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# Briefing

#### PGDP Citizens Advisory Board

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**KRCEE** 

for

PGDP TCE FT Project Team

June 19, 2008





# 4 Project Phases

Phase I - Data Evaluation

Phase II - Aerobic Degradation Investigation

Phase III - Stable Carbon Isotope Investigation

Phase IV - Abiotic Degradation Evaluation





#### Phase I - Data Evaluation

- Completed
- Screening to ID most-likely TCE attenuation processes
- Determination of TCE degradation rate in RGA
  - Utilized Existing NWP Data
  - · Calculated as "half-life"\*
  - Range of Half-Life Rates from 4 26 years

<sup>\*</sup>half-life = time required for present concentration to be reduced by 50%





# Phase II - Aerobic Degradation Investigation <u>Status of Activities</u>

- · Completed Scoping Document (May '07)
- · Completed Sampling (December '07)
- Completed Microbiological, Stable Carbon Isotope & Geochemical Laboratory Analyses (April '08)
- · SRNL Report due July 1, 2008
- · KRCEE White Paper due July 15, 2008





Phase II - Aerobic Degradation Investigation

## Goals

- To identify biological degradation mechanisms active in the RGA (degradation by bacteria)
- 2. Provide DOE with recommendations for future Biological Degradation Investigation(s)



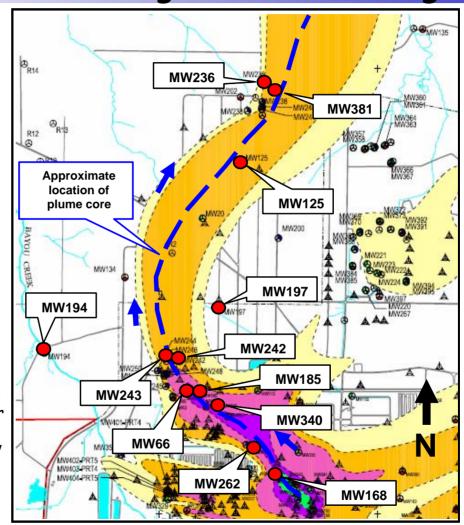


#### Phase II - Aerobic Degradation Investigation

Study Area & Well Locations

= TCE contaminant plume in Regional Gravel Aquifer

= NWP GW Flow is to NW from MW168 to MW66







Phase II - Aerobic Degradation Investigation

#### Background

- 1. The RGA is an aerobic "oxygen rich" aquifer
  - Dissolved Oxygen present in groundwater (throughout aquifer)
  - Under the right conditions, Aerobic "oxygen loving" microbes via process of Co-metabolism destruct TCE





Phase II - Aerobic Degradation Investigation

#### Background

- 2. Co-metabolism occurs in aerobic environments when a microbe produces enzyme(s) capable of TCE destruction
  - The microbe does not use TCE as a food source
  - The microbe does not benefit from the enzymatic destruction of TCE
- 3. Microbes are utilizing other substances for respiration/metabolism





Phase II - Aerobic Degradation Investigation

#### <u>Background</u>

- 4. Aerobic microbes metabolize (oxidize) naturally occurring and man-made (anthropogenic) sources of organic material
- 5. Many sources of naturally available organic material
  - Organic material deposited with aquifer sediment
  - Decaying plant matter
  - · Decaying microbial biomass





Phase II - Aerobic Degradation Investigation

#### <u>Background</u>

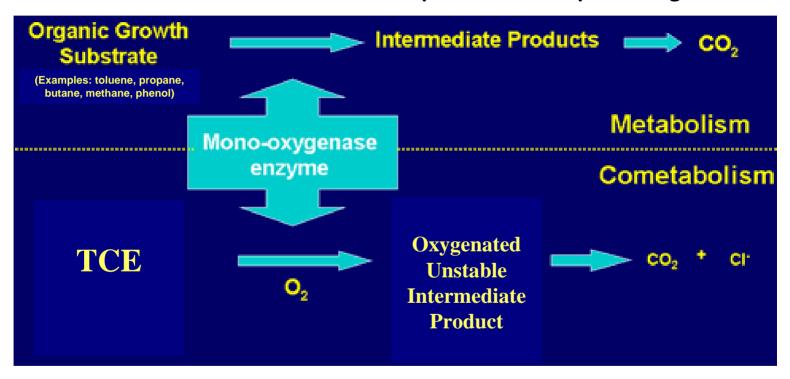
- 6. Aerobic microbes, thru their metabolic processes, <u>add oxygen</u> (oxidize) to organic compounds
- 7. Addition of oxygen into the organic substances occurs via enzyme reactions ("oxygenase" enzymes)
- 8. Oxygenase enzymes fortuitously destroy TCE
- 9. Enzyme destruction of TCE produces endproducts carbon dioxide, chloride, and water
- 10. No harmful intermediate/end products (such as vinyl chloride in anaerobic degradation)

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#### Phase II - Aerobic Degradation Investigation

Microbial metabolic process on top of diagram



Co-metabolism process on bottom of flow diagram (Source - http://wrhsrc.oregonstate.edu/briefs/brief\_8.htm)





Phase II - Aerobic Degradation Investigation

#### Background

Pseudomonas putida

(Known to metabolize toluene in soil)



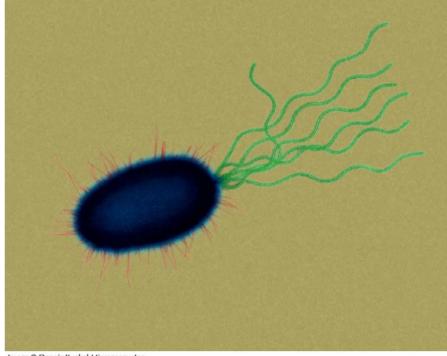


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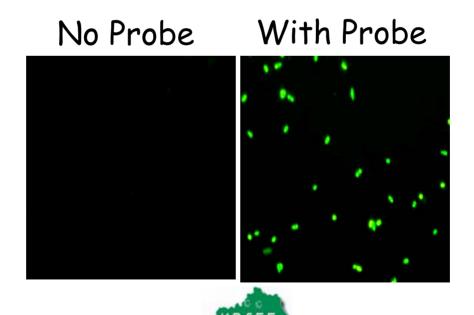




Phase II - Aerobic Degradation Investigation

#### Enzyme Activity Probes

Laboratory tools that evaluate the activity of the microbial oxygenase enzymes; cells appear fluorescent when the enzyme is active



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#### Phase II - Aerobic Degradation Investigation

Monitoring Well	Aquifer Designation	Screened Interval Depth (ft bgs)	Qualitative data (6/4/7)		Toluene probes			
			sMMO probe Coumarin	Toluene probes	Quantitative data ( fluorescent cells/mL )			Total –DAPI cells/mL
					3НРА	PA	Cinnamonitrile	
MW168	URGA	63 - 68	-	-	nd	2.41x10 <sup>3</sup>	nd	1.90x10 <sup>5</sup>
MW66		55 - 60	+	+++	1.43x10 <sup>4</sup>	2.10x10 <sup>4</sup>	9.14x10 <sup>3</sup>	3.67×10 <sup>5</sup>
MW194		47 - 52	+	+++	3.13x10 <sup>3</sup>	9.52x10 <sup>3</sup>	1.20x10 <sup>4</sup>	1.76x10 <sup>5</sup>
MW197		58 - 63	-	+	1.73x10 <sup>4</sup>	6.28x10 <sup>4</sup>	2.23x10 <sup>3</sup>	1.59×10 <sup>5</sup>
MW197 (resample)			na	na	5.03x10 <sup>3</sup>	1.20x10 <sup>4</sup>	2.04x10 <sup>3</sup>	7.05x10 <sup>5</sup>
MW185	MRGA	68 - 73	-	++	1.79x10 <sup>4</sup>	1.37x10 <sup>4</sup>	1.95x10 <sup>3</sup>	9.75x10 <sup>5</sup>
MW242		65 - 75	-	-	3.57x10 <sup>3</sup>	1.24×10 <sup>3</sup>	8.85x10 <sup>3</sup>	7.76x10 <sup>5</sup>
MW243		65 - 75	-	-	3.29x10 <sup>3</sup>	4.61x10 <sup>3</sup>	1.32x10 <sup>3</sup>	4.27×10 <sup>5</sup>
MW381		66 - 76	-	++	6.14x10 <sup>4</sup>	3.52×10 <sup>4</sup>	5.51x10 <sup>3</sup>	9.66x10 <sup>5</sup>
MW262	LRGA	90 - 95	+	+++	1.35x10 <sup>4</sup>	1.36x10 <sup>4</sup>	2.79x10 <sup>4</sup>	3.52x10 <sup>5</sup>
MW 262 (resample)			na	na	1.05x10 <sup>4</sup>	1.22x10 <sup>4</sup>	5.71x10 <sup>3</sup>	2.84x10 <sup>5</sup>
MW340		85.5 - 95.3	+	+	3.63×10 <sup>2</sup>	9.57×10 <sup>3</sup>	nd	7.25x10 <sup>5</sup>
MW236		69.5 - 79.5	+	+++	3.24×10 <sup>4</sup>	5.26x10 <sup>4</sup>	9.28x10 <sup>3</sup>	8.84x10 <sup>5</sup>
MW125		78 - 88	+	++	1.39x10 <sup>4</sup>	6.37×10 <sup>4</sup>	2.03x10 <sup>4</sup>	7.99×10 <sup>5</sup>

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)

Highlight denotes that the toluene probe response was considered moderate (fluorescent activity > 3x103 cells/mL and < 8x103 cells/mL) - see text for explanation

Highlight denotes that the sMMO probe was significantly above background or the toluene probe response was considered significant (> 8x103 cells/mL fluorescent activity)



ft bgs- feet below ground surface

μg/L - micrograms per liter

pCI/L - picocuries per liter

cells/mL - per milliliter



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

#### **EAP** Well Locations

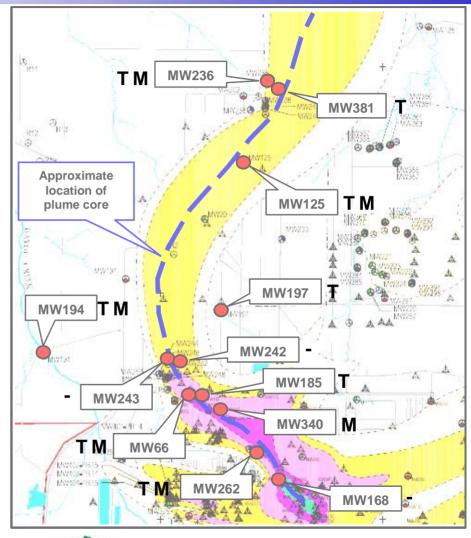
#### EAP Results Legend

T = positive for Toluene degrading enzyme

M = positive for Methane degrading enzyme

Blank = not ID'ed\*

\*not ID'ed in qualitative results and/or did not meet 10<sup>4</sup>/mL quantitative criteria







Phase III - Stable Carbon Isotope Investigation

#### Stable Carbon Isotope (SCI) Background

- 1. Carbon in TCE molecule
- 2. Carbon in TCE molecule contains <u>stable</u>\* carbon isotopes carbon-12 (12C) and carbon-13 (13C)
- 3. The weight of a  $^{13}C$  atom is greater than the weight of a  $^{12}C$  atom

\*stable" indicates isotope does not undergo radioactive decay





Phase III - Aerobic Degradation Investigation

#### Stable Carbon Isotope (SCI) Background

- 4. Ratio of carbon-13 to carbon-12 ( $^{13}C/^{12}C$ ) is specific to every material containing carbon, including TCE source material
- 5. Microbes prefer to utilize the "lighter" <sup>12</sup>C isotope in metabolic processes
- 6. If biodegradation is occurring then the ratio of carbon-13 to carbon-12 ( $^{13}C/^{12}C$ ) increases in the remaining TCE





Phase III - Stable Carbon Isotope Investigation

#### Stable Carbon Isotope (SCI) Evaluation

- 1. Pair each up-gradient well with a downgradient well along plume flowpath
- 2. Measure the ratio of carbon-13 to carbon-12 ( $^{13}C/^{12}C$ ) in up-gradient well TCE
- 3. Measure the ratio of carbon-13 to carbon-12 ( $^{13}C/^{12}C$ ) in down-gradient well TCE
- 4. Determine if the ratio of carbon-13 to carbon-12 ( $^{13}C/^{12}C$ ) in down-gradient well is greater than that of up-gradient well





Phase III - Stable Carbon Isotope Investigation

#### Stable Carbon Isotope (SCI) Results

- 70% of SCI well-pair comparisons showed an increase in the carbon-13 to carbon-12  $(^{13}C/^{12}C)$  ratio in the downgradient well
- The increase in the carbon-13 to carbon-12  $(^{13}C/^{12}C)$  ratio in the downgradient wells supports the occurrence of biodegradation along the plume flowpath
- Provides third line of evidence that biodegradation is occurring





Phase II - Aerobic Degradation Investigation

#### **Conclusions**

- Three lines of evidence for occurrence of aerobic-cometabolic TCE degradation in RGA:
  - I. Decrease in concentrations of TCE along plume along plume flowpath (decrease >99Tc)
    - · First-order degradation rate calculation
  - II. Positive Enzyme Activity Probe results provide evidence that co-metabolism is occurring & contributing to TCE degradation
  - III. Stable Carbon Isotope well-pair evaluations support occurrence of co-metabolism in Northwest Plume



Phase II - Aerobic Degradation Investigation

#### **Conclusions**

- Aerobic co-metabolic degradation of TCE is occurring in the RGA
- Rate of TCE degradation in dissolved phase NWP is attributable in some degree to cometabolism.





Phase II - Aerobic Degradation Investigation

#### Aerobic BioDegradation Recommendations

- 1. Conduct study to quantify rate of cometabolism
- 2. Expand characterization of Northwest Plume
- 3. Characterize biodegradation in Northeast and Southwest Plumes
- 4. Reflect range of degradation rates (half-lives) in groundwater modeling
- 5. Evaluate potential enhancements to environment to increase degradation rate





Phase III - Stable Carbon Isotope Investigation

#### <u>Status</u>

- 1. DQO process completed
- 2. Applied DQO to evaluation of SCI data collected in support of Phase II Aerobic Investigation
- 3. Future activities to be scoped





Phase IV - Abiotic Degradation Investigation

#### <u>Status</u>

- 1. Compiled existing site data related to abiotic degradation process from historical activities
- 2. Conducted preliminary literature review
- 3. Future activities to be scoped

