DEPARTMENT OF THE AIR FORCE
60TH CIVIL ENGINEER SQUADRON (AMC)

September 5, 2012

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411 Airmen Drive
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SUBJECT: Final Enzyme Assessment Technical Memorandum

1. The attached CD-ROM contains the final Technical Memorandum for Assessment of Aerobic Chlorinated Cometabolism Enzymes at Travis AFB, California. This technical memorandum describes the results of an investigation to determine whether microbial aerobic cometabolism is a mechanism that can break down trichloroethene in the groundwater beneath the base. The investigation was conducted in accordance with the *Work Plan for Assessment of Aerobic Chlorinated Cometabolism Enzymes at Travis Air Force Base* (CH2M HILL, 2012). This technical memorandum will support the selection of remedial actions in the upcoming Travis AFB Groundwater Record of Decision.

2. If you have any questions concerning this technical memorandum, please contact Mr. Glenn Anderson at (707) 424-4359.

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Attachment:
Final Enzyme Assessment Technical Memorandum

Distribution: (see attached)

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TRAVIS AIR FORCE BASE
ENVIRONMENTAL RESTORATION PROGRAM

Final

Technical Memorandum for Assessment of Aerobic Chlorinated Cometabolism Enzymes at Travis Air Force Base, California

USACE Contract No. W91238-06-D-0013

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Prepared for:

U.S. Army Corps of Engineers
Omaha District

60 CES
Travis Air Force Base, California

Prepared by:

CH2MHILL

September 2012

Technical Memorandum for Assessment of Aerobic Chlorinated Cometabolism Enzymes at Travis Air Force Base, California

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Introduction

This technical memorandum provides the results of an investigation that was performed in February and March 2012 to assess whether microbial aerobic cometabolism is a mechanism that can break down trichloroethene (TCE) in groundwater at Travis Air Force Base (AFB), California. The investigation was conducted in accordance with the *Work Plan for Assessment of Aerobic Chlorinated Cometabolism Enzymes at Travis Air Force Base* (CH2M HILL, 2012). Groundwater samples for the investigation were collected from monitoring wells at two (2) indicator sites (Sites FT004 and DP039), which are located in the eastern and western portions of Travis AFB. These indicator sites are considered to be representative of Travis AFB, although they are located in geographically different portions of the Base. However, it should be noted that the aerobic biotransformation rates of TCE may be location and time-dependent; depending on the concentrations and reactivity of the competing substrates. Therefore, the results of this investigation provide one (1) line of evidence supporting the occurrence of natural attenuation at Travis AFB that must be corroborated by site-specific data, including geochemical conditions, decreasing contaminant concentration trends, and reduction in plume size over time. The locations of Sites FT004 and DP039 are shown on Figures 1 and 2.

The primary purposes of the Technical Memorandum for Assessment of Aerobic Chlorinated Cometabolism Enzymes at Travis AFB are as follows:

- Describe the aerobic chlorinated cometabolism enzyme investigation conducted in February and March 2012.
- Describe the presence and activity of cometabolic enzymes at Travis AFB Sites FT004 and DP039.

- Evaluate the cometabolic enzymes present at Travis AFB Sites FT004 and DP039 as a possible biological mechanism for natural attenuation.

Investigation Scope

The scope of the investigation was to collect and analyze groundwater samples from six (6) monitoring wells located at two (2) Travis AFB groundwater sites (FT004 and DP039) in accordance with the *Work Plan for Assessment of Aerobic Chlorinated Cometabolism Enzymes at Travis Air Force Base* (CH2M HILL, 2012). Groundwater samples were analyzed for volatile organic compounds (VOCs), presence of microbes or microbial genes capable of producing cometabolic enzymes, and cometabolic enzyme activity as described in the following subsections. While this investigation did not isolate, culture, or characterize specific microorganisms, the scope of the investigation included the following:

- Determine if cometabolic enzymes are present and active in the environment.
- Identify the presence or absence of microbial genes linked to TCE cometabolic enzymes.
- Identify the presence or absence of bacteria that produce cometabolic enzymes.

Enzyme activity probes (EAPs) were used to identify the presence and activity of the cometabolic enzymes in the environment. Quantitative polymerase chain reaction (qPCR) was used to confirm the presence of the microbes or the microbial genes that produce the cometabolic enzymes. These tools are described in greater detail in the Theory section of this technical memorandum.

Investigation Objective

The objective of this investigation is to determine whether enzyme cometabolism may be a mechanism contributing to natural attenuation of chlorinated solvents in groundwater at Travis AFB. Demonstrating the presence and activity of cometabolic enzymes capable of TCE degradation is an important line of evidence supporting a biological component of natural attenuation at Travis AFB. The data obtained in this investigation also supports the evaluation of monitored natural attenuation (MNA) as a potential remedy for several sites or portions of several sites at Travis AFB.

Background

The effectiveness of MNA as a remedy at all or a part of Sites FT004, LF006, LF007, SS015, SD031, SD033, SD037, and DP039 has been evaluated over an 8- to 10-year period, as documented in the *Natural Attenuation Assessment Report* (NAAR) (CH2M HILL, 2010). The NAAR concluded that natural attenuation was occurring at Travis AFB, resulting in stable or shrinking plumes at several of the sites. Because aquifer conditions are naturally aerobic at Travis AFB, and therefore not conducive to anaerobic biodegradation, the NAAR also concluded that the primary mechanisms for MNA at Travis AFB are physical (e.g., dilution and dispersion). However, research into aerobic biodegradation has recently shown that compounds such as TCE can be broken down by aerobic microbes through enzyme cometabolism.

Theory

Cometabolism

Cometabolism is a secondary nonmetabolic enzymatic reaction occurring incidentally to a primary metabolic energy-producing reaction. For example, methanotrophs produce the enzyme soluble methane monooxygenase (sMMO) to oxidize methane (i.e., the primary substrate) for both their energy and carbon requirements. The sMMO enzyme also fortuitously degrades chlorinated ethenes (i.e., the secondary substrate) in a reaction that is of no metabolic benefit to the microorganism. To be effective, the primary substrate (e.g., methane) must be present at higher concentrations than the secondary compound (e.g., chlorinated solvent) to initiate a microbial response. In addition to methane, compounds such as propane, propene, isoprene, isopropylbenzene, toluene, phenol, butane, ethane, ethene, and ammonia have also been shown to serve as primary substrates and elicit microbial cometabolic responses. Field studies have also demonstrated that humics and other aromatic compounds can serve as primary substrates. Humics are the result of natural organic matter breakdown and are ubiquitous in most soils; thus, in most natural groundwater systems, a primary substrate is available to stimulate the production of cometabolic enzymes.

The most likely primary substrate present at Travis AFB is humics resulting from a breakdown of natural organic matter. Total organic carbon (TOC) concentrations in soil samples collected at Travis AFB range from 55 to 5,810 milligrams per kilogram (mg/kg), with an average of 910 mg/kg. Therefore, humics are usually present at concentrations greater than dilute contaminants (typically in the range of 5 to 500 micrograms per liter [µg/L]). The presence of the cometabolic enzyme demonstrates the sufficiency of the primary substrate.

A detailed description of aerobic cometabolism is presented in Attachment 1.

Cometabolism of Chlorinated Hydrocarbons

Cometabolic processes have been studied since the 1950s. These studies have focused on the degradation of industrial chemicals including aromatics, chlorinated organics, pesticides, and petroleum hydrocarbons. This research has identified several compounds that are readily cometabolized, including TCE, dichloroethene (DCE), vinyl chloride (VC), trichloroethane (TCA), dichloroethane (DCA), chloromethane (CM), dichloromethane (DCM), and chloroform. Numerous primary substrates have been shown to induce cometabolic degradation in chlorinated solvents and other contaminants, including methane, propane, isopropene, isopropylbenzene, toluene, phenol, butane, ethane, ethene, and ammonia. Examples of enzymes that degrade TCE cometabolically include toluene-2-monooxygenase, toluene-3-monooxygenase, toluene-4-monoxygenase, toluene-2,3-dioxygenase, sMMO, and particulate methane monooxygenase (pMMO).

The process of microbial aerobic cometabolism of chlorinated solvents does not result in the generation of lasting daughter products, as may occur through the anaerobic dechlorination process. Potential toxicological risk is not increased by aerobic cometabolism. Aerobic cometabolism of chlorinated solvents converts the contaminant to an organic acid (an unstable epoxide). The unstable chlorinated epoxy ethanes are completely mineralized, and the final end products are carbon monoxide, carbon dioxide, chloride, and water.

Complete mineralization occurs so rapidly that the analysis for the intermediary unstable epoxides is not feasible.

EAPs

EAPs have recently been developed that specifically assess both the presence and activity of specific enzymes in subsurface environments. These probes are innovative research tools that can provide direct evidence that the mechanism for aerobic cometabolic oxidation is present and active in an aquifer. EAPs, which serve as alternate substrates for enzymes capable of cometabolic activity, have been developed for a suite of aromatic compounds (e.g., toluene, phenol, benzene, and naphthalene) and alkane molecules (e.g., propane, butane, methane). These probes undergo transformation to yield a strongly fluorescent product only when the enzyme of interest is actively functioning. If the appropriate enzyme is not present, or is present but not active in a given sample, then the probes will not be transformed and no fluorescence will be detected. If the sample has an EAP response, then there is direct evidence of enzyme degradative activity.

Molecular Techniques and Real-time qPCR

Molecular techniques, specifically qPCR analyses of microbial deoxyribonucleic acid (DNA), can be used to investigate potential cometabolic microbial populations in an environment, thus providing context for the EAP data. Microbial DNA can be extracted out of water samples and used in molecular assays. These assays are designed to look for the genetic material, specifically, biological oxygenase genes, that are required to build cometabolic enzymes and for microorganisms that are present in the environment that have the potential for degradative activity. Although qPCR is a valuable tool for determining if genes and microorganisms capable of a cometabolic response are present, it does not indicate whether the organisms and enzymes are actually cometabolically active. Additionally, the filtering methods standard to qPCR sample preparation reduces a given sample's size by approximately 70 percent, meaning that the microbial population may not be entirely detected. As such, qPCR is a first approximation of the potential for a cometabolic response, and provides evidence supporting further enzyme activity analyses such as EAPs.

Potential Limitations

While EAP data can provide strong supporting evidence of enzymatic activity and the potential for contaminant biodegradation, it is important to note the following potential limitations:

- It is not possible to probe the entire microbial community. Many microbes act in syntrophy with each other, and changes to one (1) aspect of a community have the potential to impact the entire community. It is possible to probe a microbial community and only have a limited understanding of what the entire population is potentially capable of doing. While the probes allow a snapshot into some of the members of the microbial community, it does not provide information on the behavior of the entire community.
- Filtration of the sample by the laboratory is required to collect microbial DNA; however, this will cause a percentage of the microbial population to not be recovered because of

the nature of filtration and the breakdown of DNA. Again, certain members of the microbial community may not be identified.

- Idaho National Laboratory (INL) is the only laboratory currently performing EAP analysis and studies. The EAPs are proprietary to INL and Dr. Hope Lee.
- EAPs may demonstrate that specific enzymes capable of cometabolic degradation of chlorinated solvents are present and active in an environment. However, the EAPs alone cannot fully demonstrate the degree to which cometabolism contributes to the natural attenuation of a solvent plume. Multiple lines of evidence, including geochemical conditions, decreasing contaminant concentration trends, and reduction in plume size over time, are needed to demonstrate that natural attenuation is occurring. If contaminant concentrations are decreasing over time and cometabolic evidence indicates that cometabolic enzymes and microbes are present and active, then it can be inferred that microbial processes may be contributing to the reduction in contaminant mass.

Methods

Well Selection

Six (6) monitoring wells from two (2) ERP sites at Travis AFB, Site FT004 and Site DP039, were selected for this investigation. Well selection at each of these sites was based primarily on TCE concentration and well location. The monitoring wells selected for sampling include a background well (chlorinated solvents were not detected), plume wells (TCE concentrations are between 100 and 500 µg/L), and a distal well (TCE concentrations are less than 50 µg/L). Several interim remedial actions (IRAs), demonstration projects, and remedy optimization actions are underway at Travis AFB, which had the potential to impact the investigation results. Therefore, the monitoring wells selected for sampling are located in areas unaffected by these activities and thus represent natural aquifer conditions.

Site FT004, located in the northeastern portion of the Base, was selected as a site for the cometabolism evaluation for several reasons. The interim remedy at this site is a combination of groundwater extraction and treatment and natural attenuation assessment. Therefore the effectiveness of natural attenuation in a portion of this site was evaluated in the NAAR. Groundwater conditions are predominantly aerobic, which is conducive to aerobic cometabolism. The extraction wells have been off since December 2007 because of a rebound study and are therefore not artificially oxygenating the groundwater. Total petroleum hydrocarbons (TPH) (carbon source promoting anaerobic biological degradation) are not present at Site FT004. The following Site FT004 wells were sampled for evidence of TCE cometabolism (Figure 1, Table 1): MW264x04, MW131x04, MW266x04, and MW591x04. Physical details for the selected monitoring wells, including construction information, depth to groundwater, sample collection depth, and lithology, are also provided in Table 1. Lithologic logs for these monitoring wells are provided in Attachment 2.

Groundwater monitoring wells MW131x04 and MW266x04 were selected because they are located in the portions of the plume with the highest TCE concentrations. MW591x04 was selected because the well is located in the distal portion of the groundwater plume. Monitoring well MW264x04 was selected because it is an uncontaminated upgradient well

and should be representative of background conditions for the Base. The background well, although shallow (15 to 25 feet bgs), is screened primarily in shale bedrock, and the lithology therefore differs from the other monitoring wells included in the study, which are screened in unconsolidated sediments. As a result, differences in analytical results between the background well and the other monitoring wells included in the study may be due to both the location of the wells in relation to the groundwater plumes and the lithologic conditions.

To provide information from another geographical area of the Base, a second site was selected for inclusion in this investigation. Site DP039, located in the western portion of the Base, was selected for similar reasons as those presented for Site FT004. It is also a natural attenuation assessment site that was included in the NAAR. The portion of the plume selected for sampling is aerobic and unaffected by the emulsified vegetable oil (EVO), phytoremediation, and bioreactor treatment zones. TPH is not present in groundwater at the site. The following wells at Site DP039 were sampled (Figure 2 and Table 1): MW781x39 and MW04x39. Monitoring wells MW781x39 and MW04x39 were selected because they are located along the eastern boundary of the groundwater plume where TCE concentrations are elevated.

Groundwater Sample Collection

On February 21 and 22, 2012, seven (7) groundwater samples (including a field duplicate) were aseptically collected in the field under low-flow purge techniques in accordance with the requirements of the *Field Sampling Plan* (CH2M HILL, 2009a).

The sampling method utilized, low-flow purging, creates laminar flow through the aquifer and reduces potential for mixing groundwater from different depth intervals. The pump intake was placed at the area of highest permeability within the screened interval and at least 5 feet below the water table to avoid collecting a sample near the water table where oxygen levels may be unrepresentatively high. Table 2 specifies the pump intake depth for sample collection.

Prior to collection of the groundwater samples, the monitoring wells were purged using the low-flow purge technique until field parameters stabilized. The groundwater sampling field sheets are provided in Attachment 3. The samples were collected in sample bottles that were autoclaved for sterilization to ensure that the bottles did not contain residual microbes or other organic material that could adversely impact the results. All sampling equipment coming into contact with the groundwater was sterilized with ethyl alcohol to avoid potential sample contamination.

The groundwater samples were analyzed for VOCs by U.S. Environmental Protection Agency (EPA) Method 8260B and for qPCR and EAPs by the methods described in Attachment 1. A trip blank for each of these methods was provided by the laboratories. The trip blanks were shipped to Travis AFB along with the other sampling containers and remained in the coolers used to store the site samples. The trip blanks were sent back to the laboratories for analysis along with the field samples.

The VOC groundwater samples were collected in three (3) 40-milliliter (mL) volatile organic analysis vials (VOAs) and preserved with hydrochloric acid. The VOC samples were placed in a cooler filled with ice and shipped overnight under chain-of-custody to the Spectrum

Analytical, Inc. laboratory in Tampa, Florida. VOC analyses were conducted by the Spectrum Analytical, Inc. laboratory in accordance with the *Analytical Quality Assurance Project Plan for Remedial Design/Remedial Action, Long-term Operation Program, Revision 2* (CH2M HILL, 2009b).

The qPCR and EAP groundwater samples were collected in two (2) 1-liter (L) bottles. Both bottles were completely filled, with minimal headspace, capped, and sealed with parafilm to reduce potential exposure to atmospheric oxygen. The groundwater samples were placed in a cooler filled with frozen gel packs and shipped overnight under chain-of-custody to Dr. Hope Lee at INL in Idaho Falls, Idaho. The qPCR and EAP analyses were conducted by INL. INL was chosen to perform the qPCR and EAP analyses, because EAP analysis is a relatively new technology, and Dr. Lee is the only expert qualified to perform this analysis. The qPCR and EAP analyses were performed in accordance with the *Work Plan for Assessment of Aerobic Chlorinated Cometabolism Enzymes at Travis Air Force Base* (CH2M HILL, 2012).

EAPs and qPCR Primers for TCE

Prior to analyzing the groundwater samples by EAP analyses, the slide samples were stained with a 4',6-diamidino-2-phenylindole (DAPI) stain to identify the total biomass on the slide sample. This total biomass documentation was compared with the results of the EAP analyses.

EAP analyses were performed using five (5) EAPs, including 3-hydroxyphenylacetyle (3-HPA) for evaluation of the toluene-3-monooxygenase pathway, phenylacetylene (PA) for the toluene-2-monooxygenase pathway, trans-cinnamonitrile (CINN) for the toluene-2,3-dioxygenase pathway, coumarin for the sMMO pathway, and naphthalene for both the sMMO and pMMO pathways. The EAPs provide insight into the activity (e.g., cometabolic enzymes may be identified but may not be actively degrading TCE) of cometabolic enzymes in the environment.

A qPCR primer is a short strand of nucleic acid that serves as a starting point for DNA synthesis. It is required for DNA replication, because the enzymes that catalyze this process, DNA polymerases, can only add new nucleotides to an existing strand of DNA. Many of the laboratory techniques of biochemistry and molecular biology that involve DNA polymerase, such as DNA sequencing and PCR, require DNA primers. They are hybridized to a target DNA, which is then copied by the polymerase.

The assessment of aerobic chlorinated cometabolism enzymes at Travis AFB was conducted using seven (7) qPCR primers, including primers for toluene-2,3,4-monooxgenases (PHE), toluene-3,4-monooxygenases (RMO), toluene-2,3-dioxygenase (TOD), catechol-2,3-dioxygenase (23CAT), sMMO, pMMO, and alkane monooxygenase (alkB). The qPCR primers selected have been shown to hybridize with microbial DNA, including DNA for specific cometabolic enzymes that are linked to TCE cometabolic degradation processes. Through this analysis, it is possible to identify the presence or absence of cometabolic microorganisms and enzymes in the environment.

EAP Analyses, DNA Extraction, and qPCR Amplification

EAP analyses, DNA extraction, and qPCR amplification were performed according to the established standard protocols and quality assurance/quality control measures presented in the *Work Plan for Assessment of Aerobic Chlorinated Cometabolism Enzymes at Travis Air Force Base* (CH2M HILL, 2012).

Summary of Investigation Results

Field Parameters

Field parameters were measured with a Horiba U-22 field instrument and a flow-through cell. The Horiba U-22 was calibrated daily prior to sample collection. The field parameters recorded during groundwater sample collection are summarized in Table 3.

The pH for all of the samples had consistent results ranging from 6.69 to 6.96. The temperature ranged from 16.04 degrees Celsius (°C) in MW266x04 to 18.45°C in MW781x39. These pH and temperature ranges can support the targeted microbe population.

Conductivity was relatively low for samples MW266x04, MW591x04, MW04x39, and MW781x39 with measurements ranging from 1.24 to 1.91 millisiemens per centimeter (mS/cm). The conductivity for MW131x04 and MW264x04 was somewhat higher, ranging from 4.68 to 6.67 mS/cm. Turbidity was consistent for wells MW131x04, MW591x04, MW04x39, and MW781x39 at 0 nephelometric turbidity units (NTU). The readings from monitoring wells MW264x04 and MW266x04 were 38.8 and 102.1 NTU, respectively.

Measured concentrations of dissolved oxygen (DO) were variable in the wells sampled. The measurement of DO for monitoring wells MW131x04, MW264x04, MW266x04, and MW781x04 ranged from 0 to 0.08 milligrams per liter (mg/L). The measurement of DO for monitoring wells MW591x04 and MW04x39 were 3.63 and 3.01 mg/L, respectively. The low DO measurements were inconsistent with the redox measurements. The redox measurements were all greater than 0, ranging from +16 to +172 millivolts (mV). Five (5) of the six (6) redox measurements were greater than +50 mV. While no measurable DO was detected by the field instruments in some of the wells, the positive redox in all of the monitoring wells sampled indicate oxidizing conditions are present at both sites.

VOCs

A summary of the analytical results for TCE, DCE, and VC are presented in Table 2. A complete listing of the analytical results is provided in Attachment 4. Figures 3 and 4 provide the most recent distribution of TCE concentrations at each site.

Analytical results for TCE concentrations in the groundwater samples collected for EAP and qPCR analyses from the Site FT004 wells ranged from not detectable in monitoring well MW264x04 to 447 µg/L in monitoring well MW04x39. Well MW264x04, where TCE was not detected, is the background well located upgradient of the Site FT004 TCE groundwater plume. In the central portion of the Site FT004 TCE groundwater plume, monitoring wells MW131x04 and MW266x04 had TCE concentrations of 154 and 122 µg/L, respectively. In the distal portion of the Site FT004 TCE plume, the groundwater sample from MW591x04 had a TCE concentration of 19.7 µg/L. For each of the samples collected at Site FT004, the

highest combined concentration of DCE isomers (cis-DCE, trans-DCE, and 1,1-DCE) observed was 3.4 µg/L in both wells MW131x04 and MW264x04. None of the groundwater samples from Site FT004 had detectable concentrations of VC.

At Site DP039, the TCE concentrations were 53.5 µg/L (MW781x39) and 447 µg/L (MW04x39). Both of these wells are located along the eastern side of the groundwater plume. The combined DCE concentrations for MW781x39 and MW04x39 were 0.52 J µg/L and 10.1 µg/L, respectively. VC was not detected in the groundwater samples from Site DP039.

EAPs

The EAP results are summarized in Table 4 and described in detail in Attachment 1. Results of the EAP analyses show that there was significant EAP activity observed in the groundwater samples for three (3) of the five (5) EAPs including 3-HPA, PA, and CINN (Table 4). Significant EAP activity is considered to be greater than or equal to 8,000 cells per milliliter (cell/mL). Significant activity was observed in the 3-HPA EAP in the groundwater samples from monitoring wells MW131x04, MW266x04, MW591x04, MW04x39, and MW781x39. The groundwater samples from wells MW131x04, MW591x04, MW04x39, and MW781x39 showed significant activity with the PA EAP. Also, significant activity for the CINN EAP was observed in the groundwater samples for monitoring wells MW131x04 and MW591x04. This indicates that the enzymes toluene-2-monoxygenase and toluene-3-monooxygenase were present and active in monitoring wells MW131x04, MW266x04, MW591x04, MW04x39, and MW781x39 at the time the groundwater was sampled. This also indicates that the enzyme toluene-2,3-dioxygenase was present and active in wells MW131x04 and MW591x04 when they were sampled.

No significant EAP activity was observed in any groundwater samples for the coumarin and naphthalene EAPs. This indicates that the enzymes sMMO and pMMO were either not active or not present in the groundwater samples that were collected.

No significant activity was observed for any of the five (5) EAPs in the background well MW264x04 groundwater sample, nor in the trip blank sample. The lack of enzyme activity in the sample collected from well MW264x04 indicates that the primary substrate is insufficient to support the microbial population of interest. The most likely primary substrate present at Travis AFB is humics resulting from a breakdown of natural organic matter. The background monitoring well is screened in shale bedrock. The shale is sufficiently fractured to allow groundwater flow; however, it is likely to have lower available humic content than the overlying unconsolidated sediments. It is also possible that the chlorinated solvents themselves act as the primary substrate, although this pathway is not well understood at this time (Attachment 1).

The EAP analytical results indicate that enzymes (toluene-1,2-monooxygenase, toluene-1,3-monooxygenase, toluene-1,4-monooxygenase, and toluene-2,3-dioxygenase) that are known to cometabolically degrade TCE, DCE, tetrachloroethene (PCE), chlorobenzene, benzene, toluene, ethylbenzene, xylenes, and petroleum hydrocarbons (Table 5) are present and active at Sites FT004 and DP039. This suggests that the primary chemicals of concern (COCs) at Sites FT004 (TCE, cis-1,2-DCE, and 1,1-DCE) and DP039 (TCE, 1,1-DCE, and PCE) may be degraded by aerobic cometabolism.

qPCRs

The qPCR results are summarized in Table 6 and described in detail in Attachment 1. The results of the qPCR show that a significant number of gene copies of the RMO and the 23CAT qPCR primers were observed in the samples collected from monitoring wells MW266x04, MW591x04, MW04x39, and MW781x39. A significant number of gene copies of the 23CAT qPCR primer was observed in the samples collected from monitoring well MW131x04. A significant number of gene copies is greater than or equal to 1,000 gene copies. This confirms the presence of the toluene-3-monoxygenase and the catechol-2,3-dioxygenase enzyme genes in the wells that showed significant EAP activity for these enzymes.

A significant number of gene copies of the TOD qPCR primer were observed in samples collected from monitoring well MW131x04, confirming the presence of the enzyme toluene-2,3-dioxygenase in the well. However, a significant number of gene copies were not observed in monitoring well MW591x04 for the qPCR primer TOD. Thus the presence of the enzyme toluene-2,3-dioxygenase in MW591x04 was not confirmed by the qPCR. However, according to the INL, because of biases associated with extracting DNA and the amplification process, it is not unexpected or uncommon to have one (1) groundwater sample show EAP activity, although the DNA is not quantifiable (Attachment 1).

Significant gene copies were not observed in any of the groundwater samples for the qPCR primers sMMO, pmoA, and alkB. This is consistent with the EAP results.

Additionally, the groundwater sample from the background monitoring well MW264x04 and the trip blank sample did not have significant gene copies for any of the qPCRs. Again, this is consistent with the EAP results for these samples.

The qPCR results confirm that the enzymes (toluene-1,2-monooxygenase, toluene-1,3-monooxygenase, toluene-1,4-monooxygenase, and toluene-2,3-dioxygenase) that were observed to be active in the EAP analyses are present at Sites FT004 and DP039 (Table 7).

Plume Attenuation

As previously stated, one of the limitations of the EAP and qPCR data is that demonstration of the presence and activity of cometabolic enzymes alone cannot fully show the degree to which cometabolism contributes to the natural attenuation of a solvent plume. It is also necessary to have data that demonstrate plume attenuation to support the conclusion that cometabolism may be one (1) process contributing to natural attenuation. If contaminant concentrations are decreasing over time and cometabolic evidence indicates that cometabolic enzymes and microbes are present and active, then it can be inferred that microbial processes may be contributing to the reduction in contaminant mass. Plume attenuation at both Sites FT004 and DP039 has been evaluated for more than a decade. The monitoring data from both sites indicate that plume attenuation is occurring at both sites. A detailed summary of plume attenuation at Sites FT004 and DP039 is provided in Attachment 5.

Conclusions

Based on the results of this investigation, the following conclusions can be drawn:

- With the exception of the background well MW264x04, at least one (1) aromatic EAP showed significant activity in groundwater samples collected from each of the monitoring wells at both of the sites studied.
- The qPCR data supported the EAP results. Positive amplifiable gene targets corresponded well with EAP results that showed significant enzyme activity.
- The EAP and qPCR results indicate the presence and activity of the toluene enzymes, but not the methane enzymes. Methanotropic bacteria appear to not be present at the site. Therefore, methane is not a primary substrate of the bacteria.
- The EAP and qPCR results provide evidence of potential for intrinsic aerobic biodegradation at Sites FT004 and DP039. However, only a few locations (MW131x04 and MW591x04) showed a diversity of active cometabolic enzymes under in situ conditions.
- The negative EAP and qPCR results for background well MW264x04 indicate that there are insufficient amounts of the primary substrate at this location to support the cometabolic microorganisms targeted by this study. The primary substrate at Travis AFB is likely to be humics. This well is screened in shale bedrock, which is likely to have lower available humic substrate than the overlying unconsolidated sediments.
- The results of the investigation are indicative of enzymatic cometabolic activity at similar but geographically distant indicator groundwater sites (FT004 and DP039) and provide one (1) line of evidence supporting the occurrence of natural attenuation at Travis AFB. It is likely that cometabolic enzymes are widespread at Travis AFB sites, assuming site geochemical and contaminant histories are consistent. It follows, therefore, that cometabolic activity may be contributing to contaminant natural attenuation not only at Sites FT004 and DP039, but also at other Travis AFB sites.

References

CH2M HILL. 2012. *Work Plan for Assessment of Aerobic Chlorinated Cometabolism Enzymes at Travis Air Force Base*. Prepared for Travis Air Force Base, California. Final. February.

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CH2M HILL. 2009b. *Analytical Quality Assurance Project Plan for Remedial Design/Remedial Action, Long-term Operation Program, Revision 2*. Prepared for Travis Air Force Base, California. Final. July.

Interstate Technology and Regulatory Council (ITRC). 2011. Enzyme Activity Probes EMD Team Fact Sheet. November.

TABLE 1
Physical Details of Wells Sampled
Technical Memorandum for Assessment of Aerobic Chlorinated Cometabolism Enzymes, Travis Air Force Base, California

Well ID	Date Installed	February 2012 TCE (µg/L)	February 2012 Depth to Water (feet btoc)	February 2012 Groundwater Elevation (feet amsl)	Well Completion Depth (feet bgs)	Top of Casing (feet amsl)	Screen Interval (feet bgs)	Screen Lithology	Description of Lithology	Pump Intake Depth (feet bgs)	Comments
Site FT004											
MW131x04	1/18/1985	154	9.77	52.86	31.5	62.63	10.00–30.00	A	10–11.5 Sandy clay, 15–16.5 clayey sand, 20–21.5 gravelly sand with gravel, 25–26.5 well graded sand	15	Uncontaminated upgradient well representative of background conditions
MW264x04	4/4/1991	ND (<0.5)	11.10	57.49	26	68.59	15.00–25.00	A/B	15–15.6 Sandy lean clay, 15.6–16.3 lean clay, 16.3–16.5 shale, 18–19.5 shale, 20–21.5 shale	15	Within plume hot spot; rebound study in progress and groundwater extraction has stopped
MW266x04	4/8/1991	122	9.76	52.51	17.3	62.27	6.00–16.00	A	6–6.5 Lean clay, 10–11.5 silt with sand, 15–15.5 silty sand, 15.5–16 lean clay	15	Within plume hot spot; rebound study in progress and groundwater extraction has stopped
MW591x04	9/9/1999	19.7	10.92	51.03	44	61.95	15.00–35.00	A	15–16.5 Sandy silt, 17–19 silty sand, 20–21.5 silt, 23.5–29 silty sand, 30–31.5 poorly graded sand with silt, 32–34 silty sand	30	Within distal portion of plume; rebound study in progress and groundwater extraction has stopped
Site DP039											
MW04x39	4/12/1996	477	14.40	40.5	30	54.90	16.00–26.00	A	16–18 Sandy silt, 18–19 silty sand, 19–22.5 sandy silt, 22.5–24 silty sand, 24–25.5 silt, 25.5–26 poorly graded sand with silt	23	Within plume; outside area of influence of interim remedial action and treatability studies
MW781x39	11/5/2004	53.5	27.11	43.67	37.5	70.78	27.00–37.00	A	27–37 Silty sand	32	Within plume; outside area of influence of interim remedial action and treatability studies

Notes:

µg/L = microgram(s) per liter
A = alluvium
amsl = above mean sea level
B = bedrock
bgs = below ground surface
btoc = below top of casing
ND = not detected

TABLE 2
VOC Analytical Results
Technical Memorandum for Assessment of Aerobic Chlorinated Cometabolism Enzymes, Travis Air Force Base, California

Well	Well Position	Depth (feet)	Date	TCE (µg/L)	DCE* (µg/L)	Vinyl Chloride (µg/L)
Site FT004						
MW131x04	Plume	10–30	2/21/2012	154	3.4	ND
MW264x04	Background	15–25	2/21/2012	ND	ND	ND
MW266x04	Plume	6–16	2/21/2012	122	3.4	ND
MW591x04	Distal	15–35	2/21/2012	19.7	1.82	ND
Site DP039						
MW04x39	Plume	16–26	2/21/2012	447	10.1	ND
MW781x39	Plume	27–37	2/21/2012	53.5	0.52 J	ND
			2/21/2012 (Dup)	49.9	0.58 J	ND

* Total concentration of cis-, trans-, and 1,1-DCE isomers

Notes:

µg/L= microgram(s) per liter
DCE = dichloroethene
J = estimated concentration
ND = not detected
TCE = trichloroethene

TABLE 3
Field Parameters
Technical Memorandum for Assessment of Aerobic Chlorinated Cometabolism Enzymes, Travis Air Force Base, California

Well	Sample Date	pH (units)	Conductivity (mS/cm)	Temperature (°C)	DO (mg/L)	Redox (mV)	Turbidity (NTU)
Site FT004							
MW131x04	2/21/2012	6.88	4.68	17.99	0	76	0
MW264x04	2/21/2012	6.85	6.67	17.86	0	62	38.8
MW266x04	2/21/2012	6.96	1.91	16.04	0.08	16	102.1
MW591x04	2/22/2012	6.85	1.87	16.54	3.63	130	0
Site DP 039							
MW04x39	2/22/2012	6.69	1.24	17.12	3.01	172	0
MW781x39	2/22/2012	6.77	1.53	18.45	0	126	0

Notes:

°C = degree(s) Celsius
DO = dissolved oxygen
mg/L = milligram(s) per liter
mS/cm = millisiemen(s) per centimeter
mV = millivolt(s)
NTU = nephelometric turbidity unit(s)

TABLE 4
Enzyme Activity Probe Results
Technical Memorandum for Assessment of Aerobic Chlorinated Cometabolism Enzymes, Travis Air Force Base, California

Well	Well Position	DAPI (cells/mL) Total	3-HPA (cells/mL) T3-mono	PA (cells/mL) T2-mono	CINN (cells/mL) TOD	Coumarin (cells/mL) sMMO	Naphthalene (cells/mL) sMMO and pMMO
MW131x04	Plume	4.52E+04	**1.17E+04**	**3.55E+04**	**4.17E+04**	Neg	Neg
MW264x04	Background	7.64E+04	0.00E+0	0.00E+0	0.00E+00	Neg	Neg
MW266x04	Plume	3.67E+05	**1.01E+04**	5.48E+03	0.00E+00	Neg	Neg
MW591x04	Distal	2.23E+04	**1.62E+04**	**1.54E+04**	**1.68E+04**	Neg	Neg
MW04x39	Plume	1.11E+05	**1.87E+04**	**1.78E+04**	0.00E+00	Neg	Neg
MW781x39	Plume	8.65E+04	**1.59E+04**	0.00E+00	0.00E+00	Neg	Neg
MW781x39 (Dup)	Plume	5.74E+04	**1.48E+04**	**9.89E+03**	5.48E+03	Neg	Neg
MWTB	Tripblank	0.00E+00	0.00E+00	0.00E+00	0.00E+00	Neg	Neg

Notes:

Significant values are **bolded** (≥ 8.0×10^3 cells/mL)

3-HPA = 3-hydroxyphenylacetyle
cells/mL = cell(s) per milliliter
CINN = trans-cinnamonitrile
DAPI = 4',6-diamidino-2-phenylindole
PA = phenylacetylene
pMMO = particulate methane monooxygenase
sMMO = soluble methane monooxygenase
T2-mono = toluene-2-monooxygenase
T3-mono = toluene-3-monooxygenase
TOD = toluene-2,3-dioxygenase

TABLE 5
Contaminants Cometabolized by Active Enzymes at Travis Air Force Base
Technical Memorandum for Assessment of Aerobic Chlorinated Cometabolism Enzymes, Travis Air Force Base, California

Enzyme Activity Probe	Target Enzymes	Contaminants Degraded by Target Enzymes
3-HPA	T2-mono, T3-mono, T4-mono	TCE, DCE, PCE, benzene, toluene, ethylbenzene, and xylenes
PA	T2-mono, T3-mono, T4-mono	TCE, DCE, PCE, benzene, toluene, ethylbenzene, and xylenes
CINN	TOD	TCE, DCE, chlorobenzene, and petroleum hydrocarbons

Source: ITRC, 2011

Notes:

3-HPA = 3-hydroxyphenylacetyle
CINN = trans-cinnamonitrile
DCE = dichloroethene
PA = phenylacetylene
PCE = tetrachloroethene
T2-mono = toluene-2-monooxygenase
T3-mono = toluene-3-monooxygenase
T4-mono = toluene-4-monooxygenase
TCE = trichloroethene
TOD = toluene-2,3-dioxygenase

TABLE 6
Quantitative PCR Results
Technical Memorandum for Assessment of Aerobic Chlorinated Cometabolism Enzymes, Travis Air Force Base, California

Well	Well Position	PHE	RMO	TOD	23CAT	sMMO	pMMO	alkB
MW131x04	Plume	**+**	740	**1,701**	**6,100**	ND	ND	ND
MW264x04	Background	ND	ND	ND	ND	ND	ND	ND
MW266x04	Plume	**+**	**3,340**	ND	**405,000**	ND	ND	ND
MW591x04	Distal	**+**	**4,020**	ND	**37,500**	ND	ND	ND
MW04x39	Plume	**+**	**5,840**	ND	**43,600**	ND	ND	ND
MW781x39	Plume	**+**	**8,440**	ND	**6,350**	ND	ND	ND
MW781x39 (Dup)	Plume	**+**	**8,440**	ND	**19,400**	ND	ND	ND
MWTB	Tripblank	ND	ND	ND	ND	ND	ND	ND

Notes:

Because of high background noise, the PHE results are shown as positive or negative rather than as numerical results. Positive results are **bolded**.

Numerical results reported in the gene copy numbers. Significant number of gene copies are bolded (≥ 1,000 gene copies).

23CAT = catechol-2,3-dioxygenase
alkB = alkane monooxygenase
ND = no product detected within range of standard curve
PHE = toluene-2,3,4-monooxgenase
pMMO = particulate methane monooxygenase
RMO = toluene-3,4-monooxygenase
sMMO = soluble methane monooxygenase
TOD = toluene-2,3-dioxygenase

TABLE 7
Targeted Enzymes for Quantitative PCR with Positive Results at Travis Air Force Base
Technical Memorandum for Assessment of Aerobic Chlorinated Cometabolism Enzymes, Travis Air Force Base, California

Quantitative PCR	Target Enzymes
PHE	T2-mono, T3-mono, T4-mono
RMO	T3-mono, T4-mono
TOD	TOD
CAT	Catechol-2,3-dioxygenase

Notes:

T2-mono = toluene-2-monooxygenase
T3-mono = toluene-3-monooxygenase
T4-mono = toluene-4-monooxygenase
TOD = toluene-2,3-dioxygenase

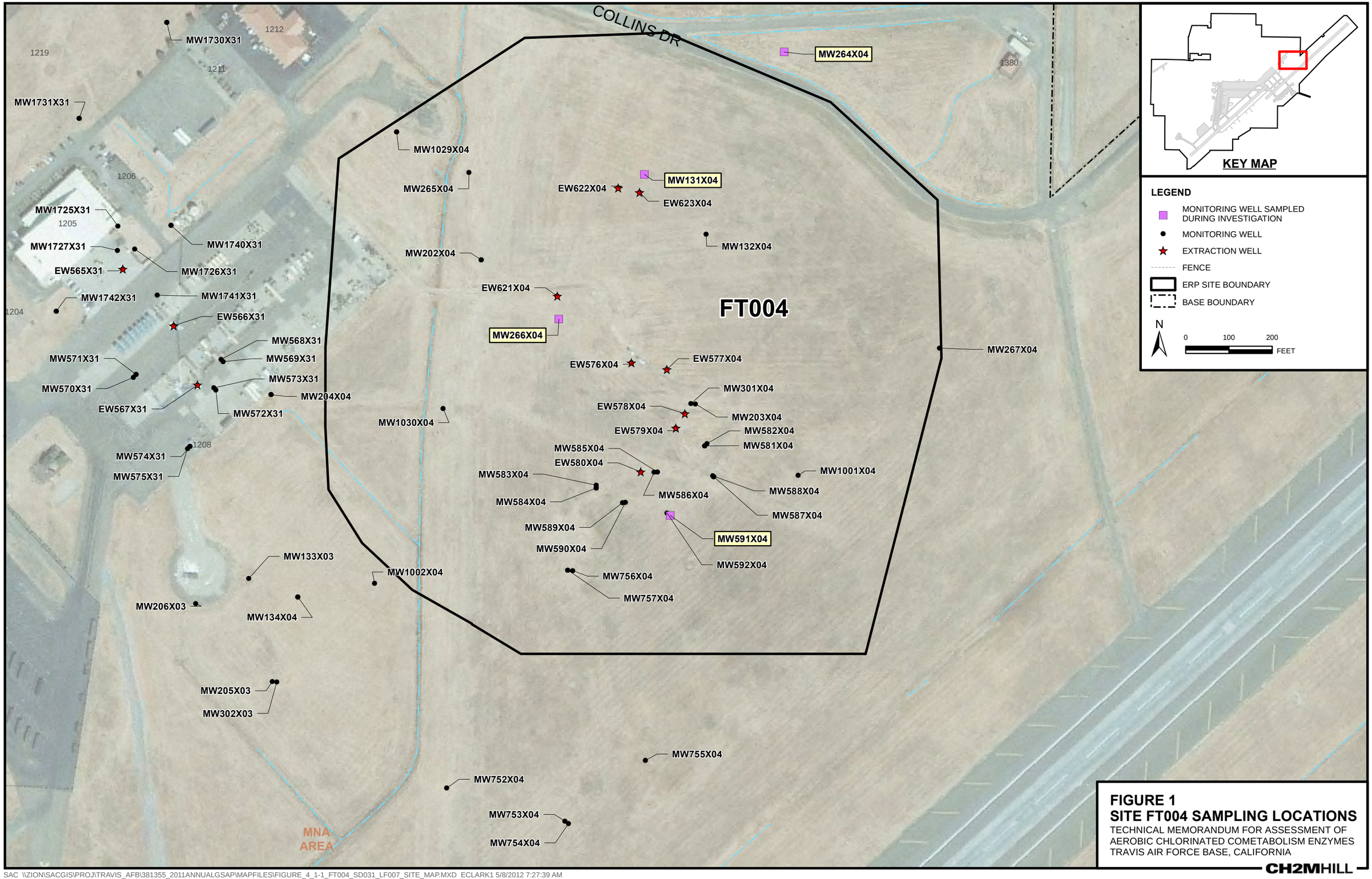

FIGURE 1
SITE FT004 SAMPLING LOCATIONS
TECHNICAL MEMORANDUM FOR ASSESSMENT OF AEROBIC CHLORINATED COMETABOLISM ENZYMES
TRAVIS AIR FORCE BASE, CALIFORNIA

CH2MHILL

SAC \\ZION\SACGIS\PROJ\TRAVIS_AFB\381355_2011ANNUALGSAP\MAPFILES\FIGURE_4_1-1_FT004_SD031_LF007_SITE_MAP.MXD ECLARK1 5/8/2012 7:27:39 AM

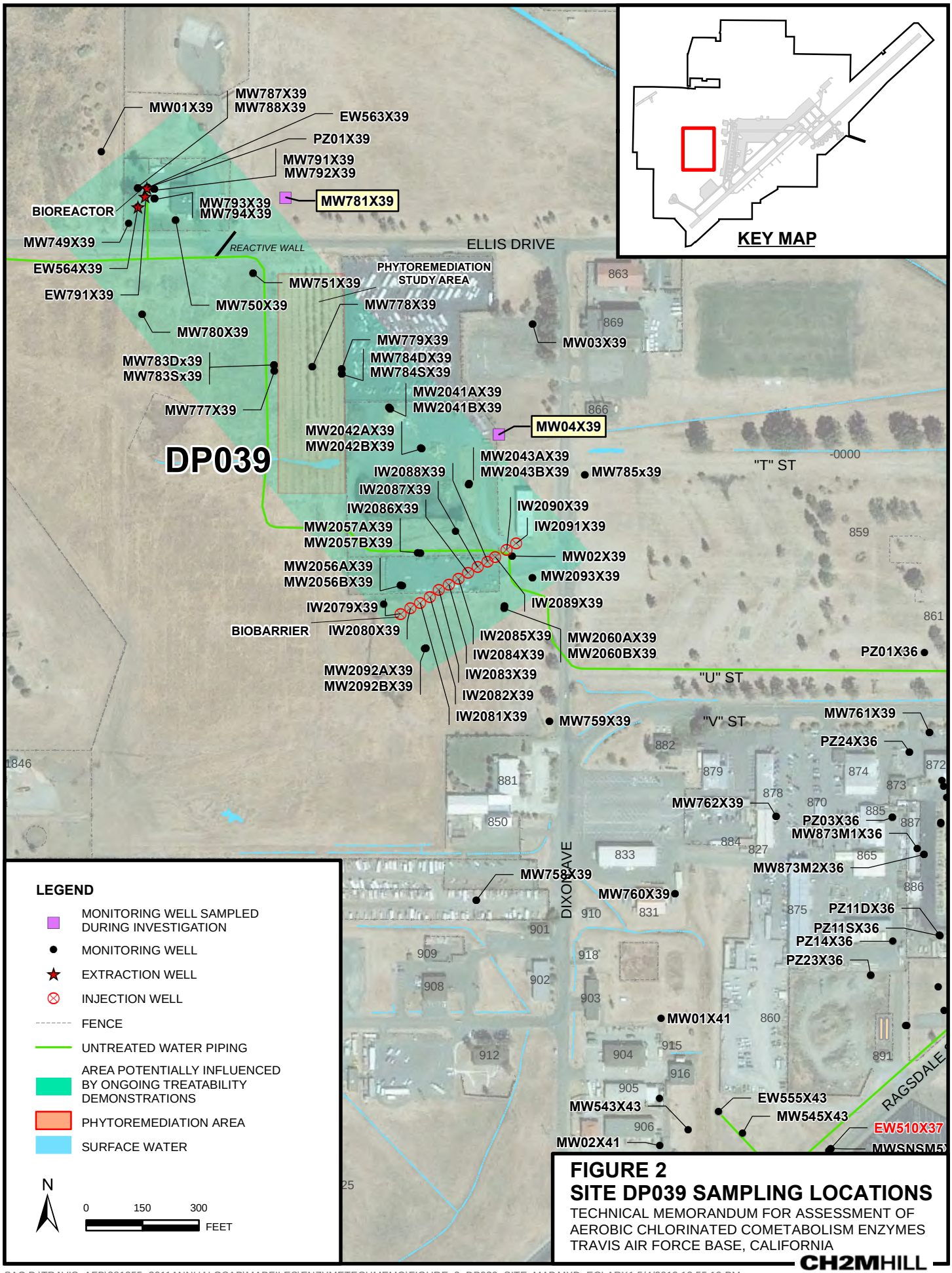

FIGURE 2
SITE DP039 SAMPLING LOCATIONS
TECHNICAL MEMORANDUM FOR ASSESSMENT OF AEROBIC CHLORINATED COMETABOLISM ENZYMES
TRAVIS AIR FORCE BASE, CALIFORNIA

CH2MHILL

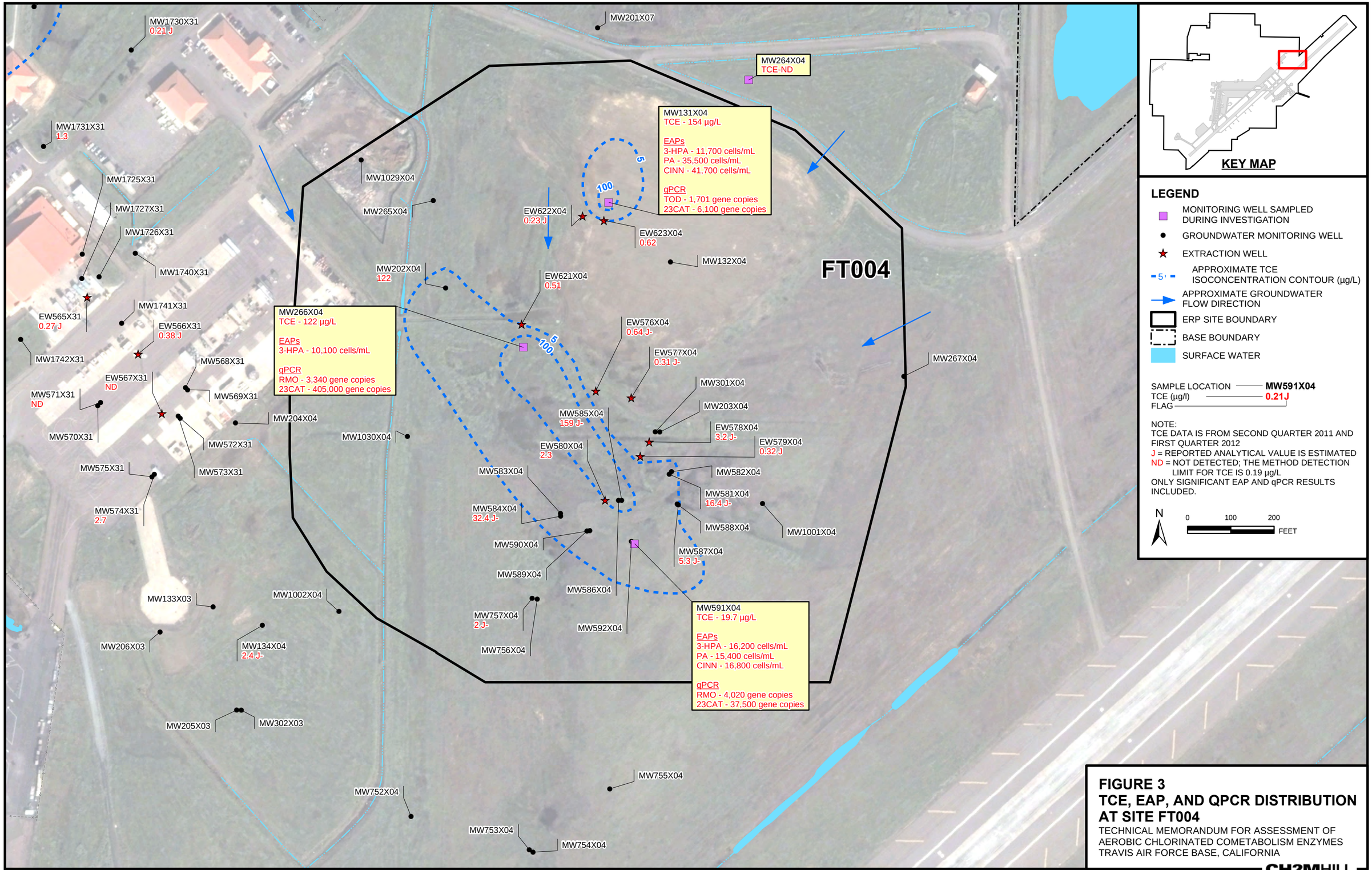

FIGURE 3
TCE, EAP, AND QPCR DISTRIBUTION AT SITE FT004
TECHNICAL MEMORANDUM FOR ASSESSMENT OF AEROBIC CHLORINATED COMETABOLISM ENZYMES
TRAVIS AIR FORCE BASE, CALIFORNIA

CH2MHILL

SAC \\ZION\SACGIS\PROJ\TRAVIS_AFB\381355_2011ANNUALGSAP\MAPFILES\FIGURE_4_1-3_FT004_SD031_LF007_TCE_2011.MXD ECLARK1 5/14/2012 3:06:17 PM

MW03X44

MW01X39
0.33 J

MW788X39
0.25

MW787X39
ND

EW563X39
ND

MW791X39
0.97

MW792X39
65.9

MW793X39
214

MW794X39
38.9

MW781X39
TCE - 53.5 µg/L

EAPs
3-HPA - 14,800 cells/mL
PA - 9,890 cells/mL

qPCR
RMO - 8,440 gene copies
23CAT - 19,400 gene copies

BIOREACTOR

100

5

MW750X39
1170

MW751X39
1350

EW782X39
0.23

100

500

1000

EW564X39

MW779X39
434

MW03X39

MW778X39
782

MW784SX39

MW784DX39
1470

MW780X39

MW783SX39

MW749X39

MW783DX39
33.2

MW2041AX39
71.5

MW777X39
1280

MW2041BX39
701

5

1000

DP039

MW04X39
TCE - 477 µg/L

EAPs
3-HPA - 18,700 cells/mL
PA - 17,800 cells/mL

qPCR
RMO - 5,840 gene copies
23CAT - 43,600 gene copies

MW2042AX39
670

MW2043AX39*
52.8

MW2042BX39
1740

MW2043BX39*
1020

500

MW2057AX39*
6.1 J

IW2089X39

IW2090X39*
0.24 J

MW785X39
108

IW2088X39

MW2057BX39*
832 J

IW2091X39

PHYTOREMEDIATION
STUDY AREA

MW02X39*
37.2

MW2056AX39*
681

IW2086X39

100

5

MW2056BX39*
61.7

MW2093X39*
ND

IW2087X39

100

IW2080X39

EVO PRB

MW2060BX39*
166

IW2085X39*
0.45 J

PZ01X36

IW2079X39

MW2060AX39*
163

IW2084X39

IW2081X39*
0.97

IW2083X39

IW2082X39

PZ02X36

MW761X39
ND

MW2092AX39*
413 J

MW2092BX39*
482 J

MW759X39
ND

PZ24X36

PZ04X36

MW872X36

EW593X36

MW762X39
ND

PZ03X36

MW873M1X36

MW873M2X36

PZ11SX36

PZ11DX36

MW758X39
2.2

MW760X39
8.8

5

PZ14X36

PZ23X36

PZ15X36

PZ18X36

MW01X41

PZ19SX36

PZ19DX36

PZ02X41

EW555X43

MW543X43

MW545X43

MWSNSM5X37

MW02X41

EW510X37

MW544X43

MW521X37

MW520X37

MW01X13

KEY MAP

LEGEND

- MONITORING WELL SAMPLED DURING INVESTIGATION
- GROUNDWATER MONITORING WELL
- PIEZOMETER
- EXTRACTION WELL
- INJECTION WELL
- 5 — APPROXIMATE TCE ISOCONCENTRATION CONTOURS (µg/L)
- APPROXIMATE GROUNDWATER FLOW DIRECTION
- PHYTOREMEDIATION AREA
- SURFACE WATER

SAMPLE LOCATION — MW591X04
TCE (µg/l) — 0.21J
FLAG

NOTES:
TCE DATA IS FROM SECOND QUARTER 2011, FOURTH QUARTER 2011, AND FIRST QUARTER 2012
J = REPORTED ANALYTICAL DATA IS ESTIMATED
ND = NOT DETECTED; THE METHOD DETECTION LIMIT FOR TCE IS 0.19 µg/L
ONLY SIGNIFICANT EAP AND qPCR RESULTS INCLUDED.

N

0 150 300 FEET

FIGURE 4
TCE, EAP, AND QPCR DISTRIBUTION AT SITE DP039

TECHNICAL MEMORANDUM FOR ASSESSMENT OF AEROBIC CHLORINATED COMETABOLISM ENZYMES
TRAVIS AIR FORCE BASE, CALIFORNIA

CH2MHILL

SAC P:\TRAVIS_AFB\381355_2011ANNUALGSAP\MAPFILES\ENZYMETECHMEMO\FIGURE_4_TCE_DP039.MXD ECLARK1 5/10/2012 3:01:04 PM

Attachment 1
INL qPCR and EAP Report

Idaho National Laboratory

Travis AFB

April 2012

M. Hope Lee

Brady Lee

1. OVERVIEW: BIODEGRADATION

Introduction to Aerobic Cometabolism

Much of the relevant literature has been extensively reviewed and summarized (Semprini, 1997; Arp, 2001; Alvarez-Cohen and Speitel, 2001; Mattes et al., 2010; Penny et al., 2010; Cecen, 2010); what is provided here is a high level summary of this body of work.

Microbial metabolism is the means by which a microbe obtains the energy and carbon it needs to live and reproduce. Microbes use many different types of metabolic strategies and species can often be differentiated from each other based on metabolic characteristics. The specific metabolic characteristics of a microbe are the major factors in determining that microbe's ecological niche, and often allow for that microbe to be useful in industrial processes, breaking down anthropogenic compounds, or responsible for biogeochemical cycles. All microbial metabolisms can be arranged according to three groups based on the primary carbon metabolized: (1) autotrophic organisms obtain carbon from carbon dioxide (CO_2), (2) heterotrophic organisms obtain carbon from organic compounds, and (3) mixotrophic organisms obtain carbon from both organic compounds and by fixing carbon dioxide. Most microbes, particularly environmentally relevant microbes, are heterotrophic, using organic compounds as both carbon and energy sources. These microbes are extremely abundant in nature and are responsible for the breakdown of large organic polymers such as cellulose, chitin or lignin which are generally indigestible to larger organisms. Some heterotrophic organisms are even able to degrade more recalcitrant compounds such as petroleum compounds or pesticides, making them useful in bioremediation.

There is a diversity of compounds that can serve as carbon for microbial metabolism. One subgroup of organisms is those that biodegrade one organic substance to obtain carbon and energy for growth, simultaneously transforming other compounds that cannot be used for growth (Thomas and Ward, 1989). This process is known as ***cometabolism*** and it describes the ability of microorganisms to fortuitously transform non-growth-supporting substrates, such as pollutants. Cometabolic transformations are catalyzed by existing microbial enzymes and yield no carbon or energy benefits to the transforming cells (Horvath, 1972), thus a growth substrate must be available at least periodically to grow new cells, provide an energy source, and induce production of the cometabolic enzymes. Although some studies have found that naturally occurring humic substances (an organic residue of decaying organic matter; Wymore et al., 2007; Lee et al., 2008) and the chlorinated solvents themselves can act as inducers of the cometabolic enzymes, the extent of and time for activation, as well as the mechanism for cell energy and growth in these instances, are poorly understood (Shingleton et al., 1998; Park et al., 2002; Yeager et al., 2004).

Cometabolic processes were first studied in the 1950s and 60s and focused on the microbial degradation of important classes of industrial chemicals including aromatics (Dagley and Pate, 1957), chlorinated organics (Jensen, 1957, 1963), pesticides (Alexander, 1967) and petroleum hydrocarbons (Foster, 1962). Decades of research have concluded that the following compounds are among those that are readily cometabolized: trichloroethene (TCE), dichloroethene (DCE), vinyl chloride (VC), trichloroethane (TCA), dichloroethane (DCA), chloromethane (CM), dichloromethane (DCM), and chloroform (CF) (Vandenwijngaard, 1992; Hartmans, 1985; Hartmans and Debont, 1992; Munakata-Marr, 1997; Vannelli, 1998; McCarty et al., 1998; Braus-Stromeyer, 1993; Gisi, 1998; Edwards and Cox, 1997; McCarty, 1997a; Bradley and Chapelle, 1998; Travis and Rosenberg, 1997). Collectively, these studies established that microorganisms could transform many compounds/contaminants without

concurrent microbial growth on those compounds, and the enzymes responsible for these transformations are mono- and dioxygenases.

Oxygenase enzymes in general are a subset of the enzymes classified as oxidoreductases, one of the six major classes of enzymes. Oxygenases serve a myriad of functions in cells including biosynthesis, detoxification, and catabolism (metabolic breakdown of complex compounds). Oxygenases catalyze the reduction of O_2 with incorporation of one (monooxygenases) or two (dioxygenases) of the oxygen atoms into the substrate that is being oxidized. In the context of contaminant degradation, the oxygenase reaction generates chlorinated solvent oxidation products that may react with cellular macromolecules or may be hydrolyzed spontaneously into carbon dioxide, chloride, or other non-volatile products that are easily mineralized by microorganisms (Little et al., 1988; Tsien et al., 1989; Oldenhuis et al., 1989; Fox et al., 1990; Nelson et al., 1986, 1987; Rasche et al., 1991). Unlike anaerobic reductive dechlorination, aerobic cometabolism does not proceed through sequential dechlorination steps producing daughter products such as DCE, VC, and ethene; thus, signature aerobic degradation products, such as Cl^- and CO_2, are difficult to attribute to aerobic degradation in situ using geochemistry alone. The end result is that the only evidence for aerobic cometabolism is the disappearance of the contaminants themselves.

Chlorinated solvents and other contaminants can be oxidized by a wide range of oxygenase-expressing microorganisms including those that utilize **methane** (Wilson & Wilson, 1985; Strand & Shippert, 1986; Fogel et al., 1986; Little et al., 1988; Tsien et al., 1989; Oldenhuis et al., 1989), **propane** (Fliermans et al., 1988; Wackett et al., 1989; Phelps et al., 1990; Malachowsky et al., 1994), **propene** (Ensign et al., 1992; Saeki et al., 1999), **isoprene** (Ewers et al., 1990), **isopropylbenzene** (Pflugmacher et al., 1996; Dabrock et al., 1992; Kesseler et al., 1996), **toluene** (Nelson et al., 1986;Wackett et al., 1988; Zylstra et al., 1989; Shields et al., 1989), **phenol** (Folsom et al., 1990; Harker & Kim, 1990; Segar, 1995), **butane** (Kim et al., 1997; 2000), **ethene and ethane** (Freedman & Herz, 1996; Koziollek et al., 1999), and **ammonia** (Arciero et al, 1989; Vannelli et al., 1990; Rasche et al., 1991; Hyman et al., 1995) as energy and/or carbon sources.

Representative cultured organisms, their primary growth substrates, and kinetic data with regards to TCE are included in Table 1 (modified from Arp review 2001). The enzyme responsible for TCE oxidation in these organisms is also listed. The majority of these organisms are capable of growth on many substrates, several of which may stimulate expression of the TCE-degrading oxygenase enzymes. While some oxygenase enzymes are very specific for particular substrates, others oxygenase enzymes have remarkably broad substrate ranges. *It is important to note that the TCE oxidation rates presented in Table 1 are based on cultured organisms maintained and evaluated under controlled laboratory settings, and as such may not reflect the true potential for degradation under field conditions.*

In order to monitor aerobic cometabolism given the challenge of monitoring the process through groundwater chemistry, subsurface microbial communities have been interrogated using validated biomarkers including enzyme activity probes (EAP) and quantitative polymerase chain reaction (qPCR). EAPs have been applied at almost 20 aerobic contaminated sites for their reliability, reproducibility and sensitivity in evaluating aerobic cometabolic enzymes, while qPCR has been evaluated for at least a subset of the potential aerobic oxygenase genes (McDonald et al., 1995; Baldwin et al., 2003; 2005; 2008; 2009; Bowman et al., 1993; Hendrickx et al., 2006a; 2006b; Domiguez et al., 2008). These approaches provide information about both the presence of the genes of interest, which is important if evaluating the potential for enhanced attenuation of the contaminant in situ, and the activity of the oxygenases, which is important in evaluating degradation capacity and long-term sustainability. When these technologies are simultaneously evaluated and compared with more traditional approaches such as geochemical analyses, they provide a comprehensive assessment that can potentially quantitatively relate the qPCR and EAP results to contaminant biotransformation.

Table 1. Cometabolic enzyme systems with respective organisms and TCE oxidation rates.

Growth Substrate	Organism	Enzyme	TCE oxidation rate (nmol min-1 mg of protein -1)	Reference
Ethene/ propene	*Xanthobacter Py2*	Alkene monooxygenase	8.6 16-95	Ensign, 1992; Reij, 1995
Propene	*Rhodococcus corallimus*	Alkene monooxygenase	2.4	Saeki, 1999
Isopropylbenzene	*Pseudomonas sp strain JP1* *Rhodococcus erythropolis BD2*	Isopropylbenzene dioxygenase; Toluene dioxygenase	0.5-2	Pflugmacher et al., 1996; Dabrock et al., 1992; Kesseler et al., 1996
Ammonia	*Nitrosomonas europaea*	Ammonia monooxygenase	10.9	Bedard, 1989; Ely, 1995b; Hyman, 1995; Rasche, 1991
Phenol	*JMP 134*	Phenol monooxygenase	0.2	Harker , 1990
Butane	*Pseuodomonas butanavora*	Butane monooxygenase	.06	Hamamura, 1997
Propane	*Mycobacterium vaccae JOB5*	propane monooxygenase		Wackett, 1989
Methane	*Methylosinus trichosporium OB3b*	Particulate methane monooxygenase	4.1	DiSpirito, 1992; Lontoh, 1998
Methane	*Methylosinus trichosporium OB3b*	Soluble methane monooxygenase	16.6 37.5	Koh, 1993; Oldenhuis, 1989; 1991; Tsien, 1989
Methane	*Methylosinus methanica*	Soluble methane monooxygenase	38.8	Koh, 1993
Toluene	*Pseudomonas putida F1*	Toluene dioxygenase	8 1.8 0.5	Heald, 1994; Leahy, 1996; Wackett, 1988; Zylstra, 1989
Toluene	*Burkholderia cepacia G4*	Toluene-2-monooxygenase	8 10 9 3	Folsom, 1990; Landa, 1994; Leahy, 1996; Shields, 1991
Toluene	*Pseudomonas mendocina KR1*	Toluene-4-monooxygenase	20 2.4	Leahy, 1996; Winter 1989
Toluene	*Ralstonia picketti PK01*	Toluene-3-monooxygenase	2.4	Leahy, 1996

2. MOLECULAR TOOLS: EAP & QPCR

2.1.1 Enzyme activity probes.

Several methods assess the in-situ activity of microbes in the subsurface (Keift and Phelps, 1997); however, these methods can be time consuming and frequently provide overestimates of the actual rates of activity (Phelps et al., 1994). The recent design of a suite of EAPs has permitted the determination of specific aerobic cometabolism of chlorinated ethenes, most notably TCE. EAPs that serve as alternate substrates for TCE cometabolizing enzymes have been developed for four separate aromatic oxygenases (Keener et al., 1998; 2001; Miller et al., 2001; Clingenpeel et al., 2005), and for the soluble methane monooxygenase (sMMO; Miller et al., 2001). These non-fluorescent probes are transformed by the enzymes into a quantifiable fluorescent signal upon transformation, thus providing direct evidence of cometabolic enzyme activity. Enzyme probes have been evaluated at a number of DOE and DoD sites over the last five years (Lee et al., 2005; Lee et al., 2007; Wymore et al., 2007). Based on these analyses of contaminated groundwater, ranging in TCE concentrations from <100 µg/L to over 10,000 µg/L, it appears that enzyme probes provide a direct estimate of aerobic cometabolic enzyme activity for subsurface populations. Application of EAP's at contaminated sites can provide valuable information regarding the presence and activity of in situ microbial enzyme systems important for aerobic cometabolism for plume-wide assessment of intrinsic assessment of degradation.

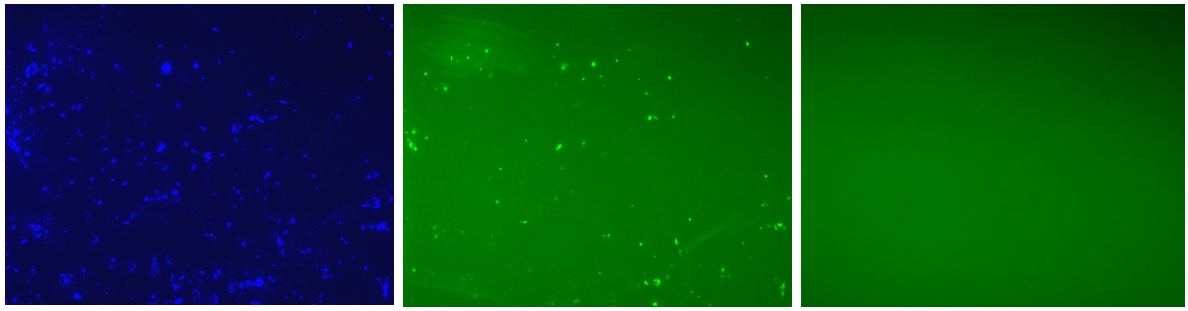

Figure 1. The micrograph on the left represents the total number of microbial cells (DAPI-stained); the center micrograph represents the cells that transformed the probe into a fluorescent product. The right micrograph shows a negative response with the probe.

2.1.2 Quantitative PCR (qPCR) for oxygenase and dioxygenase genes.

In general, qPCR provides an opportunity to identify specific microorganisms or even specific genes in a microbial community in order to assess the potential for that community to carry out a desired biotransformation process. As noted above, aerobic cometabolism of TCE is a fortuitous reaction catalyzed by diverse monooxygenases and dioxygenases with somewhat broad substrate ranges (Arp et al., 2001). Because of the phylogenetic diversity of these enzymes (and the genes that code for them), it is impossible to develop a single nucleic acid-based biomarker for TCE-cometabolizing microorganisms. However, many of these enzymes do share structural similarities that are reflected in conserved stretches of their encoding DNA sequences, against which broadly-specific, degenerate PCR primers have been constructed. From environmental studies performed with these primers, it is known that there is abundant diversity in the recovered sequences (Futamata et al., 2005;Baldwin et al., 2003; 2005; 2008; 2009; Erwin et al., 2005; Nebe et al., 2009; Mesarch et al., 2000). Application of qPCR at contaminated sites will provide quantification of diverse range of known organisms and genes that are

relevant to aerobic cometabolism. To date qPCR is the only method that can identify the broad diversity of oxygenase genes and/or organisms known to possess those genes. In particular, qPCR allows for the assessment of genes and/or organisms relevant for aerobic cometabolism of chlorinated solvents. The qPCR results can be related back to the EAP results to provide a comprehensive assessment of changes in number and activity of genes along the midline of a contaminant plume or over spatial and/or temporal scales.

2.1.3 Relationship between EAP and qPCR targets

The enzyme probe data relates to the qPCR as shown in the table below (Table 2). Some of the targets for the qPCR analyses do not directly correspond to EAP analyses and are completed in order to target other oxygenase enzymes which are known to also cometabolize contaminants such as chlorinated solvents. While there are dozens of known enzymes, some are more commonly found in environmental systems and/or are potential targets for remediation strategies such as bioaugmentation or biostimulation (propane, methane, benzene etc.). Table 3 provides a list of all of the qPCR targets considered for the current site and completed herein.

Probe	**Pathway**	**qPCR**
3-hydroxyphenylacetylene	toluene-2-monooxygenase toluene-3-monooxygenase toluene-2,3-dioxygenase	 RMO, PHE TOD
Phenylacetylene 3-ethynylbenzoate	toluene-2,3-dioxygenase toluene-3-monooxygenase toluene-2-monooxygenase toluene-side-chain-monooxygenase	TOD RMO, PHE TOL
trans-cinnamonitrile	toluene-2,3-dioxygenase	TOD
Coumarin, naphthalene	Soluble methane monooxygenase	mmoX

Table 2. Relationship between the EAP, the oxygenase(s)/ pathway and the qPCR methods completed within.

SEQUENCE	**CITATION**	**OXYGENASE**
CGACCTGATCWSCATGACCGA	Mesarch et al., 2000	Catechol 2,3 dioxygenase (CAT)
TYAGGTCAKMACGGTCA		
ACCGATGARGAYCTGTACC	Baldwin et al., 2003	Toluene 2,3 dioxygenase (TOD)
CTTCGGTCMAGTAGCTGGTG		
TGAGGCTGAAACTTTACGTAGA	Baldwin et al., 2003	Toluene side chain monooxygenase (TOL)
CTCACCTGGAGTTGCGTAC		
TCTCVAGCATYCAGACVGACG	Baldwin et al., 2003	Toluene 3,4 monooxygenases (RMO)
TTKTCGATGATBACRTCCCA		
TYTCVAGCATHCARACVGAYGA	Baldwin et al., 2003	Toluene 3,4 monooxygenases (RDEG)
TTDTCGRTRATBACRTCCCA		

GTGCTGACSAAYCTGYTGTTC	Baldwin et al., 2003	Toluene 2,3,4 monooxygenases (PHE)
CGCCAGAACCAYTTRTC		
TCCCTCACACAGCTGGAACTC	Sharp et al, 2007	Alkane monooxygenase (alkB)
TCGCTGTGACGCTGCAA		
GGCTCCAAGTTCAAGGTCGAGC	McDonald et al., 1995; 1997	soluble Methane monooxygenase (sMMO)
TGGCACTCGTAGCGCTCCGGCTCG		
GGNGACTGGGACTTCTGG	Costello and Lidstrom, 1999	particulate Methane monooxygenase (pmoA)
CCGGMGCAACGTCYTTACC		

Table 3. List of quantitative PCR analyses completed on sampled from Travis AFB. Listed are the oxygenase targets, the sequences of the primers and the most relevant reference for the work.

3. METHODS

3.1.1 Toluene Enzyme Activity Determination

10 mL of groundwater was filtered onto black polycarbonate filters and exposed to ~500 µL of 5 mM of an EAP (PA, 3hpa, or CINN) in 40 mM phosphate buffer or DAPI (4,6-diaminophenylindole). Three separate filters were made for each stain/probe. The probe was exposed to the sample for 10 minutes and the vacuum was reapplied to remove the solution. The filter was mounted on a glass microscope slide with non-fluorescent citifluor and a cover slip. The filters were examined for fluorescent cells by epifluorescent microscopy using a 100X oil-immersion fluorescent objective and a filter set for blue excitation wavelengths. Probe response was visualized on a Nikon Eclipse fluorescence microscope equipped with a PL APO (plan apochromatic) 100x 1.40 oil objective. An longpass orange-red filter set was used (excitation 535 ± 17.5 nm, dichroic mirror 570 nm, emission 590 nm) to visualize probe positive cells (Omega Optical, Inc., Brattleboro, VT, USA). The imaging system included a color digital camera and commercial imaging software (Diagnostic Instruments, Inc., Sterling Heights, MI, USA).

3.1.2 DNA extraction and PCR amplification

Samples ~500 mL were filtered through sterile glass towers onto 0.22µm, 47 mm Supor filters immediately upon arrival in the laboratory. Filters were then placed inside 15 or 50 mL falcon tubes, being careful to not let the filter to overlap, labeled and placed immediately in a whirlpack bag and into the -80 C freezer until removed to be extracted. A minimum of 2 filters were stored for each sample; in many cases, 3, 500 mL filters were stored. Once removed from the freezer, using sterile technique, Supor filters were cut into pieces and placed directly into two commercially available DNA extraction kits. In this method, DNA is isolated from the cells/material trapped on filters. DNA extractions were performed using both Bio 101 and the MoBio UltraClean Soil DNA kit, as described by the manufacturers. Two kits were used to ensure that biases associated with one kit or another did not provide a false positive or negative for the presence of the gene of interest. The samples consistently yielded high-quality bacterial DNA, based on gel electrophoresis.

Polymerase chain reaction (PCR) amplification reactions for the 16S rRNA gene were performed in 25 µL (total volume) reaction mixtures in 0.2 mL thin-walled tubes using a DNA thermocycler. The PCR experiments were performed using the Jumpstart Ready Mix PCR kit from Invitrogen. PCR products were separated and visualized using an Agilent 2100 Bioanalyzer and DNA 1000 LabChips.

Quantitative PCR (qPCR) was performed using a Rotor-Gene 3000 real-time PCR instrument. The PCR conditions for the toluene oxygenase primers were as stated in Baldwin et al. (2003). The PCR primers were designated: RMO-F/R, which amplify the toluene-3 and -4-monooxygenase genes, TOD-F/R which amplifies the toluene 2,3-dioxygenase gene, and PHE-F/R which amplifies the toluene-2, -3, -4-monooxygenase genes (Baldwin et al., 2003). PCR conditions for the catechol 2,3-dioxygenase gene were taken from Mesarch et al. (2004). PCR primers were designated RDEG F/R. Alkane hydrolase genes were monitored using alkB F/R primers and PCR conditions outlined by Alonso-Gutierrez et al. (2011). PCR conditions for particulate and soluble methane monooxygenase genes were performed using pmoA and mmoX F/R primers with conditions from (McDonald et al., 1995; 2008; Costello and Lidstrom, 1999).

4. RESULTS

The evaluation of the potential for intrinsic attenuation by biological processes was addressed through (a) analysis of samples with a suite of EAP and (b) qPCR for identification of the potential of the community as well as supporting evidence for the EAP analysis. Table 4 provides a summary of the results for this sampling event and includes data with five independent enzyme probes: 3-hydroxyphenylacetylene (3-HPA), phenylacetylene (PA), and trans-cinnamonitrile (CINN), which target aromatic oxygenase enzymes, coumarin, which interrogates the activity of the soluble form of the methane monooxygenase (sMMO), and naphthalene which also assesses the methane monooxygenase.

	DAPI Total	*stdev*	3hpa T3-mono	*Stdev*	PA T2-mono	*stdev*	Cinn T23-di	stdev	Coumarin sMMO	Naphthalene MMOs
	Cells mL^{-1}		*Cells mL^{-1}*		*Cells mL^{-1}*		*Cells mL^{-1}*		*RFU*	*RFU*
MW131	4.52E+04	*1.02E+04*	1.17E+04	*4.87E+03*	3.55E+04	*7.56E+03*	4.17E+04	*7.99E+03*	Neg	Neg
MW591	2.23E+04	*6.19E+03*	1.62E+04	*6.11E+03*	1.54E+04	*4.25E+03*	1.68E+04	*5.72E+03*	Neg	Neg
MW266	3.67E+05	*1.22E+05*	1.01E+04	*3.70E+03*	5.48E+03	*2.30E+03*	0.00E+00	*0.00E+00*	Neg	Neg
MW264	7.64E+04	*1.59E+04*	0.00E+00	*0.00E+00*	0.00E+00	*0.00E+00*	0.00E+00	*0.00E+00*	Neg	Neg
MW781B	5.74E+04	*1.37E+04*	1.48E+04	*6.04E+03*	9.89E+03	*4.76E+03*	5.48E+03	*1.97E+03*	Neg	Neg
MW04	1.11E+05	*2.04E+04*	1.87E+04	*2.50E+03*	1.78E+04	*5.65E+03*	0.00E+00	*0.00E+00*	Neg	Neg
MW781	8.65E+04	*2.54E+04*	1.59E+04	*6.40E+03*	0.00E+00	*0.00E+00*	0.00E+00	*0.00E+00*	Neg	Neg
MWTB	0.00E+00	*0.00E+00*	0.00E+00	*0.00E+00*	0.00E+00	*0.00E+00*	0.00E+00	*0.00E+00*	Neg	Neg

Table 4. Results of EAP analysis of groundwater samples from Travis AFB site. Significant values are highlighted (8.0E+03). Coumarin and naphthalene responses (RFU, relative fluorescence units) were recorded as NEG as they were not significant above background fluorescence.

Based on these results, all of the locations at this site showed significant activity with at least one EAP. DNA was amplifiable from every sample and provided sufficient template for qPCR or traditional PCR analyses (Table 5). The PHE assay was repeated (report v3) and results are presented here as positive and negative rather than quantified as gene copies per cell. These primers were reordered and the assays were repeated, however there was still high background noise with this assay. The data correlate well with other qPCR assays and as such, the assay was not continuously repeated to provide quantified gene copy results. The 2,3 catechol dioxygenase assay was also repeated due to amplification efficiency. While this assay was satisfactory and passed QA/QC, it was repeated with modifications in order to minimize inefficient amplification and to minimize background and non-specific fluorescence interference.

	16S	PHE	RMO	TOD	23CAT	mmoX	pmoA	alkB
MW131	+	+	7.40E+2	1.70E+3	6.10E+3	ND	ND	ND
MW591	+	+++	4.02E+3	ND	3.75E+4	ND	ND	ND
MW266	+	+++	3.34E+3	ND	4.05E+5	ND	ND	ND
MW264	+	ND	ND	ND	ND	ND	ND	ND
MW781	+	++	8.44E+3	ND	6.35E+3	ND	ND	ND
MW04	+	+	5.84E+3	ND	4.36E+4	ND	ND	ND
MW781B	+	+++	8.44E+3	ND	1.94E+4	ND	ND	ND
MW-TB	-	ND	ND	ND	ND	ND	ND	ND
EC	-	ND	ND	ND	ND	ND	ND	ND

Table 5. Quantitative PCR results from the analysis of groundwater samples from Travis AFB. Data represents copies of gene target present in sample. Significant values are highlighted (1.0E+03). ND: Non Detect. EC: extraction control.

5. SIGNIFICANCE

- At least one aromatic EAP showed significant activity at all but one of the locations sampled; MW 264 is the only monitoring well location which did not have any significant enzyme activity;

- qPCR data supported many of the EAP conclusions with positive amplifiable gene targets corresponding to significant EAP results; however, one of the groundwater samples which showed EAP activity, did not show positive results with quantitative or traditional PCR amplification of genes;

  a. One exception: toluene dioxygenase: TOD corresponds to the dioxygenase enzyme and cinnamonitrile; two samples were positive with cinnamonitrile (MW131, MW591), however only one samples had quantifiable product (MW 131). In this case, the activity of the enzyme was detected but the DNA was quantified; due to the biases associated with extracting DNA and the amplification process, this is not unexpected or uncommon. EAP is a whole cell assay and there and as such the biases are limited (sample filtered and cells stained).

  b. Remaining qPCR data correlates well with EAP; RMO resulted in quantifiable DNA in all of the samples which also showed enzyme activity with PA, or 3hpa. The 23CAT and PHE analyses supported these data as well.

  c. The majority of the samples (6/7) showed the potential for aerobic degradation of contaminants, based on qPCR results. These samples also had positive amplifications with at least two different gene targets, a diversity of enzymes.

  d. The only sample without any quantifiable oxygenase genes was MW 264.

- Based on the EAP and qPCR data, there is evidence that there is potential for ***significant intrinsic aerobic biodegradation across the site***; however only a few locations show a diversity of enzymes (2+ enzymes) and activities (all three aromatic EAPs) under *in situ* conditions (MW131, MW591).

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APPENDIX 1. OXYGENASE BIOCHEMISTRY

Action of Oxygenases

Oxygenases serve a myriad of functions in cells including biosynthesis, detoxification, and catabolism. They catalyze the reduction of O_2 with incorporation of one (monooxygenases) or two (dioxygenases) of the O atoms into the substrate that is being oxidized. Monooxygenases require an input of reductant, which is used to reduce the second atom of O to H_2O. Dioxygenases do not necessarily require reductant as both atoms of O_2 are reduced upon incorporation into the substrate; however, some use reductant to further reduce the substrate (e.g., toluene dioxygenase). Although all oxygenases have in common the need to activate O_2, the chemical mechanisms can be quite variable.

The activation of O_2 to a reactive state is typically facilitated by prosthetic groups in the enzymes. A variety of active site groups have been recognized including flavin, heme, binuclear iron clusters, mononuclear iron centers, and Cu. The protein composition of oxygenases can be relatively simple or quite complex. Some oxygenases are soluble while others are associated with the membrane. While some monooxygenases are very specific for particular substrates, others have remarkably relaxed substrate ranges. In some cases, this relaxed substrate range appears to be by design (example: P450 monooxygenases), while in other cases, the broad substrate range does not appear to serve any particular purpose. For example, ammonia monooxygenase has a very broad substrate range, which extends to several classes of compounds; however it appears to derive an energetic benefit only from the growth-supporting substrate, ammonia.

Genetics

Based on the analysis of both cultured and natural organisms (gene sequences), studies have found that the genes coding for aromatic degradation genes are present as plasmids, transposons, as well as integrative genetic elements. There are even cases in which the genes conferring the ability to grow on substrates are located on a chromosome; those elements are almost identical to that found on plasmids conferring different substrate utilization in other organsims (pWWO; Sinclair et al., 1986). In general, it appears that the majority of organisms that exhibit the targeted activities (aromatic, methane) have conserved genes present on the bacterial chromosome; only a small fraction of the targeted organisms have the genes present on a plasmid, which could be transferred or lost over time.

Induction

Induction of oxygenase synthesis occurs when the cell responds to the presence of an inducer, which is typically a substrate for the enzyme. In the presence of an appropriate inducer molecule, transcription of the structural genes for the oxygenase is up-regulated, resulting in a significant increase in the synthesis of the oxygenase and/or other enzymes involved in a specific catabolic pathway. Recent studies have found that both naturally occurring humic substances and the chlorinated solvents themselves can act as inducers of the cometabolic enzymes, although the extent of and time for activation, as well as the mechanism for cell energy and growth in these instances are poorly understood.

Attachment 2
Monitoring Well Boring Logs

WESTON

DRILLING LOG

WELL NUMBER: MW-131
LOCATION: North Landfill Zone, Fire Training Area #3
SURFACE ELEVATION: _____
DRILLING COMPANY: Datum Exploration
DRILLER: Robin David
LOG BY: G. D. Witmer

OWNER: USAF Travis
ADDRESS: Fairfield, Ca. 94533
TOTAL DEPTH: 29'
WATER LEVEL: 15'
DRILLING METHOD: Hollow Auger
DATE DRILLED: 1/18/85
HELPER: Frank Grossman

SKETCH MAP

NOTES:

DEPTH (FEET)	HNU / OVA	SAMPLE NUMBER	% RECOVERY	SAMPLE BLOWS*	DESCRIPTION / SOIL CLASSIFICATION (COLOR, TEXTURE, STRUCTURES)
0	0	1	80	1 1 4	0-1.5' Dark brown (10 yr 3/3) Very wet silty CLAY cohesive, crumb structure.
2.5	0	2	100	4 6 11	2.5-4.0' Dark brown (10 yr 3/3) Very wet, cohesive, CLAY. Grading to yellowish brown (10 yr 5/6) moist silty CLAY, very cohesive, crumb structure.
5.0	0	3	100	8 9 11	5.0-6.5' Yellowish brown (10 yr 5/6) Moist silty CLAY, very cohesive, crumb structure.
7.5	0	4	100	6 6 9	7.5-9.0' Yellowish brown (10 yr 5/6) Moist silty CLAY, very cohesive, crumb structure, mottles present.

* A.S.T.M. D1586

WESTON

DRILLING LOG

WELL NUMBER: MW-131(cont'd) OWNER:
LOCATION: ADDRESS:
TOTAL DEPTH
SURFACE ELEVATION: WATER LEVEL:
DRILLING COMPANY: DRILLING METHOD: DATE DRILLED:
DRILLER: HELPER:
LOG BY:

SKETCH MAP

NOTES:

DEPTH (FEET)	HNU / OVA	SAMPLE NUMBER	% RECOVERY	SAMPLE BLOWS*	DESCRIPTION / SOIL CLASSIFICATION (COLOR, TEXTURE, STRUCTURES)
10	0	5	100	9 12 15	10.0-11.5' Yellowish brown (10 yr 5/6) Very sandy CLAY cohesive, moist, ribbons, mottles present.
15	0	6	100	N/A	15.0-16.5' Yellowish brown (10 yr 5/6) Clayey SAND, moist, cohesive. Grading to yellowish brown (10 yr 5/8) med. SAND with trace clay and some coarse fragments, moist to wet, non-cohesive. Mottles present.
20	0	7	100	16 36 40	20.0-21.5' Yellowish brown (10 yr 5/8) highly compacted gravelly SAND, with green sand vien, dry non cohesive.
25	0	8	47	50/6" refusal	25.0-26.5' Brownish yellow (10 yr 6/6) med. variegated SAND with some gravel, moist, non cohesive.

* A.S.T.M. D1586

WESTON

DRILLING LOG

WELL NUMBER: MW-131(cont'd)
LOCATION: _____
_____
_____
SURFACE ELEVATION: _____
DRILLING COMPANY: _____
DRILLER: _____
LOG BY: _____

OWNER: _____
ADDRESS: _____
_____
TOTAL DEPTH _____
WATER LEVEL: _____
DRILLING METHOD: _____
HELPER: _____
DATE DRILLED: _____

SKETCH MAP

NOTES:

DEPTH (FEET)	HNU / OVA	SAMPLE NUMBER	% RECOVERY	SAMPLE BLOWS*	DESCRIPTION / SOIL CLASSIFICATION (COLOR, TEXTURE, STRUCTURES)
30	0	9	40	50 /6" refusal	30.0-31.5' Brownish yellow (10 yr 6/6) and red (2.5 yr 4/6) med. SAND, wet, non cohesive.
					- End of Boring -

* A.S.T.M. D1586

SHEET 3 OF 3

Borehole Log

ROY F. WESTON, Inc.

CLIENT	: TRAVIS AFB	TOTAL DEPTH	: 26.50
SITE NAME	: LF-2	LOGGER	: ROY MARION
WELL ID	: 04-0264	DRILLING COMPANY	: WDC
NORTHING	: 221350.3100 surveyed	DRILLING RIG	: CME 75 (HSA)
EASTING	: 2024076.0000 surveyed	DATE STARTED	: 04/03/91
ELEVATION	: 64.400 surveyed	DATE COMPLETED	: 04/04/91

ELEVATION	DEPTH	MATERIAL	% RECOVERY	CLASSIFICATION	COLOR	STRENGTH	MOISTURE	BLOW COUNT	FIELD INSTRUMENT READING	COMMENTS
			40	Fill	REDDISH BROWN	SFT	DRY	8 9 9 0	OVM 0.0 CGI 0.0	FILL IS 40% SAND & GRAVEL 60% SILT & CLAY. 2.5Y-5/4.
				No Sample Recovered						
63	1									
				Interval Not Sampled						
62	2									
61	3									
60	4									
59	5		20	Fat clay, CH	DK YEL. BROWN	FRM	DRY		OVM 4.0 CGI 0.0	10YR - 4/6.
				No Sample Recovered						
58	6									
				Fat clay, CH	DK YEL. BROWN	FRM	DRY		OVM 0.0 CGI 0.0	LOGGED BASED ON CUTTINGS. 10YR - 4/6.
57	7									
56	8									
55	9									
54	10		100	Fat clay, CH	LT BROWN. GREY	FRM	DRY	11 13 15 0	OVM 5.9 CGI 0.0	40-50% MOTTLED WITH STRONG BROWN, YELLOW-BROWN, & BLACK STAIN. 2.5Y-6/2.

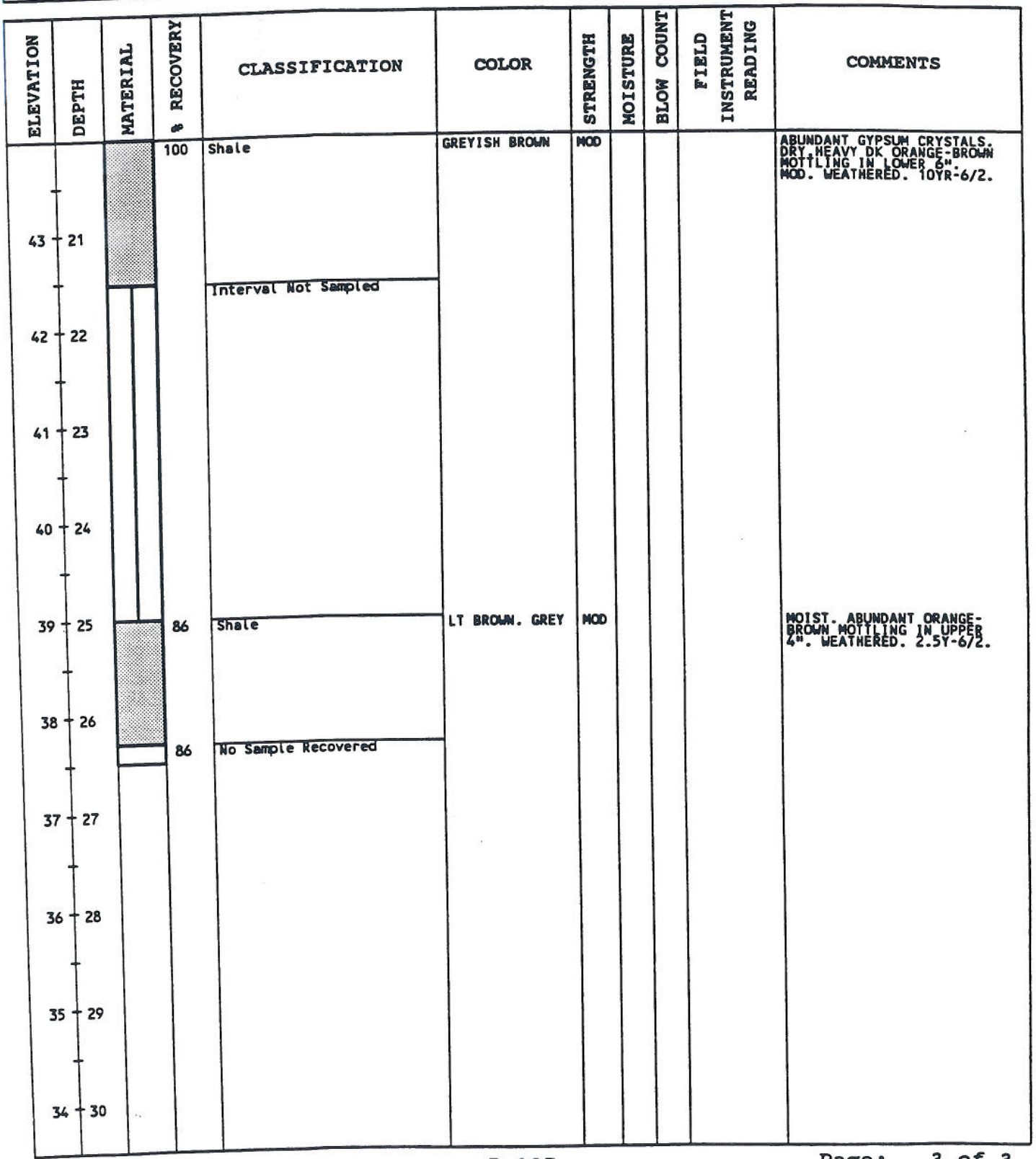
CLIENT	: TRAVIS AFB	TOTAL DEPTH	: 26.50
SITE NAME	: LF-2	LOGGER	: ROY MARION
WELL ID	: 04-0264	DRILLING COMPANY	: WDC
NORTHING	: 221350.3100 surveyed	DRILLING RIG	: CME 75 (HSA)
EASTING	: 2024076.0000 surveyed	DATE STARTED	: 04/03/91
ELEVATION	: 64.400 surveyed	DATE COMPLETED	: 04/04/91

ELEVATION	DEPTH	MATERIAL	% RECOVERY	CLASSIFICATION	COLOR	STRENGTH	MOISTURE	BLOW COUNT	FIELD INSTRUMENT READING	COMMENTS
53	11			Fat clay, CH	LT BROWN. GREY	FRM	DRY		OVM 5.9 CGI 0.0	40-50% MOTTLED WITH STRONG BROWN, YELLOW-BROWN,& BLACK STAIN. 2.5Y-6/2.
52	12			Interval Not Sampled						
51	13									
50	14									
49	15		100	Sandy lean clay, CL	DK YELLOW BROWN	FRM	MST		NA 0.0	10YR - 4/6.
				Lean clay, CL	LT BROWN. GREY	FRM	DRY		NA 0.0	MOTTLED W/ORANGE-BROWN & OCCASIONAL BLACK. 2.5Y-6/2.
48	16		100	Shale	GREYISH BROWN					OCCASIONAL BLACK MOTTLING HIGHLY WEATHERED. 10YR - 5/2.
47	17			Interval Not Sampled						
46	18		100	Shale	GREYISH BROWN	WEK		11 12 14 0		HIGHLY WEATHERED. ABUNDANT (30-40%) ORANGE-BROWN MOTTLING. 10YR-5/2.
45	19		100	Shale	GREYISH BROWN	MOD				30% ORANGE & BLACK MOTTLING & 5-10% GYPSUM CRYSTALS. WEATHERED. 10YR - 5/2.
				Interval Not Sampled						
44	20		100	Shale	GREYISH BROWN	MOD		15 13 18 0		ABUNDANT GYPSUM CRYSTALS. DRY. HEAVY DK ORANGE-BROWN MOTTLING IN LOWER 6". MOD. WEATHERED. 10YR-6/2.

Borehole Log

ROY F. WESTON, Inc.

CLIENT	: TRAVIS AFB	TOTAL DEPTH	: 26.50
SITE NAME	: LF-2	LOGGER	: ROY MARION
WELL ID	: 04-0264	DRILLING COMPANY	: WDC
NORTHING	: 221350.3100 surveyed	DRILLING RIG	: CME 75 (HSA)
EASTING	: 2024076.0000 surveyed	DATE STARTED	: 04/03/91
ELEVATION	: 64.400 surveyed	DATE COMPLETED	: 04/04/91



Borehole Log

ROY F. WESTON, Inc.

CLIENT	: TRAVIS AFB	TOTAL DEPTH	: 21.50
SITE NAME	: FTA-3	LOGGER	: MARK DOMINICK
WELL ID	: 02-0266	DRILLING COMPANY	: WDC
NORTHING	: 220732.8500 surveyed	DRILLING RIG	: CME 750 (HSA)
EASTING	: 2023555.0600 surveyed	DATE STARTED	: 04/08/91
ELEVATION	: 57.800 surveyed	DATE COMPLETED	: 04/08/91

ELEVATION	DEPTH	MATERIAL	% RECOVERY	CLASSIFICATION	COLOR	STRENGTH	MOISTURE	BLOW COUNT	FIELD INSTRUMENT READING	COMMENTS
			100	Lean clay, CL	VDK GREY. BROWN	FRM	DRY	3 7 10 0	OVM 0.0	OCCASIONAL ROOTS PRESENT; 10YR - 3/2.
56	1									
				Interval Not Sampled						
55	2									
54	3									
53	4									
52	5			Lean clay, CL	DK YEL. BROWN	FRM	DRY	7 12 16 0	OVM 0.0	IRON OXIDE MOTTLING PRESENT. 10YR - 4/6.
51	6									
				Interval Not Sampled						
50	7									
49	8									
48	9									
47	10		100	Silt with sand, ML	DK YEL. BROWN	FRM	MST	11 13 17 0	OVM 0.0	IRON OXIDE MOTTLING PRESENT. 10YR - 4/6.

CLIENT	: TRAVIS AFB	TOTAL DEPTH	: 21.50
SITE NAME	: FTA-3	LOGGER	: MARK DOMINICK
WELL ID	: 02-0266	DRILLING COMPANY	: WDC
NORTHING	: 220732.8500 surveyed	DRILLING RIG	: CME 750 (HSA)
EASTING	: 2023555.0600 surveyed	DATE STARTED	: 04/08/91
ELEVATION	: 57.800 surveyed	DATE COMPLETED	: 04/08/91

ELEVATION	DEPTH	MATERIAL	% RECOVERY	CLASSIFICATION	COLOR	STRENGTH	MOISTURE	BLOW COUNT	FIELD INSTRUMENT READING	COMMENTS
				Silt with sand, ML	DK YEL. BROWN	FRM	MST		OVM 0.0	IRON OXIDE MOTTLING PRESENT. 10YR - 4/6.
46	11									
				Interval Not Sampled						
45	12									
44	13									
43	14									
42	15		100	Silty sand, SM	DK YEL. BROWN	LSE	SAT	9 10 10 0	OVM 0.0	10YR - 4/4.
				Lean clay, CL	DK YEL. BROWN	STF	MST		OVM 0.0	10YR - 4/4.
41	16									
				Interval Not Sampled						
40	17									
39	18									
38	19									
37	20		100	Silty sand, SM	DK GREY. BROWN	LSE	SAT	3 7 14 0	OVM 0.0	SATURATION AT -21.0FT BGS 10YR - 4/2.

Borehole Log

ROY F. WESTON, Inc.

CLIENT	: TRAVIS AFB	TOTAL DEPTH	: 21.50
SITE NAME	: FTA-3	LOGGER	: MARK DOMINICK
WELL ID	: 02-0266	DRILLING COMPANY	: WDC
NORTHING	: 220732.8500 surveyed	DRILLING RIG	: CME 750 (HSA)
EASTING	: 2023555.0600 surveyed	DATE STARTED	: 04/08/91
ELEVATION	: 57.800 surveyed	DATE COMPLETED	: 04/08/91

ELEVATION	DEPTH	MATERIAL	% RECOVERY	CLASSIFICATION	COLOR	STRENGTH	MOISTURE	BLOW COUNT	FIELD INSTRUMENT READING	COMMENTS
36	21			Silty sand, SM	DK GREY. BROWN	LSE	SAT		OVM 0.0	SATURATION AT -21.0FT BGS 10YR - 4/2.
35	22									
34	23									
33	24									
32	25									
31	26									
30	27									
29	28									
28	29									
27	30									

SHEET 1 of 2	PROJECT NUMBER:	BORING NUMBER: *MW591X04*

SOIL BORING LOG

PROJECT NAME:		HOLE DEPTH (ft): 44.00	DRILLING CONTRACTOR:	
SURFACE ELEVATION: 59.78 ft. MSL	NORTHING (CCS NAD83 Z2): 1860669.65	EASTING (CCS NAD83 Z2): 6585170.22	DATE STARTED:	DATE COMPLETED:
WATER LEVEL: --- ft. bgs	DRILLING METHOD:		DRILLING EQUIPMENT:	
LOCATION: FT004			LOGGED BY:	

DEPTH (ft bgs)	INTERVAL (feet)	RECOVERY (%)	#TYPE	SPT RESULTS 6"-6"-6" (N)	SIZE DISTRIBUTION %G	%S	%F	SOIL DESCRIPTION USCS GROUP NAME (USCS GROUP SYMBOL): color, moisture, mineralogy, density, structure, cementation, staining/odor, reaction with HCL. COARSE FRACTION: grain size, angularity, hardness, shape. FINE FRACTION: plasticity, dry strength, toughness, dilatancy. Additional comments.	OVM (ppm): BREATHING ZONE	HEAD SPACE	COMMENTS (e.g.: DRILLING FLUID LOSS, TESTS, OR DRILLER COMMENTS, ETC.)
1–35								**SILT (ML)**: 40 % clay and 60% silt, dark gray to light brown, low plasticity, medium soft, moist, no odor, older alluvium. **SILT (ML)**: 10% fine sand, 30% clay, and 60% silt, light brown to yellowish orange, moist, soft, no odor, older alluvium. **SILT WITH SAND (ML)**: 20% fine sand, 20% clay, and 60% silt, light brown to yellowish orange, moist, soft, low plasticity, no odor, older alluvium. **SANDY SILT (ML)**: 40% fine sand and 60% silt, yellowish orange and greenish gray, soft, moist to wet, no odor, older alluvium, sand is subrounded. **SANDY SILT (ML)**: 50% sand and 50% silt, light brown, nonplastic, soft to medium soft, moist to wet, sand is poorly graded, loose to medium dense, no odor, older alluvium. **SILT (ML)**: 10% fine sand, 30% clay, and 60% silt, mottled - light brown to yellowish orange and greenish gray, low pasticity, soft to medium soft, moist to wet, no odor, older alluvium. **SILTY SAND (SM)**: 30% silt and 70% sand, light brown to yellowish orange and greenish gray, medium, poorly graded, subrounded, loose, wet, some organics and oxide staining (rust and black), no odor, older alluvium. **SILTY SAND (SM)**: 40% silt and 60% sand, brown to yellowish orange and greenish gray, fine to medium, poorly graded, rounded, loose, wet, no odor, older alluvium. **SILTY SAND (SM**: 40% silt and 60% sand, light brown to reddish brown and greenish gray, fine, loose, moist, no hydrocarbon odor, slight organic odor, older alluvium. **POORLY GRADED SAND WITH SILT (SP-SM)**: 10% silt and 90% sand, dark brown, poorly graded, subrounded, loose, wet, no odor, some organics, older alluvium. **SILTY SAND (SM)**: 30% silt and 70% sand, light brown, yellowish orange and greenish gray, poorly graded, subrounded, loose, wet, no odor, older alluvium.			

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SHEET 2 of 2	PROJECT NUMBER:	BORING NUMBER: *MW591X04*

SOIL BORING LOG

PROJECT NAME:		HOLE DEPTH (ft): 44.00	DRILLING CONTRACTOR:	
SURFACE ELEVATION: 59.78 ft. MSL	NORTHING (CCS NAD83 Z2): 1860669.65	EASTING (CCS NAD83 Z2): 6585170.22	DATE STARTED:	DATE COMPLETED:
WATER LEVEL: --- ft. bgs	DRILLING METHOD:		DRILLING EQUIPMENT:	
LOCATION: FT004			LOGGED BY:	

DEPTH (ft bgs)	INTERVAL (feet)	RECOVERY (%)	#TYPE	SPT RESULTS 6"-6"-6" (N)	SIZE DISTRIBUTION %G	%S	%F	SOIL DESCRIPTION USCS GROUP NAME (USCS GROUP SYMBOL): color, moisture, mineralogy, density, structure, cementation, staining/odor, reaction with HCL. COARSE FRACTION: grain size, angularity, hardness, shape. FINE FRACTION: plasticity, dry strength, toughness, dilatancy. Additional comments.	OVM (ppm): BREATHING ZONE	HEAD SPACE	COMMENTS (e.g.: DRILLING FLUID LOSS, TESTS, OR DRILLER COMMENTS, ETC.)
36								**SILTY SAND (SM)**: same as above.			
37											
38								**SILTSTONE**: brown, purple, yellowish orange, and greenish gray, moist, siltstone chips, weathered bedrock contact.			
39											
40											
41								**SILTSTONE**: same as above.			
42											
43								**SILTSTONE**: bluish gray to olive and greenish gray, dry, no odor, nortonville shale.			
44											
								Boring Terminated at 44 feet bgs			

CH2MHILL

MW04x39

PROJECT NUMBER	BORING NUMBER
134853	28-MW04 Page 1

SOIL BORING LOG

PROJECT : Travis AFB WABOU RI ROUND 2 LOCATION : Building 755

ELEVATION : 55.52 DRILLING CONTRACTOR : WTRD

DRILLING METHOD AND EQUIPMENT USED : Drill Rig

WATER LEVELS : START : 4/12/96 END : LOGGER : KROOK

DEPTH BELOW SURFACE (FT)	INTERVAL (FT)	RECOVERY ft.	#/TYPE	Sample Field ID Number	CORE DESCRIPTION: SOIL NAME, USCS GROUP SYMBOL, COLOR, MOISTURE CONTENT, RELATIVE DENSITY, OR CONSISTENCY, SOIL STRUCTURE, MINERALOGY.	COMMENTS: DEPTH OF CASING, DRILLING RATE, DRILLING FLUID LOSS, TESTS, AND INSTRUMENTATION.
0					Clayey Silt with fine sand (ML), soft saturated, moderately plastic, dark yellowish	
0.5						
1						
1.5						
2					Clay (CL), with fine to medium sand, stiff, moist, dark yellowish - brown	
2.5						
3						
3.5		3.5			Clayey Silt (ML), friable, moist, with fine sand, stiff yellowish-brown	
4						
4.5						
5						OVM = 0.0 ppm
5.5						
6						
6.5						
7						
7.5		4				
8						
8.5					Clayey Silt (ML), friable, moist, with fine sand, stiff yellowish-brown, but hard	
9					Silty Sand (SM), very dense fine to medium, moist, yellowish-brown	
9.5						
10						OVM = 0.0 ppm
10.5					Sandy Silt (ML), with fine to medium sand, moist, friable, moderately plastic, yellow-brown	

MW04x39

PROJECT NUMBER	BORING NUMBER
134853	28-MW04 Page 2

SOIL BORING LOG

PROJECT : Travis AFB WABOU RI ROUND 2 LOCATION : Building 755

ELEVATION : 55.52 DRILLING CONTRACTOR : WTRD

DRILLING METHOD AND EQUIPMENT USED : Drill Rig

WATER LEVELS : START : 4/12/96 END : LOGGER : KROOK

DEPTH BELOW SURFACE (FT)	INTERVAL (FT)	RECOVERY ft.	#/TYPE	Sample Field ID Number	CORE DESCRIPTION: SOIL NAME, USCS GROUP SYMBOL, COLOR, MOISTURE CONTENT, RELATIVE DENSITY, OR CONSISTENCY, SOIL STRUCTURE, MINERALOGY.	COMMENTS: DEPTH OF CASING, DRILLING RATE, DRILLING FLUID LOSS, TESTS, AND INSTRUMENTATION.
11						
11.5						
12						
12.5					Sandy Silt (ML), with fine to medium sand, moist, friable, moderately plastic, yellow-brown	
13						
13.5					Silty Clay (ML-CL), with fine sand, moist, moderately friable yellowish - brown with olive mottling	
14						
14.5						
15				B755-1303	Sandy Silt (ML), fine, moist, friable, with some clay	OVM = 0.0 ppm
15.5						
16				B755-3704		
16.5						
17						
17.5		5				
18					Silty Sand (SM), fine to medium, moderately dense, wet to saturated	
18.5						
19					Sandy Silt (ML), with clay, fine to medium, sand, moist, moderately plastic	Possible water producer
19.5						
20						OVM = 0.0 ppm
20.5						
21					Sandy Silt (ML), with clay, fine to medium, sand, moist, moderately plastic, more hard	
21.5						

MW04x39

PROJECT NUMBER	BORING NUMBER
134853	28-MW04 Page 3

SOIL BORING LOG

PROJECT : Travis AFB WABOU RI ROUND 2 LOCATION : Building 755

ELEVATION : 55.52 DRILLING CONTRACTOR : WTRD

DRILLING METHOD AND EQUIPMENT USED : Drill Rig

WATER LEVELS : START : 4/12/96 END : LOGGER : KROOK

DEPTH BELOW SURFACE (FT)	INTERVAL (FT)	RECOVERY ft. #/TYPE	Sample Field ID Number	CORE DESCRIPTION: SOIL NAME, USCS GROUP SYMBOL, COLOR, MOISTURE CONTENT, RELATIVE DENSITY, OR CONSISTENCY, SOIL STRUCTURE, MINERALOGY.	COMMENTS: DEPTH OF CASING, DRILLING RATE, DRILLING FLUID LOSS, TESTS, AND INSTRUMENTATION.
22					
22.5		4.5		Silty Sand (SM), fine to medium, moderately dense, saturated to wet	
23					
23.5					Possible water producer, a little water at bottom of the auger after 5 minutes
24				Clayey Silt (ML), with fine to medium sand, moderately friable, moist	
24.5					
25					OVM = 0.0 ppm, quarter inch sand seem.
25.5				Sand (SP), with silt, well-graded, saturated, moderately dense	
26					
26.5				Silty Sand (SM), fine to medium, with some clay, moist to wet, dense	
27					
27.5		5			
28					
28.5					
29					
29.5					
30				Total Depth = 30.0'	Bottom of hole at 30.0', OVM 0.0 ppm
30.5					
31					
31.5					
32					
32.5					

SHEET 1 of 2

PROJECT NUMBER:

BORING NUMBER: *MW781X39*

SOIL BORING LOG

PROJECT NAME:		HOLE DEPTH (ft): 37.00	DRILLING CONTRACTOR:
SURFACE ELEVATION: 71.07 ft. MSL	NORTHING (CCS NAD83 Z2): 1857134.40	EASTING (CCS NAD83 Z2): 6573405.60	DATE STARTED: / DATE COMPLETED:
WATER LEVEL: --- ft. bgs	DRILLING METHOD:		DRILLING EQUIPMENT:
LOCATION: DP039			LOGGED BY:

DEPTH (ft bgs)	INTERVAL (feet)	RECOVERY (%)	SAMPLE #	SPT RESULTS 6"-6"-6" (N)	SIZE DISTRIBUTION %G	%S	%F	SOIL DESCRIPTION	OVM (ppm): BREATHING ZONE	HEAD SPACE	COMMENTS (e.g.: DRILLING FLUID LOSS, TESTS, OR DRILLER COMMENTS, ETC.)
1–4								LEAN CLAY WITH SAND (CL), DARK YELLOWISH BROWN 10YR 4/4, MOIST, VERY STIFF CLAY WITH SILT AND FINE SAND			
4–10								SILTY SAND (SM) VERY PALE BROWN 10YR 7/4, DRY, DENSE, WEAKLY CEMENTED FINE SAND WITH SILT			
10–14								SILTY SAND (SM) BROWNISH YELLOW 10YR 6/6, DRY, DENSE, FINE SAND WITH SILT			
14–15								SILTY SAND (SM) DARK YELLOWISH BROWN 10YR 4/4, DRY, VERY DENSE, FINE SAND WITH SILT			
15–20								WELL GRADED SAND (SW) BROWNISH YELLOW 10YR 6/6, DRY, VERY DENSE, FINE TO COARSE SAND			
20–25								SILTY SAND (SM) PALE YELLOW 5Y 8/2, MOIST, VERY DENSE, FINE SAND WITH SILT			
25–30								SILTY SAND (SM) PALE YELLOW 5Y 8/2, MOIST, VERY DENSE, FINE SAND WITH SILT			
31–35								SILTY SAND (SM) PALE YELLOW 5Y 8/1, MOIST, VERY DENSE, FINE SAND WITH SILT			

CH2MHILL

SHEET 2 of 2	PROJECT NUMBER:	BORING NUMBER: *MW781X39*

SOIL BORING LOG

PROJECT NAME:		HOLE DEPTH (ft): 37.00	DRILLING CONTRACTOR:	
SURFACE ELEVATION: 71.07 ft. MSL	NORTHING (CCS NAD83 Z2): 1857134.40	EASTING (CCS NAD83 Z2): 6573405.60	DATE STARTED:	DATE COMPLETED:
WATER LEVEL: --- ft. bgs	DRILLING METHOD:		DRILLING EQUIPMENT:	
LOCATION: DP039			LOGGED BY:	

DEPTH (ft bgs)	INTERVAL (feet)	RECOVERY (%)	SAMPLE #	SPT RESULTS 6"-6"-6"-6" (N)	SIZE DISTRIBUTION %G	%S	%F	SOIL DESCRIPTION USCS GROUP NAME (USCS GROUP SYMBOL): color, moisture, mineralogy, density, structure, cementation, staining/odor, reaction with HCL. COARSE FRACTION: grain size, angularity, hardness, shape. FINE FRACTION: plasticity, dry strength, toughness, dilatancy. Additional comments.	OVM (ppm): BREATHING ZONE	OVM (ppm): HEAD SPACE	COMMENTS (e.g.: DRILLING FLUID LOSS, TESTS, OR DRILLER COMMENTS, ETC.)
36	–							SILTY SAND (SM) PALE YELLOW 5Y 8/1, MOIST, VERY DENSE, FINE SAND WITH SILT			
37	–							*Boring Terminated at 37 feet bgs*			

CH2MHILL

Attachment 3
Field Documentation

Field Worksheet *Travis AFB*

Project: Travis Enzymes Study	**DO/TO:** 1
PM: Mike Wray	**Field Phone:** 530-604-4129

Site: FT004 **Location** MW591x04 **Sample Date:**

Verified: ☐ Plant operation required: ☑ Northing Easting:

Sample ID **MW591X04-140** **GROUNDWATER** Depth From **15** To: **35** Total Depth **FT**

Lab Name	Methods	Filter	Count	Container	Preservative	QA/QC	By/Time/Date
INL	EAP	☐	1	1L HDPE	4'C	N	KR / 09:45 / 2-21-12
INL	QPCR	☐	1	1L HPDE	4'C	N	/ /
PEL	SW8260B	☐	3	40ml Glass Vial	HCl, pH<2, 4'C	N	/ /

Pump Number: _____

End of Sample ID

End of Location

	Signatures	Date/Time
Sampled by		
Relinquished by		
Received by		
Relinquished by		
Received by		

Travis AFB - Enzyme Study
SAMPLING
LOW-FLOW PURGE FIELD DATA SHEET

SITE ID FT004 **LOCATION ID** MW59/X04

DATE 2.21.12 **JOB NUMBER** 381355.01.01.03.MN

GENERAL INFORMATION

WEATHER (circle) (SUN) CLEAR OVERCAST RAIN

WIND DIRECTION Ø AMBIENT TEMPERATURE 63°

FIELD CONDITIONS Dry

INITIAL PID: WELL CASING Ø BREATHING ZONE Ø

PURGE INFORMATION

START TIME 09:00 END TIME 0950

INITIAL DEPTH TO WATER 10.92 (BTOC) SCREEN INTERVAL 15 TO 35

PUMP (circle one) Portable ~~Dedicated~~ PORTABLE PUMP DEPTH (ft) 30 (BGS)

TOTAL VOLUME PURGED (gal) 1.2 PURGE RATE (~~gpm~~ LPM) 1.0

Stabalization Criteria		+/- .1	+/- 3%	+/- 5	+/- 10%	+/- ~~.2~~ .05	+/- 10%	NA
TIME	VOLUME PURGED (gal)	pH	COND (mS/cm µS/cm) *Circle Units Used*	TURBIDITY (ntu)	DO (mg/L)	TEMP (°C)	ORP (mV)	WATER LEVEL (ft btoc)
9:13	.05	5.95	1.87	0.1	3.57	16.60	187	10.97
9:16	.2	6.43	1.87	0	4.09	16.71	152	10.96
9:19	.4	6.64	1.88	0	3.89	16.60	140	10.96
9:22	.6	6.69	1.87	0	3.84	16.60	138	10.96
9:25	.8	6.78	1.87	0	3.72	16.62	134	10.96
9:28	1.0	6.83	1.87	0	3.63	16.58	132	10.96
9:31	1.2	6.85	1.87	0	3.63	16.54	130	10.96

ADDITIONAL FIELD COMMENTS

TIME	COMMENTS
	Stick up = 24" Above ground surface to TOC

FIELD TEAM (initials) EP, LR

Travis AFB - Enzyme Study
SAMPLING
LOW-FLOW PURGE FIELD DATA SHEET

SITE ID FT004 **LOCATION ID** MW591

DATE 2.21.12 **JOB NUMBER** 381355.01.01.03.MN

FIELD MEASUREMENT/
COLLECTION EQUIPMENT MAKE/MODEL SERIAL/ID #

PID METER _____

HORRIBA U-22 C101991

WATER LEVEL INDICATOR C101970

PUMP TYPE (circle) Grundfos Bladder Barcad Peristaltic Other (specify)

DECOMTAMINATION (Y) N **~~ALCONOX WASH~~** Denatured Alcohol **DISTILLED RINSE** _____

SAMPLING INFORMATION

SAMPLE FIELD ID MW591x04-140

SAMPLE TIME 09:45

SAMPLING WATER LEVEL 10.96

ANALYSIS (circle)

SW9060	SW9056	E160.1	SW8260B	E300.0	E300.0M
SW6010BF	SW6010B	SW8015-D	SW8015-P	SW6850	RSK-175
E310.1	HACH				

Other (specify) qPCR, EAP

FIELD FILTERED Y (N) if yes, for which analysis _____

EQUIPMENT BLANK Y (N)

QA/QC FIELD ID _____ QA/QC TYPE _____

QA/QC FIELD ID _____ QA/QC TYPE _____

QA/QC analysis different from original analysis? (circle) Y N If Yes, specify: _____

COMMENTS/FIELD NOTES: _____

FIELD TEAM (initials) EP, KR

Checklist for Travis AFB Enzyme Sampling Procedures

Date 2-21-12

Travis AFB Site # FT004

Well Number MW591x04

Sample Depth 30 ft bgs/ 30 ft below TOC

Field Duplicate to be Collected No

___ 1) Make sure that at least two coolers are being used. One cooler will be for the volatile organic compound (VOC) samples and the other cooler will be for the quantitative polymerase chain reaction (qPCR) and enzyme activity probe (EAP) samples. Check each cooler to make sure that frozen blue gel packs are being used to keep the groundwater samples cold instead of wet ice.

___ 2) Make sure the VOC, qPCR, and EAP trip blanks are in the coolers.

___ 3) Layout new plastic bag on the ground for a sterilized surface to work on and as a secondary containment.

___ 4) Samplers put on nitrile gloves to protect hands and collect sterile samples.

___ 5) Blot some ethyl alcohol on Kim Wipes and wipe down all instruments, exterior of sample bottles and caps, the gloved hands, the end of the sample tube, and anything else that may come into contact with the sample to sterilize the equipment. The ethyl alcohol will denature and kill any bacteria but is harmless to us (unless ingested or set afire).

___ 6) Measure the depth to groundwater in the well to be sampled. Confirm that there is at least 5 feet of groundwater above the depth that the sample is to be collected. If less than 5 feet of groundwater is present please call Leslie Royer (916-320-3038) or Tony Chakurian (916-468-9447) to get a new sampling depth. Record the depth to water on the sampling field data sheet.

___ 7) Measure and place into the well the appropriate length (the sample depth written above or the depth given by Leslie or Tony) of new poly tubing that will be used to collect the groundwater sample.

___ 8) Change out the motor tubing that is in the head of the peristaltic pump.

___ 9) Start pumping with a peristaltic pump using low groundwater flow procedures with the pumping rate between 1 and 4 liters per minute (L/min).

___ 10) Purge and collect field parameters at intervals of every 3 minutes for a period of at least 15 minutes or when field parameters have stabilized, whichever is longer. Stabilization of field parameters include a maximum change over a 3 minute interval of: +/- 0.1 standard units for pH, +/- 3% for conductivity, +/- 0.5°C, +/- 10% for dissolved oxygen (DO) > 1.0 milligrams per liter (mg/L) or +/- 0.1 mg/L for DO <1.0 mg/L, and +/- 10 mv for redox potential. Document the field parameters on the sampling field sampling sheet.

___ 11) Collect groundwater samples for VOC, qPCR, and EAP analyses. Collect the VOC groundwater sample in three (3) 40-mL VOA viles preserved with hydrochloric acid. Collect the groundwater qPCR sample in one (1) 1-L HDPE bottle. Collect the groundwater EAP sample in one (1) 1-L HDPE bottle. Fill the bottles and VOA viles to form a meniscus at the top of the bottle. Then fill the cap with groundwater and screw the cap on the bottle cap tightly, so as to fill the bottle with no headspace or bubbles. **Make sure that there is no headspace in each of the containers that groundwater samples were collected in**. There can be no head space in the VOC, qPCR, and EAP samples.

___ 12) Seal the bottle caps of the 1-L HDPE bottles that were sampled for qPCR and EAP analyses with parafilm to minimize potential for exposure to air.

___ 13) Label each bottle with a unique sample number, the sample location, date and time of sample collection, sampling depth, groundwater temperature, client, and sampler. Complete the chain-of-custody forms for the sample collected.

___ 14) Place each bottle into a self-sealing plastic bag. Make sure the plastic bag is sealed shut (e.g. Ziploc bag).

___ 15) Place the bottles UPRIGHT on ice for storage prior to packing for shipping.

___ 16) When packing for shipping replace the gel packs that have been used for storage of the samples in the cooler with freshly frozen gel packs as the gel packs melt quickly. Gel packs don't keep as cold as ice. Put gel packs on top, bottom, and sides of cooler with the qPCR and EAP samples. Pack tightly in the UPRIGHT position. If necessary, use packing materials to prevent movement, but be sure ice packs are next to the bottles. Include in the shipping package the chain-of-custody form for the appropriate laboratory. For the cooler that is being sent to the Idaho National Laboratory, a copy of the sampling field data sheets for each groundwater sample needs to be sent with the shipment.

___ 17) Send samples for the Idaho National Laboratory by Priority Overnight Express (arrival by 10 am the following day). No more than four (4) qPCR and EAP samples can be sent to the Idaho National Laboratory each day. Contact either M. Hope Lee (cell 1 [240-818-2987] and cell 2 [208-351-8148]) or Brady Lee (office [208-526-0981] and cell [208-520-1617]) of the Idaho National Laboratory with the courier reference number for each sample shipment on the day of the shipment. The personnel from Idaho National Laboratory will track the shipment and will investigate if the shipment does not arrive at the expected time so that the appropriate corrective action can be initiated. Samples can be only accepted Monday through Thursday from 8 am -5 pm MST, except on holidays. Contact if you have questions.

___ 18) Send samples to Spectrum Analytical by Standard Overnight Express (arrival by 3 pm the following day).

___ 19) Send the trip blanks for the qPCR and the EAP samples to the Idaho National Laboratory with the second shipment. Send a trip blank for VOCs to Spectrum Analytical with each

Field Worksheet *Travis AFB*

Project: Travis Enzymes Study	**DO/TO:** 1
PM: Mike Wray	**Field Phone:** 530-604-4129

Site: FT004 **Location** MW131x04 **Sample Date:**

Verified: ☐ Plant operation required: ☑ Northing Easting:

Sample ID **MW131X04-140** **GROUNDWATER** Depth From **10** To: **30** Total Depth **FT**

Lab Name	Methods	Filter	Count	Container	Preservative	QA/QC	By/Time/Date
INL	EAP	☐	1	1L HDPE	4'C	N	KR / 1150 / 12.21.12
INL	QPCR	☐	1	1L HPDE	4'C	N	/ /
PEL	SW8260B	☐	3	40ml Glass Vial	HCl, pH<2, 4'C	N	/ /

Pump Number: _____

End of Sample ID

End of Location

	Signatures	Date/Time
Sampled by		
Relinquished by		
Received by		
Relinquished by		
Received by		

Travis AFB - Enzyme Study
SAMPLING
LOW-FLOW PURGE FIELD DATA SHEET

SITE ID FT004 LOCATION ID MW131

DATE 2.21.12 JOB NUMBER 381355.01.01.03.MN

GENERAL INFORMATION

WEATHER (circle) (SUN) (CLEAR) OVERCAST RAIN

WIND DIRECTION 0 AMBIENT TEMPERATURE 67

FIELD CONDITIONS DRY

INITIAL PID: WELL CASING 0 BREATHING ZONE 0

PURGE INFORMATION

START TIME 1115 END TIME 1155

INITIAL DEPTH TO WATER 9.77 SCREEN INTERVAL 10 TO 30

PUMP (circle one) Portable (Dedicated) PORTABLE PUMP DEPTH (ft) 16.6' BTOC

TOTAL VOLUME PURGED (gal) 1.2 PURGE RATE (~~gpm~~ LPM) 1.0

Stabalization Criteria		+/- .1	+/- 3%	+/- 5	+/- 10%	+/- .2	+/- 10%	NA
TIME	VOLUME PURGED (gal)	pH	COND (~~mS/cm~~ µS/cm) *Circle Units Used*	TURBIDITY (ntu)	DO (mg/L)	TEMP (°C)	ORP (mV)	WATER LEVEL (ft btoc)
1118	.05	6.84	4.78	12.5	2.45	18.30	115	9.88
1121	.2	6.85	4.84	0.6	0.02	17.95	101	9.96
1124	.4	6.86	4.70	1.3	0.00	17.97	91	9.90
1127	.6	6.90	4.67	0	0	17.82	80	9.89
1130	.8	6.89	4.67	0	0	17.91	79	9.89
1133	1.0	6.88	4.67	0	0	17.98	76	9.89
1136	1.2	6.88	4.68	0	0	17.99	76	9.89

ADDITIONAL FIELD COMMENTS

TIME	COMMENTS
	Stick up = 1.6' above Ground surface to TOC

FIELD TEAM (initials) EP, KR

Travis AFB - Enzyme Study
SAMPLING
LOW-FLOW PURGE FIELD DATA SHEET

SITE ID ~~ET004~~ **LOCATION ID** MW131

DATE 2-21-12 **JOB NUMBER** 381355.01.01.03.MN

FIELD MEASUREMENT/
COLLECTION EQUIPMENT MAKE/MODEL SERIAL/ID #

PID METER _____

HORRIBA U-22 C101991

WATER LEVEL INDICATOR C101970

PUMP TYPE (circle) **Grundfos** **Bladder** **Barcad** **Peristaltic** **Other (specify)**

DECOMTAMINATION Y N ~~**ALCONOX WASH**~~ DENATURED ALCOHOL ~~**DISTILLED RINSE**~~ _____

SAMPLING INFORMATION

SAMPLE FIELD ID MW131x04-140

SAMPLE TIME 1150

SAMPLING WATER LEVEL 9.89

ANALYSIS (circle)

SW9060	SW9056	E160.1	~~SW8260B~~	E300.0	E300.0M
SW6010BF	SW6010B	SW8015-D	SW8015-P	SW6850	RSK-175
E310.1	HACH				

Other (specify) EAP, qPCR

FIELD FILTERED Y (N) if yes, for which analysis _____

EQUIPMENT BLANK Y (N)

QA/QC FIELD ID _____ QA/QC TYPE _____

QA/QC FIELD ID _____ QA/QC TYPE _____

QA/QC analysis different from original analysis? (circle) Y N If Yes, specify: _____

COMMENTS/FIELD NOTES: _____

FIELD TEAM (initials) EP, RR

Checklist for Travis AFB Enzyme Sampling Procedures

Date 2-21-12

Travis AFB Site # FT004

Well Number MW131x04

Sample Depth 15 ft bgs/ 18.43 ft below TOC

Field Duplicate to be Collected No

1) Make sure that at least two coolers are being used. One cooler will be for the volatile organic compound (VOC) samples and the other cooler will be for the quantitative polymerase chain reaction (qPCR) and enzyme activity probe (EAP) samples. Check each cooler to make sure that frozen blue gel packs are being used to keep the groundwater samples cold instead of wet ice.

2) Make sure the VOC, qPCR, and EAP trip blanks are in the coolers.

3) Layout new plastic bag on the ground for a sterilized surface to work on and as a secondary containment.

4) Samplers put on nitrile gloves to protect hands and collect sterile samples.

5) Blot some ethyl alcohol on Kim Wipes and wipe down all instruments, exterior of sample bottles and caps, the gloved hands, the end of the sample tube, and anything else that may come into contact with the sample to sterilize the equipment. The ethyl alcohol will denature and kill any bacteria but is harmless to us (unless ingested or set afire).

6) Measure the depth to groundwater in the well to be sampled. Confirm that there is at least 5 feet of groundwater above the depth that the sample is to be collected. If less than 5 feet of groundwater is present please call Leslie Royer (916-320-3038) or Tony Chakurian (916-468-9447) to get a new sampling depth. Record the depth to water on the sampling field data sheet.

7) Measure and place into the well the appropriate length (the sample depth written above or the depth given by Leslie or Tony) of new poly tubing that will be used to collect the groundwater sample.

8) Change out the motor tubing that is in the head of the peristaltic pump.

9) Start pumping with a peristaltic pump using low groundwater flow procedures with the pumping rate between 1 and 4 liters per minute (L/min).

10) Purge and collect field parameters at intervals of every 3 minutes for a period of at least 15 minutes or when field parameters have stabilized, whichever is longer. Stabilization of field parameters include a maximum change over a 3 minute interval of: +/- 0.1 standard units for pH, +/- 3% for conductivity, +/- 0.5°C, +/- 10% for dissolved oxygen (DO) > 1.0 milligrams per liter (mg/L) or +/- 0.1 mg/L for DO <1.0 mg/L, and +/- 10 mv for redox potential. Document the field parameters on the sampling field sampling sheet.

___ 11) Collect groundwater samples for VOC, qPCR, and EAP analyses. Collect the VOC groundwater sample in three (3) 40-mL VOA viles preserved with hydrochloric acid. Collect the groundwater qPCR sample in one (1) 1-L HDPE bottle. Collect the groundwater EAP sample in one (1) 1-L HDPE bottle. Fill the bottles and VOA viles to form a meniscus at the top of the bottle. Then fill the cap with groundwater and screw the cap on the bottle cap tightly, so as to fill the bottle with no headspace or bubbles. **Make sure that there is no headspace in each of the containers that groundwater samples were collected in**. There can be no head space in the VOC, qPCR, and EAP samples.

___ 12) Seal the bottle caps of the 1-L HDPE bottles that were sampled for qPCR and EAP analyses with parafilm to minimize potential for exposure to air.

___ 13) Label each bottle with a unique sample number, the sample location, date and time of sample collection, sampling depth, groundwater temperature, client, and sampler. Complete the chain-of-custody forms for the sample collected.

___ 14) Place each bottle into a self-sealing plastic bag. Make sure the plastic bag is sealed shut (e.g. Ziploc bag).

___ 15) Place the bottles UPRIGHT on ice for storage prior to packing for shipping.

___ 16) When packing for shipping replace the gel packs that have been used for storage of the samples in the cooler with freshly frozen gel packs as the gel packs melt quickly. Gel packs don't keep as cold as ice. Put gel packs on top, bottom, and sides of cooler with the qPCR and EAP samples. Pack tightly in the UPRIGHT position. If necessary, use packing materials to prevent movement, but be sure ice packs are next to the bottles. Include in the shipping package the chain-of-custody form for the appropriate laboratory. For the cooler that is being sent to the Idaho National Laboratory, a copy of the sampling field data sheets for each groundwater sample needs to be sent with the shipment.

___ 17) Send samples for the Idaho National Laboratory by Priority Overnight Express (arrival by 10 am the following day). No more than four (4) qPCR and EAP samples can be sent to the Idaho National Laboratory each day. Contact either M. Hope Lee (cell 1 [240-818-2987] and cell 2 [208-351-8148]) or Brady Lee (office [208-526-0981] and cell [208-520-1617]) of the Idaho National Laboratory with the courier reference number for each sample shipment on the day of the shipment. The personnel from Idaho National Laboratory will track the shipment and will investigate if the shipment does not arrive at the expected time so that the appropriate corrective action can be initiated. Samples can be only accepted Monday through Thursday from 8 am -5 pm MST, except on holidays. Contact if you have questions.

___ 18) Send samples to Spectrum Analytical by Standard Overnight Express (arrival by 3 pm the following day).

___ 19) Send the trip blanks for the qPCR and the EAP samples to the Idaho National Laboratory with the second shipment. Send a trip blank for VOCs to Spectrum Analytical with each

shipment. The VOC samples can be held until all of the samples are collected and shipped to Spectrum Analytical at the same time.

Laboratory Shipping Addresses:

Attn: Hope or Brady Lee
Idaho National Laboratory
1765 North Yellowstone Highway
IF 603
Lab 103
Idaho Falls, ID 83402

Receiving Sample
Spectrum Analytical, Inc.
8405 Benjamin Rd
Suite A
Tampa, FL 33634

(813) 888-9507

Field Worksheet *Travis AFB*

Project: Travis Enzymes Study	**DO/TO:** 1
PM: Mike Wray	**Field Phone:** 530-604-4129

Site: FT004 **Location** MW264x04 **Sample Date:**

Verified: ☐ Plant operation required: ☑ Northing Easting:

Sample ID **MW264X04-140** **GROUNDWATER** Depth From **15** To: **25** Total Depth FT

Lab Name	Methods	Filter	Count	Container	Preservative	QA/QC	By/Time/Date
INL	EAP	☐	1	1L HDPE	4'C	N	RR / 1250 / 2-21-12
INL	QPCR	☐	1	1L HPDE	4'C	N	/ /
PEL	SW8260B	☐	3	40ml Glass Vial	HCl, pH<2, 4'C	N	/ /

Pump Number:

End of Sample ID

End of Location

	Signatures	Date/Time
Sampled by		
Relinquished by		
Received by		
Relinquished by		
Received by		

Travis AFB - Enzyme Study
SAMPLING
LOW-FLOW PURGE FIELD DATA SHEET

SITE ID FZ004 LOCATION ID MW26

DATE 2-21-12 JOB NUMBER 381355.01.01.03.MN

GENERAL INFORMATION

WEATHER (circle) (SUN) (CLEAR) OVERCAST RAIN

WIND DIRECTION 0 AMBIENT TEMPERATURE 70°F

FIELD CONDITIONS DRY

INITIAL PID: WELL CASING 0 BREATHING ZONE 0

PURGE INFORMATION

START TIME 1217 END TIME 1300

INITIAL DEPTH TO WATER 11.10 SCREEN INTERVAL 15 TO 25

PUMP (circle one) Portable (Dedicated) PORTABLE PUMP DEPTH (ft) 16.1' (BTOC)

TOTAL VOLUME PURGED (gal) 1.2 PURGE RATE (~~gpm~~ LPM) 1.0

Stabalization Criteria		+/- .1	+/- 3%	+/- 5	+/- 10%	+/- 2.5°C	+/- 10%	NA
TIME	VOLUME PURGED (gal)	pH	COND (~~mS/cm~~ µS/cm) *Circle Units Used*	TURBIDITY (ntu)	DO (mg/L)	TEMP (°C)	ORP (mV)	WATER LEVEL (ft btoc)
1220	.05	6.90	6.80	89.9	1.81	18.17	130	11.31
1223	.2	6.87	6.78	69.9	0.35	17.96	97	11.51
1226	.4	6.85	6.65	62.5	0.0	17.84	64	11.69
1229	.6	6.85	6.56	42.2	0.0	17.85	61	11.79
1232	.8	6.85	6.55	40.8	0	17.83	61	11.83
1235	1.0	6.85	6.59	40.0	0	17.88	62	11.83
1238	1.2	6.85	6.67	38.8	0	17.86	62	11.83

ADDITIONAL FIELD COMMENTS

TIME	COMMENTS
	Stick up = 1.1' Above ground surface to TOC
	Water cloudy (slightly)

FIELD TEAM (initials) EP, LR

Travis AFB - Enzyme Study
SAMPLING
LOW-FLOW PURGE FIELD DATA SHEET

SITE ID ~~MW26~~ FD004 **LOCATION ID** MW264

DATE 2-21-12 **JOB NUMBER** 381355.01.01.03.MN

FIELD MEASUREMENT/
COLLECTION EQUIPMENT MAKE/MODEL SERIAL/ID #

PID METER _____

HORRIBA U-22 C101991

WATER LEVEL INDICATOR C101970

PUMP TYPE (circle) Grundfos Bladder Barcad Peristaltic Other (specify)

DECOMTAMINATION (Y) N ~~ALCONOX WASH~~ Denatured Alcohol ~~DISTILLED RINSE~~

SAMPLING INFORMATION

SAMPLE FIELD ID MW264x04-140

SAMPLE TIME 1250

SAMPLING WATER LEVEL 11.83

ANALYSIS (circle)

SW9060	SW9056	E160.1	~~SW8260B~~	E300.0	E300.0M
SW6010BF	SW6010B	SW8015-D	SW8015-P	SW6850	RSK-175
E310.1	HACH				

Other (specify) qPCR, EAP

FIELD FILTERED Y (N) if yes, for which analysis _____

EQUIPMENT BLANK Y (N)

QA/QC FIELD ID _____ QA/QC TYPE _____

QA/QC FIELD ID _____ QA/QC TYPE _____

QA/QC analysis different from original analysis? (circle) Y N If Yes, specify: _____

COMMENTS/FIELD NOTES: _____

FIELD TEAM (initials) EP, KR

Checklist for Travis AFB Enzyme Sampling Procedures

Date 2-21-12

Travis AFB Site # FT004

Well Number MW264x04

Sample Depth 15 ft bgs / 19.19 ft below TOC

Field Duplicate to be Collected No

___ 1) Make sure that at least two coolers are being used. One cooler will be for the volatile organic compound (VOC) samples and the other cooler will be for the quantitative polymerase chain reaction (qPCR) and enzyme activity probe (EAP) samples. Check each cooler to make sure that frozen blue gel packs are being used to keep the groundwater samples cold instead of wet ice.

___ 2) Make sure the VOC, qPCR, and EAP trip blanks are in the coolers.

___ 3) Layout new plastic bag on the ground for a sterilized surface to work on and as a secondary containment.

___ 4) Samplers put on nitrile gloves to protect hands and collect sterile samples.

___ 5) Blot some ethyl alcohol on Kim Wipes and wipe down all instruments, exterior of sample bottles and caps, the gloved hands, the end of the sample tube, and anything else that may come into contact with the sample to sterilize the equipment. The ethyl alcohol will denature and kill any bacteria but is harmless to us (unless ingested or set afire).

___ 6) Measure the depth to groundwater in the well to be sampled. Confirm that there is at least 5 feet of groundwater above the depth that the sample is to be collected. If less than 5 feet of groundwater is present please call Leslie Royer (916-320-3038) or Tony Chakurian (916-468-9447) to get a new sampling depth. Record the depth to water on the sampling field data sheet.

___ 7) Measure and place into the well the appropriate length (the sample depth written above or the depth given by Leslie or Tony) of new poly tubing that will be used to collect the groundwater sample.

___ 8) Change out the motor tubing that is in the head of the peristaltic pump.

___ 9) Start pumping with a peristaltic pump using low groundwater flow procedures with the pumping rate between 1 and 4 liters per minute (L/min).

___ 10) Purge and collect field parameters at intervals of every 3 minutes for a period of at least 15 minutes or when field parameters have stabilized, whichever is longer. Stabilization of field parameters include a maximum change over a 3 minute interval of: +/- 0.1 standard units for pH, +/- 3% for conductivity, +/- 0.5°C, +/- 10% for dissolved oxygen (DO) > 1.0 milligrams per liter (mg/L) or +/- 0.1 mg/L for DO <1.0 mg/L, and +/- 10 mv for redox potential. Document the field parameters on the sampling field sampling sheet.

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___ 12) Seal the bottle caps of the 1-L HDPE bottles that were sampled for qPCR and EAP analyses with parafilm to minimize potential for exposure to air.

___ 13) Label each bottle with a unique sample number, the sample location, date and time of sample collection, sampling depth, groundwater temperature, client, and sampler. Complete the chain-of-custody forms for the sample collected.

___ 14) Place each bottle into a self-sealing plastic bag. Make sure the plastic bag is sealed shut (e.g. Ziploc bag).

___ 15) Place the bottles UPRIGHT on ice for storage prior to packing for shipping.

___ 16) When packing for shipping replace the gel packs that have been used for storage of the samples in the cooler with freshly frozen gel packs as the gel packs melt quickly. Gel packs don't keep as cold as ice. Put gel packs on top, bottom, and sides of cooler with the qPCR and EAP samples. Pack tightly in the UPRIGHT position. If necessary, use packing materials to prevent movement, but be sure ice packs are next to the bottles. Include in the shipping package the chain-of-custody form for the appropriate laboratory. For the cooler that is being sent to the Idaho National Laboratory, a copy of the sampling field data sheets for each groundwater sample needs to be sent with the shipment.

___ 17) Send samples for the Idaho National Laboratory by Priority Overnight Express (arrival by 10 am the following day). No more than four (4) qPCR and EAP samples can be sent to the Idaho National Laboratory each day. Contact either M. Hope Lee (cell 1 [240-818-2987] and cell 2 [208-351-8148]) or Brady Lee (office [208-526-0981] and cell [208-520-1617]) of the Idaho National Laboratory with the courier reference number for each sample shipment on the day of the shipment. The personnel from Idaho National Laboratory will track the shipment and will investigate if the shipment does not arrive at the expected time so that the appropriate corrective action can be initiated. Samples can be only accepted Monday through Thursday from 8 am -5 pm MST, except on holidays. Contact if you have questions.

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___ 19) Send the trip blanks for the qPCR and the EAP samples to the Idaho National Laboratory with the second shipment. Send a trip blank for VOCs to Spectrum Analytical with each

Field Worksheet *Travis AFB*

Project: Travis Enzymes Study	**DO/TO:** 1
PM: Mike Wray	**Field Phone:** 530-604-4129

Site: FT004 **Location** MW266x04 **Sample Date:**

Verified: ☐ Plant operation required: ☑ Northing Easting:

Sample ID **MW266X04-140** **GROUNDWATER** Depth From **6** To: **16** Total Depth **FT**

Lab Name	Methods	Filter	Count	Container	Preservative	QA/QC	By/Time/Date
INL	EAP	☐	1	1L HDPE	4'C	N	KR / 1050 / 2-21-12
INL	QPCR	☐	1	1L HPDE	4'C	N	/ /
PEL	SW8260B	☐	3	40ml Glass Vial	HCl, pH<2, 4'C	N	/ /

Pump Number: _____

End of Sample ID

End of Location

	Signatures	Date/Time
Sampled by		
Relinquished by		
Received by		
Relinquished by		
Received by		

Travis AFB - Enzyme Study
SAMPLING
LOW-FLOW PURGE FIELD DATA SHEET

SITE ID FB004 LOCATION ID MW266

DATE 2.21.12 JOB NUMBER 381355.01.01.03.MN

GENERAL INFORMATION

WEATHER (circle) (SUN) CLEAR OVERCAST RAIN

WIND DIRECTION 0 AMBIENT TEMPERATURE 65°F

FIELD CONDITIONS DRY

INITIAL PID: WELL CASING 0 BREATHING ZONE 0

PURGE INFORMATION

START TIME 1012 END TIME 11:00

INITIAL DEPTH TO WATER 9.76 (BTOC) SCREEN INTERVAL 6 TO 16

PUMP (circle one) Portable (Dedicated) PORTABLE PUMP DEPTH (ft) 17.4 (BTOC)

TOTAL VOLUME PURGED (gal) 1.8 PURGE RATE ~~(gpm)~~ LPM 1.0

Stabalization Criteria		+/- .1	+/- 3%	+/- 5	+/- 10%	+/- 2.5°C	+/- 10%	NA
TIME	VOLUME PURGED (gal)	pH	COND (~~mS/cm~~ µS/cm) *Circle Units Used*	TURBIDITY (ntu)	DO (mg/L)	TEMP (°C)	ORP (mV)	WATER LEVEL (ft btoc)
1015	.05	7.02	1.96	164.	2.27	15.76	-22	9.91
1018	.2	6.97	1.92	95.7	0.44	15.78	-2	9.96
1021	.4	6.96	1.96	82.0	0.32	15.73	8	9.99
1024	.6	6.95	1.91	96.2	0.10	15.82	11	10.08
1027	.8	6.95	1.92	96.4	0.16	15.86	14	10.08
1030	1.0	6.95	1.91	91.0	0.20	15.91	10	10.10
1033	1.2	6.95	1.91	105.0	0.20	15.87	13	10.12
1036	1.4	6.96	1.92	104.0	0.08	15.87	15	10.16
1039	1.6	6.95	1.91	102.0	0.07	16.07	16	10.17
1041	1.8	6.96	1.91	102.1	0.08	16.04	16	10.17

ADDITIONAL FIELD COMMENTS

TIME	COMMENTS
	Stick up 2.4' Above Ground Surface
	light brown particles seen in water

FIELD TEAM (initials) EP KR

Travis AFB - Enzyme Study
SAMPLING
LOW-FLOW PURGE FIELD DATA SHEET

SITE ID FT004 **LOCATION ID** MW266

DATE 2-21-17 **JOB NUMBER** 381355.01.01.03.MN

FIELD MEASUREMENT/
COLLECTION EQUIPMENT MAKE/MODEL SERIAL/ID #

PID METER _____

HORRIBA U-22 C101991

WATER LEVEL INDICATOR C101970

PUMP TYPE (circle) Grundfos **Bladder** Barcad ~~Peristaltic~~ **Other (specify)**

DECOMTAMINATION Y N ~~ALCONOX WASH~~ Dea. Alcohol ~~DISTILLED RINSE~~

SAMPLING INFORMATION

SAMPLE FIELD ID MW266x04

SAMPLE TIME 1050

SAMPLING WATER LEVEL 10.17'

ANALYSIS (circle)

SW9060	SW9056	E160.1	~~SW8260B~~	E300.0	E300.0M
SW6010BF	SW6010B	SW8015-D	SW8015-P	SW6850	RSK-175
E310.1	HACH				

Other (specify) qPCR, EAP

FIELD FILTERED Y N if yes, for which analysis _____

EQUIPMENT BLANK Y N

QA/QC FIELD ID _____ QA/QC TYPE _____

QA/QC FIELD ID _____ QA/QC TYPE _____

QA/QC analysis different from original analysis? (circle) Y N If Yes, specify: _____

COMMENTS/FIELD NOTES: _____

FIELD TEAM (initials) EP, KL

Checklist for Travis AFB Enzyme Sampling Procedures

Date 2-21-12

Travis AFB Site # FT004

Well Number MW266x04

Sample Depth 15 ft bgs / 19.47 ft below TOC

Field Duplicate to be Collected No

___ 1) Make sure that at least two coolers are being used. One cooler will be for the volatile organic compound (VOC) samples and the other cooler will be for the quantitative polymerase chain reaction (qPCR) and enzyme activity probe (EAP) samples. Check each cooler to make sure that frozen blue gel packs are being used to keep the groundwater samples cold instead of wet ice.

___ 2) Make sure the VOC, qPCR, and EAP trip blanks are in the coolers.

___ 3) Layout new plastic bag on the ground for a sterilized surface to work on and as a secondary containment.

___ 4) Samplers put on nitrile gloves to protect hands and collect sterile samples.

___ 5) Blot some ethyl alcohol on Kim Wipes and wipe down all instruments, exterior of sample bottles and caps, the gloved hands, the end of the sample tube, and anything else that may come into contact with the sample to sterilize the equipment. The ethyl alcohol will denature and kill any bacteria but is harmless to us (unless ingested or set afire).

___ 6) Measure the depth to groundwater in the well to be sampled. Confirm that there is at least 5 feet of groundwater above the depth that the sample is to be collected. If less than 5 feet of groundwater is present please call Leslie Royer (916-320-3038) or Tony Chakurian (916-468-9447) to get a new sampling depth. Record the depth to water on the sampling field data sheet.

___ 7) Measure and place into the well the appropriate length (the sample depth written above or the depth given by Leslie or Tony) of new poly tubing that will be used to collect the groundwater sample.

___ 8) Change out the motor tubing that is in the head of the peristaltic pump.

___ 9) Start pumping with a peristaltic pump using low groundwater flow procedures with the pumping rate between 1 and 4 liters per minute (L/min).

___ 10) Purge and collect field parameters at intervals of every 3 minutes for a period of at least 15 minutes or when field parameters have stabilized, whichever is longer. Stabilization of field parameters include a maximum change over a 3 minute interval of: +/- 0.1 standard units for pH, +/- 3% for conductivity, +/- 0.5°C, +/- 10% for dissolved oxygen (DO) > 1.0 milligrams per liter (mg/L) or +/- 0.1 mg/L for DO <1.0 mg/L, and +/- 10 mv for redox potential. Document the field parameters on the sampling field sampling sheet.

11) Collect groundwater samples for VOC, qPCR, and EAP analyses. Collect the VOC groundwater sample in three (3) 40-mL VOA viles preserved with hydrochloric acid. Collect the groundwater qPCR sample in one (1) 1-L HDPE bottle. Collect the groundwater EAP sample in one (1) 1-L HDPE bottle. Fill the bottles and VOA viles to form a meniscus at the top of the bottle. Then fill the cap with groundwater and screw the cap on the bottle cap tightly, so as to fill the bottle with no headspace or bubbles. **Make sure that there is no headspace in each of the containers that groundwater samples were collected in**. There can be no head space in the VOC, qPCR, and EAP samples.

12) Seal the bottle caps of the 1-L HDPE bottles that were sampled for qPCR and EAP analyses with parafilm to minimize potential for exposure to air.

13) Label each bottle with a unique sample number, the sample location, date and time of sample collection, sampling depth, groundwater temperature, client, and sampler. Complete the chain-of-custody forms for the sample collected.

14) Place each bottle into a self-sealing plastic bag. Make sure the plastic bag is sealed shut (e.g. Ziploc bag).

15) Place the bottles UPRIGHT on ice for storage prior to packing for shipping.

16) When packing for shipping replace the gel packs that have been used for storage of the samples in the cooler with freshly frozen gel packs as the gel packs melt quickly. Gel packs don't keep as cold as ice. Put gel packs on top, bottom, and sides of cooler with the qPCR and EAP samples. Pack tightly in the UPRIGHT position. If necessary, use packing materials to prevent movement, but be sure ice packs are next to the bottles. Include in the shipping package the chain-of-custody form for the appropriate laboratory. For the cooler that is being sent to the Idaho National Laboratory, a copy of the sampling field data sheets for each groundwater sample needs to be sent with the shipment.

17) Send samples for the Idaho National Laboratory by Priority Overnight Express (arrival by 10 am the following day). No more than four (4) qPCR and EAP samples can be sent to the Idaho National Laboratory each day. Contact either M. Hope Lee (cell 1 [240-818-2987] and cell 2 [208-351-8148]) or Brady Lee (office [208-526-0981] and cell [208-520-1617]) of the Idaho National Laboratory with the courier reference number for each sample shipment on the day of the shipment. The personnel from Idaho National Laboratory will track the shipment and will investigate if the shipment does not arrive at the expected time so that the appropriate corrective action can be initiated. Samples can be only accepted Monday through Thursday from 8 am -5 pm MST, except on holidays. Contact if you have questions.

18) Send samples to Spectrum Analytical by Standard Overnight Express (arrival by 3 pm the following day).

19) Send the trip blanks for the qPCR and the EAP samples to the Idaho National Laboratory with the second shipment. Send a trip blank for VOCs to Spectrum Analytical with each

shipment. The VOC samples can be held until all of the samples are collected and shipped to Spectrum Analytical at the same time.

Laboratory Shipping Addresses:

Attn: Hope or Brady Lee
Idaho National Laboratory
1765 North Yellowstone Highway
IF 603
Lab 103
Idaho Falls, ID 83402

Receiving Sample
Spectrum Analytical, Inc.
8405 Benjamin Rd
Suite A
Tampa, FL 33634

(813) 888-9507

id Worksheet Travis AFB

Project: Travis Enzymes Study	DO/TO: 1
PM: Mike Wray	Field Phone: 530-604-4129

Site: DP039 **Location MW781x39** **Sample Date:**

Verified: ☐ Plant operation required: ☑ Northing Easting:

Sample ID **MW781X39-140** **GROUNDWATER** Depth From **27** To: **37** Total Depth **FT**

Lab Name	Methods	Filter	Count	Container	Preservative	QA/QC	By/Time/Date
INL	EAP	☐	1	1L HDPE	4'C	N	KR 0755 / 2-22-12
INL	QPCR	☐	1	1L HPDE	4'C	N	/ /
PEL	SW8260B	☐	3	40ml Glass Vial	HCl, pH<2, 4'C	N	/ /

Pump Number:

End of Sample ID

Sample ID **MW781X39-140B** **GROUNDWATER** Depth From **27** To: **37** Total Depth **FT**

Lab Name	Methods	Filter	Count	Container	Preservative	QA/QC	By/Time/Date
INL	EAP	☐	1	1L HDPE	4'C	FD	KR 0755 / 2-22-12
INL	QPCR	☐	1	1L HPDE	4'C	FD	/ /
PEL	SW8260B	☐	3	40ml Glass Vial	HCl, pH<2, 4'C	FD	/ /

Pump Number:

End of Sample ID

End of Location

	Signatures	Date/Time
Sampled by		
Relinquished by		
Received by		
Relinquished by		
Received by		

Travis AFB - Enzyme Study
SAMPLING
LOW-FLOW PURGE FIELD DATA SHEET

SITE ID DP039 **LOCATION ID** MW781

DATE 2-22-12 **JOB NUMBER** 381355.01.01.03.MN

GENERAL INFORMATION

WEATHER (circle) ~~SUN~~ CLEAR OVERCAST RAIN

WIND DIRECTION 0 AMBIENT TEMPERATURE 58°F

FIELD CONDITIONS DRY

INITIAL PID: WELL CASING 0 BREATHING ZONE 0

PURGE INFORMATION

START TIME 0717 END TIME 0805

INITIAL DEPTH TO WATER 27.11 SCREEN INTERVAL 27 TO 37

PUMP (circle one) Portable ~~Dedicated~~ PORTABLE PUMP DEPTH (ft) 32' (BGS)

TOTAL VOLUME PURGED (gal) 1.2 PURGE RATE (~~gpm~~ LPM) 1.0

Stabalization Criteria		+/- .1	+/- 3%	+/- 5	+/- 10%	+/- .2	+/- 10%	NA
TIME	VOLUME PURGED (gal)	pH	COND (~~mS/cm~~ µS/cm) *Circle Units Used*	TURBIDITY (ntu)	DO (mg/L)	TEMP (°C)	ORP (mV)	WATER LEVEL (ft btoc)
0720	.05	5.88	1.23	0	4.01	16.52	219	27.12
0723	.2	6.11	1.23	0	2.76	16.95	200	27.25
0726	.4	6.33	1.22	0	2.87	17.23	187	27.29
0729	.6	6.47	1.22	0	2.94	17.49	180	27.31
0732	.8	6.58	1.22	0	2.91	17.27	175	27.36
0735	1.0	6.65	1.23	0	3.05	17.17	173	27.41
0738	1.2	6.69	1.24	0	3.01	17.12	172	27.41

ADDITIONAL FIELD COMMENTS

TIME	COMMENTS
	Flush mount MW

FIELD TEAM (initials) EP, KR

Travis AFB - Enzyme Study
SAMPLING
LOW-FLOW PURGE FIELD DATA SHEET

SITE ID 0039 **LOCATION ID** MW781

DATE 2-22-12 **JOB NUMBER** 381355.01.01.03.MN

FIELD MEASUREMENT/
COLLECTION EQUIPMENT MAKE/MODEL SERIAL/ID #

PID METER _____

HORRIBA U-22 C161991

WATER LEVEL INDICATOR _____

PUMP TYPE (circle) Grundfos Bladder Barcad (Peristaltic) Other (specify)

DECOMTAMINATION (Y) N ~~ALCONOX WASH~~ Den. Alcohol _____ ~~DISTILLED RINSE~~ _____

SAMPLING INFORMATION

SAMPLE FIELD ID MW781x39-140

SAMPLE TIME 0755

SAMPLING WATER LEVEL 27.41

ANALYSIS (circle)

SW9060	SW9056	E160.1	(~~SW8260B~~)	E300.0	E300.0M
SW6010BF	SW6010B	SW8015-D	SW8015-P	SW6850	RSK-175
E310.1	HACH				

Other (specify) EAP, qPCR

FIELD FILTERED Y (N) if yes, for which analysis _____

EQUIPMENT BLANK Y (N)

QA/QC FIELD ID MW781x39-140B QA/QC TYPE F.D.

QA/QC FIELD ID _____ QA/QC TYPE _____

QA/QC analysis different from original analysis? (circle) Y (N) If Yes, specify: _____

COMMENTS/FIELD NOTES: _____

FIELD TEAM (initials) EP, KR

Checklist for Travis AFB Enzyme Sampling Procedures

Date 2-22-12

Travis AFB Site # DP039

Well Number MW781x39

Sample Depth 32

Field Duplicate to be Collected Yes

✓ 1) Make sure that at least two coolers are being used. One cooler will be for the volatile organic compound (VOC) samples and the other cooler will be for the quantitative polymerase chain reaction (qPCR) and enzyme activity probe (EAP) samples. Check each cooler to make sure that frozen blue gel packs are being used to keep the groundwater samples cold instead of wet ice.

✓ 2) Make sure the VOC, qPCR, and EAP trip blanks are in the coolers.

✓ 3) Layout new plastic bag on the ground for a sterilized surface to work on and as a secondary containment.

✓ 4) Samplers put on nitrile gloves to protect hands and collect sterile samples.

✓ 5) Blot some ethyl alcohol on Kim Wipes and wipe down all instruments, exterior of sample bottles and caps, the gloved hands, the end of the sample tube, and anything else that may come into contact with the sample to sterilize the equipment. The ethyl alcohol will denature and kill any bacteria but is harmless to us (unless ingested or set afire).

✓ 6) Measure the depth to groundwater in the well to be sampled. Confirm that there is at least 5 feet of groundwater above the depth that the sample is to be collected. If less than 5 feet of groundwater is present please call Leslie Royer (916-320-3038) or Tony Chakurian (916-468-9447) to get a new sampling depth. Record the depth to water on the sampling field data sheet.

✓ 7) Measure and place into the well the appropriate length (the sample depth written above or the depth given by Leslie or Tony) of new poly tubing that will be used to collect the groundwater sample.

✓ 8) Change out the motor tubing that is in the head of the peristaltic pump.

✓ 9) Start pumping with a peristaltic pump using low groundwater flow procedures with the pumping rate between 1 and 4 liters per minute (L/min).

✓ 10) Purge and collect field parameters at intervals of every 3 minutes for a period of at least 15 minutes or when field parameters have stabilized, whichever is longer. Stabilization of field parameters include a maximum change over a 3 minute interval of: +/- 0.1 standard units for pH, +/- 3% for conductivity, +/- 0.5°C, +/- 10% for dissolved oxygen (DO) > 1.0 milligrams per liter (mg/L) or +/- 0.1 mg/L for DO <1.0 mg/L, and +/- 10 mv for redox potential. Document the field parameters on the sampling field sampling sheet.

___ 11) Collect groundwater samples for VOC, qPCR, and EAP analyses. Collect the VOC groundwater sample in three (3) 40-mL VOA viles preserved with hydrochloric acid. Collect the groundwater qPCR sample in one (1) 1-L HDPE bottle. Collect the groundwater EAP sample in one (1) 1-L HDPE bottle. Fill the bottles and VOA viles to form a meniscus at the top of the bottle. Then fill the cap with groundwater and screw the cap on the bottle cap tightly, so as to fill the bottle with no headspace or bubbles. **Make sure that there is no headspace in each of the containers that groundwater samples were collected in**. There can be no head space in the VOC, qPCR, and EAP samples.

___ 12) Seal the bottle caps of the 1-L HDPE bottles that were sampled for qPCR and EAP analyses with parafilm to minimize potential for exposure to air.

___ 13) Label each bottle with a unique sample number, the sample location, date and time of sample collection, sampling depth, groundwater temperature, client, and sampler. Complete the chain-of-custody forms for the sample collected.

___ 14) Place each bottle into a self-sealing plastic bag. Make sure the plastic bag is sealed shut (e.g. Ziploc bag).

___ 15) Place the bottles UPRIGHT on ice for storage prior to packing for shipping.

___ 16) When packing for shipping replace the gel packs that have been used for storage of the samples in the cooler with freshly frozen gel packs as the gel packs melt quickly. Gel packs don't keep as cold as ice. Put gel packs on top, bottom, and sides of cooler with the qPCR and EAP samples. Pack tightly in the UPRIGHT position. If necessary, use packing materials to prevent movement, but be sure ice packs are next to the bottles. Include in the shipping package the chain-of-custody form for the appropriate laboratory. For the cooler that is being sent to the Idaho National Laboratory, a copy of the sampling field data sheets for each groundwater sample needs to be sent with the shipment.

___ 17) Send samples for the Idaho National Laboratory by Priority Overnight Express (arrival by 10 am the following day). No more than four (4) qPCR and EAP samples can be sent to the Idaho National Laboratory each day. Contact either M. Hope Lee (cell 1 [240-818-2987] and cell 2 [208-351-8148]) or Brady Lee (office [208-526-0981] and cell [208-520-1617]) of the Idaho National Laboratory with the courier reference number for each sample shipment on the day of the shipment. The personnel from Idaho National Laboratory will track the shipment and will investigate if the shipment does not arrive at the expected time so that the appropriate corrective action can be initiated. Samples can be only accepted Monday through Thursday from 8 am -5 pm MST, except on holidays. Contact if you have questions.

___ 18) Send samples to Spectrum Analytical by Standard Overnight Express (arrival by 3 pm the following day).

___ 19) Send the trip blanks for the qPCR and the EAP samples to the Idaho National Laboratory with the second shipment. Send a trip blank for VOCs to Spectrum Analytical with each

Field Worksheet *Travis AFB*

Project: Travis Enzymes Study	**DO/TO:** 1
PM: Mike Wray	**Field Phone:** 530-604-4129

Site: DP039 **Location** MW04x39 **Sample Date:**

Verified: ☐ Plant operation required: ☑ Northing Easting:

Sample ID **MW04X39-140** **GROUNDWATER** Depth From **16** To: **26** Total Depth FT

Lab Name	Methods	Filter	Count	Container	Preservative	QA/QC	By/Time/Date
INL	EAP	☐	1	1L HDPE	4'C	N	KR / 1900 / 2-22-12
INL	QPCR	☐	1	1L HPDE	4'C	N	/ /
PEL	SW8260B	☐	3	40ml Glass Vial	HCl, pH<2, 4'C	N	/ /

Pump Number: _____

End of Sample ID

End of Location

	Signatures	Date/Time
Sampled by		
Relinquished by		
Received by		
Relinquished by		
Received by		

Printed on Thursday, February 02, 2012 5:16:14 PM

Travis AFB - Enzyme Study
SAMPLING
LOW-FLOW PURGE FIELD DATA SHEET

SITE ID DP039 **LOCATION ID** MW04

DATE 2-22-12 **JOB NUMBER** 381355.01.01.03.MN

GENERAL INFORMATION

WEATHER (circle) (SUN) CLEAR OVERCAST RAIN

WIND DIRECTION Ø AMBIENT TEMPERATURE 65°F

FIELD CONDITIONS DRY

INITIAL PID: WELL CASING Ø BREATHING ZONE Ø

PURGE INFORMATION

START TIME 0828 END TIME 0910

INITIAL DEPTH TO WATER 14.40 SCREEN INTERVAL 16 TO 26

PUMP (circle one) Portable (Dedicated) PORTABLE PUMP DEPTH (ft) 23' BGS

TOTAL VOLUME PURGED (gal) 1.2 PURGE RATE ~~(gpm)~~ LPM 1.0

Stabalization Criteria		+/- .1	+/- 3%	+/- 5	+/- 10%	+/- .2	+/- 10%	NA
TIME	VOLUME PURGED (gal)	pH	COND ~~(mS/cm µS/cm)~~ *Circle Units Used*	TURBIDITY (ntu)	DO (mg/L)	TEMP (°C)	ORP (mV)	WATER LEVEL (ft btoc)
0831	.05	6.65	1.56	109.	2.97	17.30	164	14.64
0834	.2	6.69	1.55	0	0	18.20	152	14.70
0837	.4	6.70	1.56	0	0	18.12	145	14.72
0840	.6	6.74	1.55	0	0	18.35	133	14.76
0843	.8	6.73	1.54	0	0	18.32	128	14.80
0846	1.0	6.76	1.553	0	0	18.43	126	14.84
0849	1.2	6.77	1.553	0	0	18.45	126	14.86

ADDITIONAL FIELD COMMENTS

TIME	COMMENTS
	Flush mount MW

FIELD TEAM (initials) EP, RR

Travis AFB - Enzyme Study
SAMPLING
LOW-FLOW PURGE FIELD DATA SHEET

SITE ID DP039 **LOCATION ID** MW04

DATE 2-22-12 **JOB NUMBER** 381355.01.01.03.MN

FIELD MEASUREMENT/ COLLECTION EQUIPMENT MAKE/MODEL SERIAL/ID #

PID METER _____

HORRIBA U-22 C101991

WATER LEVEL INDICATOR _____

PUMP TYPE (circle) Grundfos Bladder Barcad (Peristaltic) Other (specify)

DECOMTAMINATION (Y) N ~~ALCONOX WASH~~ Den. Alcohol ~~DISTILLED RINSE~~

SAMPLING INFORMATION

SAMPLE FIELD ID MW04x39-140

SAMPLE TIME 0900

SAMPLING WATER LEVEL 14.86

ANALYSIS (circle)

SW9060	SW9056	E160.1	(~~SW8260B~~)	E300.0	E300.0M
SW6010BF	SW6010B	SW8015-D	SW8015-P	SW6850	RSK-175
E310.1	HACH				

Other (specify) EAA, qPCR

FIELD FILTERED Y (N) if yes, for which analysis _____

EQUIPMENT BLANK Y (N)

QA/QC FIELD ID _____ QA/QC TYPE _____

QA/QC FIELD ID _____ QA/QC TYPE _____

QA/QC analysis different from original analysis? (circle) Y N If Yes, specify: _____

COMMENTS/FIELD NOTES: _____

FIELD TEAM (initials) EP, KF

Checklist for Travis AFB Enzyme Sampling Procedures

Date 2-22-12

Travis AFB Site # DP039

Well Number MW04x39

Sample Depth 23 ft bgs/ 22.4 ft below TOC

Field Duplicate to be Collected No

___ 1) Make sure that at least two coolers are being used. One cooler will be for the volatile organic compound (VOC) samples and the other cooler will be for the quantitative polymerase chain reaction (qPCR) and enzyme activity probe (EAP) samples. Check each cooler to make sure that frozen blue gel packs are being used to keep the groundwater samples cold instead of wet ice.

___ 2) Make sure the VOC, qPCR, and EAP trip blanks are in the coolers.

___ 3) Layout new plastic bag on the ground for a sterilized surface to work on and as a secondary containment.

___ 4) Samplers put on nitrile gloves to protect hands and collect sterile samples.

___ 5) Blot some ethyl alcohol on Kim Wipes and wipe down all instruments, exterior of sample bottles and caps, the gloved hands, the end of the sample tube, and anything else that may come into contact with the sample to sterilize the equipment. The ethyl alcohol will denature and kill any bacteria but is harmless to us (unless ingested or set afire).

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___ 15) Place the bottles UPRIGHT on ice for storage prior to packing for shipping.

___ 16) When packing for shipping replace the gel packs that have been used for storage of the samples in the cooler with freshly frozen gel packs as the gel packs melt quickly. Gel packs don't keep as cold as ice. Put gel packs on top, bottom, and sides of cooler with the qPCR and EAP samples. Pack tightly in the UPRIGHT position. If necessary, use packing materials to prevent movement, but be sure ice packs are next to the bottles. Include in the shipping package the chain-of-custody form for the appropriate laboratory. For the cooler that is being sent to the Idaho National Laboratory, a copy of the sampling field data sheets for each groundwater sample needs to be sent with the shipment.

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shipment. The VOC samples can be held until all of the samples are collected and shipped to Spectrum Analytical at the same time.

Laboratory Shipping Addresses:

Attn: Hope or Brady Lee
Idaho National Laboratory
1765 North Yellowstone Highway
IF 603
Lab 103
Idaho Falls, ID 83402

Receiving Sample
Spectrum Analytical, Inc.
8405 Benjamin Rd
Suite A
Tampa, FL 33634

(813) 888-9507

Attachment 4
Complete VOC Analytical Results

Location	Field ID	Sample Date	QA/QC Type	Method	Analyte	Result[a]	Flag	Units	IRG
Site: Site FT004									
MW131X04									
MW131x04-140		2/21/2012	N	SW8260	1,1,1,2-Tetrachloroethane	1	U	µg/L	
		2/21/2012	N	SW8260	1,1,1-Trichloroethane	1	U	µg/L	200
		2/21/2012	N	SW8260	1,1,2,2-Tetrachloroethane	1	U	µg/L	
		2/21/2012	N	SW8260	1,1,2-Trichloroethane	1	U	µg/L	0.5
		2/21/2012	N	SW8260	1,1-DCA	1	U	µg/L	
		2/21/2012	N	SW8260	1,1-DCE	0.5	U	µg/L	6
		2/21/2012	N	SW8260	1,2,3-Trichloropropane	3.2	U	µg/L	
		2/21/2012	N	SW8260	1,2-DCA	0.25	J	µg/L	0.5
		2/21/2012	N	SW8260	1,2-DCB	0.5	U	µg/L	
		2/21/2012	N	SW8260	1,2-Dibromoethane	1	U	µg/L	
		2/21/2012	N	SW8260	1,2-Dichloropropane	0.5	U	µg/L	5
		2/21/2012	N	SW8260	1,3-DCB	0.5	U	µg/L	
		2/21/2012	N	SW8260	1,4-DCB	0.5	U	µg/L	5
		2/21/2012	N	SW8260	2-Hexanone	5	U	µg/L	
		2/21/2012	N	SW8260	Acetone	5	U	µg/L	5110
		2/21/2012	N	SW8260	Benzene	0.5	U	µg/L	1
		2/21/2012	N	SW8260	Bromobenzene	1	U	µg/L	
		2/21/2012	N	SW8260	Bromodichloromethane	0.5	U	µg/L	100
		2/21/2012	N	SW8260	Bromoform	1.2	U	µg/L	
		2/21/2012	N	SW8260	Bromomethane	1.1	U	µg/L	
		2/21/2012	N	SW8260	Carbon disulfide	1	U	µg/L	
		2/21/2012	N	SW8260	Carbon tetrachloride	0.5	U	µg/L	0.5
		2/21/2012	N	SW8260	Chlorobenzene	0.5	U	µg/L	70
		2/21/2012	N	SW8260	Chloroethane	1	U	µg/L	
		2/21/2012	N	SW8260	Chloroform	1	U	µg/L	100
		2/21/2012	N	SW8260	Chloromethane	1	U	µg/L	1.5
		2/21/2012	N	SW8260	cis-1,2-DCE	3.4		µg/L	6
		2/21/2012	N	SW8260	cis-1,3-Dichloropropene	1	U	µg/L	
		2/21/2012	N	SW8260	Dibromochloromethane	0.5	U	µg/L	
		2/21/2012	N	SW8260	Dibromomethane	2.4	U	µg/L	
		2/21/2012	N	SW8260	Dichlorodifluoromethane	1	U	µg/L	
		2/21/2012	N	SW8260	Ethylbenzene	0.5	U	µg/L	700
		2/21/2012	N	SW8260	m,p-Xylene	0.6	U	µg/L	1750
		2/21/2012	N	SW8260	Methyl ethyl ketone	5	U	µg/L	

Note: Grouped by Site and Location, sorted by Field ID and Analyte

[a] Bold values indicate result greater than IRGs

Location	Field ID	Sample Date	QA/QC Type	Method	Analyte	Result[a]	Flag	Units	IRG
Site: Site FT004									
MW131X04									
MW131x04-140		2/21/2012	N	SW8260	Methyl isobutyl ketone	5	U	µg/L	
		2/21/2012	N	SW8260	Methylene chloride	2	U	µg/L	5
		2/21/2012	N	SW8260	MTBE	1	U	µg/L	13
		2/21/2012	N	SW8260	o-Xylene	0.5	U	µg/L	1750
		2/21/2012	N	SW8260	PCE	0.5	U	µg/L	5
		2/21/2012	N	SW8260	Styrene	1	U	µg/L	
		2/21/2012	N	SW8260	**TCE**	**154**		µg/L	5
		2/21/2012	N	SW8260	Toluene	0.5	U	µg/L	150
		2/21/2012	N	SW8260	trans-1,2-DCE	0.54	U	µg/L	
		2/21/2012	N	SW8260	trans-1,3-Dichloropropene	1	U	µg/L	
		2/21/2012	N	SW8260	Trichlorofluoromethane	1	U	µg/L	
		2/21/2012	N	SW8260	Vinyl acetate	1	U	µg/L	
		2/21/2012	N	SW8260	Vinyl chloride	0.5	UJ	µg/L	0.5
MW264X04									
MW264x04-140		2/21/2012	N	SW8260	1,1,1,2-Tetrachloroethane	1	U	µg/L	
		2/21/2012	N	SW8260	1,1,1-Trichloroethane	1	U	µg/L	200
		2/21/2012	N	SW8260	1,1,2,2-Tetrachloroethane	1	U	µg/L	
		2/21/2012	N	SW8260	1,1,2-Trichloroethane	1	U	µg/L	0.5
		2/21/2012	N	SW8260	1,1-DCA	1	U	µg/L	
		2/21/2012	N	SW8260	1,1-DCE	0.5	U	µg/L	6
		2/21/2012	N	SW8260	1,2,3-Trichloropropane	3.2	U	µg/L	
		2/21/2012	N	SW8260	1,2-DCA	0.5	U	µg/L	0.5
		2/21/2012	N	SW8260	1,2-DCB	0.5	U	µg/L	
		2/21/2012	N	SW8260	1,2-Dibromoethane	1	U	µg/L	
		2/21/2012	N	SW8260	1,2-Dichloropropane	0.5	U	µg/L	5
		2/21/2012	N	SW8260	1,3-DCB	0.5	U	µg/L	
		2/21/2012	N	SW8260	1,4-DCB	0.5	U	µg/L	5
		2/21/2012	N	SW8260	2-Hexanone	5	U	µg/L	
		2/21/2012	N	SW8260	Acetone	5	UJ	µg/L	5110
		2/21/2012	N	SW8260	Benzene	0.5	U	µg/L	1
		2/21/2012	N	SW8260	Bromobenzene	1	U	µg/L	
		2/21/2012	N	SW8260	Bromodichloromethane	0.5	U	µg/L	100
		2/21/2012	N	SW8260	Bromoform	1.2	U	µg/L	
		2/21/2012	N	SW8260	Bromomethane	1.1	U	µg/L	

Note: Grouped by Site and Location, sorted by Field ID and Analyte

[a] Bold values indicate result greater than IRGs

Location	Field ID	Sample Date	QA/QC Type	Method	Analyte	Result[a]	Flag	Units	IRG
Site:	**Site FT004**								
MW264X04									
MW264x04-140		2/21/2012	N	SW8260	Carbon disulfide	1	U	µg/L	
		2/21/2012	N	SW8260	Carbon tetrachloride	0.5	U	µg/L	0.5
		2/21/2012	N	SW8260	Chlorobenzene	0.5	U	µg/L	70
		2/21/2012	N	SW8260	Chloroethane	1	U	µg/L	
		2/21/2012	N	SW8260	Chloroform	1	U	µg/L	100
		2/21/2012	N	SW8260	Chloromethane	1	U	µg/L	1.5
		2/21/2012	N	SW8260	cis-1,2-DCE	1	U	µg/L	6
		2/21/2012	N	SW8260	cis-1,3-Dichloropropene	1	U	µg/L	
		2/21/2012	N	SW8260	Dibromochloromethane	0.5	U	µg/L	
		2/21/2012	N	SW8260	Dibromomethane	2.4	U	µg/L	
		2/21/2012	N	SW8260	Dichlorodifluoromethane	1	U	µg/L	
		2/21/2012	N	SW8260	Ethylbenzene	0.5	U	µg/L	700
		2/21/2012	N	SW8260	m,p-Xylene	0.6	U	µg/L	1750
		2/21/2012	N	SW8260	Methyl ethyl ketone	5	U	µg/L	
		2/21/2012	N	SW8260	Methyl isobutyl ketone	5	U	µg/L	
		2/21/2012	N	SW8260	Methylene chloride	2	U	µg/L	5
		2/21/2012	N	SW8260	MTBE	1	U	µg/L	13
		2/21/2012	N	SW8260	o-Xylene	0.5	U	µg/L	1750
		2/21/2012	N	SW8260	PCE	0.5	U	µg/L	5
		2/21/2012	N	SW8260	Styrene	1	U	µg/L	
		2/21/2012	N	SW8260	TCE	0.5	U	µg/L	5
		2/21/2012	N	SW8260	Toluene	0.5	U	µg/L	150
		2/21/2012	N	SW8260	trans-1,2-DCE	0.54	U	µg/L	
		2/21/2012	N	SW8260	trans-1,3-Dichloropropene	1	U	µg/L	
		2/21/2012	N	SW8260	Trichlorofluoromethane	1	U	µg/L	
		2/21/2012	N	SW8260	Vinyl acetate	1	U	µg/L	
		2/21/2012	N	SW8260	Vinyl chloride	0.5	UJ	µg/L	0.5
MW266X04									
MW266x04-140		2/21/2012	N	SW8260	1,1,1,2-Tetrachloroethane	1	U	µg/L	
		2/21/2012	N	SW8260	1,1,1-Trichloroethane	1	U	µg/L	200
		2/21/2012	N	SW8260	1,1,2,2-Tetrachloroethane	1	U	µg/L	
		2/21/2012	N	SW8260	1,1,2-Trichloroethane	1	U	µg/L	0.5
		2/21/2012	N	SW8260	1,1-DCA	1	U	µg/L	
		2/21/2012	N	SW8260	1,1-DCE	0.5	U	µg/L	6

Note: Grouped by Site and Location, sorted by Field ID and Analyte

[a] Bold values indicate result greater than IRGs

Location	Field ID	Sample Date	QA/QC Type	Method	Analyte	Result[a]	Flag	Units	IRG
Site: Site FT004									
MW266X04									
MW266x04-140		2/21/2012	N	SW8260	1,2,3-Trichloropropane	3.2	U	µg/L	
		2/21/2012	N	SW8260	1,2-DCA	0.5	U	µg/L	0.5
		2/21/2012	N	SW8260	1,2-DCB	0.5	U	µg/L	
		2/21/2012	N	SW8260	1,2-Dibromoethane	1	U	µg/L	
		2/21/2012	N	SW8260	1,2-Dichloropropane	0.5	U	µg/L	5
		2/21/2012	N	SW8260	1,3-DCB	0.5	U	µg/L	
		2/21/2012	N	SW8260	1,4-DCB	0.5	U	µg/L	5
		2/21/2012	N	SW8260	2-Hexanone	5	U	µg/L	
		2/21/2012	N	SW8260	Acetone	5	UJ	µg/L	5110
		2/21/2012	N	SW8260	Benzene	0.5	U	µg/L	1
		2/21/2012	N	SW8260	Bromobenzene	1	U	µg/L	
		2/21/2012	N	SW8260	Bromodichloromethane	0.5	U	µg/L	100
		2/21/2012	N	SW8260	Bromoform	1.2	U	µg/L	
		2/21/2012	N	SW8260	Bromomethane	1.1	U	µg/L	
		2/21/2012	N	SW8260	Carbon disulfide	1	U	µg/L	
		2/21/2012	N	SW8260	Carbon tetrachloride	0.5	U	µg/L	0.5
		2/21/2012	N	SW8260	Chlorobenzene	0.5	U	µg/L	70
		2/21/2012	N	SW8260	Chloroethane	1	U	µg/L	
		2/21/2012	N	SW8260	Chloroform	1	U	µg/L	100
		2/21/2012	N	SW8260	Chloromethane	1	U	µg/L	1.5
		2/21/2012	N	SW8260	cis-1,2-DCE	3.4		µg/L	6
		2/21/2012	N	SW8260	cis-1,3-Dichloropropene	1	U	µg/L	
		2/21/2012	N	SW8260	Dibromochloromethane	0.5	U	µg/L	
		2/21/2012	N	SW8260	Dibromomethane	2.4	U	µg/L	
		2/21/2012	N	SW8260	Dichlorodifluoromethane	1	U	µg/L	
		2/21/2012	N	SW8260	Ethylbenzene	0.5	U	µg/L	700
		2/21/2012	N	SW8260	m,p-Xylene	0.6	U	µg/L	1750
		2/21/2012	N	SW8260	Methyl ethyl ketone	5	U	µg/L	
		2/21/2012	N	SW8260	Methyl isobutyl ketone	5	U	µg/L	
		2/21/2012	N	SW8260	Methylene chloride	2	U	µg/L	5
		2/21/2012	N	SW8260	MTBE	1	U	µg/L	13
		2/21/2012	N	SW8260	o-Xylene	0.5	U	µg/L	1750
		2/21/2012	N	SW8260	PCE	0.5	U	µg/L	5
		2/21/2012	N	SW8260	Styrene	1	U	µg/L	

Note: Grouped by Site and Location, sorted by Field ID and Analyte

[a] Bold values indicate result greater than IRGs

Location	Field ID	Sample Date	QA/QC Type	Method	Analyte	Result[a]	Flag	Units	IRG
Site: Site FT004									
MW266X04									
MW266x04-140		2/21/2012	N	SW8260	**TCE**	**122**		µg/L	5
		2/21/2012	N	SW8260	Toluene	0.5	U	µg/L	150
		2/21/2012	N	SW8260	trans-1,2-DCE	0.54	U	µg/L	
		2/21/2012	N	SW8260	trans-1,3-Dichloropropene	1	U	µg/L	
		2/21/2012	N	SW8260	Trichlorofluoromethane	1	U	µg/L	
		2/21/2012	N	SW8260	Vinyl acetate	1	U	µg/L	
		2/21/2012	N	SW8260	Vinyl chloride	0.5	UJ	µg/L	0.5
MW591X04									
MW591x04-140		2/21/2012	N	SW8260	1,1,1,2-Tetrachloroethane	1	U	µg/L	
		2/21/2012	N	SW8260	1,1,1-Trichloroethane	0.76	J	µg/L	200
		2/21/2012	N	SW8260	1,1,2,2-Tetrachloroethane	1	U	µg/L	
		2/21/2012	N	SW8260	1,1,2-Trichloroethane	1	U	µg/L	0.5
		2/21/2012	N	SW8260	1,1-DCA	1	U	µg/L	
		2/21/2012	N	SW8260	1,1-DCE	1.5		µg/L	6
		2/21/2012	N	SW8260	1,2,3-Trichloropropane	3.2	U	µg/L	
		2/21/2012	N	SW8260	1,2-DCA	0.5	U	µg/L	0.5
		2/21/2012	N	SW8260	1,2-DCB	0.5	U	µg/L	
		2/21/2012	N	SW8260	1,2-Dibromoethane	1	U	µg/L	
		2/21/2012	N	SW8260	1,2-Dichloropropane	0.5	U	µg/L	5
		2/21/2012	N	SW8260	1,3-DCB	0.5	U	µg/L	
		2/21/2012	N	SW8260	1,4-DCB	0.5	U	µg/L	5
		2/21/2012	N	SW8260	2-Hexanone	5	U	µg/L	
		2/21/2012	N	SW8260	Acetone	5	UJ	µg/L	5110
		2/21/2012	N	SW8260	Benzene	0.5	U	µg/L	1
		2/21/2012	N	SW8260	Bromobenzene	1	U	µg/L	
		2/21/2012	N	SW8260	Bromodichloromethane	1.4		µg/L	100
		2/21/2012	N	SW8260	Bromoform	1.2	U	µg/L	
		2/21/2012	N	SW8260	Bromomethane	1.1	U	µg/L	
		2/21/2012	N	SW8260	Carbon disulfide	1	U	µg/L	
		2/21/2012	N	SW8260	Carbon tetrachloride	0.5	U	µg/L	0.5
		2/21/2012	N	SW8260	Chlorobenzene	0.5	U	µg/L	70
		2/21/2012	N	SW8260	Chloroethane	1	U	µg/L	
		2/21/2012	N	SW8260	Chloroform	5.4		µg/L	100
		2/21/2012	N	SW8260	Chloromethane	1	U	µg/L	1.5

Note: Grouped by Site and Location, sorted by Field ID and Analyte

[a] Bold values indicate result greater than IRGs

Location	Field ID	Sample Date	QA/QC Type	Method	Analyte	Result[a]	Flag	Units	IRG
Site: Site FT004									
MW591X04									
MW591x04-140		2/21/2012	N	SW8260	cis-1,2-DCE	0.32	J	µg/L	6
		2/21/2012	N	SW8260	cis-1,3-Dichloropropene	1	U	µg/L	
		2/21/2012	N	SW8260	Dibromochloromethane	0.53		µg/L	
		2/21/2012	N	SW8260	Dibromomethane	2.4	U	µg/L	
		2/21/2012	N	SW8260	Dichlorodifluoromethane	1	U	µg/L	
		2/21/2012	N	SW8260	Ethylbenzene	0.5	U	µg/L	700
		2/21/2012	N	SW8260	m,p-Xylene	0.6	U	µg/L	1750
		2/21/2012	N	SW8260	Methyl ethyl ketone	5	U	µg/L	
		2/21/2012	N	SW8260	Methyl isobutyl ketone	5	U	µg/L	
		2/21/2012	N	SW8260	Methylene chloride	2	U	µg/L	5
		2/21/2012	N	SW8260	MTBE	1	U	µg/L	13
		2/21/2012	N	SW8260	o-Xylene	0.5	U	µg/L	1750
		2/21/2012	N	SW8260	PCE	0.5	U	µg/L	5
		2/21/2012	N	SW8260	Styrene	1	U	µg/L	
		2/21/2012	N	SW8260	**TCE**	**19.7**		µg/L	5
		2/21/2012	N	SW8260	Toluene	0.5	U	µg/L	150
		2/21/2012	N	SW8260	trans-1,2-DCE	0.54	U	µg/L	
		2/21/2012	N	SW8260	trans-1,3-Dichloropropene	1	U	µg/L	
		2/21/2012	N	SW8260	Trichlorofluoromethane	1	U	µg/L	
		2/21/2012	N	SW8260	Vinyl acetate	1	U	µg/L	
		2/21/2012	N	SW8260	Vinyl chloride	0.5	UJ	µg/L	0.5
Site: Site DP039									
MW04X39									
MW04x39-140		2/22/2012	N	SW8260	1,1,1,2-Tetrachloroethane	1	U	µg/L	
		2/22/2012	N	SW8260	1,1,1-Trichloroethane	1	U	µg/L	200
		2/22/2012	N	SW8260	1,1,2,2-Tetrachloroethane	1	U	µg/L	
		2/22/2012	N	SW8260	1,1,2-Trichloroethane	1	U	µg/L	0.5
		2/22/2012	N	SW8260	1,1-DCA	1	U	µg/L	
		2/22/2012	N	SW8260	**1,1-DCE**	**8.3**		µg/L	6
		2/22/2012	N	SW8260	1,2,3-Trichloropropane	3.2	U	µg/L	
		2/22/2012	N	SW8260	1,2-DCA	0.5	U	µg/L	0.5
		2/22/2012	N	SW8260	1,2-DCB	0.5	U	µg/L	
		2/22/2012	N	SW8260	1,2-Dibromoethane	1	U	µg/L	
		2/22/2012	N	SW8260	1,2-Dichloropropane	0.5	U	µg/L	5
		2/22/2012	N	SW8260	1,3-DCB	0.5	U	µg/L	

Note: Grouped by Site and Location, sorted by Field ID and Analyte

[a] Bold values indicate result greater than IRGs

Location	Field ID	Sample Date	QA/QC Type	Method	Analyte	Result[a]	Flag	Units	IRG
Site: Site DP039									
MW04X39									
MW04x39-140		2/22/2012	N	SW8260	1,4-DCB	0.5	U	µg/L	5
		2/22/2012	N	SW8260	2-Hexanone	5	U	µg/L	
		2/22/2012	N	SW8260	Acetone	5	UJ	µg/L	5110
		2/22/2012	N	SW8260	Benzene	0.5	U	µg/L	1
		2/22/2012	N	SW8260	Bromobenzene	1	U	µg/L	
		2/22/2012	N	SW8260	Bromodichloromethane	0.5	U	µg/L	100
		2/22/2012	N	SW8260	Bromoform	1.2	U	µg/L	
		2/22/2012	N	SW8260	Bromomethane	1.1	U	µg/L	
		2/22/2012	N	SW8260	Carbon disulfide	1	U	µg/L	
		2/22/2012	N	SW8260	Carbon tetrachloride	0.5	U	µg/L	0.5
		2/22/2012	N	SW8260	Chlorobenzene	0.5	U	µg/L	70
		2/22/2012	N	SW8260	Chloroethane	1	U	µg/L	
		2/22/2012	N	SW8260	Chloroform	0.18	J	µg/L	100
		2/22/2012	N	SW8260	Chloromethane	1	U	µg/L	1.5
		2/22/2012	N	SW8260	cis-1,2-DCE	1.8		µg/L	6
		2/22/2012	N	SW8260	cis-1,3-Dichloropropene	1	U	µg/L	
		2/22/2012	N	SW8260	Dibromochloromethane	0.5	U	µg/L	
		2/22/2012	N	SW8260	Dibromomethane	2.4	U	µg/L	
		2/22/2012	N	SW8260	Dichlorodifluoromethane	1	U	µg/L	
		2/22/2012	N	SW8260	Ethylbenzene	0.5	U	µg/L	700
		2/22/2012	N	SW8260	m,p-Xylene	0.6	U	µg/L	1750
		2/22/2012	N	SW8260	Methyl ethyl ketone	5	U	µg/L	
		2/22/2012	N	SW8260	Methyl isobutyl ketone	5	U	µg/L	
		2/22/2012	N	SW8260	Methylene chloride	2	U	µg/L	5
		2/22/2012	N	SW8260	MTBE	0.72	J	µg/L	13
		2/22/2012	N	SW8260	o-Xylene	0.5	U	µg/L	1750
		2/22/2012	N	SW8260	PCE	0.5	U	µg/L	5
		2/22/2012	N	SW8260	Styrene	1	U	µg/L	
		2/22/2012	N	SW8260	**TCE**	**477**		µg/L	5
		2/22/2012	N	SW8260	Toluene	0.5	U	µg/L	150
		2/22/2012	N	SW8260	trans-1,2-DCE	0.54	U	µg/L	
		2/22/2012	N	SW8260	trans-1,3-Dichloropropene	1	U	µg/L	
		2/22/2012	N	SW8260	Trichlorofluoromethane	1	U	µg/L	
		2/22/2012	N	SW8260	Vinyl acetate	1	U	µg/L	

Note: Grouped by Site and Location, sorted by Field ID and Analyte

[a] Bold values indicate result greater than IRGs

Location	Field ID	Sample Date	QA/QC Type	Method	Analyte	Result[a]	Flag	Units	IRG
Site: Site DP039									
MW04X39									
MW04x39-140		2/22/2012	N	SW8260	Vinyl chloride	0.5	UJ	µg/L	0.5
MW781X39									
MW781x39-140		2/22/2012	N	SW8260	1,1,1,2-Tetrachloroethane	1	U	µg/L	
		2/22/2012	N	SW8260	1,1,1-Trichloroethane	1	U	µg/L	200
		2/22/2012	N	SW8260	1,1,2,2-Tetrachloroethane	1	U	µg/L	
		2/22/2012	N	SW8260	1,1,2-Trichloroethane	1	U	µg/L	0.5
		2/22/2012	N	SW8260	1,1-DCA	1	U	µg/L	
		2/22/2012	N	SW8260	1,1-DCE	0.26	J	µg/L	6
		2/22/2012	N	SW8260	1,2,3-Trichloropropane	3.2	U	µg/L	
		2/22/2012	N	SW8260	1,2-DCA	0.5	U	µg/L	0.5
		2/22/2012	N	SW8260	1,2-DCB	0.5	U	µg/L	
		2/22/2012	N	SW8260	1,2-Dibromoethane	1	U	µg/L	
		2/22/2012	N	SW8260	1,2-Dichloropropane	0.5	U	µg/L	5
		2/22/2012	N	SW8260	1,3-DCB	0.5	U	µg/L	
		2/22/2012	N	SW8260	1,4-DCB	0.5	U	µg/L	5
		2/22/2012	N	SW8260	2-Hexanone	5	U	µg/L	
		2/22/2012	N	SW8260	Acetone	5	UJ	µg/L	5110
		2/22/2012	N	SW8260	Benzene	0.5	U	µg/L	1
		2/22/2012	N	SW8260	Bromobenzene	1	U	µg/L	
		2/22/2012	N	SW8260	Bromodichloromethane	0.5	U	µg/L	100
		2/22/2012	N	SW8260	Bromoform	1.2	U	µg/L	
		2/22/2012	N	SW8260	Bromomethane	1.1	U	µg/L	
		2/22/2012	N	SW8260	Carbon disulfide	1	U	µg/L	
		2/22/2012	N	SW8260	Carbon tetrachloride	0.5	U	µg/L	0.5
		2/22/2012	N	SW8260	Chlorobenzene	0.5	U	µg/L	70
		2/22/2012	N	SW8260	Chloroethane	1	U	µg/L	
		2/22/2012	N	SW8260	Chloroform	1.2		µg/L	100
		2/22/2012	N	SW8260	Chloromethane	1	U	µg/L	1.5
		2/22/2012	N	SW8260	cis-1,2-DCE	0.26	J	µg/L	6
		2/22/2012	N	SW8260	cis-1,3-Dichloropropene	1	U	µg/L	
		2/22/2012	N	SW8260	Dibromochloromethane	0.5	U	µg/L	
		2/22/2012	N	SW8260	Dibromomethane	2.4	U	µg/L	
		2/22/2012	N	SW8260	Dichlorodifluoromethane	1	U	µg/L	
		2/22/2012	N	SW8260	Ethylbenzene	0.5	U	µg/L	700

Note: Grouped by Site and Location, sorted by Field ID and Analyte

[a] Bold values indicate result greater than IRGs

Location	Field ID	Sample Date	QA/QC Type	Method	Analyte	Result[a]	Flag	Units	IRG
Site:	**Site DP039**								
MW781X39									
MW781x39-140		2/22/2012	N	SW8260	m,p-Xylene	0.6	U	µg/L	1750
		2/22/2012	N	SW8260	Methyl ethyl ketone	5	U	µg/L	
		2/22/2012	N	SW8260	Methyl isobutyl ketone	5	U	µg/L	
		2/22/2012	N	SW8260	Methylene chloride	2	U	µg/L	5
		2/22/2012	N	SW8260	MTBE	1	U	µg/L	13
		2/22/2012	N	SW8260	o-Xylene	0.5	U	µg/L	1750
		2/22/2012	N	SW8260	PCE	0.5	U	µg/L	5
		2/22/2012	N	SW8260	Styrene	1	U	µg/L	
		2/22/2012	N	SW8260	**TCE**	**53.5**		µg/L	5
		2/22/2012	N	SW8260	Toluene	0.5	U	µg/L	150
		2/22/2012	N	SW8260	trans-1,2-DCE	0.54	U	µg/L	
		2/22/2012	N	SW8260	trans-1,3-Dichloropropene	1	U	µg/L	
		2/22/2012	N	SW8260	Trichlorofluoromethane	1	U	µg/L	
		2/22/2012	N	SW8260	Vinyl acetate	1	U	µg/L	
		2/22/2012	N	SW8260	Vinyl chloride	0.5	UJ	µg/L	0.5
MW781x39-140B		2/22/2012	FD	SW8260	1,1,1,2-Tetrachloroethane	1	U	µg/L	
		2/22/2012	FD	SW8260	1,1,1-Trichloroethane	1	U	µg/L	200
		2/22/2012	FD	SW8260	1,1,2,2-Tetrachloroethane	1	U	µg/L	
		2/22/2012	FD	SW8260	1,1,2-Trichloroethane	1	U	µg/L	0.5
		2/22/2012	FD	SW8260	1,1-DCA	1	U	µg/L	
		2/22/2012	FD	SW8260	1,1-DCE	0.27	J	µg/L	6
		2/22/2012	FD	SW8260	1,2,3-Trichloropropane	3.2	U	µg/L	
		2/22/2012	FD	SW8260	1,2-DCA	0.5	U	µg/L	0.5
		2/22/2012	FD	SW8260	1,2-DCB	0.5	U	µg/L	
		2/22/2012	FD	SW8260	1,2-Dibromoethane	1	U	µg/L	
		2/22/2012	FD	SW8260	1,2-Dichloropropane	0.5	U	µg/L	5
		2/22/2012	FD	SW8260	1,3-DCB	0.5	U	µg/L	
		2/22/2012	FD	SW8260	1,4-DCB	0.5	U	µg/L	5
		2/22/2012	FD	SW8260	2-Hexanone	5	U	µg/L	
		2/22/2012	FD	SW8260	Acetone	5	U	µg/L	5110
		2/22/2012	FD	SW8260	Benzene	0.5	U	µg/L	1
		2/22/2012	FD	SW8260	Bromobenzene	1	U	µg/L	
		2/22/2012	FD	SW8260	Bromodichloromethane	0.5	U	µg/L	100
		2/22/2012	FD	SW8260	Bromoform	1.2	U	µg/L	

Note: Grouped by Site and Location, sorted by Field ID and Analyte

[a] Bold values indicate result greater than IRGs

Location	Field ID	Sample Date	QA/QC Type	Method	Analyte	Result[a]	Flag	Units	IRG
Site:	**Site DP039**								
MW781X39									
MW781x39-140B		2/22/2012	FD	SW8260	Bromomethane	1.1	U	µg/L	
		2/22/2012	FD	SW8260	Carbon disulfide	1	U	µg/L	
		2/22/2012	FD	SW8260	Carbon tetrachloride	0.5	U	µg/L	0.5
		2/22/2012	FD	SW8260	Chlorobenzene	0.5	U	µg/L	70
		2/22/2012	FD	SW8260	Chloroethane	1	U	µg/L	
		2/22/2012	FD	SW8260	Chloroform	1.2		µg/L	100
		2/22/2012	FD	SW8260	Chloromethane	1	U	µg/L	1.5
		2/22/2012	FD	SW8260	cis-1,2-DCE	0.31	J	µg/L	6
		2/22/2012	FD	SW8260	cis-1,3-Dichloropropene	1	U	µg/L	
		2/22/2012	FD	SW8260	Dibromochloromethane	0.5	U	µg/L	
		2/22/2012	FD	SW8260	Dibromomethane	2.4	U	µg/L	
		2/22/2012	FD	SW8260	Dichlorodifluoromethane	1	U	µg/L	
		2/22/2012	FD	SW8260	Ethylbenzene	0.5	U	µg/L	700
		2/22/2012	FD	SW8260	m,p-Xylene	0.6	U	µg/L	1750
		2/22/2012	FD	SW8260	Methyl ethyl ketone	5	U	µg/L	
		2/22/2012	FD	SW8260	Methyl isobutyl ketone	5	U	µg/L	
		2/22/2012	FD	SW8260	Methylene chloride	2	U	µg/L	5
		2/22/2012	FD	SW8260	MTBE	1	U	µg/L	13
		2/22/2012	FD	SW8260	o-Xylene	0.5	U	µg/L	1750
		2/22/2012	FD	SW8260	PCE	0.5	U	µg/L	5
		2/22/2012	FD	SW8260	Styrene	1	U	µg/L	
		2/22/2012	FD	SW8260	**TCE**	**49.9**		µg/L	5
		2/22/2012	FD	SW8260	Toluene	0.5	U	µg/L	150
		2/22/2012	FD	SW8260	trans-1,2-DCE	0.54	U	µg/L	
		2/22/2012	FD	SW8260	trans-1,3-Dichloropropene	1	U	µg/L	
		2/22/2012	FD	SW8260	Trichlorofluoromethane	1	U	µg/L	
		2/22/2012	FD	SW8260	Vinyl acetate	1	U	µg/L	
		2/22/2012	FD	SW8260	Vinyl chloride	0.5	UJ	µg/L	0.5

Note: Grouped by Site and Location, sorted by Field ID and Analyte

[a] Bold values indicate result greater than IRGs

Location	Field ID	Sample Date	QA/QC Type	Method	Analyte	Result[a]	Flag	Units	IRG

Notes:
DCA = dichloroethane
DCB = dichlorobenzene
DCE = dichloroethene
FD = field duplicate
IRG = Interim Remediation Goal
N = normal
PCE = tetrachloroethene
QA/QC = quality assurance/quality control
TCA = trichloroethane
TCE = trichloroethene
µg/L = microgram(s) per liter
Qualifier Description:
J = The analyte was present but reported value may not be accurate or precise.
U = The analyte was analyzed for but not detected.
UJ = The analyte was analyzed for but not detected, the reported value may not be accurate or precise.

Note: Grouped by Site and Location, sorted by Field ID and Analyte

[a] Bold values indicate result greater than IRGs

Attachment 5
Plume Attenuation

ATTACHMENT 5

Plume Attenuation

Natural attenuation of the solvent plumes at Sites FT004 and DP039 has been evaluated for more than a decade. Evaluations of the effectiveness of monitored natural attenuation (MNA) as a remedy in the downgradient portions of Sites FT004 and DP039 are documented in the *Natural Attenuation Assessment Report* (NAAR) (CH2M HILL, 2010). At the time the NAAR was written, both sites had active interim remedies in the plume source areas, and the effectiveness of MNA was evaluated only for the downgradient portion of the plume. Based on historical analytical, hydrogeologic, and geochemical data, the NAAR concluded that natural attenuation was occurring at both sites. At Site FT004, the NAAR concluded that the plume had remained stable over the interim period leading up to the groundwater record of decision (ROD), so MNA was an appropriate remedy for the distal portion of the plume. At Site DP039, the NAAR concluded that the southern toe of the plume remained stable, but increasing contaminant concentrations in some areas within the plume indicated that MNA alone may not be sufficient to prevent plume migration at the site.

Since the NAAR was completed, changes have been made to the interim remedies at both sites. At Site FT004, the groundwater extraction and treatment (GET) system was shut down in phases to support a rebound study. A portion of the Site FT004 GET was shut down in December 2007, and the rest of the GET system was shut down in March 2009. At Site DP039, an emulsified vegetable oil permeable reactive biobarrier (EVO PRB) was installed in June and July 2010 as a technology demonstration to treat trichloroethene (TCE) concentrations higher than 500 micrograms per liter (µg/L). The EVO PRB supports natural attenuation by reducing mass loading to the downgradient portion of the plume. Groundwater monitoring data have continued to be collected and evaluated for evidence of plume stability at both sites. Detailed evaluation of plume stability at Sites FT004 and DP039 is provided in the NAAR (CH2M HILL, 2010), the *Groundwater Sampling and Analysis Program 2010-2011 Annual Report* (CH2M HILL, 2012a), and the *2011 Remedial Process Optimization Report for the Central Groundwater Treatment Plant, North Groundwater Treatment Plant, and South Base Boundary Groundwater Treatment Plant* (2011 RPO Report) (CH2M HILL, 2012b). The following subsections summarize the evidence for plume attenuation at these sites.

Site FT004

Figure 1 shows the current distribution of TCE at Site FT004. Chemical time-series plots for the routinely sampled Site FT004 wells are provided on Figure 2. The results of the Mann-Kendall TCE concentration trend analysis for routinely sampled wells are presented in Table 1. Note that in this table, trends were assumed to be significant for p-values less than 0.05 (95 percent confidence level).

Chemical time-series plots (Figure 2) indicate decreasing TCE concentrations at two (2) of the four (4) Site FT004 monitoring wells sampled as part of this investigation (MW131x04, MW264x04, MW266x04, and MW591x04). TCE concentrations are decreasing at wells MW131x04 and MW266x04, both of which are located in the source area. TCE

concentrations have continued to decline in these wells after groundwater extraction had ceased. No trend in TCE concentrations is identifiable in well MW264x04, which is an upgradient background well where TCE has never been detected. TCE concentrations have increased at monitoring well MW591x04 to a maximum concentration of 19.7 µg/L in February 2012. TCE concentrations at this well have been somewhat variable since groundwater extraction ceased but show a general increasing trend; the Mann-Kendall trend analysis also indicates an increasing TCE trend.

Some rebound in volatile organic compound (VOC) concentrations is expected following shutdown of the GET system, because in high clay content sediments, such as those found at Site FT004, the cessation of groundwater extraction allows VOCs sorbed to clay particles the time to desorb and accumulate in groundwater, resulting in increased VOC concentrations. However, the preponderance of data obtained at Site FT004 over the rebound study period (between 4Q07and 1Q12) indicate that significant rebound of the Site FT004 TCE plume is not occurring. Chemical time-series plots (Figure 2) and Mann-Kendall concentration trend analysis (Table 1) indicate that since groundwater extraction has ceased, TCE concentrations have remained stable or decreased in 14 of the 19 extraction and monitoring wells sampled at Site FT004 for rebound.

The five (5) monitoring wells where TCE concentrations have increased since the rebound study began in 2007 are MW134x04, MW581x04, MW585x04, MW587x04, and MW591x04. The TCE concentration increase at well MW581x04 since the cessation of groundwater extraction is within the typical historical variation of TCE concentrations detected at this well and does not represent significant rebound. The increase in TCE concentrations detected at wells MW134x04, MW585x04, MW587x04, and MW591x04 since the cessation of groundwater extraction indicates that some rebound has occurred at these wells. However, the continued decreasing concentrations in the Site FT004 extraction wells and source area wells MW131x04 and MW266x04 over the course of the rebound study (since groundwater extraction has ceased) indicate that the Site FT004 source area has been effectively addressed by groundwater extraction and is not continuing to release mass to the groundwater plume. As a whole, the groundwater plume has remained stable since the cessation of groundwater extraction as evidenced by continued decreasing TCE concentration trends in most of the extraction and monitoring wells and the continuing decline in TCE concentrations at downgradient well MW757x04.

The lack of significant rebound within the Site FT004 TCE plume, and continued declining concentrations within the plume source area and the majority of routinely monitored wells, are consistent with the NAAR conclusion that natural attenuation is occurring at the site.

Site DP039

Figure 3 shows the current distribution of TCE at Site DP039. As depicted on Figure 3, much of the Site DP039 plume is potentially influenced by ongoing technology demonstrations (bioreactor, phytoremediation, and the EVO PRB). Data collected from the areas potentially impacted by the technology demonstrations are not representative of natural attenuation, but rather enhanced attenuation. Therefore, data from the area potentially impacted by the technology demonstrations are not discussed here (refer to the 2011 RPO Report [CH2M HILL, 2012b] for data analysis pertaining to the technology demonstrations).

The data discussed in this technical memorandum are restricted to the areas considered representative of the natural attenuation capacity of the aquifer. Routinely sampled monitoring wells considered to be representative of the natural attenuation capacity of the aquifer are MW03x39, MW04x39, MW758x39, MW759x39, MW760x39, MW761x39, MW762x39, MW781x39, and MW785x39. Chemical time-series plots for these nine (9) wells are provided on Figure 4. The results of the Mann-Kendall TCE concentration trend analysis for these wells are presented in Table 1.

Two (2) Site DP039 wells (MW04x39 and MW781x39) were sampled during this investigation. Both of these wells are located on the northern edge of the plume, crossgradient of the source area. These wells were selected because the TCE concentrations were relatively elevated (50- to 400-µg/L range) and they were outside the expected area of influence of the technology demonstration projects. Chemical time-series plots (Figure 4) indicate recently increasing TCE concentrations at both MW781x39 and MW04x39. The increase in TCE concentrations at well MW781x39 began in 2008. However, the TCE concentration detected at MW781x39 has declined over the last two (2) sampling events and was 53.5 µg/L in 1Q12, lower than the maximum detection of 75.2 µg/L in 2Q11. It is unclear whether the TCE concentrations at this well have stabilized.

TCE concentrations at MW04x39 have historically been variable. TCE concentrations increased from 2000 through 2003 and then declined from 2003 to 2009. TCE concentrations increased significantly again in 2010 to 2011. The maximum TCE concentration detected at this well was 772 µg/L, detected in 4Q11. The TCE concentration detected during the investigation was 477 µg/L. While this concentration is lower than the maximum detection, it is still significantly higher than historical concentrations typical for this well (100 to 200 µg/L). It appears that TCE concentrations near this well exceed the attenuation capacity of the aquifer in this area.

The potential for natural attenuation at Site DP039 is better represented by the furthest downgradient monitoring wells. Well MW785x39 is located downgradient of MW04x39. After a brief period of increasing TCE concentrations when MW785x39 was installed in 2006, TCE concentrations have stabilized and begun to decline. The maximum TCE concentration detected at this well was 151 µg/L in 2009. In 2011, the TCE concentration detected was 108 µg/L.

Well MW759x39 is located immediately downgradient of the Site DP039 groundwater plume. Historically, the TCE plume exceeding the interim remediation goal (IRG) (5 µg/L) extended beyond this monitoring well. The maximum TCE concentration detected at this location was 46 µg/L in 2002. Since then, TCE concentrations have declined to non-detect at this location. The TCE concentrations had declined to non-detect prior to the installation of the EVO PRB in 2010. Thus, the decline in TCE concentrations at this well is due to natural attenuation. It should be noted that TCE concentrations in the untreated portion of the plume upgradient of this well exceeded 1,000 µg/L while TCE concentrations were declining at MW759x39.

Downgradient of well MW759x39 are four (4) routinely sampled Site DP039 wells (MW758x39, MW760x39, MW761x39, and MW762x39). TCE has never been detected in downgradient well MW761x39. TCE was historically detected at trace concentrations (less than 1 µg/L) at well MW762x39 but has not been detected since 2002. The downgradient plume has remained stable in this area.

After several years of trace TCE concentration detections, TCE concentrations have increased slightly above the IRG (5 µg/L) at the southernmost downgradient monitoring wells (MW758x39 and MW760x39). Although TCE concentrations have continued to increase at MW760x39 (to a maximum of 8.8 µg/L in 4Q11), the TCE concentration detected at MW758x39 fell below the IRG in 4Q11. The increases in TCE concentrations in these two (2) wells, along with increasing trends within the core of the plume, led to the conclusion, documented in the NAAR, that natural attenuation alone may be insufficient to prevent plume migration. Consequently, to reduce mass loading on the distal portion of the plume, the EVO PRB was installed in June and July 2010.

In summary, stable or declining TCE concentrations in five (5) of the nine (9) wells located outside of the area potentially impacted by the technology demonstrations (MW03x39, MW759x39, MW761x39, MW762x39, and MW785x39) indicate that natural attenuation is occurring at Site DP039. However, the recent increases in TCE concentrations in the other four (4) wells (MW04x39, MW758x39, MW760x39, and MW781x39) indicate that the mass loading to the aquifer exceeds the aquifer attenuation capacity in portions of the plume.

References

CH2M HILL. 2012a. *Groundwater Sampling and Analysis Program 2010-2011 Annual Report*. Prepared for Travis Air Force Base, California. Final. April.

CH2M HILL. 2012b. *2011 Remedial Process Optimization Report for the Central Groundwater Treatment Plant, North Groundwater Treatment Plant, and South Base Boundary Groundwater Treatment Plant.* Prepared for Travis Air Force Base, California. Draft. April.

CH2M HILL. 2010. *Natural Attenuation Assessment Report*. Prepared for Travis Air Force Base, California. Final. July.

TABLE 1

Mann-Kendall TCE Trend Analysis Results for Sites FT004 and DP039

Technical Memorandum for Assessment of Aerobic Chlorinated Cometabolism Enzymes, Travis Air Force Base, California

Location	Analyte	Count	S-Statistic	p-Value	Trend	Significant
Study Area: FT004						
Site: Site FT004						
EW576X04	TCE	22	-198	0.00	DECREASING	YES
EW577X04	TCE	19	-82	0.00	DECREASING	YES
EW578X04	TCE	22	-124	0.00	DECREASING	YES
EW579X04	TCE	22	-177	0.00	DECREASING	YES
EW580X04	TCE	23	-177	0.00	DECREASING	YES
EW621X04	TCE	17	-113	0.00	DECREASING	YES
EW622X04	TCE	18	-3	0.47	DECREASING	NO
EW623X04	TCE	15	-95	0.00	DECREASING	YES
MW131X04	TCE	27	-89	0.03	DECREASING	YES
MW134X04	TCE	18	100	0.00	INCREASING	YES
MW202X04	TCE	22	-166	0.00	DECREASING	YES
MW264X04	TCE	20	0	0.50	NO TREND	NO
MW266X04	TCE	26	-310	0.00	DECREASING	YES
MW581X04	TCE	22	29	0.21	INCREASING	NO
MW584X04	TCE	19	-125	0.00	DECREASING	YES
MW585X04	TCE	21	-97	0.00	DECREASING	YES
MW587X04	TCE	21	-38	0.13	DECREASING	NO
MW591X04	TCE	25	136	0.00	INCREASING	YES
MW757X04	TCE	17	-52	0.02	DECREASING	YES
Study Area: DP039						
Site: Site DP039						
MW03X39	TCE	20	35	0.14	INCREASING	NO
MW04X39	TCE	25	127	0.00	INCREASING	YES
MW758X39	TCE	20	109	0.00	INCREASING	YES
MW759X39	TCE	20	-66	0.02	DECREASING	YES
MW760X39	TCE	20	122	0.00	INCREASING	YES
MW761X39	TCE	14	0	0.50	NO TREND	NO
MW762X39	TCE	18	-51	0.03	DECREASING	YES
MW781X39	TCE	12	40	0.00	INCREASING	YES
MW785X39	TCE	11	13	0.18	INCREASING	NO

Note:
p-value indicates the probability that a trend exists. A lower p-value indicates a higher probability that a trend exists. All p-values less than 0.05 (95 percent confidence level) were assumed to be significant.

MW1730X31
0.21 J

MW201X07

MW264X04
TCE-ND

MW1731X31
1.3

MW1725X31

MW1727X31

MW1726X31

MW1740X31

MW1029X04

MW265X04

MW131X04
TCE - 154 µg/L

5

100

EW622X04
0.23 J

EW623X04
0.62

MW132X04

FT004

MW202X04
122

EW621X04
0.51

EW565X31
0.27 J

MW1741X31

EW566X31
0.38 J

MW266X04
TCE - 122 µg/L

5

100

EW576X04
0.64 J-

MW1742X31

MW568X31

EW577X04
0.31 J-

MW267X04

EW567X31
ND

MW571X31
ND

MW569X31

MW301X04

MW203X04

MW585X04
159 J-

MW204X04

EW578X04
3.2 J-

MW570X31

MW1030X04

EW579X04
0.32 J

MW572X31

EW580X04
2.3

MW575X31

MW573X31

MW583X04

MW582X04

MW581X04
16.4 J-

MW574X31
2.7

MW584X04
32.4 J-

MW588X04

MW1001X04

MW590X04

MW587X04
5.3 J-

MW589X04

MW586X04

MW591X04
TCE - 19.7 µg/L

MW133X03

MW1002X04

MW757X04
2 J-

MW592X04

MW206X03

MW134X04
2.4 J-

MW756X04

MW205X03

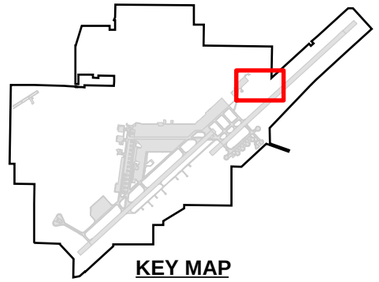
MW302X03

MW755X04

MW752X04

MW753X04

MW754X04



KEY MAP

LEGEND

- MONITORING WELL SAMPLED DURING INVESTIGATION
- GROUNDWATER MONITORING WELL
- EXTRACTION WELL
- 5 — APPROXIMATE TCE ISOCONCENTRATION CONTOUR (µg/L)
- APPROXIMATE GROUNDWATER FLOW DIRECTION
- ERP SITE BOUNDARY
- BASE BOUNDARY
- SURFACE WATER

SAMPLE LOCATION — **MW591X04**
TCE (µg/l) — **0.21J**
FLAG

NOTE:
TCE DATA IS FROM SECOND QUARTER 2011 AND FIRST QUARTER 2012
J = REPORTED ANALYTICAL VALUE IS ESTIMATED
ND = NOT DETECTED; THE METHOD DETECTION LIMIT FOR TCE IS 0.19 µg/L
ONLY SIGNIFICANT EAP AND qPCR RESULTS INCLUDED.

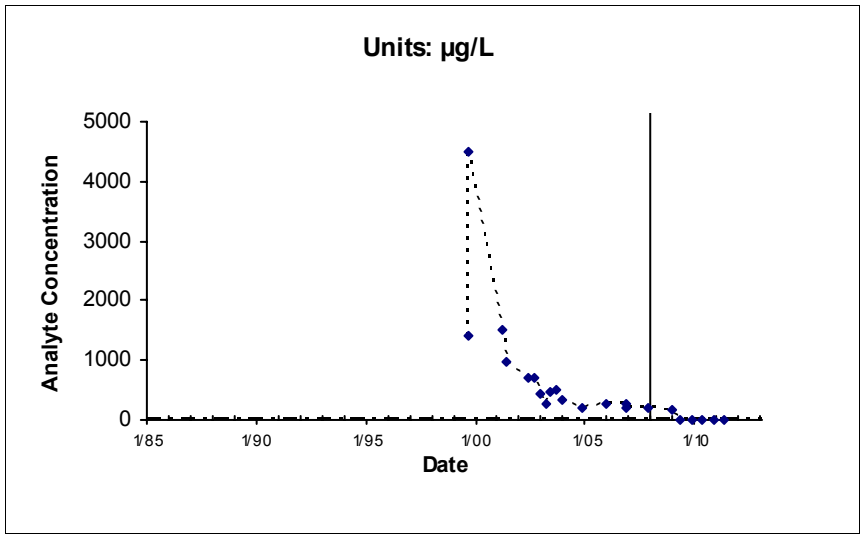
N

0 100 200
FEET

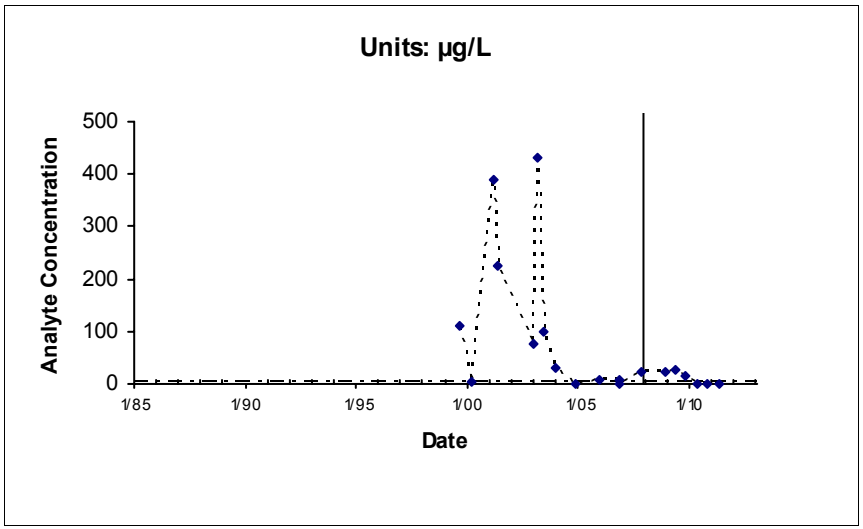
FIGURE 1
TCE DISTRIBUTION AT SITE FT004
TECHNICAL MEMORANDUM FOR ASSESSMENT OF AEROBIC CHLORINATED COMETABOLISM ENZYMES
TRAVIS AIR FORCE BASE, CALIFORNIA

CH2MHILL

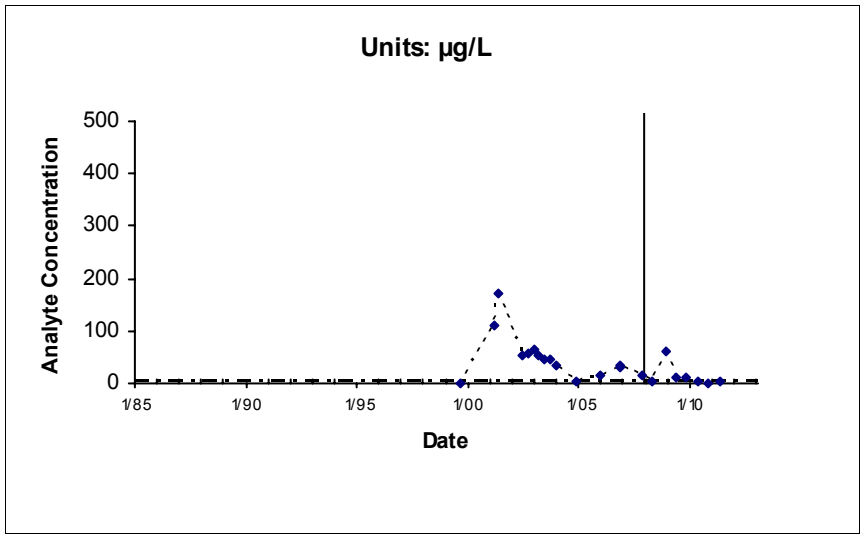
SAC U:\TRAVIS_AFB\381355_2011ANNUALGSAP\MAPFILES\ENZYMETECHMEMO\ATTACH\FIGURE_1_ATTACH_FT004__TCE_2011.MXD MSCHROCK 6/12/2012 5:06:42 PM



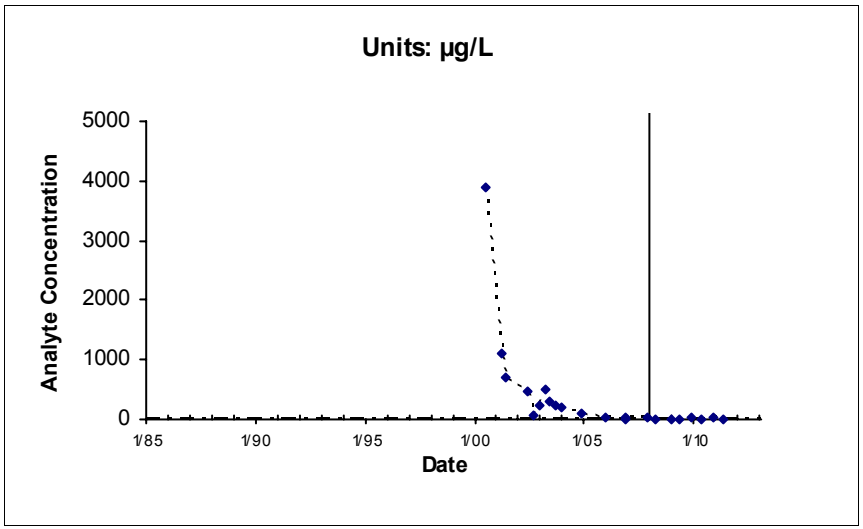
Location: EW576X04 Maximum: 4500



Location: EW577X04 Maximum: 430



Location: EW578X04 Maximum: 170



Location: EW579X04 Maximum: 3900

† Monitoring Well Sampled During Investigation

———— Rebound Study Initiated

— - — - — IRG (5 µg/L)

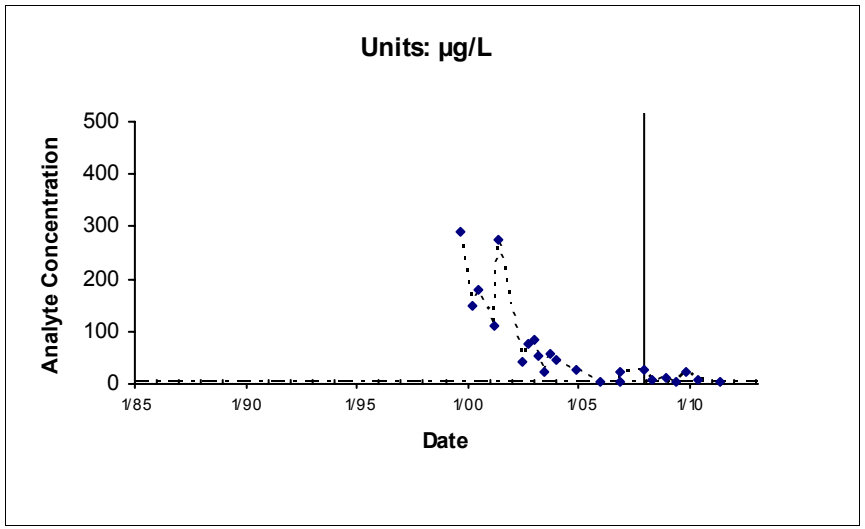
*Nondetects shown as the Method Detection Limit (0.03 µg/L)

FIGURE 2

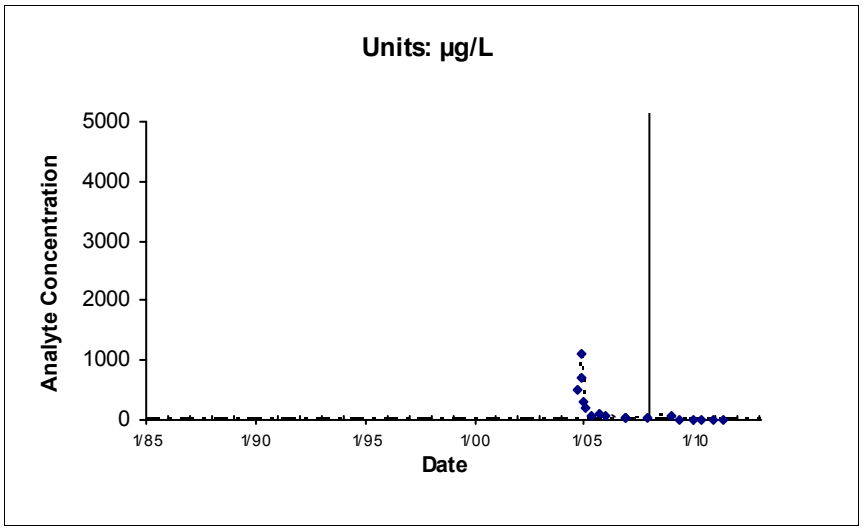
Site FT004

TCE

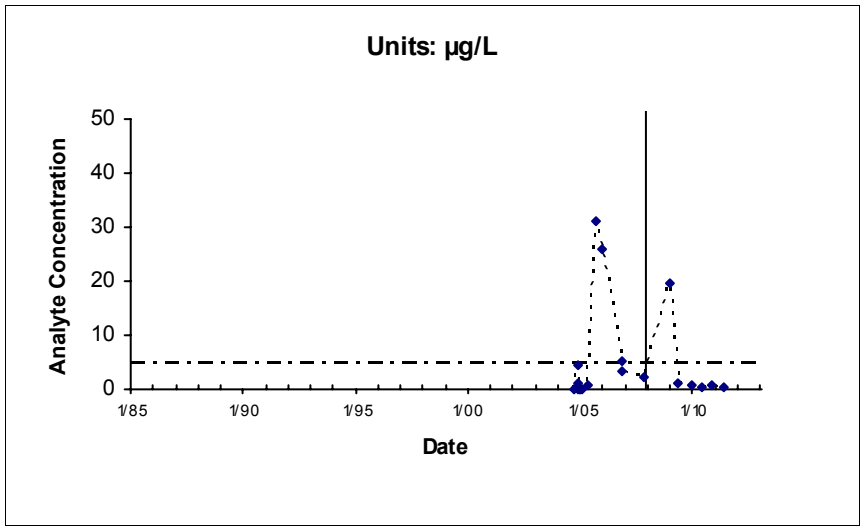
Chemical Time-series Plots



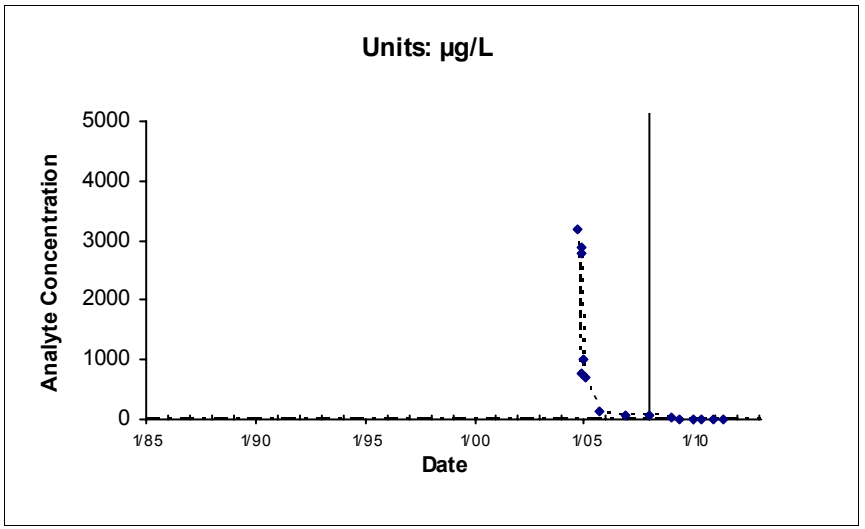
Location: EW580X04 Maximum: 290



Location: EW621X04 Maximum: 1100



Location: EW622X04 Maximum: 31



Location: EW623X04 Maximum: 3200

† Monitoring Well Sampled During Investigation

———— Rebound Study Initiated

— - — - — IRG (5 µg/L)

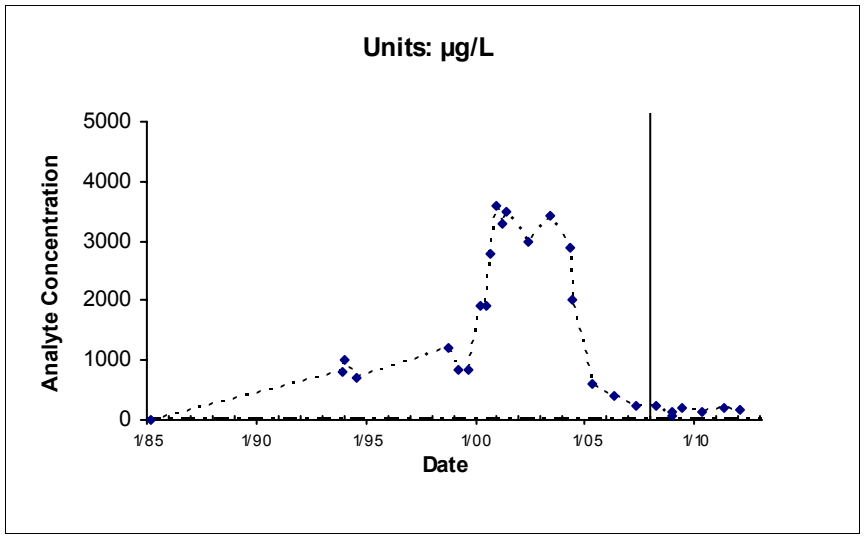
*Nondetects shown as the Method Detection Limit (0.03 µg/L)

FIGURE 2

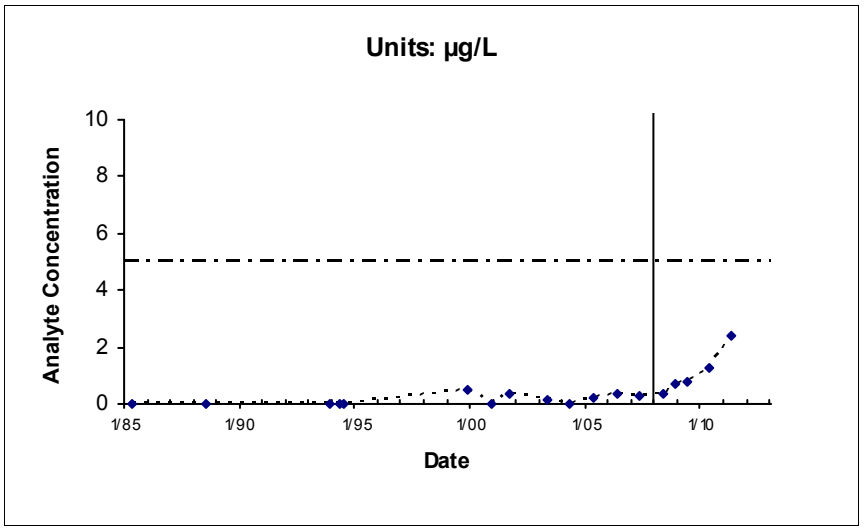
Site FT004

TCE

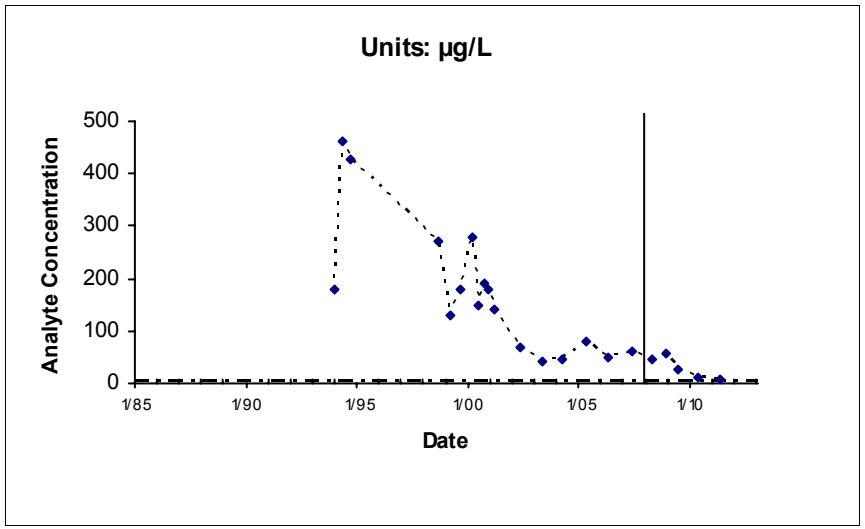
Chemical Time-series Plots



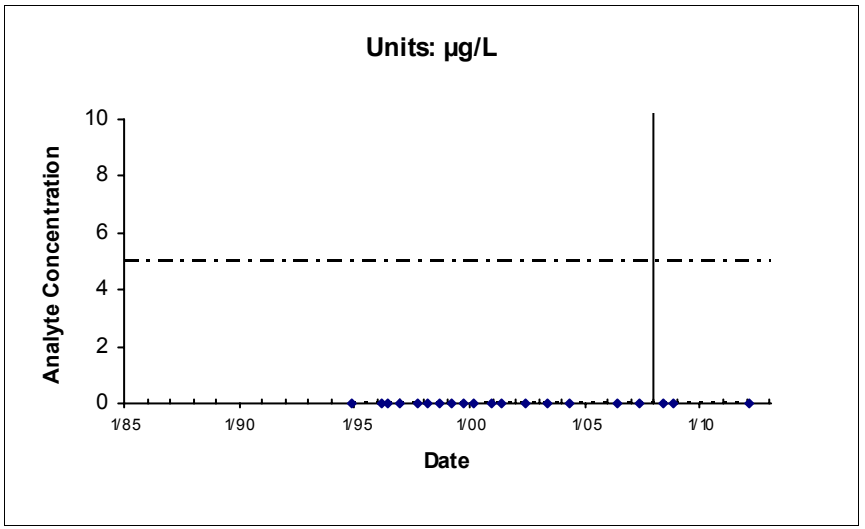
Location: MW131X04† Maximum: 3600



Location: MW134X04 Maximum: 2.4



Location: MW202X04 Maximum: 460



Location: MW264X04† Maximum: 0.03

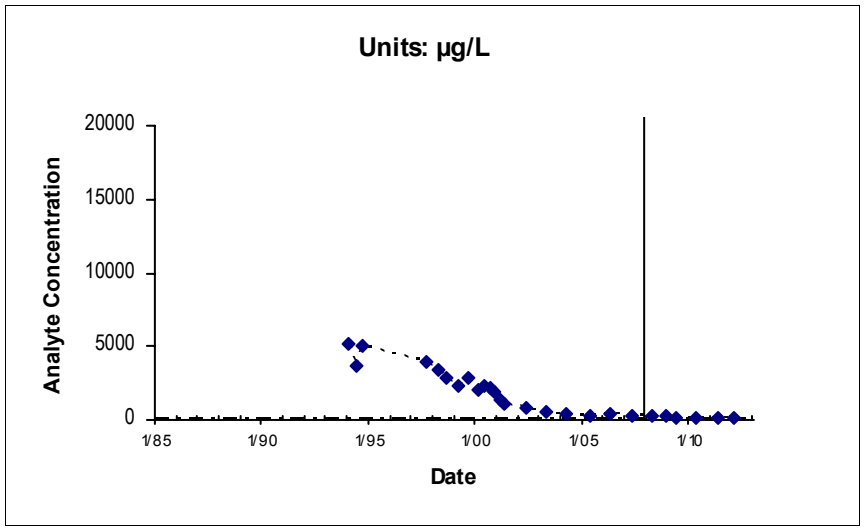
† Monitoring Well Sampled During Investigation

———— Rebound Study Initiated

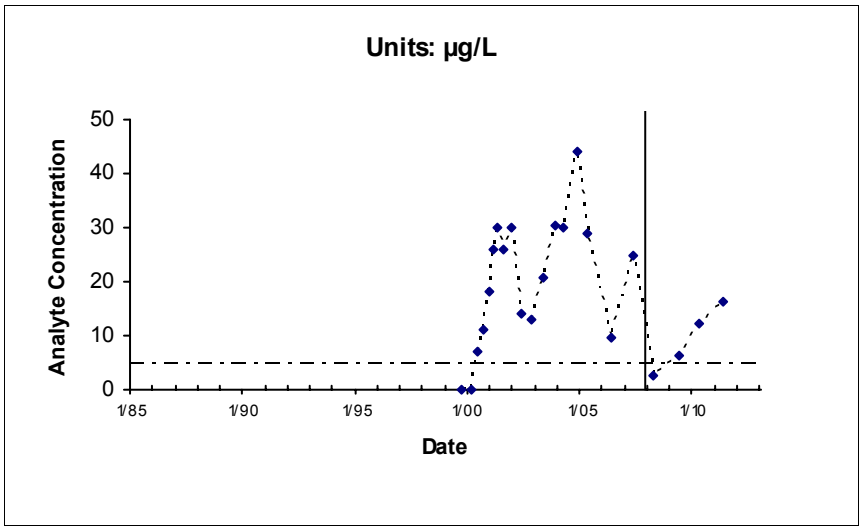
— - — - — IRG (5 µg/L)

*Nondetects shown as the Method Detection Limit (0.03 µg/L)

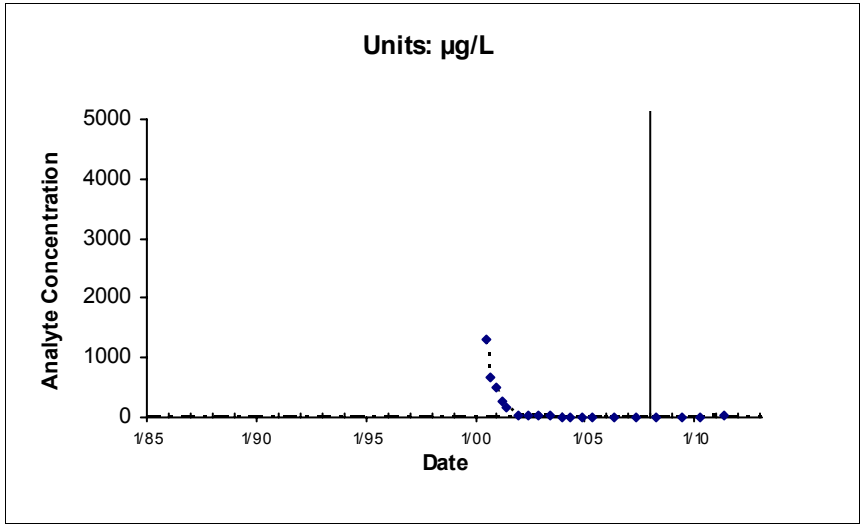
FIGURE 2
Site FT004
TCE
Chemical Time-series Plots



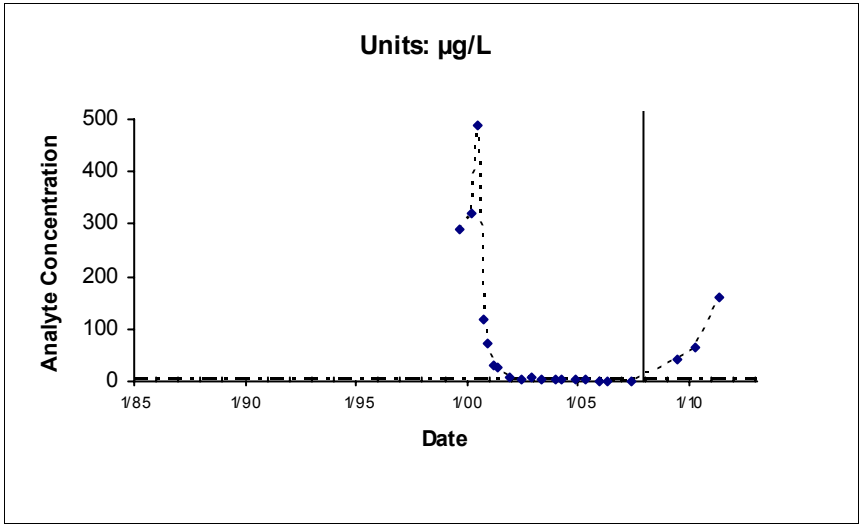
Location: MW266X04† Maximum: 5200



Location: MW581X04 Maximum: 44



Location: MW584X04 Maximum: 1300



Location: MW585X04 Maximum: 490

† Monitoring Well Sampled During Investigation

———— Rebound Study Initiated

— - — - — IRG (5 µg/L)

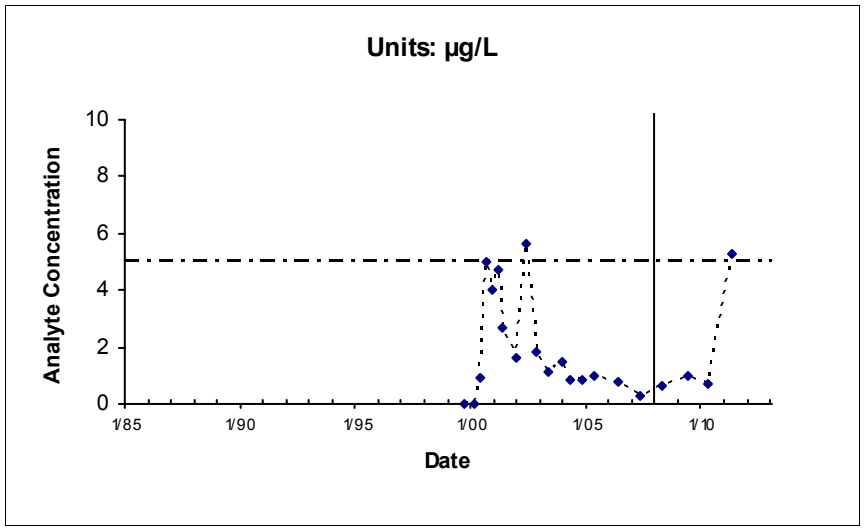
*Nondetects shown as the Method Detection Limit (0.03 µg/L)

FIGURE 2

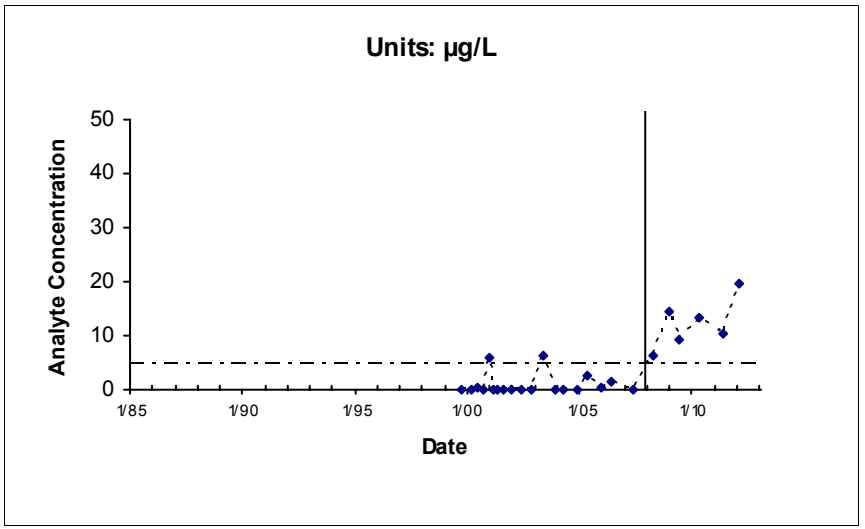
Site FT004

TCE

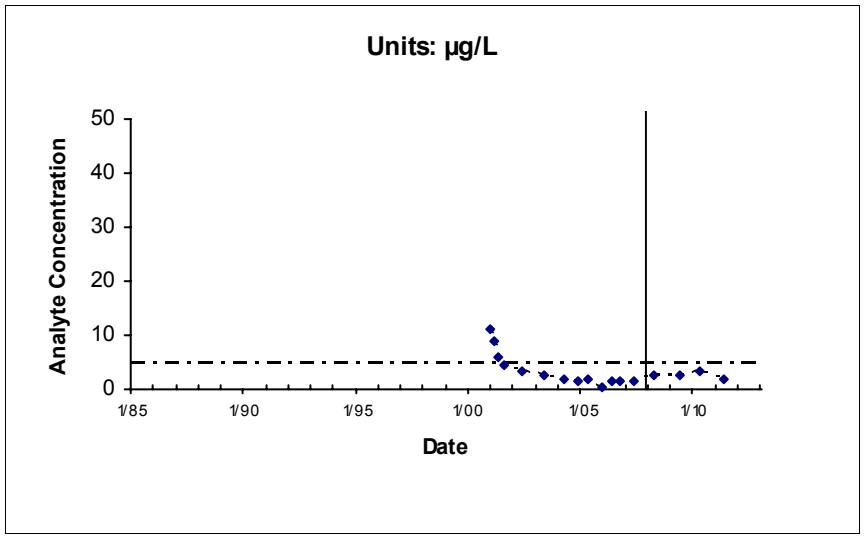
Chemical Time-series Plots



Location: MW587X04 Maximum: 5.6



Location: MW591X04† Maximum: 19.7



Location: MW757X04 Maximum: 11

† Monitoring Well Sampled During Investigation

———— Rebound Study Initiated

— - — - — IRG (5 µg/L)

*Nondetects shown as the Method Detection Limit (0.03 µg/L)

FIGURE 2
Site FT004
TCE
Chemical Time-series Plots

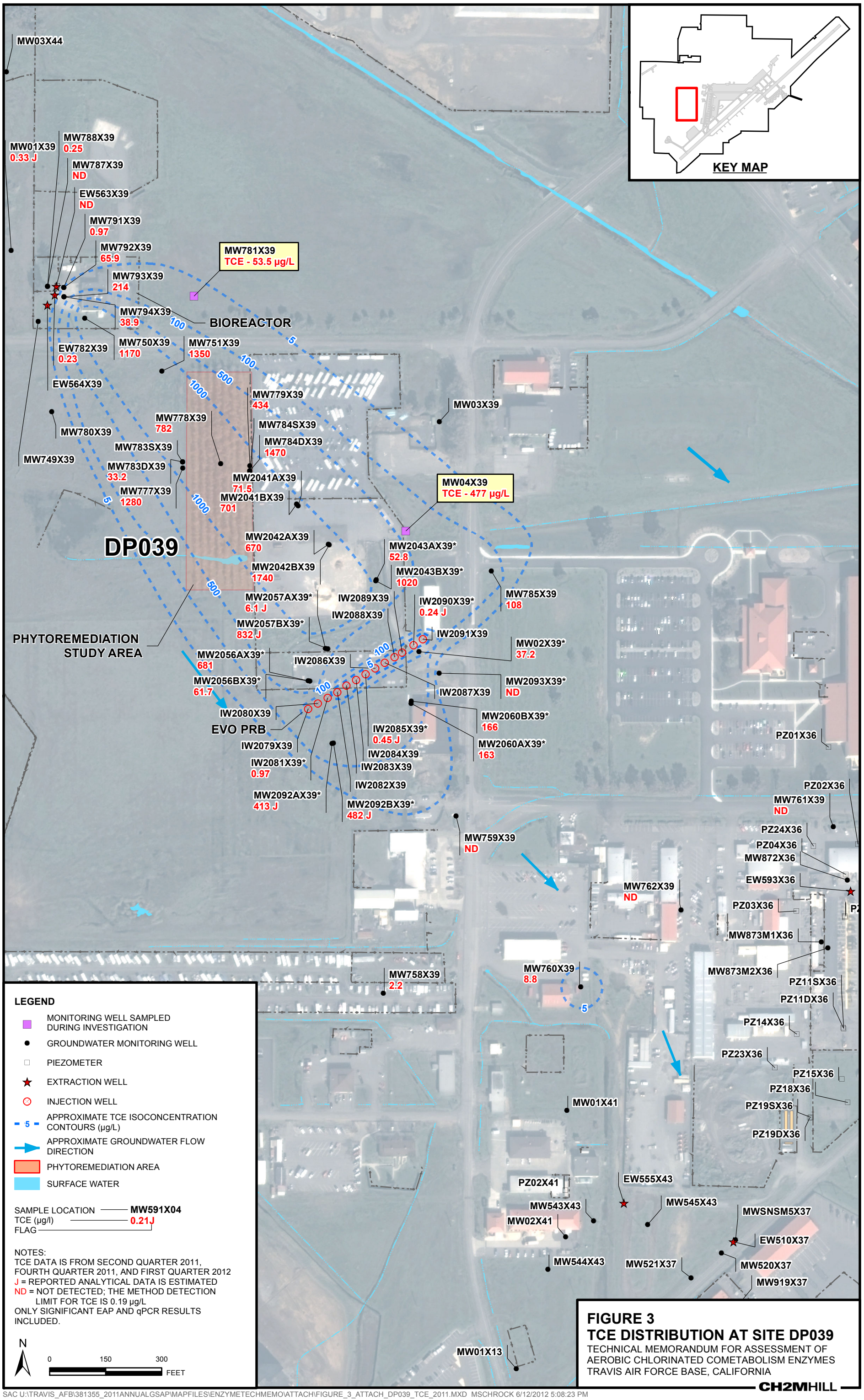
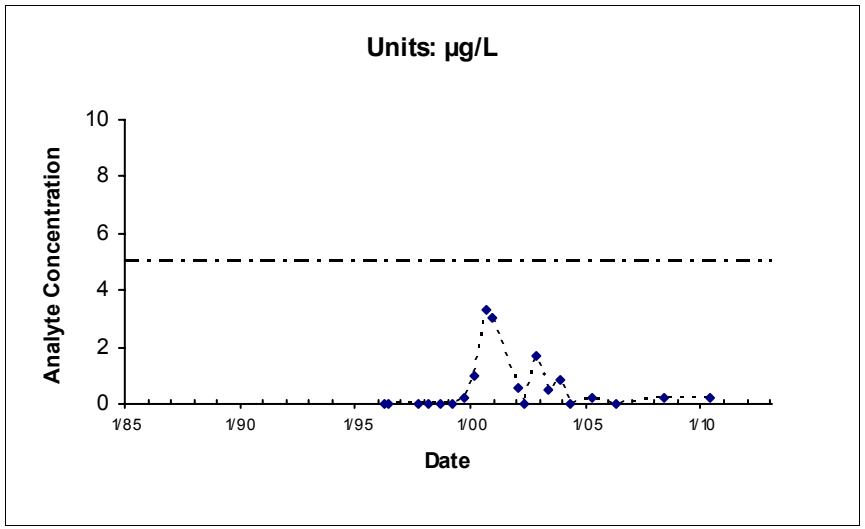


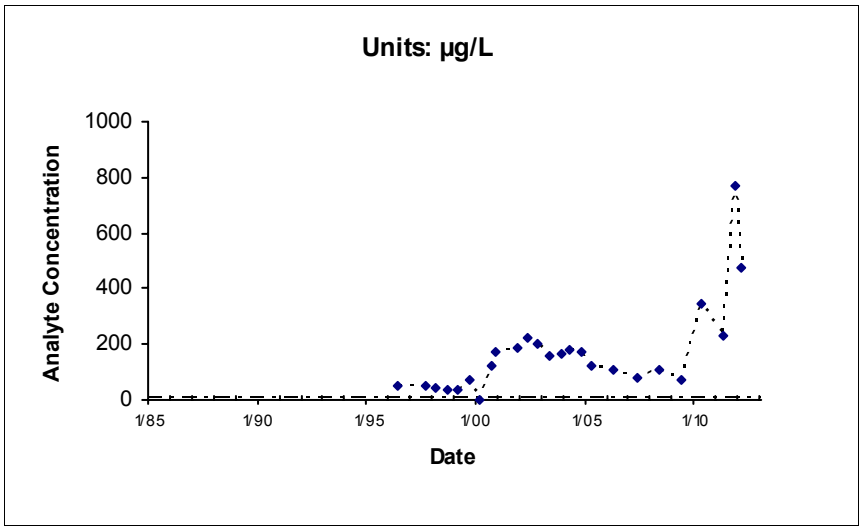
FIGURE 3
TCE DISTRIBUTION AT SITE DP039
TECHNICAL MEMORANDUM FOR ASSESSMENT OF AEROBIC CHLORINATED COMETABOLISM ENZYMES
TRAVIS AIR FORCE BASE, CALIFORNIA

CH2MHILL

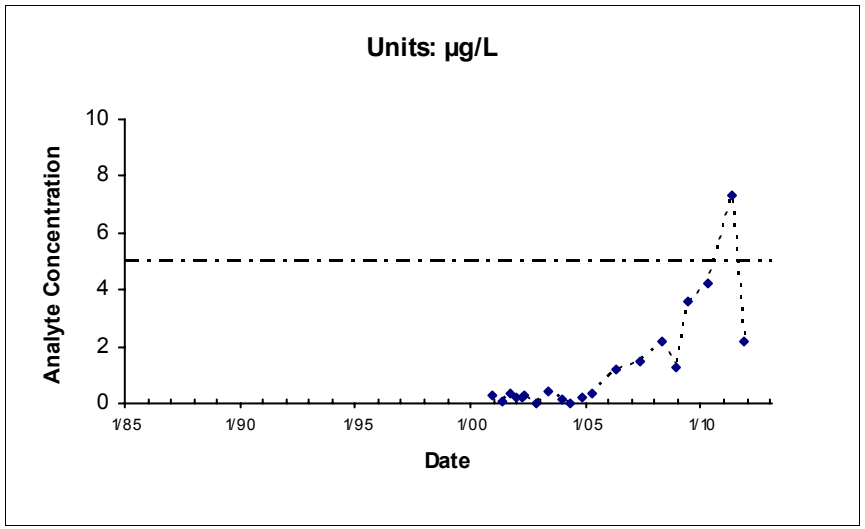
SAC U:\TRAVIS_AFB\381355_2011ANNUALGSAP\MAPFILES\ENZYMETECHMEMO\ATTACH\FIGURE_3_ATTACH_DP039_TCE_2011.MXD MSCHROCK 6/12/2012 5:08:23 PM



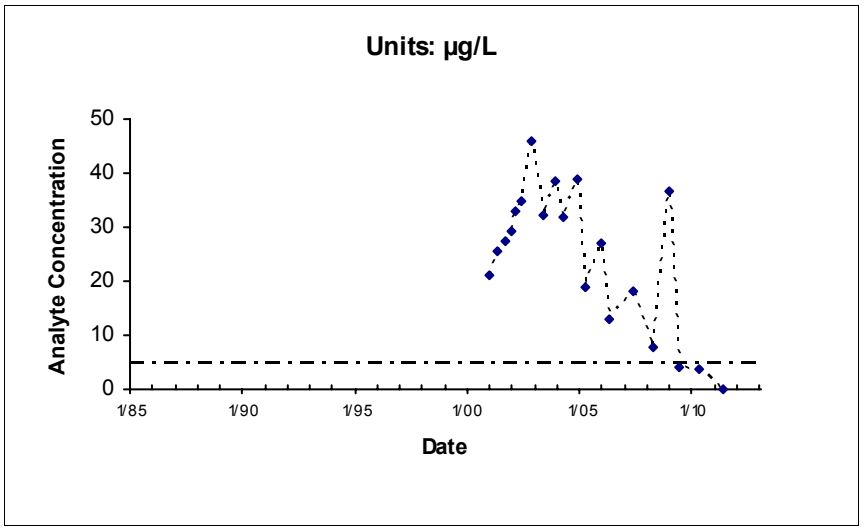
Location: MW03X39 Maximum: 3.3



Location: MW04X39† Maximum: 772



Location: MW758X39 Maximum: 7.3



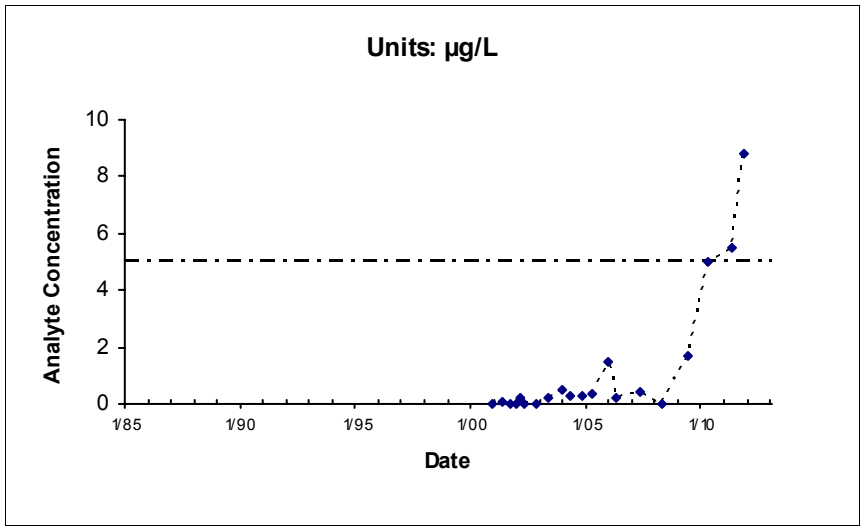
Location: MW759X39 Maximum: 46

FIGURE 4
Site DP039
TCE
Chemical Time-series Plots

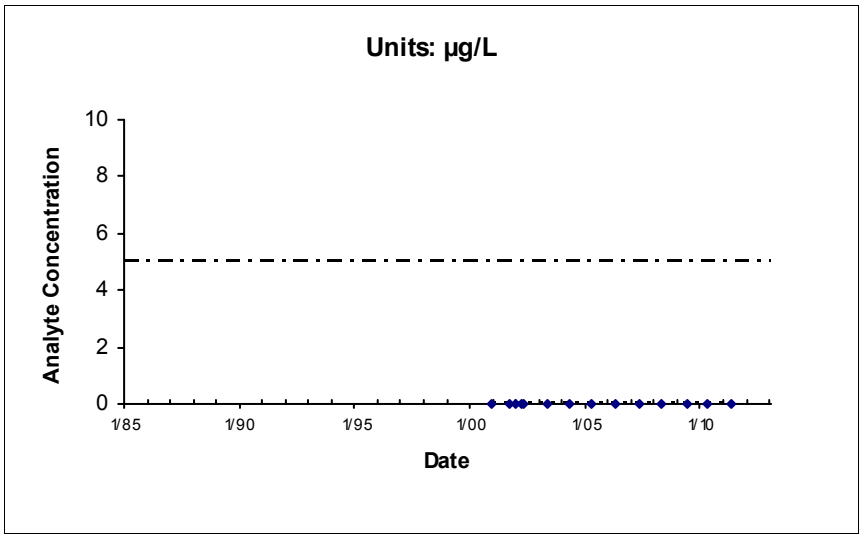
† Monitoring Well Sampled During Investigation

— - — - — IRG (5 µg/L)

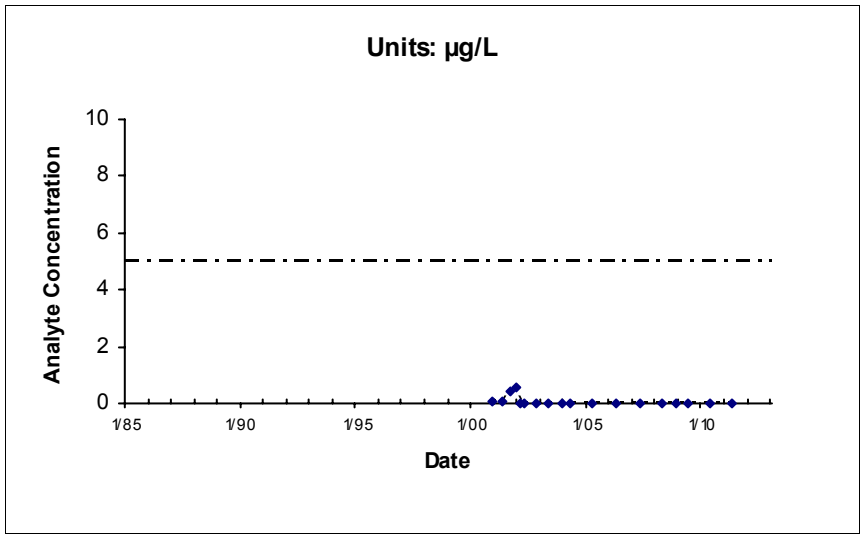
*Nondetects shown as the Method Detection Limit (0.03 µg/L)



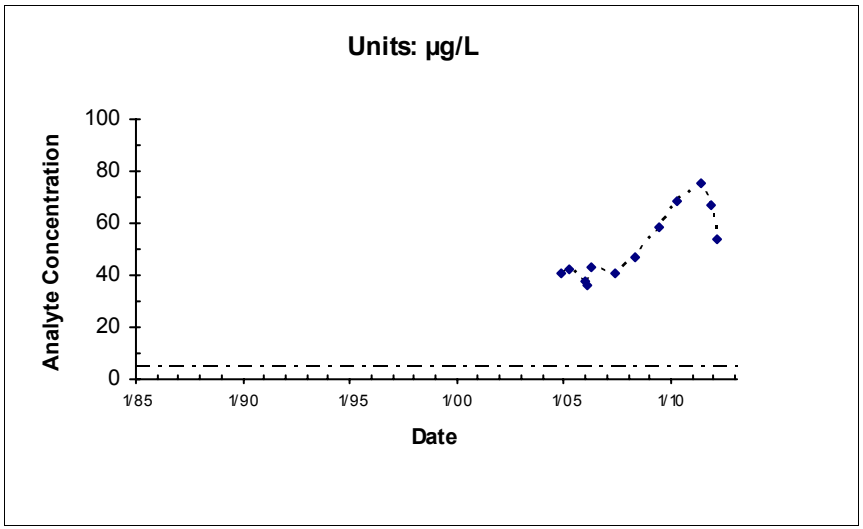
Location: MW760X39 Maximum: 8.8



Location: MW761X39 Maximum: 0.03



Location: MW762X39 Maximum: 0.54



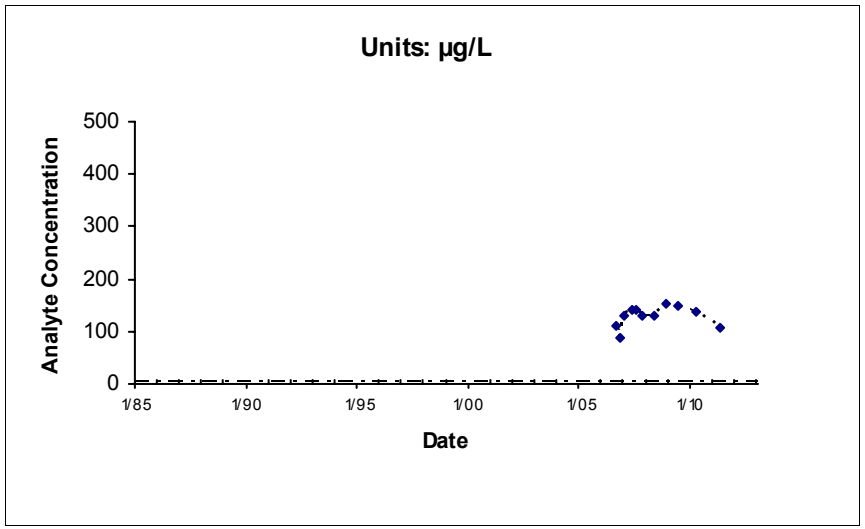
Location: MW781X39† Maximum: 75.2

† Monitoring Well Sampled During Investigation

— - — - — IRG (5 µg/L)

*Nondetects shown as the Method Detection Limit (0.03 µg/L)

FIGURE 4
Site DP039
TCE
Chemical Time-series Plots



Location: MW785X39 Maximum: 151

† Monitoring Well Sampled During Investigation

— - — - — IRG (5 µg/L)

*Nondetects shown as the Method Detection Limit (0.03 µg/L)

FIGURE 4
Site DP039
TCE
Chemical Time-series Plots

Attachment 6
Response to Comments

Responses to Comments on the
Draft Technical Memorandum for Assessment of Aerobic Chlorinated Cometabolism Enzymes,
Travis Air Force Base, California

EPA Region IX

No.	Comments	Responses
REVIEW COMMENTS – Nadia Hollan Burke, EPA Region IX dated July 17, 2012		
GENERAL COMMENTS		
1.	It is difficult to relate the results of this Tech Memo with potential clean-up processes via Natural Attenuation for specific contaminants of concern COCs at Travis AFB. For example, there is no information in the main text of the Tech Memo to directly correlate the specific positive enzyme activity probe (EAP) and quantitative polymerase chain reaction (qPCR) results to COCs that may be addressed by the positive probe or reaction responses. For example, the Tech Memo indicates that there was a positive response for 3-Hydroxyphenylacetylene (3-HPA) but no clear reference is made that this EAP result can be associated with the cometabolism of perchloroethene (PCE), trichloroethene (TCE), dichloroethene (DCE), benzene, toluene, ethylbenzene, and/or xylenes; per Interstate Technology Regulatory Council (ITRC), Enzyme Activity Probes EMD [Environmental Molecular Diagnostics] Team Fact Sheet, November 2011. Please provide additional detail to support how the results can be related to site specific COCs at Travis AFB.	We have added Tables 5 and 7 to correlate the specific positive enzyme activity probe results and quantitative polymerase chain reaction results to the COCs at Sites FT004 and DP039. We have added the following paragraph after paragraph 3 of the EAPs subsection of the Summary of Investigation Results section: "The EAP analytical results indicate that enzymes (toluene-1,2-monooxygenase, toluene-1,3-monooxygenase, toluene-1,4-monooxygenase, and toluene-2,3-dioxygenase) that are known to cometabolically degrade TCE, DCE, tetrachloroethene (PCE), chlorobenzene, benzene, toluene, ethylbenzene, xylenes, and petroleum hydrocarbons (Table 5) are present and active at Sites FT004 and DP039. This suggests that the primary chemicals of concern (COCs) at Sites FT004 (TCE, cis-1,2-DCE, and 1,1-DCE) and DP039 (TCE, 1,1-DCE, and PCE) may be degraded by aerobic cometabolism." We have added the following paragraph after paragraph 4 of the qPCRs subsection of the Summary of Investigation Results section: "The qPCR results confirm that the enzymes (toluene-1,2-monooxygenase, toluene-1,3-monooxygenase, toluene-1,4-monooxygenase, and toluene-2,3-dioxygenase) that were observed to be active in the EAP analyses are present at Sites FT004 and DP039 (Table 7)."

No.	Comments	Responses
SPECIFIC COMMENTS		
1.	**Introduction, paragraph 1, Page 1:** The representativeness or applicability of the results from this Tech Memo to other groundwater plumes at Travis AFB appear to be over stated by the following text, "These indicator sites are considered to be representative of Travis AFB, although they are located in geographically different portions of the Base." It should be noted that there is a proximal and temporal component of cometabolism enzyme studies that should be recognized in the Tech Memo. Please revise the introductory paragraph to acknowledge the temporal and proximal limitation to the results presented in this Tech Memo.	We have added the following text to the first paragraph of the Introduction: "However, it should be noted that the aerobic biotransformation rates of TCE may be location and time-dependent; depending on the concentrations and reactivity of the competing substrates. Therefore, the results of this investigation provide one (1) line of evidence supporting the occurrence of natural attenuation at Travis AFB that must be corroborated by site specific data including: geochemical conditions, decreasing contaminant concentration trends, and reduction in plume size over time."
2.	**EAPs, paragraph 3, Page 9:** Based on the information provided in the Tech Memo, the background well location does not appear to be correlative to conditions at the wells, as the text indicates that the background well is located in bedrock shale. Please discuss the implications of the background well not having comparable conditions to the other wells utilized in this investigation.	We have added the following text to the third paragraph of the Well Selection subsection: "The background well, although shallow (15 to 25 feet bgs), is screened primarily in shale bedrock, and the lithology therefore differs from the other monitoring wells included in the study, which are screened in unconsolidated sediments. As a result, differences in analytical results between the background well and the other monitoring wells included in the study may be due to both the location of the wells in relation to the groundwater plumes and the lithologic conditions." The sampling results from this background well were informative as they are consistent with the conclusion that the primary substrate at Travis AFB is likely to be humics, as described in the first bullet on page 11 (conclusions section): "The negative EAP and qPCR results for background well MW264x04 indicate that there are insufficient amounts of the primary substrate at this location to support the cometabolic microorganisms targeted by this study. The primary substrate at Travis AFB is likely to be humics. This well is screened in shale bedrock, which is likely to have lower available humic substrate than the overlying unconsolidated sediments."
	References Interstate Technology Regulatory Council (ITRC), 2011. Enzyme Activity Probes EMD Team Fact Sheet. November.	We have added this reference to the technical memorandum.

Responses to Comments on the
Draft Technical Memorandum for Assessment of Aerobic Chlorinated Cometabolism Enzymes,
Travis Air Force Base, California

Department of Toxic Substances Control

No.	Comments	Responses
REVIEW COMMENTS – Jose Salcedo, P.E., Department of Toxic Substances Control		
GENERAL COMMENTS		
1.	DTSC reviewed this document and had no comments.	No response necessary.

Responses to Comments on the Draft Technical Memorandum for Assessment of Aerobic Chlorinated Cometabolism Enzymes, Travis Air Force Base, California

Regional Water Quality Control Board

No.	Comments	Responses
REVIEW COMMENTS – Alan D. Friedman, P.E., Regional Water Quality Control Board		
GENERAL COMMENTS		
1.	RWQCB reviewed this document and had no comments.	No response necessary.