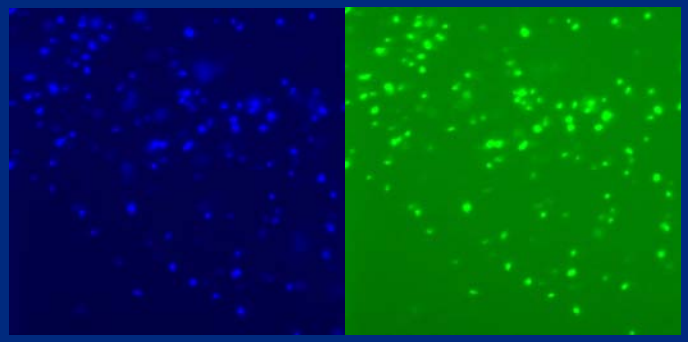
Application of Enzyme Activity Probes to Characterize Aerobic Microorganisms

in Groundwater at PGDP, INL, & elsewhere



M. Hope Lee

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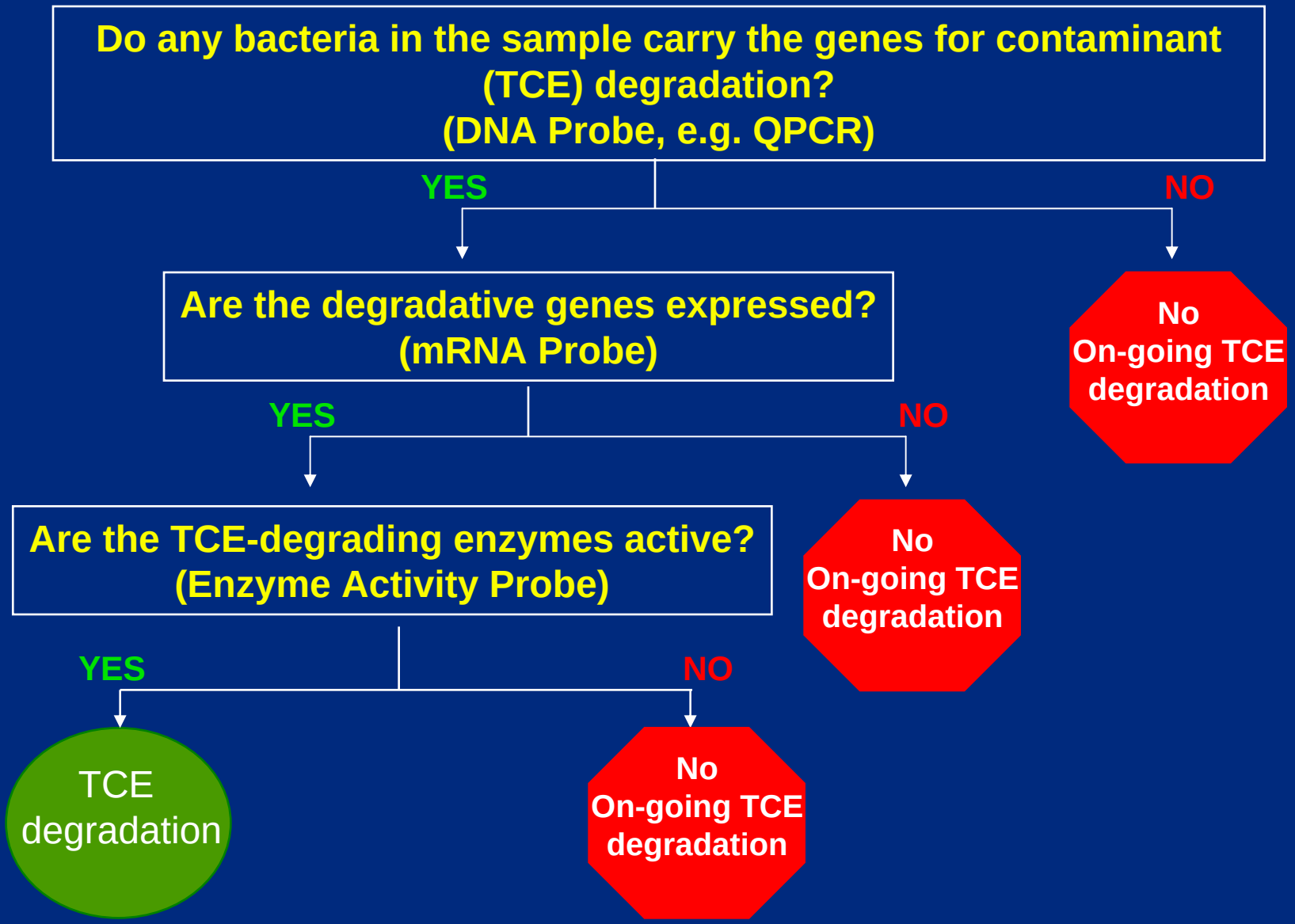
Biodegradation / Bioremediation of TCE

- TCE can be degraded by bacteria
- Several different mechanisms, including two primary ones:
    1. Anaerobic reductive dechlorination (ARD)
    2. *Aerobic cometabolic oxidation*
- Bioremediation technology can be based on microbial degradation capacity
- Tools are *needed* to detect appropriate enzyme systems and assess their *activity* in the environment

Molecular Data: What do they tell you?



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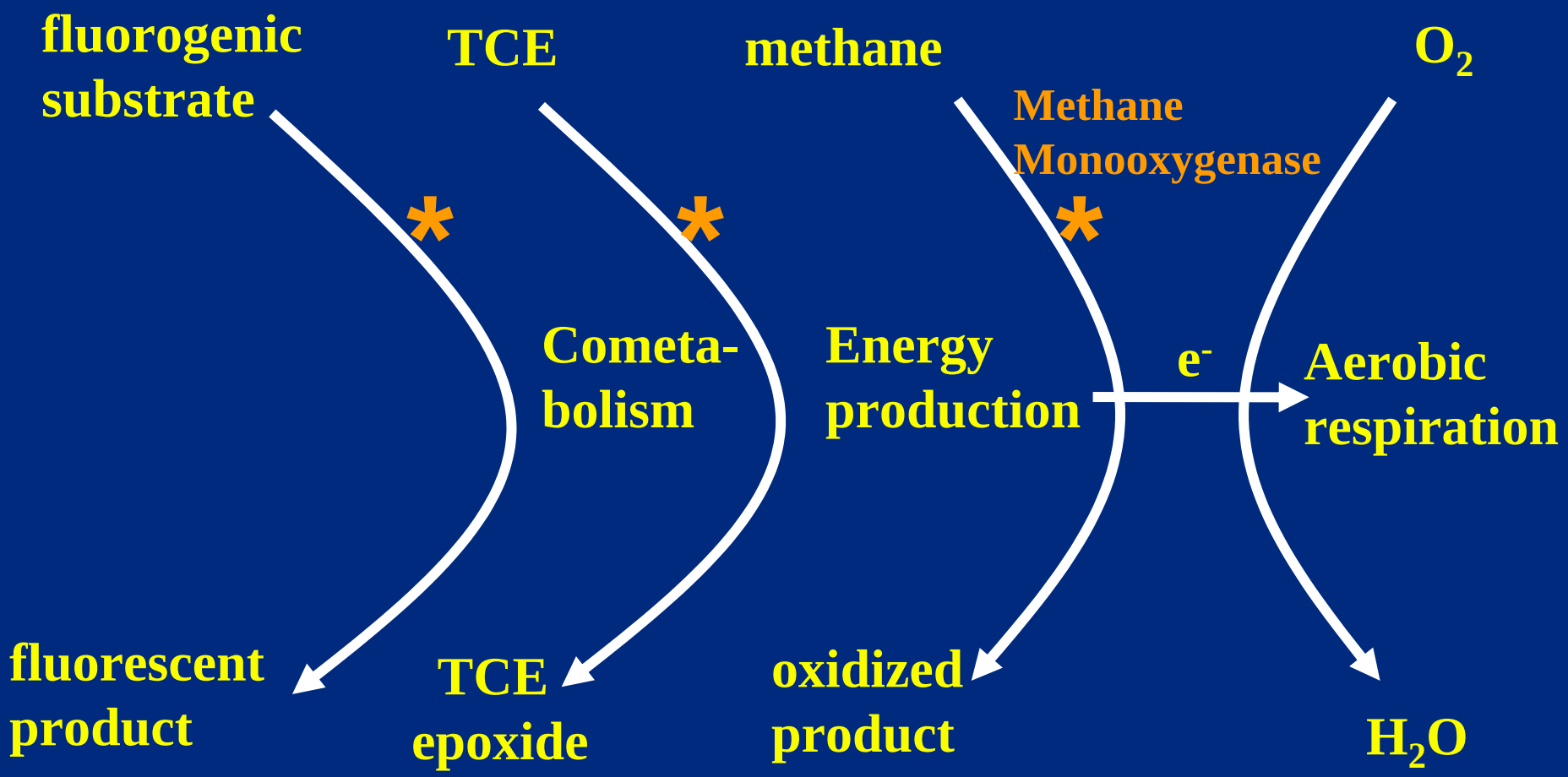
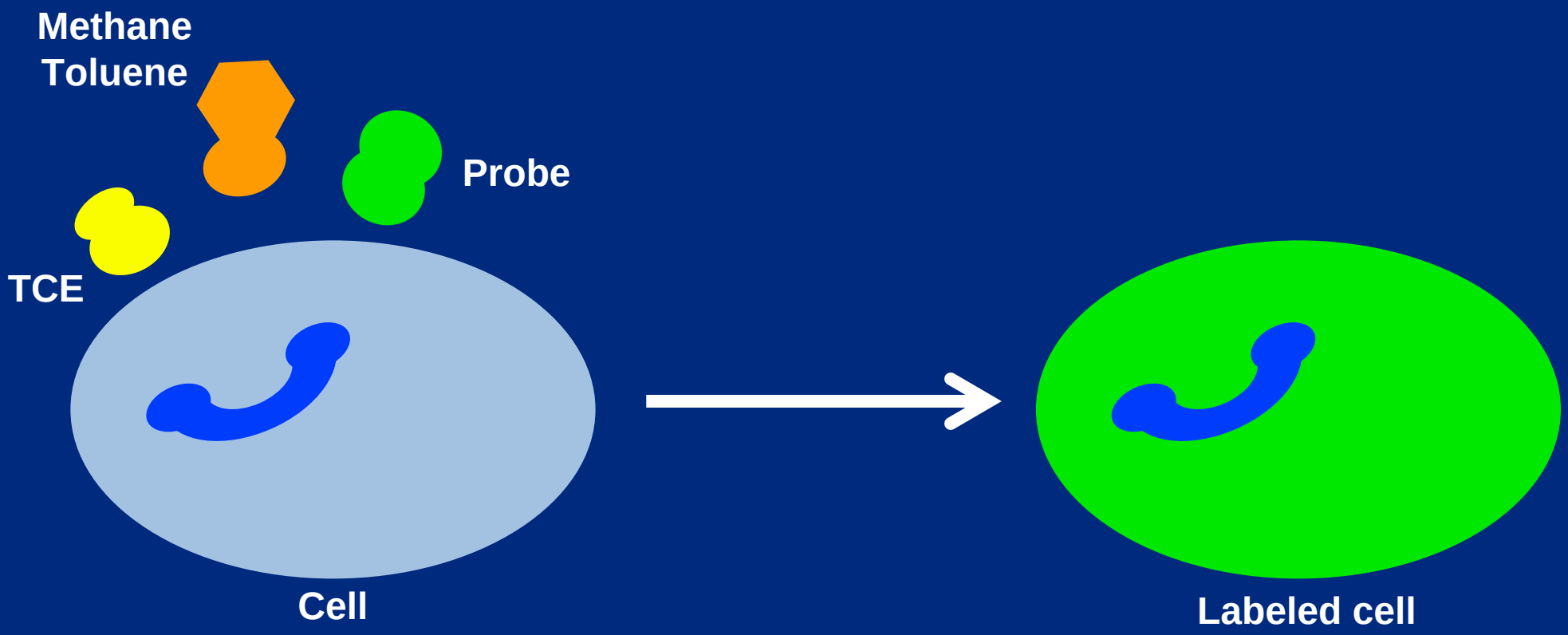
Microbial metabolism, cometabolism, and enzyme activity probes



* Other enzymes include propane, ammonium, toluene oxygenases, etc.

What is an Enzyme Activity Probe?



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

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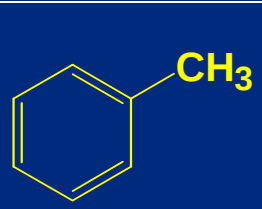
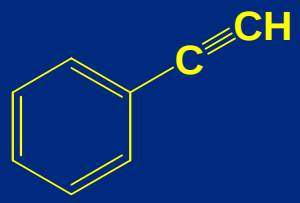
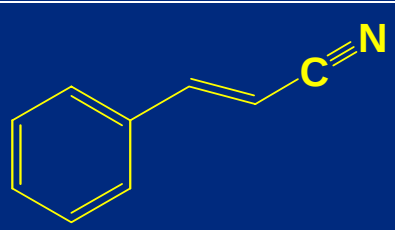
Representative Enzyme Probes and Target Systems:



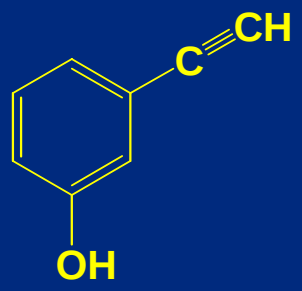
toluene
(natural
substrate)



phenylacetylene
(2,3-TDO)
(2-TMO, 3-TMO)

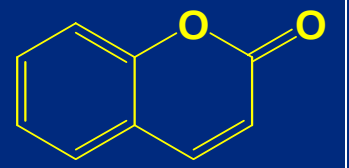


trans-cinnamonitrile
(2,3-TDO)



3-hydroxy-phenylacetylene
(2-TMO)
(3-TMO)

3-ethynyl-benzoate
(side chain MO)



coumarin
(sMMO)

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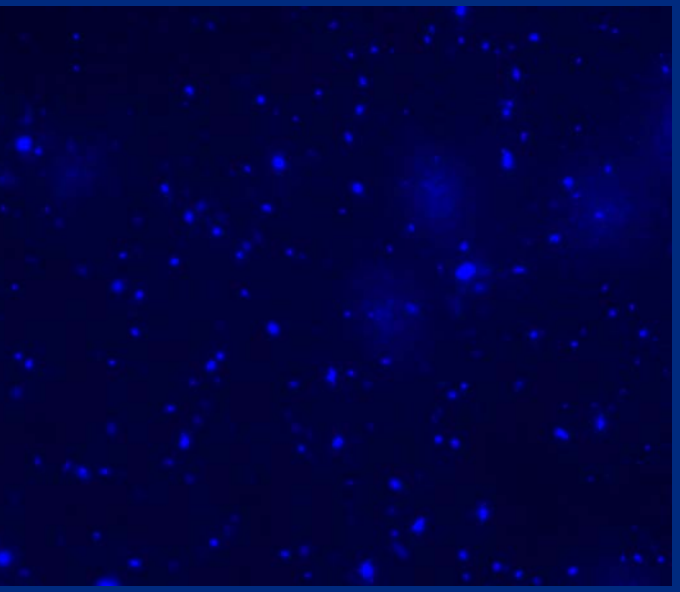
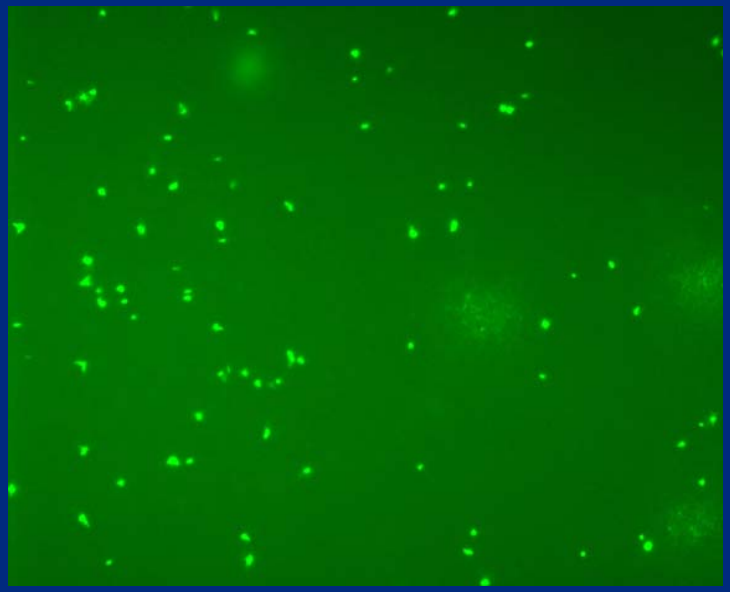
Results of applying Enzyme Activity Probes to groundwater



Total bacterial cell count



Positive probe response

Negative probe response

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Control Assays

Purpose: To ensure that the measured degradation is attributable to the organisms of interest; *verify that either the sMMO or toluene enzymes are responsible for any observed positive response to the assay.*

(a) Acetylene: irreversible inhibitor of sMMO

(b) Methane: competitive reversible inhibitor

(c) 1-pentyne (3.5%): irreversible inhibitor for the 2-monooxygenase pathways

(d) 3-hexyne (2%): irreversible inhibitor for the 3-and 4-monooxygenases

(e) Phenylacetylene (10-15%): dioxygenase

(f) DNA…

Additional Control Assays

To offer supporting evidence for the enzyme activity probes.

PCR *characterize the potential of the microbial community*

TOD: toluene 2,3-dioxygenase
TOL: xylene monooxygenase
RMO: toluene-3,-4-monooxygenase
PHE: toluene-2, -3, -4-monooxygenase
sMMO: mmoX (f882 & r1403)
Universal: (8F and 907R).

FISH *characterize the activity of the microbial community*

Eubacteria
Cytophaga-Flavobacterium (most common toluene degrading organisms),
type I and type II methanotrophs
component B of the sMMO

Common Myths, dismissed…

Early studies (1980-mid-90s) determined that:

(a) Cometabolism requires a natural substrate induction (methane, phenol, other aromatic)

(b) Cometabolic degradation of TCE results in TCE epoxides and/or oxygen radicals which inactivate the active site of the oxygenase

(c) Growth on non-inducing substrates will result in an enzyme that will not cometabolize chlorinated solvents (TCE)

Recent studies have shown that under natural conditions:

(a) Non-aromatic substrates can induce activity (naturally occurring phenolic compounds e.g. humics); TCE itself can induce cometabolic activity

(b) Studies have shown that TCE epoxides do not cause significant decreases in TCE cometabolizing abilities or rates

(c) Growth on non-inducing substrates results in TCE degradation

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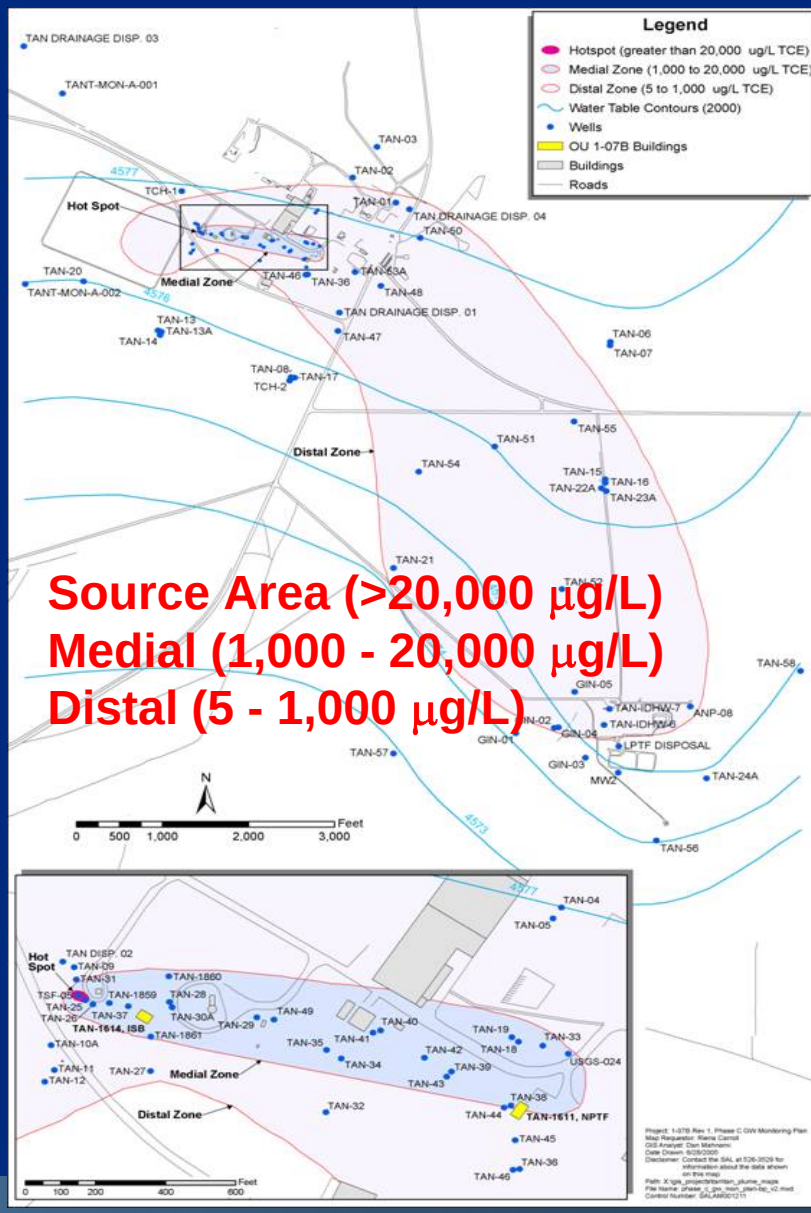
Heald and Jenkins, 1994; McClay et al., 1995; Leahy et al., 1996; Shingleton et al., 1998; Ryoo et al., 2000, 2001; Lee et al., 2002; Yeager et al., 2004

Test Area North, Idaho National Lab

- Past waste injections into the deep, fractured basalt aquifer have resulted in a nearly 2-mile long TCE plume at the TAN facility of the INL.
- 1995 ROD selected 30 years of pump and treat as the default remedy, but allowed for innovative technology evaluation.
- Zones within the plume are defined by concentration of TCE, which decreases with distance from the source area.



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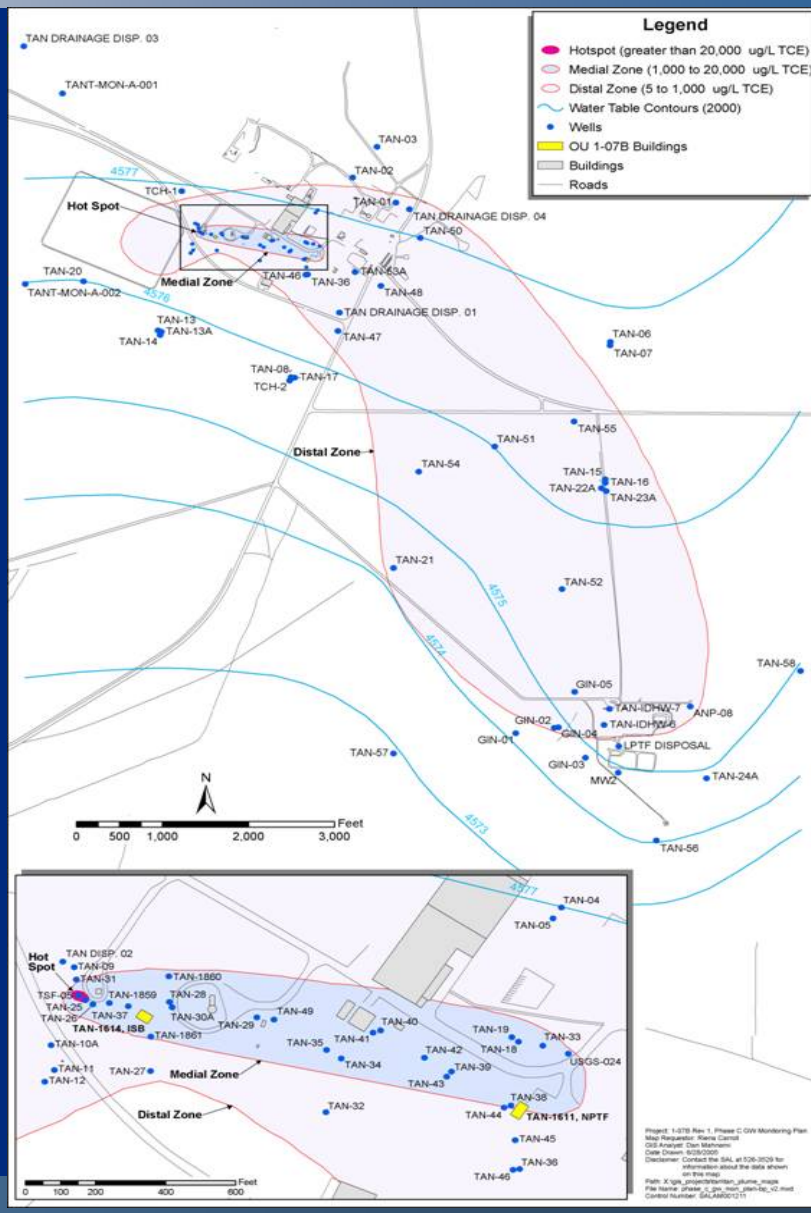
General Geochemical Conditions at TAN

TCE concentrations decrease with distance from the source area.

The source area has significant DNAPL; this zone is actively enhanced to remove oxygen and create anaerobic conditions (contaminants degraded by ARD)

The medial zone of the plume, down gradient of the source area, is generally aerobic. However this zone also contains a transition zone from anaerobic to aerobic conditions that is not well understood. This zone has variable concentrations of methane in groundwater.

The distal portion of the plume has saturated oxygen conditions and variable methane, primarily based on natural sources.

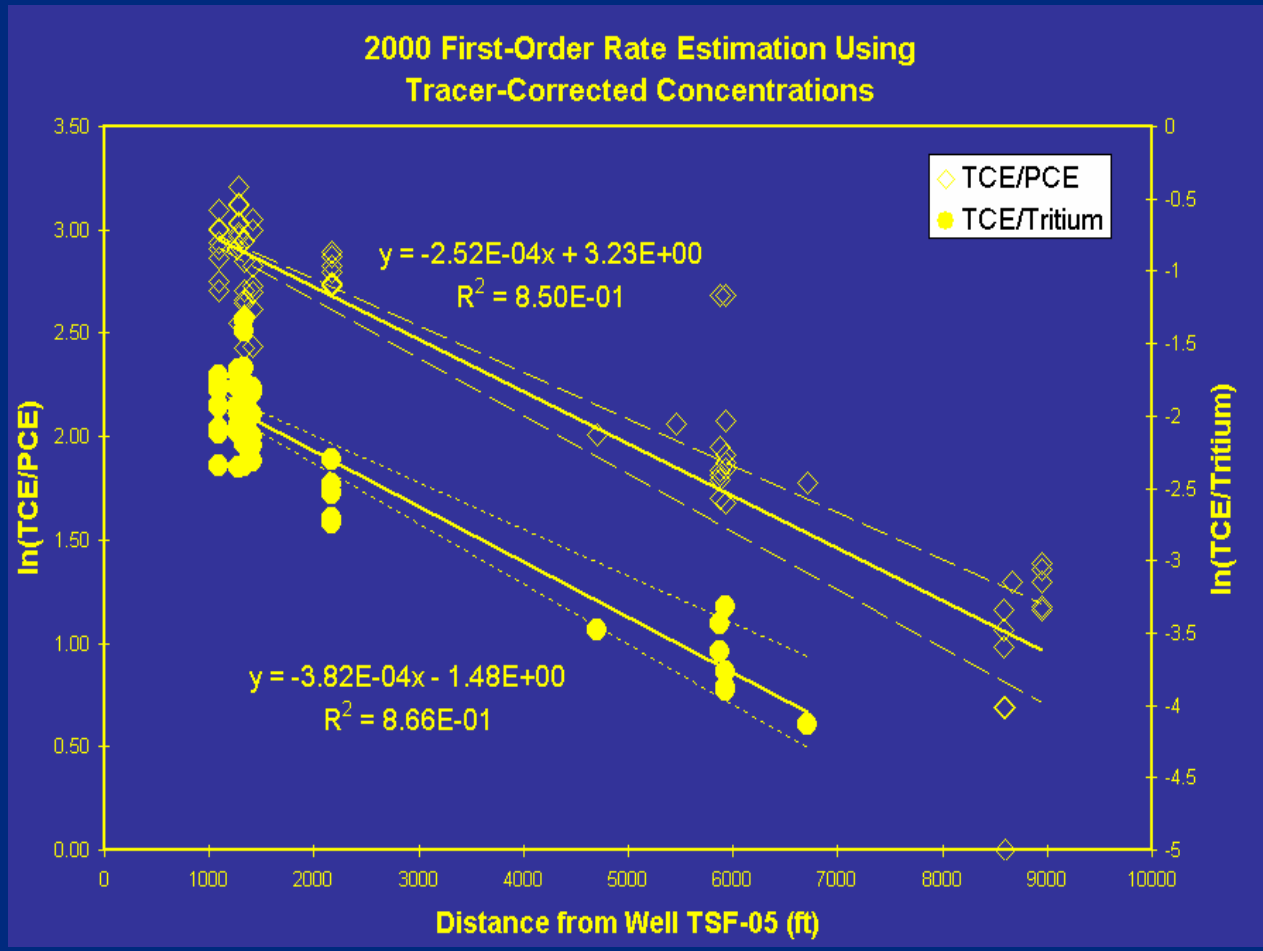


Tracer Corrected Method: Indirect Evidence of Attenuation

Attenuation mechanisms for TCE in groundwater :

1. Anaerobic reductive dechlorination
2. *Aerobic cometabolism*
3. Non-degradative mechanisms (e.g. dispersion)

Results showed that TCE was decreasing with distance from the source area, and attenuating relative to two internal tracers, PCE and tritium with a half-life of 9-21 years.



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9/11/02	TAN 7	Filter 1, 50L		Filter 2, 20 L		Filter 3, 60L	
EB			2.8E+04	+	4.E+05	+	5.5E+05
HPA		+	1.1E+06	+	5.E+05	+	8.4E+05
CINN		+	7.8E+05	+	3.E+05		4.7E+05
PA		+	4.3E+04	+	4.E+05	+	4.8E+05

9/16/02	TAN 55	317 FT		424 FT		461 FT	
EB		+	2.5E+05	+	3.7E+05	+	8.7E+05
HPA		+	2.4E+05	+	4.0E+05	+	4.8E+05
CINN		+	2.4E+05	+	5.8E+05	+	4.8E+05
PA		+	4.7E+05	+	2.7E+05	+	5.3E+05

9/23/02	TAN 52	266 FT		373 FT		456 FT	
EB			3.4E+05		3.0E+05		7.3E+05
HPA			3.0E+05		2.8E+05		2.8E+05
CINN			1.8E+05		3.6E+05		2.3E+05
PA		+	2.9E+05	+	3.6E+05	+	1.2E+05

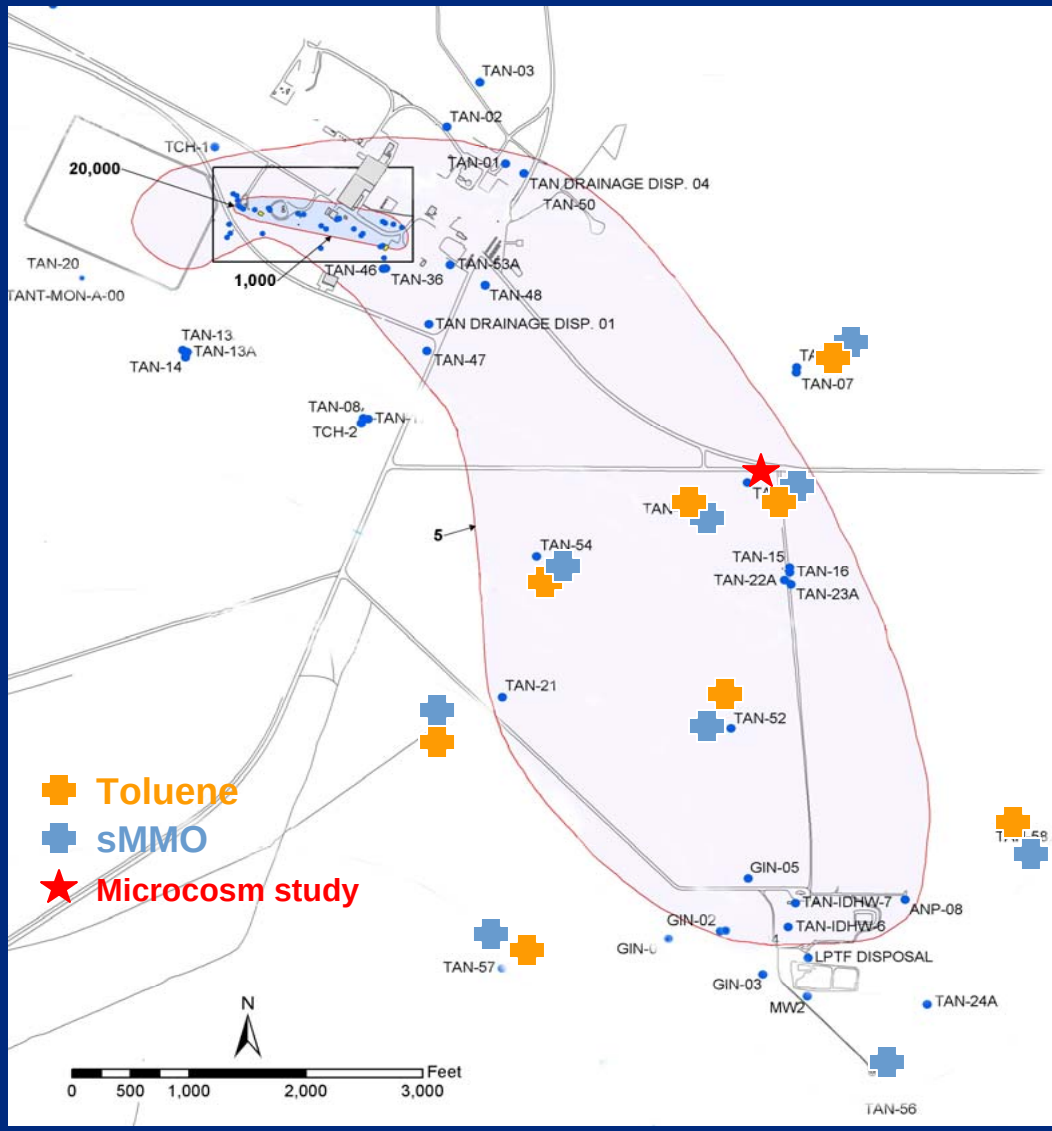
9/25/02	TAN 51	263 FT		342 FT		460 FT	
EB			4.8E+05		1.4E+06		5.2E+05
HPA		+	2.7E+05	+	7.5E+05		3.4E+05
CINN			2.7E+05	+	8.8E+05		3.8E+05
PA		+	3.7E+05	+	1.2E+06		7.0E+05

Field application of enzyme activity probes at TAN (INL): 2001-2003

Demonstrated methane- and toluene-oxygenase activity at TAN

sMMO and toluene oxygenase activity were noted for wells both inside and outside of TCE plume and at all of the wells sampled

Based on this two year study, our results confirm that the degradation mechanisms at TAN include aerobic cometabolism by indigenous subsurface microbial communities



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Conclusions/Accomplishments

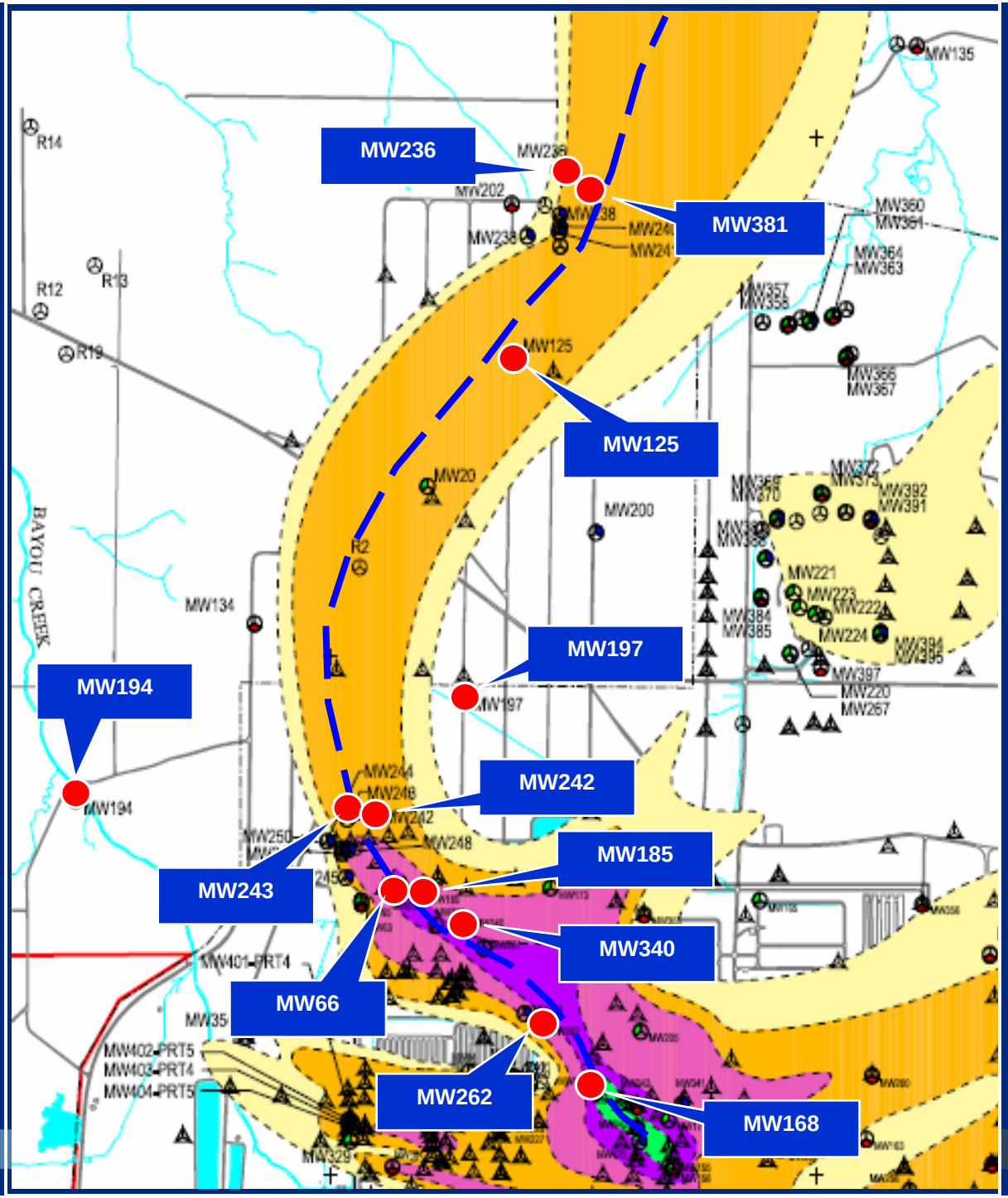
- Results suggest aerobic cometabolic degradation of TCE is an attenuation mechanism, both in the dissolved phase and medial zone.
- Degradation rate evaluations provide a study of indigenous organisms and their capability to degrade TCE under un-induced conditions; the results from these studies suggest that natural populations are actively degrading the contaminant TCE.
- These data can be used to support a MNA remedial strategy for this plume, can provide significant cost savings over the lifetime of monitoring of the plume (estimated for the TAN plume, in excess of $8M)

Sampling locations in PGDP NW plume

- MW were chosen based on location within the plume (upper, middle or lower regional gravel aquifer)

AND location along the flow path of the contamination, i.e. plume core

- MW within the high concentration “source” area as well as wells outside the contaminant plume were also chosen for evaluation



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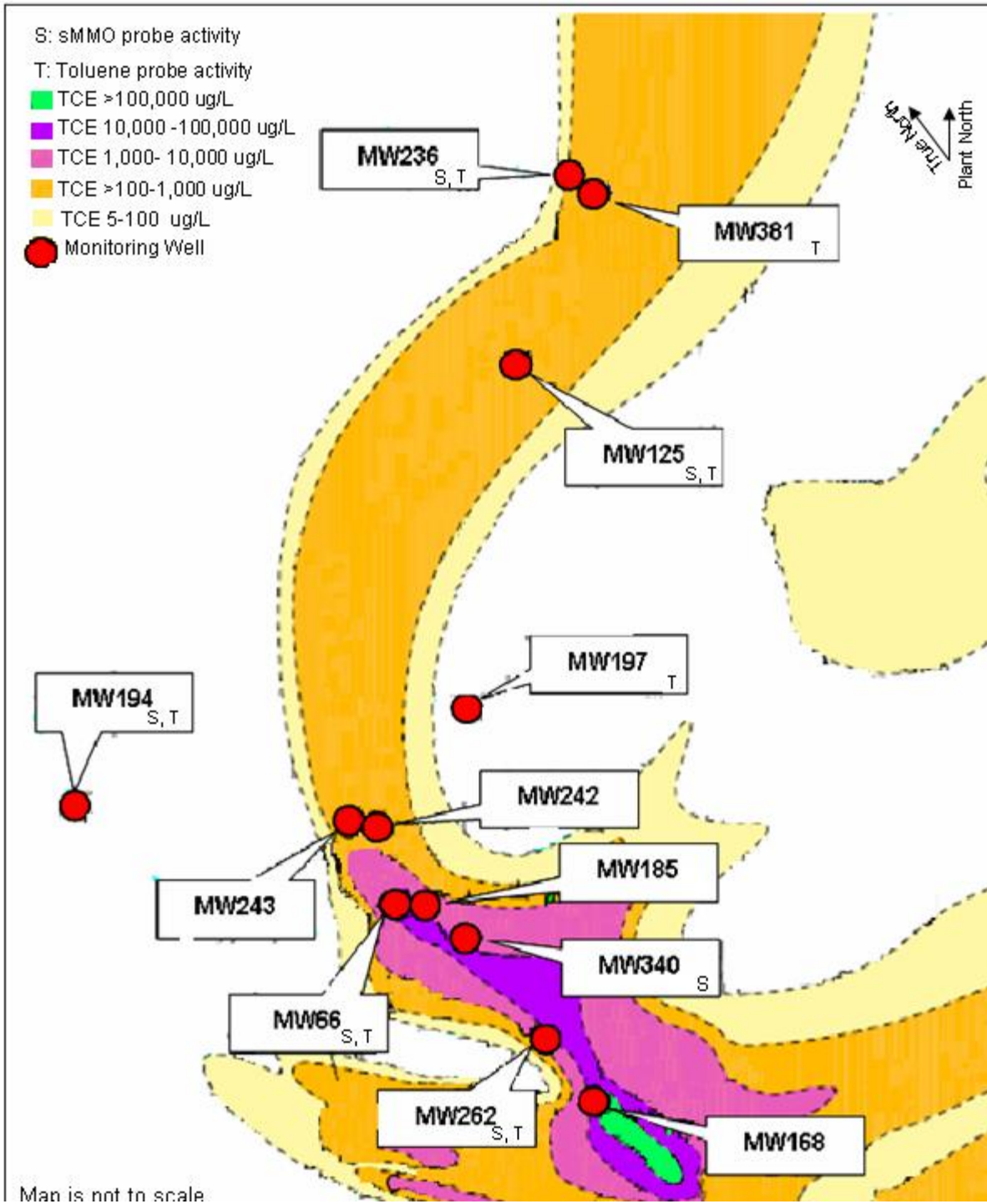
Enzyme Probe results for NW plume

MW	Aquifer Designation	Tc-99 (pCi/L)	Coumarin	3HPA	PA	Cinn	DAPI cells/mL
MW168	URGA	3260					1.90E+05
MW66		3670	+	+	+		3.67E+05
MW194		17	+	+	+	+	1.76E+05
MW197		283			+		1.59E+06
MW185	MRGA	1260					9.75E+05
MW242		341					7.76E+05
MW243		3860					4.27E+05
MW381		329		+	+		9.66E+05
MW262	LRGA	4178	+	+	+	+	3.52E+05
MW340		747	+				7.25E+05
MW236		936	+	+	+		8.84E+05
MW125		273	+		+		7.99E+05

General Activity Results at PGDP NW plume



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DNA gene results for NW plume

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		+	+	+	+

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Results for application of probes at NW plume

Activity *(significant)*

sMMO activity was detected in 6 out of the 12 MW

toluene activity was detected in 7 out of the 12 MW

DNA

MW168, MW242 and MW243 positive for sMMO DNA, not activity.

MW1168, MW197, MW185, MW242, MW243, and MW340 positive for toluene oxygenase genes, not activity.

- 3 out of 4 URGA wells showed enzyme activity with either toluene or sMMO probes
- Only one of the MRGA wells showed any enzyme activity (toluene)
- All of the LRGA wells showed enzyme activity with either toluene or sMMO probes

More than 50% of the wells sampled in the NW plume showed aerobic activity.

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Results continued …

- Activity was not detected using the sMMO or toluene probes at MW168, the well with the highest concentration of TCE
  (There were hits with DNA amplification)

- There does not appear to be a direct relationship between Tc-99 and enzyme activity; MW262 and 66 showed aerobic enzyme activity with both methane and toluene probes, while MW243 and MW168 showed minimal enzyme activity

In general, enzyme activity probes suggest that aerobic cometabolic degradation is occurring in groundwater in the NW plume. These results also suggest that aerobic cometabolism may be contributing to the natural attenuation of the contaminant TCE within this plume.

TCE Fate & Transport Project
Phase II - Aerobic Degradation DQOs
Range of Decision / Estimation Statements Developed

- #1 Based on the use of "oxygenase" specific enzyme activity probes, determine whether bacteria capable of aerobically biodegrading TCE are present in the RGA. They are present

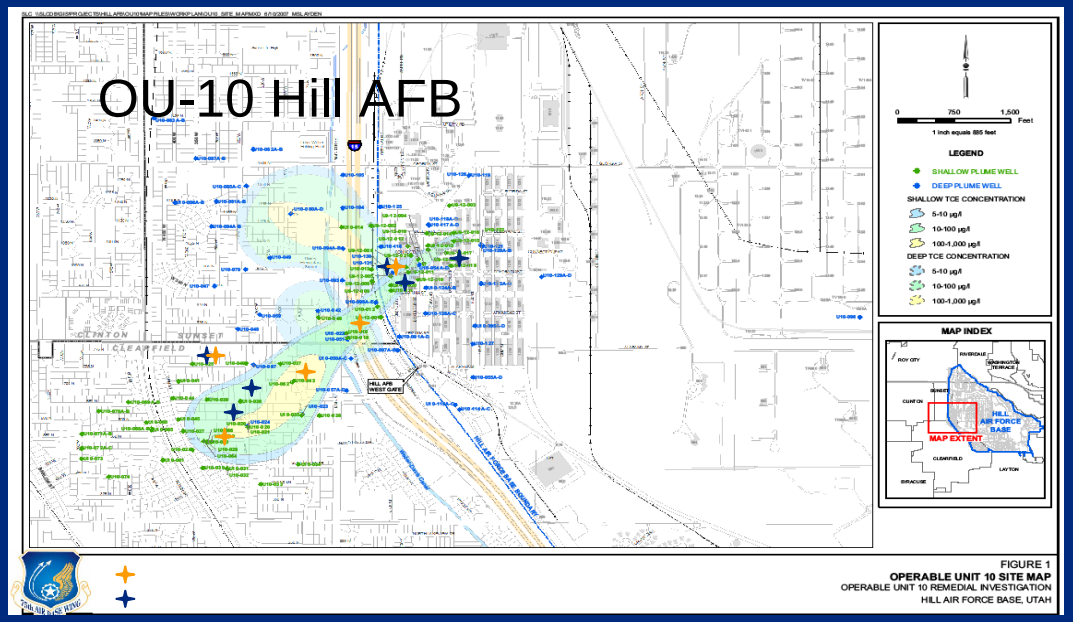
- #2 Based on the use of stable carbon isotope (SCI) fractionation tests, determine whether SCI supports the occurrence of aerobic degradation and/or other biotic/abiotic degradation processes.

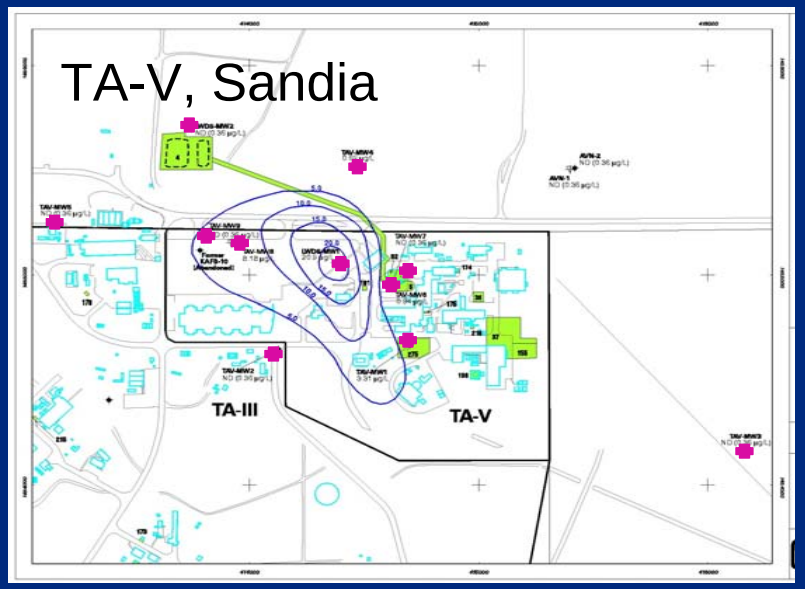
  In Progress

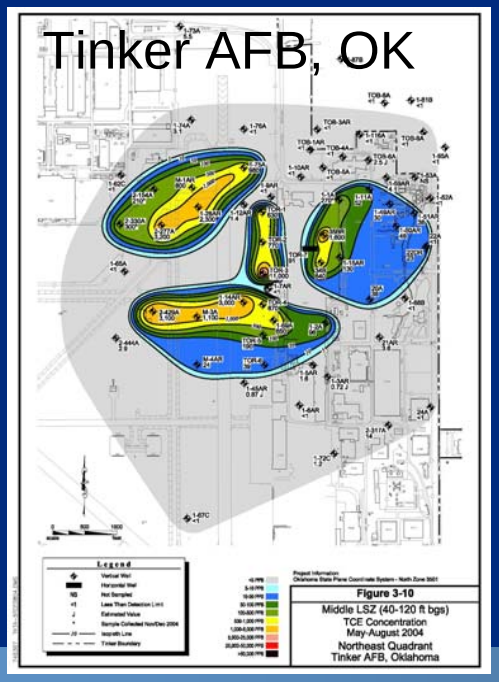
- #3 Estimate whether the distribution and number of bacteria are sufficient to significantly biodegrade the plumes Present along plume core, inside and outside the plume

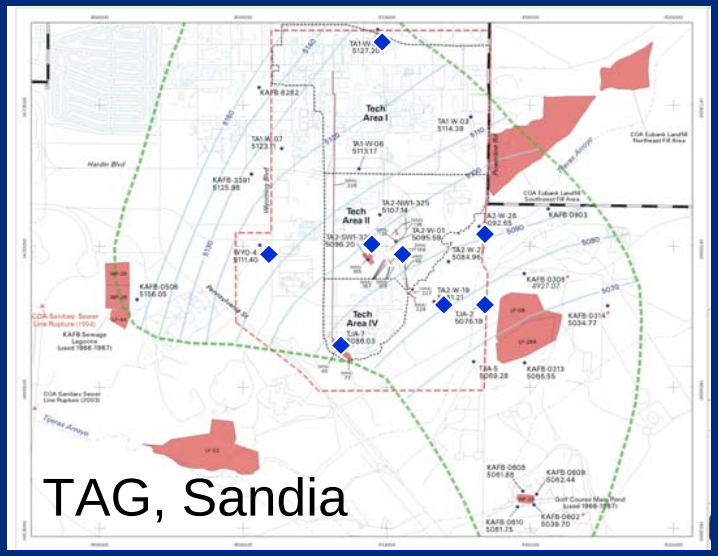
- #4 Determine whether conditions (e.g., bioavailable and sustainable substrates) in the RGA are conducive for ongoing and sustainable aerobic biodegradation of TCE In Progress

- #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 Decision yet to be made









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Enzyme probe application elsewhere …

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