# Investigating Biodegradation of Chlorofluorocarbons Using Compound Specific Isotope Analysis (CSIA)

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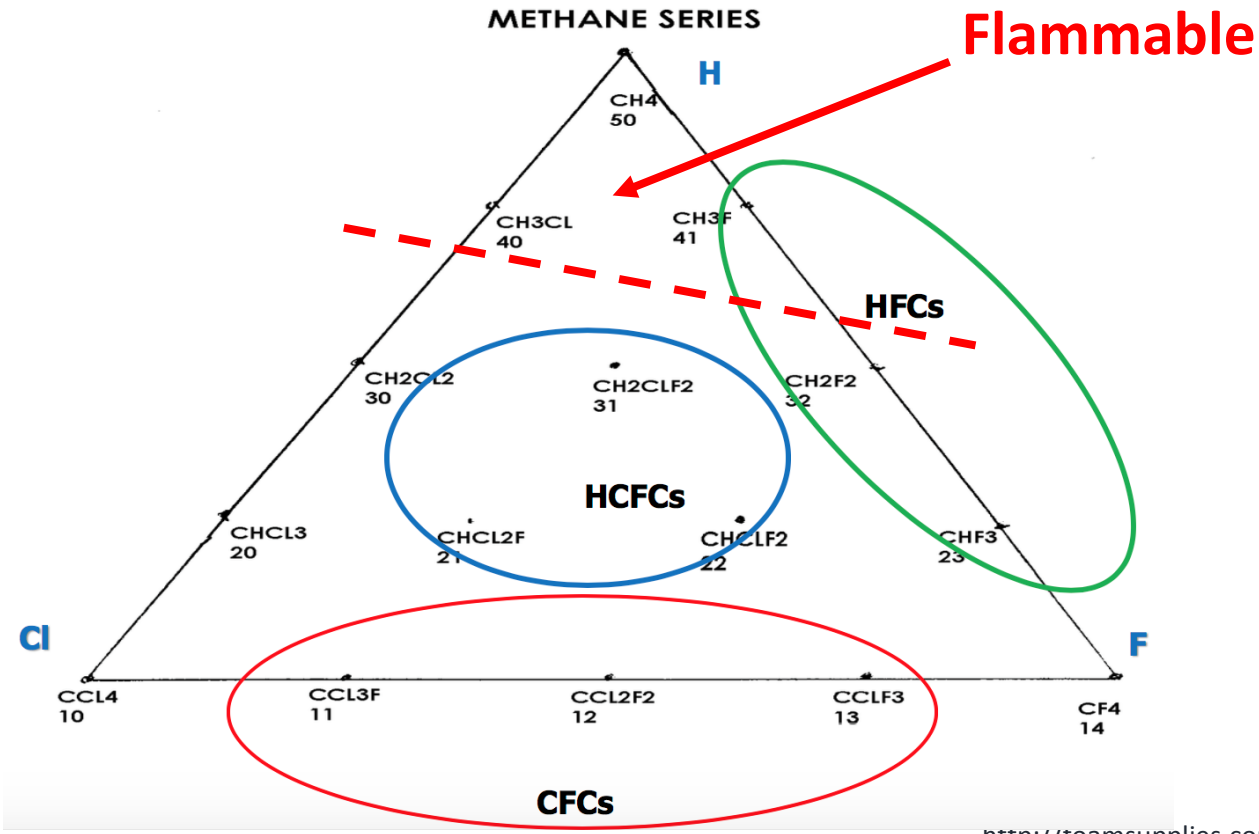
# Presentation Outline

- CFCs Information and History
- Introduction to Isotope Studies
- CSIA in Contaminant Hydrogeology
- Experimental Method
- CFC-113 and CFC-11 Experimental Results
- Field Application

# Chlorofluorocarbons (CFCs)

- Anthropogenically produced aliphatic organic compounds part of the CH4 series.

- Low-flammability, low toxicity, and are extremely volatile and persistent.

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http://foamsupplies.com/resources-list/papers/2012-10-Newark_NJ_USA.pdf

# Chlorofluorocarbons (CFCs)

## Applications:

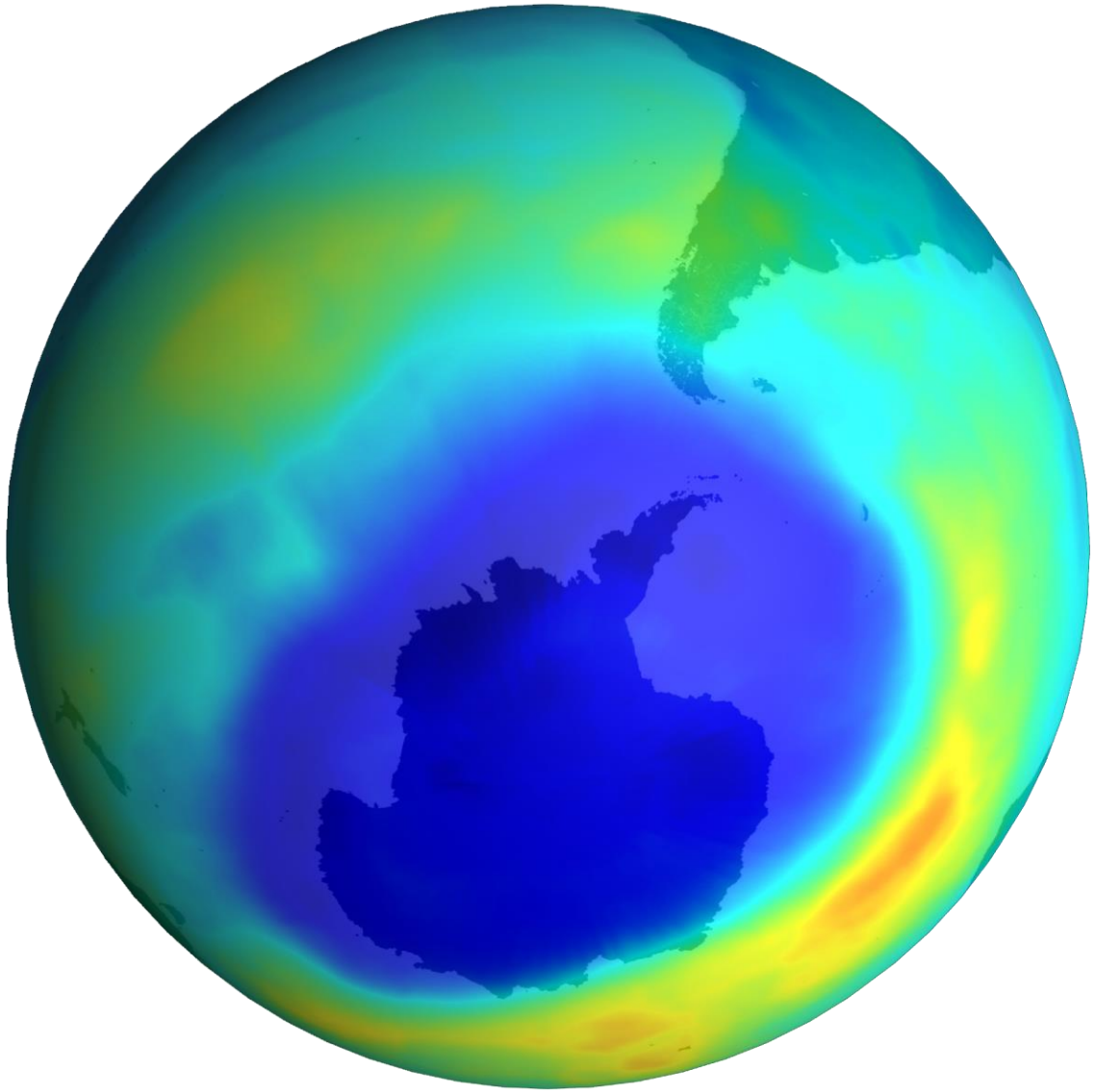
- Refrigerants
- Air conditioning systems
- Foaming agents
- Propellants (aerosols) etc.

Montzka et al. 2011

http://inhabitat.com/the-earths-ozone-layer-increases-for-the-first-time-in-35-years/

# Stratospheric Ozone Hole

**Frank Sherwood Rowland**



Retrieved from https://www.nobelprize.org/nobel_prizes/chemistry/laureates/1995/rowland-bio.html

Retrieved from http://earthobservatory.nasa.gov/IOTD/view.php?id=38835

# CFCs Also an Important Issue in Groundwater

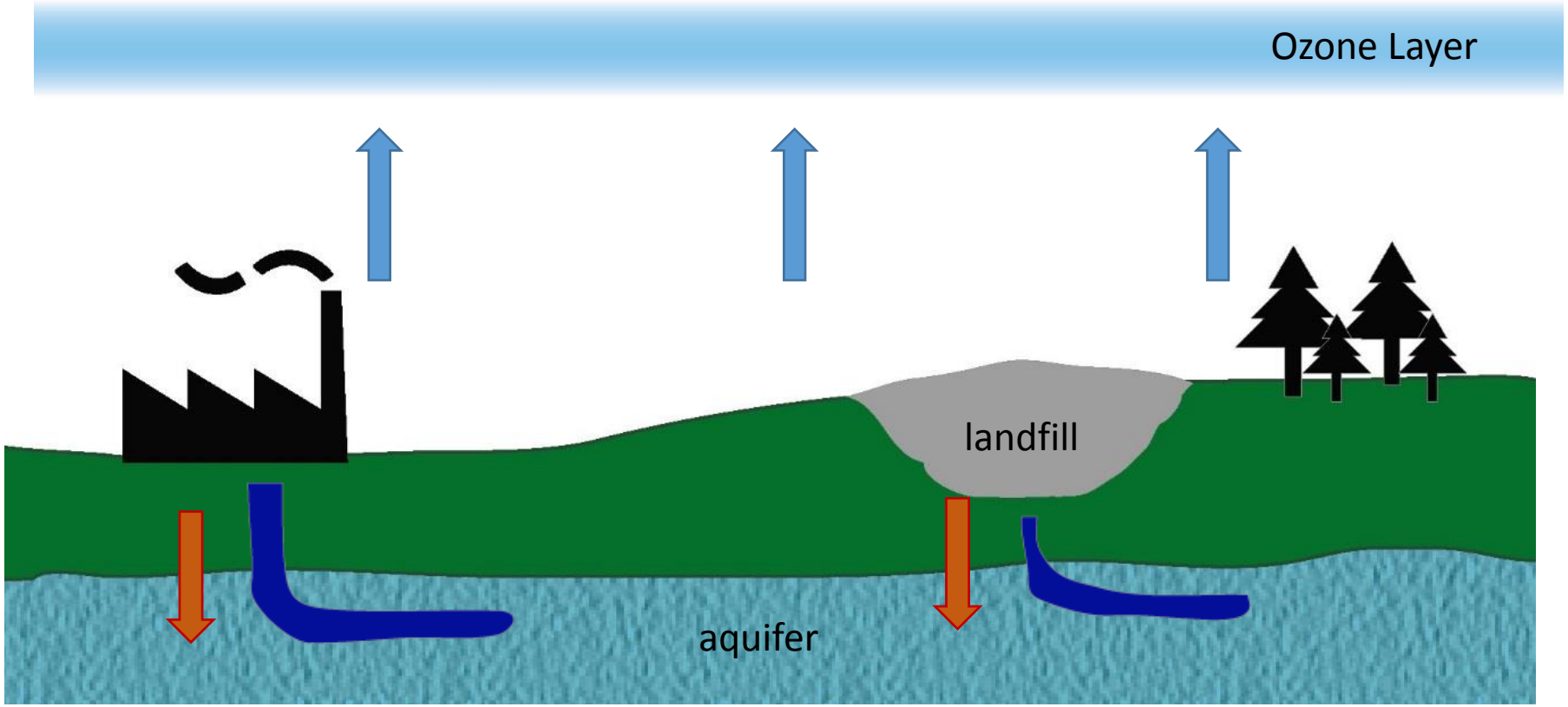
**Groundwater contaminants:**

- Production sites
- Landfill sites
- Foam materials
- Solvent spills

**Concentrations in groundwater:**

- Up to 40 µg/L (non-point sources)
- Up to 10 µg/L (point sources)

(Squillace et al., 1999)



# Chlorofluorocarbons (CFCs)

**Remediation strategies:**

- Abiotic degradation: reaction with ZVI
- Biotic degradation: microbial dechlorination

Scheutz et al (2000), Loveley and Woodward, 1992

**Limitation of concentration-only studies: distinguish degradation from:**

- Dilution (mixing or dispersion)
- Volatilization
- Sorption

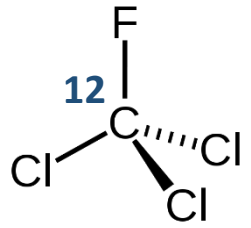
EPA/600/R-08/148 (Hunkeler et al., 2009)

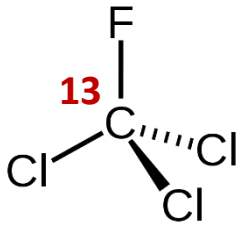
**Concentration decreases do not necessarily confirm transformation and remediation**

# Compound Specific Isotope Analysis (CSIA)

- Naturally occurring differences in $^{13}C$ vs $^{12}C$

Delta value ($\delta^{13}C$):




$$\delta^{13}C = \frac{\frac{^{13}C}{^{12}C}_{sample}}{\frac{^{13}C}{^{12}C}_{standard}} - 1$$

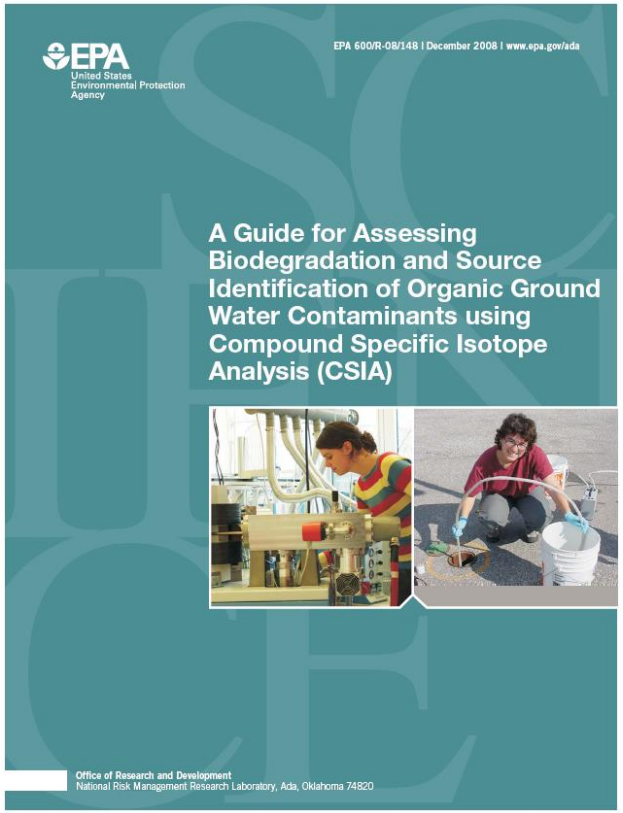
$$\delta^{13}C \text{ in } ‰ = \frac{(^{13}C/\,^{12}C_{\,sample} - {}^{13}C/\,^{12}C_{standard})}{^{13}C/\,^{12}C_{\,standard}} \times 1000$$

# CSIA – Applications to Contaminant Hydrogeology

- **Kinetic Isotope Effect:** The reaction rate for molecules containing exclusively $^{12}C$ atoms ($k_{12}$) is slightly faster than the reaction rate for molecules containing $^{13}C$ atoms ($k_{13}$).

- The reaction rate during chemical degradation or biodegradation **potentially causes measurable changes in $\delta^{13}C$ signatures of hydrocarbon contaminants in groundwater.**

→ **Isotope Fractionation**

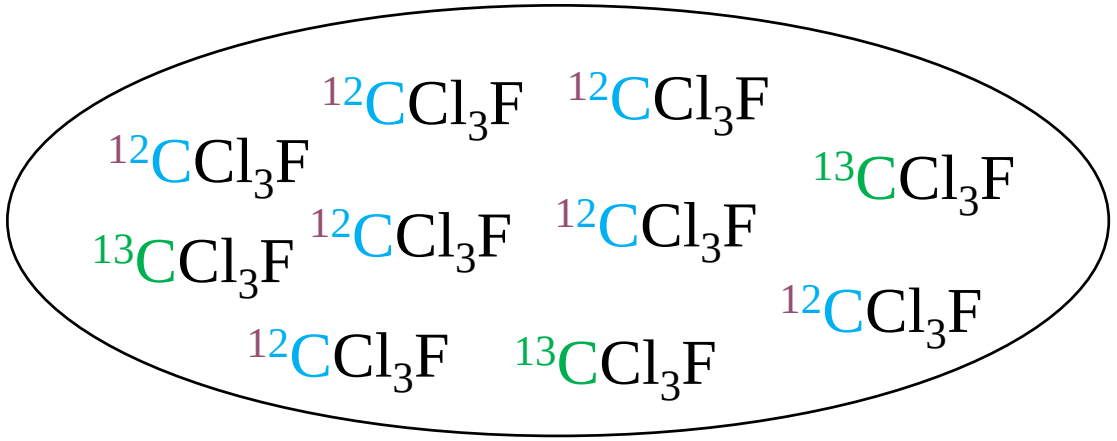




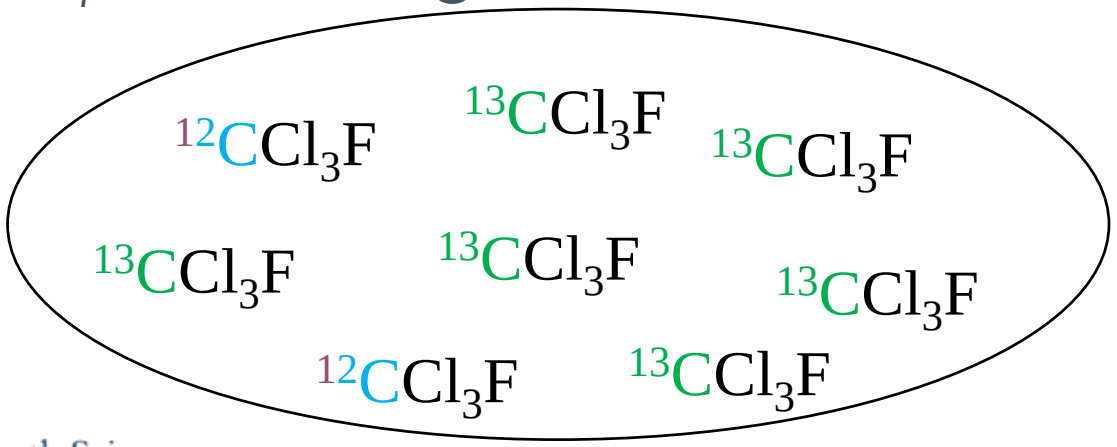

(Sherwood Lollar et al., 1999; Meckenstock, Richnow *et* al. 1999; Bloom *et al*. 2000; Hunkeler *et* al. 1999)

# Theory of Isotope Fractionation

*$T_o$ - Before degradation*




*$T_1$ - Post degradation*




Preferential degradation of $CCl_3F$ (CFC11)

$k_{12C} > k_{13C}$

Remaining $CCl_3F$ progressively isotopically enriched in $^{13}C$ i.e. **less negative $\delta^{13}C$ value**

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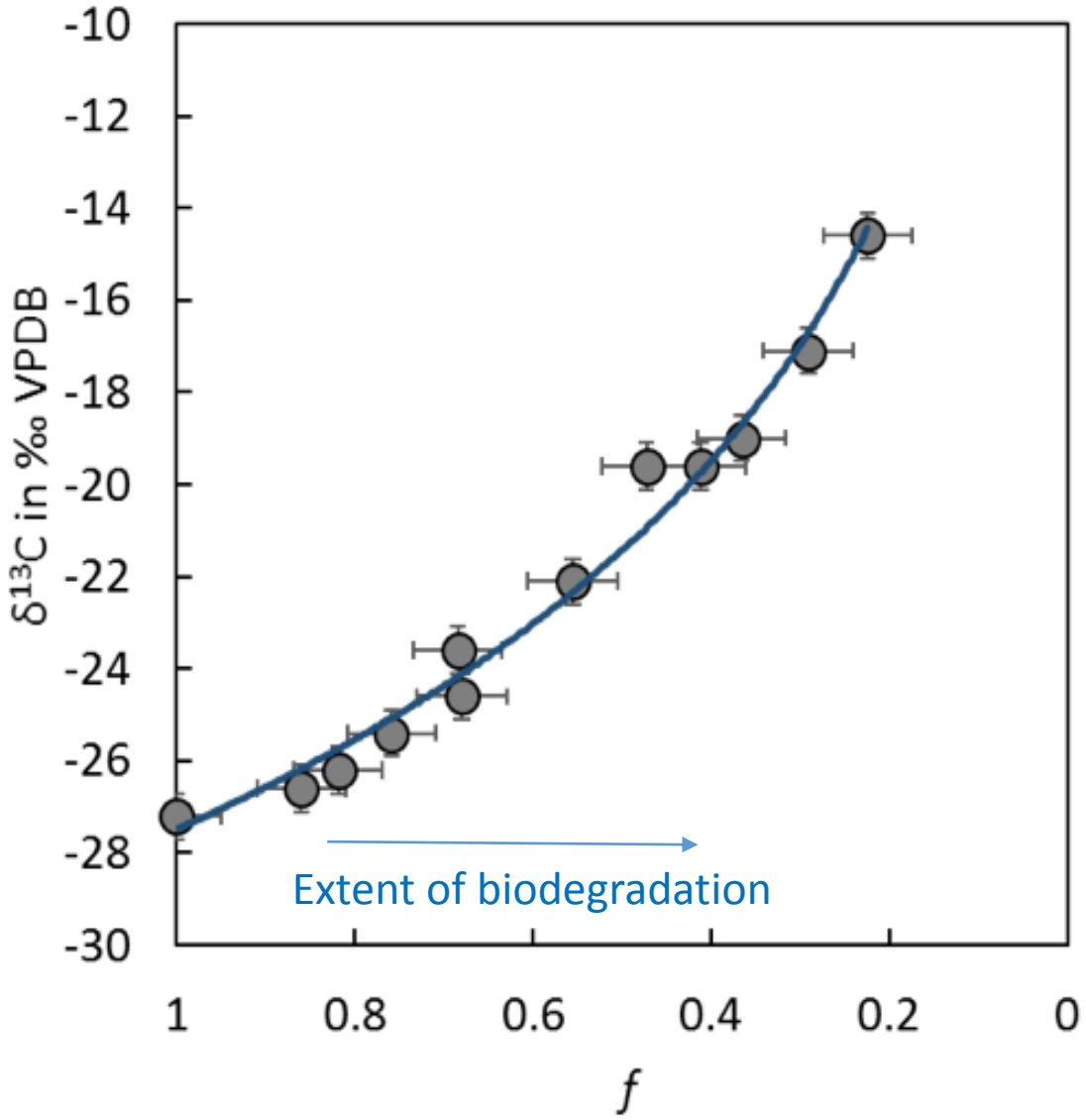
# Enrichment factor (ε) – a measure of fractionation




1. As biodegradation increases, $\delta^{13}C$ gets less negative (enrichment trend).

2. The signal is typically reproducible.

3. Expressed as an enrichment factor (ε).

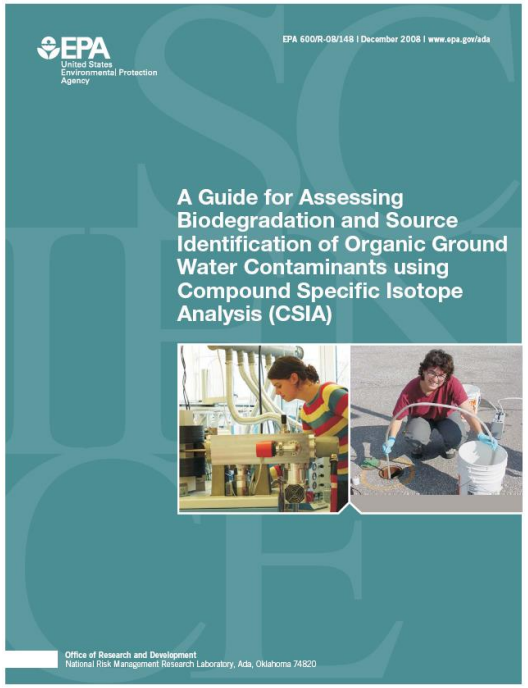
# CSIA – Typical Framework

- **Enrichment Trend** in $^{13}C$ in remaining contaminant (less negative $^{13}C$ values) as indicator of bond cleavage during degradation

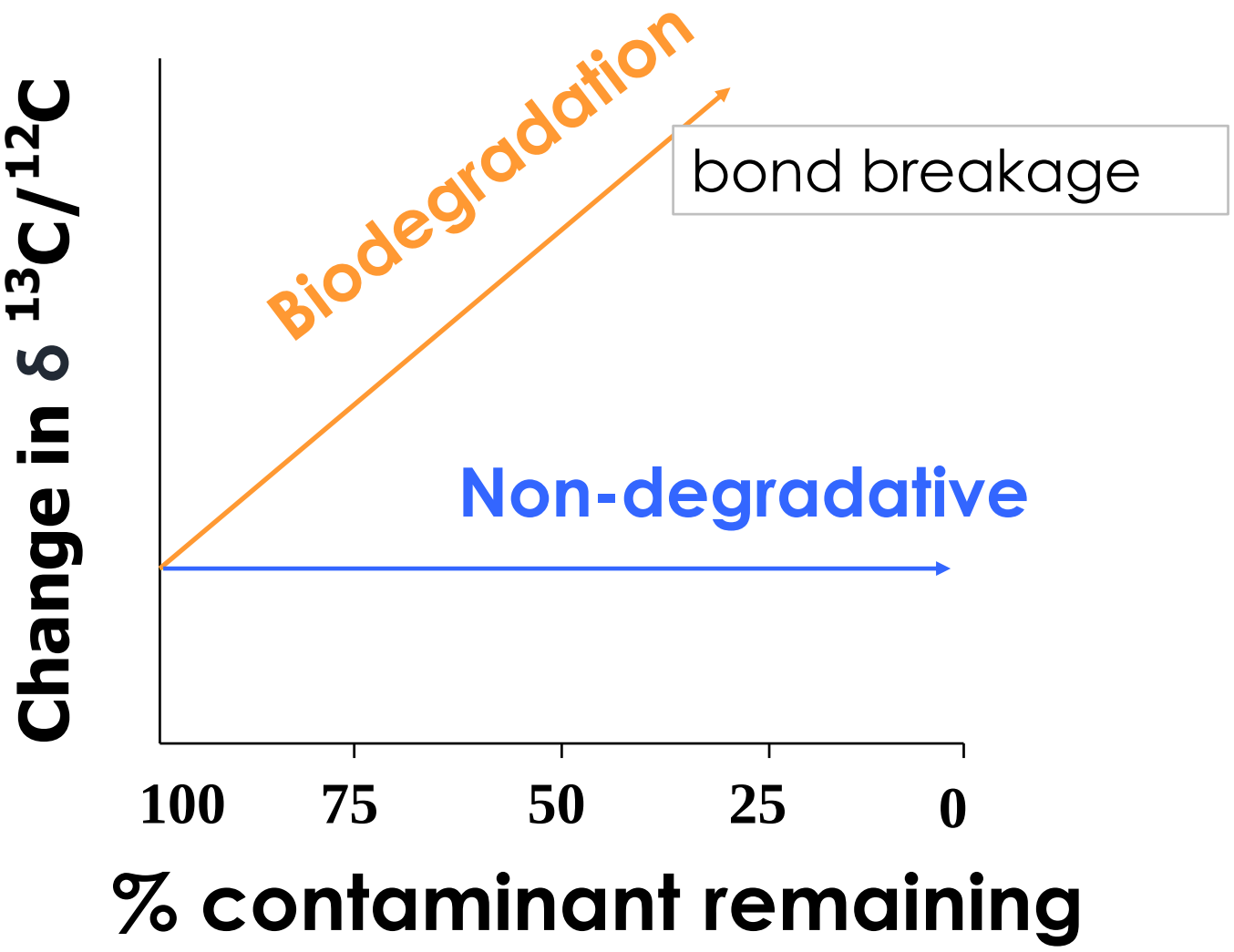
- CSIA can distinguish mass loss due to **strong carbon isotope fractionation associated with biodegradation → Non-conservative behavior**

- Versus small- or relatively non-fractionating processes such as **volatilization, diffusion, sorption , etc. → conservative behavior**

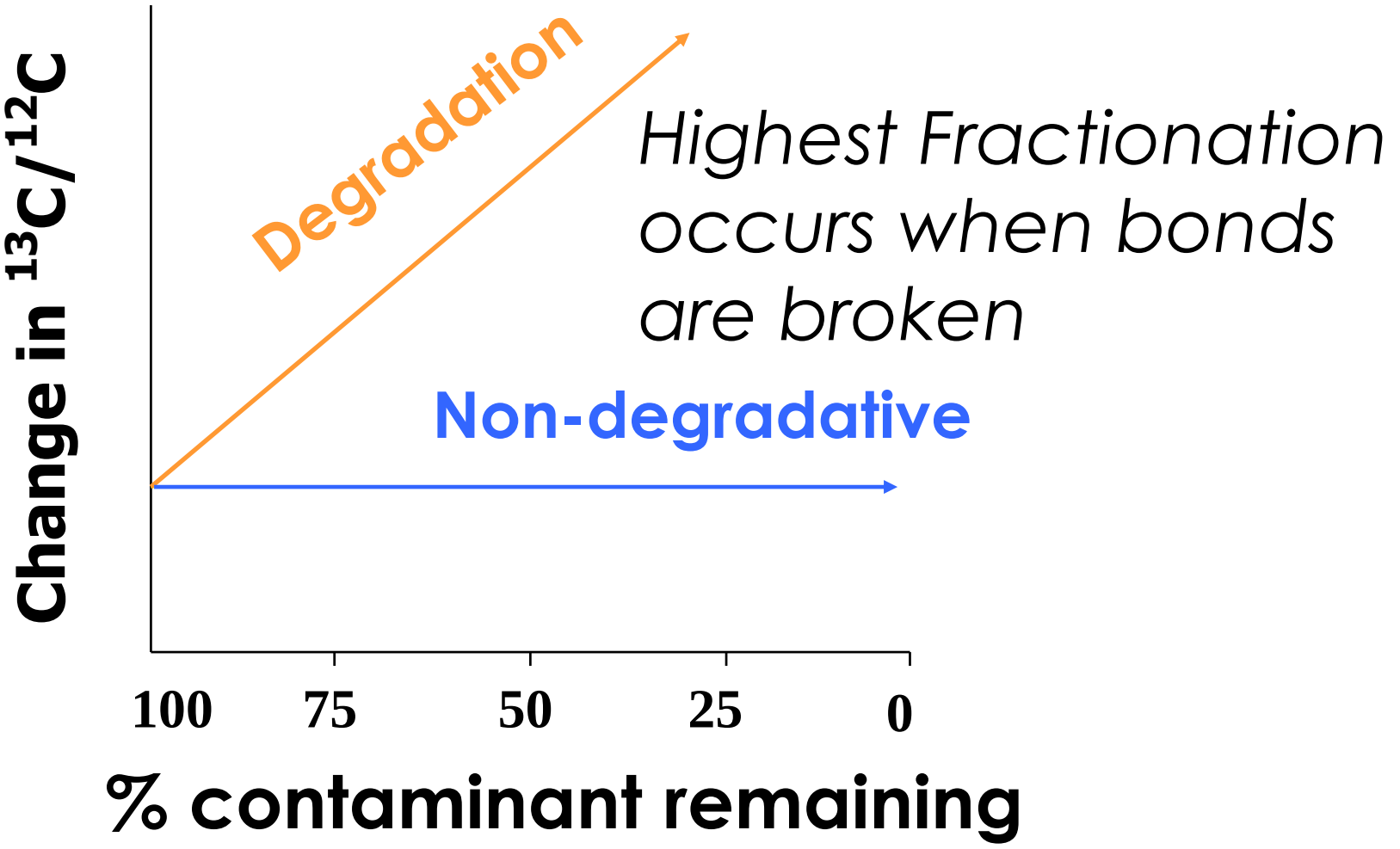
*(Slater et al., 1998; 2000; Harrington et al., 1999; Poulson & Drever, 1999; Wang & Huang, 2001; Hunkeler et al. 2004; Kopinke et al., 2005; Elsner at al., 2007; Bouchard et al. 2007)*



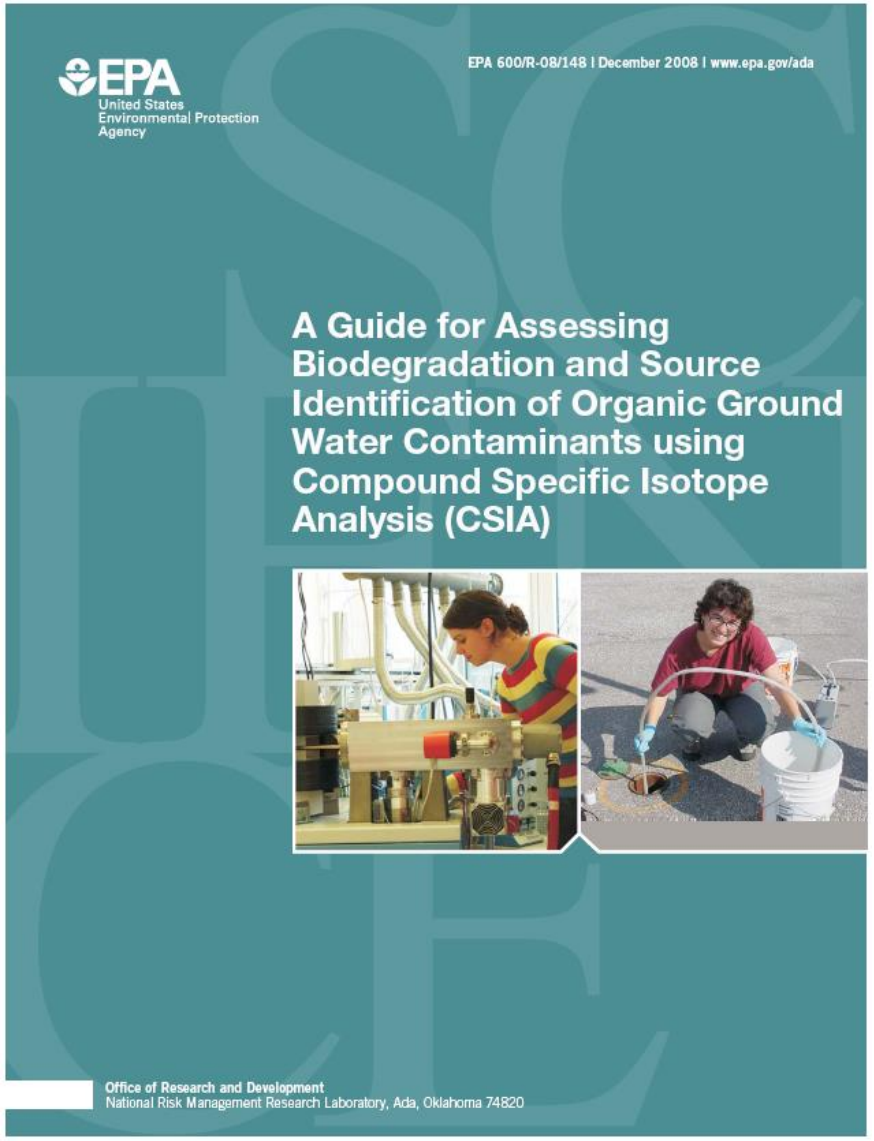
# Non-conservative vs. Conservative



# Non-conservative vs. Conservative



# Much of this information has been gathered for:




**Chlorinated ethenes**
**Chlorinated ethanes**
**BTEX, alkanes, MTBE**

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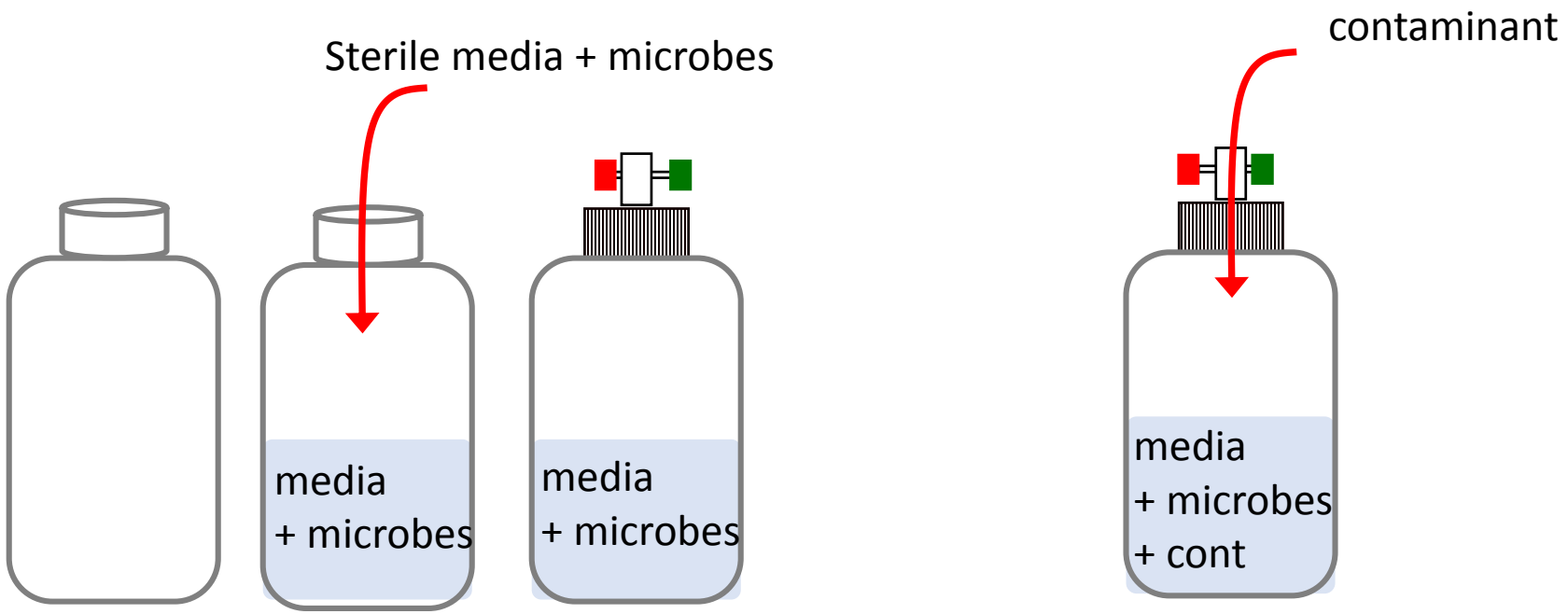
**EPA 600/R-08/148**

# Chlorofluorocarbons (CFCs) Experimental Method

# EXPERIMENTAL PROCEDURE

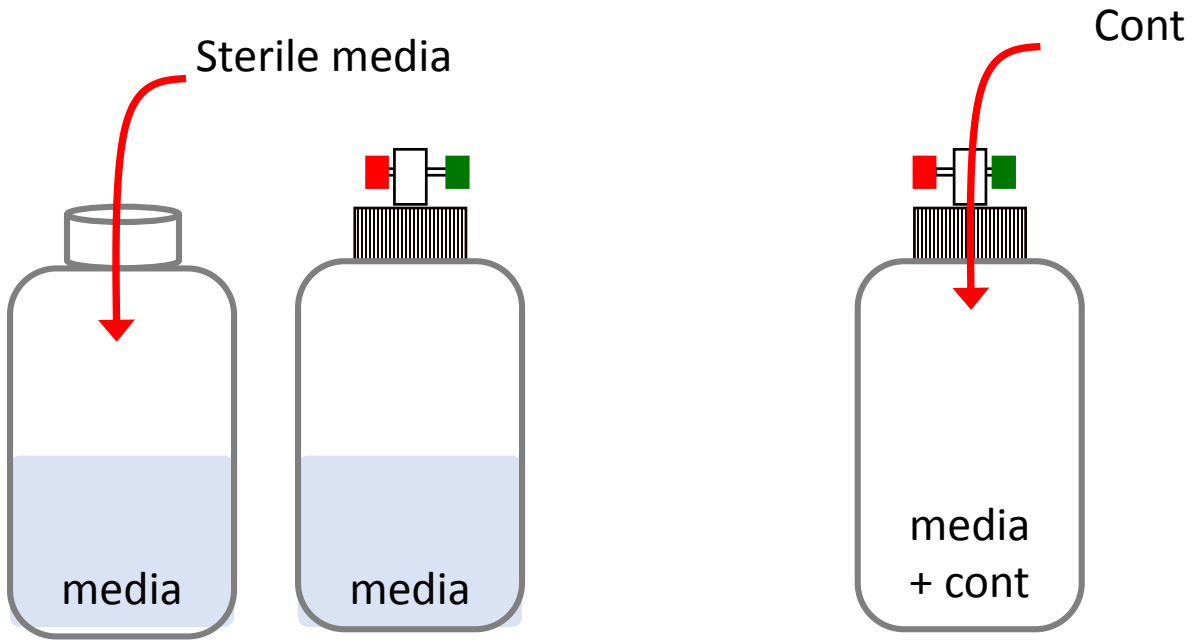
- **Anaerobic biodegradation experiment with SiREM Culture 01 (SC01) known to degrade CFCs.**

**Sample Bottle preparation:** (prepare several (3) replicates)

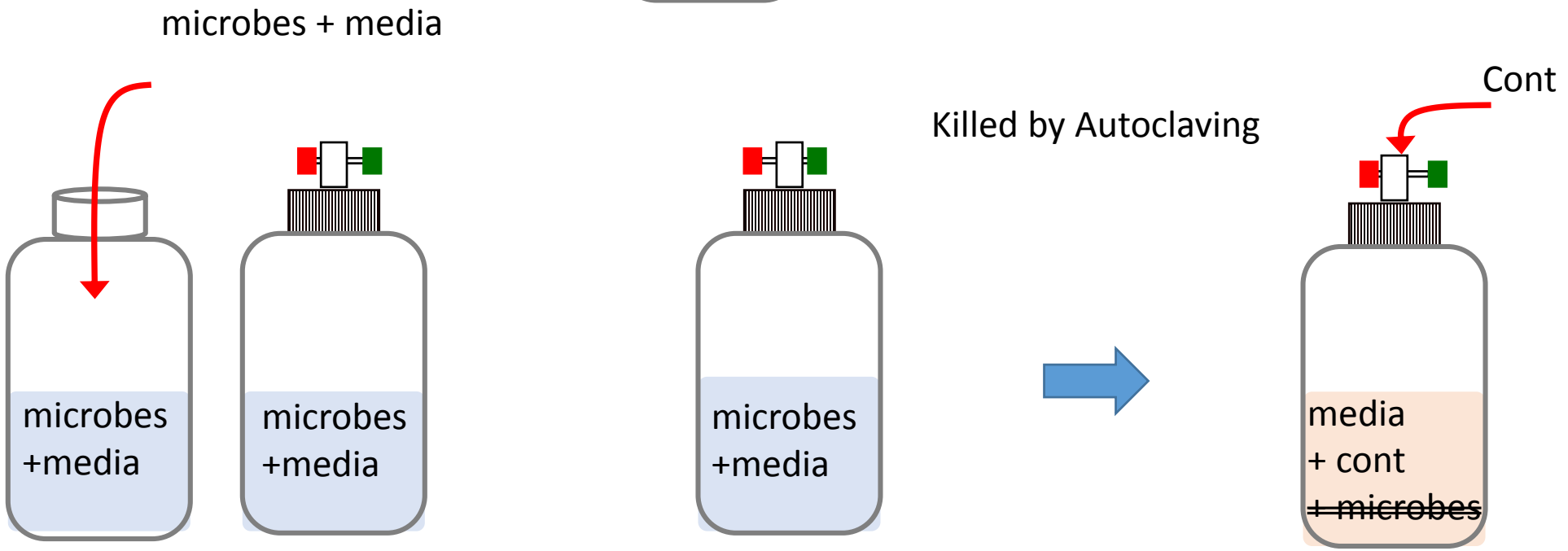


# Control Bottle Preparation

## MEDIA CONTROL




## KILLED CONTROL



# Analysis

## Gas Chromatography (GC) for Concentration Analysis

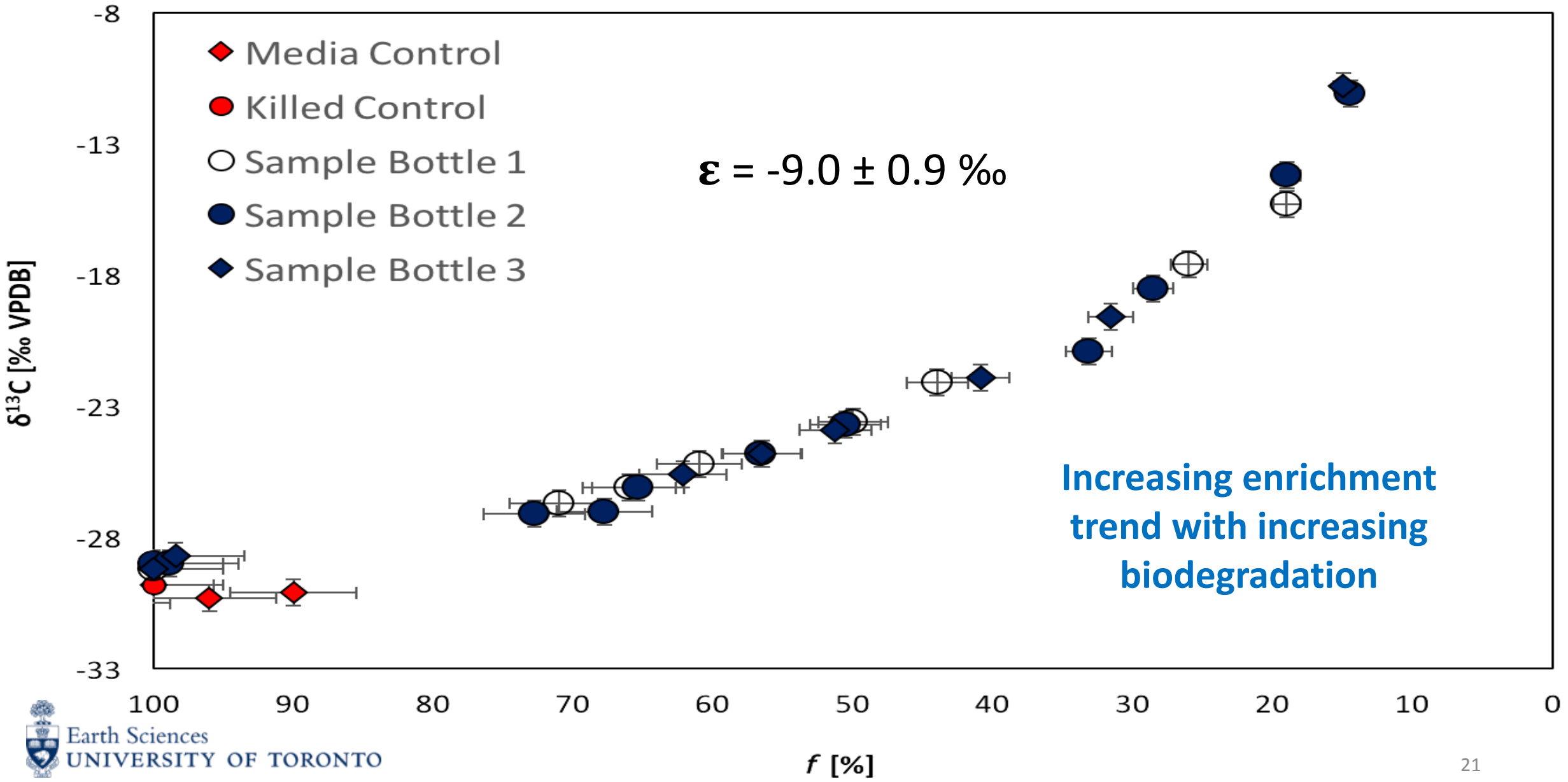
## Gas Chromatography Combustion Mass Spectrometry (GC-C-MS) for Isotope Analysis



Retrieved from http://www.speciation.net/Database/Instruments/Varian-Inc-Part-A/3380-Gas-Chromatograph-;i386

# Chlorofluorocarbons (CFCs)
# Experimental Results

# CFC-113 Experiment Results

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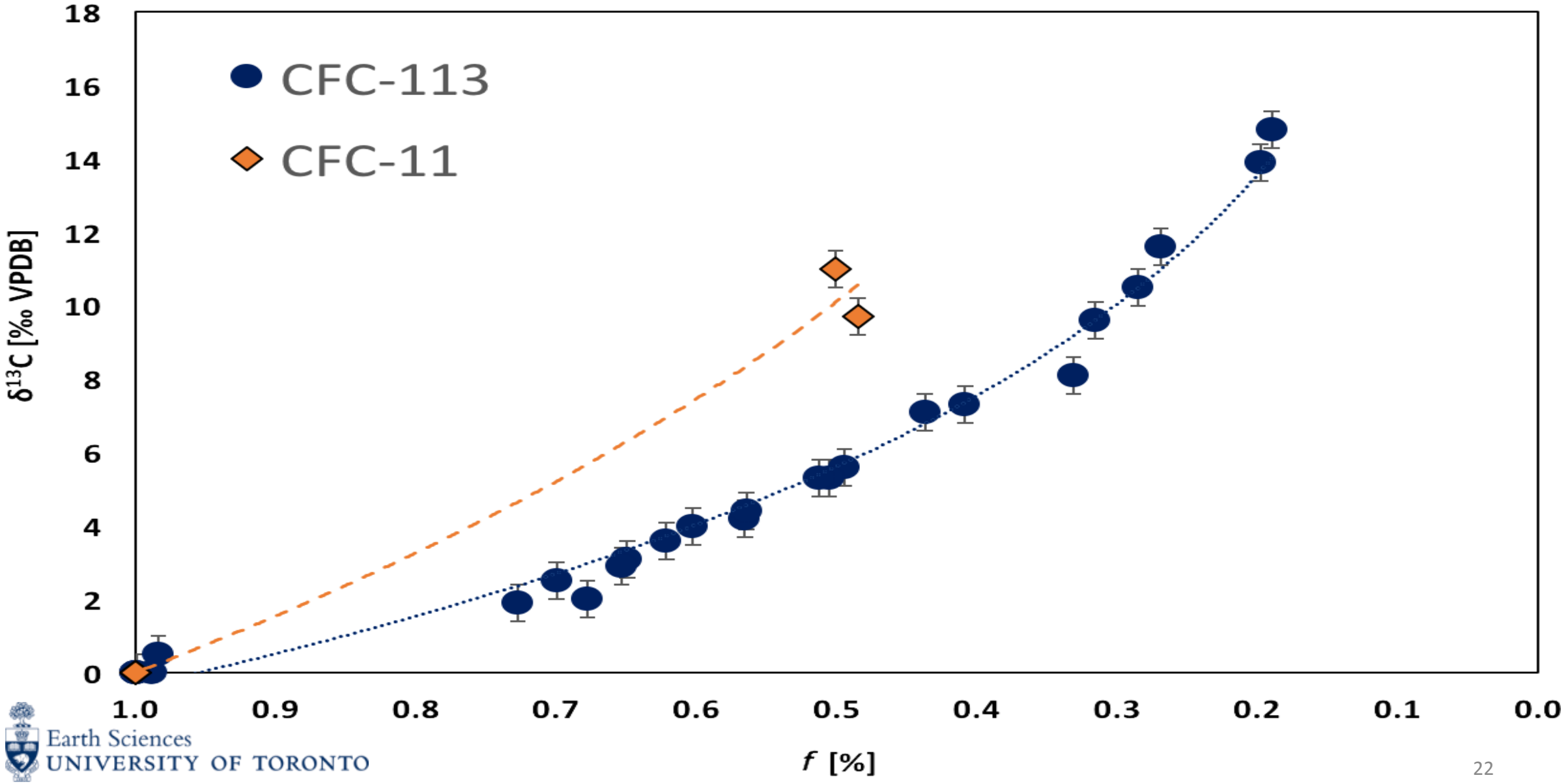
# CFC-11 Experimental Results

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# Field Application

## Poster 68

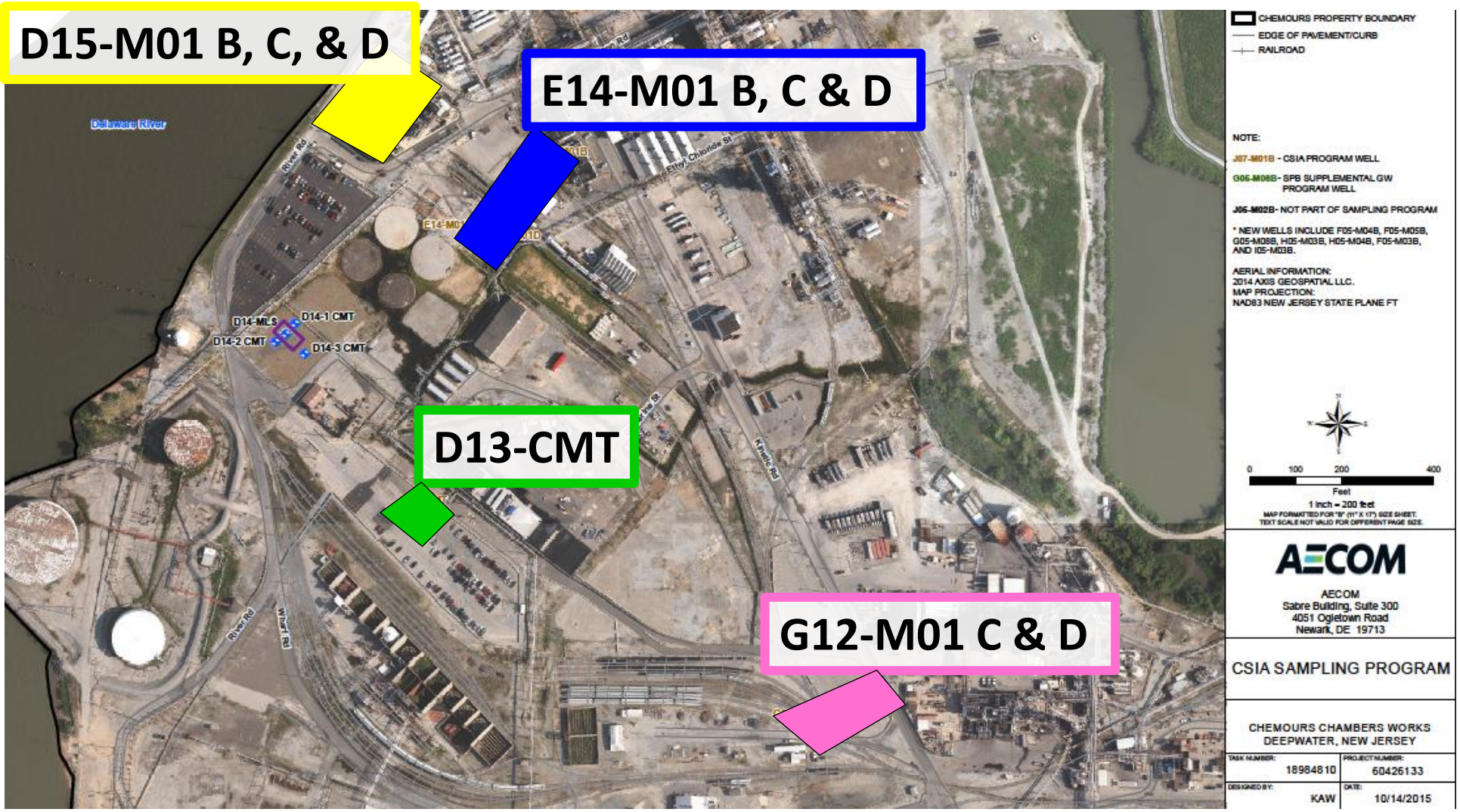
## Investigation of In Situ Bioremediation of Chlorofluorocarbons at a Contaminated Field Site via Compound Specific Isotope Analysis (CSIA)

T. Gilevska[1], A. Horst[1], B. Sherwood Lollar[1], E. Lutz[2], E. Seger[2], S. Norcoss[3], S.A. Morgan [3,] , K.A. West [3] & E.E.Mack[4]

1. Earth Sciences Centre, University of Toronto, 22 Russell Street, Toronto, Ontario. 2. The Chemours Company, Wilmington, DE, USA. 3. AECOM, Deepwater, NJ, USA. 4. Dupont, Newark, DE, USA.

Tuesday, May 23 @ 5:45-7:00 p.m. (Today)

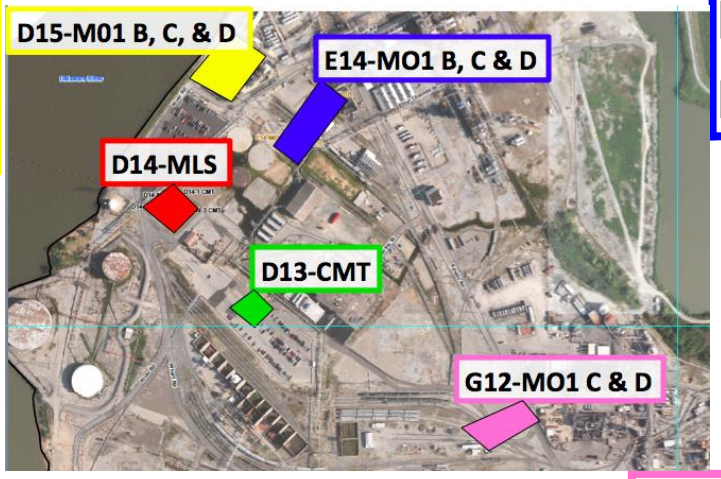
# Chemours Chambers Site



# CFC-113

**D15-M01 B, C, & D**

| | µg/L | $\delta^{13}C$ |
|---|---|---|
| D15-M01B | 7600 | **-31.7** |
| D15-M01C | 11000 | **-31.6** |
| D15-M01D | 3200 | **-31.4** |




**E14-M01 B, C & D**

| | µg/L | $\delta^{13}C$ |
|---|---|---|
| E14-M01B | 36000 | **-30.1** |
| E14-M01C | 12000 | **-31.0** |
| E14-M01D | 5400 | **-30.7** |

**D13-CMT**

| | µg/L | $\delta^{13}C$ |
|---|---|---|
| D13-CMT-Port3 | 65000 | **-29.4** |
| D13-CMT-Port4 | 8300 | **-29.4** |
| D13-CMT-Port5 | 790 | **-28.8** |
| D13-CMT-Port6 | 250 | **-28.6** |

**G12-M01 C & D**

| | µg/L | $\delta^{13}C$ |
|---|---|---|
| G12-M01C | 14000 | **-24.8** |
| G12-M01D | 9900 | **-25.7** |

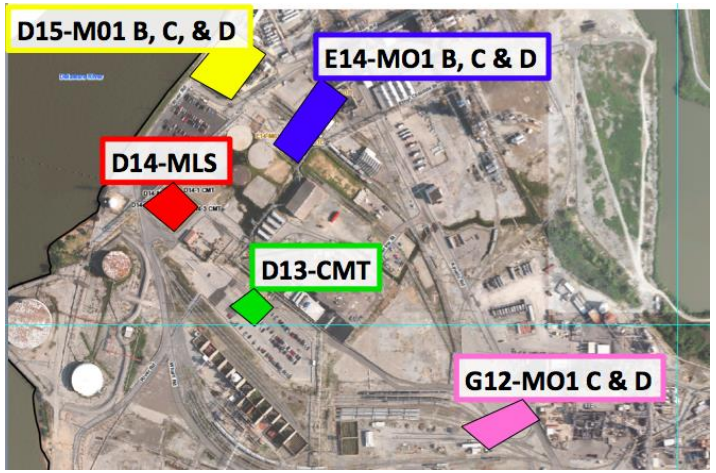
# CFC-113

D15-M01 B, C, & D

-31.4 to -31.7 ‰

E14-M01 B, C & D

-30.1 to -31.0 ‰




D13-CMT

-28.6 to -29.4 ‰

G12-M01 C & D

-24.8 to -25.7 ‰

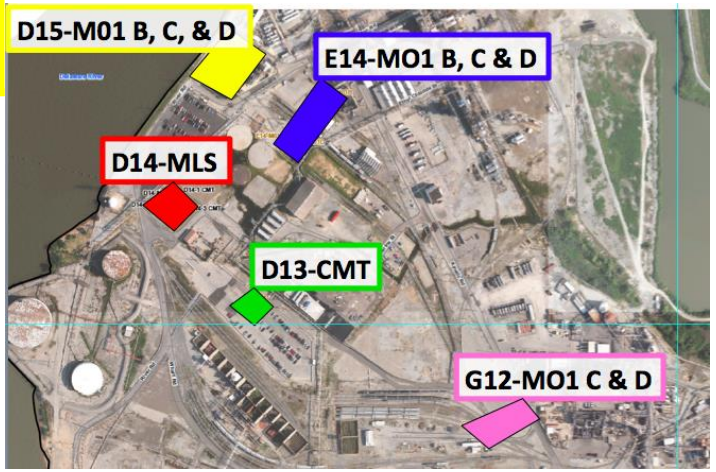
➔ Less than 1‰ variation within each well so no evidence of biodegradation

➔ Significant variation between wells may reflect differences in source or in hydrogeologic flowpath.

# CFC-11

**D15-M01 B, C, & D**

| | µg/L | $\delta^{13}C$ |
|---|---|---|
| D15-M01B | 1800 | **-30.8** |
| D15-M01C | 2100 | **-30.7** |




**E14-M01 B, C & D**

| | µg/L | $\delta^{13}C$ |
|---|---|---|
| E14-M01B | 380 | ***-46.9*** |
| E14-M01C | 12000 | **-26.7** |
| E14-M01D | 9600 | **-23.4** |

**D13-CMT**

| | µg/L | $\delta^{13}C$ |
|---|---|---|
| D13-CMT-Port3 | 12000 | **-31.7** |
| D13-CMT-Port4 | 3100 | **-24.4** |
| D13-CMT-Port5 | 590 | **-25.8** |
| D13-CMT-Port6 | 250 | **-24.9** |

**G12-M01 C & D**

| | µg/L | $\delta^{13}C$ |
|---|---|---|
| G12-M01C | 6100 | ***-38.2*** |
| G12-M01D | 4700 | **-26.3** |

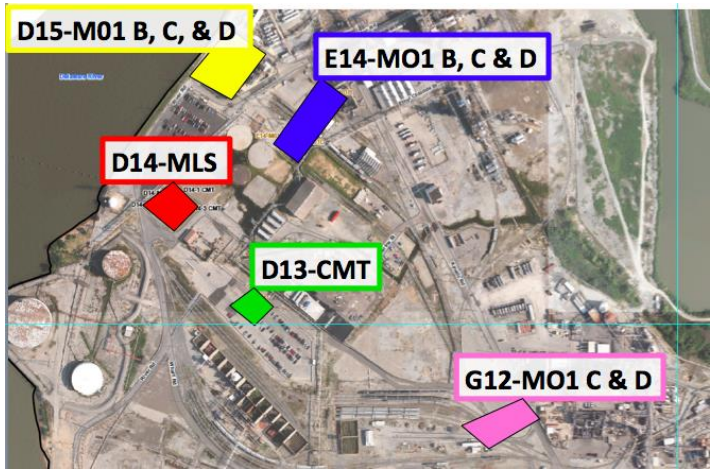
# CFC-11

D15-M01 B, C, & D

-30.7 to -30.8 ‰

E14-M01 B, C & D

-23.4 to *-46.9* ‰




D13-CMT

-24.4 to -31.7 ‰

G12-M01 C & D

-26.3 to *-38.2* ‰

➔ Significant variation within well

➔ Significant variation between wells

# CFC Summary

## CFC-113

- Values are very consistent within each well
- Values differ between locations

**Conclusion:** These uniform signatures are inconsistent with biodegradation; possibly differences due to source variation or different hydrogeological pathways.

## CFC-11

- Values vary significantly within most wells and between locations.

**Conclusion**:

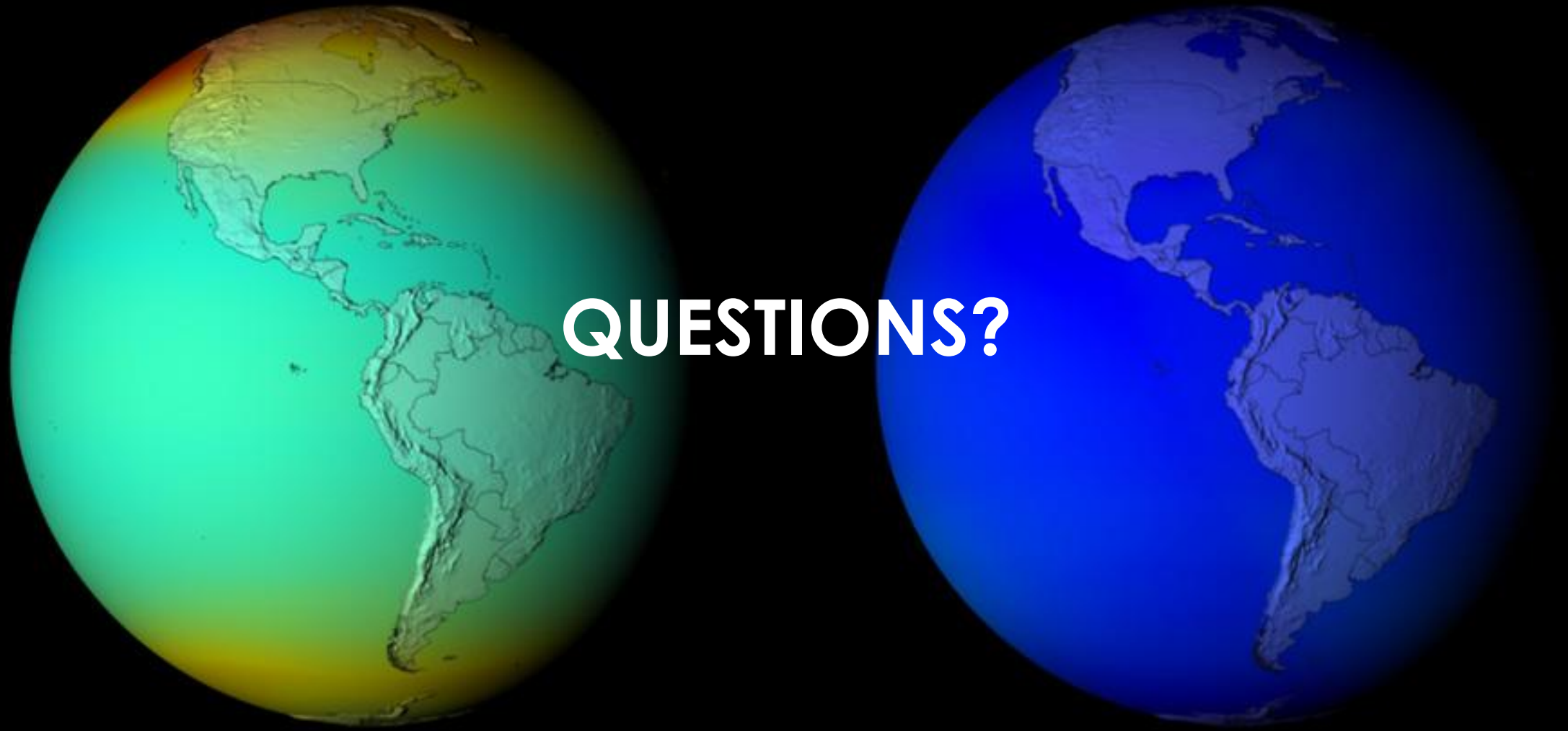
- May still be a sign of differences in source material at different locations.
- Signs of variability within a given well are suggestive of *in situ* biodegradation.

# Acknowledgements


- Funding for this study was provided by a Natural Sciences and Engineering Research Council of Canada (NSERC) Collaborative Research and Development grant and by the E.I. DuPont Canada Co., and the DuPont Corporate Remediation Group (CRG).

- Additional funding was provided by the Canada Research Chair Program, NSERC Discovery

# A World Without the Montreal Protocol




QUESTIONS?



http://svs.gsfc.nasa.gov/vis/a010000/a010800/a010824/index_svs.html