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# TCE Fate and Transport Evaluation for Paducah Groundwater: Attenuation Mechanisms

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# Outline



- Monitored Natural Attenuation: Then and Now
- Mechanisms for Attenuation
- DQO for Paducah
- Lines of evidence
  - Enzyme Activity Probes
  - Compound Specific Isotope Analysis, CSIA
  - qPCR
  - Microcosm Rate studies
  - Terminal Restriction Fragment Length Polymorphism, tRFLP
- Broader Implications for these approaches

# Monitored Natural Attenuation 1998



*EPA defines the term MNA .. Refers to reliance on natural attenuation processes to achieve site-specific remedial objectives within time frame which is reasonable compared to that offered by other more active methods. The natural attenuation processes that are at work in such a remediation approach include a variety of physical, chemical, or biological processes that under favorable conditions, act without human intervention to reduce mass, toxicity, mobility, volume, and bioconcentration of contaminants in soil or groundwater.*

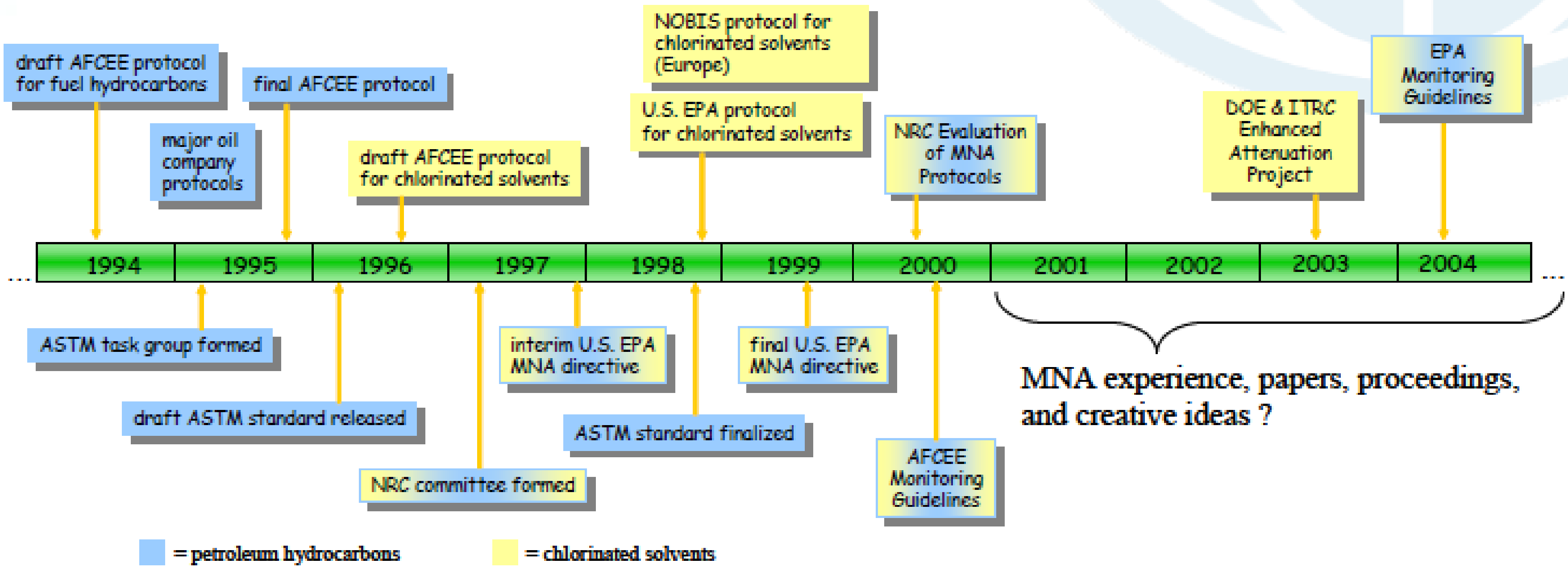
- **BIODEGRADATION**
- **DISPERSION**
- **DILUTION**
- **SORPTION**
- **VOLATILIZATION**
- **BIOLOGICAL OR CHEMICAL STABILIZATION**
- **TRANSFORMATION**
- **DESTRUCTION**

# Biological Mechanisms



| Degradation Process | Reaction Process |
| --- | --- |
| ** Aerobic Oxidation | Compound is oxidized (electron donor). Yields energy to the microorganism |
| Aerobic Cometabolism | Compound is oxidized by an enzyme or co-factor produced during microbial metabolism of another compound; No carbon or energy to microorganism |
| Anaerobic Oxidation | Compound is oxidized by electron acceptors other than oxygen. Yields energy to microorganism |
| ** Direct ARD | Compound is reduced (electron acceptor). Yields energy to microorganism |
| Cometabolic ARD | Compound is reduced by an enzyme or co-factor produced during microbial metabolism of another compound; No carbon or energy to microorganism |
| ** Abiotic | Compound is reduced by chemical reaction. |

# Natural Attenuation of hydrocarbons and chlorinated solvents




1990s- So why did virtually all natural attenuation and bioremediation research for chlorinated solvents shift to anaerobic?

- aerobic slow, indirect process
- difficult/challenging to design
- intermediates, other inhibitors

**2000s -** As result of large plumes, emerging contaminants, and our understanding aerobic potential for direct metabolism ... Focus is back to the aerobic community.

*** Lots of examples of successful application of MNA at DOE sites (SRNL, INL) ***

# AEROBIC Microbial Abundance and Activity … Why do we care?



- Large number of sites are:
  aerobic, large (extent or depth), low biomass and low organic matter

- Many sites will remain above MCLs after the remediation strategy/treatment is complete:
  source as result of less transmissible layers (clays, silts)
  immobilized forms (metals and radionuclides)
  return on investments and/or cost of aggressive treatments

- ***Guidance, technical protocols, acceptable standards are lacking in how to transition these sites (within and between agencies)***

# Background: Historical TCE Attenuation Activities/Information



- **PGDP Groundwater Flow & Transport Models**
  - MODFLOW & MODFLOWT
  - Development 1990 – 1999
  - Applied TCE half-life of 26.7 years to sources and all dissolved phase plume concentrations

- **Evaluation of Natural Attenuation Processes for TCE and $^{99}$Tc in the Northwest and Northeast Plumes** (Lockheed Martin Energy Systems,1997)
  - Evaluated RGA Geochemistry
  - Evaluated Biological and Abiotic Processes based on existing site monitoring data
  - Estimated TCE half-life range from 9.4 to 26.7 years

- **Chlorine Isotope Investigation of Natural Attenuation in an Aerobic Aquifer** (Sturchio, Claussen, et.al.,1999)

- ***Southwest Plume Site Investigation*** (DOE, 2004)
  - 1$^{st}$ Order Decay Calculations revisited
  - Used $^{99}$Tc to estimate TCE half-life range from 3.2 to 11.3 years.

# Path Forward

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- **Regulators and technical community concerned degradation rates for TCE attenuation/degradation developed thru 2005 not well supported**
- **Need for site to identify & quantify TCE Fate & Transport parameters in order to proceed with assessment of:**
  - Long term environmental impacts
  - Long term risks
  - Remedial options
- **KRCEE asked by DOE-Portsmouth/Paducah Project Office (PPPO) to assemble a Project Team to address TCE Fate and Transport**
  - Use DQO Process
  - Discussions with project team participants started Summer 2005
  - Degradation rate of TCE in the RGA is only one of several parameters affecting fate and transport being addressed

# TCE Fate & Transport Project Team: 2005



| Organization | Representatives |
|---|---|
| DOE-PPPO | Rich Bonczek (PPPO Tech Lead)<br>Dave Dollins (PGDP GWOU PM) |
| KRCEE | Steve Hampson, John Volpe |
| USEPA Region IV | David Williams |
| Kentucky Division of Waste Mgmt | Ed Winner, Todd Mullins |
| DOE-EM | Beth Moore |
| Savannah River National Laboratory | Brian Looney |
| North Wind Environmental | Hope Lee |
| Paducah Remediation Services | Bryan Clayton, Ken Davis |
| Navarro Engineering | Bruce Phillips, Tracey Fitzgerald |

# TCE Fate & Transport Project

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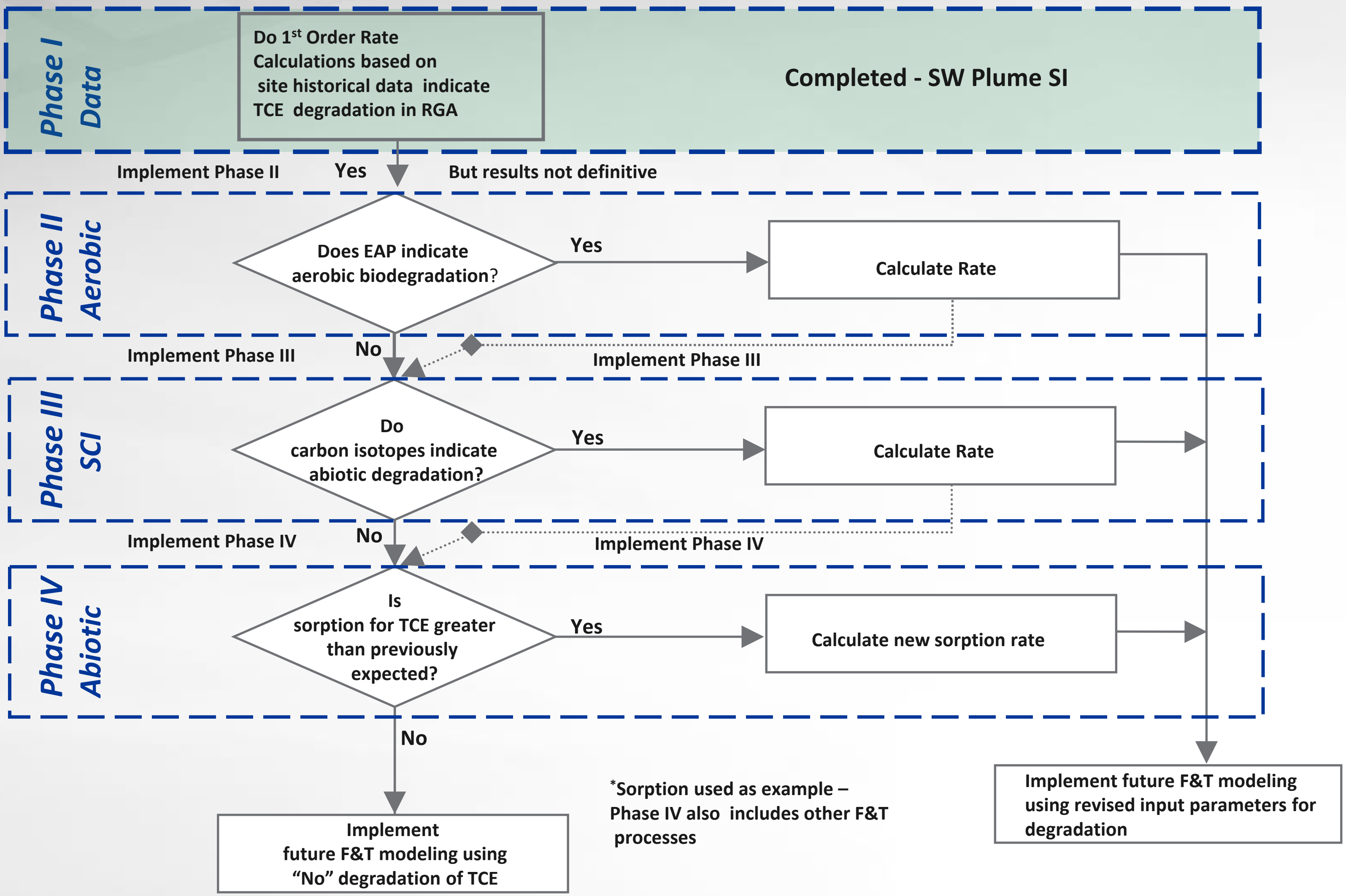
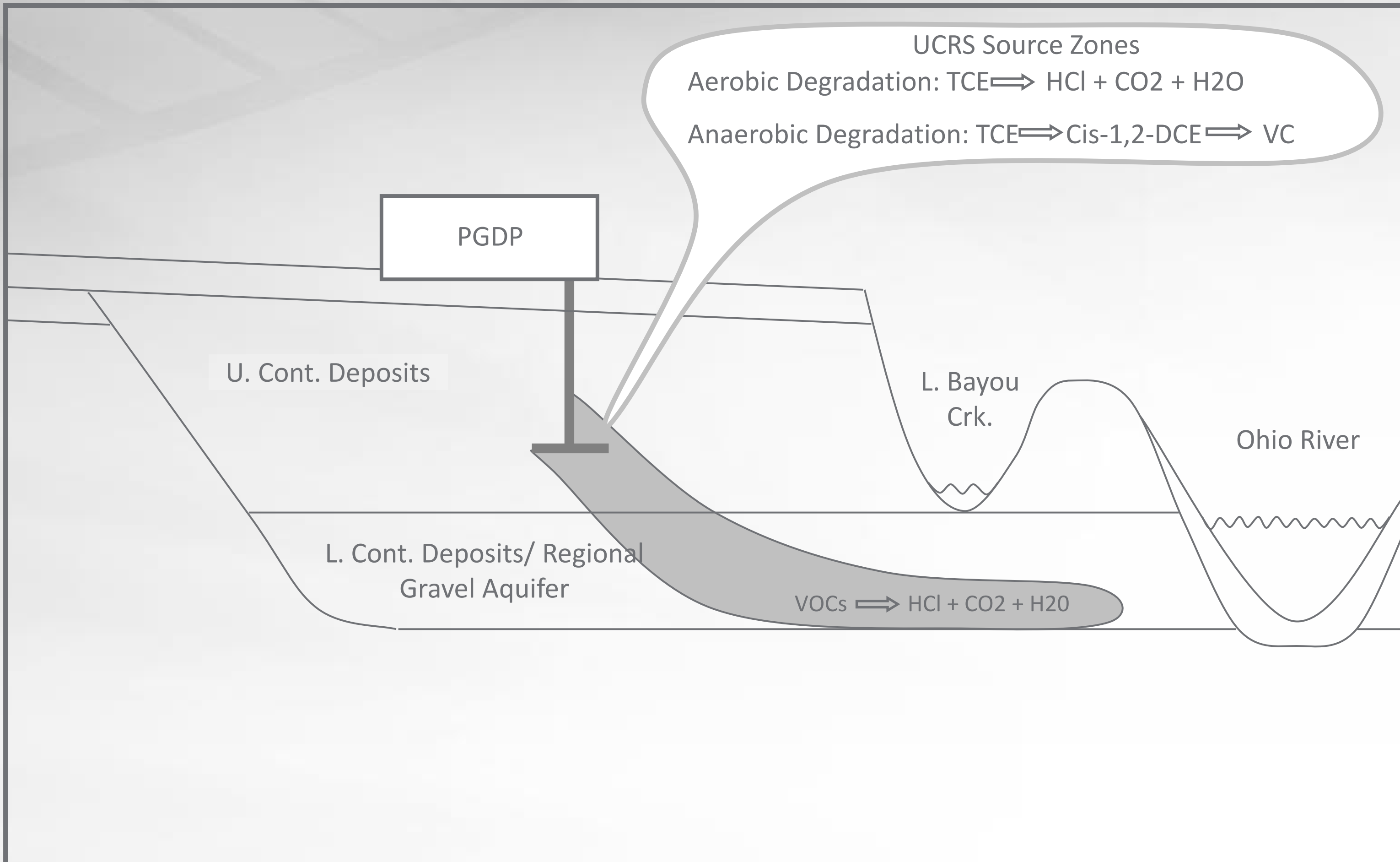
## 4 Phased Project Approach



# Background GW Conceptual Model






Trichloroethene Migration (Primary Source and Dissolved-Phase Plume)

Regional Gravel Aquifer: Aerobic Environment

# TCE Fate & Transport Project, Phase II - Aerobic Degradation



**Why investigate the presence and activity of aerobic microorganisms (bacteria) in the RGA?**

- RGA Groundwater Evaluation - RGA scored poorly on ranking for anaerobic degradation occurrence based on hydro-geochemical conditions
- RGA Is an Aerobic Aquifer (in and outside of plumes) - contains sufficient levels of Dissolved Oxygen to support/sustain aerobic microorganisms
- Dissolved Oxygen trends indicate possible relationship to respiration processes
- Redox conditions in the RGA strongly indicate the potential for aerobic microorganisms & aerobic TCE degradation
  - *Would not support/sustain anaerobic microorganisms*

# TCE Fate & Transport Project, Phase II - Aerobic Degradation DQOs



## Problem Statement
## (Phase II – Aerobic Biodegradation)

- The Paducah site has contaminated groundwater. The purpose of the proposed work is to demonstrate whether sustainable trichloroethene (TCE) biodegradation occurs within the RGA under aerobic aquifer conditions. Biodegradation needs to be characterized and assessed, and the resources necessary to evaluate this process needs to be identified.

# TCE Fate & Transport Project, Phase II - Aerobic Degradation DQOs



## Decision / Estimation Statements

1. Based on the use of "oxygenase" specific enzyme activity probes, determine whether bacteria capable of aerobically biodegrading TCE are present in the RGA.
2. Based on the use of stable carbon isotope (SCI) fractionation tests, determine whether SCI supports the occurrence of aerobic degradation and/or other biotic/abiotic degradation processes.
3. Estimate whether the distribution and number of bacteria are sufficient to significantly biodegrade the plumes
4. Determine whether conditions (e.g., bioavailable and sustainable substrates) in the RGA are conducive for ongoing and sustainable aerobic biodegradation of TCE
5. Based upon a comparison of the calculated biodegradation rate, or rate range, to values in literature, either accept the calculated rate for future modeling or assess the team's confidence in the unsupported results

# TCE Fate & Transport Project, Phase II – Aerobic Degradation DQOs

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## Draft Decision Rules

- Greater than or equal to half (50%) of the wells in the plume must contain bacteria having an “oxygenase” enzyme capable of aerobically degrading TCE in order to conclude that aerobic processes are occurring throughout the plume.

- If greater than 50% of the EAP analyses indicate bacteria having an “oxygenase” enzyme capable of degrading TCE, then the spatial relationship between the monitoring wells with positive results will be examined to estimate the impact of biodegradation on the plume.

- If the 50% level is not reached, it will be assumed that aerobic bacteria are not appreciably contributing to degradation in the plume from which the samples were collected (does not mean that biodegradation is not occurring, but biodegradation alone is insignificant in it’s impact on the areal extent of the plume.

- The bacterial cell count per well must be greater than $10^3$/ml. If the cell count in any well is less than $10^3$/ml, the well is considered to have no aerobic bacteria activity capable of TCE degradation.

# Enzyme Activity Probes: EAP

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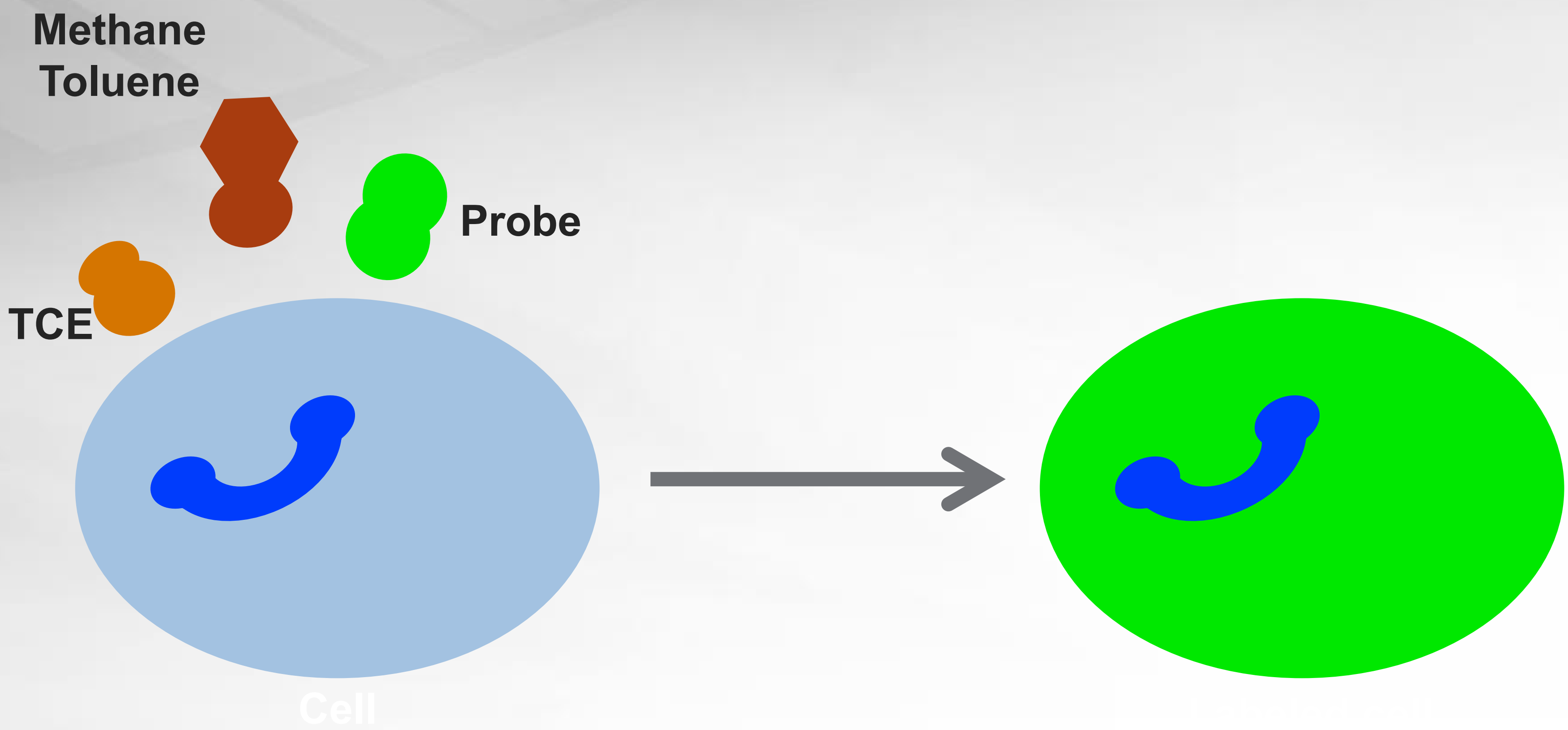
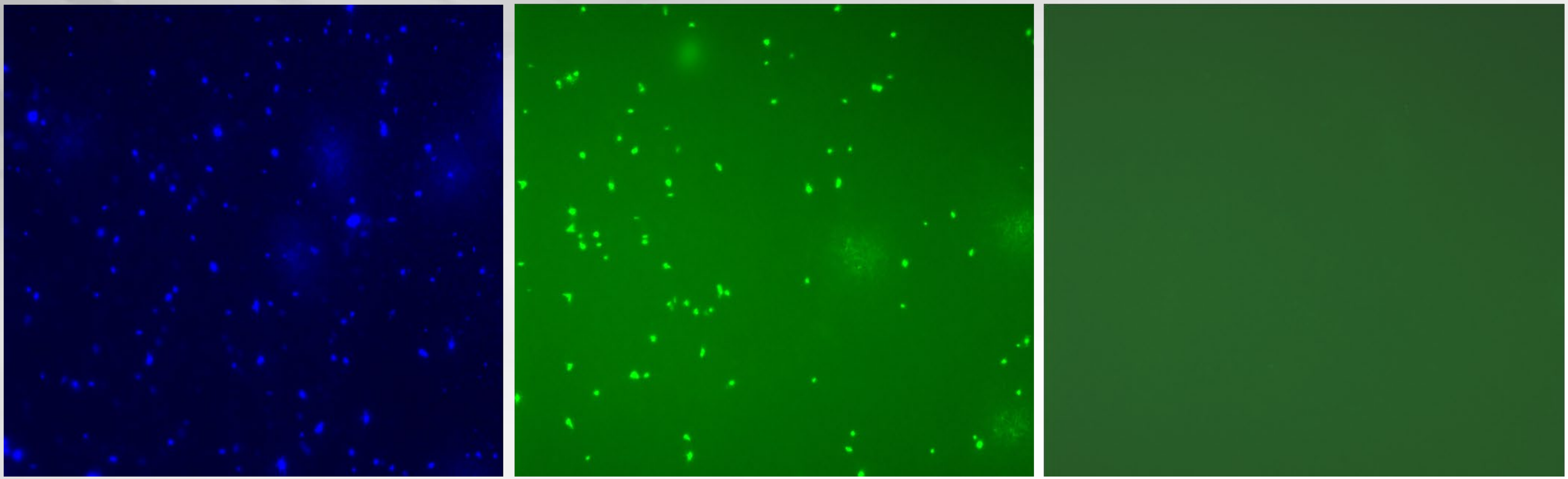



Other natural substrates that support TCE oxidation: benzene, ammonia, phenol, naphthalene, propane

# EAP micrographs





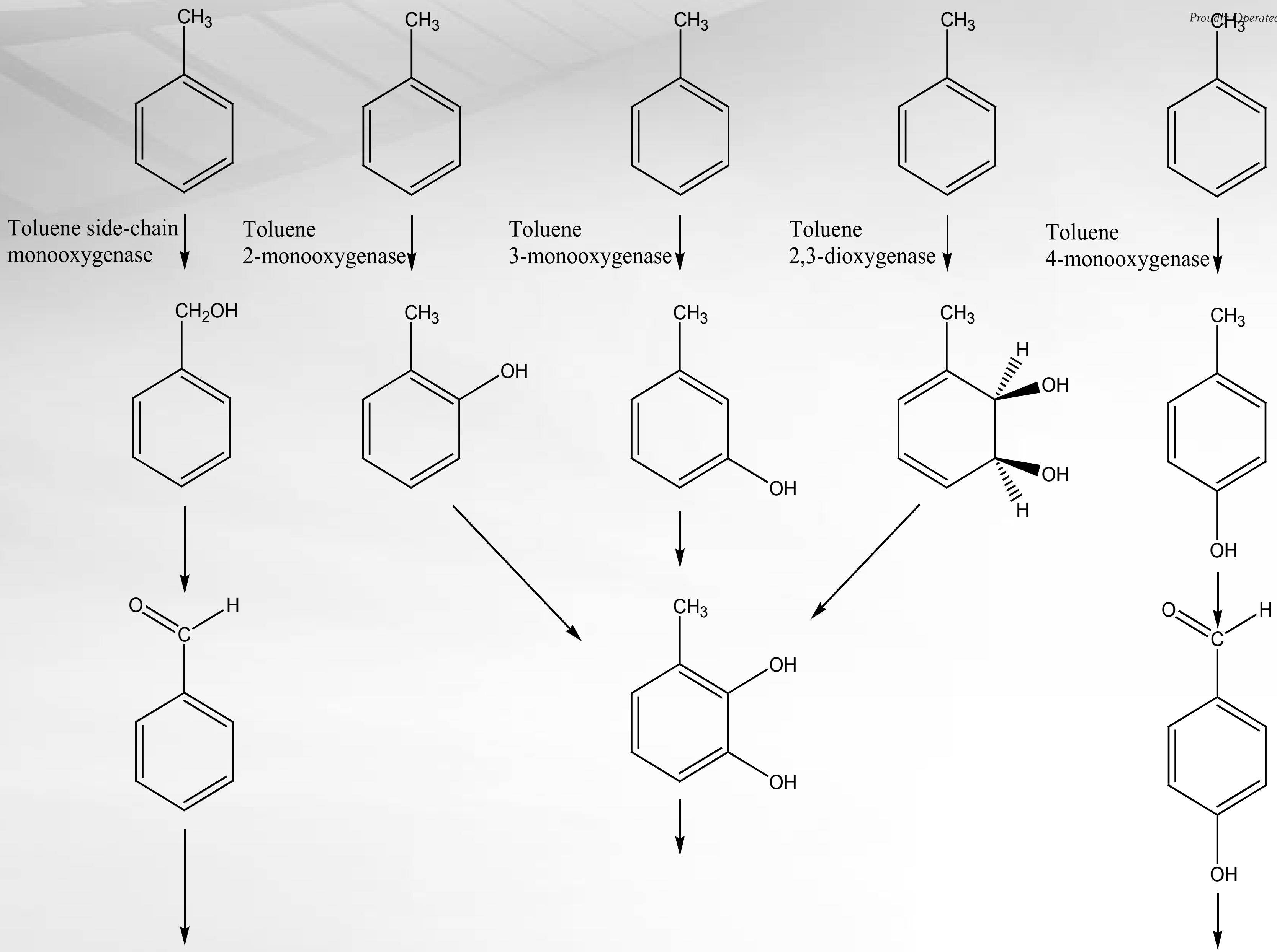
*DAPI*- total cells

*Enzyme probes*- positive response

*Enzyme probes*- negative response

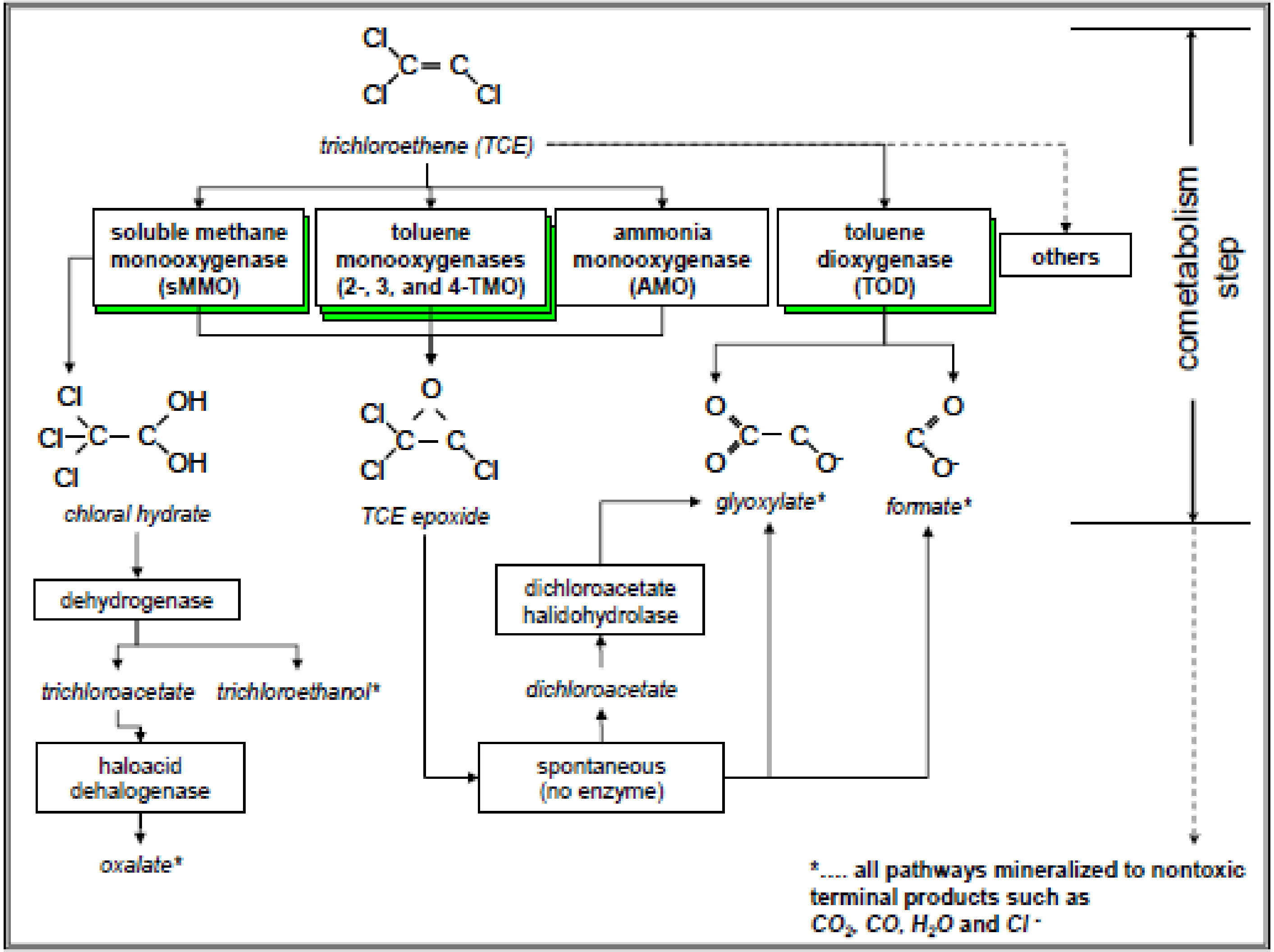
# Aromatic Oxygenase Pathways





# Cometabolic Pathways for TCE, PCE, petroleum hydrocarbons …





# Correlation: qPCR & EAP



| Pathway | Probe | PCR/qPCR |
|---|---|---|
| side-chain monooxygenase | 3EB | TOL |
| 2-monooxygenase | 3HPA<br>PA | PHE |
| 3-monooxygenase | 3HPA<br>*maybe* PA | RMO, PHE |
| 4-monooxygenase | NO EAP *currently validated* | RMO, PHE |
| 2,3-dioxygenase | trans-cinnamonitrile | TOD |
| soluble methane monooxygenase (sMMO) | Coumarin | mmoX |

*New quantitative assays developed & validated:*
*Naphthalene, ammonia, particulate methane, alkane, benzene, propane oxygenases, catechol dioxygenase*

# TCE Fate & Transport Project, Phase II – Aerobic Degradation DQOs

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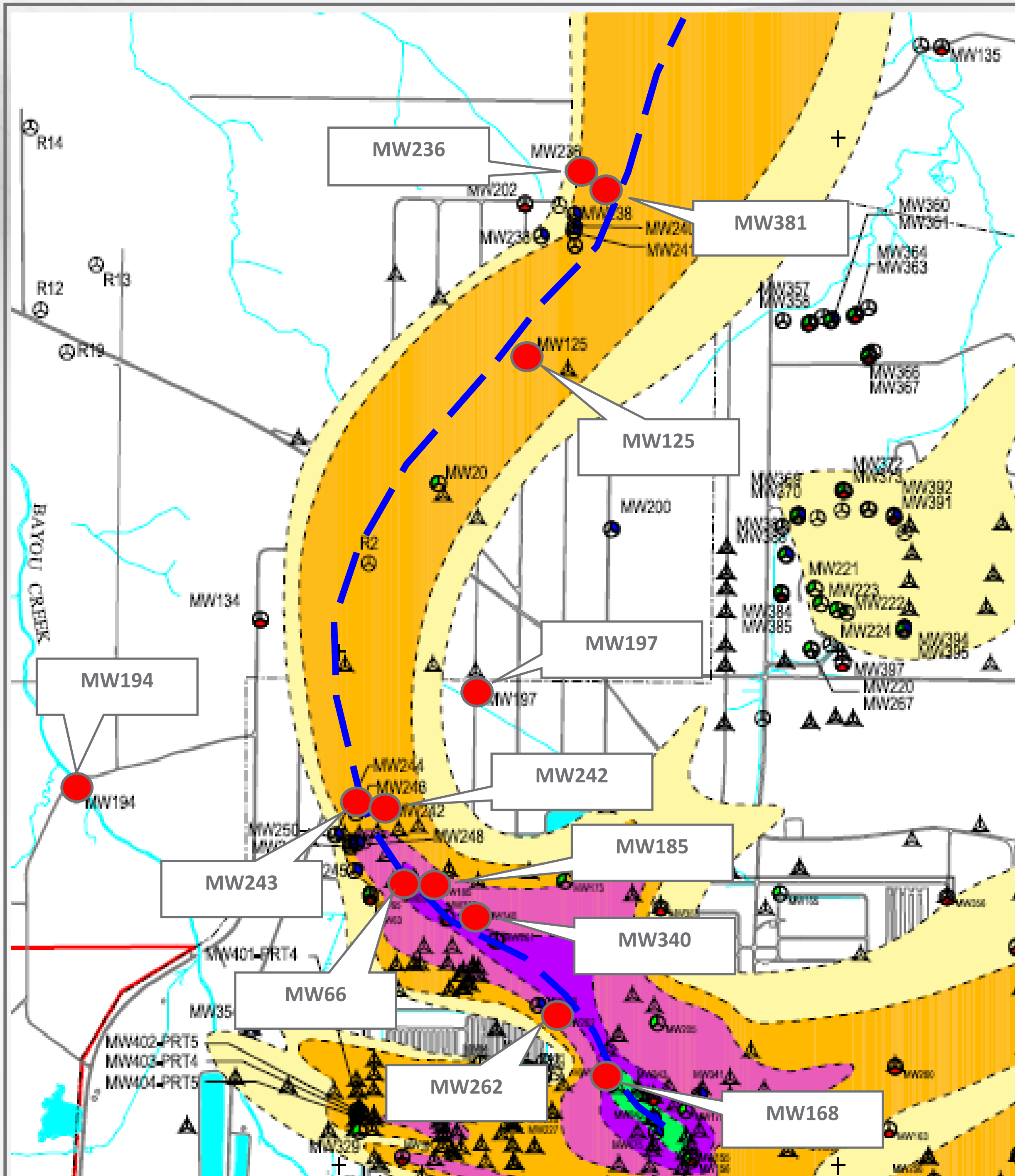
## Monitoring Wells Proposed for Sampling

| Well ID | Screen Interval | Approx. Screen Depth (ft bgs) | Next Scheduled Sample Date | Priority | Comments |
|---|---|---|---|---|---|
| **MW66** | URGA | 55 - 60 | March | 2 | Near SWMU 7/30 Source |
| **MW125** | LRGA | 78 - 88 | March | 1 | |
| **MW168** | URGA | 63 - 68 | March | 1 | |
| **MW185** | MRGA | 68 - 73 | March | 1 | |
| **MW194** | URGA | 47 - 52 | March | 2 | Control Well - outside of Plume |
| **MW197** | URGA | 58 - 63 | March | 3 | Control Well - outside of Plume |
| **MW236** | LRGA | 69.5 - 79.5 | May | 2 | |
| **MW381** | MRGA | 66 - 76 | May | 3 | |
| **MW242** | MRGA | 65 - 75 | May | 3 | |
| **MW243** | MRGA | 65 - 75 | May | 3 | Downgradient of South Well Field; initially >10 mg/L, been at 1 mg/L for last 10 years |
| **MW262** | LRGA | 90 - 95 | March | 1 | |
| **MW340** | LRGA | 85.5 - 95.3 | March | 2 | |

**"Priority" based on sampling schedule dates only**



MW236
MW381
MW125
MW197
MW194
MW242
MW185
MW243
MW340
MW66
MW262
MW168
MW135
R14
R12
R13
R19
BAYOU CREEK
MW134
MW20
MW200
R2
MW202
MW360
MW364
MW363
MW357
MW366
MW367
MW221
MW222
MW224
MW387
MW220
MW267
MW244
MW248
MW250
MW401-PRT4
MW402-PRT5
MW403-PRT4
MW404-PRT5
MW329

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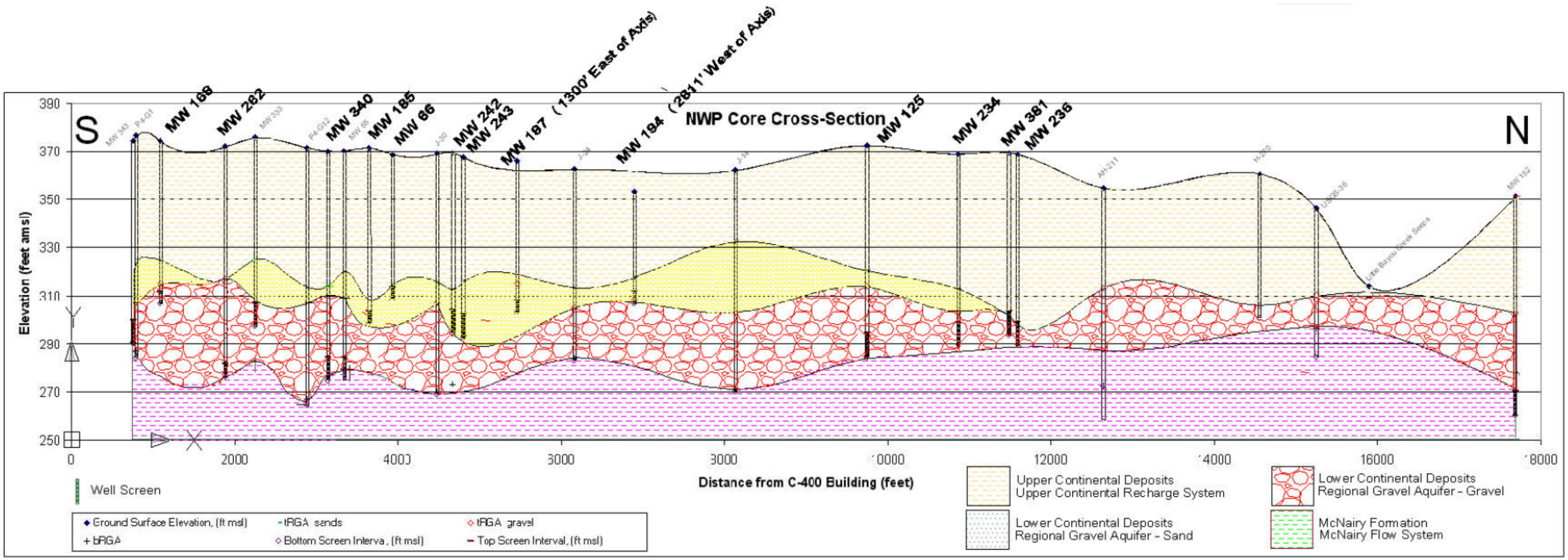

# Well Characterization Data: May 2007

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| Monitoring Well | Aquifer Designation | Screened Interval Depth (ft) | TCE (µg/L) | DCE (µg/L) | technetium (pCi / L) | | dissolved oxygen (mg/L) | pH (std units) |
|---|---|---|---|---|---|---|---|---|
| | | | | | result | error | | |
| MW168 | URGA | 63 - 68 | 110 | < 100 | -- | -- | 2.5 | 5.76 |
| MW66 | URGA | 55 - 60 | 700 | < 5 | -- | -- | 5.8 | 6.01 |
| MW194 | URGA | 47 - 52 | 1 | < 5 | -- | -- | 5.4 | 5.98 |
| MW197 | URGA | 58 - 63 | 3.9 | < 5 | -- | -- | 0.6 | 6.01 |
| MW185 | MRGA | 68 - 73 | 3300 | 140 | -- | -- | 2.0 | 6.08 |
| MW242 | MRGA | 65 - 75 | 110 | < 5 | -- | -- | 1.5 | 5.62 |
| MW243 | MRGA | 65 - 75 | 100 | < 5 | -- | -- | 5.9 | 6.22 |
| MW381 | MRGA | 66 - 76 | 50 | < 5 | -- | -- | 3.2 | 6.18 |
| MW262 | LRGA | 90 - 95 | 950 | < 50 | -- | -- | 0.6 | 5.89 |
| MW340 | LRGA | 85.5 - 95.3 | 6500 | < 250 | -- | -- | 3.5 | 5.94 |
| MW236 | LRGA | 69.5 - 79.5 | 21 | < 5 | -- | -- | 3.4 | 6.19 |
| MW125 | LRGA | 78 - 88 | 700 | < 25 | -- | -- | 2.8 | 6.05 |

| Monitoring Well | oxidation - reduction potential (mV) | specific conductivity (umhos/cm) | chloride (mg/L) | nitrate (mg/L) | sulfate (mg/L) | iron (II) (mg/L) | total organic carbon (mg/L) | alkalinity (mg/L as $CaCO_3$) |
|---|---|---|---|---|---|---|---|---|
| MW168 | 428 | 533 | 92 | 17 | 11 | 0.035 | < 1 | 77 |
| MW66 | 304 | 213 | 13 | 5.8 | 11 | < 0.02 | < 1 | 72 |
| MW194 | 367 | 249 | 27 | 7.0 | 6.5 | < 0.02 | < 1 | 72 |
| MW197 | -7 | 440 | 65 | < 4.4 | 16 | 23.9 | 2.3 | 78 |
| MW185 | 527 | 437 | 57 | 7.5 | 12 | < 0.02 | < 1 | 109 |
| MW242 | 166 | 358 | 63 | < 4.4 | 12 | 8.13 | < 1 | 55 |
| MW243 | 252 | 459 | 12 | < 4.4 | 67 | 0.046 | < 1 | 113 |
| MW381 | 286 | 372 | 41 | 6.7 | 24 | < 0.02 | < 1 | 98 |
| MW262 | 339 | 679 | 110 | 5.6 | 39 | < 0.02 | < 1 | 105 |
| MW340 | 367 | 460 | 61 | 7.2 | 28 | < 0.02 | < 1 | 109 |
| MW236 | 332 | 321 | 31 | 7.3 | 21 | < 0.02 | < 1 | 90 |
| MW125 | 303 | 302 | 33 | 5.8 | 19 | < 0.02 | < 1 | 91 |

# Well Characterization Data: December 2007

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| Monitoring Well | Aquifer Designation | Screened Interval Depth (ft) | TCE (µg/L) | DCE (µg/L) | technetium (pCi / L) | | dissolved oxygen | pH (std units) | oxidation - reduction potential (mV) | specific conductivity (umhos/cm) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | result | error | | | | |
| MW 168 | URGA | 63 - 68 | 110 | < 1.2 | 2400 | 45 | 3.1 | 5.87 | 233 | 492 |
| MW66 | | 55 - 60 | 930 | < 5 | 530 | 24 | 5.7 | 6.01 | 285 | 190 |
| MW 194 | | 47 - 52 | 1 | < 1 | ND | -- | 3.6 | 6.20 | 114 | 251 |
| MW 197 | | 58 - 63 | 3.5 | < 1 | ND | -- | 0.7 | 6.13 | 2 | 424 |
| MW 185 | MRGA | 68 - 73 | 3600 | 76 | 696 | 26 | 1.7 | 6.10 | 269 | 382 |
| MW 242 | | 65 - 75 | 150 | 4.4 | 110 | 15 | 0.8 | 6.09 | 63 | 395 |
| MW 243 | | 65 - 75 | 590 | < 5 | 306 | 19 | 3.8 | 5.96 | 150 | 378 |
| MW 381 | | 66 - 76 | 47 | < 1 | 21.5 | 12.5 | 6.1 | 6.65 | 261 | 502 |
| MW 262 | LRGA | 90 - 95 | 1400 | 11 | 519 | 23 | 0.8 | 5.97 | 218 | 601 |
| MW 340 | | 85.5 - 95.3 | 9700 | < 80 | 647 | 26 | 3.2 | 6.04 | 254 | 453 |
| MW 236 | | 69.5 - 79.5 | 72 | < 1 | 29.1 | 12.7 | 6.1 | 6.65 | 261 | 502 |
| MW 125 | | 78 - 88 | 620 | < 5 | 220 | 18 | 2.9 | 6.11 | 400 | 310 |

# Enzyme Activity Probes



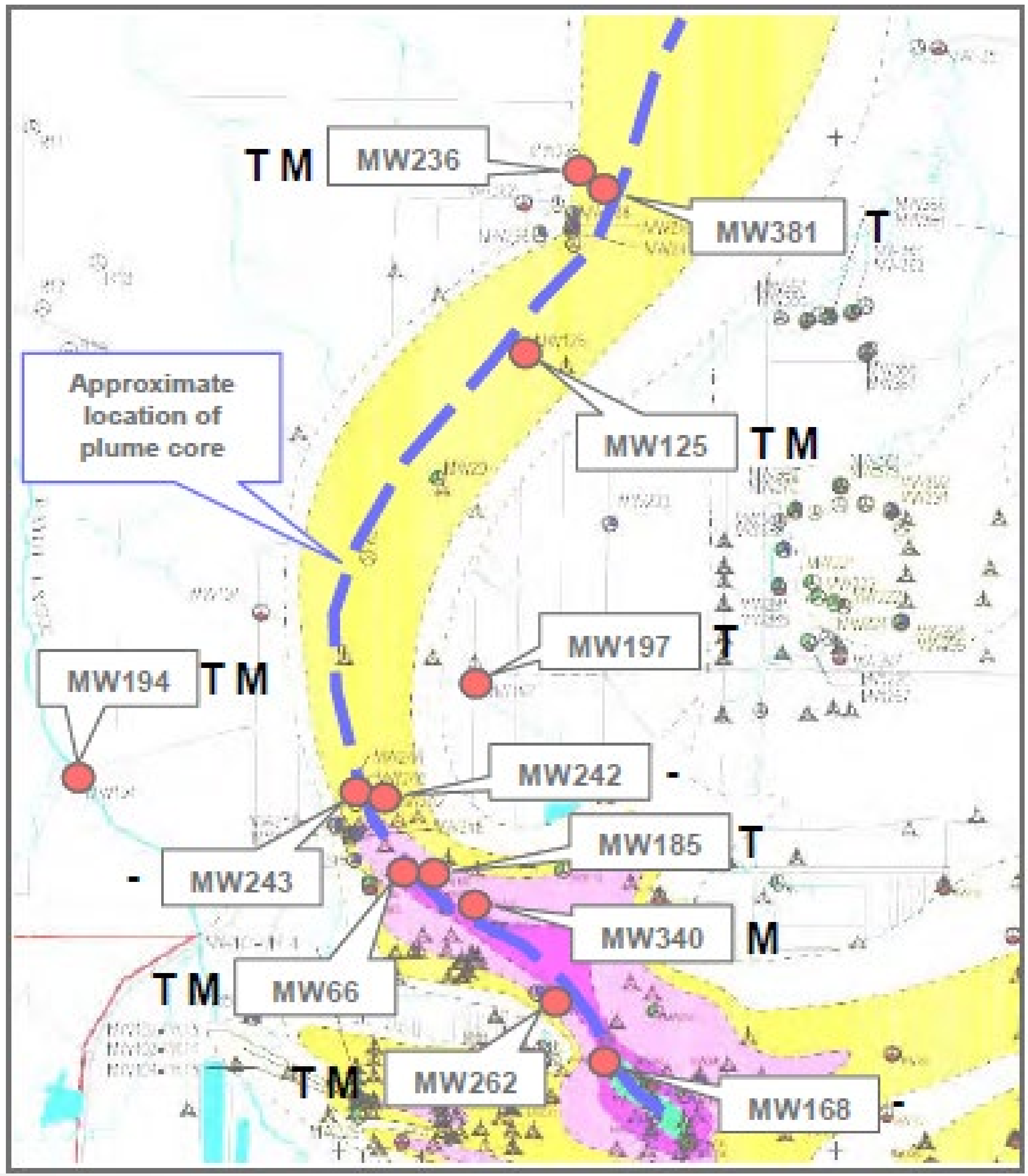
| Monitoring Well | Aquifer Designation | Screened Interval Depth (ft bgs) | Qualitative data (6/4/7) | | Toluene probes | | | Total –DAPI cells/mL |
|---|---|---|---|---|---|---|---|---|
| | | | sMMO probe Coumarin | Toluene probes | Quantitative data ( fluorescent cells/mL ) | | | |
| | | | | | 3HPA | PA | Cinnamonitrile | |
| MW168 | URGA | 63 - 68 | - | - | nd | $2.41x10^3$ | nd | $1.90x10^5$ |
| MW66 | URGA | 55 - 60 | + | +++ | $1.43x10^4$ | $2.10x10^4$ | $9.14x10^3$ | $3.67x10^5$ |
| MW194 | URGA | 47 - 52 | + | +++ | $3.13x10^3$ | $9.52x10^3$ | $1.20x10^4$ | $1.76x10^5$ |
| MW197 | URGA | 58 - 63 | - | + | $1.73x10^4$ | $6.28x10^4$ | $2.23x10^3$ | $1.59x10^5$ |
| MW197 (resample) | URGA | | na | na | $5.03x10^3$ | $1.20x10^4$ | $2.04x10^3$ | $7.05x10^5$ |
| MW185 | MRGA | 68 - 73 | - | ++ | $1.79x10^4$ | $1.37x10^4$ | $1.95x10^3$ | $9.75x10^5$ |
| MW242 | MRGA | 65 - 75 | - | - | $3.57x10^3$ | $1.24x10^3$ | $8.85x10^3$ | $7.76x10^5$ |
| MW243 | MRGA | 65 - 75 | - | - | $3.29x10^3$ | $4.61x10^3$ | $1.32x10^3$ | $4.27x10^5$ |
| MW381 | MRGA | 66 - 76 | - | ++ | $6.14x10^4$ | $3.52x10^4$ | $5.51x10^3$ | $9.66x10^5$ |
| MW262 | LRGA | 90 - 95 | + | +++ | $1.35x10^4$ | $1.36x10^4$ | $2.79x10^4$ | $3.52x10^5$ |
| MW 262 (resample) | LRGA | | na | na | $1.05x10^4$ | $1.22x10^4$ | $5.71x10^3$ | $2.84x10^5$ |
| MW340 | LRGA | 85.5 - 95.3 | + | + | $3.63x10^2$ | $9.57x10^3$ | nd | $7.25x10^5$ |
| MW236 | LRGA | 69.5 - 79.5 | + | +++ | $3.24x10^4$ | $5.26x10^4$ | $9.28x10^3$ | $8.84x10^5$ |
| MW125 | LRGA | 78 - 88 | + | ++ | $1.39x10^4$ | $6.37x10^4$ | $2.03x10^4$ | $7.99x10^5$ |

URGA: Upper Regional Gravel Aquifer
MRGA: Middle Regional Gravel Aquifer
LRGA: Lower Regional Gravel Aquifer
3HPA: 3-hydroxy-phenylacetylene --> probe for toluene oxidase and related activity
PA: Phenylacetylene --> probe for toluene oxidase and related activity
cinnamonitrile: probe for tolulene dioxygenase and related activity
DAPI: 4',6-Diamidino-2-Phenylindole (double stranded DNA staining)

ft bgs– feet below ground surface
µg/L – micrograms per liter
pCi/L – picocuries per liter
cells/mL – per milliliter

Highlight denotes that the toluene probe response was considered moderate (fluorescent activity > $3x10^3$ cells/mL and < $8x10^3$ cells/mL) – see text for explanation
Highlight denotes that the sMMO probe was significantly above background or the toluene probe response was considered significant (> $8x10^3$ cells/mL fluorescent activity)

# EAP data for the NW plume




*T: Toluene Oxygenase activity*
*S: sMMO activity*

# PCR: Aromatic Genes of Interest



| Monitoring Well | Aquifer Designation | Genes amplified | | | |
|---|---|---|---|---|---|
| | | sMMO | RMO | PHE | TOD |
| MW168 | URGA | + | - | + | - |
| MW66 | | + | + | + | + |
| MW194 | | + | + | + | + |
| MW197 | | - | + | + | + |
| MW185 | MRGA | - | - | + | + |
| MW242 | | + | - | + | + |
| MW243 | | + | - | + | + |
| MW381 | | - | + | + | + |
| MW262 | LRGA | + | + | + | + |
| MW340 | | + | - | + | + |
| MW236 | | + | + | + | + |
| MW125 | | + | + | + | + |

sMMO: soluble methane monooxygenase
RMO: Ringhydroxylation toluene monooxygenase
PHE: Phenol monooxygenase
TOD: toluene/xylene monooxygenase

# TCE Fate & Transport Project, Phase II – Aerobic Degradation DQOs

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## Draft Decision Rules

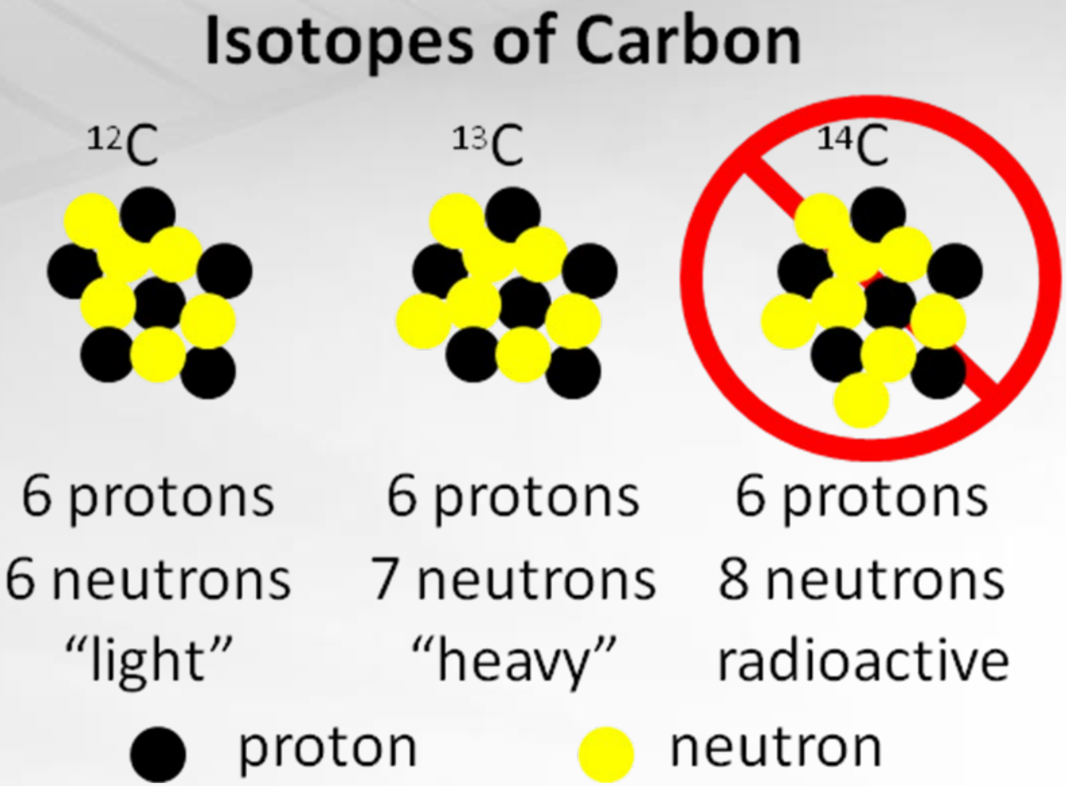
- Greater than or equal to half (50%) of the wells in the plume must contain bacteria having an “oxygenase” enzyme capable of aerobically degrading TCE in order to conclude that aerobic processes are occurring throughout the plume.

- If greater than 50% of the EAP analyses indicate bacteria having an “oxygenase” enzyme capable of degrading TCE, then the spatial relationship between the monitoring wells with positive results will be examined to estimate the impact of biodegradation on the plume.

- If the 50% level is not reached, it will be assumed that aerobic bacteria are not appreciably contributing to degradation in the plume from which the samples were collected (does not mean that biodegradation is not occurring, but biodegradation alone is insignificant in it’s impact on the areal extent of the plume.

- The bacterial cell count per well must be greater than $10^3$/ml. If the cell count in any well is less than $10^3$/ml, the well is considered to have no aerobic bacteria activity capable of TCE degradation.

# Compound Specific Isotope Analysis




- Changes in isotopic ratios are caused by the breaking of bonds between atoms. Physical processes do not change the ratios in compounds to the same extent as bio-geochemical processes.
- It takes slightly less energy to break a bond between a light isotope and another atom than between heavy isotope and the same atom; the reaction rates of the heavier isotope are slightly slower so the percentage of heavy isotopes increases as the contaminant is degraded

# Enrichment factors for degradation pathways

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| Pathway | c-DCE | TCE |
|---|---|---|
| Anaerobic | −12.0 to −25.5 | −2.5 to −31.1 |
| sMMO | −0.4±0.5 | −1.1±0.3 |
| T2MO | | −19.3±1.8 |
| T3MO | −0.89±0.51 | −11.60±4.11 |
| T4MO | | −14.40±6.44 |
| TDO | −1.17±0.60 | −14.31±2.38 |
| aerobic oxidation | −8.5±0.10 | −7.2±0.07 |

*Published values for specific pathways (summary of 10 studies).*
*Problems: Wide RANGE of values for aerobic degradation of chlorinated solvents. MANY aerobic pathways.*

# CSIA data: Third line of evidence, biodegradation IS occurring

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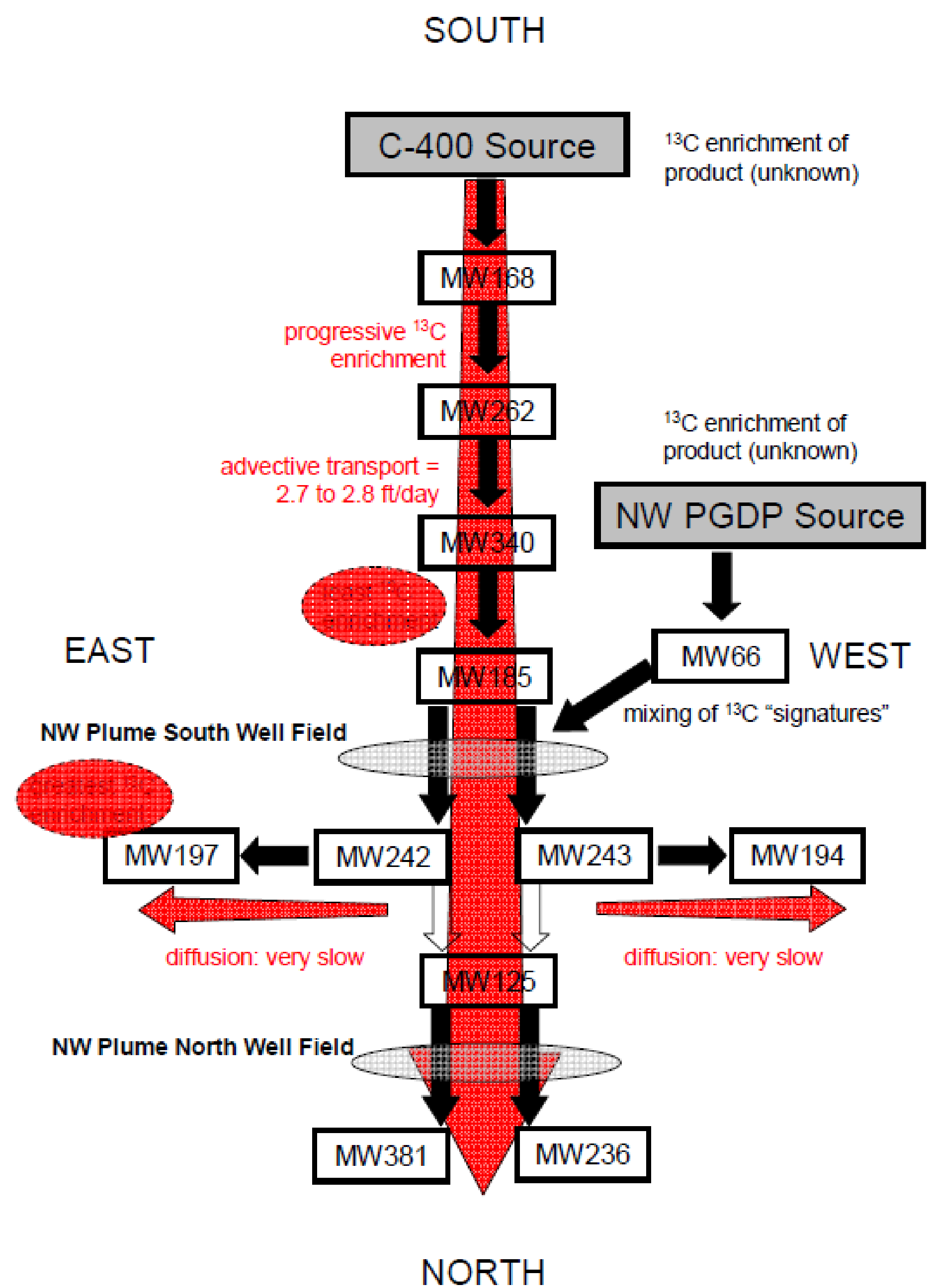
- 70% of SCI well-pair comparisons showed an increase in the carbon-13 to carbon-12 ($^{13}C/^{12}C$) ratio in the down gradient well
- The increase in the carbon-13 to carbon-12 ($^{13}C/^{12}C$) ratio in the down gradient wells supports the occurrence of biodegradation along the plume flowpath

| Sample ID | TCE d13C (permil) | |
|---|---|---|
| **PGDP NW plume wells along flow path** | | |
| MW-168 | -24.8 | near source |
| MW-262 | -25.8 | |
| MW-340 | -25.9 | |
| MW-185 | -25.9 | |
| MW-242 | -24.6 | |
| MW-243 | -25.3 | |
| MW-125 | -25.6 | |
| MW-381 | -25.4 | |
| MW-236 | -25.3 | distal portion of plume |
| **PGDP well near downgradient source** | | |
| MW-66 | -25.3 | |
| **PGDP control wells outside plume** | | |
| MW-194 | na | |
| MW-197 | -23.1 | |

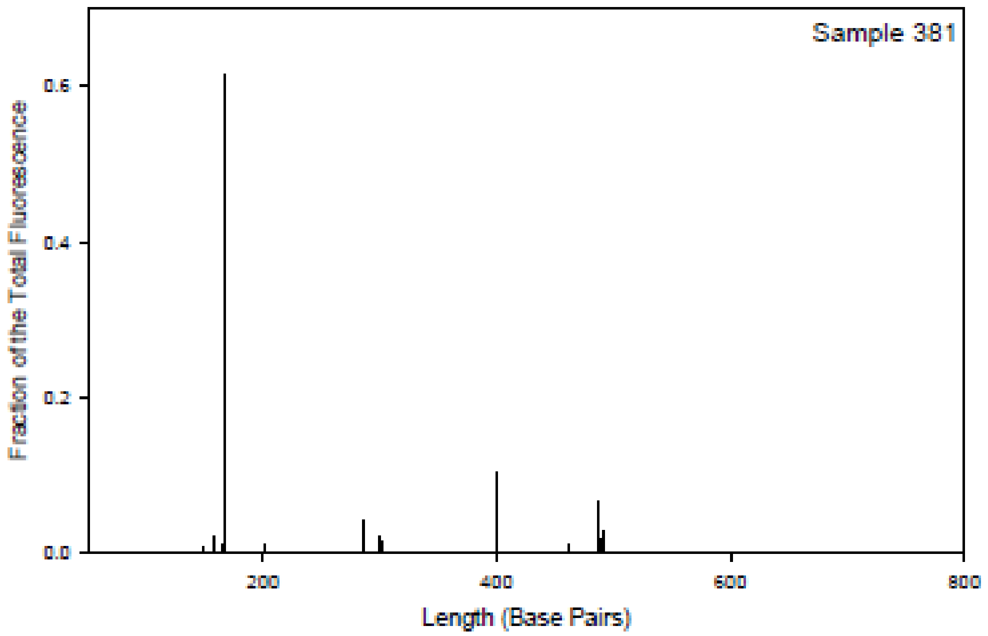
# CSIA data: Monitoring Well Pairs

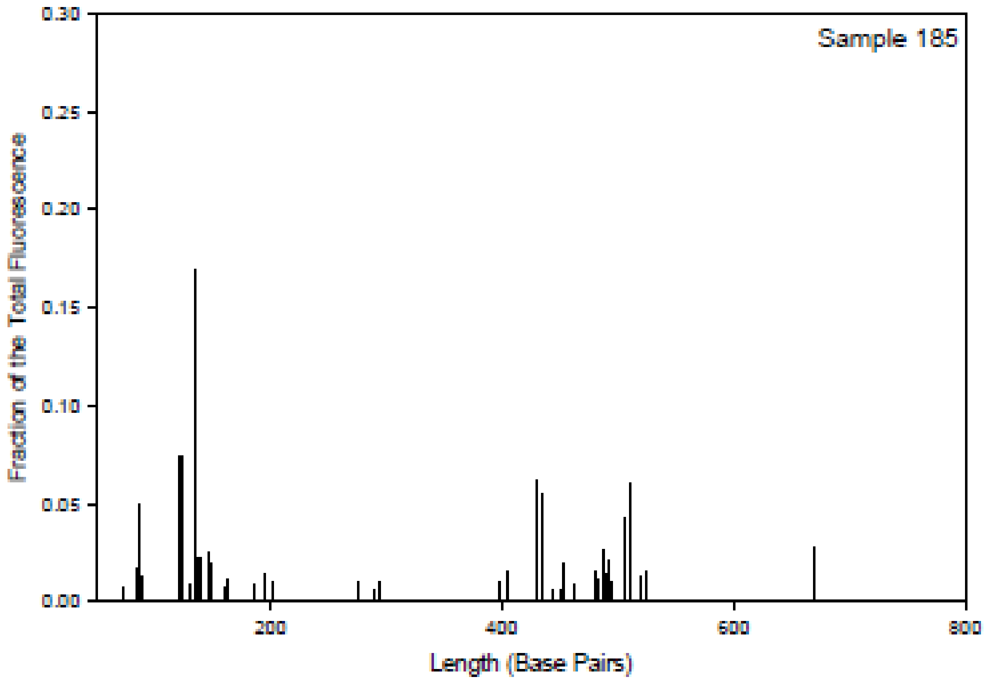


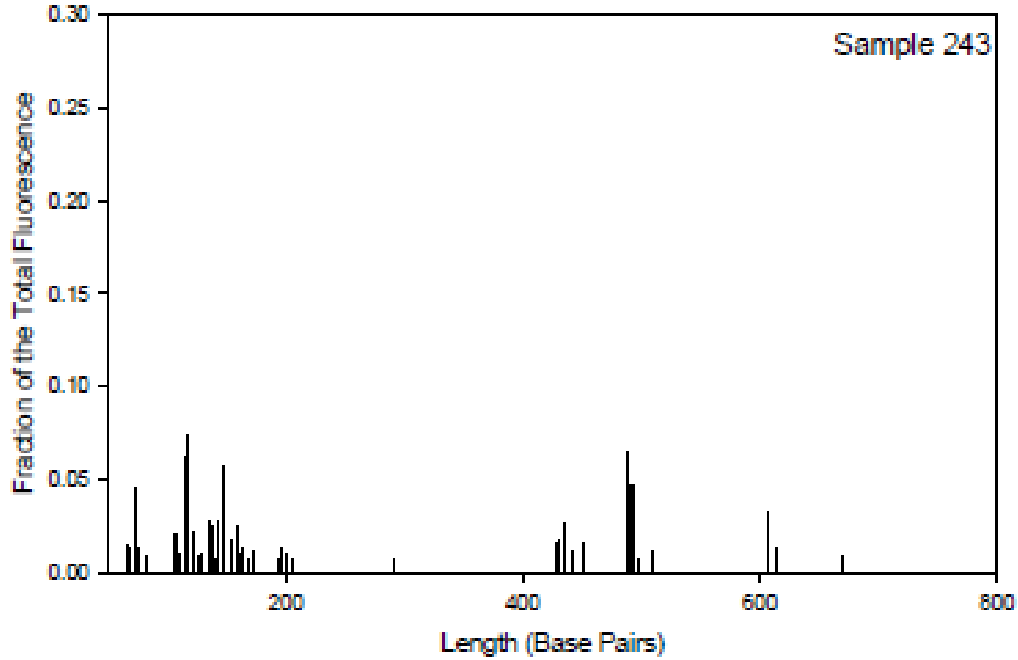
| | Original Data Set, mean Epsilon -1.1 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Decision Rule 4 | | Decision Rule 5 | | | | Decision Rule 6 |
| Well Pair | Mean Epsilon (ε) | SCIR ln(C/$C_0$) | ln(C/Co) SCIR < ln(C/C0) TCE | ln(C/Co) SCIR < 0.33 * ln(C/Co) TCE | ln(C/C0) SCIR not < 0.33 * ln(C/Co) TCE | TCE $\delta^{13}C$ (permil) Up-gradient + SDev | TCE $\delta^{13}C$ (permil) Down-gradient - SDev | Mean ln (C/Co) (TCE $\delta^{13}C$ (permil) +/- Sdev) Significant | ln(C/Co) SCIR significant < 0.10 * ln(C/Co) TCE |
| MW262-236 | -1.1 | -0.45 | Yes | | | -25.6 | -25.5 | -0.091 | Yes |
| MW340-236 | -1.1 | -0.55 | | | Yes | -25.7 | -25.5 | -0.182 | |
| MW340-125 | -1.1 | -0.27 | | | Yes | -25.7 | -25.8 | 0.091 | |
| MW185-242 | -1.1 | -1.18 | | Yes | | -25.7 | -24.8 | -0.818 | Yes |
| MW185-243 | -1.1 | -0.55 | | Yes | | -25.7 | -25.5 | -0.182 | Yes |
| MW185-125 | -1.1 | -0.27 | | Yes | | -25.7 | -25.8 | 0.091 | No |
| MW185-381 | -1.1 | -0.45 | | Yes | | -25.7 | -25.6 | -0.091 | Yes |
| MW185-236 | -1.1 | -0.55 | | Yes | | -25.7 | -25.5 | -0.182 | Yes |
| MW66-242 | -1.1 | -0.64 | Yes | | | -25.1 | -24.8 | -0.273 | Yes |
| MW125-236 | -1.1 | -0.27 | Yes | | | -25.4 | -25.5 | 0.091 | No |

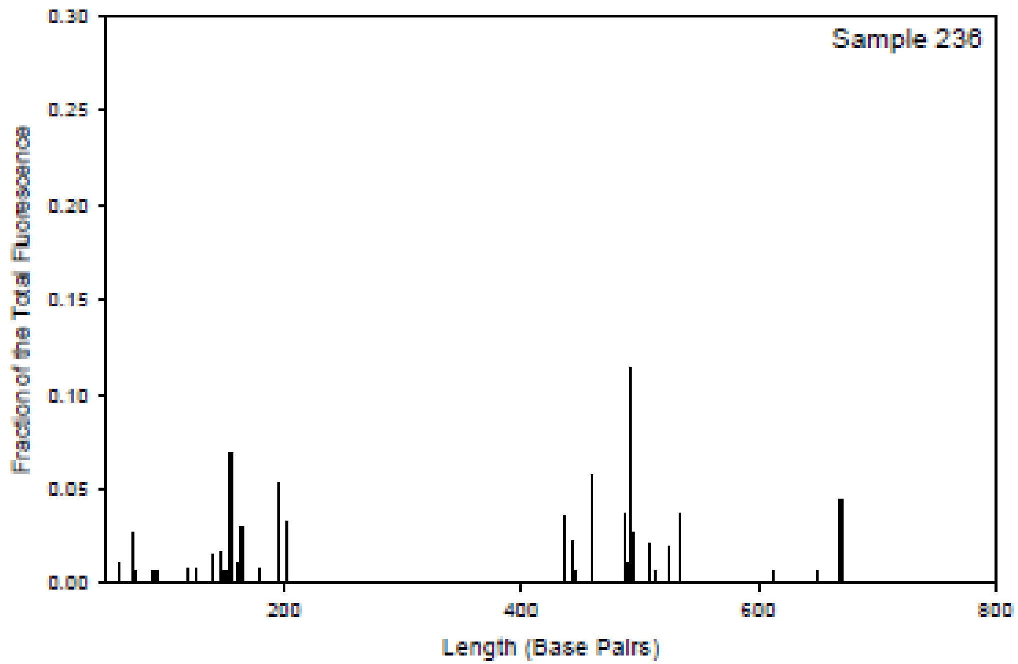

SOUTH
C-400 Source
13C enrichment of product (unknown)
MW168
progressive 13C enrichment
MW262
13C enrichment of product (unknown)
advective transport = 2.7 to 2.8 ft/day
NW PGDP Source
MW340
EAST
MW185
MW66
WEST
mixing of 13C "signatures"
NW Plume South Well Field
MW197
MW242
MW243
MW194
diffusion: very slow
diffusion: very slow
MW125
NW Plume North Well Field
MW381
MW236
NORTH

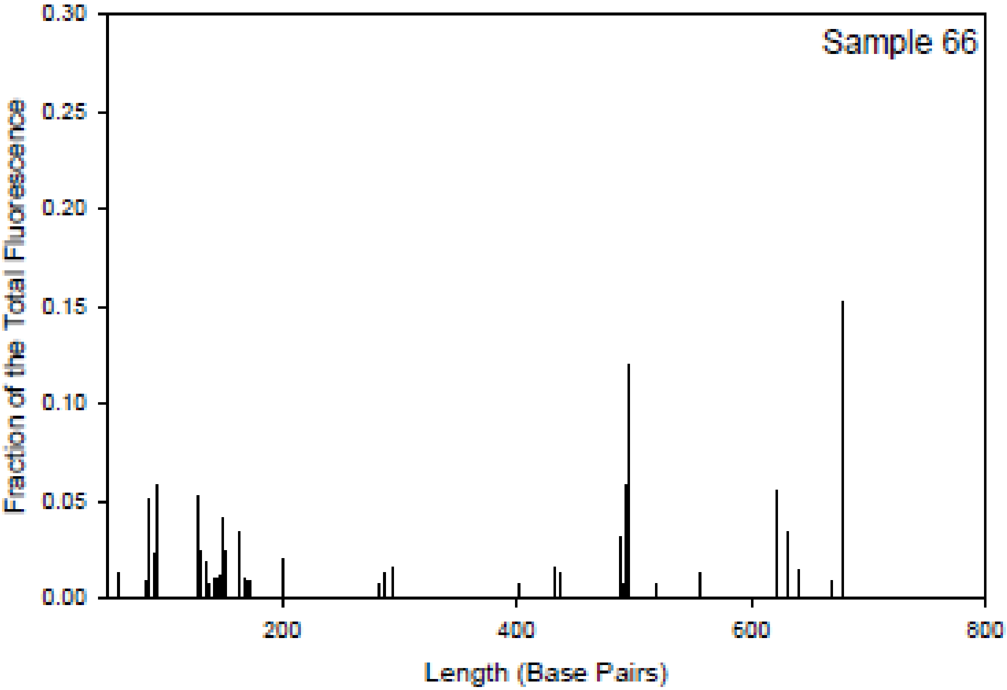
# tRFLP profiles for groundwater



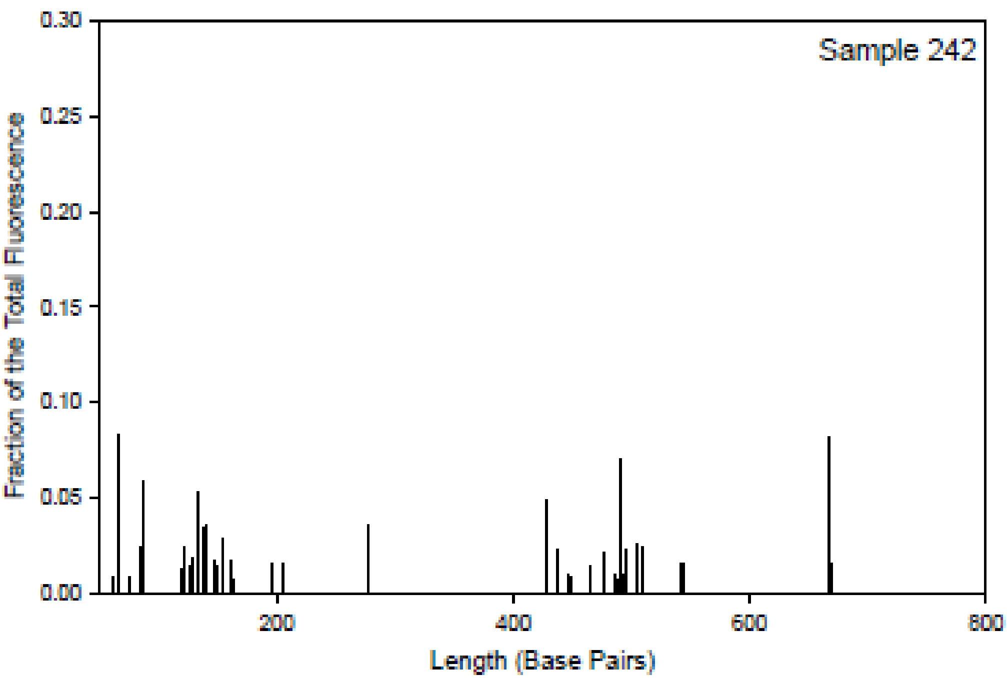




Sample 243
Fraction of the Total Fluorescence
0.30
0.25
0.20
0.15
0.10
0.05
0.00
200
400
600
800
Length (Base Pairs)


Sample 236
Fraction of the Total Fluorescence
0.30
0.25
0.20
0.15
0.10
0.05
0.00
200
400
600
800
Length (Base Pairs)



Sample 66
Fraction of the Total Fluorescence
Length (Base Pairs)
0.00
0.05
0.10
0.15
0.20
0.25
0.30
200
400
600
800

Sample 242
Fraction of the Total Fluorescence
Length (Base Pairs)
0.00
0.05
0.10
0.15
0.20
0.25
0.30
200
400
600
800

# DOE, Paducah, Kentucky

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| | | | | | |
|---|---|---|---|---|---|
| **COCs+** | TCE < 1,000 - 8,000 μg $L^{-1}$ | $^{99}Tc$ ND- < 3,000 pCi $L^{-1}$ | | | |
| **General Geochemistry** | Aerobic; anaerobic near source | Carbon: natural organic matter | No detection of daughter or end products (e.g. DCE, VC, ethene and ethane) | High iron and other metals near source | Near neutral pH; above average tempera tures |
| **EAPs*** | Aromatic: 85% active | sMMO: 25% active | | | |
| **qPCR** | Aromatic: 95% | Methane (sMMO + pmoA): 80% | DHC: ND | vcrA, bvcA, tceA: ND | |
| **CSIA** | Mean episilon value of -1.1‰ | Significant aerobic degradation in 6/8 well pairs examined | | | |

*KRCEE FFY 09-10 Project Status*
*TCE FT Project: 80% Complete*
*TCE FT Ph 2 Report Submission – December 2010*

# WP RECOMMENDATIONS

1. **Collect off-site NEP microbial samples for to ensure presence and abundance of microbial populations similar to NWP**
    a. DAPI
    b. RNA/DNA
    c. Enzyme Probe analyses
    d. Collect microbial samples from NEP at PGDP east security fence to determine impacts of near site low DO anomaly
2. **Conduct biodegradation modeling for NEP, NWP, and SnT**
    a. Develop matrix of probable future conditions at site relative to:
        i. Plant shutdown
        ii. Biotic and Abiotic Remedial Implementations
3. **Establish near site and offsite transects for continuous monitoring of mass flux**
    a. Recommended as metric to identify loss of contaminants in plume
    b. Encompass plume bounds
    c. Establish baseline ASAP
    d. Obtain technical concurrence on mass flux transect locations relative to P & T and other facilities
    e. Obtain technical concurrence on NW 99Tc mass flux relative to NW TCE Plume
4. **Identify appropriate field techniques for evaluating microorganisms responsible for aerobic co-metabolism of TCE**
    a. Confirm field results with lab results
5. **Collect REDOX condition and process geochemical parameters to establish on and off-site baselines**
    1. Sulfide (solid, groundwater)
    2. H2S/HS/S (groundwater)
    3. S (solid and solid surface)
    4. Fe3+ (solid and solid surface)

# WP RECOMMENDATIONS (cont'd)

6. **Collect additional geochemical parameters on-site to account for occurrence of multiple degradation processes**
    1. H (gas, groundwater)
    2. $CO_2$ (gas, groundwater)
    3. Methane (gas, groundwater)
    4. Ethene (gas, groundwater
    5. $NH_4$ (groundwater)
    6. DOC (groundwater)
7. **Evaluate water levels and Redox process geochemistry in the vicinity of facilities that are likely to impact the RGA**
    1. Sewage treatment system facilities (basins and lagoons)
8. **Collect soil and groundwater REDOX Geochemical data beneath, adjacent to, and downgradient of the C-616 lagoons**
    1. Sulfide (solid, groundwater)
    2. H2S/HS/S (groundwater)
    3. S (solid and solid surface)
    4. Fe3+ (solid and solid surface)
9. **Confirm/deny the occurrence of intrinsic Biotic/Abiotic degradation relative to NW 99Tc Plume and TCE Plumes**
    1. Collect water level and REDOX geochemical data in the vicinity of the C-616 Lagoons in order to evaluate potential impacts of C-616 lagoons on the UCRS in the vicinity of SWMUs 7/30

# WP RECOMMENDATIONS (cont'd)

10. **Collect geochemical parameter data in the area bifurcating the NW and SW Plumes**
    a. Determine if anthropogenic losses from C-400 & Steam Facilities are cause of plume bifurcation
    b. Consider relative to costs of long-term costs of treating/controlling/monitoring 2 plumes
    c. Confirm/modify flow field in updated PGDP Groundwater Flow Model
11. **Conduct continuous flow column tests to evaluate enhancement of aerobic co-metabolism in DPP's.**
    a. Utilize readily available & economic Carbon source
12. **Identify and instrument on-site and DPP Pilot test plot(s) to evaluate performance of potential applications of biological and abiotic remedial options**
    a. Conduct pilot tests for DPP aerobic co-metabolic enhancement (biostimulation)
    b. Conduct pilot test for biogeochemical transformation process

# Site characteristics



- **Co-contaminants**
  - Carbon tetrachloride
  - $^{99}$Tc, Sr, I, U, Cr
  - PCE, DCE, VC
  - 1,1,1 TCA
  - Petroleum hydrocarbons
  - Nitrate
  - NDMA
  - 1,4 dioxane
  - MTBE

- **Depth, extent of plume**
  - Surface to 500 ft bgs
  - > 3 miles in length

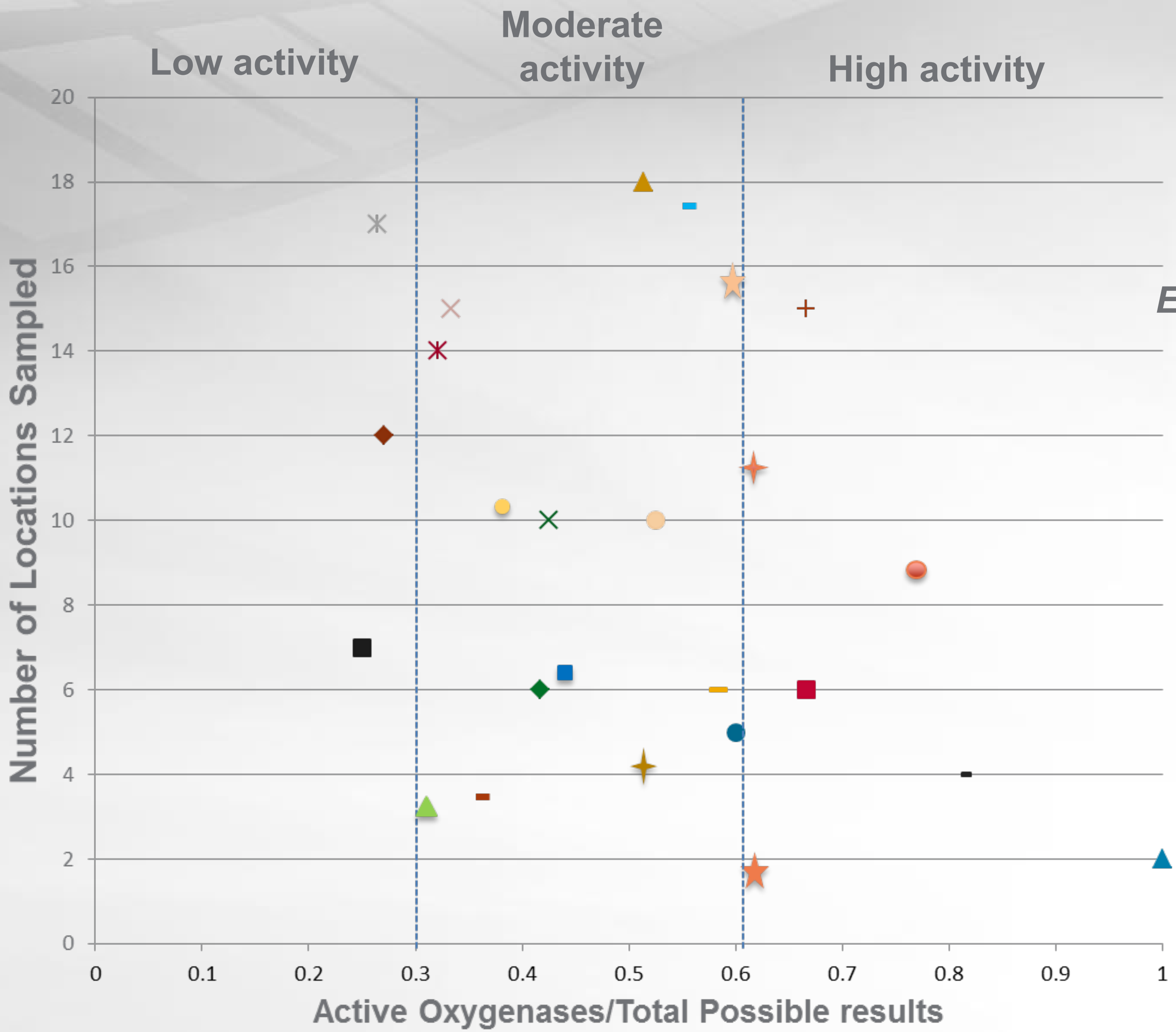
- **EAPs & qPCR**:

  9 DOE, 15 DoD, 4 EPA, 8 Industrial sites (400 + MW locations)

  3 vapor impacted sites
- Rates (microcosms) @ 12 sites
- Coupled with CSIA @ 7 sites
- Line of evidence for MNA @ >20 sites

# EAP data …






*Each data point= site*

# Enzyme activity (PA) & TCE concentration

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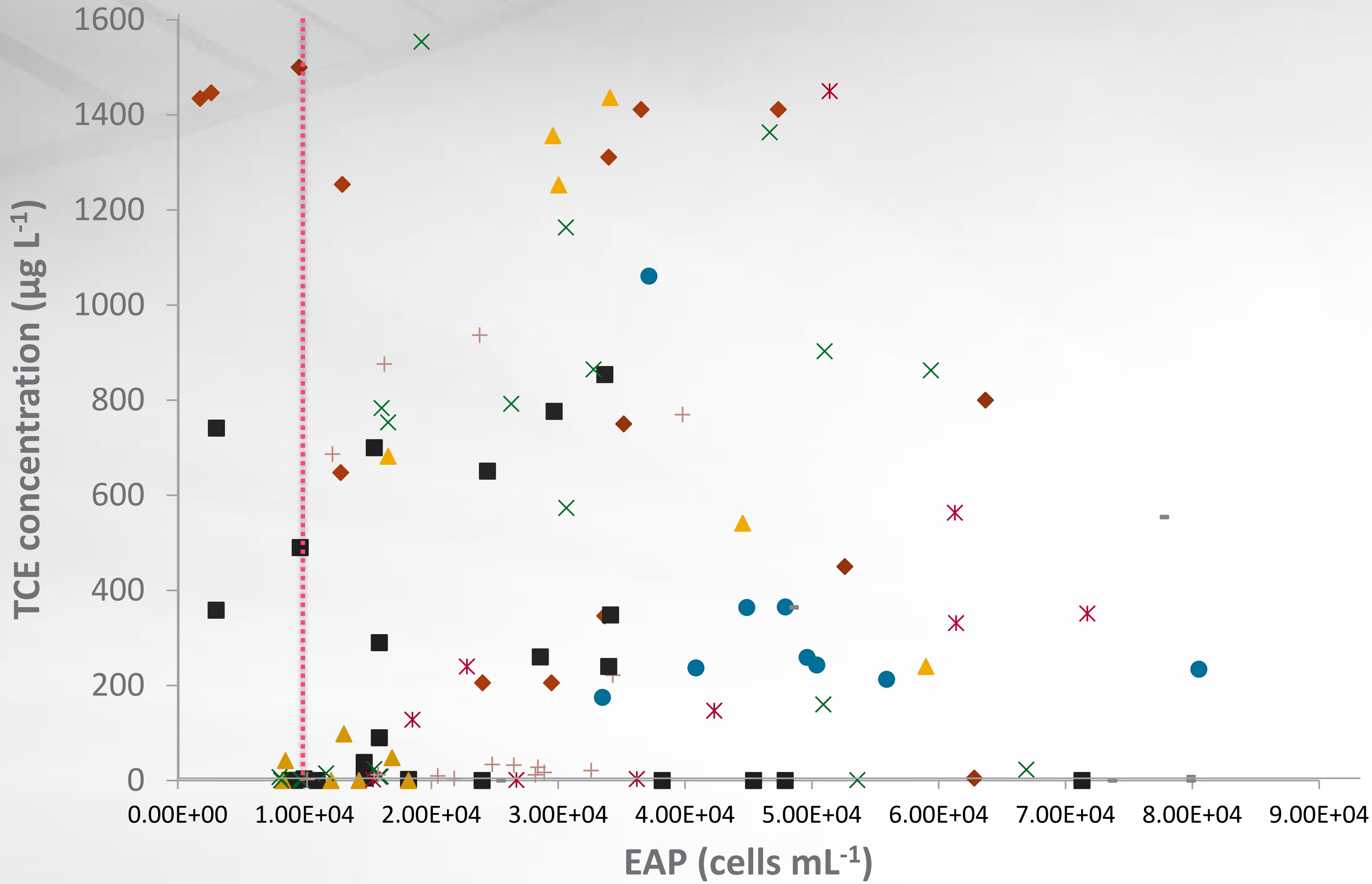



*8*10³ = activity level of significance*

| MW | EAP | DO | Methane | TCE |
|---|---|---|---|---|
| MW-9 | 1.55E+04 | 0.5 | 0 | 700 |
| MW197 | 6.28E+04 | 0.62 | 0.00 | 5 |
| MW262 | 1.36E+04 | 0.6 | 0.00 | 4000 |
| 1-45B | 5.14E+04 | 0.03 | 0.29 | 1450 |
| M-2BR | 1.54E+04 | 0.61 | 0.001 | 2.3 |
| 2-429A | 4.23E+04 | 0.15 | 0.002 | 147 |
| TAN-28 | 3.71E+04 | 0.17 | 7185 | 1061 |
| TAN-29 | 4.49E+04 | 0.33 | 5968 | 364 |
| TAN-41 | 4.96E+04 | 0.95 | 1624 | 259 |
| CRP-41B | 1.63E+04 | 0.23 | 5.09 | 875.9 |
| SSM-16C | 1.46E+04 | 0 | 0 | 3383.3 |
| SSM-11B | 2.41E+04 | 0 | 0 | 8297.2 |
| TCM-5 | 3.26E+04 | 0.54 | 16.74 | 20.9 |
| MSG-05-01 | 2.52E+04 | 0 | 0.45 | 0 |
| MSG-05-04 | 7.96E+04 | 0 | 0.45 | 7 |
| MSG-05-05 | 7.96E+04 | 0.1 | 5 | 0 |
| 1-12BR | 1.94E+04 | 1.18 | 0.001 | 5920 |
| MW185 | 1.37E+04 | 1.96 | 0.00 | 3750 |
| SSL-13B | 2.48E+04 | 1.7 | 0 | 33.7 |
| MW-7 | 1.59E+04 | 2.5 | 0 | 90 |
| MW-10 | 1.59E+04 | 2 | 0 | 290 |
| MW125 | 6.37E+04 | 2.77 | 0.00 | 800 |
| MSG-05-11 | 7.34E+04 | 2.55 | 35 | 0 |
| MW-4 | 1.47E+04 | 3.4 | 0 | 38 |
| MW236 | 5.26E+04 | 3.36 | 0.00 | 450 |
| MW340 | 9.57E+03 | 3.51 | 0.00 | 1500 |
| MW381 | 3.52E+04 | 3.23 | 0.00 | 750 |
| MSG-05-02 | 7.75E+04 | 3.1 | 0.7 | 554 |
| MW-13 | 1.47E+04 | 4.5 | 0 | 5.3 |
| MW66 | 9.66E+03 | 4.1 | 0 | 490 |
| M-4B | 2.67E+04 | 4.02 | 0.11 | 1 |
| 1-8A | 1.56E+04 | 4.3 | 0.001 | 3240 |
| MW04I | 2.40E+04 | 4.2 | | 0 |
| MW05 | 1.82E+04 | 4.2 | | 2.2 |
| MW06S | 2.12E+05 | 4.2 | | 2500 |
| MW06I | 9.96E+03 | 4.2 | | 3.5 |
| MW07S | 7.13E+04 | 4.2 | | 0 |
| MW07I | 3.82E+04 | 4.2 | | 0 |
| MW08S | 4.54E+04 | 4.2 | | 0 |
| MW08I | 4.79E+04 | 4.2 | | 0 |
| MW10 | 9.25E+03 | 4.2 | | 0.16 |
| MW12 | 8.84E+03 | 4.2 | | 0.16 |
| MW15 | 1.10E+04 | 4.2 | | 0 |
| MW194 | 9.52E+03 | 5.43 | 0.00 | 1 |
| 2-444A | 3.62E+04 | 5.61 | 0.001 | 3 |
| P-2U | 1.19E+04 | 5.58 | 0 | 8129.2 |
| PGW-25DU | 2.89E+04 | 6.6 | 0 | 17.0 |
| P-3[illegible] | 1.56E+04 | 6.16 | 0 | 8394.8 |
| SB 7-21 | 1.39E+05 | 6.3 | 4.2 | 0 |
| SB 7-23 | [illegible] | 6.9 | 1800 | 8 |
| M-1CR | 1.85E+04 | 6.8 | 0.001 | 128 |
| TAN-4[illegible] | [illegible] | 6.98 | 175 | 213 |
| TAN-44 | 8.05E+04 | 6.91 | 110 | 234 |
| CRP-42B | 3.98E+04 | 7.62 | 4.8 | 769.6 |
| CRP-3D | 3.43E+04 | 7.72 | 0.15 | 221.4 |
| TBG-3 | 1.58E+04 | 7.34 | 12.47 | 12.1 |
| TRW-2 | 2.05E+04 | 7 | 7.8 | 9.8 |
| P-3L | 2.65E+04 | 8.68 | 0 | 32.3 |
| TNX-3D | 2.84E+04 | 8.47 | 0 | 28.1 |
| P-2L | 1.22E+04 | 8.84 | 0.23 | 686.7 |
| SSL-13C | 1.02E+04 | 8.23 | 0 | 2.1 |

**DO < 1 – 8 mg/L ++**

**Methane 0 – 1800 mg/L**

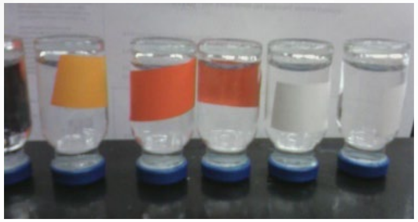
**TCE 0 – 8500 µg/L**

# Microcosm Studies



**Purpose:** *Obtain a degradation rate based on natural populations and a laboratory microcosm.*

These are focused on overcoming the shortcomings of traditional laboratory microcosms. The innovative aspect is the ability to: (a) directly measure enzyme activity and (b) simultaneously measure enzyme activity and TCE degradation.



Microcosms:

- 10 to 20mL crimp top vials
- Un-amended groundwater or spiked with TCE
- No "excess" headspace (*In situ conditions are mimicked as closely as possible)*
- Treatments are performed in triplicate (60-80 vials, sampled destructively over time)
- In situ temperatures in the dark for up to 60 days.
- Samples taken for
  GC (TCE, aromatics, methane), enzyme probes
  Oxygen, Molecular

*VOC/contaminants: (1) MS for characterization and baseline concentrations (2) SPME (Solid Phase Microextraction)*

# Select microcosms to date …



| TCE Concentration | Co-contaminant | Half Life |
|---|---|---|
| <5–170 µg/L | PCE | ~ 19.2 y |
| <5–5,900 µg/L | Chromium, acetone, benzene, toluene, xylene, metals | ~ 15.1 y |
| <5-250 µg/L | None | ~ 30.8 y |
| <5–6,000 µg/L | PCE, strontium, sewage | ~ 6.7- 22.3 y |
| <5-30 µg/L | PCE, nitrate | ~ 19.7 y |
| <5-250 µg/L | PCE, 111 TCA, 111DCA | ~ 27.5 y |
| <5-8,600 µg/L | PCE, $^{99}$Tc | ~ 31.4 y |
| <5-50 µg/L | PCE, nitrate, metals | ~ 15.4 y |
| <5-1600 µg/L | Petroleum hydrocarbons | ~ 17.6 y |
| <5-2700 µg/L | MTBE, PCE | ~ 24.5 y |
| <5-10,000 µg/L | Carbon tetrachloride, nitrate | ~ 38.4 y |
| <5-100 µg/L | Metals | ~ 26.6 y |
| <5-2,800 µg/L | 1,4-dioxane, hydrocarbons, PCR, DCE, VC | ~ 17.4 y |

# Conclusions …

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- 80 to 100% of the samples analyzed are positive for organisms that are expressing the enzymes necessary for cometabolism
- EAP data to date DO NOT correlate with concentrations of carbon, oxygen, or contaminants
- EAP DO correlate well with other measurements of biological degradation: CSIA, FISH, qPCR HOWEVER EAP provide a measurement of activity that other tools cannot
- Fingerprinting and other tools can be used to quantify the proportion of the population that have the genes of interest and provide some level of prediction capability

# Conclusions continued…



- Cometabolism is occurring at some rate in all of the aerobic plumes tested to date

- RATE studies suggest ½ life for TCE of 15-40 years … when compared with CSMs, the data match plume behavior in the real world

- These tools provide clear evidence that degradation of contaminants can occur in situ, and when coupled with other tools/measurements, is occurring in situ

- EAPs are powerful for evaluating long term attenuation, as well as performance over time of EA or MNA remedies and approaches

# Where do we go from here …



- Develop more sensors or probes for other contaminants

- Continue to push the envelope for developing methods for estimating rates- aerobic may be the turtle in the race  but it continues to be an important degradation pathway as
  - (a) Primary means of degradation for many emerging contaminants AND
  - (b) the sustainable, ‘green’, long term management strategy for reducing or attenuating contaminants

- Develop better understanding of how tools inform one another (e.g. CSIA, geophysical)

- Start looking at the system and how MBTs can provide metrics of valuable, predictive information when plume and the dynamics are viewed as a system rather than its parts- INTEGRATION

# **ESTCP**: Providing Additional Support for MNA by Including Quantitative Lines of Evidence for Abiotic Degradation and Co-metabolic Oxidation of Chlorinated Ethylenes (2015-2018)

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The overarching objectives of the work described herein are to:

(1) Provide a method to readily and inexpensively acquire the data on magnetic susceptibility that is required to evaluate the abiotic degradation of chlorinated ethylenes on magnetite.

(2) Provide a method to readily and inexpensively acquire the data required to evaluate and quantify the aerobic degradation of TCE.

- Todd Wiedemeier
- John Wilson
- David Freedman
- Brady Lee
- Hope Lee

# THANK YOU!

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