Case Studies of Advances in Bioremediation of Organics: Part 1

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Outline

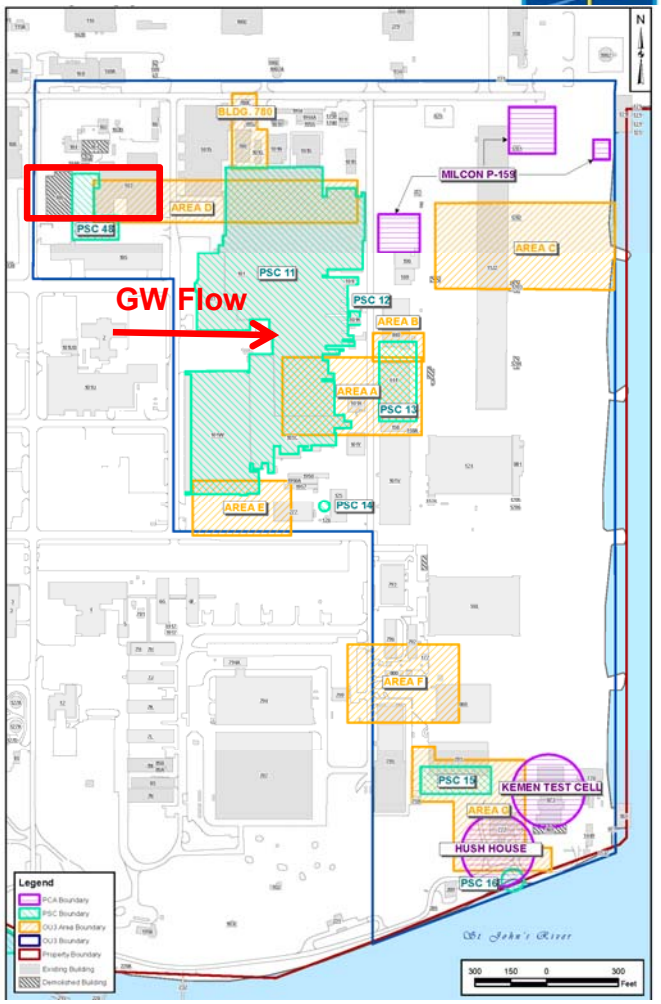
- **Chlorinated Solvents**
  - **Molecular Tools**
  - **Electrokinetic Bioremediation**

- **1,4-Dioxane**
  - **^{14}C Assay**
  - **Aerobic Cometabolism using Multiple Primary Substrates**

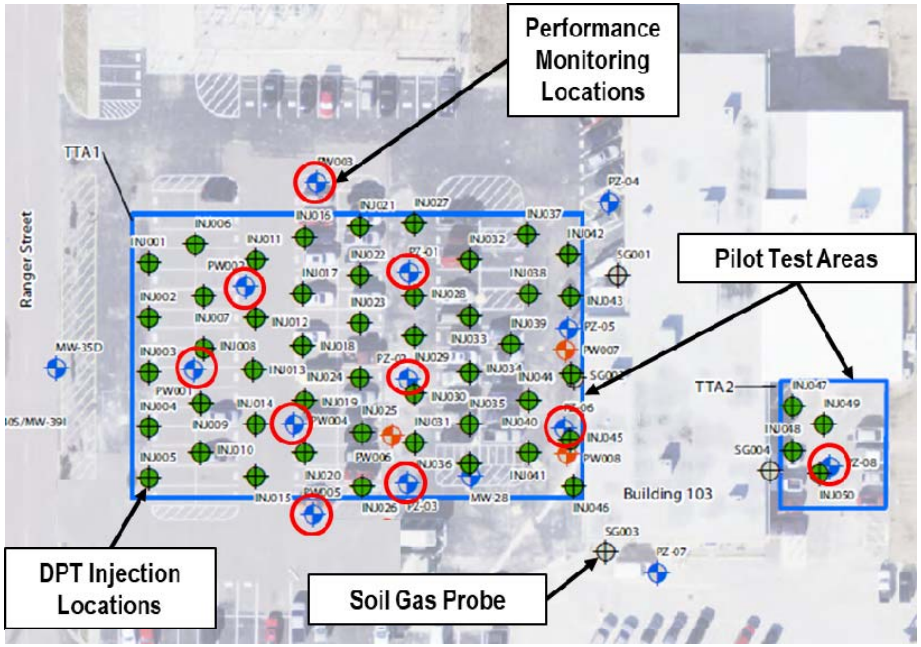
Case Study – qPCR and 16S Sequencing at NAS JAX

Site Background

- **Operable Unit 3 (OU3) occupies 134 acres on eastern side of installation**
- **Industrial/commercial land use**
- **Fleet Readiness Command (FRC) (formerly NADEP) primary tenant on installation since 1940s**
- **Former dry cleaner facility located within OU3 property**
- **7 identified groundwater plumes (Areas A – G)**
- **Buildings 780 and 106 also sources of contamination**



Case Study – qPCR and 16S Sequencing at NAS JAX
Enhanced *In Situ* Bioremediation Application



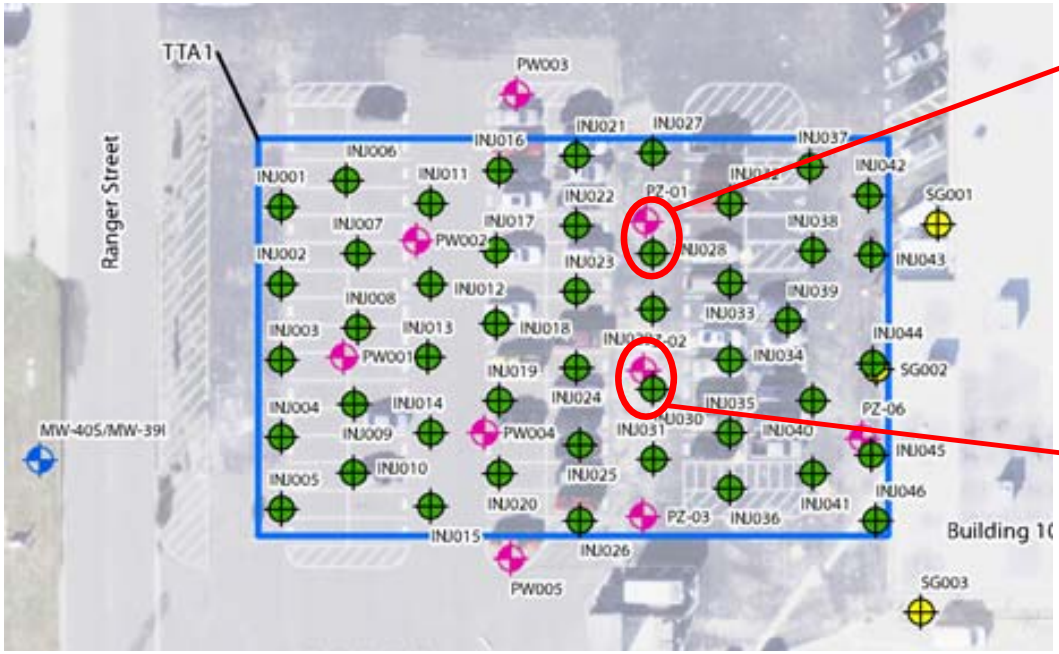
Legend

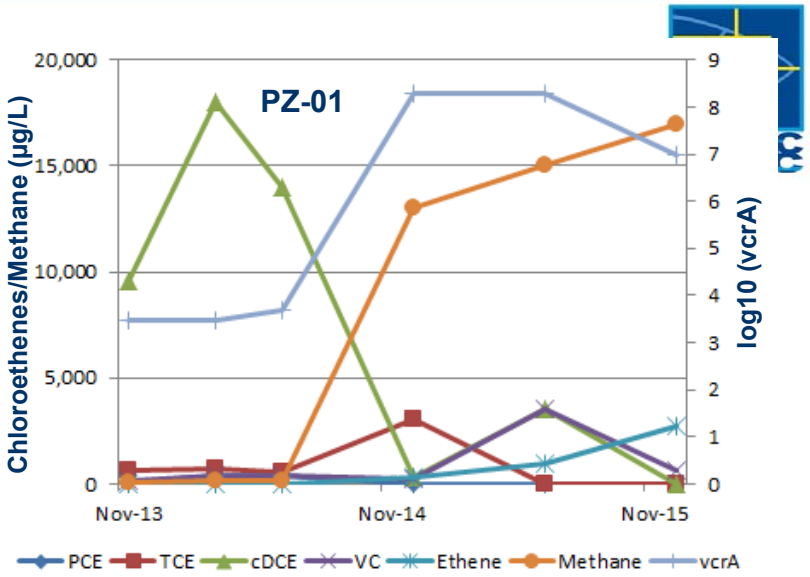
- Temporary DPT Location
- Existing Soil Gas Probe
- Existing Sand Well
- Existing Sand/Clay Well
- arget Treatment Area (TTA)

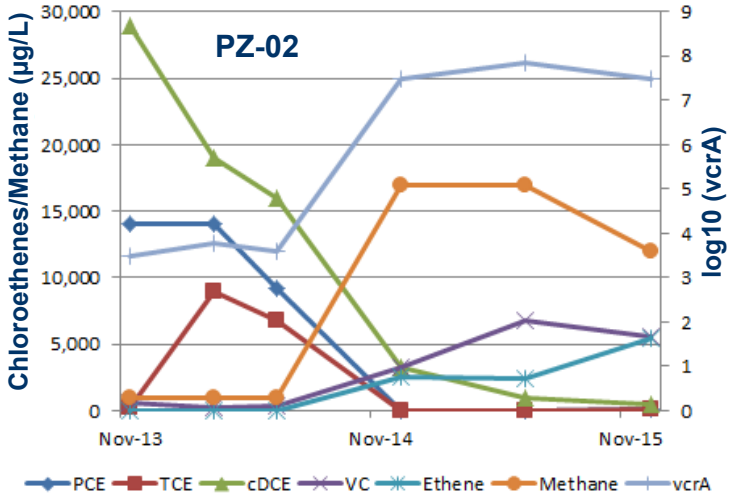
- 50 DPT injection locations
- 2 injection intervals per location
- 145,000 gallons of an 0.7% emulsified vegetable oil solution (EDS-ERTM)
- Average flow rate of 1.8 gpm
- 100 liters of KB-1® and KB-1® Plus injected
- Bromide tracer used
- 10 performance monitoring wells
- 4 soil gas probes

Case Study – qPCR & 16S Sequencing at NAS JAX

CVOC Results







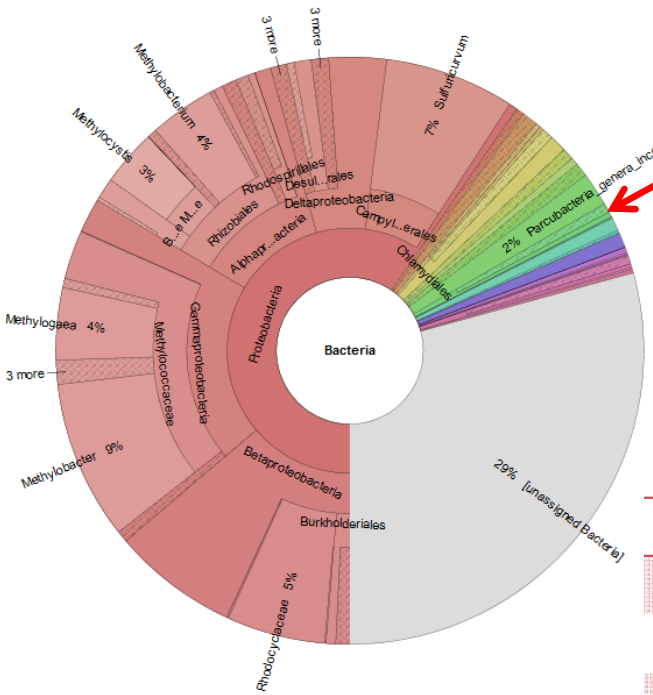
Legend

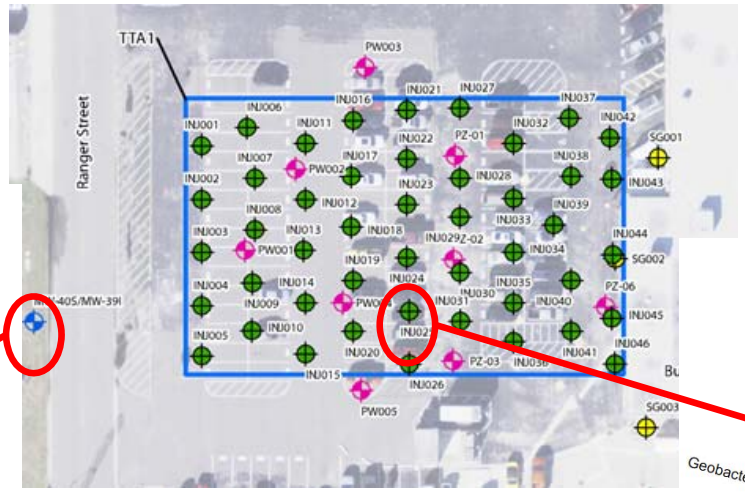
- Performance Monitoring Well
- UIC Monitoring Well
- Soil Gas Probe
- Temporary DPT Injection Location
- arget Treatment Area (TTA)

Case Study – CVOC qPCR & 16S Sequencing at NAS JAX

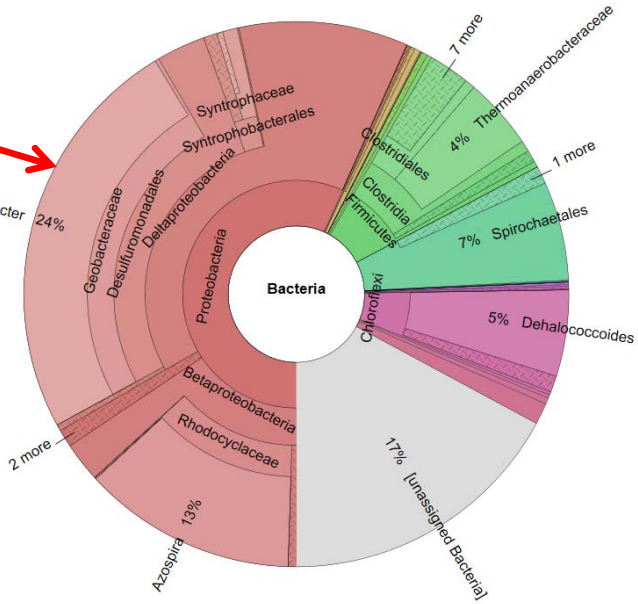
Microbial Composition

Upgradient Well MW-40S





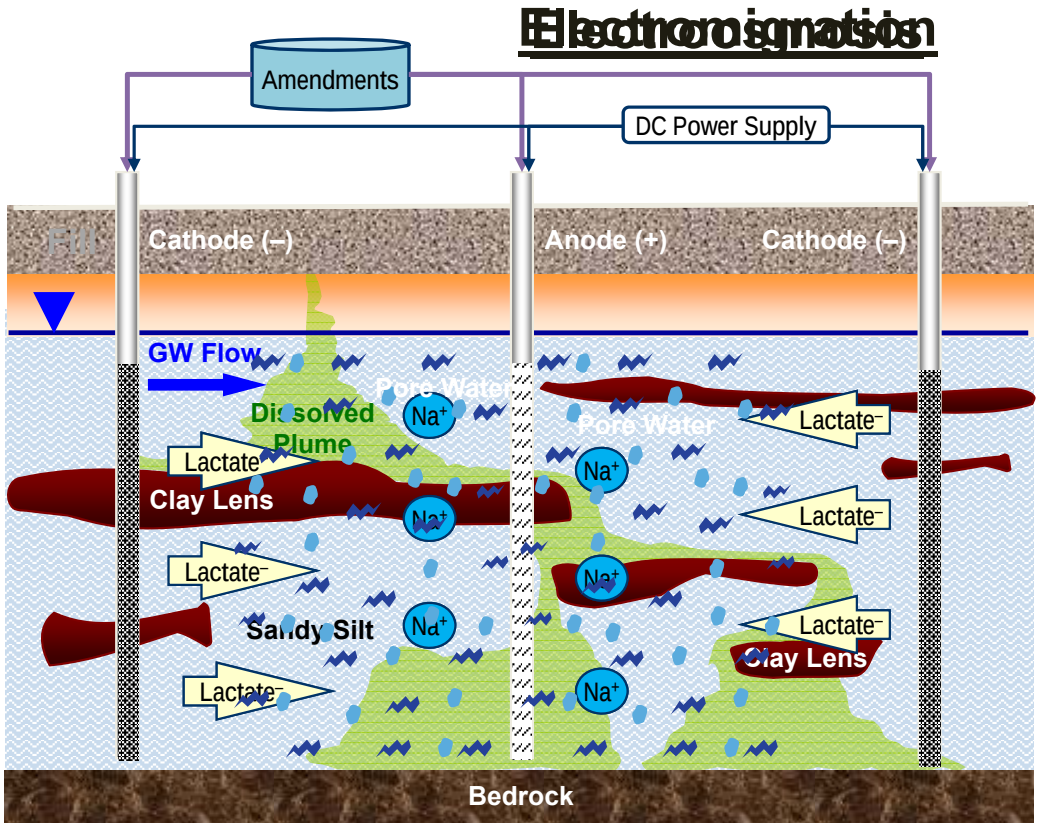
Pilot Test Well PZ-02



	% of Bacteria	
Bacteria	**MW-40S**	**PZ-02**
Dehalococcoides	0.01%	5%
Geobacter	0.09%	24%
Methylobacter	9%	9%

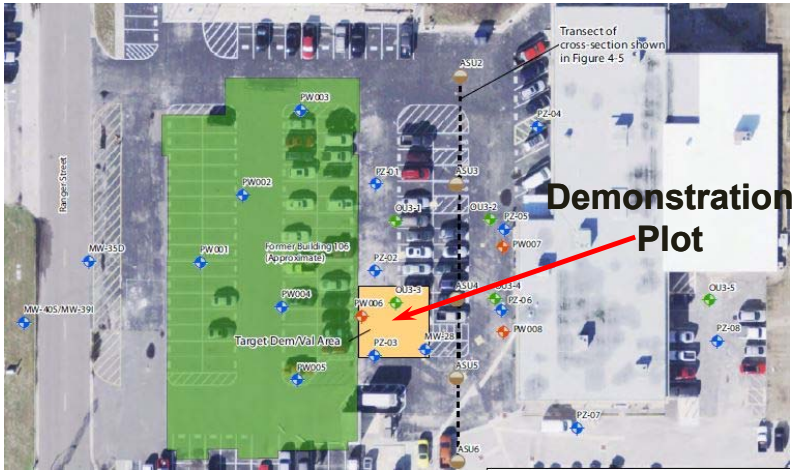
Electrokinetic (Ek) Bioremediation

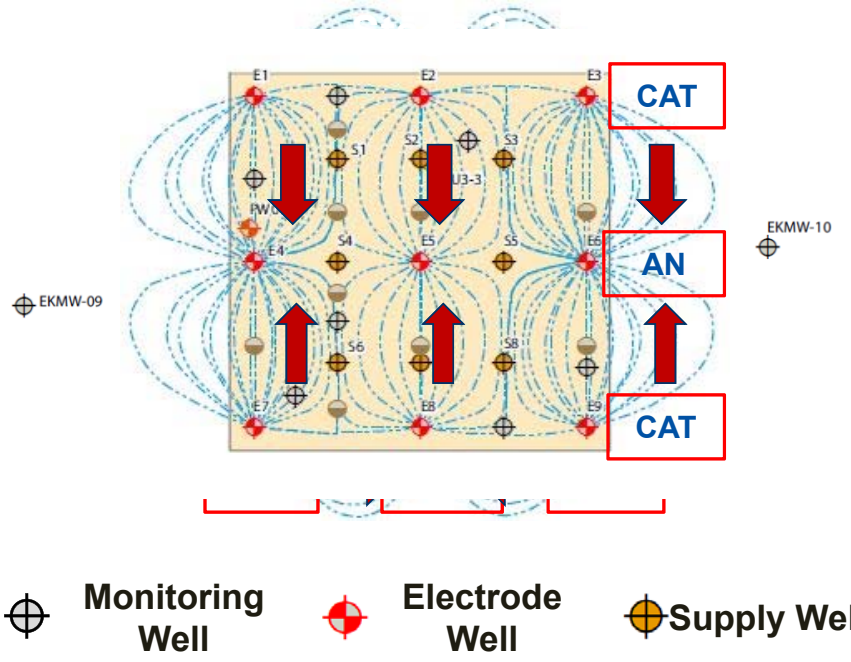
- Low permeability silts and clays (K <10^{-7} m/s) present challenge for amendment distribution
- This technology leverages the electrical properties of the amendments to promote distribution
- Technology applies an electric current to facilitates electromigration and electro-osmosis
- Successfully demonstrated in Denmark to treat PCE
  - Generated lactate flow of 3 to 5 cm/day through clay



Ek-Bio Demonstration at NAS Jacksonville, FL (ESTCP ER-201325)

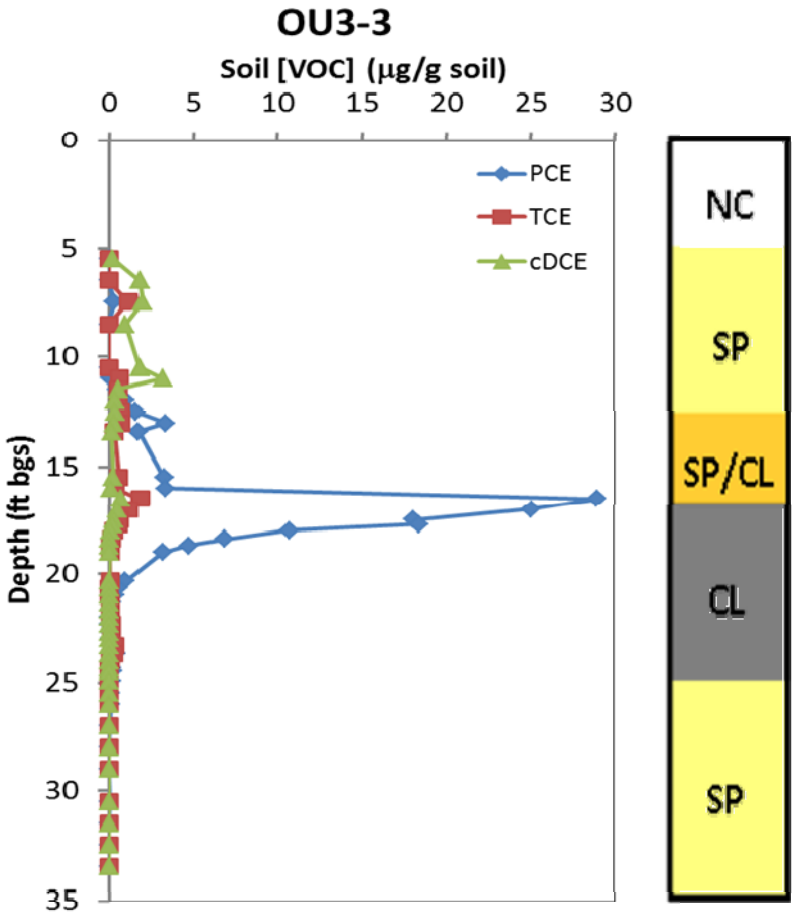
- **Potassium lactate and KB-1 (2 Stages)**
- **Stage 1**
  - **100 gallons, 60% lactate**
  - **4 L KB-1 into each of 8 supply wells & 2 L KB-1 into each of 9 electrode wells**
  - **Introduced over 6 months**

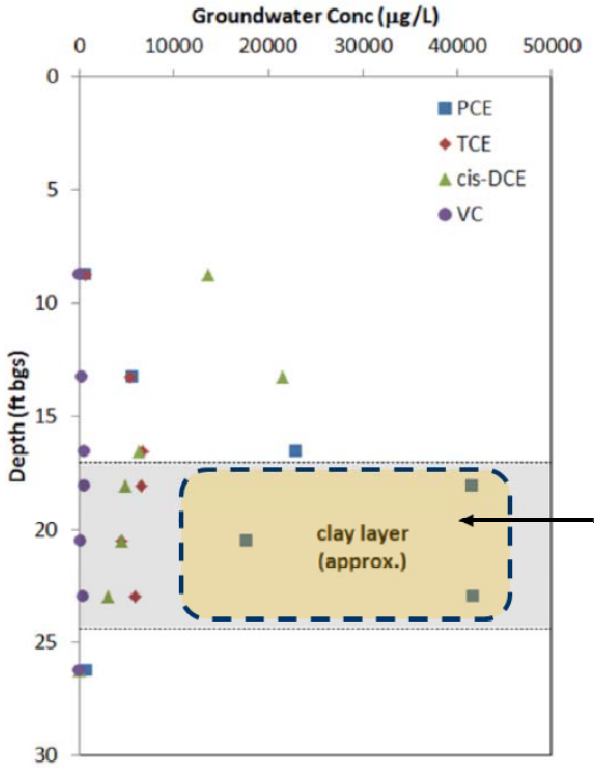




Ek-Bio Demonstration at NAS Jacksonville, FL (ESTCP ER-201325)

Results (cont'd)



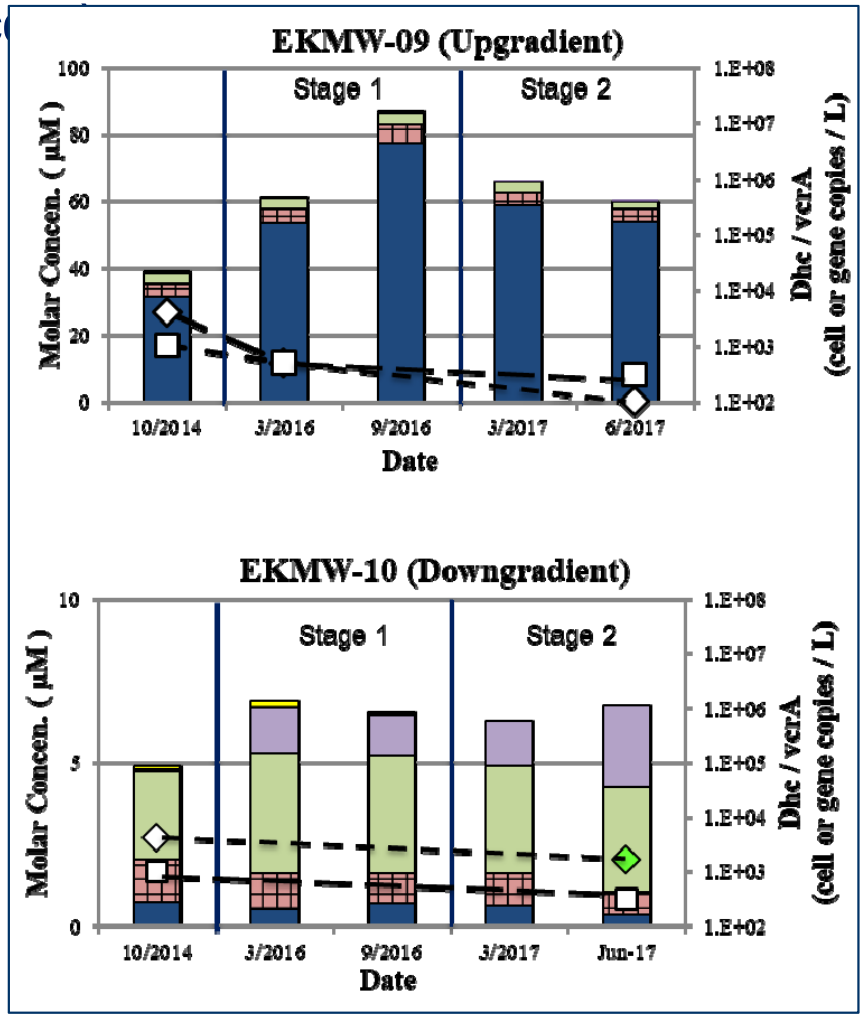


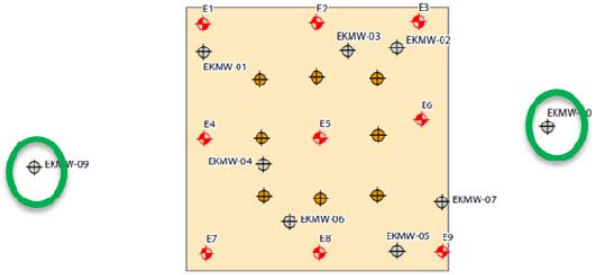
PCE at 15 – 40 mg/L in clay

NC – not characterized; SP – sand; CL – clay

Ek-Bio Demonstration at NAS Jacksonville, FL (ESTCP ER-201325)

Results (c





Ethene

VC

cDCE

TCE

PCE

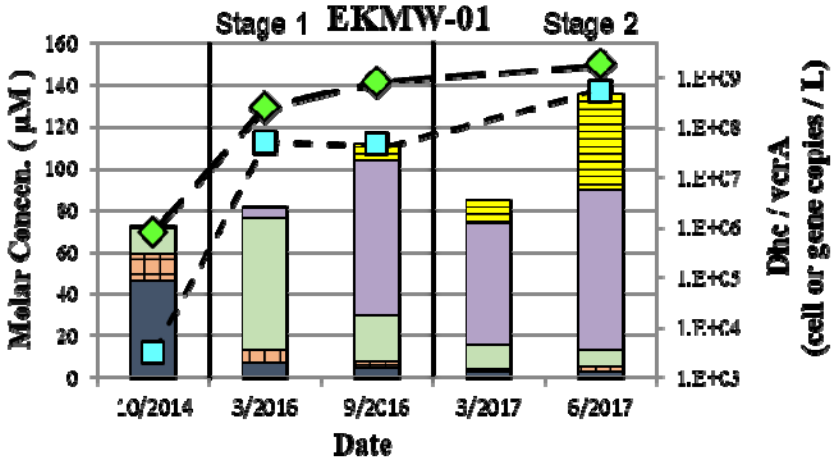
Dhc

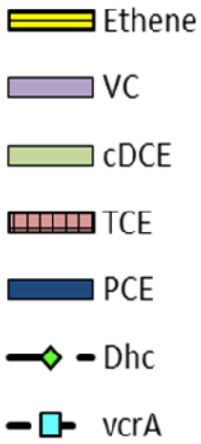
vcrA

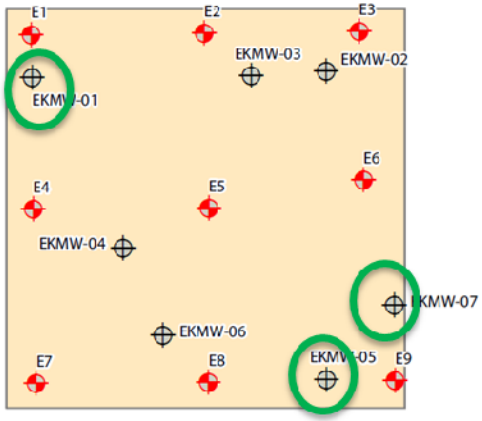
Electrode Wells in **RED**

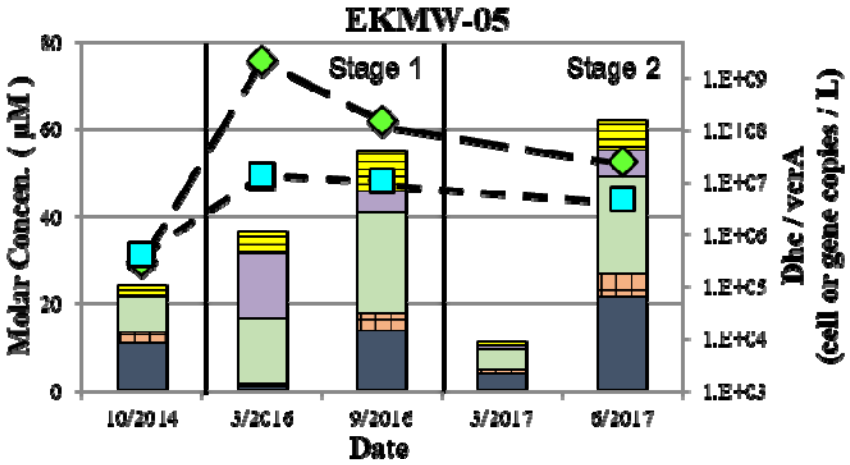
Monitoring Well in **Grey**

Ek-
Resu


Stage 1
EKMW-01
Stage 2
Molar Concen. (µM)
Dhc / vcrA (cell or gene copies / L)
Date
10/2014
3/2016
9/2016
3/2017
6/2017


Ethene
VC
cDCE
TCE
PCE
Dhc
vcrA


E1
E2
E3
EKMW-03
EKMW-02
EKMW-01
E6
E4
E5
EKMW-04
EKMW-07
EKMW-06
E7
E8
EKMW-05
E9


EKMW-05
Stage 1
Stage 2
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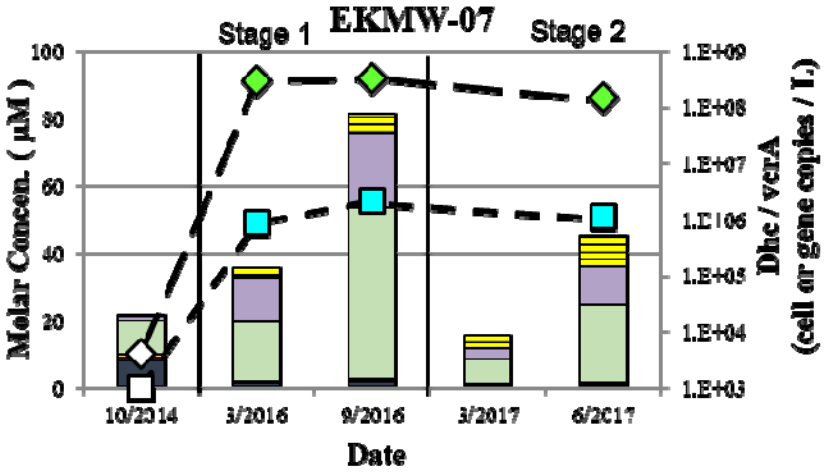
Dhc / vcrA (cell or gene copies / L)
Date
10/2014
3/2016
9/2016
3/2017
6/2017


Stage 1
EKMW-07
Stage 2
Molar Concen. (µM)
Dhc / vcrA (cell or gene copies / L)
Date
10/2014
3/2016
9/2016
3/2017
6/2017

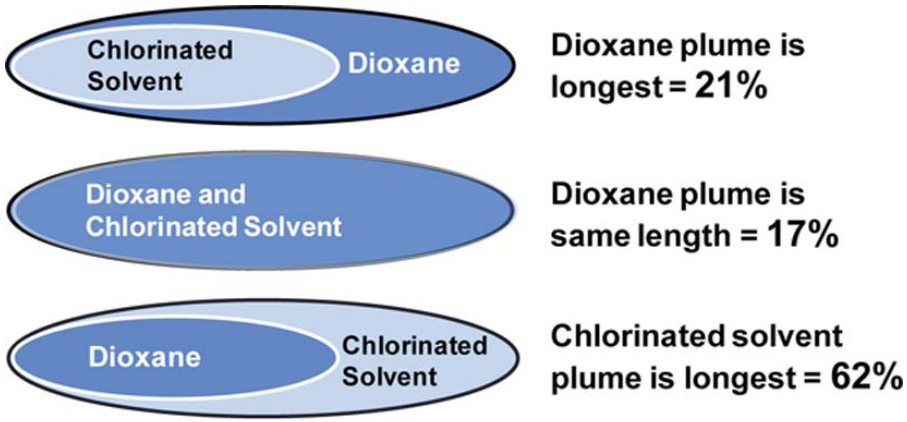
Outline

- **Chlorinated Solvents**
  - **Molecular Tools**
  - **Electrokinetic Bioremediation**

- **1,4-Dioxane**
  - **^{14}C Assay**
  - **Aerobic Cometabolism using Multiple Primary Substrates**

1,4-Dioxane – ESTCP 201730

- **Many 1,4-dioxane plumes appear to attenuate; how to prove?**
- **Multiple lines of evidence approach, including:**
  - **Concentration trend analysis; plume mass estimates; CSIA; biomarkers**
- **More direct evidence of aerobic biodegradation may be needed**
  - **Challenge: Aerobic biodegradation of 1,4-dioxane yields CO_2, biomass, and possibly soluble intermediates; *how to document product formation?***
- **^{14}C assays quantify products and allow for measurement of a rate coefficient**



n = 103 sites where dioxane and chlorinated solvents co-occur

Adamson et al., 2014

What is BioPIC (ESTCP ER-201129)?

- **Bioremediation Pathway Identification Criteria**
- **Updated protocol for evaluating natural attenuation**
- **Guides users in the selection of MNA, biostimulation and/or bioaugmentation or other remedial technology**
- **Spreadsheet driven (Excel™)**
- **Currently limited to chlorinated ethenes**
  - **Development of 'BioPic 2.0' for 1,4-Dioxane and associated cVOCs (ESTCP 201730)**

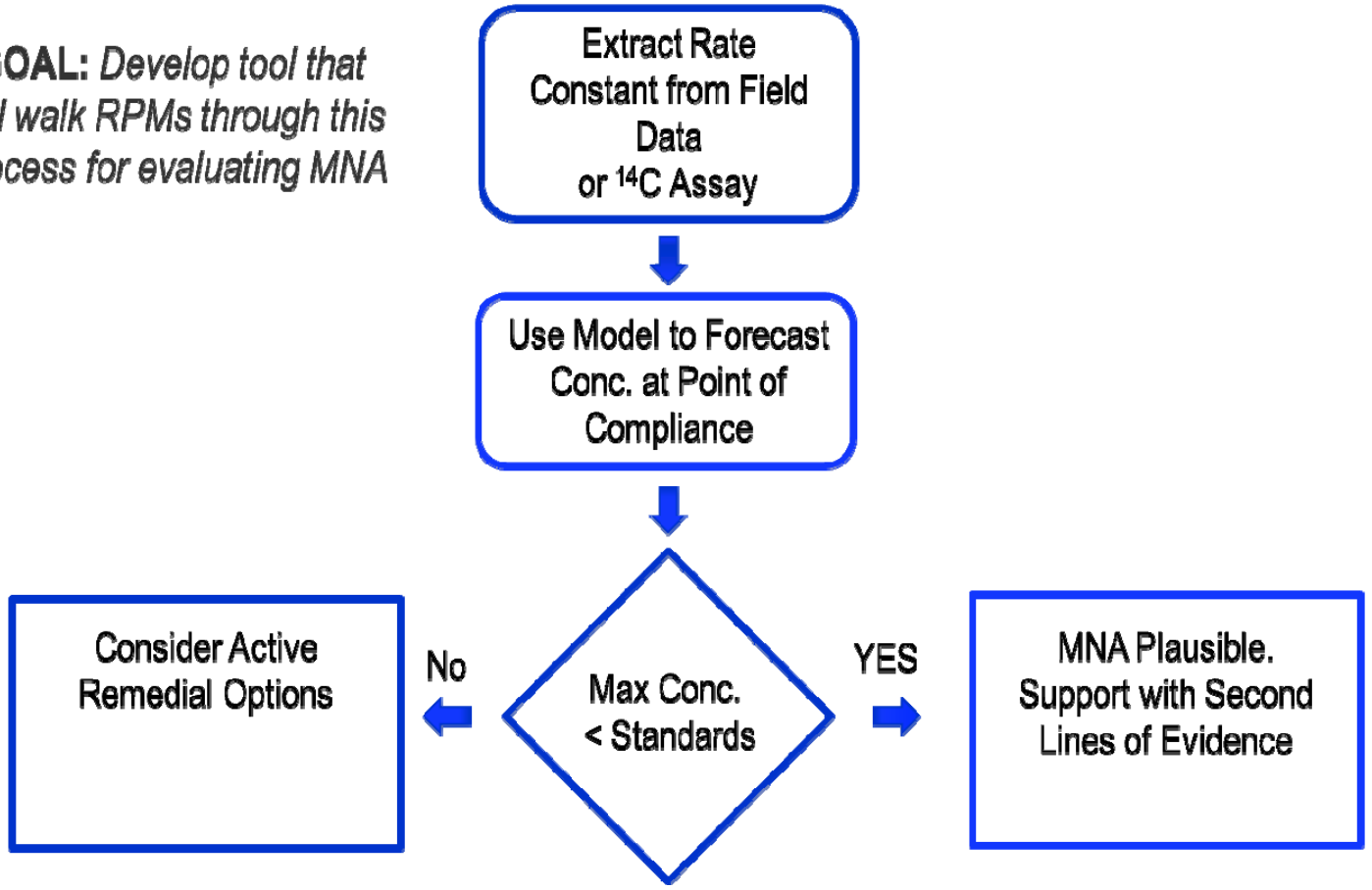
Based on EPA/600/R-98/128 (Sep 1998)

To Obtain BioPIC

Search under ER-201129 Report at SERDP-ESTCP

1,4-Dioxane – ESTCP 201730

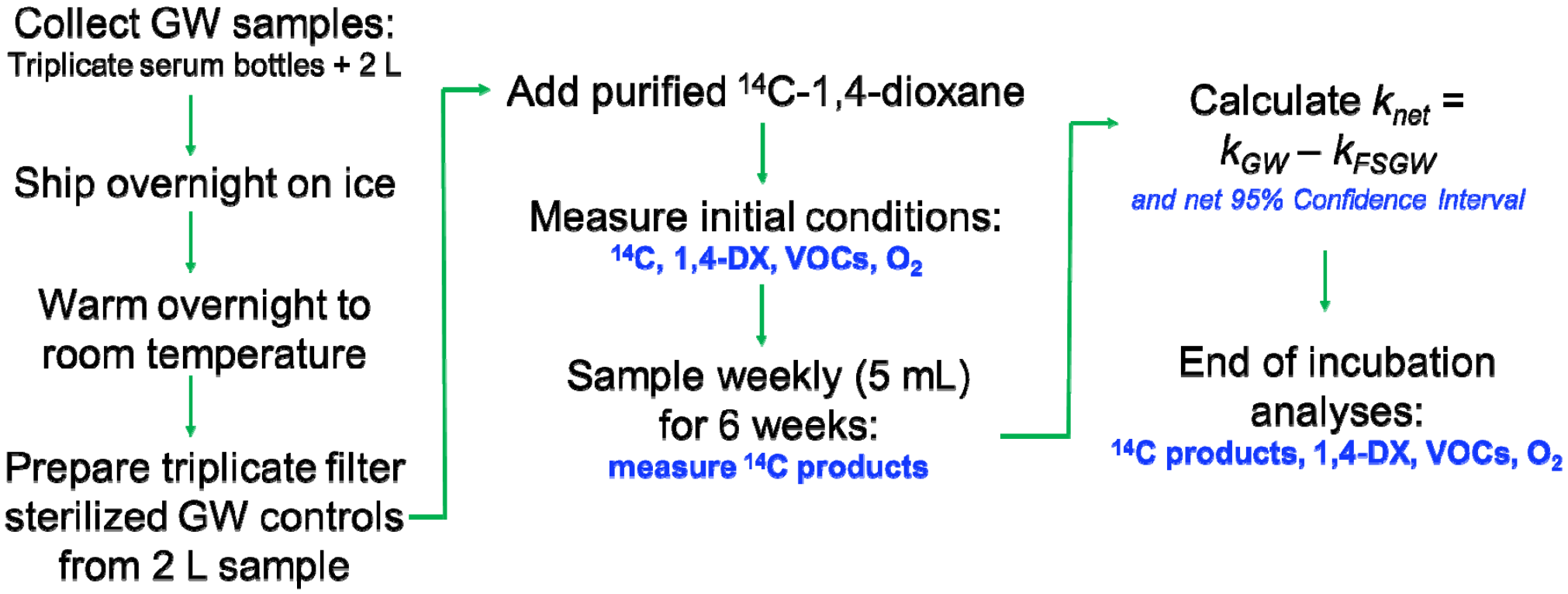
GOAL: *Develop tool that will walk RPMs through this process for evaluating MNA*



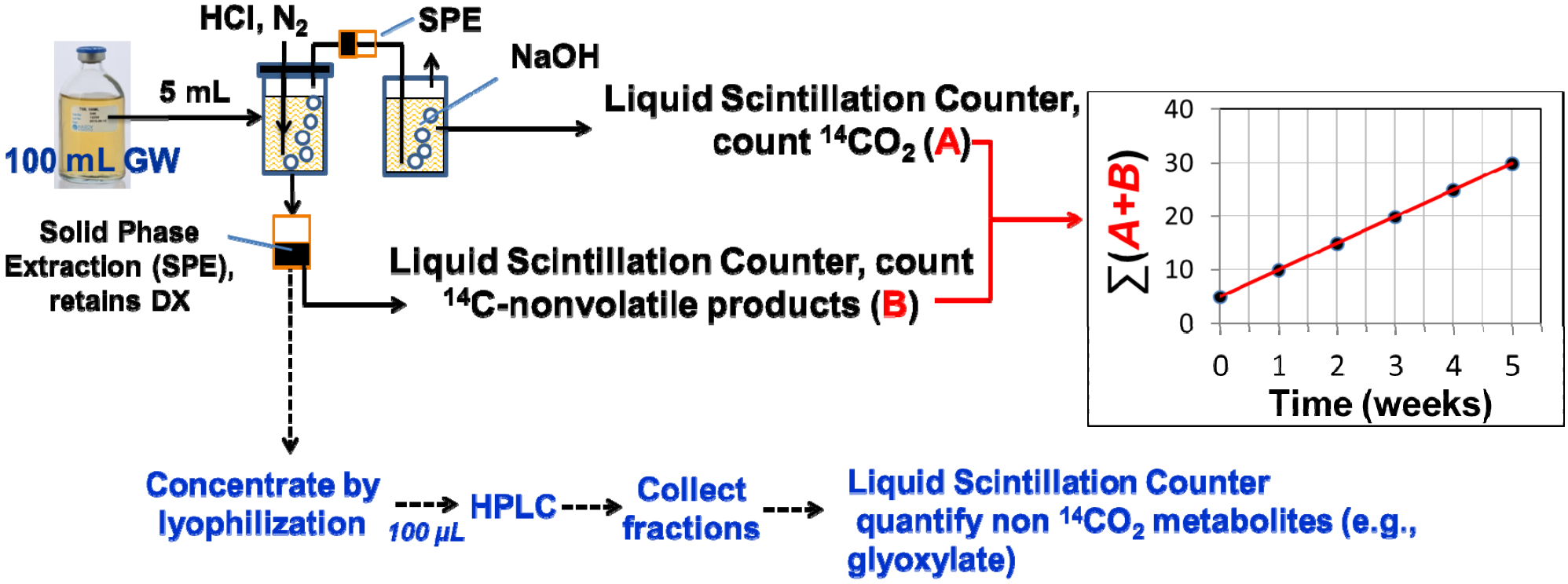
1,4-Dioxane – ESTCP 201730

- **Geographic diversity**
  - **≥ 4 states; East coast, West coast, Midwest**
- **Mix of Department of Defense and industrial sites (7 sites)**
- **All exhibit a decrease in C/C_o along plume axis**
  - **Range of 1,4-dioxane concentrations: 163-11,000 µg/L; median = 169 µg/L**
  - **Range of VOC co-contaminant concentrations: non-detect to 6 mg/L; 1,1-DCE from non-detect to 162 µg/L**
- **3-5 wells sampled per site; repeat samples for 2 sites**
- **Monitored: $\Delta^{14}C$ products; Δ1,4-dioxane; VOCs; ΔO_2**
  - **Also included CSIA and relevant biomarkers**

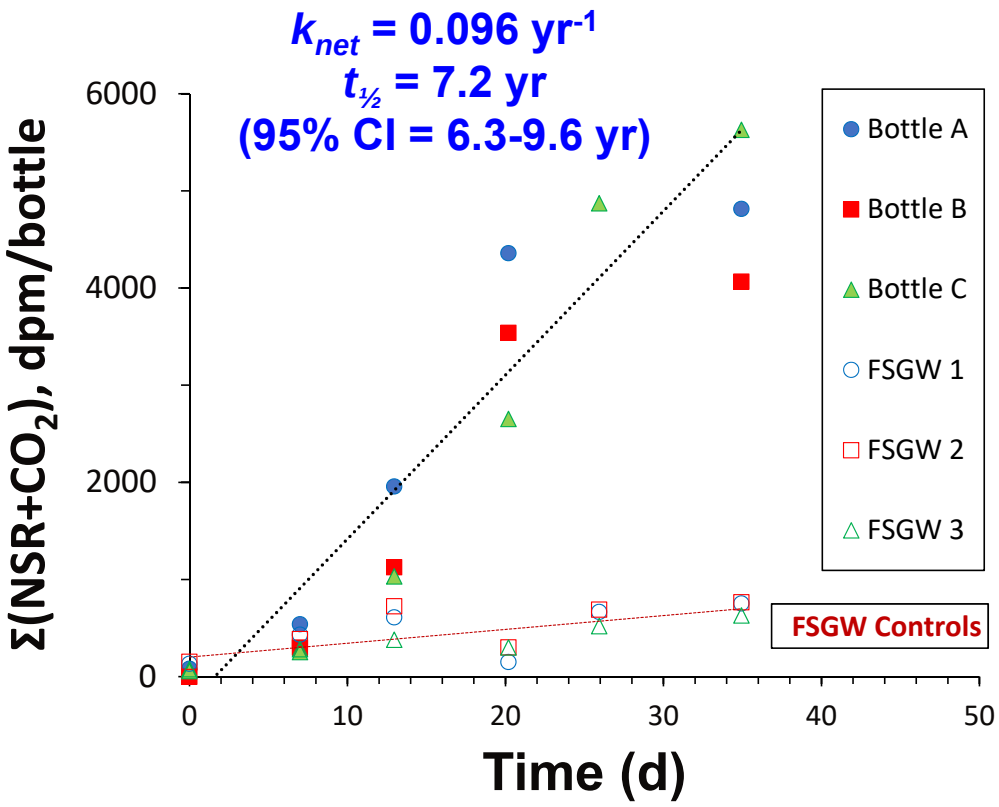
Basic Test Procedure: ^{14}C Assay



1,4-Dioxane – ESTCP 201730



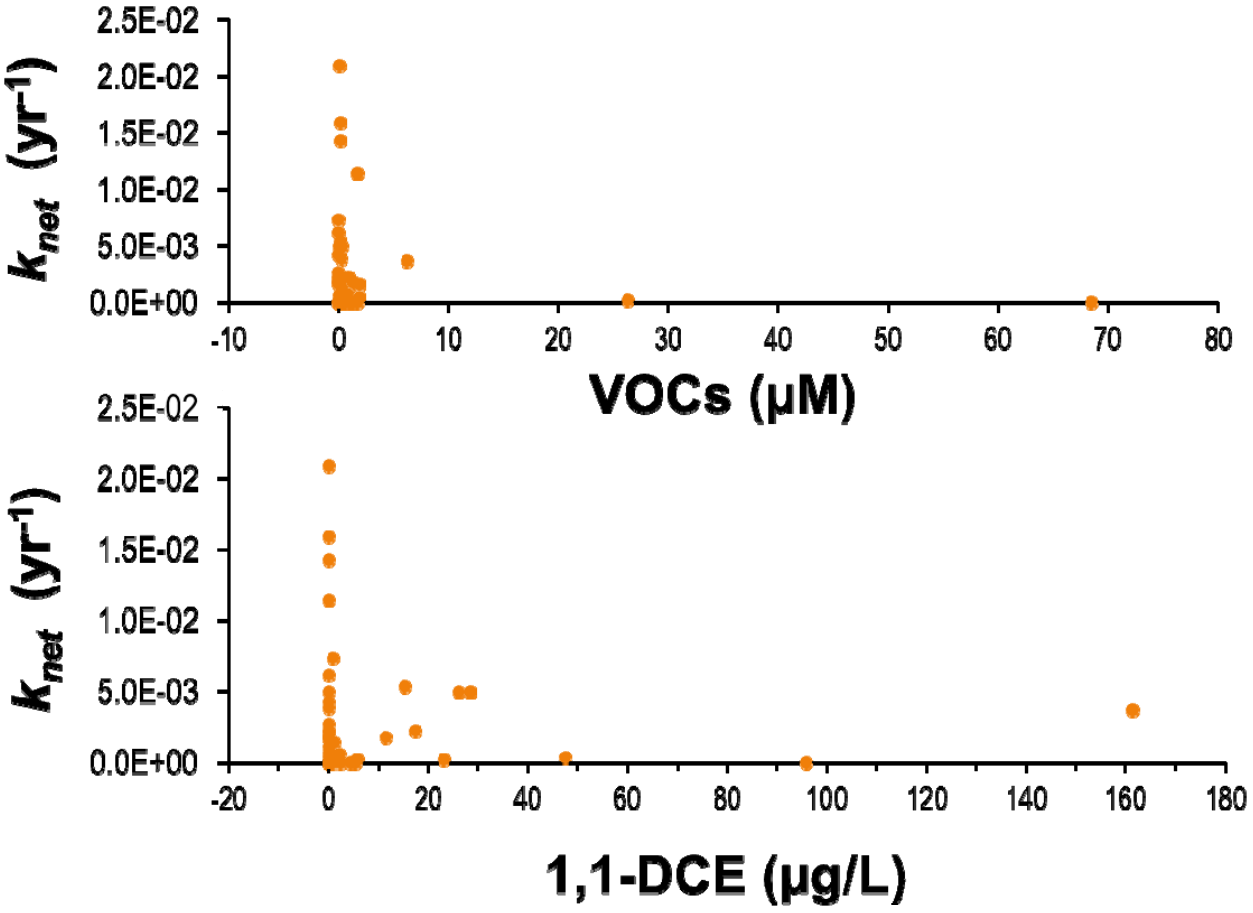
1,4-Dioxane – ESTCP 201730



Overall

- 36 well samples analyzed
- 12 have statistically significant rate coefficients
- Maximum rate coefficient* = 0.096 yr^{-1}
  Median rate coefficient = 0.0061 yr^{-1}

1,4-Dioxane – ESTCP 201730



- Highest rate constants at the lowest VOCs and 1,1-DCE
- Rate constants found at
  - 6.3 µM VOCs
  - 29 µg/L 1,1-DCE (0.30 µM)
- VOCs likely slow in situ rates

1,4-Dioxane – ESTCP 201733

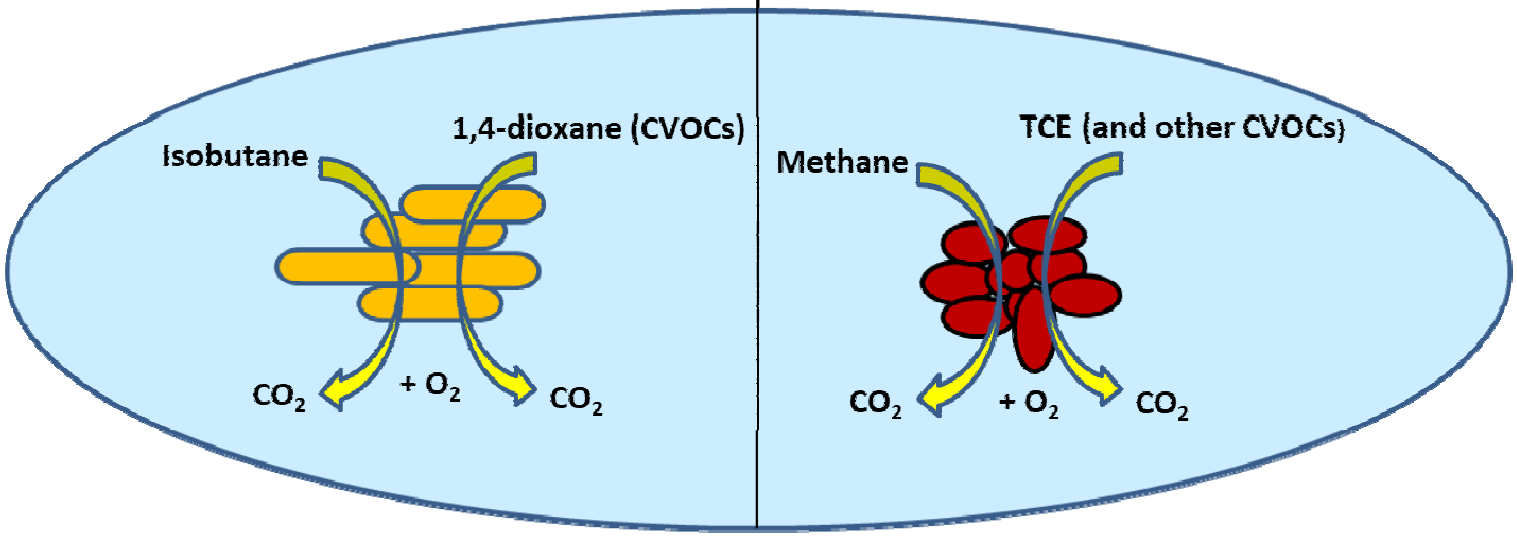
Multiple Primary Substrates: Isobutane + Methane

Process A: Isobutane + Oxygen

- Stimulate isobutane degraders
- 1,4-D targeted
- 1,1-DCE and select other CVOCs

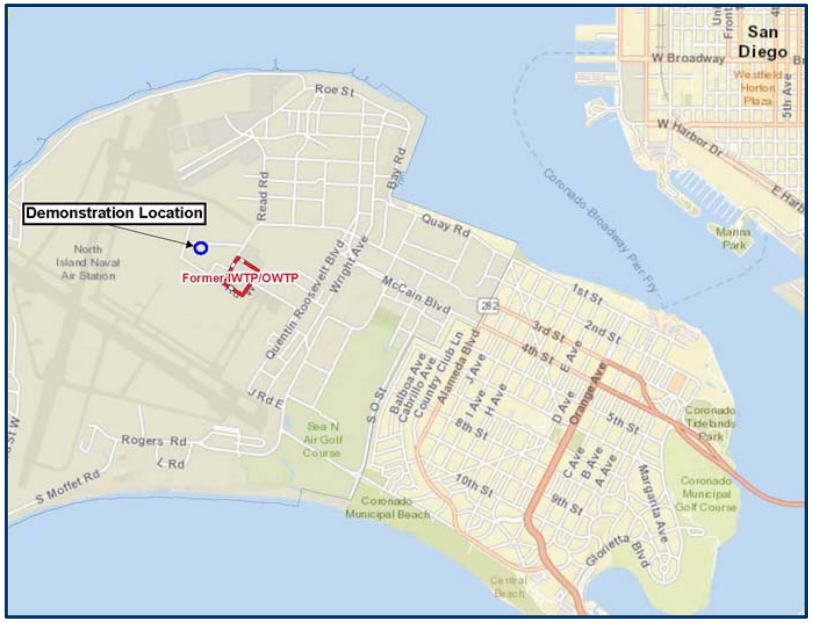
Process B: Methane + Oxygen

- Stimulate methanotrophs
- TCE targeted
- Select other CVOCs



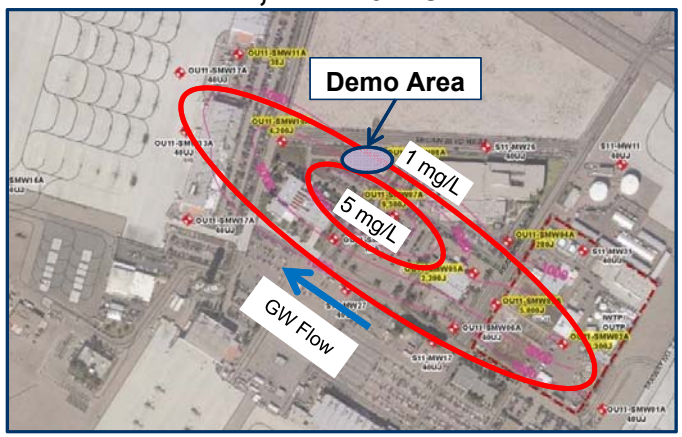
1,4-Dioxane – ESTCP 201733

NAS North Island: Operable Unit 11

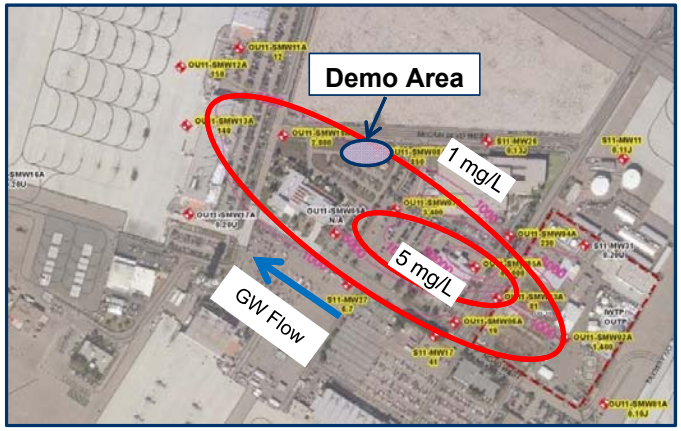


1. Waste disposal–surface impoundments
2. Shallow aquifer (25’-40’ BGS)
3. Neutral pH
4. Comingled 1,4-D and CVOCs (mg/L)

1,4-D Plume

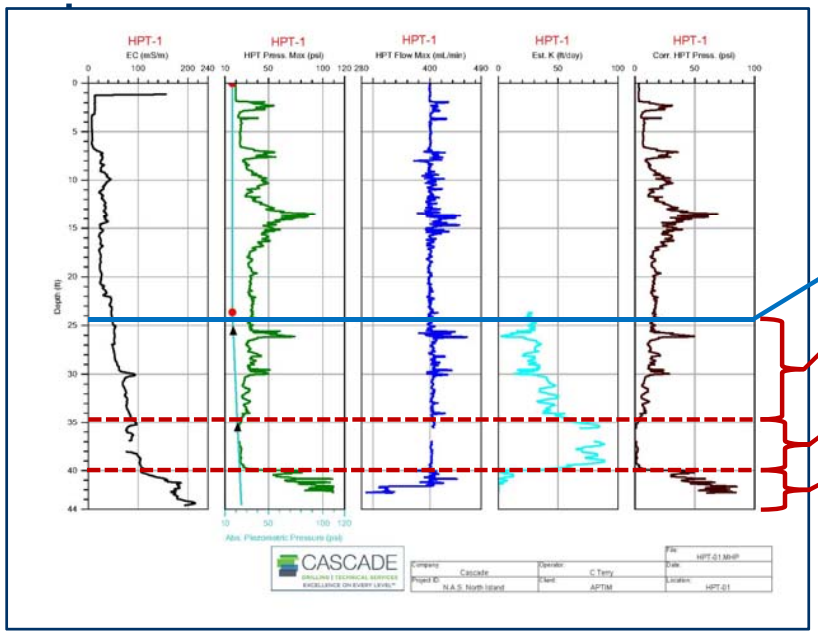


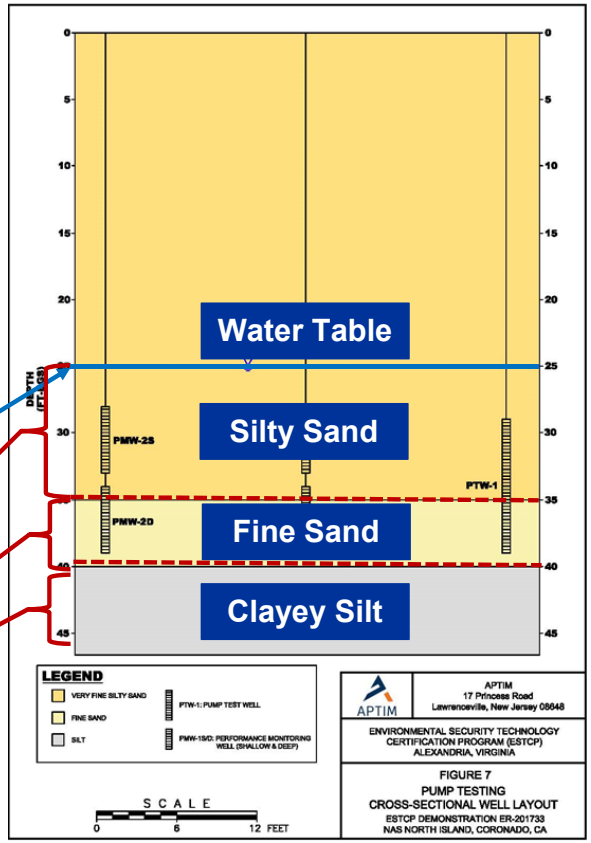
TCE Plume



1,4-Dioxane – ESTCP 201733

- Sandy Aquifer: 25’- 40’ bgs
- Low permeability unit at 40’





1,4-Dioxane – ESTCP 201733

Discrete groundwater sampling results

- Concentrations generally increasing with depth
- Plume located from ~30’ to 40’ bgs

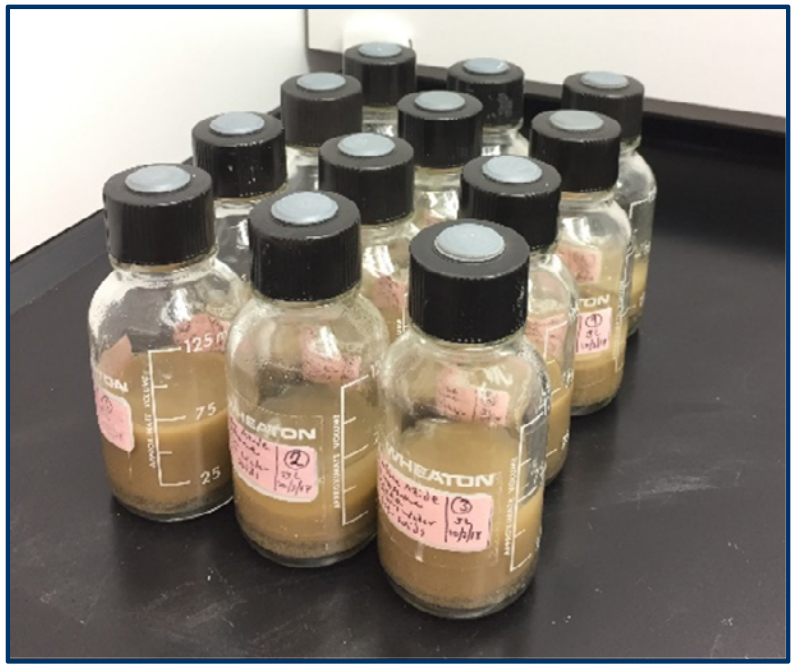
Boring ID	HPT-1			HPT-2			HPT-3		
Depth (ft, bgs)	29	34	39	27.5-29	32.5-34	38-39.5	25.5-28	32-33.5	38-39.5
Compound/Concentration	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
TCE	<5	155	1360	<5	323	851	7	210	2108
1,1-DCE	<5	135	1168	<5	311	667	15	339	2140
1,4-Dioxane	0.94J	69.8	427	234	107	240	50.2	415	1107
cis-1,2-DCE	<5	72	328	<5	100	89	30	293	613
1,1-DCA	<5	13	136	<5	34	58	<5	34	198
Vinyl Chloride	<5	<5	6	<5	<5	<5	<5	<5	15
Chloroform	<5	<5	5	2J	1J	3J	<5	2J	7
1,2-DCA	<5	<5	<5	<5	<5	<5	<5	1J	6
PCE	<5	<5	4J	<5	<5	<5	<5	<5	6
trans, 1,2-DCE	<5	<5	3J	<5	<5	<5	<5	<5	3J
1,1,2-TCA	<5	<5	<5	<5	<5	<5	<5	<5	1J

1,4-Dioxane – ESTCP 201733

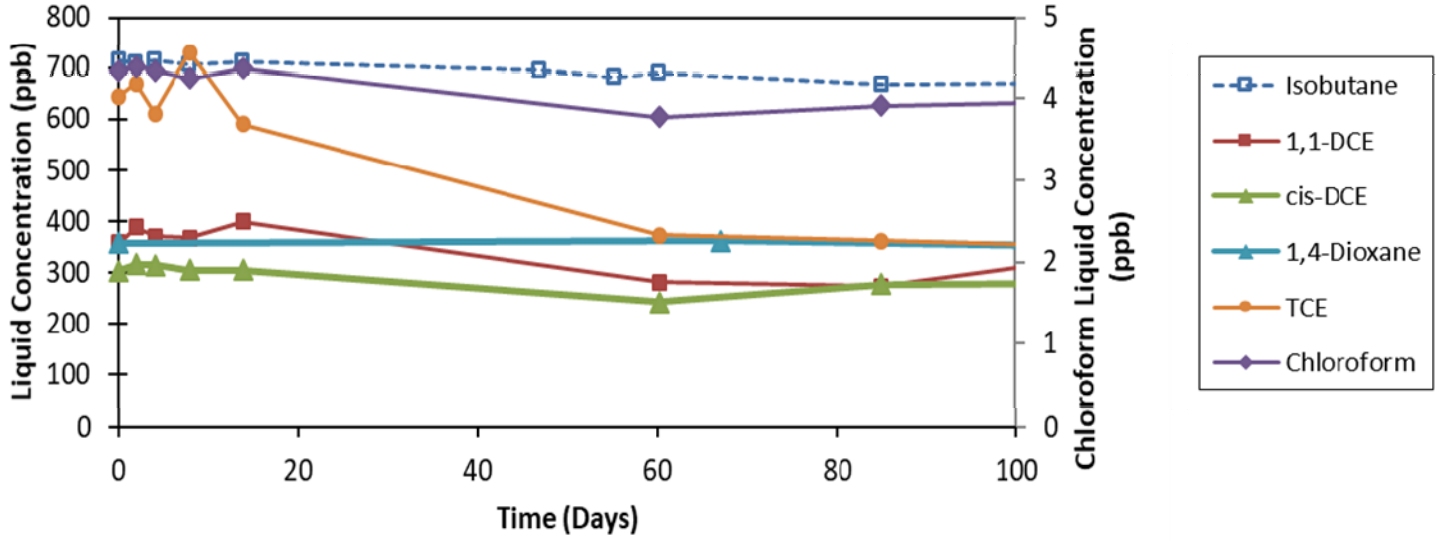
Microcosm Test

Objectives

1. Can indigenous organisms be stimulated to degrade 1,4-D and target cVOCs?
   - ➢Evaluating several alkane/alkene gases
2. Are low levels achievable?
3. Nutrients required/beneficial?
4. Bioaugmentation required?

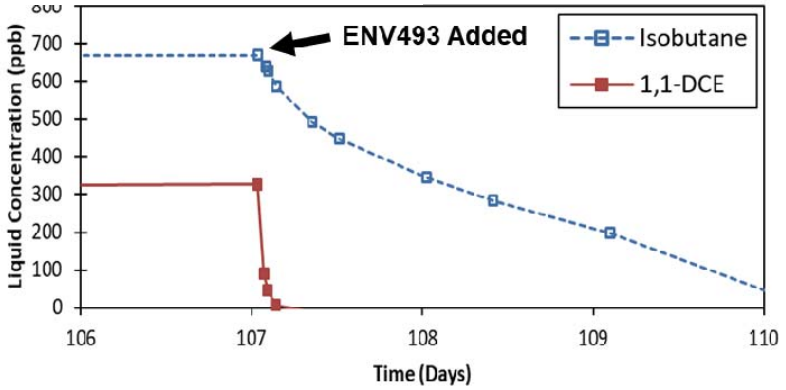


1,4-Dioxane – ESTCP 201733

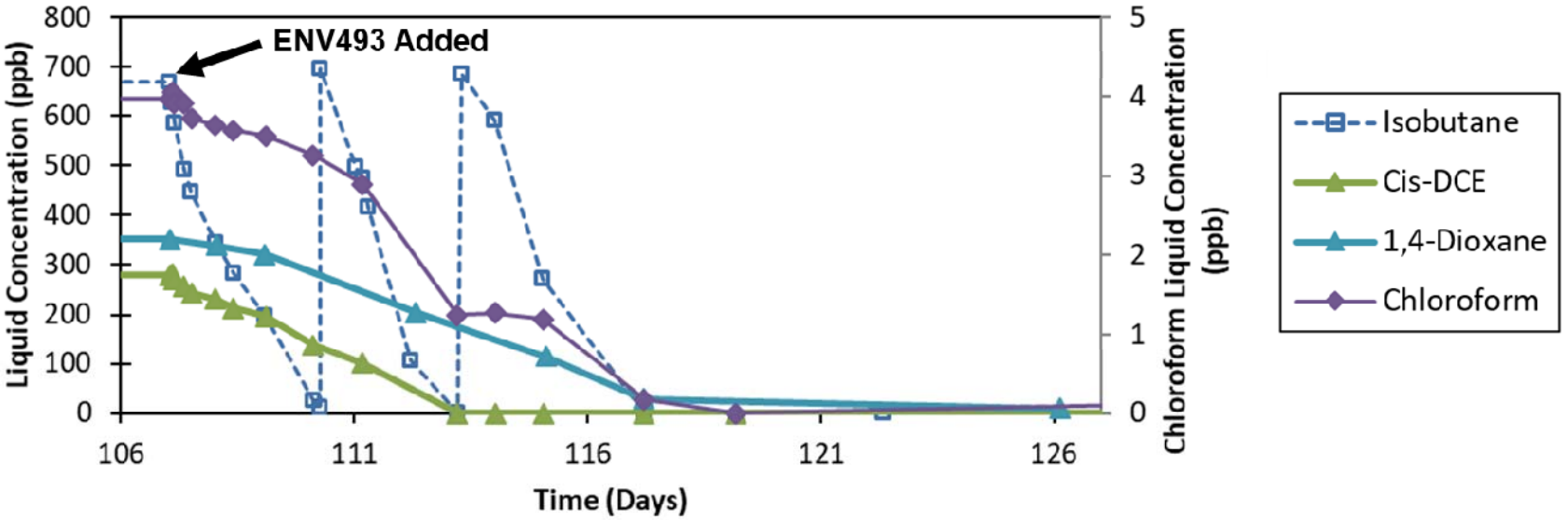


Isobutane Treatments

- No isobutane uptake in 107 days
- Suspect 1,1-DCE toxicity
- ENV493 added on day 107
- Rapid transformation of 1,1-DCE
- Uptake of isobutane (3 days)



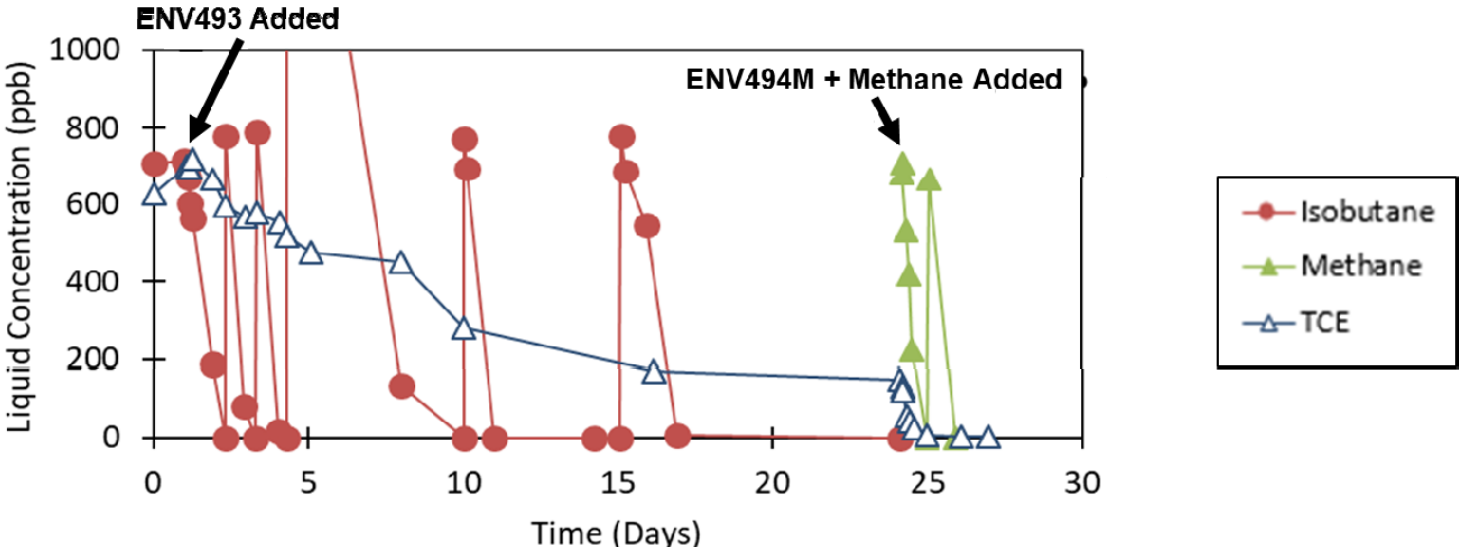
1,4-Dioxane – ESTCP 201733



Isobutane Treatments

- ENV493 added on day 107
- 3 successive spikes of isobutane
- Rapid transformation of 1,4-dioxane, *cis*-DCE and chloroform

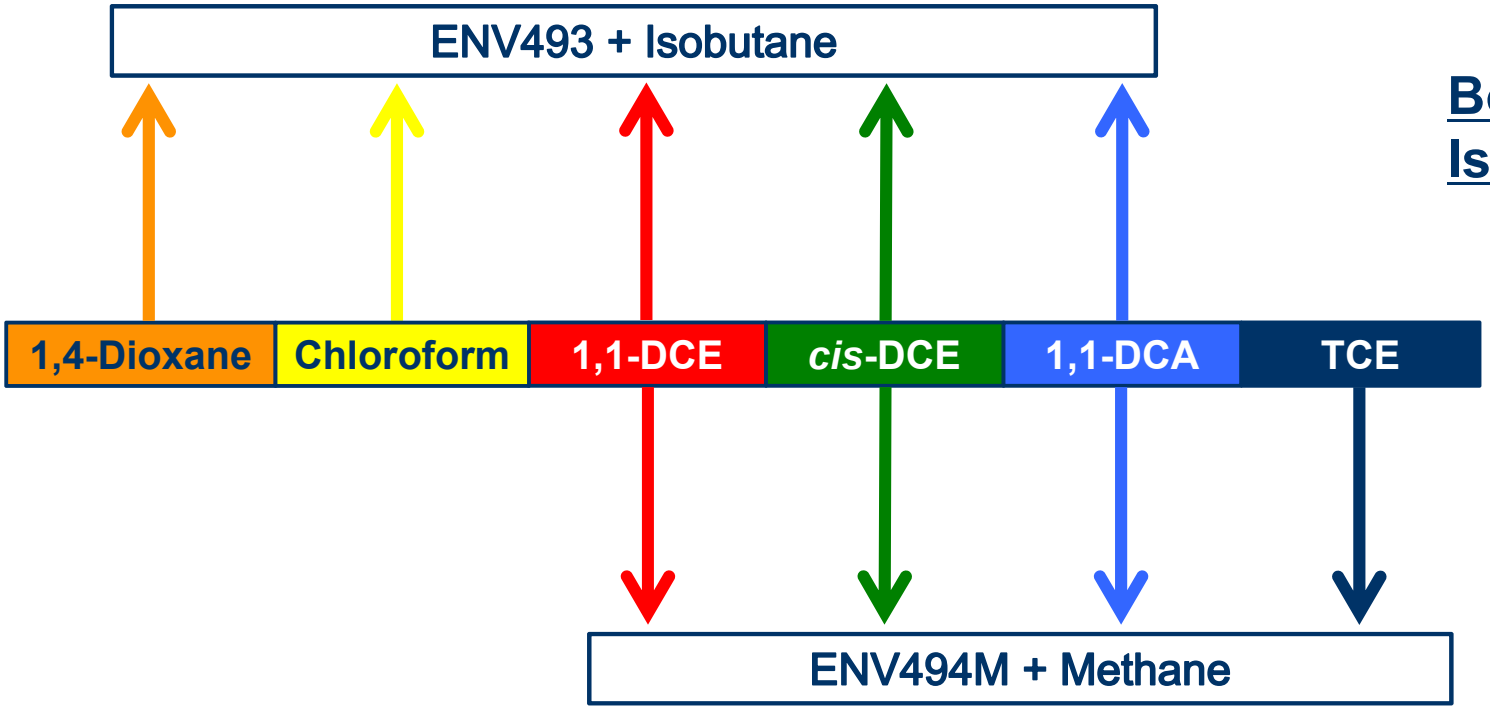
1,4-Dioxane – ESTCP 201733



Isobutane Treatments

- Limited cometabolism of TCE by ENV493
- Mixed methanotroph culture ENV494M and methane added
- TCE degraded and methane uptake observed

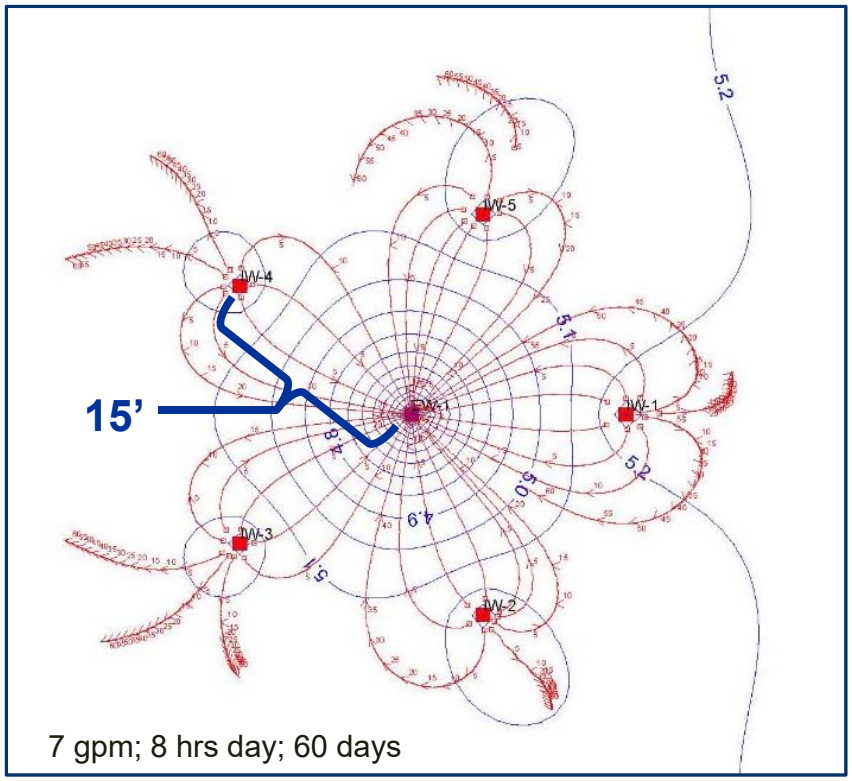
1,4-Dioxane – ESTCP 201733



Both Cultures Isolated from the Site

1,4-Dioxane – ESTCP 201733

Preliminary MODFLOW Modeling Simulation



Groundwater Modeling

- Groundwater recirculation
  - ➢1 extraction well
  - ➢5 injection wells
  - ➢15’ well spacing
- ~8-10 gpm – 8 hr/day
- Substrate gas & nutrient addition
- Bioaugmentation

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Thank you!