**UK/KRCEE Doc #: P22.3 2007**

# TCE Fate & Transport Project Evaluation of Aerobic Degradation Enzyme Activity Probe Sampling Scoping Document

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Prepared for
United States Department of Energy Portsmouth/Paducah Project Office
Acknowledgment: This material is based upon work supported by the Department of Energy under Award Number DE-FG05-03OR23032.

**March 2007**

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Prepared by
TCE Fate and Transport Project Scoping Team
&
University of Kentucky
Kentucky Research Consortium for Energy and Environment
March 16, 2007

**March 2007**

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# TCE Fate & Transport– Enzyme Activity Probe Sampling Scoping

## *Background*

This document summarizes the scoping process related to the implementation of activities to identify and quantify degradation processes that may be actively influencing Trichloroethene (TCE) fate and transport in the three (3) Regional Groundwater Aquifer (RGA) contaminant plumes at the Paducah Gaseous Diffusion Plant (PGDP) and it's environs *(Figure 1)*. A Data Quality Objectives process was employed to ensure that project activities identified the problem and relevant information necessary to address the problem.

The TCE Biodegradation Investigation includes four (4) topics of investigation relative to biodegradation of TCE in the RGA: 1) Derivation of a first order rate constant by normalizing against $Tc^{99}$ or chloride which was completed as part of the Southwest Plume Investigation; 2) Identification of the presence of microbes capable of aerobic biodegradation using enzyme activity probes; 3) Stable Carbon Isotope (SCI) ratio analysis to support biotic and abiotic degradation process; and 4) TCE sorption rates. Each topic of investigation will have its own DQO process. The results of each investigation will be employed in the development of TCE degradation rates for the groundwater plumes at the PGDP.

The derivation of a first order rate constant has been completed and was included in the *Site Investigation Report for the Southwest Groundwater Plume at the Paducah Gaseous Plant (DOE, May 2006)*. The three remaining topics *(Figure 2)*, 1) enzyme activity probe analysis, 2) stable carbon isotope ratio analysis, and a final step addressing TCE sorption rates are intended to serve as parallel lines of support for determination of a TCE degradation rate. The stable carbon isotope DQOs are in progress.

## *Scoping Team*

The project scoping team *(Table 1)* consists of representatives from; DOE, DOE contractors PRS and Navarro, the State of Kentucky Division of Waste Management, EPA Region 4, and the Kentucky Research Consortium for Energy and Environment (KRCEE).

**Table 1. Project Scoping Team Representatives**

| Organization | Representative |
|---|---|
| DOE-PPPO | Rich Bonczek |
| Paducah Remediation Services | Bryan Clayton, Ken Davis |
| Navarro Engineering | Bruce Phillips |
| Kentucky Division of Waste Mgmt | Ed Winner, Todd Mullins |
| USEPA Region IV | David Williams |
| KRCEE | John Volpe, Steve Hampson |
| DOE-EM | Beth Moore |
| Savannah River Laboratory | Bryan Looney |
| Idaho National Engineering Laboratory | Hope Lee |

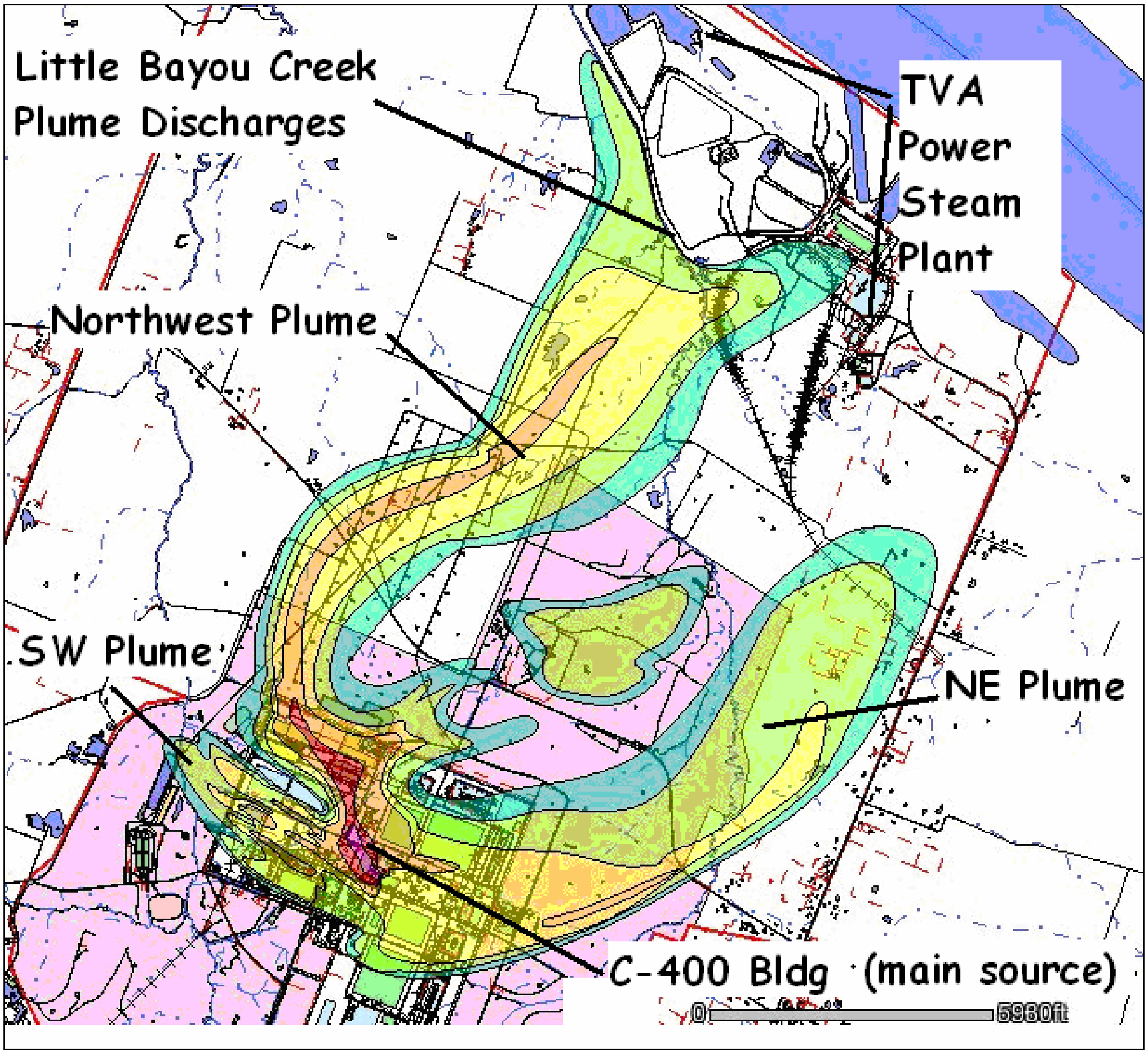



**Figure 1. Composite TCE Plume map depicting the maximum extent of contamination in the Upper, Middle and Lower portions of the Regional Gravel Aquifer at the PGDP.**

## *TCE Fate and Transport Conceptual Model*

The conceptual site model developed for this project specifically addresses TCE in the RGA.

### *Geology and Hydrogeology*

The geology underlying the PGDP consists of sequences of unconsolidated clays, silts, sands, and gravels deposited on limestone bedrock. The unconsolidated materials above the limestone bedrock are grouped into three major stratigraphic units: 1) loess, 2) Continental Deposits, and 3) the McNairy Formation (see Figure 3).

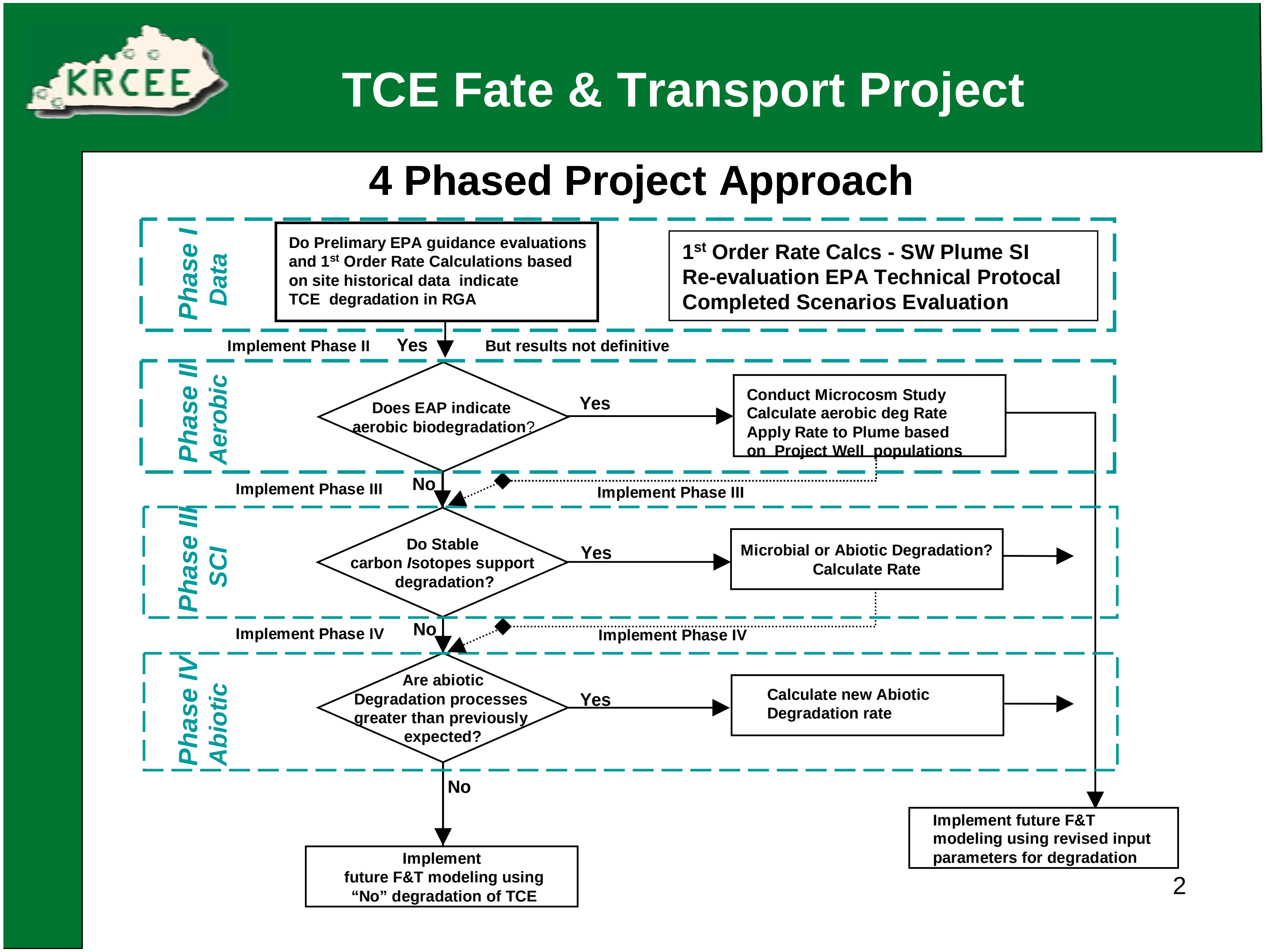



**Figure 2. Flowchart for phases of PGDP TCE Fate and Transport Project**

The upper-most stratigraphic unit, loess, is predominated by wind-deposited silty clay. The loess extends from ground surface to approximately 6.1 m (20 ft) below ground surface (bgs). Underlying the loess, from approximately 6.1 m to 16.8 m bgs (20 – 55 ft.), are the Upper Continental Deposits which consist of discontinuous interbedded sand and gravel layers in a predominantly silt and clay matrix. The Lower Continental Deposits consist of highly permeable sands and gravels extending from approximately 16.8 to 28.0 m (55 to 92 ft) bgs. Below the Continental Deposits is the McNairy Formation which consists of sequences of silts, clays, and fine sands that extend from approximately 28.0 to 106.7 m (92 to 350 ft) bgs.

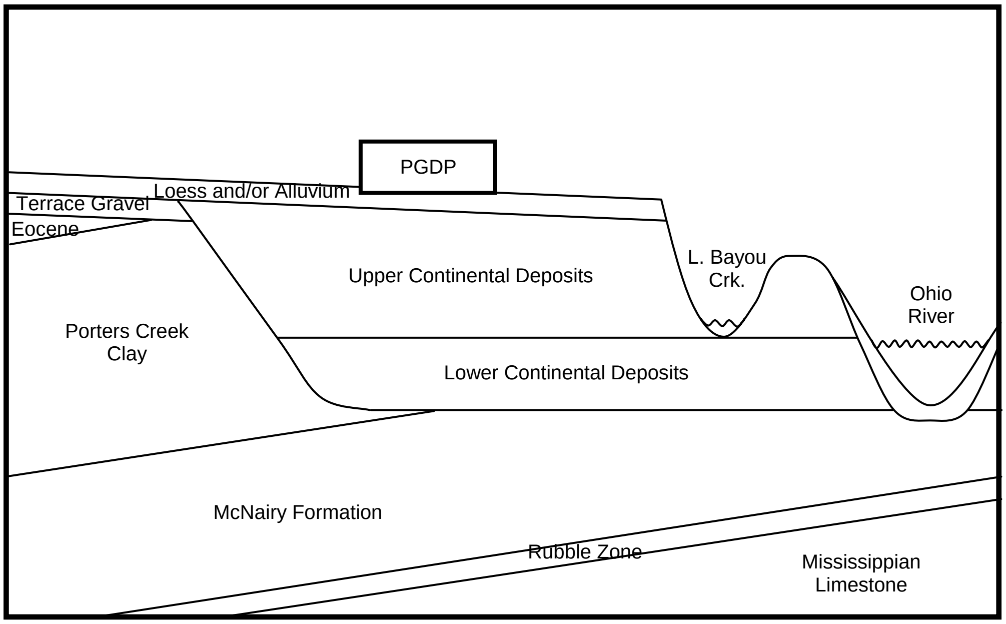



**Figure 3** ***Conceptual Geologic Model for the PGDP and it's environs.***

Groundwater flow through the Upper Continental Deposits is primarily downward into the Lower Continental Deposits *(Figure 4)*. The groundwater flow system associated with the Upper Continental Deposits is called the Upper Continental Recharge System (UCRS). The Regional Gravel Aquifer (RGA) occurs in the Lower Continental Deposits and is the shallowest aquifer underlying the PGDP. The McNairy Flow System occurs in the McNairy Formation which underlies the Lower Continental Deposits and RGA.

The high contrast of hydraulic conductivities between the conductive Lower Continental Deposits and relatively nonconductive McNairy Formation limits flow between the Lower Continental Deposits and the McNairy Formation and directs flow laterally within the RGA. Groundwater flow in the RGA is generally northward toward the Ohio River although local flow-direction variability exists as indicated by the orientations of the Northwest, Northeast and Southwest Plumes within the industrialized portion of the PGDP (see Figure 1).

Dissolved oxygen concentrations for UCRS, RGA, and McNairy Flow System groundwater at the PGDP range from 20 to 289,000 ug/L. Dissolved oxygen concentrations in RGA groundwater, both within and outside of the three PGDP contaminant plumes, range from approximately 1,000 to 8,000 ug/L. RGA dissolved oxygen concentrations indicate that aerobic conditions predominate in the aquifer and that those conditions could support microbial populations capable of aerobic co-metabolic biodegradation of TCE. (See Attachment 1 – Descriptive statistics for PGDP and RGA geochemical parameters).

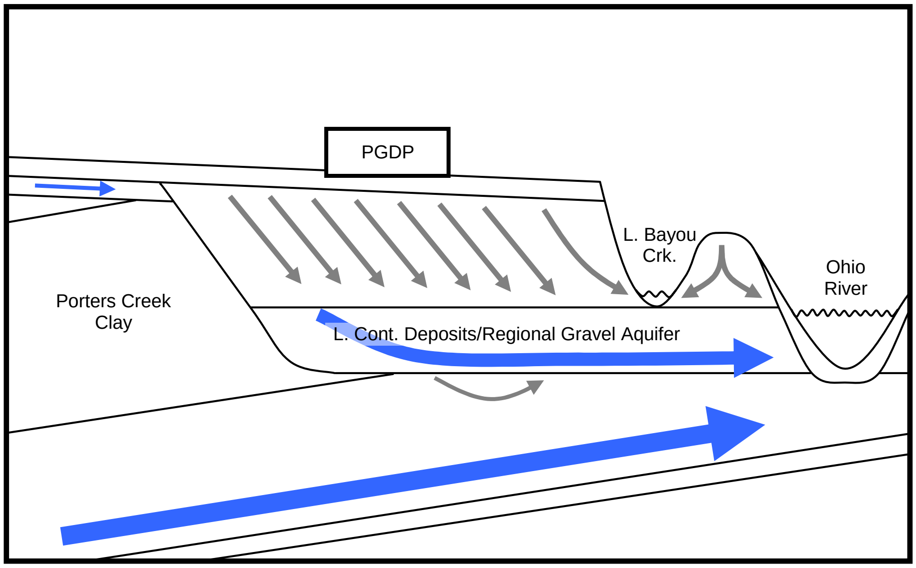



**Figure 4.** ***Conceptual Groundwater Flow Model for the PGDP and it's environs.***

## *Sources*

The Groundwater Operable Unit (GWOU) is comprised of facilities/solid waste management units (SWMUs) with impacted groundwater along with facilities and SWMUs that are sources of contamination to groundwater. Table 2 identifies the facilities and SWMUs that are characterized as sources to PGDP groundwater contamination and the Northwest, Southwest, and Northeast plumes:

The C-400 Cleaning Building located near the center of the industrialized section of PGDP, is the primary source area for TCE. Primary industrial activities conducted in the C-400 Building have included; cleaning machinery parts, disassembling and testing cascade components, and laundering plant clothes. Suspected sources of leaks and spills at the C-400 Building include degreaser and cleaning tank pits, drains and sewers, the east side plenum/fan room basement, tanks and sumps outside the building, and various other processes. According to the WAG 6 Remedial Investigation the most significant TCE leaks and spills occurred at the southeast corner

**Table 2. GWOU Facilities and Solid Waste Management Units**

| |
|---|
| C-720 Maintenance and Storage Building |
| C-400 Cleaning Facility |
| SWMU 1 - C-747-C Oil Land Farm |
| SWMU 2 - C-749 Uranium Burial Ground |
| SWMU 4 - C-747 Contaminated Burial Ground |
| SWMU 201 - Northwest Groundwater Plume |
| SWMU 202 - Northeast Groundwater Plume |
| SWMU 210 - Southwest Groundwater Plume |
| Little Bayou Creek Groundwater Plume Seeps |

of the C-400 Building. The southeast corner of the C-400 building includes SWMU 11 where a drain line from a degreaser sump was poorly connected to a storm sewer, and SWMU 533 where transfer pumps and piping moved TCE to and from a storage area associated with the building.

The highest concentrations of TCE in PGDP soil and groundwater were found in the UCRS and RGA to the southeast and southwest of the C-400 Building. Elevated concentrations of TCE and its breakdown products in subsurface soils and groundwater suggest the presence of TCE DNAPL. In subsurface soil to the southeast of the C-400 Building, TCE has been detected at 11,055 ppm, trans-1,2-DCE was detected at 102 ppm; and VC was detected at 29 ppm. Cis-1,2-DCE and 1,1,1-trichloroethane (l,l,l-TCA) have also been detected at 2 ppm. To the southwest of the C-400 building, TCE has been detected in subsurface soil at 168 ppm, trans-1,2-DCE at 15 ppm, and cis-1,2-DCE at 1 ppm. The presence of the TCE degradation products trans-1,2-DCE, Cis-1,2-DCE, and VC indicate that anaerobic degradation processes may occur locally within the Upper Continental Deposits/UCRS (see Figure 5).

The maximum TCE concentration detected in the RGA in the vicinity of the C-400 Building is 701 ppm or 64% of the maximum solubility of TCE in water. The high concentration suggests that DNAPL has penetrated the RGA and is acting as a secondary source of groundwater contamination to the three PGDP contaminant plumes. DNAPL sources and high TCE concentrations indicative of DNAPL sources are generally believed to act as biocides on microorganisms that could degrade TCE via aerobic co-metabolism.

### *Dissolved Phase TCE Plumes*

The Northwest (NWP), Southwest (SWP), and Northeast (NEP) TCE plumes appear to originate from the C-400 Building near the center of the PGDP industrial facility and burial grounds or disposal areas within the PGDP security fence. TCE concentrations indicative of the presence of primary DNAPL sources in the UCRS and secondary DNAPL sources in the RGA are generally limited to areas within the PGDP security fence.

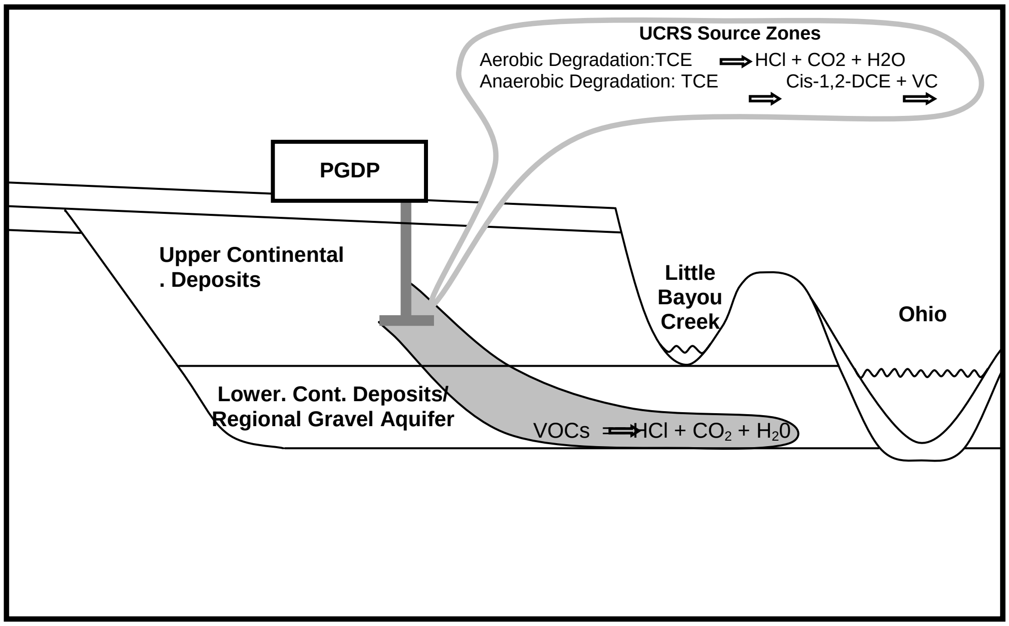
Dissolved phase TCE contamination is typical of all three (3) PGDP groundwater plumes once away from the immediate vicinity of UCRS primary and RGA secondary TCE sources. Redox conditions and the availability of dissolved oxygen in the plumes are conditions that could support aerobic co-metabolic biodegradation of TCE at the PGDP (Figure 5).

## *The DQO Process*

### *Step 1. State the Problem*

#### <u>*Description of the Problem*</u>

Groundwater underlying and downgradient of the PGDP is contaminated by two (2) primary constituents, TCE and Technetium-99 ($^{99}$Tc). The need to evaluate the fate of TCE in the RGA can be defined by several observations about the characteristics of the RGA and the behavior of




**(will use the other csm with UCRS aerobe/anaerobe added as above)**

**Figure 5.** ***Conceptual TCE Contaminant Transport Model for the PGDP and it's environs.***

TCE and $^{99}$Tc in RGA contaminant plumes: 1) Comparison of TCE concentrations to $^{99}$Tc concentrations along NWP flowpaths suggest that TCE concentrations exhibit greater decreases along a given section of the plume than do $^{99}$Tc concentrations; 2) Calculation of first-order rate constants indicate that TCE degradation may occur at rates greater than those currently accepted for application to groundwater fate and transport characterization; 3) The RGA is characterized as an aerobic aquifer based on dissolved oxygen concentrations and the absence of TCE degradation products typically found in anaerobic groundwater environments; and 4) Aerobic groundwater conditions preclude the widespread occurrence of anaerobic microbial populations that require anaerobic groundwater conditions for survival and metabolic breakdown of TCE.

An evaluation of the PGDP hydrogeological setting, geochemical setting, strength of TCE sources and plume stability was conducted utilizing recently published "Scenarios" guidance for assessing microbial degradation potential in a variety of groundwater and source settings (*Analysis for PGDP Groundwater Plumes Utilizing the Scenarios Evaluation Tool for Chlorinated Solvent MNA*" (WSRC-STI-2006-00096, Rev. 1, Savannah River National Laboratory, 2001). The results of the evaluation indicate that groundwater conditions at the PGDP include an aerobic groundwater environment and relatively fast groundwater flow rates. The Scenarios evaluation in provided in Attachment 2.

The purpose of the proposed work for this project is to demonstrate whether sustainable aerobic biodegradation of TCE is occurring in the RGA. Because the occurrence of aerobic biodegradation needs to be characterized and assessed, the resources necessary to evaluate this process need to be identified. This scoping document summarizes necessary resources and activities to be conducted to characterize possible aerobic co-metabolic biodegradation of TCE in PGDP groundwater.

The proposed characterization will support ongoing and future groundwater characterizations and remedial assessments at the PGDP. The information generated by the project may provide for improved decision-making related to remedial options and monitoring, shortened time frames for compliance, and the minimization of impacts on public health.

## *Step 2. Identify the Goals of the Study*

The following principal study questions were developed through application of the DQO process:

*Identify the Principal Study Questions*

1. Is aerobic biodegradation, co-metabolism employing an appropriate oxygenase enzyme, occurring in the RGA plumes? (Ph II sampling and evaluation)
    - Are the appropriate bacteria present in the aquifer? (Ph II sampling and evaluation)
    - Do TCE carbon isotopic fractionation support the biodegradation hypothesis?(Ph II data collection, ph II and ph IV evaluations)

2. Are the bacteria present in sufficient numbers to impact the plumes? (Ph II)
    - Are the total cell counts high enough to support biodegradation?
    - Does the distribution of the biodegradation process in RGA wells support the conclusion that the plume is being temporally and spatially impacted? (Ph II)

3. Are conditions in the RGA conducive for ongoing and sustainable aerobic biodegradation?
    - Is a primary bioavailable substrate present for co-metabolic reactions?
    - Does bacterial detritus provide a carbon source for the co-metabolic reaction?
    - If one or more bioavailable substrates are present, are the substrates sustainable?
    - If one or more bioavailable substrates are present, are they available in sufficient quantities to sustain co-metabolic reactions indefinitely?
    - Are nutrients present for co-metabolic reactions?

4. If aerobic biodegradation is occurring, what is the rate?
    - What are the probe-specific bacterial cell counts determined for the sampled wells?
    - What are the physiological parameters of the aquifer that are to be replicated in the microcosm study (Dark, groundwater temperature, TCE concentration, other parameters)? Not defined in document.
    - What are the rates of biodegradation based on a microcosm study? (Ph II)
    - Can TCE carbon isotopic fractionation results be used to estimate a rate? (Ph II)

5. Is the calculated biodegradation rate or rates qualitatively supported by literature values? (Ph II)
    - Are rates generally supported based on similar studies for a variety of bacteria and their co metabolites? (Ph II)
    - Are rates generally supported based on the type of “oxygenase” enzyme known to be bound by the probes employed? (Ph II)

*Develop Decision / Estimation Statements (based on Principle Study Questions)*

Decision / Estimation Statement #1. Based on use of specific "oxygenase" probes, determine whether bacteria capable of aerobically biodegrading TCE are present and therefore require an estimation of their impact on the plumes or recommend that other mechanisms of TCE degradation/attenuation be evaluated.

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

Decision / Estimation Statement #3. Estimate whether the distribution and number of bacteria are sufficient to significantly biodegrade TCE in RGA groundwater.. If the distribution and number of microorganisms are sufficient to biodegrade TCE in RGA groundwater, determine whether biodegradation is sustainable. If it is determined that biodegradation is not sustainable, recommend that other mechanisms of TCE degradation/ attenuation be evaluated.

Decision / Estimation Statement #4. Determine whether conditions including, but not limited to, the existence of a bioavailable and sustainable substrate in the RGA are conducive for ongoing and sustainable aerobic biodegradation of TCE. If conditions are determined to be ongoing and sustainable, conduct an evaluation of the biodegradation rate using a multiple lines of evidence approach. If conducive conditions are not determined to be present, recommend that other mechanisms of TCE degradation/attenuation be evaluated.

Decision / Estimation Statement #5. Based upon a comparison to the calculated biodegradation *rate* or range of rates *to values* in the literature, either accept the calculated rate(s) for use in future fate-and-transport modeling or access the team's confidence in the unsupported results.

## *Step 3. Identify Information Inputs (for each decision/estimation statement)*

***Decision / Estimation Statement #1.*** Based on use of specific "oxygenase" probes, determine whether bacteria capable of aerobically biodegrading TCE are present and therefore require an estimation of their impact on the plumes or recommend that other mechanisms of TCE degradation/attenuation be evaluated.

- Representative groundwater samples from the Northwest Plume will be collected and analyzed for "oxygenase" containing bacteria using oxygenase-specific enzyme activity probes.

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

- SCI sampling and analysis will be conducted on a path parallel to oxygenase-specific enzyme activity probe sampling and analysis.

*Decision / Estimation Statement #3.* Estimate whether the distribution and number of bacteria are sufficient to significantly biodegrade TCE in RGA groundwater. If the distribution and number of microorganisms are sufficient to biodegrade TCE in RGA groundwater, determine whether biodegradation is sustainable. If it is determined that biodegradation is not sustainable, recommend that other mechanisms of TCE degradation/attenuation be evaluated.

- Representative RGA groundwater samples from the Northwest Plume will be collected and analyzed for "oxygenase" containing bacteria using oxygenase-specific enzyme activity probes.
  - Use on-site enzyme probes to address bacterial quantity (bacteria per liter), or
  - Containerize, package, and ship samples according to sampling protocols (Attachment 6) for laboratory enzyme probe evaluation at Idaho National Environmental and Engineering Laboratory (INEEL-NORTH WIND).
  - Obtain bacterial information for each well location.
- Utilize professional judgment and literature values to determine if the cell counts and the distribution of organisms are sufficient to identify the occurrence of aerobic TCE biodegradation.
- Conduct Microcosm Studies
  - Collect a representative RGA groundwater sample from one of the NWP enzyme probe analysis wells according to INEEL-NORTH WIND sampling protocols (Attachment 6).
  - Containerize, package, and ship samples for microcosm studies according to INEEL-NORTH WIND sampling protocols
  - Conduct the Microcosm Study for up to two (months) to observe changes in TCE concentrations.
  - Establish biodegradation rates from the microcosm study.
  - Representative samples and direct measurements will be required of sufficient quantity and quality to satisfy measurement of rate.

*Decision / Estimation Statement #4.* Determine whether conditions including, but not limited to, the existence of a bioavailable and sustainable substrate in the RGA and the presence of other geochemical parameters are conducive for ongoing and sustainable aerobic biodegradation of TCE (see Table 3). If conditions are determined to be ongoing and sustainable, recommend that an evaluation of the biodegradation rate using a multiple lines of evidence approach be applied at the PGDP. If conditions are not determined to be ongoing and sustainable, recommend that other mechanisms of TCE degradation/attenuation be evaluated immediately.

- Targeted geochemical parameters (see Table 3; Attachment 1) will be assessed by historical data evaluation and from data generated from split samples collected for enzyme probe analysis. Assessment of historical data sets for a number of the targeted geochemical parameters indicate that it may be necessary to collect additional geochemical data to address data gaps.
- Geochemical samples will be collected from the identified wells (see Table 3) on a path parallel to the enzyme specific probe samples

- Based on the *"FY07 PGDP Environmental Monitoring Plan"*, wells on the proposed sampling list for this project (MW125, etc) may also sampled for the following parameters on an annual basis: sulfate, nitrate, total organic carbon, chloride, total dissolved solids, silica, fluoride, phosphate, ferrous iron, alkalinity, methane, ethene,

**Table 3. Groundwater geochemical parameters for biodegradation evaluation.**

| |
|---|
| **Volatile Organics** |
| Trichloroethene (TCE) |
| Dichloroethene (DCE) |
| Vinyl Chloride (VC) |
| Dissolved oxygen (DO) |
| pH |
| Eh |
| Temperature |
| Specific conductance |
| **Other parameters/analytes:** |
| Total organic carbon |
| Nitrate |
| Sulfate |
| Ferrous iron |
| Phosphate as Phosphorous |
| Copper, Dissolved |
| Copper |
| Methane |
| **Major cations/anions** |
| **K** |
| **Ca** |
| **Na** |
| **Mg** |
| **CO3** |
| **HCO3** |
| **SO4** |
| **Cl** |
| **CO2** |

ethane, calcium, copper, magnesium, potassium, and sodium. Sampling results from annual and or quarterly PGDP sampling events that include the parameters in Table 3 will be utilized for this study if available

*Decision / Estimation Statement #5.* Based upon a comparison to the calculated biodegradation rates (or range of rates) to those supported in literature, either accept the calculated rate(s) for use in future fate-and-transport modeling or assess the team's confidence in the unsupported results. If biodegradation is deemed to be sustainable, calculate a degradation rate using the following method(s):

- First order rate constant calculations (completed)
- Microcosm studies –
- Specific Carbon Isotopic Fractionation – Estimate the degradation rate using data obtained during the carbon isotopic fractionation testing.
- Compare the calculated biodegradation rates to values available in literature.

## *Step 4. Define the Boundaries of the Study*

The spatial boundaries of this study include: 1) The areal extent of RGA groundwater; 2) The vertical extents of the RGA groundwater; 3) The screened interval of monitoring wells in the RGA within and outside of the NWP; 4) Spatial distribution of NWP TCE concentrations less than 1000 ug/L; 5) The location of NWP wells relative to potential source areas; and 6) NWP flowpaths/flowlines relative to on-site NWP primary and secondary sources.

Temporal boundaries of this study include: 1) Dates that annual groundwater sampling is conducted for NWP sampling locations; 2) The availability of INEEL-North Wind labs to conduct enzyme probe analyses; 3) The number of samples that the INEEL-North Wind lab can process relative to a designated sampling date; 4) Dates that degradation rates are needed to support ongoing work in PGDP environmental projects; and 5) The availability of organizations and personnel to conduct field sample collection activities.

*Monitoring Well Selection*

The factors considered for identification of monitoring wells suited for this study included: 1) relative position of monitoring wells to the centerline of the NWP; 2) Relative location of monitoring wells relative to TCE sources and high TCE concentrations that could induce biocide effects on microbial populations; 3) Relative location of suited wells to one another; 4) Screened interval of wells; 5) General geochemical characteristics of each well including alkalinity, pH, dissolved oxygen, TCE concentration. TCE trend analysis, scheduled well sampling dates, and costs for additional analytes or special sampling were also considered in the identification of wells for this project. Geochemical characteristics of the wells were evaluated relative to each parameters potential to support or inhibit microbial populations capable of TCE degradation.

The NWP was identified by the Project Team as the focus of sampling and characterization activities for this project because NWP wells were used for first-order rate constant tracer normalization analyses and because the greatest number and areal distribution of RGA monitoring wells are available in the NWP. Sampling of the suitable NWP RGA wells is intended to provide a profile of potential aerobic microbial degradation along the plume axis.

Dissolved Oxygen and TCE temporal and spatial data (see Attachment 3 & Attachment 4) was generated for all of the wells at the PGDP, including those identified for sampling in this project. Dissolved oxygen and TCE spatial and temporal trends were evaluated relative to potential impacts on field sampling locations prior to final identification of the wells to be sampled.

PGDP datasets for all project geochemical parameters are summarized in Attachment 1. Geochemical parameters were evaluated relative to potential impacts on sample locations prior to final identification of sampling wells. Factors such as pH and copper concentrations will be evaluated following receipt of EAP sample results.

*Table 4* identifies the initial list or “population” of *sixteen (16)* NWP wells suitable for oxygenase-specific enzyme probe analysis, stable carbon isotope analysis and geochemical sampling based on an evaluation of TCE concentrations, well screen depths, and well locations relative to the centroid of the plume.

**Table 4. Wells Suitable for enzyme probe, stable carbon isotope, and geochemical sampling**

| Plume | Well | Screen Interval |
|---|---|---|
| Available Northwest Plume Wells | ***MW65*** | ***LRGA*** |
| | ***MW66*** | ***URGA*** |
| | MW125* | LRGA |
| | MW168 | URGA |
| | MW185 | MRGA |
| | MW194 | MRGA |
| | MW197 | URGA |
| | MW234 | LRGA |
| | MW236 | LRGA |
| | MW238 | LRGA |
| | MW242 | LRGA |
| | MW243 | MRGA |
| | MW262 | LRGA |
| | MW340 | LRGA |
| | MW381/235 | LRGA |

* = Wells in routine PGDP geochemical sampling schedule.
URGA = upper RGA, MRGA = middle RGA, LRGA = lower RGA
Bold Italics = wells to be sampled as “special cases” because of proximity to source areas.

## *Step 5. Develop Decision Rules*

- A minimum of 8 NWP wells and 2 control wells outside of the NWP must be sampled and analyzed for the presence of aerobic-oxygenase containing bacteria in RGA groundwater.
- Greater than or equal to half (50%) of the minimum number of sampled NWP wells must contain bacteria having an “oxygenase” capable of aerobically degrading TCE in order conclude that aerobic degradation processes are occurring throughout the plume.
    - If greater than 50% of the samples contain bacteria having an “oxygenase” capable of degrading TCE, then the spatial relationship between the wells having positive samples will be examined to estimate the areal extents and impact of biodegradation upon the plume.
    - If 50% of the samples do not indicate the presence of oxygenase containing aerobic microbes, it will be concluded that aerobic bacteria are not present in significantly distributed populations capable of contributing to aerobic degradation across the plume.
    - When the 50% or more of the samples do not indicate the presence of oxygenase and aerobic microbes, it is not automatically assumed that biodegradation is not occurring. However, the Project Team will conclude that biodegradation is not significant throughout the dissolved portions of the plume and the project team

will evaluate whether areas of the plume are being impacted by aerobic degradation.

- 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 will be considered to have no activity of aerobic bacteria that degrade TCE. Any specific well or wells that do not indicate the presence of aerobic bacteria populations greater than $10^3$/ml will not be evaluated for the required 50% of the wells.
- If this study shows that aerobic degradation is occurring in the NWP, additional field sampling and analysis may be required to refine the temporal and spatial extents of biodegradation.

### *Step 6. Limits of the Decision*

- The failure to meet the criteria set forth in Step 5 will support the conclusion that aerobic biodegradation by means of bacteria containing the oxygenase genes considered is not occurring at levels sufficient to impact the plume.

## *Summary - Monitoring Well Selection for Enzyme Activity Probe Analysis*

The most important factors in the selection of sampling wells for this project included: (1) the location of wells relative to the plume core and plume flowpaths; (2) location of wells relative to TCE sources; (3) TCE concentrations in the well (4) the date when the well could be sampled. The wells suitable for sampling were identified through the evaluation of each well relative to the parameters cited above and the parameter cited in the preceding text for "Step 4. Define the Boundaries of the Study Monitoring Well Selection." Sample collection will focus on twelve (12) of the sixteen (16) NWP wells identified in Table 4 as suitable for sampling (see Figure 6; Table 4).

- Monitoring wells MW194 and MW197 will be sampled and evaluated as control wells to identify the presence of aerobic microbial populations outside of the PGDP contaminant plumes.

- MW66 is being evaluated in this project as a "special case. MW 66 will be sampled in order to evaluate the presence and level of activity of aerobic biodegradation relative to high dissolved concentrations of TCE in the vicinity of suspected DNAPL sources.

Based on evaluation of the original sixteen (16) wells in Table 4, the twelve wells retained for sampling are identified in Table 5. The priority for sampling the wells is provided in Table 5 along with the screened interval of the wells below ground surface and the RGA interval associated with the well screen.

## Project Schedule

A pro-forma project schedule is provided in Figure 7. Project field activities are projected to start in March 2007 and be completed no later than October 2007.

The duration of the project may be dependent upon PGDP sampling schedules that have not been disclosed to the Project Team. Field activities may be expedited through the use of resources coordinated independently by the Project Team and DOE PPPO.

**Table 5. Final wells selected for enzyme probe, stable carbon isotope, and geochemical 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 | |

### Project Reports

A written summary report for the project will be completed by SRNL within 30 days of the completion of laboratory microbial, chemical, and geochemical analytical work. Results of SCI analyses will be included in the report relative to the assessment of biodegradation.

## Project Resources

### Project Funding

<u>*DOE HQ*</u>

DOE HQ will provide financial support to SRNL and INEEL-NORTH WIND for enzyme activity probe analytical and microcosm study evaluations.

*DOE-PPPO*

DOE-PPPO has assigned responsibility for execution of field sampling and analytical project needs to Paducah Remediation Services. Project funding will support execution and completion of microbial, chemical, geochemical, and TCE stable carbon isotopic fractionation sample collection, analytical procedures and supporting work.

*PRS*

PRS will provide resources for coordination and execution of field sampling activities.

*KRCEE*

KRCEE will continue to provide project support services including administration and field support for sample collection and shipping.

*KDWM*

KDWM will provide personnel for its role in field sample collection. Additional funding for Project analytical costs may be available if additional Project analytical costs are identified.

## *Manpower & Project Management Resources*

*PRS*

PRS will provide site coordination, personnel, supplies and equipment for field sampling events.

*SRNL*

SRNL will provide data coordination services with DOE HQ, INEEL-NORTH WIND and the Project Team.

*KDWM*

KDWM will provide personnel to conduct field sampling activities.

*KRCEE*

KRCEE will provide project coordination with the Project Team through the conclusion of field sampling activities and receipt and disposition of the SRNL/INEEL-North Wind project report.

If necessary, KRCEE will provide the services of TRICORD, Inc. to support fieldwork. Tasks would include physical support for field sampling events and shipment of samples to laboratories.

## *Analytical Services*

*Microbial Laboratory*

Microbial laboratory services will be provided by INEEL-North Wind Environmental through a contract with DOE-HQ and SRNL. Project data generated from enzyme specific oxygenase probe analyses will be provided to SRNL and, in turn, to DOE-HQ and the Project Team.

*Geochemical/VOC Laboratory*

General sample analyses, geochemical analyses, and VOC analyses will be conducted by DOE contract laboratories at the PGDP, the University of Oklahoma, and/or other laboratories as necessary.

*Stable Carbon Isotope Laboratory*

Stable carbon isotope analyses will be conducted at the University of Oklahoma.

*Savannah River National Laboratory*

SRNL will coordinate INEEL-North Wind Environmental probe and microcosm study data with the Project Team.

*Field Analytical Measurement Services*

Field measurements, for project sample parameters that can be measured in the field, will be accomplished by PRS as identified by Project Team recommendation.

## *Estimated Project Costs*

Individual sample costs addressing the collection and associated analytical costs are provided in Table 6. Implementation of field measurements for parameters identified in Table 6, where available, could significantly lower project analytical costs.

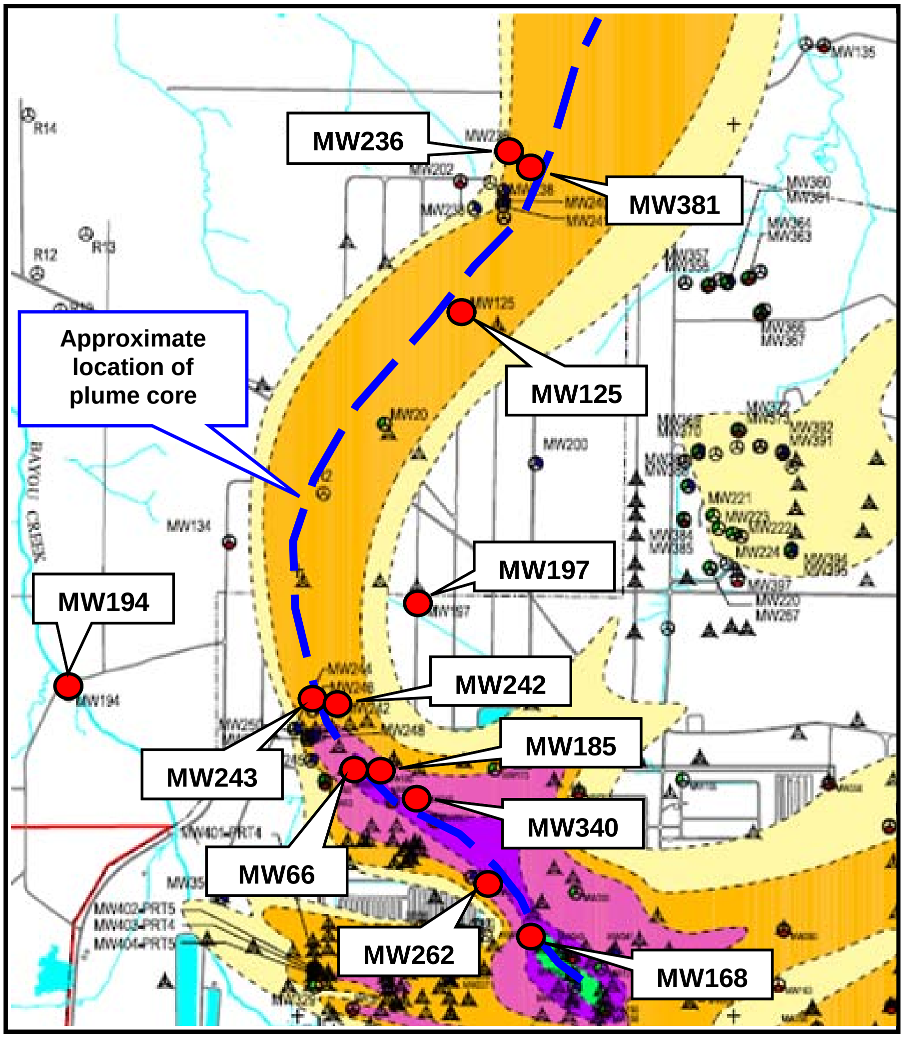


Figure 6. Monitoring well locations for enzyme activity probe sampling, stable carbon isotope sampling and geochemical sampling.

| ID | Task Name | Start | End | Duration |
|---|---|---|---|---|
| 1 | **Project Background** | **9/19/2005** | **6/16/2006** | **39w** |
| 2 | Project Start re: anaerobic degradation +/or TCE degradation Processes | 9/19/2005 | 9/19/2005 | 0.20w |
| 3 | Discuss Project Needs with UK faculty: UK fac & KRCEE to State EPA | 10/17/2005 | 10/28/2005 | 2w |
| 4 | Discuss Project Needs with KDWM | 11/2/2005 | 11/15/2005 | 2w |
| 5 | Project Need Conference Call with KDWM | 11/15/2005 | 11/15/2005 | 0.20w |
| 6 | Follow up with DOE, KDWM, EPA | 11/15/2005 | 1/13/2006 | 8.80w |
| 7 | Project on hold pending SW Plume Degradatio Publication | 1/16/2006 | 5/16/2006 | 17.40w |
| 8 | Dsicussions with DOE-PPPO re Project Restart | 5/1/2006 | 6/16/2006 | 7w |
| 9 | **Project Initiation** | **5/15/2006** | **8/15/2006** | **13.40w** |
| 10 | Call INEEL, SRS, USGS, per Discussions with KDWM & UK faculty | 5/15/2006 | 6/15/2006 | 4.80w |
| 11 | Project Initiation Meeting 1 | 6/16/2006 | 6/16/2006 | 0.20w |
| 12 | Initial Discussions with EPA, KDWM | 6/16/2006 | 7/14/2006 | 4.20w |
| 13 | Develop CSM, Initial Site Deg Screening | 6/19/2006 | 7/28/2006 | 6w |
| 14 | Initial Project Activities (Scope, Background Docs, Mtg Planning) | 6/19/2006 | 8/15/2006 | 8.40w |
| 15 | **Project Kickoff Meeting** | **8/16/2006** | **8/16/2006** | 0.20w |
| 16 | **Activities pursuant to Kickoff Meeting (Action Items)** | 8/16/2006 | 9/18/2006 | 4.80w |
| 17 | Revisions to CSM, Develop Study Questions/DQOs | 8/16/2006 | 8/29/2006 | 2w |
| 18 | Conference Call to Revise DQOs from 8/18 Mtg | 8/29/2006 | 8/29/2006 | 0.20w |
| 19 | Preparation for September Project Team Meeting | 8/29/2006 | 9/18/2006 | 3w |
| 20 | **September Project Team Meeting (Paducah-FFA)** | 9/21/2006 | 10/13/2006 | 3.40w |
| 21 | **Activities Pursuant to September PTM (Action Items)** | **9/21/2006** | **10/13/2006** | **3.40w** |
| 22 | Monitoring & Geochemical Sampling Workgroup CC to Develop Locations & Geochemical Sampling Parameters | 9/21/2006 | 9/21/2006 | 0.20w |
| 23 | DQO Workgroup activities & CC for mtg. modifications | 9/21/2006 | 10/13/2006 | 3.40w |
| 24 | **Project Mgmt Mtg with SRNL** | ***9/25/2006*** | ***9/25/2006*** | 0.20w |
| 25 | **October SRNL Briefing & Meeting** | ***10/16/2006*** | ***10/16/2006*** | 0.20w |
| 26 | **October Project Team Meeting (SRS Presentation) FFA Managers** | ***10/16/2006*** | ***10/18/2006*** | 0.60w |
| 27 | Oct. Activities and Action Items Addressed | 10/16/2006 | 11/2/2006 | 2.80w |
| 28 | **November 2 Project Team Meeting Videoconference (INEEL Presentation)** | 11/2/2006 | 11/2/2006 | 0.20w |
| 29 | SRNL, INEEL, & DOE-EM Added to PT | 11/2/2006 | 11/2/2006 | 0.20w |
| 30 | **November Project Team Meeting 1** | **11/15/2006** | **11/15/2006** | **0.20w** |
| 31 | November Activities Pursuant to PT Mtg Addressed 1 | 11/22/2006 | 12/20/2006 | 4.20w |
| 32 | **November Project Team Meeting 2** | 11/29/2006 | 11/29/2006 | 0.20w |
| 33 | November Activities Pursuant to PT Mtg Addressed 2 | 11/30/2006 | 12/20/2006 | 3w |
| 34 | **December Project Team Meeting** | ***12/20/2006*** | ***12/20/2006*** | **0.20w** |
| 35 | December Activities and Action Items Addressed | 12/21/2006 | 1/19/2007 | 4.40w |
| 36 | **January Project Team Meeting 1** | **1/22/2007** | **1/22/2007** | **0.20w** |
| 37 | January 1 Activities and Action Items Addressed | 1/23/2007 | 1/29/2007 | 1w |
| 38 | **January Project Team Meeting 2** | **1/29/2007** | **1/29/2007** | **0.20w** |
| 39 | ***Finalize DQO & Field Sampling Plan 1*** | **2/2/2007** | **3/14/2007** | **5.80w** |
| 40 | March well sampling locs/logic to Project Team | 2/2/2007 | 2/2/2007 | 0.20w |
| 41 | Initial Identification of PGDP et.al. resources & schedule | 2/2/2007 | 2/13/2007 | 1.50w |
| 42 | Finalize February Well Sampling Locations | 2/13/2007 | 2/19/2007 | 1w |
| 43 | Confirmation of PGDP support with resources for Ph 1 & 2 to HQ | 2/21/2007 | 2/27/2007 | 1w |
| 44 | Submmit Final DQO/ FSAP | 2/28/2007 | 3/14/2007 | 2.20w |
| 45 | **Field and Analytical Work** | **3/12/2007** | **10/26/2007** | **33w** |
| 46 | ***Final Planning for EP/Geochemical Sampling 1*** | ***3/12/2007*** | ***3/22/2007*** | ***1.80w*** |
| 47 | Sampling logistics (time, place, contact, method, COC, H&S, shipping, labs&analyses) | 3/12/2007 | 3/16/2007 | 1w |
| 48 | CC to coordinate field & lab event | 3/12/2007 | 3/22/2007 | 1.80w |
| 49 | ***Field EP & Geochemical Sample Collection 1*** | **3/23/2007** | **5/7/2007** | **6.40w** |
| 50 | Field Sample Collection | 3/23/2007 | 4/12/2007 | 3w |
| 51 | Red Light/Green Light | 3/23/2007 | 4/12/2007 | 3w |
| 52 | Field 1 Sample Results (30 days) | 4/9/2007 | 5/7/2007 | 4.20w |
| 53 | **Planning for Abiotic Degradation Activites** | 4/2/2007 | 5/1/2007 | 4.40w |
| 54 | ***Planning for Microcosm/Rate Sample Collection 1*** | **5/8/2007** | **5/14/2007** | **1w** |
| 55 | Finalize sample locations and schedule | 5/8/2007 | 5/14/2007 | 1W |
| 56 | ***Field EP & Geochemical Sample Collection 2*** | **6/1/2007** | **7/31/2007** | **8.60w** |
| 57 | Field Sample Collection | 6/1/2007 | 6/28/2007 | 4w |
| 58 | Field 2 Sample Results (30 days) | 6/8/2007 | 7/31/2007 | 7.60w |
| 59 | ***Final Planning for Microcosm/Rate Sampling 1*** | 8/1/2007 | 10/1/2007 | 8.80w |
| 60 | ***Field Microcosm/Rate Sampling 1*** | 10/2/2007 | 10/15/2007 | 2w |
| 61 | Field Microcosm Sampling | 6/1/2007 | 6/14/2007 | 2w |
| 62 | Microcosm Sampling Results 1 | 6/21/2007 | 7/18/2007 | 4w |
| 63 | ***Field Microcosm/Rate Sampling 2*** | 10/16/2007 | 11/13/2007 | 4.20w |
| 64 | Field Microcosm Sampling 2 | 8/1/2007 | 8/29/2007 | 4.20w |
| 65 | Microcosm Sampling Results 2 | 8/29/2007 | 9/28/2007 | 4.60w |
| 66 | **Abiotic Degradation DQOs/Scope** | **4/3/2007** | **5/30/2007** | 8.40w |
| 67 | **SRNL/INEEL Report to DOE-EM** | **8/1/2007** | **10/1/2007** | 8.80w |
| 68 | | | | |
| 69 | **SRNL, INEEL** | 9/15/2005 | 9/15/2005 | 0w |
| 70 | **TBD** | 9/15/2005 | 9/15/2005 | 0w |
| 71 | **PRS, Navarro** | 9/15/2005 | 9/15/2005 | 0w |

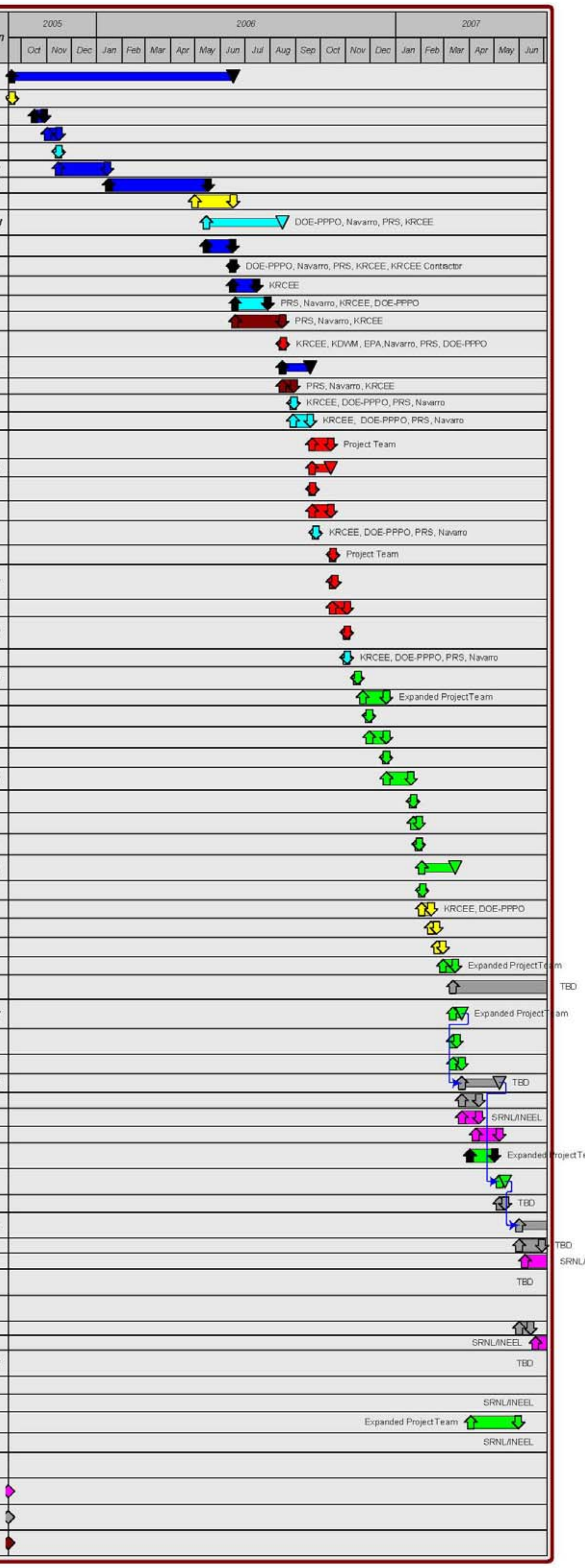



**Figure 7. Project Schedule**

| Analyte | Cost | Method | Cost | Method | Cost | Method |
|---|---|---|---|---|---|---|
| DO | $ - | Field Measurement | $ - | Field Measurement | $ - | Field Measurement |
| pH | $ - | Field Measurement | $ - | Field Measurement | $ - | Field Measurement |
| ORP | $ - | Field Measurement | $ - | Field Measurement | $ - | Field Measurement |
| Temperature | $ - | Field Measurement | $ - | Field Measurement | $ - | Field Measurement |
| eH | $ - | Field Measurement | $ - | Field Measurement | $ - | Field Measurement |
| Specific Conductance | $ - | Field Measurement | $ - | Field Measurement | $ - | Field Measurement |
| DOC | $70.00 | PGDP - 9060 | $70.00 | PGDP - 9060 | $70.00 | PGDP - 9060 |
| TOC | $90.00 | | $90.00 | | $90.00 | |
| VOCs (PCE, TCE, DCE, VC) | $190.00 | PGDP - 624/8260//U.Oklahoma | $190.00 | PGDP - 624/8260//U.Oklahoma | $190.00 | PGDP - 624/8260//U.Oklahoma |
| Alkalinity | $45.00 | PGDP 310.1 | $45.00 | PGDP 310.1 | $45.00 | PGDP 310.1 |
| Nitrate | $65.00 | PGDP - 300.0/9056 | $65.00 | PGDP - 300.0/9056 | $65.00 | PGDP - 300.0/9056 |
| Sulfate | (with nitrate) | PGDP - 300.0/9056 | (with nitrate) | PGDP - 300.0/9056 | (with nitrate) | PGDP - 300.0/9056 |
| Ferrous Iron | $125.00 | SWRI - 315B/4500FE | $125.00 | SWRI - 315B/4500FE | $125.00 | SWRI - 315B/4500FE |
| Ortho Phosphate | $55.00 | PGDP - 365.3/9056 | $55.00 | PGDP - 365.3/9056 | $55.00 | PGDP - 365.3/9056 |
| Copper | $200.00 | PGDP - 200.8/6010 | $200.00 | PGDP - 200.8/6010 | $200.00 | PGDP - 200.8/6010 |
| Methane | $250.00 | Field SWRI - RSK175 (bubble strip) | $250.00 | Field SWRI - RSK175 (bubble strip) | $250.00 | Field SWRI - RSK175 (bubble strip) |
| Stable C Isotope Ratio - low conc's | $500.00 | SWRI/U.Oklahoma | $500.00 | SWRI/U.Oklahoma | $500.00 | SWRI/U.Oklahoma |
| Stable C Isotope Ratio - high conc's | $250.00 | SWRI/U.Oklahoma | $250.00 | SWRI/U.Oklahoma | $250.00 | SWRI/U.Oklahoma |
| C-12/C-14 Ratio | TBD | SWRI/U.Oklahoma | | SWRI/U.Oklahoma | | SWRI/U.Oklahoma |
| H2S | TBD | | TBD | | TBD | |
| H isotopes | TBD | | TBD | | TBD | |
| Cl isotpes | TBD | | TBD | | TBD | |
| Mn | TBD | | TBD | | TBD | |
| *CATION/ANION | $150.00 | PRS | $150.00 | PRS | $150.00 | PRS |
| K | | PGDP, ICP as Cations | | PGDP, ICP as Cations | | PGDP, ICP as Cations |
| Ca | | PGDP, ICP as Cations | | PGDP, ICP as Cations | | PGDP, ICP as Cations |
| Na | | PGDP, ICP as Cations | | PGDP, ICP as Cations | | PGDP, ICP as Cations |
| Mg | | PGDP, ICP as Cations | | PGDP, ICP as Cations | | PGDP, ICP as Cations |
| CO3 | $45.00 | PGDP, 2330 | $45.00 | PGDP, 2330 | $45.00 | PGDP, 2330 |
| HCO3 | incl above | PGDP, 2330 | incl above | PGDP, 2330 | incl above | PGDP, 2330 |
| SO4 | $65.00 | PGDP, 300.0/9056 | $65.00 | PGDP, 300.0/9056 | $65.00 | PGDP, 300.0/9056 |
| Cl | incl above | PGDP, 300.0/9056 | incl above | PGDP, 300.0/9056 | incl above | PGDP, 300.0/9056 |
| **Total Costs per Sample** | | | | | | |
| Low SIC cost | $2,090.00 | | $2,090.00 | | $1,590.00 | |
| High SIC cost | $2,840.00 | | $2,340.00 | | $2,090.00 | |
| **Sample Collection Cost** | | | | | | |
| Low | 500 | | 250 | | 0 | Others collect samples |
| High | 1000 | PRS collects samples | 500 | | 250 | |
| Shipping Costs (Ship Splits) | $100.00 | 4 samples/location(EP, SCI, Geochem/VOC, split) | 100 | 4 samples/location(EP, SCI, Geochem/VOC, split) | 100 | 4 samples/location(EP, SCI, Geochem/VOC, split) |
| Total Samples (13) | | | | | | |
| Low Sample Collection and SCI | **$27,170.00** | | **$27,170.00** | | **$20,670.00** | |
| High Sample Collection and SCI | **$36,920.00** | | **$30,420.00** | | **$27,170.00** | |

**Table 6. Pro Forma Project Costs**