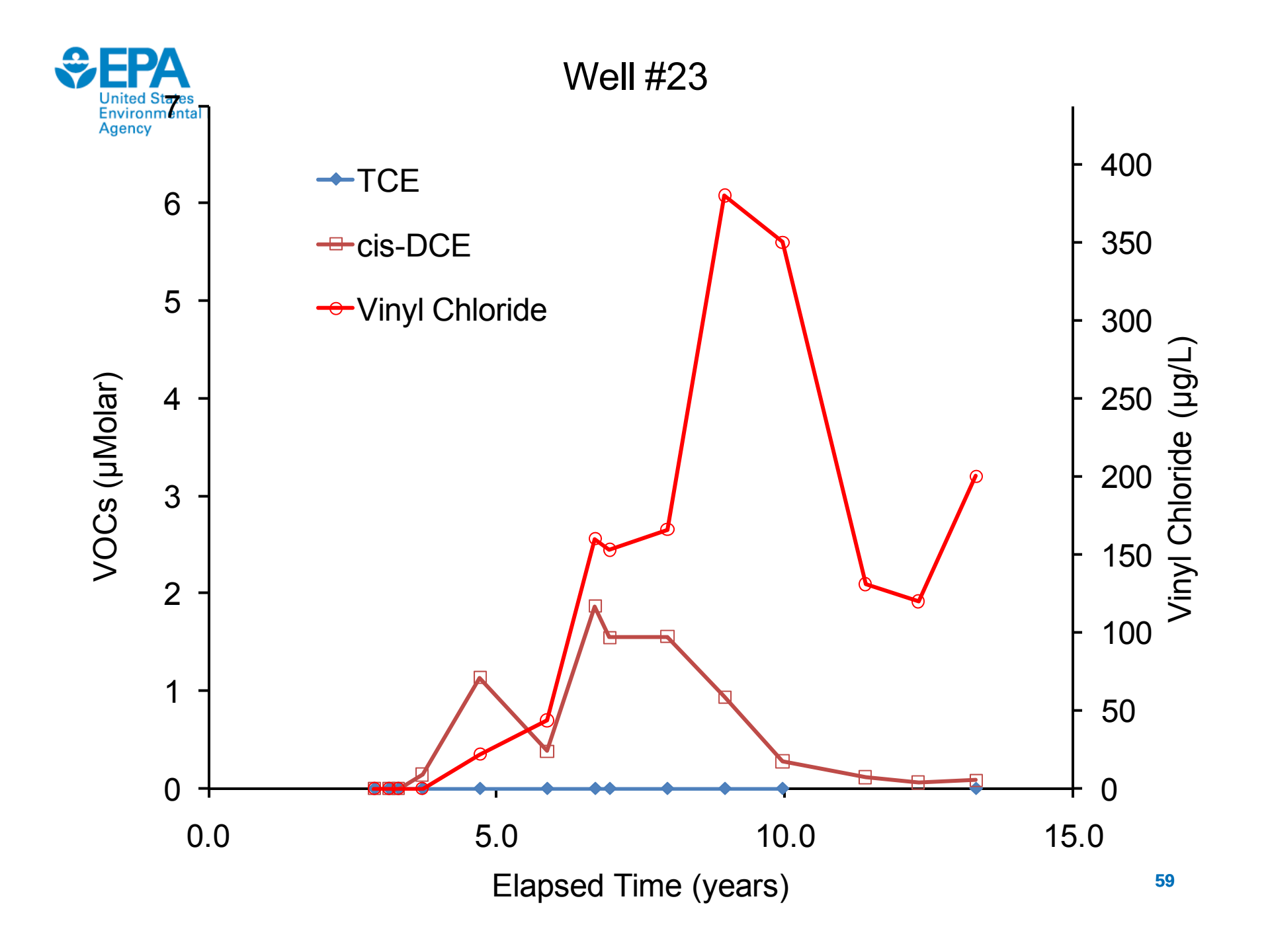

Natural Logarithm [Concentration TCE (µg/L)]
9
8
7
6
5
4
3
2
1
0
y = -3.75x + 3.10
R² = 0.83
1.0
-4.0
-9.0
-14.0
-19.0
-24.0
-29.0
-34.0
δ13C TCE
Range expected for TCE that was originally spilled
10000
1000
100
10
1
Concentration TCE (µg/L)

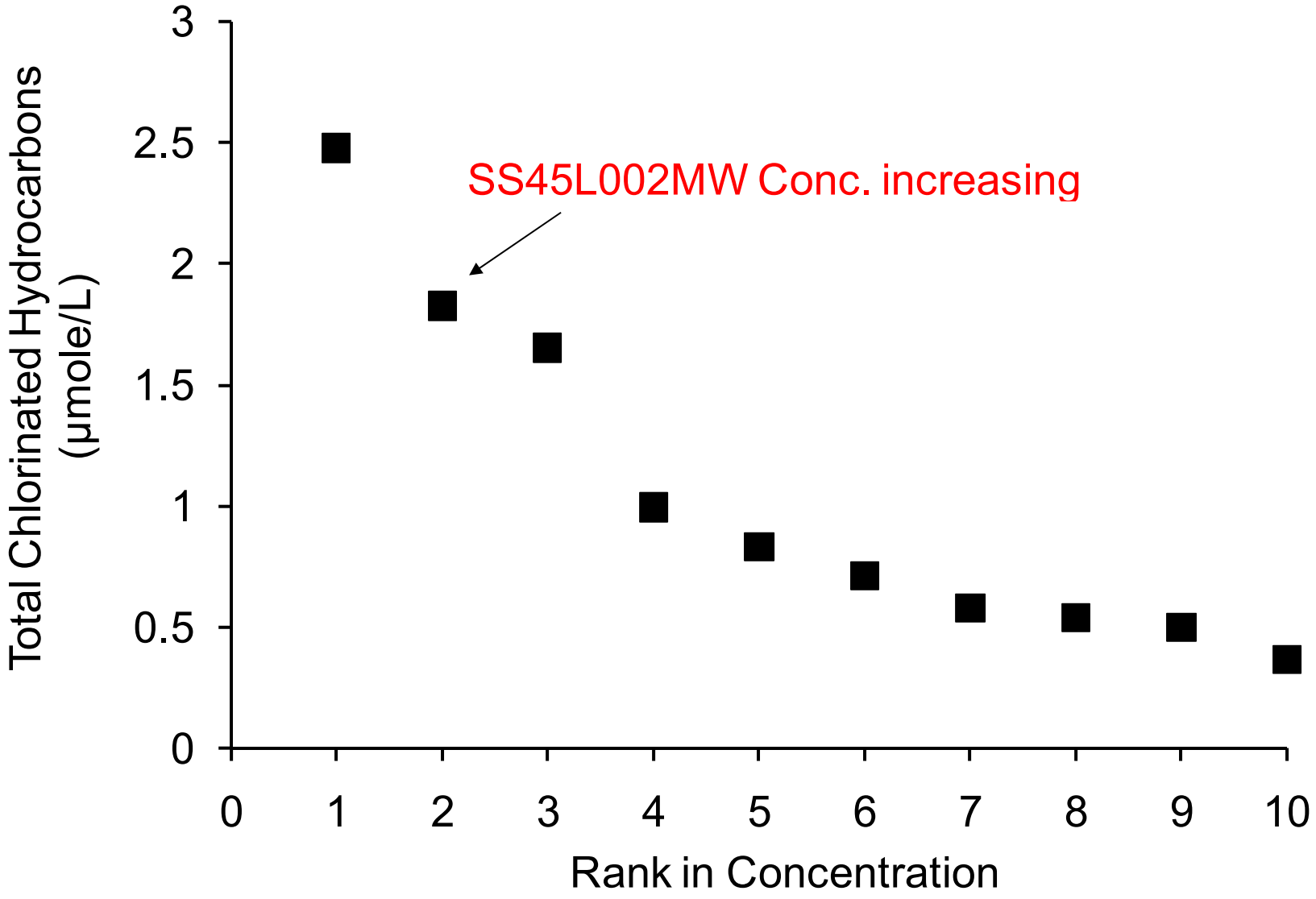

cis-DCE
Degradation
Dilution
10
5
0
-5
-10
-15
-20
-25
-30
-35
δ13C
1
0.1
0.01
0.001
Fraction of max. conc. of TCE and all products any time

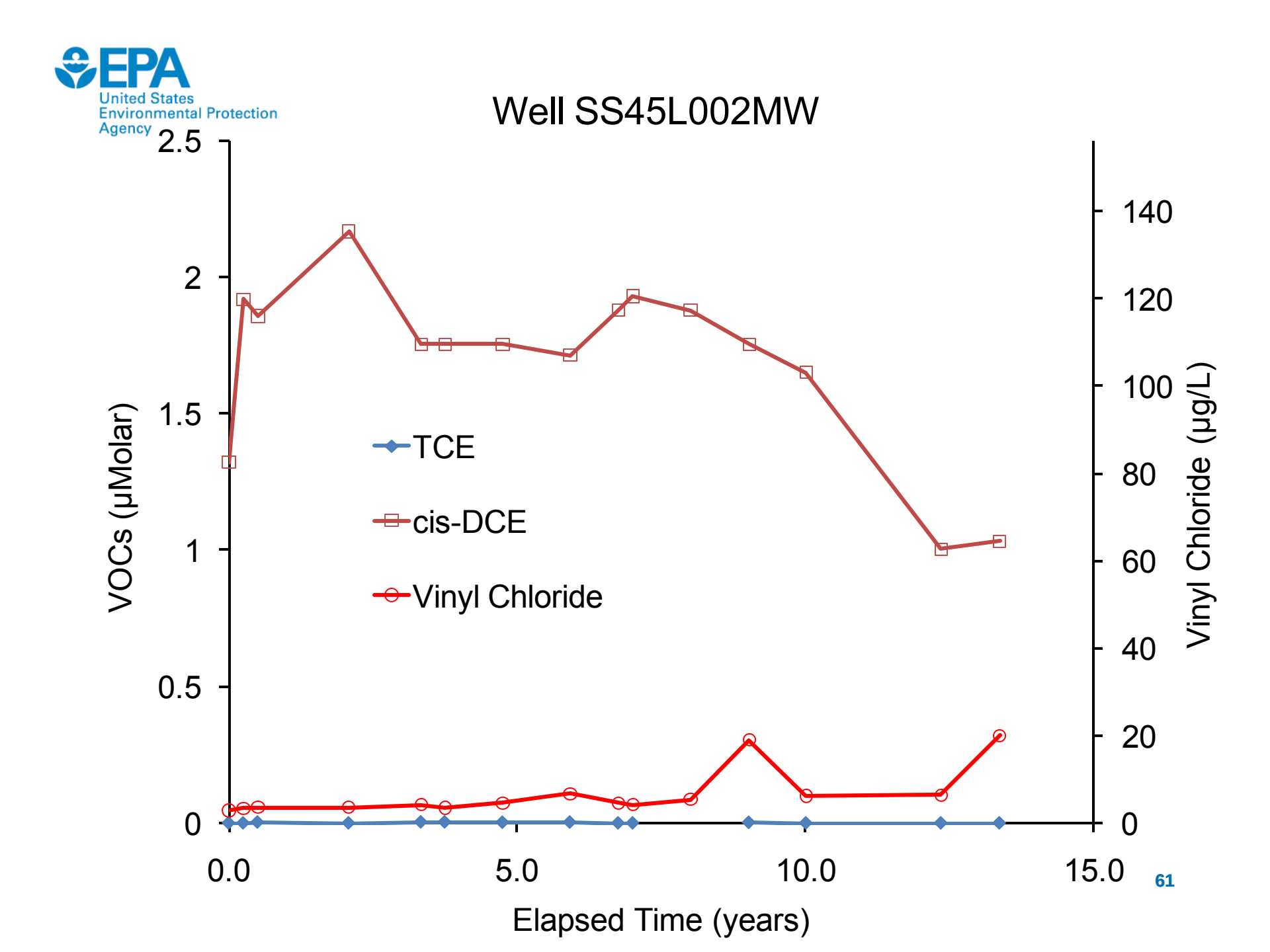

EPA
United States
Environmental Protection
Agency
Well #23
Vinyl Chloride
Total VOCs
cis-DCE
VOCs (µMolar)
Vinyl Chloride (µg/L)
Elapsed Time (years)
0
1
2
3
4
5
6
7
8
9
10
0
50
100
150
200
250
300
350
400
0.0
5.0
10.0
15.0
56

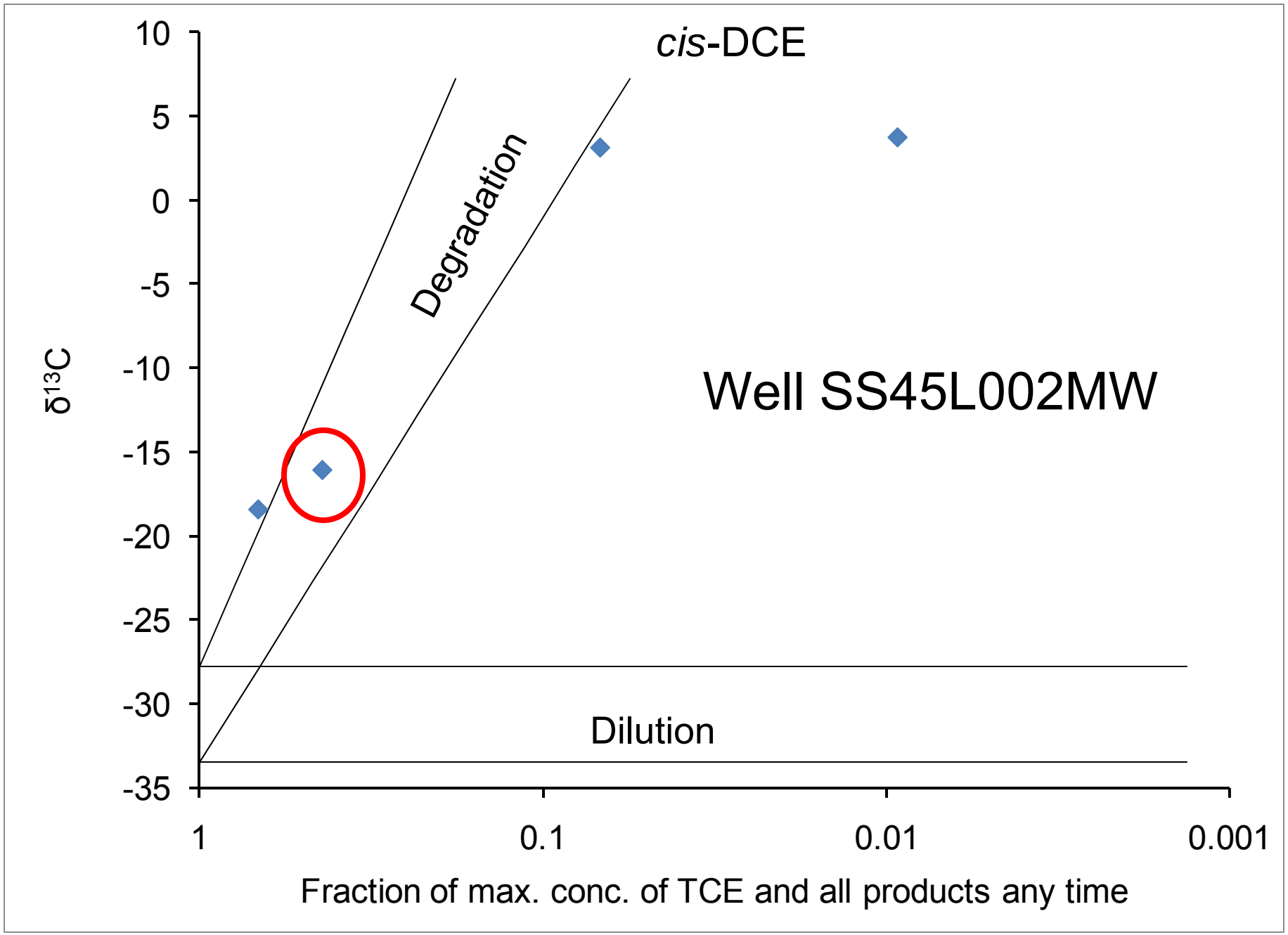

cis-DCE
Degradation
Well #23
Dilution
δ13C
10
5
0
-5
-10
-15
-20
-25
-30
-35
1
0.1
0.01
0.001
Fraction of max. conc. of TCE and all products any time

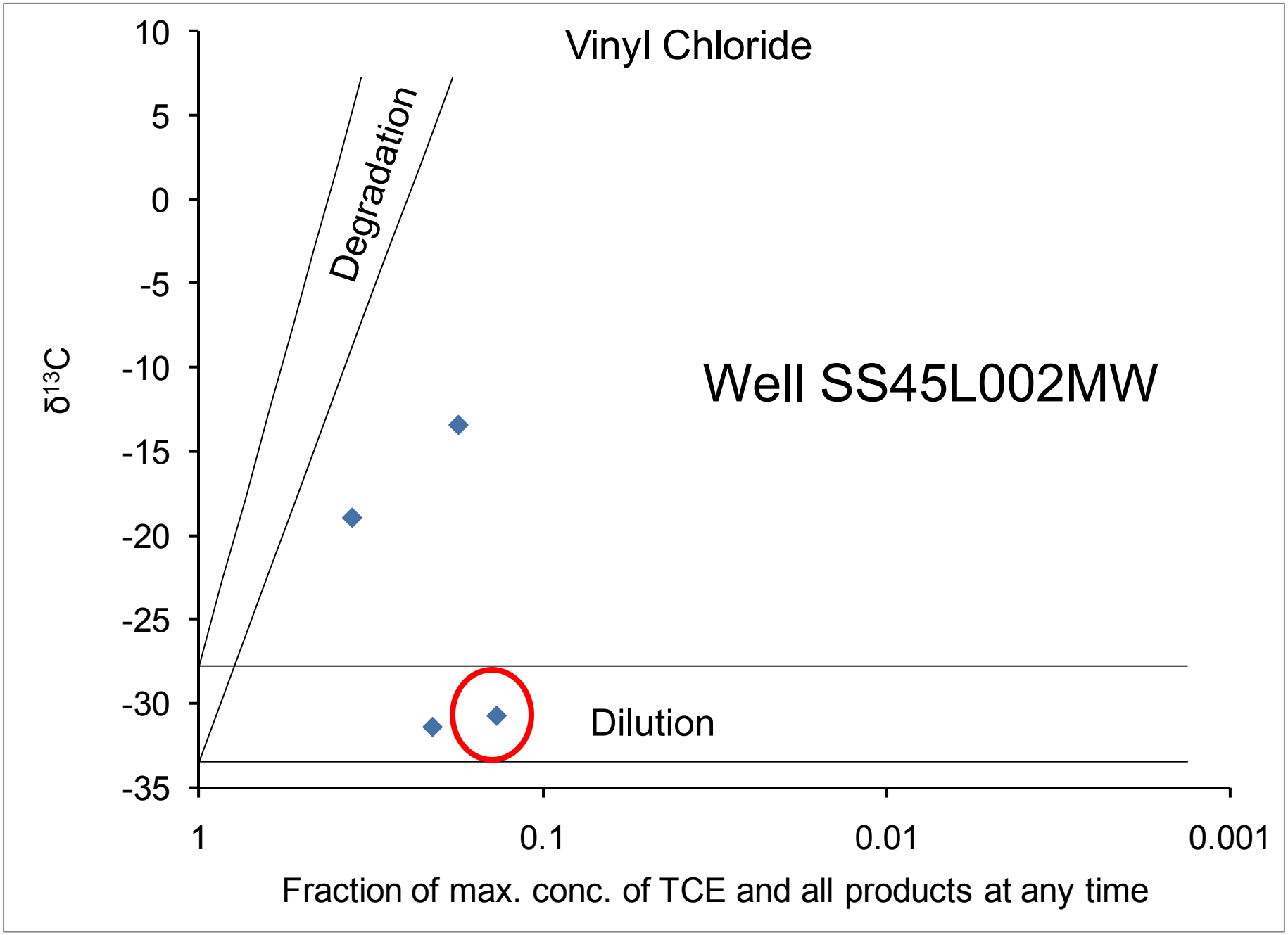

Vinyl Chloride
Degradation
Well #23
Dilution
10
5
0
-5
-10
-15
-20
-25
-30
-35
δ13C
1
0.1
0.01
0.001
Fraction of max. conc. of TCE and all products at any time

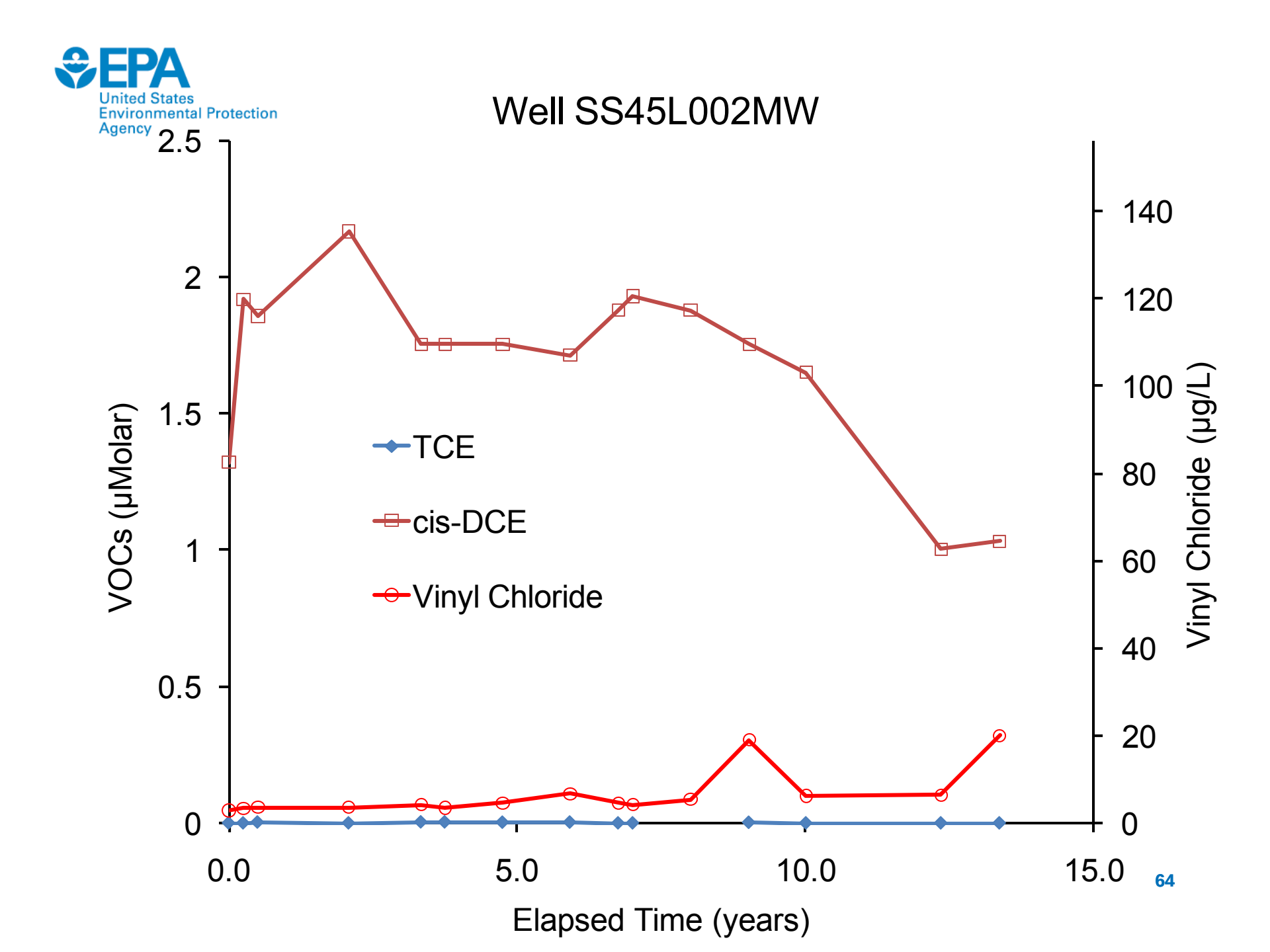

Well #23
TCE
cis-DCE
Vinyl Chloride
VOCs (µMolar)
Vinyl Chloride (µg/L)
Elapsed Time (years)
0
1
2
3
4
5
6
7
0
50
100
150
200
250
300
350
400
0.0
5.0
10.0
15.0

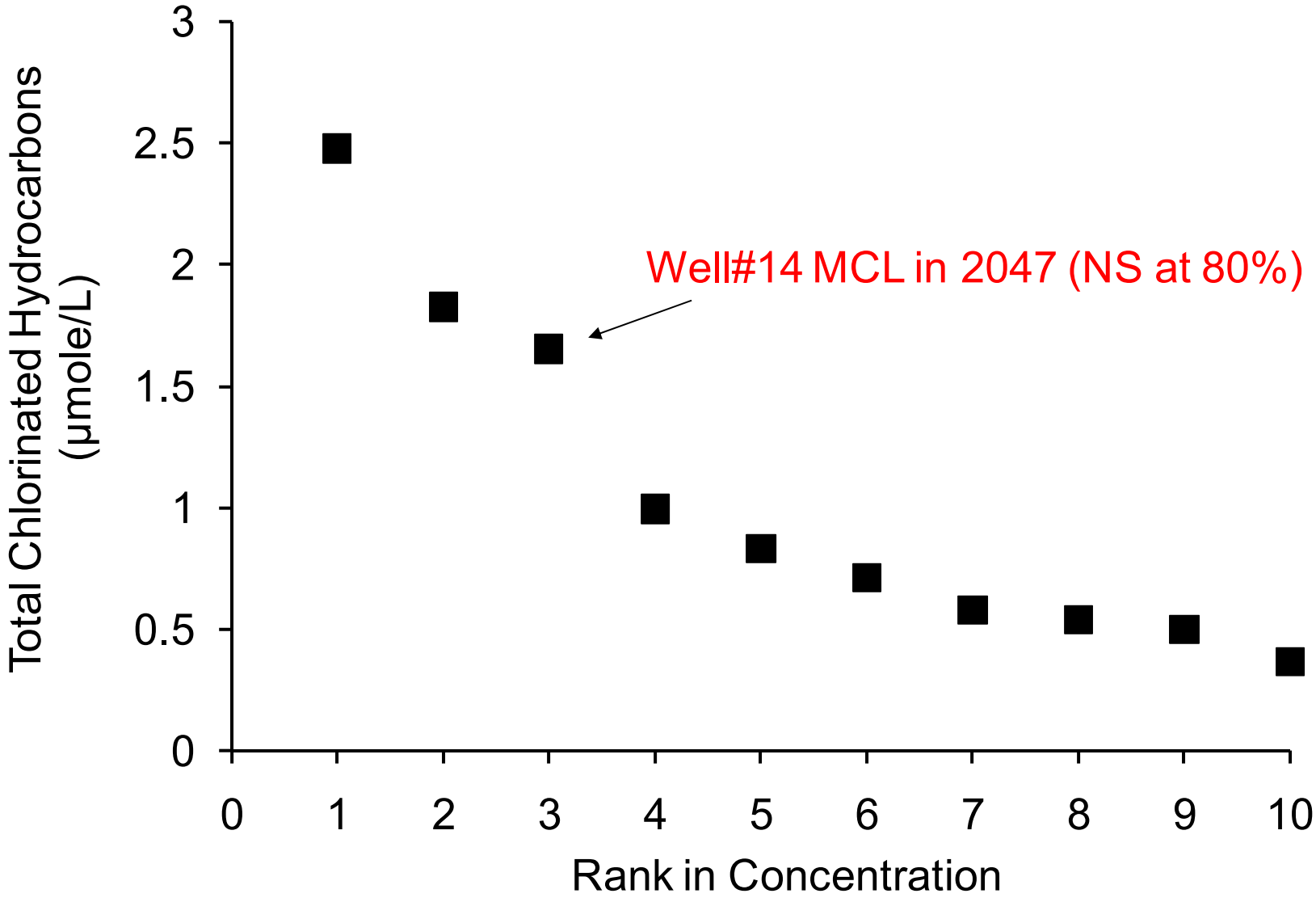

Total Chlorinated Hydrocarbons (µmole/L)
3
2.5
2
1.5
1
0.5
0
SS45L002MW Conc. increasing
0
1
2
3
4
5
6
7
8
9
10
Rank in Concentration

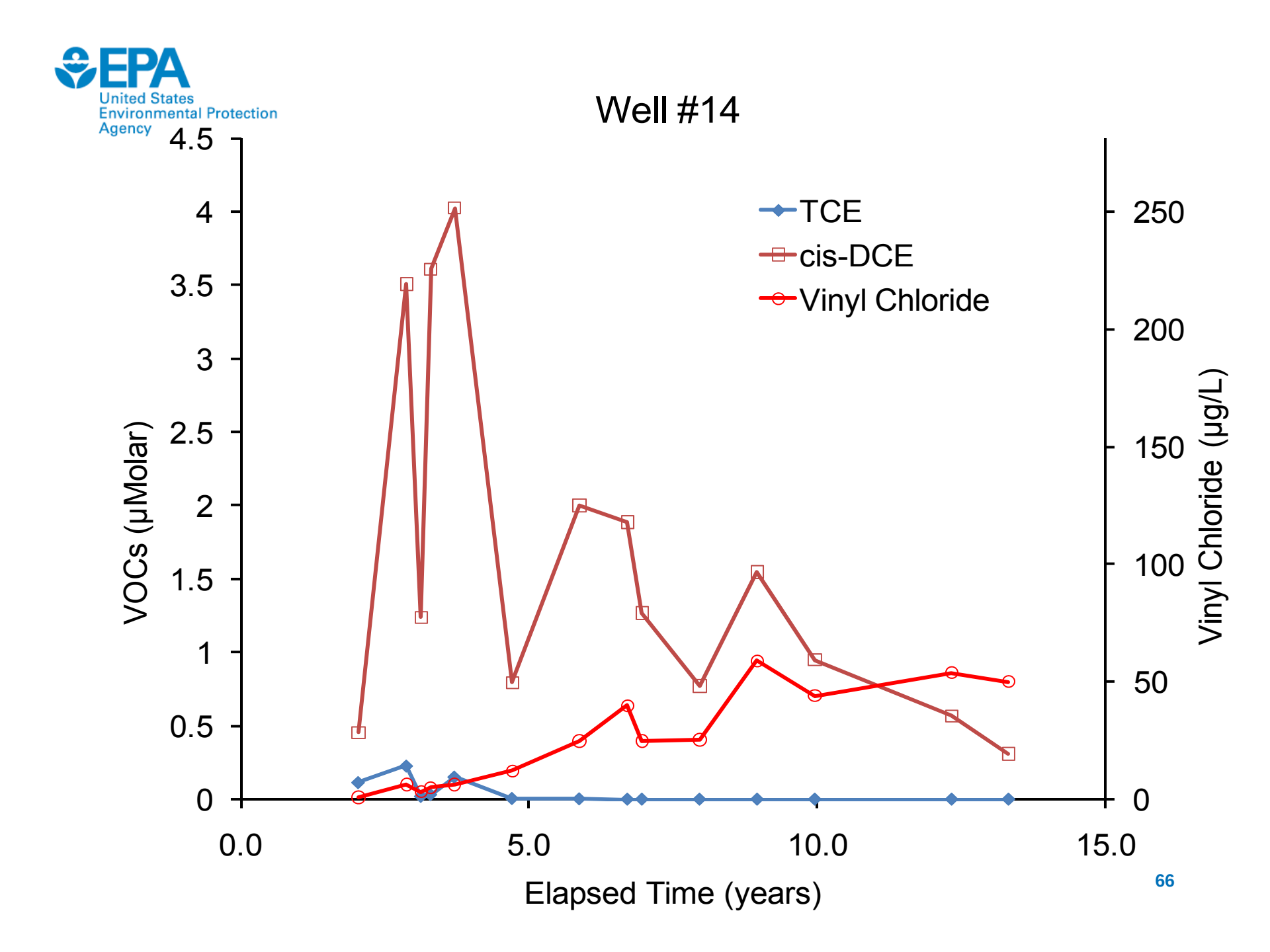

Well SS45L002MW
VOCs (µMolar)
Vinyl Chloride (µg/L)
Elapsed Time (years)
TCE
cis-DCE
Vinyl Chloride
2.5
2
1.5
1
0.5
0
140
120
100
80
60
40
20
0
0.0
5.0
10.0
15.0

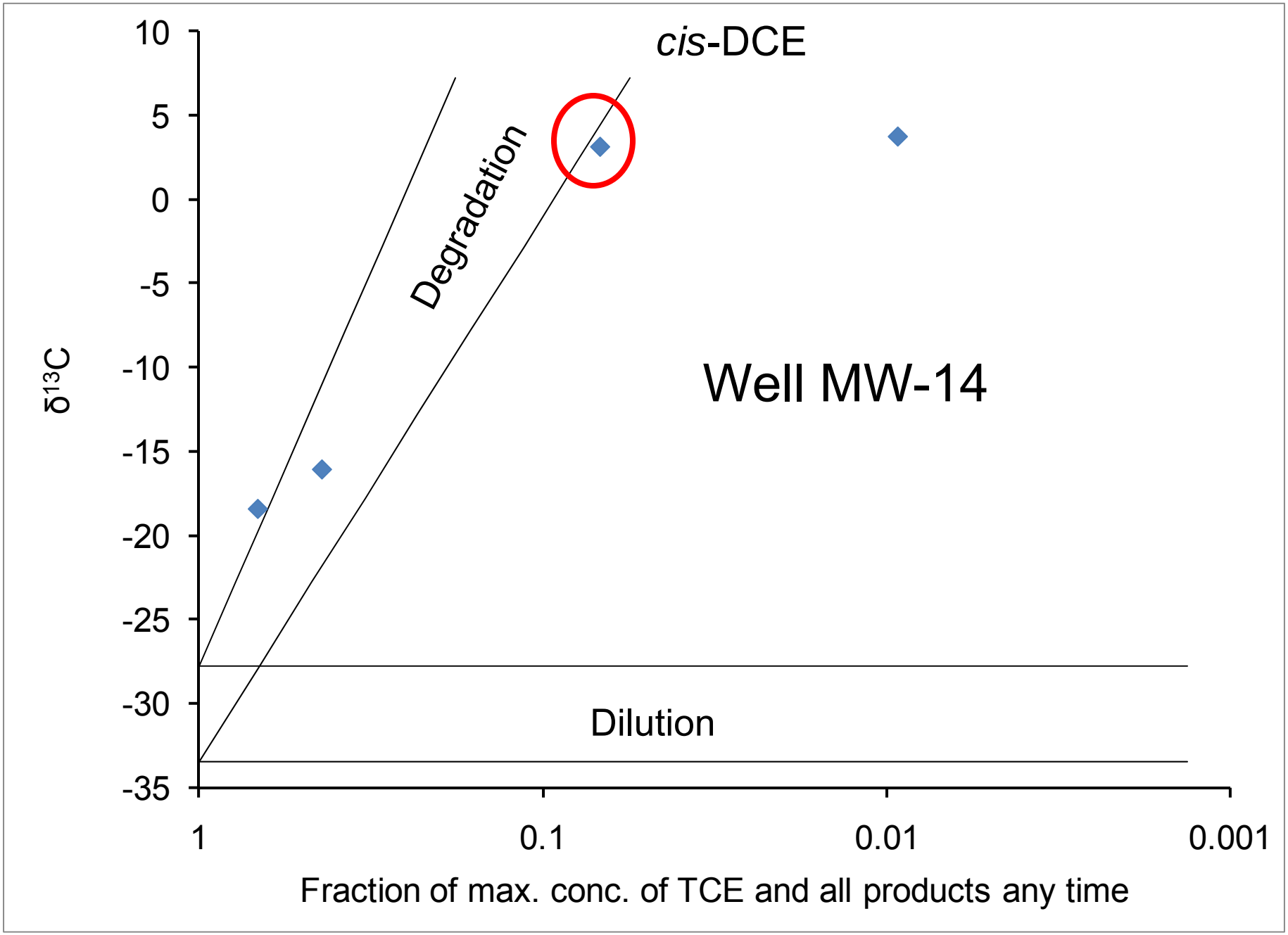

cis-DCE
Degradation
Well SS45L002MW
Dilution
δ13C
10
5
0
-5
-10
-15
-20
-25
-30
-35
1
0.1
0.01
0.001
Fraction of max. conc. of TCE and all products any time

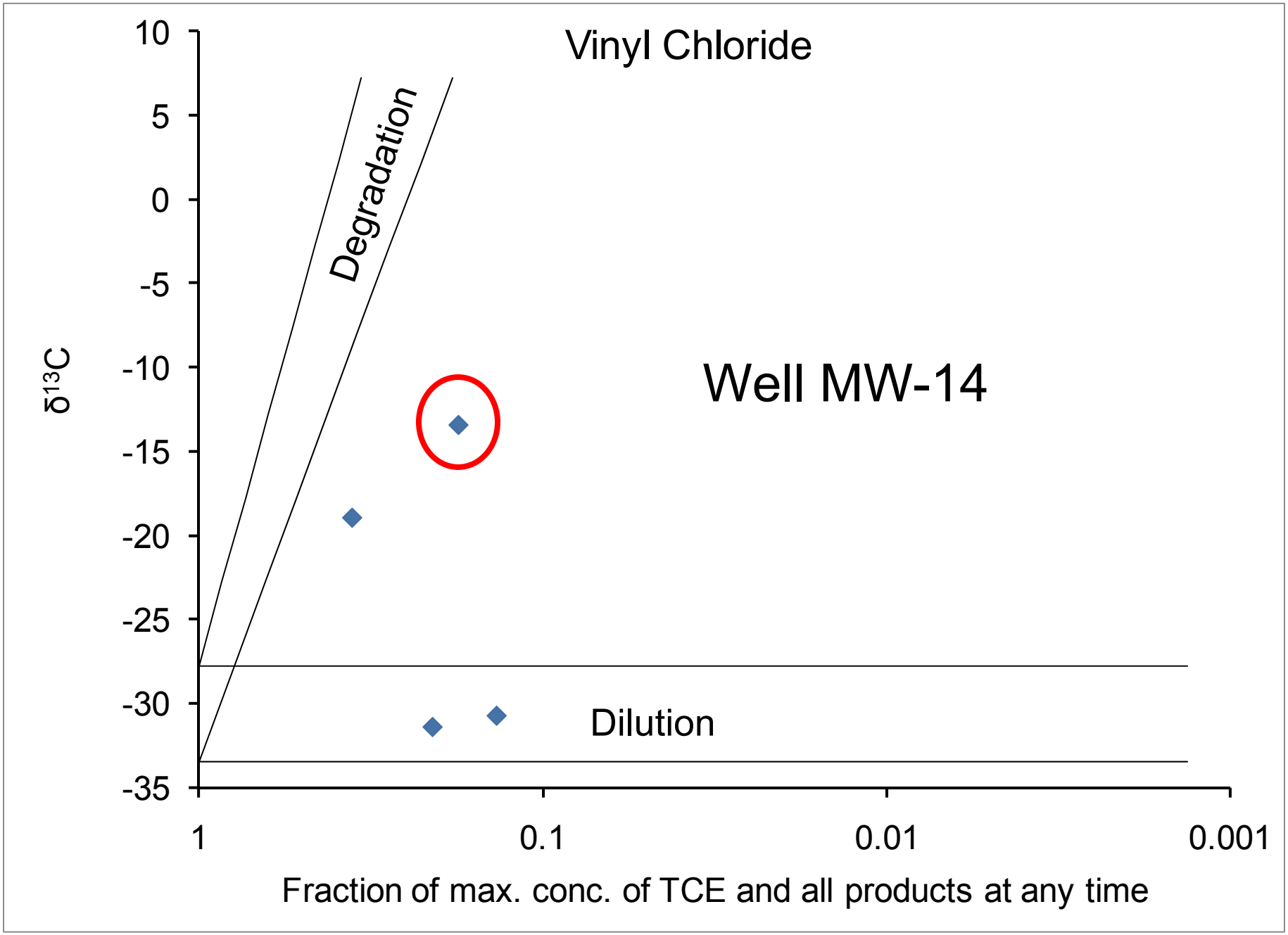

Vinyl Chloride
Degradation
Dilution
Well SS45L002MW
δ13C
10
5
0
-5
-10
-15
-20
-25
-30
-35
1
0.1
0.01
0.001
Fraction of max. conc. of TCE and all products at any time

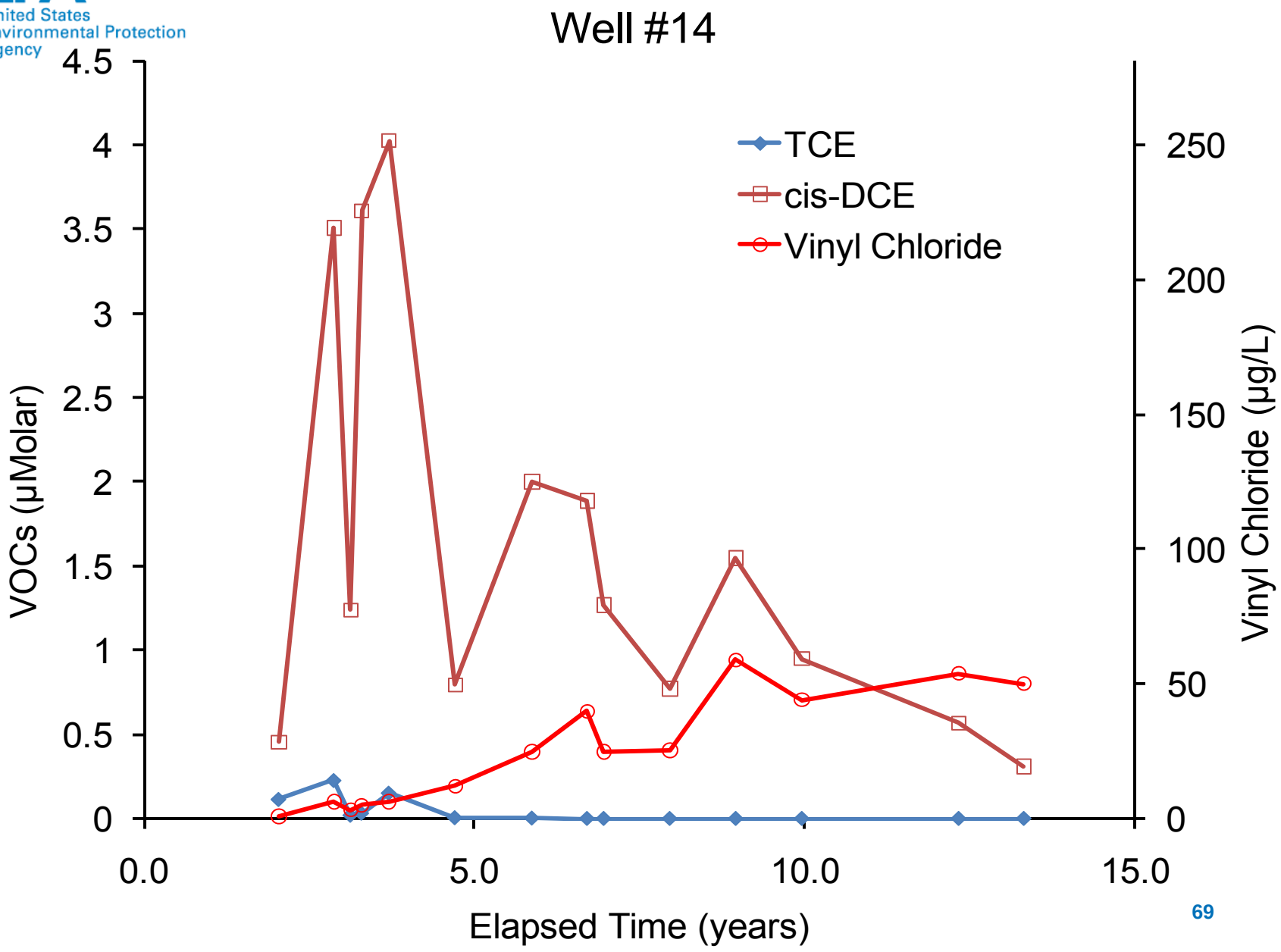

Well SS45L002MW
VOCs (µMolar)
Vinyl Chloride (µg/L)
Elapsed Time (years)
TCE
cis-DCE
Vinyl Chloride
2.5
2
1.5
1
0.5
0
140
120
100
80
60
40
20
0
0.0
5.0
10.0
15.0

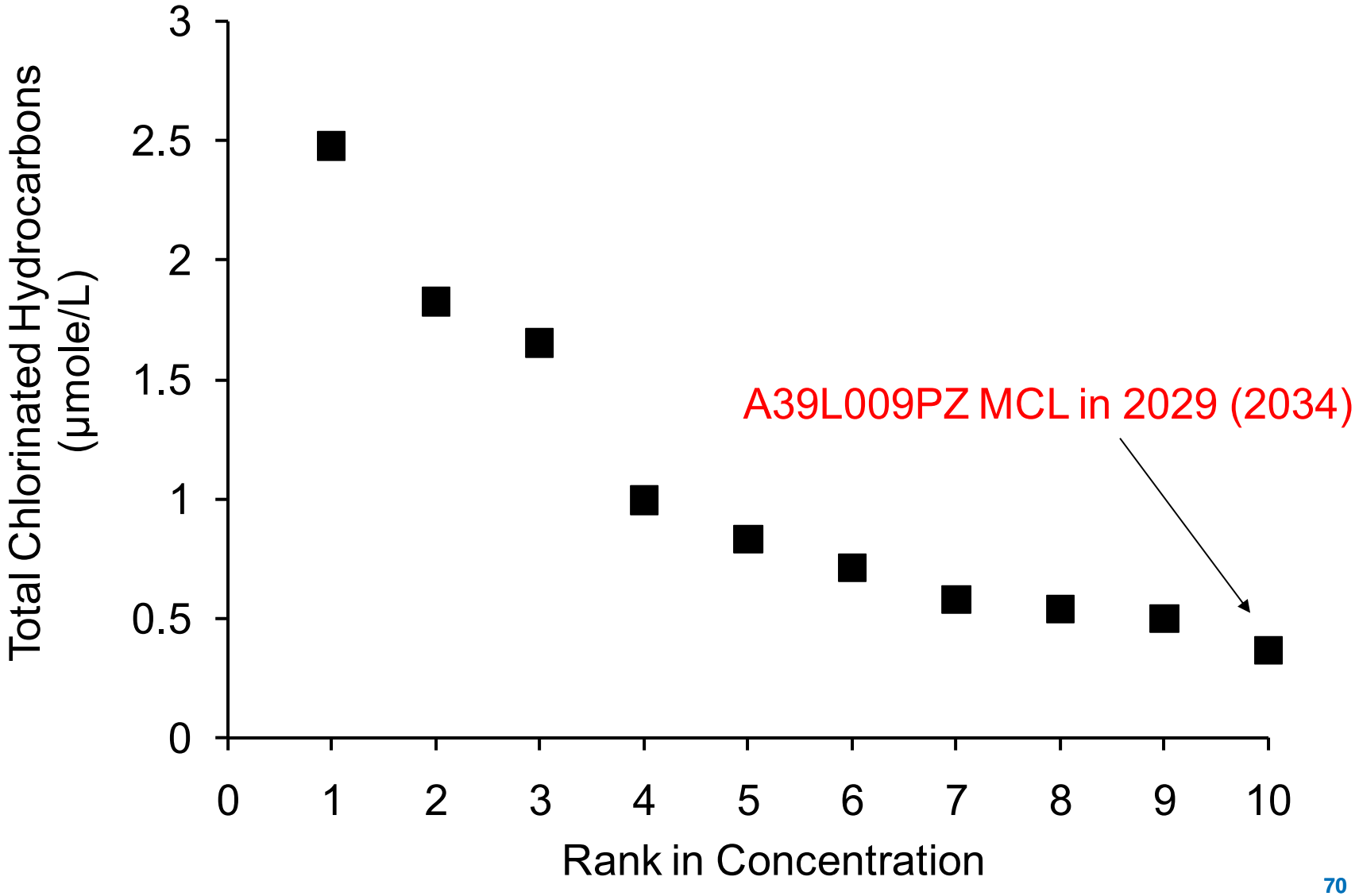

Total Chlorinated Hydrocarbons (µmole/L)
3
2.5
2
1.5
1
0.5
0
Well#14 MCL in 2047 (NS at 80%)
0
1
2
3
4
5
6
7
8
9
10
Rank in Concentration

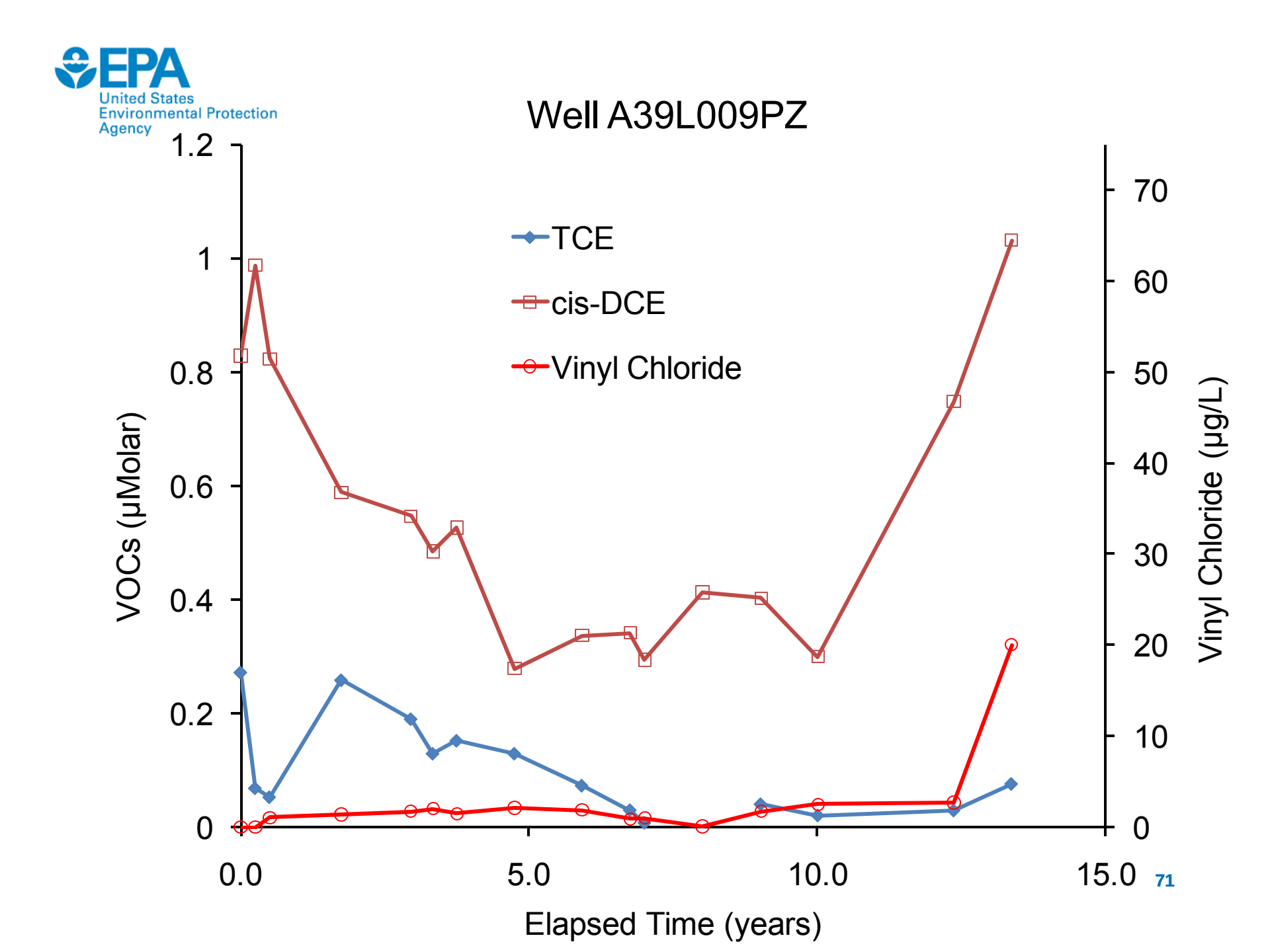

Well #14
TCE
cis-DCE
Vinyl Chloride
VOCs (µMolar)
Vinyl Chloride (µg/L)
Elapsed Time (years)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
0
50
100
150
200
250
0.0
5.0
10.0
15.0

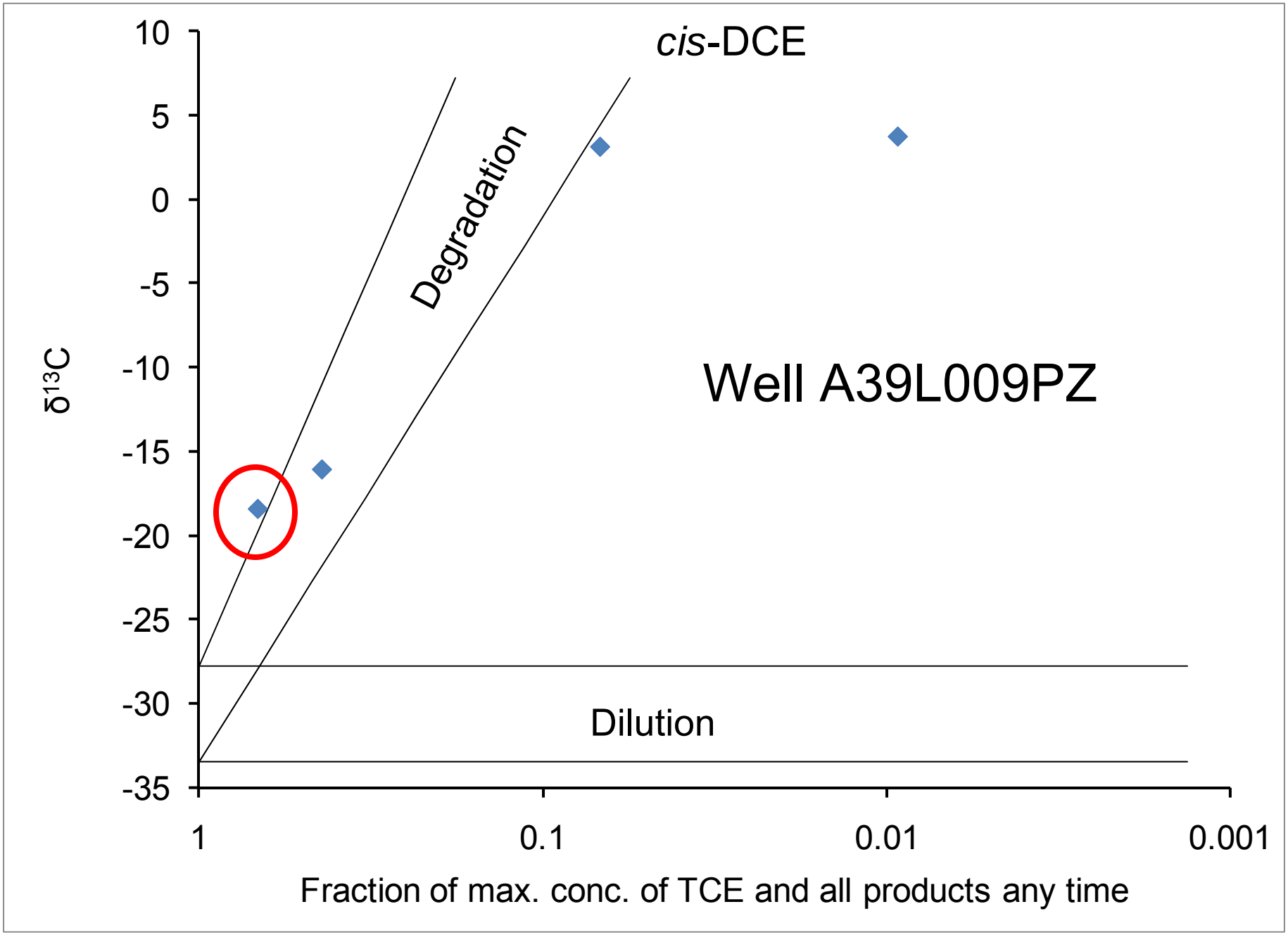

cis-DCE
Degradation
Well MW-14
Dilution
δ13C
10
5
0
-5
-10
-15
-20
-25
-30
-35
1
0.1
0.01
0.001
Fraction of max. conc. of TCE and all products any time

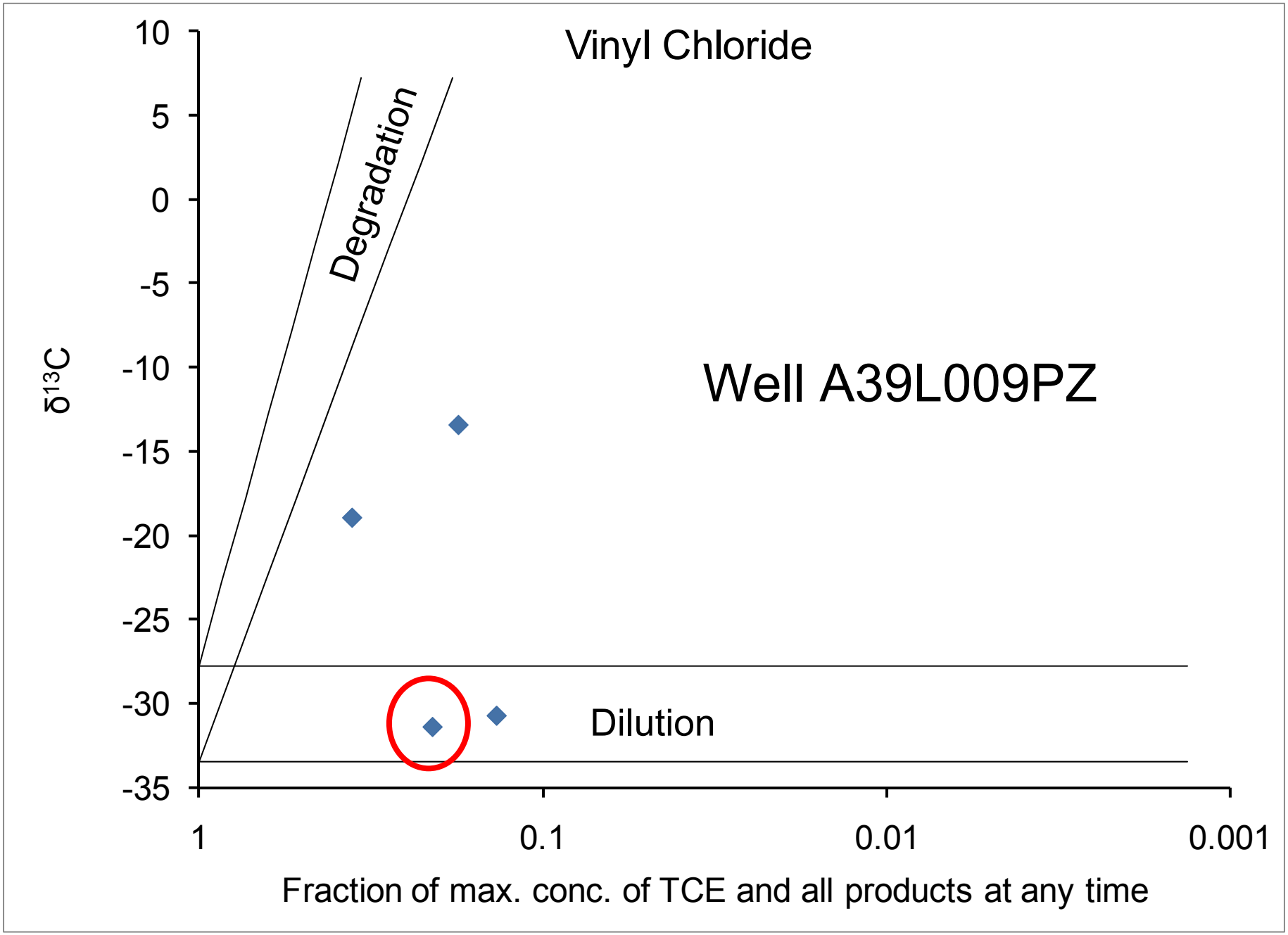

Vinyl Chloride
Degradation
Well MW-14
Dilution
10
5
0
-5
-10
-15
-20
-25
-30
-35
δ13C
1
0.1
0.01
0.001
Fraction of max. conc. of TCE and all products at any time

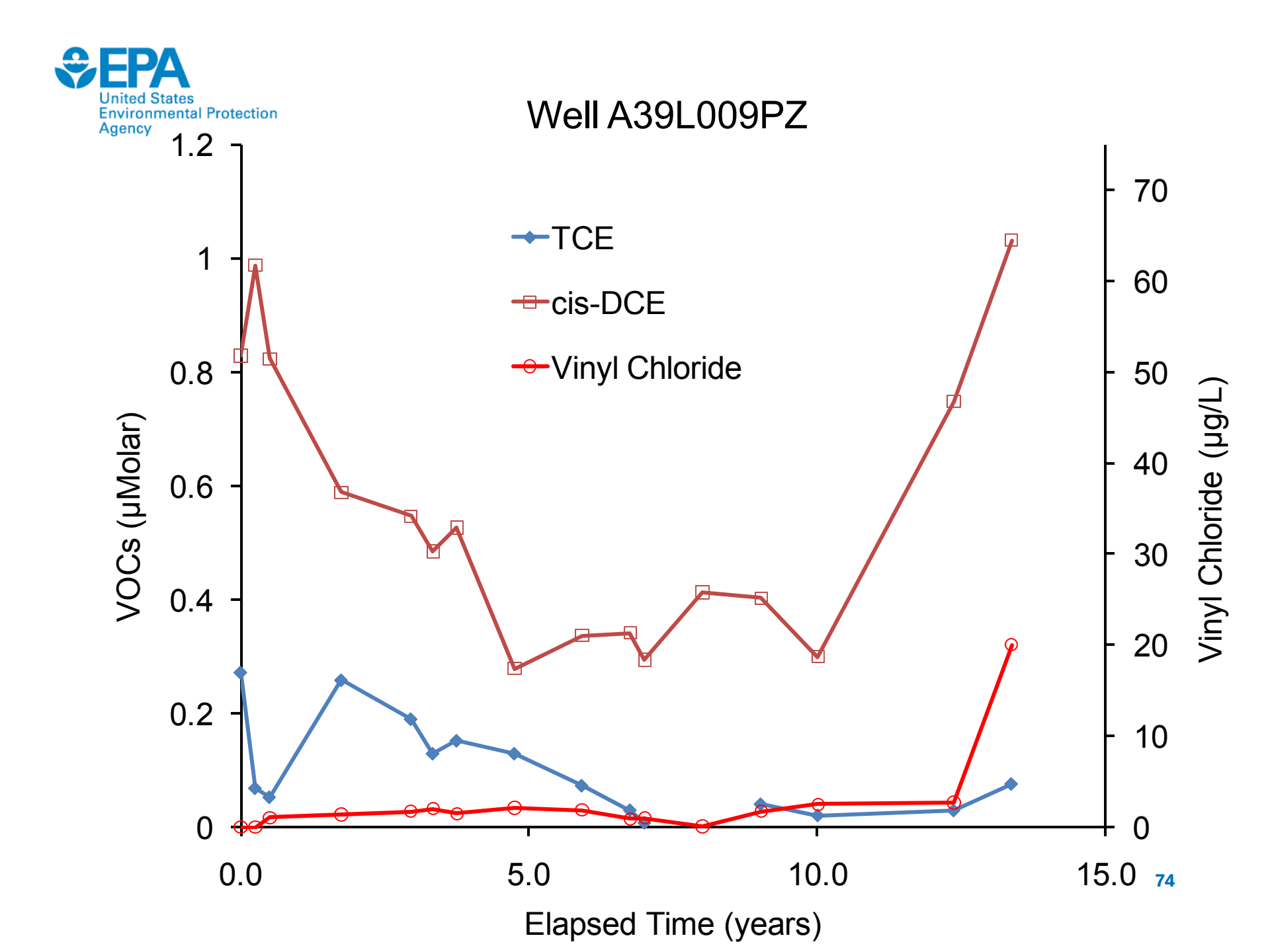

Well #14
TCE
cis-DCE
Vinyl Chloride
VOCs (µMolar)
Vinyl Chloride (µg/L)
Elapsed Time (years)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
0
50
100
150
200
250
0.0
5.0
10.0
15.0


Total Chlorinated Hydrocarbons (µmole/L)
3
2.5
2
1.5
1
0.5
0
0
1
2
3
4
5
6
7
8
9
10
Rank in Concentration
A39L009PZ MCL in 2029 (2034)


Well A39L009PZ
TCE
cis-DCE
Vinyl Chloride
VOCs (µMolar)
Vinyl Chloride (µg/L)
Elapsed Time (years)
0
0.2
0.4
0.6
0.8
1
1.2
0
10
20
30
40
50
60
70
0.0
5.0
10.0
15.0


cis-DCE
Degradation
Well A39L009PZ
Dilution
δ13C
10
5
0
-5
-10
-15
-20
-25
-30
-35
1
0.1
0.01
0.001
Fraction of max. conc. of TCE and all products any time


Vinyl Chloride
Degradation
Well A39L009PZ
Dilution
10
5
0
-5
-10
-15
-20
-25
-30
-35
δ13C
1
0.1
0.01
0.001
Fraction of max. conc. of TCE and all products at any time


Well A39L009PZ
TCE
cis-DCE
Vinyl Chloride
VOCs (µMolar)
Vinyl Chloride (µg/L)
Elapsed Time (years)
0
0.2
0.4
0.6
0.8
1
1.2
0
10
20
30
40
50
60
70
0.0
5.0
10.0
15.0

Commercial Source of Analytical Services

PCR

SiREM Labs
130 Research Lane, Suite 2
Guelph, Ontario
Canada, N1G 5G3

Phil Dennis
1-866-251-1747 ext. 238
pdennis@siremlab.com

United States Environmental Protection Agency

Commercial Source of Analytical Services

PCR, PLFA, Stable Isotope Probes

Microbial Insights, Inc.
2340 Stock Creek Blvd.
Rockford, TN
37853
United States

Greg Davis
Tel: 865-573-8188,
Fax: 865-573-8133,
Email: gdavis@microbe.com

Commercial Source of Analytical Services

CSIA

Patrick McLoughlin
pmcloughlin@microseeps.com
Microseeps Inc.
University of Pittsburgh Applied Research Center
220 William Pitt Way
Pittsburgh, PA 15238
412 826 5245 ph
412 826 3433 fax

Commercial Source of Analytical Services

CSIA

Paul Philp
Department of Geology and Geophysics
100 East Boyd Avenue
University of Oklahoma
Norman, Oklahoma 73019
405 325 4469
fax (405)-325-3140
pphilp@ou.edu

Commercial Source of Analytical Services

CSIA

Zymax Forensics
Yi Wang
Director, Zymax Forensics Isotope
600 South Andreasen Drive
Suite B,
Escondido, California
92029
yi.wang@zymaxUSA.com

Commercial Source of Analytical Services

CSIA

Barbara Sherwood Lollar
Department of Geology
University of Toronto
22 Russell Street, Toronto, Ontario
M5S 3B1

Phone: (416) 978-0770
Fax: (416) 978-3938
E-mail: bslollar@chem.utoronto.ca