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Title: Application of Nucleic Acid-Based Tools for Monitoring Monitored Natural Attenuation (MNA), Biostimulation and Bioaugmentation at Chlorinated Solvent Sites

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FINAL REPORT

Environmental Restoration Project ER-0518

Application of Nucleic Acid-Based Tools for Monitoring Monitored Natural Attenuation (MNA), Biostimulation and Bioaugmentation at Chlorinated Solvent Sites

December 2010

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ACRONYMS

amsl	Above mean sea level
AOC	Area of Concern
cis-DCE	*cis*-1,2-Dichloroethene
DCEs	Dichloroethenes
Dem/Val	Demonstration/validation (demonstrate/validate)
Dhc	*Dehalococcoides*
DHGs	Dissolved hydrocarbon gases
DNA	Deoxyribonucleic acid
DNAPL	Dense non-aqueous phase liquid
DO	Dissolved oxygen
DoD	Department of Defense
DoE	Department of Energy
ESTCP	Environmental Security Technology Certification Program
ETH	Ethene
FDEP	Florida Department of Environmental Protection
FFA	Federal Facility Agreement
HASP	Health and Safety Plan
HDPE	High-density polyethylene
IM	Interim Measure
MCL	Maximum contaminant level
MDEQ	Michigan Department of Environmental Quality
mg/L	Milligrams per liter
MBT	Molecular biological tool
MNA	Monitored natural attenuation
mRNA	Messenger ribonucleic acid
MSDS	Material safety data sheet
ORP	Oxidation reduction potential
OSHA	Occupational Safety and Health Administration
PCE	Tetrachloroethene
qPCR	Quantitative Real-Time Polymerase Chain Reaction
QAPP	Quality Assurance Project Plan
RDase	Reductive Dechlorinase
RFI	RCRA Facility Investigation
RNA	Ribonucleic acid
RPD	Relative Percent Difference
SERDP	Strategic Environmental Research and Development Program
SIA	Southeast Industrial Area
TCE	Trichloroethene
TEAP	Terminal electron accepting process
trans-DCE	*trans*-1,2-dichloroethene
USEPA	United States Environmental Protection Agency
VC	Vinyl chloride
VFAs	Volatile fatty acids
VOCs	Volatile organic compounds
µg/L	Micrograms per liter

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EXECUTIVE SUMMARY

Successful anaerobic bioremediation at chlorinated solvent sites relies on the presence of bacteria, such as *Dehalococcoides* (*Dhc)*, capable of organohalide respiration (i.e., respiratory reductive dechlorination or "[de]chlororespiration"). Nucleic acid-based assays like the quantitative real time Polymerase Chain Reaction (qPCR) technique detect and enumerate *Dhc* in soil or groundwater samples by targeting *Dhc*-specific biomarker genes including the 16S rRNA gene and the *tceA*, *bvcA*, and *vcrA* reductive dechlorinase (RDase) genes implicated in chlorinated ethene reductive dechlorination.

The results of nucleic acid-based tests, like the qPCR approach, is expected to assist site managers and practitioners in making site management decisions. The qPCR data can reduce remediation time and costs by helping site managers identify sites:

- Where long-term Monitored Natural Attenuation (MNA) will be effective;
- Where biostimulation will achieve complete dechlorination without DCE/VC "stall"; and/or
- Where bioaugmentation may be required.

This project's goals included: (1) demonstrating correlations between dechlorination of chlorinated ethenes and the presence and abundance of *Dhc* biomarker genes; (2) defining limitations of the DNA biomarker-based approach and specifying conditions where qPCR assay offers or fails to provide meaningful information; and (3) developing a guidance protocol for practitioners to apply this tool.

The project was conducted in two phases. In the first phase, a standard operating procedure (SOP) was developed for collecting groundwater samples. To avoid problems associated with contemporary procedures that rely on shipment of large volumes of contaminated groundwater, on-site biomass collection using sterile filter cartridges for *Dhc* biomarker quantification was developed and validated. Optimization of laboratory methods resulted in highly reproducible DNA recoveries of 94% compared to standard vacuum filtration methods. In the second phase, this SOP was used to collect groundwater samples from nine chlorinated ethene-impacted sites, including sites undergoing MNA and enhanced bioremediation (biostimulation bioaugmentation). The data were managed in a central database containing groundwater geochemical and microbial data. Incorporation of the data in a single database generated a platform for identifying and evaluating correlations of chlorinated ethene and ethene concentration data, with geochemical data and *Dhc* 16S rRNA gene and RDase gene quantities. Data were evaluated using the Spearman correlation, a nonparametric statistical test.

As a result of this effort, the following performance objectives were met:

Validation of Use of RDase Gene Targets

To date, the four functional genes *pceA* (presumably encoding a PCE-to-TCE RDase), *tceA* (encoding a TCE-to-VC RDase), and *bvcA and vcrA* (both encoding VC-to-ethene RDases) have been identified in chlorinated ethene-dechlorinating *Dhc* strains. At each site included in this study, groundwater samples were collected for qPCR analysis of the RDase gene targets *tceA*, *bvcA*, and/or *vcrA*. The gene copy numbers were correlated to concentrations of PCE, TCE,

dechlorination intermediates (*cis*-DCE and VC), and/or ethene, the nontoxic dechlorination end product, as well as contaminant/product ratios using the Spearman Correlation approach. Strong Spearman Correlations (greater than 0.66) were obtained consistently using *vcrA* as a predictor of ethene production. The *vcrA* and *bvcA* genes are both implicated in VC-to-ethene reductive dechlorination. Selection of an appropriate functional gene target(s) will be governed by site-specific conditions and objectives; however, based on the results of this study, the quantitative analysis of *Dhc* 16S rRNA genes and the VC RDase genes *vcrA* and *bvcA* at chlorinated solvent sites is anticipated to provide useful, reliable information describing complete reductive dechlorination to ethene.

RDase gene copy number correlations to daughter product concentration ratios or concentrations of individual dechlorination intermediates provided site-specific information about the relationship between these variables, but were not consistent from site to site.

Identification of Minimum Number of Dhc Gene Copies Indicative of Ethene Formation

Groundwater samples were collected from all sites for *Dhc*16S rRNA gene and/or RDase gene analysis and results were correlated to ethene concentrations in the sample. Strong Spearman correlations (greater than 0.66) were observed when *Dhc* cell titers or *vcr*A gene copies exceeded 10^6 to 10^7 per liter of groundwater.

Correlation of Dhc Cell Titers to Dechlorination Rates

Groundwater samples were collected from the NASA MLP/VAB site for *Dhc* 16S rRNA gene and RDase gene analysis. First-order dechlorination rates were calculated from chlorinated ethene data collected from wells inside the plume. The first-order dechlorination rates were correlated to *Dhc* and *vcr*A abundance using the Spearman Correlation. Strong correlations were established between TCE, *cis*-DCE and VC dechlorination rates and *Dhc* cell titers, while medium correlations were observed between VC dechlorination rates and *vcr*A gene copy numbers. The analysis was limited by the use of only three monitoring well locations for rate calculations.

Influence of Alternative TEAPs on Dhc Abundance

Geochemical data for identifying terminal electron accepting processes (TEAPs) were obtained for monitoring locations where *Dhc* analyses were conducted. These data were reviewed qualitatively, since mixed TEAPs are typically observed in contaminated aquifers. *Dhc* cell titers above the detection limit of 10^3 gene copies per liter were generally observed when conditions were reducing (anaerobic), as reflected in dissolved oxygen concentrations of less than 0.5 mg/L or redox potentials below -75 mV.

Evaluation of False Positive and False Negative Dhc Detections

Biomarker loss during sample handling may result in false negative results. This issue was addressed by improving sampling and handling procedures to obtain *Dhc* biomarker recoveries of greater than 90%. False positive results were eliminated by optimized qPCR protocols and appropriate controls. Further, the simultaneous quantification of *Dhc* 16S rRNA gene and RDase gene targets in undiluted and 10-fold diluted samples enabled the detection of PCR irregularities, including the presence of PCR inhibitors.

Evaluation of Sample Collection Methods
The groundwater sampling procedure was optimized and applied throughout this project to ensure sample consistency (i.e., minimize the effect of sampling procedures on the results) and quality (i.e., avoid biomarker loss). A study comparing off site (in the lab) to on site (in the field) groundwater filtration and biomass collection indicated that the *Dhc* yield of field-filtered samples exceeded 90% with high precision.

Evaluation of Analytical Sensitivity **-** A reliable limit for *Dhc* 16S rRNA or RDase gene detection is 10^3 cells (i.e., gene copies) per L of groundwater. The quantification limit (i.e., the minimum gene target number that can be reliably quantified) is about 5-fold greater than the method detection limit. Greater sensitivity is not needed, as reductive dechlorination is not observed in the field at gene copy abundances below 10^3 per L.

Evaluate Analytical Sample Reproducibility **-** The qPCR technique is highly reproducible. All qPCR data were generated with at least two replicate DNA extractions, each analyzed for at least two dilutions in triplicate qPCR runs. Differences between replicate samples analyzed in terms of DNA yields and biomarker gene quantification using the same biomass collection method were less than two fold.

Ease of Using On Site Filtration Methods **-** Groundwater sampling methods included attaching a sterile filter cartridge to low flow discharge tubing, measuring the discharge volume during sampling, and packaging the cartridge. This method added 15 to 30 minutes to the time needed to sample a monitoring well for VOCs.

The additional cost of performing nucleic acid-based (qPCR) tests for *Dhc* biomarker gene targets is currently $400 to $485 per sample, including labor and analytical laboratory expenses. Although the analysis requires additional costs, considerable savings can be realized by using this technology through improvements in the selection, design and operation of biologically-based remedies, including MNA, biostimulation and bioaugmentation.

A Guidance Protocol was developed as part of the effort. The protocol presents an SOP for groundwater sampling, as well as guidelines for sampling locations, sampling frequency and data interpretation. Flowcharts are provided for use of *Dhc* biomarker gene data to support decision making at sites where MNA is being evaluated, to predict sites where biostimulation will be successful, and to identify sites where bioaugmentation is required. The Guidance Protocol will be delivered as a separate document.

1. INTRODUCTION

1.1 BACKGROUND

The chlorinated solvents tetrachloroethene (PCE), trichloroethene (TCE), and their anaerobic dechlorination intermediates (daughter products) *cis*-1,2-dichloroethene (*cis*-DCE), *trans*-DCE, 1,1-DCE, and vinyl chloride (VC) are prevalent groundwater contaminants at many Department of Defense (DoD) sites. PCE and TCE are resistant to metabolic degradation under aerobic conditions but can be reductively dechlorinated stepwise to lesser chlorinated ethenes under anaerobic conditions. DCEs and VC can be completely dechlorinated to ethene, and sometimes transformed to ethane, by anaerobic microorganisms. Alternatively, these compounds can be mineralized to carbon dioxide and inorganic chloride under aerobic conditions (Coleman et al. 2002, Singh et al. 2004) and possibly anoxic conditions (Bradley & Chapelle 1998).

Laboratory findings and field studies indicate that reductive dechlorination can be an effective process for transforming chlorinated ethenes under anaerobic conditions (Löffler et al. 2003, ESTCP, 2004). However, at many PCE/TCE contaminated sites, MNA or injection of organic substrates (i.e., electron donor) to stimulate the reductive dechlorination process (i.e., biostimulation) leads to the accumulation of *cis*-DCE and VC with limited or no ethene formation. The accumulation of VC is of particular concern because VC is classified as a human carcinogen. Incomplete dechlorination lengthens remediation times and increases costs before site closure and/or redevelopment of DoD property can be achieved.

Complete reduction of chlorinated ethenes to the environmentally benign products ethene (or ethane) and inorganic chloride is required to achieve detoxification and successful anaerobic remediation of chlorinated ethenes. In addition to biostimulation, bioaugmentation with consortia containing dechlorinating *Dhc* bacteria has been implemented to address incomplete dechlorination and accumulation of toxic intermediates (Ellis et al. 2000, Major et al. 2002, Lendvay et al. 2003, Scheutz et al. 2008). In order to ensure successful application of both biostimulation and bioaugmentation, nucleic acid-based tools were designed (Löffler et al. 2000, Hendrickson et al. 2002, He et al. 2003 a,b, Müller et al. 2004, Krajmalnik-Brown et al. 2004, Ritalahti et al. 2006; Holmes et al. 2006, Smits et al. 2004) for qualitative and quantitative assessment of the dechlorinating bacterial community. Biomarker identification and the refinement of procedures and tools are ongoing activities in laboratories worldwide.

Although some of the available nucleic acid-based tools have been rigorously tested in laboratory settings and are commercially available, the beneficial use of these approaches had not been established in field studies. For example, little was known about the minimum number of bacterial (i.e., *Dhc*) cells needed per volume of groundwater for sustained reductive dechlorination activity. A sufficient database providing quantitative information on key dechlorinating microbes (i.e., *Dhc*), geochemistry, and dechlorination activity was not available for making generalized or site-specific recommendations. Further, no standardized groundwater sampling procedures were applied, *Dhc* biomarker loss during sample handling, shipping to the analytical laboratory and storage were not known and guidance documents for the application of nucleic acid-based tools and interpretation of the results were not available. To promote a more widespread application of MBTs and enhance implementation of bioremediation technologies at

chlorinated solvent sites, standardized protocols are needed. With validated protocols in place, quality and uniformity of test results can be ensured, which in turn will allow comparisons of data obtained from different sites and generated in different laboratories.

The use of molecular biological tools (MBTs), including the use of nucleic acid-based tools in support of environmental bioremediation, was addressed in a SERDP and ESTCP Expert Panel Workshop (2005). This workshop presented a comprehensive summary of MBT techniques, applications, issues, and research questions required to further understand and utilize these tools by the bioremediation industry. The panel concluded that there is insufficient confidence in the current MBT results and there is a need to better understand and measure the microbial activity *in situ*. The panel also confirmed that there is a tremendous potential for these tools to support and improve site assessment and the implementation and performance monitoring of enhanced bioremediation technologies to remediate chlorinated ethene-contaminated sites.

Scientific and technological advances over the past decade generated a plethora of new and sensitive tools applicable to detect and monitor microbes of interest in environmental samples. The potential of MBTs to characterize natural microbial communities taxonomically (i.e. who is there?) and functionally (i.e. who is active?) has been broadly recognized by scientists; however, these new tools have penetrated the applied community to a far lesser extent. This is unfortunate because new MBT tools can provide relevant information about the presence, abundance and activity of microbes contributing to the detoxifcation of chlorinated ethenes. This issue will be addressed by an easy to understand Guidance Protocol that educates practitioners and regulators of the new technologies and their potential to advance contaminated site remediation.

The guidance developed in this study is expected to provide remediation project managers with the background to understand the value of MBT application, to judge what information the MBT approach can/cannot provide, and to interpret MBT data. In other words, the Guidance Protocol promotes a more widespread application of MBTs and results in significant cost reductions and reduced project timelines. The remediation project manager end user will be provided with additional relevant information to interpret site conditions to select:

- Sites where implementation of long-term MNA will be effective;
- Sites where biostimulation will achieve complete dechlorination without DCE/VC “stall”; and/or
- Sites where bioaugmentation is required, ultimately shortening remediation times; or
- identify sites where the conditions (e.g., low pH, insufficient supply of electron donor, unfavorable geochemical conditions) are limiting biodegradation activity.

By clearly understanding how site geochemistry and the presence and abundance of key microbes (i.e., *Dhc*) affect contaminant detoxification, investments in the technology could focus on those sites amenable to bioremediation, and a more efficient and rapid transition from system design to full-scale remediation is expected. This could save months to years on a given remediation project time line, would achieve more rapid site closures, and save the DOD resources that can be invested elsewhere.

1.2 OBJECTIVES OF THE DEMONSTRATION

During this demonstration, we evaluated and validated the use of nucleic acid-based tools for site assessment and bioremediation process monitoring at chlorinated solvent sites undergoing MNA, biostimulation, and/or bioaugmentation treatment. Use of these tools is anticipated to reduce remediation costs by i) supporting identification of sites amenable to MNA; ii) predicting sites where biostimulation can be successfully implemented; iii) identifying sites where bioaugmentation is required early in the design process, and (iv) recognizing sites where the reductive dechlorination process cannot be productively implemented. The specific project objectives included:

1. Evaluating groundwater sampling methods that collect planktonic (i.e., unattached) cells on membrane filters on-site to avoid shipping of groundwater to the analytical laboratory.

2. Applying nucleic acid-based tools to assess the distribution and abundance of *Dhc* biomarker genes at 12 sites at different stages of bioremediation treatment.

3. Integrating the MBT information with data typically collected (e.g., contaminant concentration data, geochemical data) at bioremediation sites to develop correlations between reductions in contaminant concentrations and the abundance of specific *Dhc* biomarker genes.

4. Evaluating if qPCR data are useful predictors for the feasibility of MNA, biostimulation, and/or bioaugmentation as productive cleanup remedies at a given site contaminated with chlorinated ethenes.

5. Developing a guidance document for application of nucleic acid-based qPCR tools at chlorinated solvent sites where MNA, biostimulation, or bioaugmentation are being considered or have been implemented.

6. Identifying the limitations of the qPCR approach for the analysis of groundwater samples, and specifying the site conditions where this tool can/cannot provide useful information.

The approach was demonstrated at 12 selected DoD sites that are contaminated with chlorinated ethenes and where MNA, biostimulation, and/or bioaugmentation treatments have been implemented. Groundwater samples were collected during routine monitoring efforts by the respective site responsible parties. Samples were forwarded to the Georgia Institute of Technology for qPCR analysis.

The 2005 SERDP and ESTCP Expert Panel identified numerous research needs for MBTs application at contaminated DOD sites. The significance of these knowledge gaps is well understood by the project team and findings, materials, tools and procedures used or developed in other on-going research projects were incorporated into this evaluation to the extent possible. Because sampling procedures affect sample (i.e., biomarker) integrity, significant effort was expended to identify and minimize the impacts of sampling biases on MBT analysis and results. A major outcome of this project is a new on-site sampling procedure for collecting microbial biomass from groundwater for subsequent microbial biomarkers analysis.

1.3 REGULATORY DRIVERS

The USEPA MCL for PCE and TCE in drinking water is 5 µg/L. This concentration is considerably lower than the concentrations present in groundwater at many DoD sites. The MCLs for *cis*-DCE and VC are 70 µg/L and 2 µg/L, respectively. MNA and enhanced bioremediation have been shown to be cost-effective technologies for remediating chlorinated ethene-contaminated sites. Therefore, this demonstration sought to improve the selection, design and implementation of MNA and bioremediation treatment to achieve cleanup goals and site closures. Importantly, the findings communicated in the Guidance Protocol will assist regulators to better understand and judge the meaning of qPCR data as a relevant component for predicting contaminant concentrations and future plume behaviour.

2. TECHNOLOGY

The following sections provide: an overview of technology history and application (Section 2.1); a description of technology development (Section 2.2); and a description of the potential advantages and limitations of the technology (Section 2.3).

2.1 TECHNOLOGY DESCRIPTION

Discoveries over the past decade significantly advanced our understanding of microbial processes that contribute to the fate of chlorinated ethenes in contaminated subsurface environments. Although not all processes contributing to chlorinated ethene detoxification are fully understood, there is conclusive evidence that reductive dechlorination plays a major role in anaerobic aquifers where MNA, biostimulation and/or bioaugmentation are implemented. The complete dechlorination of PCE to ethene is a multi-step process and is most effectively carried out by more than one microbial population (reviewed in Major et al. 2003, Löffler et al. 2003, Smidt and de Vos 2004).

Several bacterial groups are involved in partial reductive dechlorination of PCE and TCE to DCEs (e.g., *Dehalobacter, Desulfitobacterium, Desulfuromonas, Geobacter,* and *Sufurospirillum* species), but *Dhc* are the key players involved in complete reductive dechlorination and detoxification (i.e., ethene formation) (Löffler et al. 2003, Smidt and de Vos 2004). Since complete reductive dechlorination is firmly linked to *Dhc* bacteria, evidence for *Dhc* presence and abundance will guide the decision making process on treatment options. The current knowledge of the detoxification process (i.e., the link between ethene formation and the presence of *Dhc*) justifies that site assessment and bioremediation monitoring focuses on members of this bacterial group.

Polymerase Chain Reaction (PCR)

DNA biomarkers in environmental samples are typically present in concentration too low for direct analysis. A milestone discovery was PCR that allowed the amplification of a specific DNA target sequence to generate enough identical copies that can be easily analyzed. PCR has revolutionized forensics applications and is now being applied to evaluate the presence/absence of target DNA sequences of interest in environmental samples. Polymerase Chain Reaction (PCR) techniques use unique, thermostable bacterial DNA-dependent DNA polymerases that produce large numbers of identical copies of target DNA. In addition to the DNA polymerase, PCR requires small DNA fragments called oligonucleotide primers (or simply "primers) in order to amplify specific DNA sequences (i.e., the target DNA) of interest within an undefined DNA mixture that represents the genomes of all the microorganisms present in the sample.

Quantitative real-time PCR (qPCR) functions in a manner similar to PCR, but through the use of fluorophore chemistry, monitors each round of amplification, thus allowing visualization of amplification in real time. Using standard curves established with known target gene copy numbers allows quantification of the target gene copies (i.e, the amplicons) in unknown samples. A common amplicon detection chemistry uses linear hybridization probes (also called Taqman probes). A Taqman qPCR probe is similar to a PCR primer but contains a fluorophore at one

end and a quenching molecule at the opposite end of the linear oligonucleotide. As long as in proximity to each other, the fluorophore and the quencher interact, thus preventing fluorescent light emissions when excited with light of a suitable wavelength. The Taqman probe binds to the target DNA between the primer binding sites. When the DNA polymerase adds nucleotides and moves along the target DNA strand during amplification, the fluorescent reporter and the quencher are cleaved from the probe and separate. Upon excitment with a suitable laser, the free reporter fluorophore emits a fluorescent signal, which is detected and quantified in the thermocycler at the end of every amplification cycle. A larger fluorescent signal corresponds to a greater number of cleaved fluorescent reporters. Since one Taqman probe containing a single fluorescent reporter hybridizes to a single target DNA molecule, the resulting signal strength after the reporter releases from the probe is directly proportional to the number of DNA copies present at the end of a cycle. A comparison of the fluorescent signal of the samples with those generated with target DNA of known concentrations (i.e., the standard curve) permits the quantification of the target DNA sequences of unknown concentration within complex samples.

Previous Testing of the Technology

PCR, as a technology, was invented over 25 years ago (Saiki et al. 1985). Competitive PCR (quantitative endpoint titration PCR) has been in use for over 20 years mostly to quantify DNA (Becker-Andre et al. 1989, Gilliland et al. 1990, Wang et al. 1989). Quantitative real-time PCR (qPCR) was introduced over a decade ago and qPCR citations have increased steadily since then. qPCR now represents the method of choice for analyzing gene targets (Van Guilder et al. 2008, Smits et al. 2004, Ritalahti et al. 2006, Karlen et al. 2007) and evolved into a standard methodology in the medical, food microbiology and biodefence sectors.

The first application of qPCR at a chlorinated solvent site quantified and compared *Dhc* 16S rRNA gene abundance at the Bachman Road site in response to different bioremediation treatments. qPCR technology conclusively demonstrated that the rapid transformation of *cis*-DCE to ethene correlated with an increase of the *Dhc* population size following bioaugmentation and biostimulation (Lendvay et al. 2003). Similarly, at a site in Milledgeville, Georgia, a correlation between rapid and complete dechlorination of TCE and an increase in *Dhc* cell titers was demonstrated after bioaugmentation (Seguiti et al. 2005). In addition, the quantitative monitoring of the three known *Dhc* RDase genes (e.g., *tceA*, *bvcA*, and *vcrA*) provided evidence that the *Dhc* provided with the bioaugmentation inoculum were responsible for complete TCE reductive dechlorination.

Qualitative Assessment with 16S rRNA Gene-Targeted Primers

Nucleic acid-based tools have been designed to rapidly assess the presence of dechlorinating bacteria in soil or groundwater samples. Species-specific 16S rRNA gene-targeted primers have been designed and applied in endpoint PCR to detect target dechlorinators in environmental samples. For *Dhc* organisms, 16S rRNA gene copies equal the cell numbers because each *Dhc* cell has one copy of the 16S rRNA gene. Other bacteria can possess more than one copy of this gene. The detection limit of this direct PCR approach is approximately 5×10^3 gene copies per reaction mix, which equates to 10^5 gene copies per liter of groundwater. For increased sensitivity, environmental DNA can be amplified first with universal bacterial 16S rRNA gene-

targeted primers followed by a second PCR round with dechlorinator-specific primer pairs. Such a nested PCR approach increases the detection sensitivity about 1000 to 10,000-fold and as few as 1-10 target genes per PCR reaction can yield measurable signals. Nested PCR offers unsurpassed sensitivity, and detection of only a few dechlorinating bacterial cells per mL of groundwater or aquifer material is feasible (Löffler et al. 2000, Ritalahti et al. 2006). Although the direct PCR approach may be used to judge relative abundances of a target gene (Major et al. 2002), quantitative information is lost with the nested PCR approach. Another drawback of the 16S rRNA gene-targeted approach is that strains of the same species with similar or even identical 16S rRNA gene sequences do not share the ability to carry out reductive dechlorination reactions. Hence, a positive signal obtained in direct or nested PCR merely indicates that a strain or strains of a species are present but it does not prove that a dechlorinating strain is actually present. Numerous nearly complete 16S rRNA gene sequences of *Dhc* strains from chlorinated solvent-dechlorinating enrichment cultures, as well as environmental clone sequences obtained from chlorinated solvent-contaminated aquifers, are available. Although progress in strain-specific detection has been made (Ritalahti and Löffler 2004, Ritalahti et al. 2006), this approach is limited due to the high degree of 16S rRNA gene similarity of *Dhc* organisms that exhibit distinct dechlorination activities. Hence, no specific dechlorination activity can be inferred from the detection of *Dhc* 16S rRNA genes in environmental samples. However, since all known *Dhc* strains are obligate organohalide respirers, the detection of *Dhc* 16S rRNA genes at elevated levels suggest the presence of a *Dhc* stain or multiple *Dhc* strains with the ability to dechlorinate the contaminant(s) present at the site. Nevertheless, targeting specific Dhc 16S rRNA gene sequences provides a foundation for the detection of *Dhc* bacteria in environmental samples.

Quantitative Assessment with qPCR

qPCR techniques have been developed to quantify target microbes (i.e., *Dhc*) (He et al. 2003a, 2003b, Smits et al. 2004, Ritalahti et al. 2006). The qPCR approach offers sensitive detection combined with quantitative information. Thus, qPCR is useful to monitor the effects of treatment on the size of the dechlorinating *Dhc* population (i.e., the amount of catalyst present in the contaminated aquifer). qPCR has several advantages over traditional endpoint PCR. qPCR is faster and highly sensitive (>5 copies per reaction), requires no post-PCR steps (e.g., agarose gels), minimizes the risk of cross contamination, and multiplex assays are feasible. Multiplex assays allow the quantification of up to four targets in a single assay tube, thus reducing chemical and labor costs; however, multiplex assays require careful testing and optimization to avoid interferences of the multiple primers and fluorescent probes in the reaction mix. Nevertheless qPCR quantifies DNA (and possibly RNA) targets precisely and reproducibly because it relies on threshold cycle (Ct) values determined during the exponential phase of PCR rather than endpoints (e.g., competitive quantitative PCR (Cupples et al. 2003)).

An inherent weakness of the 16S rRNA gene-based approach (PCR or qPCR) is that a firm link between phylogeny and function (e.g., dechlorination activity) does not exist. Although the 16S rRNA gene approach is useful for detecting and monitoring *Dhc* bacteria, the information contained in the 16S rRNA molecule cannot distinguish *Dhc* with distinct dechlorinating abilities. For instance, strain KS responsible for 1,2-dichloropropane-to-propene dechlorination does not grow with chlorinated ethenes but shares an identical 16S rRNA gene sequence with strain BAV1 (Ritalahti and Löffler 2004). This shortcoming of the 16S rRNA gene-based approach

has been rectified by identification of specific reductive dehalogenase (RDase) genes as described below.

Additional Targets – Reductive Dehalogenase (RDase) Genes

Although the 16S rRNA gene-based approach is a powerful tool to detect, monitor and quantify *Dhc*, it is limited by its inability to distinguish *Dhc* strains with similar or identical 16S rRNA genes but different dechlorinating activities. In other words, elevated numbers of *Dhc* at a given site do not prove that dechlorination to non-toxic compounds is occurring and additional measures may be needed to draw meaningful conclusions. Additional gene targets that contain information beyond that provided by the 16S rRNA gene must be identified and analyzed. Because not all *Dhc* populations are equal, with some being more effective or more efficient in the reductive dechlorination of chlorinated ethenes, identification and quantification of individual *Dhc* strains is relevant to provide information about the key dechlorination steps (i.e., VC-to-ethene dechlorination).

To date, four functional genes (i.e., *pceA, tceA, bvcA, and vcrA)* involved in chloroethene reductive dechlorination have been identified in *Dhc* strains. The *pceA* gene presumably encodes a PCE-to-TCE RDase in *Dhc ethenogenes* strain 195, and the *tceA* gene is responsible for TCE-to-VC reductive dechlorination in *Dhc ethenogenes* strain 195 and *Dhc* sp. strain FL2. The known *Dhc* strains harbor a single copy of the aforementioned biomarker genes indicating that the number of target genes enumerated with qPCR equals the number of *Dhc* cells in the sample. It is also important to note that the known *Dhc* strains carry either the *tceA, bvcA, or vcrA* gene (i.e., these genes do not co-occur in the same *Dhc* strain), and that the *tceA, bvcA, and vcrA* genes only occur on *Dhc* genomes (i.e., no other bacteria carrying these genes have been found).

Crucial for achieving detoxification and site closure is the final reductive dechlorination step that transforms VC to environmentally benign ethene. To date, three VC-respiring *Dhc* isolates (strains BAV1, VS and GT) have been obtained. *Dhc* sp. strain BAV1 harbors the *bvcA* RDase gene (but not the *vcrA* gene), and *Dhc* sp. strain VS and *Dhc* sp. strain GT both possess the *vcrA* gene but lack the *bvcA* gene. No *Dhc* strain is known that harbors both the *vcrA* and the *bvcA* genes. Both the *vcrA* and *bvcA* gene have been implicated in VC reductive dechlorination to ethene. *vcrA* was characterized biochemically and enzyme assays demonstrated that the VcrA protein reduces all DCE isomers and VC with reduced methyl viologen as artificial electron donor (Rosner et al. 1997; Müller et al. 2004). PCE and TCE were not dechlorinated by VcrA. *bvcA* was identified as the most highly expressed RDase gene in BAV1 cultures growing with VC as electron acceptor (Krajmalnik-Brown et al. 2004). Recent biochemical studies using BAV1 cell extracts corroborated that BvcA catalyzes VC-to-ethene reductive dechlorination (Fletcher et al. 2010).

Table 2-1 Characteristics of Available VC-respiring *Dhc* Isolates

***Dhc* strain**	**# of Putative RDase Genes**	**VC RDase Gene**	**Chlorinated Ethenes Used as Electron Acceptors**
BAV1	11	*bvcA*	*cis*-DCE, *trans*-DCE, 1,1-DCE, VC
VS	36	*vcrA*	TCE, *cis*-DCE, 1,1-DCE, VC
GT	21	*vcrA*	TCE, *cis*-DCE, 1,1-DCE, VC

Strain VS and GT grow with TCE whereas strain BAV1 cannot. Therefore, it is not surprising that *Dhc* strains similar to BAV1 do not compete well against *Dhc* strains VS and GT when TCE is provided as electron acceptor. For example, the BDI consortium (Amos et al. 2008) contains *Dhc* strain GT and BAV1, and this culture is dominated by strain GT when TCE is supplied as electron acceptor. A similar effect is observed when the bioaugmentation consortium KB1 is grown with TCE, and *Dhc* strains carrying *vcrA* dominate. Therefore, it is sensible to predict that *Dhc* strains of the VS and GT type are more abundant that strain BAV1 type *Dhc* strains at sites that are impacted with TCE. Data from field sites support this conclusion. For example, the initial contamination at the Bachman Road site was PCE, which was reductively dechlorinated to *cis*-DCE by non-*Dhc* populations. At this site, *cis*-DCE remained as the main contaminant and *Dhc* sp. strain BAV1, which uses *cis*-DCE as growth-supporting electron acceptor, was the dominant *Dhc* strain contributing to ethene formation (Lendvay et al. 2003). Spatial separation of *Dhc* strains with different RDase gene was observed in a PCE-fed column, where *Dhc* strains harboring *vcrA* were more abundant near the column inflow with higher PCE and TCE concentrations. In more distant zones from the column influent with predominantly *cis*-DCE and VC, *Dhc* strains with *bvcA* were most abundant (Beherens et al. 2008). Gene expression studies demonstrated that *bvcA* was the most highly expressed RDase gene in the column, except near the influent where only PCE and TCE were available as electron acceptor (Beherens et al. 2008). Temporal changes in the abundance of *vcrA* and *bvcA* were observed at a TCE-contaminated site near Milledgeville, GA. The BDI consortium used for bioaugmentation contained *Dhc* strain GT carrying *vcrA* and strain BAV1 carrying *bvcA*. During the initial TCE dechlorination phase, *vcrA* predominated but *bvcA* increased following TCE dechlorination to *cis*-DCE and VC.

Although the current knowledge of *Dhc* RDase genes involved in chlorinated ethenes reductive dechlorination is incomplete, and many more RDase genes await discovery (Ritalahti et al. 2006, 2010), the combined quantitative assessment of *Dhc* 16S rRNA genes and the *tceA*, *bvcA, and vcrA* RDase genes are a basis to establish correlations between *Dhc* biomarker presence and complete dechlorination in groundwater samples from contaminated field sites.

2.2 TECHNOLOGY DEVELOPMENT

The application of nucleic acid-based tools at chlorinated solvent sites was investigated in SERDP Project CU-1167. Technology development under SERDP project CU-1167 evaluated the degradation of *cis*-DCE and VC under different microbial growth conditions using aquifer materials collected from different redox zones collected from numerous contaminated sites. PCR techniques, including primer and probe design, were further developed and optimized in this project.

Ethene-producing enrichment cultures were obtained from numerous dechlorinating microcosms. Four VC-to-ethene dechlorinating enrichment cultures were selected for a detailed analysis of the community structure using 16S rRNA gene-targeted PCR-based tools. The four cultures were selected because they exhibited robust dechlorination activity and were highly enriched. In addition, the cultures were derived from sites with different contamination histories, allowing comparison of dechlorinating communities derived from pristine and contaminated environments.

Following prolonged enrichment with VC as electron acceptor, a comprehensive 16S rRNA gene-based community analysis revealed only subtle differences, independent of whether sample collection from pristine sites or chlorinated ethene-contaminated aquifers (Ritalahti and Löffler 2004). Clear evidence was obtained that *Dhc* were responsible for VC-to-ethene dechlorination in all cultures.

In CU-1167, twenty sites were characterized for dechlorinating bacteria, and *Dehalococcoides* populations were detected in all ethene-producing cultures. *Dehalobacter* populations were never detected in cultures that were enriched with *cis*-DCE or VC suggesting that members of this group are not involved in reductive dechlorination in any of the ethene-producing microcosms and cultures studied. To date, no other bacteria have been found that reductively dechlorinate DCEs to VC and ethene, indicating that the focus of chlorinated ethene-dechlorinating *Dhc* is justified. Although *cis*-DCE is generally the DCE isomer of concern, pathways that led to the formation of trans-DCE were described, and sites that have significant amounts of *trans*-DCE and 1,1-DCE exist (Griffin et al. 2004, Zhang et al. 2006, Cheng et al. 2009). Although not all *Dhc* populations grow with trans-DCE and 1,1-DCE, *Dhc* strains that use all DCE isomers and VC as electron acceptors exist (He et al. 2003b).

Major findings relevant to this project include:

- The 16S rRNA molecule has insufficient information to infer dechlorination activity.
- *Dehalococcoides* populations that reduce *cis*-DCE and VC are not rare in the environment, and were detected in 75% of the aquifer and sediment materials tested.
- A link was established between the presence of *Dehalococcoides* and ethene production.

Detailed results from this SERDP study can be obtained by downloading project reports from the SERDP website (www.serdp.com).

2.3 ADVANTAGES AND LIMITATIONS OF THE TECHNOLOGY

As stated above and documented by SERDP and ESTCP (2005) and Stroo et al. (2006), the use of MBTs, specifically qPCR, offers numerous benefits to bioremediation at chlorinated ethene-contaminated sites. However, these documents also highlight limitations of the current MBT procedures. The following sections summarize the advantages and disadvantages of the MBT procedures currently applied for *Dhc* monitoring at chlorinated ethene-contaminated sites.

The use of MBTs has contributed greatly to our understanding of microbial detoxification processes and our ability to exploit naturally occurring bacteria to biodegrade DoD-relevant contaminants (SERDP and ESTCP 2005, Stroo et al. 2006). qPCR has emerged as the MBT of choice for site assessment and bioremediation monitoring. The advantages of qPCR include: (1) the technique provides quantitative information of the target gene(s) and organism(s), and hence, is a powerful tool to establish cause-and-effect relationships between treatment and contaminant detoxification; (2) provides excellent sensitivity and detects as few as 5 cells per reaction tube; (3) it is relatively inexpensive and broadly available; (4) and it is available from commercial laboratories and numerous academic laboratories.

Inherent limitations that present a challenge to the application of MBTs for the assessment of environmental samples (Stroo et al. 2006) include: (1) non-uniform distribution of microbes in the subsurface; (2) insufficient sampling technologies to retrieve representative samples from the subsurface; (3) insufficient knowledge of key biomarkers; and (4) possible presence of inhibitors (e.g., humic acids) that interfere with nucleic acid extraction procedures and PCR amplification. Understanding and quantifying the impacts of these limitations on qPCR analysis of *Dhc* biomarker genes were beyond the scope of this project; however, because each of these issues can affect qPCR data and interpretation, efforts were expended to minimize the impacts of these factors.

A recognized shortcoming of *Dhc* biomarker gene quantification is that no direct correlation between biomarker gene abundance and *Dhc* dechlorination activity can be established. The quantification of the *Dhc* 16S rRNA gene and *Dhc* RDase genes demonstrates *Dhc* presence but does not prove that reductive dechlorination is actually occurring. Procedures that measure transcription of RDase genes are available; however, messenger ribonucleic acid- (mRNA-) based methods suffer from numerous issues. The foremost issue is the labile nature of RNA, which remains an obstacle for quantitative measurements. Although stabilizing agents have been introduced commercially, their value to obtain and stabilize RNA from large volumes of groundwater is limited. Further, the correlation between RDase gene transcript abundance and dechlorination activity has not been established under in situ conditions, and it is unclear if mRNA abundance is a reliable measure of *Dhc* dechlorination activity. Although promising when applied under defined laboratory conditions, the application of mRNA-based approaches to assess activity in environmental samples has not yet matured to justify its application with environmental samples. Further, recent observation that RDase gene transcription is up-regulated in response to unfavorable environmental conditions (e.g., elevated temperatures, oxygen) casts doubt that this approach can provide reliable activity information. Hence, this project focused on DNA rather than RNA targets; however the guidance document will serve as a blueprint that allows the rapid incorporation of RNA-based protocols should future research demonstrate the value of transcript measurements for assessing *Dhc* dechlorination activity. Another issue is that sampling, sample handling and laboratory analyses are not standardized, which can lead to false positive and false negative results, erroneous data interpretation, and prohibits that data generated in different analytical laboratories can be directly compared.

Erroneous assumptions and conclusions can also result from the lack of biomarker genes that analyze the *Dhc* community at the strain level. Since *Dhc* strains with different dechlorination activities share similar or identical 16S rRNA genes, detection and enumeration of *Dhc* 16S rRNA genes can lead to erroneous assumptions and conclusions. As discussed above, *tceA*, *vcrA* and *bvcA* are key biomarker genes for monitoring reductive dechlorination beyond DCE, and this project combined 16S rRNA gene enumeration with the quantitative analysis of these RDase genes.

Another key issue involves sample collection procedures and sample handling, which can significantly impact the results (Casey, 2006), and hence, affect data analysis and interpretation. Team members explored sampling and sample handling procedures, and these results have been incorporated into this study to the extent possible. The sampling approach selected for this project provides a conservative approach to avoid false positive results and minimize the effects

of sample collection and handling procedures on the qPCR results. The sampling and shipping procedures used in this project were developed to minimize the biases introduced during sampling, and sample handling, shipping and storage.

3. PERFORMANCE OBJECTIVES

The purpose of this demonstration was to evaluate and refine the use of nucleic acid-based tools to assess chlorinated solvent bioremediation using MNA, biostimulation alone, or biostimulation combined with bioaugmentation. It is expected that the use of these tools will lead to informed remediation decisions, reduced remediation times at lower costs, and enhance the efficiency of full-scale applications towards site closure, and increase confidence in the application of MBTs for bioremediation projects.

The performance objectives are provided in **Table 3-1** and described in detail below. The objectives were to:

i. establish qualitative and quantitative criteria correlating *Dhc* target gene abundance with reductive dechlorination and contaminant concentrations,
ii. determine the minimum number of *Dhc* gene copies (i.e., cell titers) to observe reductive dechlorination to ethene,
iii. correlate *Dhc* 16S rRNA gene and RDase gene abundances with contaminant (e.g., PCE, TCE) dechlorination rates,
iv. correlate RDase gene abundance with the dominant TEAP suggested by groundwater geochemical data, and
v. identify conditions that can generate false positive and/or false negative results.

Table 3-1. Performance Objectives

Type of Objective	Performance Objective	Success Criteria	Results
Quantitative	Validate use of RDase gene targets	Correlations of functional target genes (e.g., *tceA*, *bvcA*, *vcrA*) with evidence for reductive dechlorination (e.g., change in contaminant concentration ratios)	Positive Spearman correlation > 0.34
Quantitative	Identify minimum number of *Dhc* gene copies to achieve detoxification	Minimum number of target *Dhc* 16S rRNA gene or functional gene copies observed with complete dechlorination (e.g., ethene formation)	Ethene formation always observed with *Dhc* > 10^7 cells/L
Quantitative	Evaluate correlation between *Dhc* cell titers and dechlorination rates	Correlations of *Dhc* biomarkers gene targets with contaminant dechlorination rates	Positive Spearman correlation > 0.34
Quantitative	Evaluate effects of contaminant concentrations on *Dhc* abundance	Correlation of *Dhc* biomarker gene copies with contaminant (e.g., PCE, TCE) concentrations	Weak Spearman correlations dechlorination daughter products

Table 3-1 (continued). Performance Objectives

Type of Objective	Performance Objective	Success Criteria	Results
Quantitative	Evaluate optimum sample collection method	On-site filtered or off-site, laboratory-filtered groundwater samples with *Dhc* biomarker gene copies within 50% RPD	Yield of on-site, field-filtered samples > 90%
Quantitative	Evaluate analytical sensitivity	Measure *Dhc* biomarker gene copies at 10^4 gene copies/liter	Detection and quantification limits of 10^3 and 10^4 cells/L, respectively
Quantitative	Evaluate reproducibility of analytical procedure	Does the analysis of replicates yield results within 50% RPD?	qPCR of duplicate extractions within 50% RPD (most environmental samples); <10% for standards
Qualitative	Evaluate influence of dominant TEAP on *Dhc* abundance	Identify TEAP trends associated with *Dhc* biomarker gene copy numbers	Anaerobic conditions needed for observing *Dhc* biomarker genes
Qualitative	Evaluate likelihood of false positive/ negative detections	Identification of false positive and false negative detections of *Dhc* biomarker genes and its impact on decision-making	Simultaneous quantification of phylogenetic and functional biomarker genes eliminates false positives. Analytical sensitivity reduces false negatives.
Qualitative	Evaluate implementability of on-site biomass collection	Feedback from field personnel on ease and feasibility of on-site groundwater filtration and biomass collection	Sterile filter cartridges easy to use in the field

Notes:
TEAP – terminal electron acceptor process
RPD – Relative Percent Difference

Validate Use of RDase Gene Targets

The 16S rRNA gene-based qPCR approach targets the known *Dhc* community and does not distinguish strains with different dechlorination activities. An inherent weakness of the 16S rRNA gene-based approach is the inability to link phylogenetic information with function (e.g., dechlorination activity). Although the 16S rRNA gene approach is a reliable approach for detecting and monitoring *Dhc*, the information contained in the 16S rRNA molecule fails to

distinguish *Dhc* organisms with distinct dechlorinating abilities. An objective of this work was to validate the use of *Dhc* RDase gene targets to predict in situ reductive dechlorination.

At each chlorinated ethene-contaminated site selected and included in this effort, groundwater samples were collected for qPCR analysis of the RDase gene targets *tce*A, *bvc*A, and/or *vcr*A. The gene copy number was correlated to concentrations of the PCE/TCE dechlorination products *cis*-DCE and VC, compound ratios, and concentrations of the nontoxic end product ethene. Correlations were established using Spearman statistical correlations.

Identify Minimum Number of *Dhc* Gene Copies

To evaluate bioremediation performance, practitioners need to know whether *Dhc* cell titers are sufficient to catalyze complete dechlorination to ethene at acceptable rates. An objective of this work was to identify the minimum number of *Dhc* cells and RDase gene copy numbers indicative for complete reductive dechlorination to ethene. Groundwater samples were collected from all sites for *Dhc* and/or RDase gene analysis and results were correlated to ethene concentrations measured in the well where the groundwater sample was collected.

Evaluate *Dhc* Cell Titers on Dechlorination Rates

Calculation of field dechlorination rates requires extensive spatial and temporal VOC data. Practitioners could utilize a less costly and more rapid predictor of field reductive dechlorination rates based on MBT measurements. An objective of this work was to correlate *Dhc* cell titers or RDase gene copy numbers to in situ dechlorination rates observed for TCE, *cis*-DCE and VC. Groundwater samples were collected from one site (NASA MLP/VAB) for *Dhc* 16S rRNA gene and RDase gene analysis. First-order dechlorination rates were calculated from VOC data. The dechlorination rates were correlated to *Dhc* and *vcr*A data using the Spearman correlation.

Evaluate Influence of TEAP on *Dhc* Abundance

Dhc are obligate anaerobes and, similar to methanogens, require low redox potentials for activity. An objective of this work was to identify a minimum *Dhc* cell titer in a zone dominated by reducing conditions. Geochemical data were obtained from monitoring locations where *Dhc* analyses were also conducted. These data were reviewed qualitatively, since mixed TEAPs are observed in the field.

Evaluate Likelihood of False Positive and False Negative *Dhc* Detections

A false positive *Dhc* detection occurs when an assay reveals *Dhc* detection when in fact *Dhc* is not present. For a false positive *Dhc* detection, *Dhc* would be detected above a threshold cell titer, but reductive dechlorination beyond DCE might not be observed in the field. False negative *Dhc* detections occur when *Dhc* is not detected but is in fact present. For a false negative *Dhc* detection, *Dhc* cell titers would be below a threshold cell titer, while reductive dechlorination beyond DCE might be observed in the field. A qualitative objective of this work was to evaluate the likelihood of false positive and false negative *Dhc* detections, and to assess conditions where these false detections might arise.

Evaluate Optimum Sample Collection Method

Sample collection procedures and sample handling can significantly impact *Dhc* results, skew the data (Casey, 2005), and hence, affect data interpretation and the project outcome. An objective of this work was to evaluate sampling and sample handling procedures, to the extent possible. The sampling approach selected for this project provided a conservative approach to

avoid false positive results and minimize the effect of sampling procedures on the results. A study comparing lab to field filtration methods was conducted to evaluate the precision of these techniques.

Evaluate Analytical Sensitivity

qPCR is a sensitive approach and can detect <10e3 target genes in 1 liter of groundwater. If the target genes are present in higher concentration, only a few mL of groundwater are required to for detection and quantification. An objective of this work was to evaluate the detection limit for *Dhc* 16S rRNA gene and RDase gene targets, as well as evaluate the sensitivity of the qPCR approach from a practical prospective.

Evaluate Analytical Sample Reproducibility

The qPCR technique is highly reproducible. Verification of the assay was first performed using plasmid DNA carrying the gene of interest that was diluted in triplicate to 1 ng/µL. Each aliquot was then serially diluted 10-fold, 10 times, resulting in triplicate standard curves. Each dilution of the three standard curves was assayed with three qPCR pseudoreplicates (nine total values/dilution). In general, qPCR replicates for plasmid standards were within 5%, and independent dilutions yielded less than 10% difference. The lower limits of detection were observed when the dilution series and no template controls yielded no detectable signal. This happened consistently when fewer than 2 gene copies were present per µL DNA template. For environmental samples, qPCR pseudoreplicates were typically within 30%, while replicate DNA extractions yielded values that differed by up to 50%. High quality DNA yielded more reliable/reproducible quantification. For samples with high target gene abundances (>1000 copies/reaction) and high-quality DNA, replicate extractions yielded values within 10%, while in samples with low 16S rRNA gene abundances or poorer quality DNA, the differences could be up to 75%. Although qPCR data analysis methods can differ significantly in their performance, methods are available that provide DNA quantification estimates of high precision, robustness and reliability (Karlen et al., 2007). Recent studies have also demonstrated excellent precision high throughput systems (Morrison et al., 2006). An objective of this work was to evaluate the precision of *Dhc* or RDase analysis.

Ease of Using Field Filtration Methods

Collection of groundwater samples for *Dhc* 16S rRNA gene and/or RDase gene analysis should not be overly time consuming or present technical challenges to field personnel. An objective of this work was to evaluate on site biomass collection procedures that avoid the problems associated with shipping large volumes of groundwater to the analytical laboratory. Sampling methods included attaching a sterile filter cartridge to low flow discharge tubing, measuring the discharge flow during sampling, and packaging the filter cartridge with the biomass for shipping.

4. SITE DESCRIPTIONS

4.1 SITE LOCATIONS AND HISTORIES

Numerous DoD sites were identified as potential demonstration sites. The goal was to demonstrate the value of the qPCR approach at chlorinated ethene-contaminated sites where MNA, biostimulation and/or bioaugmentation had been implemented. Each potential site was pre-screened for inclusion in the demonstration and then evaluated based on a detailed set of the following criteria. First, only those sites that had undergone a detailed site characterization including hydrogeologic and geochemical characterization and source and plume delineation were considered. Furthermore, only those sites that had been sampled and monitored in accordance with EPA guidance for MNA sites (EPA, 1998) and enhanced bioremediation guidance (Parsons, 2004) were considered. Only sites with PCE or TCE concentrations greater than 100 μg/L were considered to ensure the potential for efficient biological degradation (i.e., the dechlorinating bacteria require the chlorinated contaminants as growth substrates (Cupples et al. 2003)). However, sites with documented accumulation of DCE and/or VC (DCE-VC "stall") with respect to these parent compounds were also included to determine the impact of *Dhc* 16S rRNA gene and RDase gene abundance on the degradation of these compounds. Additional screening criteria for sites considered for MNA evaluation in this study included evidence of reducing conditions favorable for reductive dechlorination in accordance with EPA guidelines (EPA, 1998). For sites slated for the implementation of enhanced bioremediation, anaerobic conditions were not a prerequisite because reducing conditions would be achieved with the addition of electron donor(s).

This demonstration project ER-0518 was structured in a way such that sampling was performed at the site owner's expense and by their personnel or contractor; hence no funding was allocated for monitoring or sampling. Although this approach proved successful at keeping cost low, it also resulted in limited ability to select a varied repertoire of sites.

Based upon a thorough review of numerous sites, a total of five (5) MNA sites, seven (7) biostimulation, and six (6) bioaugmentation sites met the aforementioned criteria and were selected for evaluation in this demonstration. These sites are summarized in **Table 4-1**. The following subsections describe the history of operations at these test sites. Figures and other supplemental site information are provided in **Appendix B**. As described in the following sections, these sites represent a broad spectrum of chlorinated ethene-contaminated aquifers with various geologic and hydrogeologic conditions.

Table 4-1 Site Summary

Facility Name and Location	Site Name	Site Remedy
Anniston Army Depot, AL	Trench Area	MNA
	Landfill Area	MNA
	Industrial Area	MNA
	Northeast Area	MNA
Former NAS Dallas, TX	SWMU 21	MNA
NASA Cape Canaveral, FL	MLP/VAB	Biostimulation
Vandenberg AFB, CA	Site 8	Biostimulation
	Site 13/14	Biostimulation
NSA Mid-South, TN	AOC A, Sub-plume A	Biostimulation
	AOC A, Sub-plume B	Biostimulation
	AOC A, Sub-plume C	Biostimulation
	AOC A, Sub-plume D	Biostimulation
Former NAS Cecil Field, FL	Site 59	Bioaugmentation
Fort Dix, NJ	Magazine 1	Bioaugmentation
NAS North Island, CA	OU 24	Bioaugmentation
Bachman Road, MI	Plume B	Bioaugmentation
Milledgeville,GA	Plant 66	Bioaugmentation
Former NAWC Trenton, NJ	NAWC	Bioaugmentation

Notes:
AOC – Area of Concern
AFB – Air Force Base
MLP/VAB - Mobile launch platform / vehicle assembly building
NAS – Naval Air Station
NSA – Naval Support Activity
OU – Operable Unit
SWMU – Solid Waste Management Unit

MNA Sites

4.1.1 Operable Unit 1, Anniston Army Depot

Anniston Army Depot (ANAD) is an active military facility located in northeastern Alabama in Calhoun County and is approximately 10 miles west of Anniston, Alabama. Previous site investigations have identified inorganic and organic contamination throughout the Southeast Industrial Area's (SIA) Operable Unit 1 (OU1) in several locations (**Appendix B-1**). Organic contaminants present at the site are primarily chlorinated solvents, such as trichloroethene (TCE), methylene chloride, 1,2-dichloroethene (1,2-DCE), chloroform, and and other solvents including acetone. Evaluation of past SIA operations indicates that large quantities of dense non-aqueous phase liquids (DNAPL) and concentrated liquid wastes were disposed on site. Although excavation and removal actions have addressed the primary sources, secondary sources of contamination still remain, including residual and/or potentially pooled DNAPL, dissolved contamination in the residuum and rock, and sorbed contamination.

An Interim Record of Decision (IROD) was issued for this site in 1991 for the initial groundwater extraction and treatment system. This pump and treat system named the Groundwater Interceptor System (GWIS) has been operating as an interim remedy since then. Preliminary trend analysis confirms the GWIS is reducing TCE concentrations at some of the extraction wells and monitoring wells; however, the extent of the reductions varies and the GWIS's ability to meet the objectives of the IROD is questionable. Thirteen of 17 extraction wells appear to be inefficient and poorly productive in terms of mass removal. The system is also unable to contain off-site plume migration. Evaluation of the system is underway (Tetra Tech, 2009). Groundwater monitoring has been conducted biannually since 2002 and a natural attenuation assessment has been conducted recently (Tetra Tech, 2009).

The final Comprehensive Groundwater Remedial Investigation (RI) and the Comprehensive Groundwater Feasibility Study (FS) were submitted in 2008 (SAIC 2008a and 2008b) and more recently by Tetra Tech (2009). A summary of these findings is included in this and subsequent sections. These studies divided the SIA into four contaminant source areas: the Landfill Area, Trench Area, Northeast Area and Industrial Area.

Groundwater monitoring has been conducted biannually since 2002 and a natural attenuation assessment has been conducted recently (Tetra Tech 2009). This site has been included as a site where MNA is currently underway. While MNA is currently being conducted it is clear that the large amount of mass present, the complex hydrostratigraphy, and an active pump and treat system complicate a straightforward assessment at this site.

Four areas within the ANAD SIA were evaluated for the purpose of this demonstration. While located on the same facility each has different geochemistry and molecular biological conditions that warranted inclusion of all four areas. Each of these areas included multiple Solid Waste Management Units (SWMUs) that are co-located or clustered. These areas are described in the following sections.

4.1.1.1 Trench Area

There are 4 SWMUs in the Trench Area. SWMU 1, the Chemical Sludge Waste Pits, is the primary contributor to groundwater contamination (SAIC, 2008b). SWMU 1 consisted of seven trenches including the disposal of numerous types of waste, which included numerous types of sludges containing TCE. In 1982 and 1983, 52,526 tons of contaminated soil were excavated and hauled to off-site disposal areas.

4.1.1.2 Landfill Area

The Landfill source area includes eight SWMUs, with SWMU 12 (Facility 414 Old Lagoons), SWMU 22 (Block Lagoon), and SWMU 13 (SIA Acid Chemical Waste Pit) being the primary groundwater contamination contributors. Until 1978, a variety of concentrated liquid chemical wastes were disposed of at SWMU 12. The 440-ft by 220-ft lagoon complex consisted of three unlined lagoons. The combined volume of the lagoons was 1.63 million gallons. In 1978, the lagoons were emptied by pumping the contents into the A-Block Lagoon (SWMU 22), a

synthetically lined lagoon constructed in 1978 to contain liquid waste previously held at SWMU 12. At that time, Weston (1984) documented that 7,500 gallons per month of concentrated waste were disposed of in the SWMU 22 lagoon (90,000 gal/yr). SWMU 13 is the SIA Acid Chemical Waste Pit. The pit was excavated between March 1948 and October 1954.

4.1.1.3 Industrial Area

Eleven SWMUs are in this source area. SWMU 3 (Old Industrial Wastewater Treatment Plant), SWMU 31 (Metal Plating Shop - Building 114), and SWMU 25 (Building 130 Sump) are the primary sources of contamination to groundwater. The Old Industrial Wastewater Treatment Plant (SWMU 3) received industrial wastewater from various operations in the SIA, containing numerous types of contaminants. The Metal Plating Shop-Building 114 (SWMU 31) housed metal cleaning, treating, and plating operations since June 1982. A minimum of 2,000 gpd of cyanide wastewater at 61.1 ppm (0.5 lb/day of cyanide) was produced at Old Building 114. The Building 130 Sump (SWMU 25) was an 8,000-gallon concrete underground tank that was used as temporary storage for all types of waste discharges from Building 130 operations prior to discharge to Dry Creek via a storm sewer.

4.1.1.4 Northeast Area

Six SWMUs are in the Northeast Source Area. SWMU 7 (Chemical Waste Disposal Pit) and SWMU 30 (Northeast Lagoon Area) were determined to be the primary contributors to groundwater contamination (SAIC, 2008a). A variety of chemical wastes reportedly were dumped into a small pit in SWMU 7 over a 6-month period in 1960. These wastes included alkaline-corrosion removers: phosphoric acid and lead-, zinc-, and cadmium-containing compounds. The lagoon may have been used for disposal of chlorinated solvent wastes from 1954 to the early 1960s, based on aerial photographic evidence; however, the types and quantities of wastes disposed of at SWMU 30 have not been documented.

4.1.2 SWMU 21, NAS Dallas

The former NAS Dallas property is located in the cities of Grand Prairie and Dallas in Dallas County, Texas, approximately twelve miles south of the Dallas-Fort Worth Airport. The facility is located at 8100 West Jefferson Avenue and SWMU 21 is located approximately 1,000 feet from the eastern City of Dallas property boundary. Building 1406, the Non-destructive Investigation (NDI) Laboratory, was constructed in 1960 on a former parking lot. A former 500-gallon concrete underground storage tank (UST) stored liquid wastes from the building's NDI dye penetrant vats. The UST was taken out of service and removed in 1988/1989 after releases of hazardous materials were reported. This UST is the presumed source for this site (**Appendix B-2**).

A pilot study was conducted in the southwest area of the SWMU 21, near Building 1406 and included the injection of zero valent iron (ZVI) via pneumatic fracturing in the subsurface. The ZVI was to promote abiotic degradation and enhance the natural biodegradation of the chlorinated volatile organic compounds (VOCs) at the site. Monitoring of the groundwater concentration and other indicator parameters were conducted prior to and throughout the pilot

study period to assess the effectiveness of ZVI in reducing VOC concentrations in the groundwater at the site.

This site was included for the purposes of evaluating sampling methodology. It is included in the MNA section as it was intended to be used for long term MNA sampling but long term monitoring /MNA was not continued after the pilot study (TtNUS, 2007) was completed.

Biostimulation Sites

4.1.3 MLP/VAB, NASA Cape Canaveral

The Mobile Launch Platform / Vehicle Assembly Building (MLP/VAB) area is located within Kennedy Space Center (KSC), on the East Coast of Florida in Brevard County. The MLP/VAB is an active NASA-operated facility and was originally built to support Apollo/Saturn-V vehicle assembly and later modified (1975) to support Space Transportation System (STS) shuttle missions.

An undocumented TCE spill (approximately 4,000 gallons) occurred in 1966, when the VAB was in the final stages of construction. A former VAB area employee stated that the TCE spill had been washed into the floor drains and out of the VAB using high-pressure fire hoses with no documented environmental cleanup.

RCRA facility investigation (RFI) activities were performed in phases to characterize the nature and extent of contamination at the MLP site from 24 February 1997 through 11 March 1999 (**Appendix B-3**). RFI characterization activities included: (i) sampling and analysis of soil, sediment, and surface water; (ii) advancing exploratory borings to describe site lithology and evaluate hydraulic properties of clay material encountered; (iii) installing piezometers to measure water levels; (iv) collecting groundwater samples using Direct Push Technology (DPT); (v) installing and developing permanent wells to sample the surficial aquifer; (vi) performing slug tests in selected wells; (vii) performing a natural attenuation investigation; (viii) performing heat pulse flow meter testing; (ix) performing several rounds of water level measurements; and (x) performing a soil interim measure. The data gathered were used to perform human health and ecological risk assessments and to develop a groundwater flow and solute transport model to determine the need for further action at the site.

Corrective measures were implemented in 2007, including biostimulation using ethyl lactate, biosparging for containment of the downgradient plume, and MNA for low concentration plume impacts.

4.1.4 Vandenberg AFB

Two sites were included in this demonstration from Vandenberg Air Force Base (AFB) located in Santa Barbara County, California. The two sites included Site 8 and Site 13/14. Site 8 is located on South Vandenberg AFB and Site 13/14 cluster is located in the Burton Mesa physiographic region in the northern part of Vandenberg AFB. Based upon the slightly different

hydrogeologic conditions and biostimulation approaches inclusion of both sites was warranted for this demonstration (**Appendix B-4**).

4.1.4.1 Site 8

Site 8 is also referred to as Space Launch Complex [SLC] 4 East and is an inactive above ground gantry launch, support structures and chemical storage facility. At this site Atlas/Agena were launched from 1963 to 1965 and Titan vehicles from 1971 to 2005 (Tetra Tech 2007a).

Based upon microcosm studies, an in situ bioremediation pilot test was initiated via seven injection wells (8-INJ-1 through 8-INJ-7). In April 2007 two pilot tests were initiated to evaluate the distribution of sodium lactate and utilization of the native microbiota. The objective of the pilot test was to define spacing for the subsequent full-scale remediation. Sodium bromide was added to potable water to achieve approximately 3 percent solution by volume. The substrate along with a sodium bromide tracer, sodium bicarbonate buffer, yeast extract and a fluorescent dye (fluorescein) were injected into 8-MW-22 in a push-pull test and monitored in adjacent new wells (8-INJ-1 through 8-INJ-5). A second push pull test was conducted by injecting sodium lactate in (8-INJ-6 and 8-INJ-7). More recently, the pilot study was expanded and full-scale implementation was completed in 2009.

4.1.4.2 Site 13/14

Sites 13 and 14 are located adjacent to one another and are often referred to as the Cluster 13 complex or Site 13C. Site 13 includes the Advanced Ballistic Reentry System A (ABRES-A) Launch Complex and a portion of ABRES-A Canyon south and west of the launch complex. Site 14 includes ABRES-A Lake, the western portion of ABRES-A Canyon and surrounding bluffs. The ABRES-A Launch Complex consists of a control center and three launch pads (Buildings 1788, 1790, and 1797). Eighty-four Atlas missiles were launched from the ABRES-A Launch Complex between 1959 and 1974. Chlorinated solvents, primarily TCE, were used on-site for degreasing missile engines and cleaning parts. A TCE storage tank was located within the launch service building at each launch pad. Chlorinated solvents, primarily TCE, were used on-site for degreasing missile engines and cleaning parts. A TCE storage tank was located within the launch service building at each launch pad.

In December 2005, soybean oil, emulsifiers and potable water were mixed on site and injected into wells 14-INJ-7 through 14-INJ-12 both “shallow” and “deep” zones. A total of 5,251 gallons of substrate and 11,949 gallons of dilution water were injected into the shallow and deep zone aquifers via injection array 14-INJ-1 through 14-INJ-6 and 14-INJ-7. An additional 6,229 gallons of water were used to flush the substrate into the formation following the injection. In June 2007, four wells were used for evaluation of various groundwater filtration and biomass collection methods.

4.1.5 AOC A, NSA Mid-South

Naval Support Activity (NSA) Mid-South is located in Millington, Tennessee. A remedial Interim Measure (IM) was conducted at several locations in Area of Concern (AOC) A where

elevated chlorinated ethene concentrations were detected. The primary contaminant included TCE at baseline concentrations (in 2004 and 2005 or prior to or immediately after injection) ranging from 200 to 1,600 μg/L.

The contaminated groundwater within AOC A is divided into four sub-plumes (shown in Figures in **Appendix B-5**). Sub-Plume A is immediately east of Hangar N-126 and has shown the highest baseline TCE concentrations within AOC A ranging from 900 to 1,600 μg/L. Three other areas with elevated TCE concentrations also are designated as plumes: Sub-plume B (northeast of Sub-Plume A) having baseline concentrations of TCE ranging from 200 to 220 μg/L; sub-plume C, (south of sub-plume A) having baseline concentrations of PCE at 110 μg/L, and Sub-Plume D (east of sub-plume B) having baseline concentrations of TCE ranging from 600 to 1,400 μg/L and in some cases DCE and VC concentrations 230 and 270 μg/L, respectively.

The interim measure consisted of biostimulation measures at each of the four sub-plumes the most contaminated locations within AOC A, while MNA is being implemented as the long-term remedy for the less contaminated parts of the aquifer.

In accordance with the Interim Measures Work Plan (EnSafe, 2003), sodium acetate is being injected to create reducing conditions necessary for TCE biodegradation. Diammonium phosphate was added to meet the nutritional requirements of the native microbiota. In May 2004, monthly injections were initiated in all injection wells throughout Sub-Plumes A, B, and C. Thirty-five injection events have occurred throughout Sub-Plumes A, B, and C from the time interim measures began in May 2004 to the end of the last monitoring period (November 2007). During this time period, a total of 1,750 pounds of sodium acetate have been introduced into each injection well (Spectra Tech and Ensafe, 2008). In Sub-plume D, vegetable oil substrate was initially injected but after poor response (EnSafe, February 2005), monthly sodium acetate injections began in all seven injection wells beginning in May 2005. Twenty-three injection events have occurred in these additional areas since May 2005 and a total of 1,650 pounds of sodium acetate have been introduced into each injection well (Spectra Tech and Ensafe, 2008).

Based upon the moderately different baseline concentrations in each of the four sub-plumes and the likely DCE-VC stall present in baseline conditions in Sub Plume D, inclusion of all four sites were warranted for this demonstration.

Bioaugmentation Sites

4.1.6 Site 59, NAS Cecil Field

NAS Cecil Field is located west of Jacksonville, Florida. Site 59 is located in the Main Base area of NAS Cecil Field near the northern end of the north-south runways. The site consists of buildings and parking lots. The majority of Site 59 is paved, with a concrete flight line apron that covers the eastern portion of the site, and buildings and parking lots that cover most of the remainder of the site. Groundwater sampling conducted around Buildings 324/1845 under the Base Realignment and Closure (BRAC) program identified TCE contamination, and the area was designated Site 59 under Operable Unit (OU) 9. The groundwater contamination was originally discovered during a Due-Diligence investigation in 2003.

A pilot study conducted in 2006 and 2007 was conducted to determine the feasibility and effectiveness of in-situ bioremediation to treat part of the contaminated groundwater plume at Site 59 (**Appendix B-6**). In the pilot study system, sodium lactate and sodium bicarbonate were injected into the subsurface of Site 59 via a groundwater recirculation application to promote anaerobic degradation TCE. Once target geochemical conditions identified in the Work Plan (dissolved oxygen, pH, oxidation-reduction potential) of the treatment zone were established, a microbial inoculum was injected to enhance in situ bioremediation. The pilot study results were evaluated and used to support selection and ultimate implementation of this remedy in 2008 and 2009 (TtNUS, 2008).

4.1.7 Magazine 1 Area, Fort Dix

Review of historic blueprints of the area indicates that the active Magazine 1 (MAG-1) Area existed as early as 1919, along the southern side of a Penn Atlantic Railroad spur (Dames & Moore, 1993 and Shaw, 2009). The MAG-1 Area was an ammunitions and weapons magazine storage area and a vapor-degreasing operations area. From approximately 1942 through 1965, vapor-degreasing of small arms was conducted at the MAG-1 Area. The vapor-degreasing operation used TCE to remove Cosmoline, a Vaseline-type petroleum product used for packing rifles.

According to the Dames & Moore Phase II RI report (Dames & Moore, 1993), an employee at Fort Dix who participated in the degreasing operations reported that drums of TCE were used until saturated with Cosmoline. The drums of spent material then were transported to a rubble pile along the southern boundary of the MAG-1 Area, where the TCE/Cosmoline mixture was poured into holes in the rubble pile. Unconfirmed reports indicate one 55-gallon drum containing approximately 40 to 60 percent TCE was discarded each day. During busy periods, approximately two drums per day were reportedly discarded (Dames & Moore, 1993). The reliability of this historical information is suspect due to lack of free-product contamination at the site and questions regarding TCE generation rates. It is unlikely the estimated quantities of TCE were consistently generated during operations and it is possible that partially-filled drums were often emptied onto the rubble pile.

Except for one drum of TCE/Cosmoline that was spilled adjacent to the degreasing operations building, all wastes generated during this operation reportedly were disposed of in the rubble pile, approximately 100 feet south of the degreasing operations building. It is not known if any TCE was spilled inside the building. No surface ponding was reported from wastes poured into the rubble pile, and TCE was disposed of in different holes within the pile. Visible surface seepage from beneath the rubble pile reportedly occurred along its southern and western edges. Due to the porous characteristic of rubble piles, volatilization losses of TCE were likely to be significant during this disposal process.

This site was selected for a field demonstration to evaluate the amount of culture needed in a bioaugmentation remediation process to effectively remediate a chlorinated solvent contaminated plume and to determine the effect of inoculum dose on remedial time. The field demonstration, funded by the Environmental Security Technology Certification Program (ESTCP), included the

construction and operation of 4 groundwater recirculation loops, each inoculated with a different amount of Shaw's SDC-9 dechlorinating consortium (**Appendix B-7**)

4.1.8 OU 24, NAS North Island

NAS North Island is an active military base on the northern end of the Silver Strand Peninsula, which separates San Diego Bay from the Pacific Ocean. OU 24 is an area of groundwater impacted by VOCs located in the northeastern portion of NAS North Island, in the vicinity of Building 653. Investigations indicate that waste releases associated with an acid waste pump station located south of Building 653 may be the source of VOC contamination.

Environmental investigation at OU 24 began in 1994, with routine groundwater monitoring continuing since this time. The primary groundwater contaminants at the site are *cis*-DCE and VC. Remedial measures were conducted at OU 24, including active (forced gradient) enhanced in-situ bioremediation (EISB) in the source area and passive (natural gradient) EISB in the downgradient plume (**Appendix B-8**). The aggressive EISB was conducted as time critical remedial actions for chlorinated ethenes in groundwater in the source area (the Phase I remediation system), coupled with a semi-passive EISB biobarrier system for chlorinated ethenes in the downgradient plume (the Phase II remediation system). The startup of the Phase I EISB system (i.e., the recirculation of groundwater amended with electron donor) began on May 8, 2007. Direct push technology injection test and extraction test was conducted May 22 – 25, 2007 in preparation for installation of the downgradient biobarriers. On June 4, 2007 and June 15, 2007, the three source area injection wells (653-IW-1, 653-IW-2 and 653-IW-3) were augmented with dechlorinating consortium KB-1. The total volume of KB-1 injected into these wells was 70 liters (Geosyntec, 2008).

4.1.9 Plume B, Bachman Road

The Bachman Road site Plume B originates from a former American Speedy Printing (ASP) facility in Oscoda, Michigan, and discharges to Lake Huron. The site consists of buildings (residential and commercial use), grass-covered areas, and paved roadways. Historic releases of dry cleaning solvents, specifically PCE, are the suspected sources at the Bachman Road site. Previous investigations have shown PCE and its daughter products in site groundwater at concentrations exceeding relevant State screening criteria. Based on the findings of previous investigations, a dense non-aqueous phase liquid (DNAPL) containing PCE has been previously identified at the source of Plume B. Pooled or free-phase DNAPL has not been observed at the site. However, residual DNAPL has been inferred from soil (saturated zone) and groundwater concentration data collected at the site under the former ASP building at Plume B. The ASP building was demolished in 2007. Remedial measures were conducted at Bachman Road in 2008, including active (forced gradient) EISB in the source area and downgradient plume area (**Appendix B-9**).

4.1.10 Milledgeville

RFI activities at the Milledgeville site identified evidence of a TCE release in the vicinity of a sump, connected to a vapor degreasing unit. The presence of numerous subsurface obstacles (buried utilities and process lines) in the vicinity of the sump and at the edge of the main manufacturing building limited access to the subsurface. Several in-situ remedial technologies were evaluated. Bench scale testing was performed on two remedial technologies, in-situ chemical oxidation (ISCO) and enhanced anaerobic bioremediation. Based on the results of the bench scale studies, bioaugmentation was selected as the remedial technology for implementation during pilot testing (**Appendix B-10**).

4.1.11 Former NAWC Trenton

The former Naval Air War Center in West Trenton, NJ (NAWC Trenton) has been the subject of active remediation since 1993. Historical releases of chlorinated solvents, principally TCE, have occurred at the site. An existing remediation system, which has been operating since 1997, consists of the pumping and treatment of contaminated groundwater. TCE concentrations in the contaminated groundwater plume had not changed substantially following 10+ years of pump and treat system operation. This may indicate that the rate of removal of TCE by groundwater extraction is limited by two factors; the rate of diffusion of TCE from the bedrock matrix and the rate of dissolution of DNAPL into groundwater. These conditions may lead to the operation of the pump & treat system for decades. The feasibility of *in situ* bioaugmentation technology for treatment of TCE groundwater impacts at the site was investigated at the pilot scale, beginning in 2005 (**Appendix B-11**).

4.2 SITE GEOLOGY/HYDROGEOLOGY DESCRIPTIONS

The following subsections provide site geology and hydrogeology information that was relevant for the technology demonstration. Figures and other supplemental site information are provided in **Appendix B**.

MNA Sites

4.2.1 Operable Unit 1, Anniston Army Depot

Three hydrostratigraphic units (HSUs) with distinct groundwater flow characteristics have been recognized in the subsurface based on post-depositional structural deformation (e.g., structural compression and thrust faults) and differential subsurface weathering (**Appendix B**). These three units also have distinct hydrogeological and hydraulic characteristics than can differentiate them from one another. From top to bottom, the three HSUs are (1) an unconsolidated clay-dominated residuum unit, (2) a weathered bedrock unit with mud-filled (clay), epi-karst, and open cavities, and (3) an unweathered bedrock unit.

4.2.2 SWMU 21, NAS Dallas

The SWMU 21 site is underlain by fined-grained fill and alluvial sediments to a depth of approximately 24 feet. Silty clay is present from ground surface down to approximately 15 to 20 feet below ground surface (bgs). Underlying this stratum are lenses of gravelly clay present down to the top of the weathered Eagle Ford Shale. The top of the weathered Eagle Ford Shale is found at most locations at approximately 24 feet bgs.

Biostimulation Sites

4.2.3 MLP/VAB, NASA Cape Canaveral

The Mobile Launch Platform / Vehicle Assembly Building Area site geology consists of horizontal layers of sand and sand with marine shells that transition gradually into marine clays with increasing depth at about 96 ft bgs. The sand is relatively permeable (with hydraulic conductivities [K] in the range of 10^{-6} to 10^{-4} centimeters per second [cm/s]). The marine clay has a relatively low permeability (K in the range of 10^{-7} to 10^{-9} cm/s). The water table generally ranges from near surface at the wetland area to approximately 5 to 6 ft bgs near the source area.

4.2.4 Vandenberg AFB

4.2.4.1 Site 8

Site 8 is located on the southern margin of the South Ynez River groundwater basin/Lompoc Terrance Sub-basin. Groundwater at the site is present in the unconsolidated material immediately above the contact with the Sisquoc Formation bedrock. The paleo-erosional surface of Sisquoc shale bedrock forms the lower boundary of the groundwater zone. No significant groundwater flow occurs in this underlying shale bedrock. The unconsolidated unconfined aquifer is very thin (2 to 8 feet thick) and is mostly comprised of sand with underlying Diatomaceous shale underlying as a bedrock aquifer. Groundwater levels ranged from 45 to 420 feet above mean sea level. During the biostimulation demonstration, the groundwater flow direction was to the northwest.

4.2.4.2 Site 13/14

Site 13/14 is located on the drainage divide of two major groundwater basins: the Santa Ynez River Basin to the south and the San Antonio Creek Basin to the north. Perennial surface water at the Site 13 Cluster is limited to ABRES-A Lake. Hydrological investigations performed during the RI indicate there is a hydraulic connection between the lake and the subsurface canyon groundwater aquifer. When water levels rise in the lake, the groundwater in the shallow canyon water table rises at a comparable rate. Based on the study of local and regional topographic and geological maps, subsurface geophysical survey data, and drilling data obtained during the RI, a paleochannel was identified. This paleochannel is downgradient of ABRES-A Lake and extends at least as far as well 14-MW-8. Because the paleochannel would potentially provide a migration pathway for contaminants in groundwater, monitoring wells were placed along its axis.

4.2.5 AOC A, NSA Mid-South

Geology at this site includes alluvium, loess, fluvial deposits, and Cockfield Formation. Within the AOC A study area, the deposits are only comprised of fluvial deposits whereas the other unconsolidated materials have been eroded from the site. Discontinuous saturated sand lenses are present locally in the Cockfield aquifer. Within the AOC A study area, the fluvial deposits and Cockfield aquifers are hydraulically connected across an erosional scarp in the Cockfield Formation located in the northern part of the area. Groundwater beneath AOC generally flows north-northwest consistent with the contaminant plume shape.

Bioaugmentation Sites

4.2.6 Site 59, NAS Cecil Field

The majority of surficial sediments encountered at this site include fine to very fine sands with varying minor amounts of silt. Isolated, discontinuous, relatively thin clay layers (less than 5 feet, usually less than 1 or 2 feet) were encountered within the top 40 feet. Starting at approximately 90 feet bgs, but deeper at some locations, the clay content increased significantly. In approximately 20 to 30 feet above bedrock, sandy clay and clayey sand interspersed with sand layers were encountered, all of varying thicknesses and generally localized in extent. Depth to groundwater in November 2004 and February 2005 ranged from approximately 6 to 8 feet bgs. In January 2006, depth to groundwater ranged from approximately 3 to 6 feet bgs.

4.2.7 Magazine 1 Area, Fort Dix

The hydrogeologic units (sequentially, from the uppermost unit down) in the vicinity of the MAG-1 Area are the Cohansey, Kirkwood, Manasquan, Vincentown, Hornerstown-Navesink, and Wenonah-Mount Laurel Sands**.** Surficial geological maps of the area indicate that the Cohansey Sand is present east of, but not within, the MAG-1 Area. The Kirkwood formation is the uppermost unit in the immediate vicinity of MAG-1 Area, but is absent west of the site. Vertical contaminant distribution and bromide tracer results from this demonstration seem to confirm this assertion.

The MAG-1 Area is located at the base of an escarpment, over which surface elevations drop approximately 40 to 80 feet. The topography in the MAG-1 Area slopes to the west and northwest. Local groundwater discharges to ponds and wetlands and streams at this escarpment base. Groundwater in the area appears to discharge to several streams and wetlands that mainly intersect the Kirkwood and Vincentown formations.

4.2.8 OU 24, NAS North Island

The shallow unconfined aquifer at OU 24 is composed primarily of sediments dredged from San Diego Bay. Groundwater at OU 24 is encountered at approximately 5 to 7 ft bgs. At approximately 35 ft bgs, there exists a freshwater-saltwater interface. Investigation results indicate no significant migration of contaminants below this interface. Downgradient of OU 24, and adjacent to the San Diego Bay shoreline, is a quay wall system that is believed to impede the discharge of impacted groundwater to San Diego Bay.

4.2.9 Plume B, Bachman Road

The major lithology at the Bachman Road site consist of glacial outwash sand, with local lenses of lower permeability silty material in two zones, at 16 ft bgs and immediately above the underlying clay at approximately 24 ft bgs. The following hydrogeological parameters have been previously determined at the Site: average hydraulic conductivity is 55 ft/day; effective porosity of the sand is 36%; and, average groundwater flow velocity is 0.5 ft/day.

4.2.10 Milledgeville

Soil borings at the site indicated differential weathering patterns within the complex shallow lithology at the site and a high degree of heterogeneity in the upper 25 ft. The result is a predominance of very low permeability, intercalated clays, silts, and sands containing numerous rock fragments as well as zones of saprolite present from land surface to approximately 20 ft bgs, and partially weathered rock (PWR) present from about 20 ft bgs to the top of competent bedrock encountered at approximately 24 ft bgs. Weathering is typically initiated along fractures or bedding planes in the bedrock and is expanded from these zones over time. It is expected that groundwater flow, including groundwater containing dissolved VOCs, seek these preferential pathways as weathering continues. The top of the groundwater surface is approximately 9 to 12 ft bgs.

4.2.11 Former NAWC Trenton

The predominant geology at the NAWC site is a fractured-rock aquifer composed of dipping layers of sedimentary rocks. Aquifer testing and tracer tests were conducted to obtain hydraulic and solute-transport properties of the aquifer. The heterogeneous nature of the underlying geology resulted in a wide range of values of each parameter measured. Transmissivity values were obtained, ranging from 95 to 1,300 feet squared per day; storage coefficients were found to range from $9x10^{-5}$ to $5x10^{-3}$; and the effective porosity ranges from 0.0003 to 0.002.

4.3 CONTAMINANT DISTRIBUTION

For each of the following subsections, the distribution of contaminant(s) at each demonstration site is described. Figures and other supplemental site information are provided in **Appendix B**.

MNA Sites

4.3.1 Operable Unit 1, Anniston Army Depot

Although other Contaminant of Concerns (COCs) are present in SIA groundwater, TCE is the primary contaminant, and is the best indicator of the overall nature and extent of organic groundwater contamination (**Appendix B-1**). The SIA has multiple source areas for VOCs that contribute to a main plume that originates in the Northeast/Industrial Area of the SIA and migrates roughly parallel to Dry Creek, with a general flow trend to the southwest (contamination has not been detected in Dry Creek). Plumes of VOCs originate in the Trench and Landfill Areas and appear to be restrained from migration in the residuum and weathered bedrock due to localized hydrogeologic conditions. It is suspected that the Landfill source area contributes to the off-site VOC contamination, although a defined pathway has not been identified.

Contaminant sources are likely present as DNAPL in several areas of the site, including SWMUs 1, 12, 25, 30 and several spill sites within the Industrial Area (**Appendix B-1**). DNAPL's presence at the site is evaluated through several lines of evidence, including site use and history, visual observation of DNAPL in site samples, and high or persistent contaminant concentrations in soil and groundwater.

Estimates of mass have been calculated resulting in the generation of low and high TCE mass estimates. The low estimate of TCE mass in SIA is 3.6 million pounds and the high estimate is 27 million pounds. Over 99 percent of the TCE mass is present as free phase liquid, as suggested by the calculation (both high and low estimates). The Industrial Area contains nearly 50 percent of the TCE mass relative to the other three source areas. The residuum layer was estimated to contain 88 percent of the mass due to its greater porosity (SAIC 2008a, 2008b, Tetra Tech, 2009).

4.3.2 SWMU 21, NAS Dallas, TX

The groundwater data collected prior to the ZVI pilot test (February 2006) indicated maximum TCE concentrations of 0.180 mg/L in well 608D32MW, 0.290 mg/L in well 608D133MW, 0.011 mg/L in well 608D134MW, 0.250 mg/L in well 608D135MW, 0.024 mg/L in well 608D137MW, 0.290 mg/L in well 608D139MW, and 0.095 mg/L in monitoring well 608D148MW. The groundwater fate and transport modeling predicts that there will be no off-site migration of the groundwater E zones (TtNUS, 2004a). These wells are illustrated on figures in **Appendix B-2**.

Biostimulation Sites

4.3.3 MLP/VAB, NASA Cape Canaveral

Site investigation activities revealed the three primary VOCs at the site were TCE, *cis*-DCE, and VC. Site data collected prior to implementation of remedial measures indicated that TCE was transformed to VC. Evidence of dechlorination in the source zone was identified during a February 2003 sampling event where ethene concentrations were detected as high as 395 μg/L.

Prior to treatment it appeared that TCE concentrations were decreased with time downgradient of the source area. Concentrations of *cis*-DCE at depths above 50 ft near the source zone were relatively unchanged during the investigation phase. As distance away from the source zone increases, some data showed that *cis*-DCE concentrations were decreasing with time.

TCE has not been detected at the leading edge of the plume. The VC portion of the plume extended approximately 1,800 ft downgradient of the VAB. VC concentrations in this area remained relatively constant (**Appendix B-3)**.

4.3.4 Vandenberg AFB

4.3.4.1 Site 8

Based upon site operations and releases, both chlorinated solvents and perchlorate are present in groundwater (**Appendix B-4)**. The maximum detections include TCE at 1,400 µg/L, *cis*-DCE at 60 µg/L, and perchlorate at 381 µg/L based upon 2004 data. The plume is relatively long at approximately 6,000 feet and discharges at seeps leading to the Pacific Ocean. At the beginning of the pilot test the TCE concentrations had decreased to 1,000 µg/L VC was not detected but it is not clear if *cis*-DCE stall is occurring or if there is efficient degradation of *cis*-DCE to innocuous compounds.

4.3.4.2 Site 13/14

The primary contaminants in many of the wells (**Appendix B-4)** upgradient portion of the canyon include TCE (850 µg/L at 13-MW-7) *cis*-DCE (230 µg/L at 13-MW-7) and VC (less than 1 µg/L at 13-MW-7). In the study area downgradient of ABRES-A Canyon and Lake, the TCE was removed in all wells; however, *cis*-DCE (up to 203 µg/L at well 14-MW-10) and VC (up to 66 µg/L at well 14-MW-10) were detected. *cis*-DCE (stable concentrations) and VC (increasing concentrations) are both persisting and no ethene was measured, suggesting a DCE-VC stall is occurring at this site.

4.3.5 AOC A, NSA Mid-South

The TCE concentrations at the beginning of the IM (baseline sampling in 2004) in Sub-Plume A ranged from 0.11 mg/L (MW 007G65LF) to 1.4 mg/L (MW 007G57LF). Two of the wells with the highest concentrations were MW 007G57LF and MW 007G64LF. As illustrated on the figures in **Appendix B-5**, the *cis*-DCE were approximately 1 to 2 orders of magnitude lower (i.e., less than 0.20 mg/L), and VC was not detected. In Sub-Plume B, the maximum concentration of TCE was as high as 0.2 mg/L (MW 007G57LF) with *cis*-DCE again being approximately one order of magnitude lower, and VC was only sporadically detected. In Sub-Plume C, the maximum concentration of TCE was less than 0.10 mg/Land *cis*-DCE and VC less than 0.010 mg/L. In Sub-Plume D, the range in TCE concentrations was generally similar to Sub-Plume A (approximately 0.100 mg/L to 1.4 mg/L). While some wells had detections of *cis*-DCE and VC at approximately one order of magnitude lower and near non-detect levels,

respectively, some wells (e.g., MW PESMW2S and MW PESMW4S had detections of *cis*-DCE and VC at or higher concentrations than TCE. These plumes are illustrated in **Appendix B-5.**

Bioaugmentation Sites

4.3.6 Site 59, NAS Cecil Field

A tag map is provided in **Appendix B-6** showing TCE concentrations at Site 59 based on groundwater results from the RI and sampling conducted during a Bioaugmentation Pilot Study. The "hot spots", as defined by TCE concentrations greater than the FDEP Natural Attenuation Default Value (NA-DV) of 300 μg/L, are located at NG-02D, CEF-059-004-73, and CEF-59-NG-12D. In the vicinity of NG-12D, an elevated zone of contamination appears to be present in a depth interval between 30 and 78 feet bgs. Therefore, the bioaugmentation pilot study focused on this depth interval.

4.3.7 MAG-1 Area, Fort Dix

Several geologic and hydraulic investigations have been performed in the MAG-1 area (Shaw, 2009). Dames & Moore (1993) and ABB (1995) remedial investigation activities (soil gas surveys, geophysical surveys, soil and groundwater sampling) focused on the area near the MAG-1 buildings.

TCE and *cis*-DCE are the main chlorinated solvents detected in the MAG-1 Area groundwater (Shaw, 2009). The TCE plume with a maximum concentration of approximately 2,000 ug/L near Monitoring Well MAG-113P is approximately 900 feet long and 450 feet wide. The *cis*-DCE plume with a maximum concentration of approximately 1,200 ug/L, near monitoring well MAG-113P, is approximately 750 feet long by 350 feet wide. However, recent groundwater data indicated that both TCE and DCE concentrations are currently substantially lower (at least in the Demonstration Area) than those observed during and prior to June 2004.

The field demonstration area was located in the plume area with the highest VOC concentrations. Based on the total VOCs observed at wells near the demonstration site (MAG-112P, MAG-113P, MAG-66,) the highest total VOC concentrations are in the 90 to 100 foot amsl (above mean sea level) range (i.e. Kirkwood Formation). Total VOC concentrations in well MAG-113P (screen interval across the Kirkwood and Manasquan Formations: 87.5-97.5 ft amsl) in June 2004 were 2,400 ug/L, while VOC concentrations in well MAG-112P (screen interval within the Manasquan Formation: 78.2-88.2 ft amsl) were below the analytical detection limit.

4.3.8 OU 24 NAS North Island

The general extent of the VOC plume at OU 24 in the shallow unconfined aquifer is illustrated on figures in **Appendix B-8**. The primary groundwater contaminants at the site are *cis*-DCE and VC. Contaminants were detected at 5 and 40 feet below ground surface, at concentrations as high as 3,700 ppb and 1,500 ppb, respectively. Previous studies showed that natural attenuation was not occurring at a rate capable of preventing contaminant transport to San Diego Bay, where these compounds present a potential risk to ecological and human receptors. A freshwater-

saltwater interface has been encountered at approximately 35 ft bgs. Investigation results indicate no significant migration of contaminants below this interface. Downgradient of OU 24, and adjacent to the San Diego Bay shoreline, is a quay wall system that is believed to impede the discharge of impacted groundwater to San Diego Bay.

4.3.9 Plume B, Bachman Road

Multilevel (ML) samplers were installed in six transects to better define the source area, monitor remediation performance, and delineate the downgradient plume. Each ML sampler has 9-inch screens at different elevations for discrete sampling. The ML arrays consist of ¼" polyethylene tubing with screened intervals, spaced to achieve a 25-foot vertical array of sampling points. Data obtained from sampling of the ML samplers indicate dissolved chlorinated solvent impacts throughout the shallow aquifer. Greater impacts have typically been associated with two intervals: the lower permeability silty material at 16 ft bgs, and immediately above the underlying clay at approximately 24 ft bgs.

Groundwater concentrations of PCE exceeding MDEQ Generic Residential Cleanup Criteria for Drinking Water (and Surface Water Human Drinking Water Values), Indoor Air Inhalation, Groundwater Surface Water Interface (GSI), and Groundwater Contact (GC) have been detected in the Bachman Road Plume B Source Area and downgradient of the Source Area. A public water supply is available and institutional controls will be implemented at the Site and at impacted downgradient properties (prior to site closure). Accordingly, the drinking water pathway is not applicable at the Site. The indoor air pathway is complete for the Plume B Source Area and portions of the downgradient plume. The GSI pathway is complete at the downgradient end of the plume, where groundwater discharges to Lake Huron (**Appendix B-9**). GC criteria are also applicable at the Site.

4.3.10 Milledgeville

Dissolved TCE concentrations in the source area (monitor well MW-7) have routinely exceeded 10,000 micrograms per Liter (µg/L) (**Appendix B-10**). A site/source characterization investigation was conducted in October 2001 to evaluate the presence of dense non-aqueous phase liquid (DNAPL) near the exterior sump south of the main assembly building. Based on the findings of this investigation, neither contaminated soils above the water table nor DNAPL appeared to be present in this source area. Additionally, historical water quality data from the shallow well installed near the sump (i.e., MW-7) indicated decreasing VOC concentrations since the RFI work began in 1997. The VOC concentrations downgradient from this area were relatively consistent with levels observed in 1997, after an increase in concentrations was noted between 2000 and 2002.

4.3.11 Former NAWC Trenton

TCE and its degradation products DCEs and VC are the primary contaminants identified at the NAWC, West Trenton, N.J. (International Technology Corporation, 1994). The highest concentrations of TCE, *cis*-DCE, and VC detected in wells at the NAWC were 88, 52, and 21 mg/L, respectively (Lacombe, 2000). A pump-and-treat system consisting of six recovery wells and an air-stripping treatment system has been operating at the NAWC (**Appendix B-11**) since 1998. The U.S. Geological Survey (USGS), in cooperation with the U.S. Department of the Navy, conducted an 11-year, multiphase hydrogeologic investigation of the NAWC. In earlier phases of the investigation, Lacombe (2000, 2002) determined the hydrogeologic framework, and Lewis-Brown and Rice (2002) developed a digital model to simulate, and evaluate the effects of various recovery-well networks on, groundwater flow at the NAWC.

5. TEST DESIGN

This section provides the detailed description of the testing conducted during the demonstrations.

5.1 CONCEPTUAL EXPERIMENTAL DESIGN

The performance objectives are provided in **Table 3-1**. They include criteria such as:

- Correlation of *Dhc* RDase gene targets with reductive dechlorination activity, and the number of *Dhc* 16S rRNA gene copies (i.e., cells).
- Correlations of RDase gene copies with contaminant concentrations.
- Correlation of *Dhc* biomarker gene abundance with the dominant TEAP (i.e., redox condition).
- Correlation of RDase biomarker genes with stable or shrinking contaminant plume(s).
- Identification of false positive and false negative qPCR results and their impact on decision-making processes. This effort also evaluated sample collection methods and determined the sensitivity and reproducibility of the analytical procedures.

To evaluate the performance objectives of this demonstration, samples were collected from the field sites described in Section 4. The resulting chemical, geochemical, and microbial data are presented in this section and were also compiled in a central database. Various data evaluation methods (e.g., statistical correlations) were conducted to determine whether the success criteria for each performance objective were achieved. The data interpretation is presented and discussed in Section 6.

5.2 BASELINE CHARACTERIZATION

For the purposes of this study, baseline characterization is defined as site characterization prior to implementation of enhanced bioremediation. No baseline characterization was conducted by the ER-0518 team prior to sampling because baseline characterization is not required prior to implementing the use of MBTs at a site. However, baseline characterization activities were conducted by the individual site managers and data resulting from those activities, which were used to build the Conceptual Site Model (CSM), is hereby presented. Figures and other supplemental site information are provided in **Appendix B**.

MNA Sites

5.2.1 Operable Unit 1, Anniston Army Depot

Groundwater monitoring has been conducted biannually since 2002 and a natural attenuation assessment has been conducted recently (Tetra Tech, 2009). Evidence of several natural attenuation processes which include a variety of physical (e.g., advection, dilution, dispersion, diffusion, volatilization, etc.), chemical (e.g., abiotic degradation), and biological processes is

evident across the SIA. These mechanisms are evident based upon a weight-of-evidence evaluation. Biodegradation, the most desirable mechanism for MNA, is occurring in the SIA plumes based on: (1) primary evidence of a decrease in the parent compounds such as TCE and a subsequent increase and decrease in concentrations of daughter products such as *cis*-DCE and VC in groundwater data from 2002 to present, (2) secondary evidence, such as field geochemistry and terminal electron acceptor processes that are indicative of reduced oxygen conditions and are believed to be favorable for supporting biological destructive processes, and (3) tertiary evidence including the presence of *Dhc* responsible for complete reductive dechlorination to ethene. MNA including reductive dechlorination is occurring to various degrees in each of the four primary source areas of the SIA.

The plume emanating from the Trench Area shows evidence of TCE transformation but contains stable *cis*-DCE and very low VC, ethene, and ethane concentrations (**Appendix B-1**). This, in combination with low organic carbon content, suggests that *cis*-DCE "stall" or "slowdown" and thus incomplete reductive dechlorination is occurring in this area. Conversely, immediately to the south of the Trench Area, elevated levels of daughter products (*cis*-DCE, VC, and ethene), and presence of elevated levels of the *Dhc* indicates that reductive dechlorination is evident in various locations in the Landfill Area. In fact, at Solid Waste Management Unit 12 (SWMU 12), strong evidence of reductive dechlorination is evident, where elevated amounts of organic carbon, a robust *Dhc* community (e.g., at well SWMU 1201 *Dhc* was detected at up to 2.6 $x10^7$ cells/mL), and resultant ethene and ethane provide strong evidence that biological degradation processes are occurring in this area. It is not clear why there is more organic carbon at this site as compared to other sites, but one hypothesis that is being considered is that in situ chemical oxidation conducted in a pilot study there in 2001-2002 may have made the carbon more bioavailable, but this has not been proven.

As indicated in the Remedial Investigation (SAIC, 2008a) for transport analysis, the plume originating from the Northeast Area and the plume from the Industrial Area are considered one integrated plume (**Appendix B-1**). In this plume, limited reductive dechlorination is occurring. Similar to the Trench Area, there is evidence of a *cis*-DCE "stall" or a "slowdown" and thus incomplete reductive dechlorination in the source areas of the Industrial and Northeast Areas. Again, similar to the Trench Area plume there is insufficient information to determine if the "stall" or "slowdown" is due to insufficient microbial activity, the lack bioavailable carbon/electron donor or both. However, downgradient from the industrial area, reductive dechlorination occurs at locations where concentrations of TCE are much lower (less than 50 parts per billion). This is particularly evident from data collected from bedrock wells in this area. This may be a significant observation as this area is where there the long term "dilute plume" persists.

5.2.2 SWMU 21, NAS Dallas

Samples collected from this site were only for the purposes of evaluating sample and biomass collection procedures (Phase I), therefore, no baseline site characterization is provided. Samples were collected from this site during post treatment monitoring period. Following ZVI injection, quarterly groundwater sampling of the same monitoring wells surveyed during the baseline activities was conducted to monitor the performance of the ZVI injection pilot study. The

samples were analyzed for the same field parameters, VOCs, and geochemistry parameters. This sampling schedule was developed to provide sufficient, timely information regarding the success of the pilot study, while limiting the overall number of samples collected and analyses performed.

Biostimulation Sites

5.2.3 MLP/VAB, NASA Cape Canaveral

Baseline measurements determined that TCE and daughter product impacted the groundwater. Dissolved ethene was detected at concentration as high as 210 µg/L and up to 10^7 *Dhc* cells/L of groundwater were present prior to treatment, indicating that complete TCE dechlorination was occurring. Geochemical analyses indicated that groundwater conditions were anaerobic, although sulfate concentrations were elevated (450 mg/L). Source zone concentrations of TCE in groundwater revealed a maximum of 50,000 µg/L (**Appendix B-3**).

Corrective measures were implemented in 2007 to address source area TCE impacts. Biostimulation injections were performed, using ethyl lactate as an electron donor. Ethyl lactate was delivered to the subsurface at regular (every other month) intervals via temporary injection points. Performance monitoring of source area groundwater was conducted by sampling wells in the source area every other month. Samples were analyzed for key geochemical, chemical, and biological parameters.

5.2.4 Site 8 & 13-14, Vandenberg AFB

Samples collected from these sites were only for the purposes of evaluating sample and biomass collection procedures (Phase I). Therefore no interpretation of site conditions is included.

5.2.5 AOC A, NSA Mid-South

The four locations where bioremediation followed by MNA were conducted include: Sub-Plume A, where monthly sodium acetate injections occurred from May 2004 to November 2007 in injection wells; Sub-Plume B located northeast of Sub-Plume A in the area of monitoring well (MW) 007G22LF, where monthly sodium acetate injections occurred from May 2004 to November 2007 in injection wells; Sub-Plume C located (south of Sub-Plume A) in the area of MW 007G03LF, where monthly sodium acetate injections occurred from May 2004 to Nov 2007 in injection wells; and, Sub-Plume D, located on the eastern half of the site and near the former Hangar N-6 (**Appendix B-5**), where emulsified vegetable oil (EVO) injections took place from 2000 to 2002 and from May 2004 to November 2007. This site is under a long term monitoring program by Spectra Tech (2008). The contaminant data are displayed in various figures and graphic format in **Appendix B-5.**

In Sub-Plume A, VOC concentrations decreased substantially in numerous monitoring wells. More specifically, TCE concentrations in most wells had decreased below the 100 micrograms per liter (µg/L) target cleanup goal (SpectraTech, 2008). TCE concentrations have decreased over one order-of-magnitude since injections began in 2005, from a high of 1,600 µg/L in May 2005 to 260 µg/L in November 2007. In general, groundwater geochemistry confirms the

reductive geochemical nature of the aquifer within Sub-Plume A. Geochemical conditions coupled with TCE reduction and daughter-product formation indicate the substrates injected are functioning as intended. Dissolved oxygen (DO) and oxidation-reduction potential (ORP) generally indicate that the aquifer is anaerobic. This is supported by elevated ferrous iron concentrations in the aquifer (majority of the locations are less than 1,000 µg/L). A majority of Sub-Plume A monitoring wells show hydrogen concentrations greater than 1.0 nanomolar (nM). Methane concentrations are well above 1,000 µg/L, levels indicative of methanogenic conditions, which are favorable to reductive dechlorination processes.

In Sub-Plume B, PCE and TCE concentrations decreased from 51 and 100 µg/L to 4 and 2 µg/L, respectively, within 4 months following the start of substrate injections. During the same period, *cis*-DCE concentrations increased from 1 to 130 µg/L. Since then, PCE and TCE concentrations in this well generally have remained less than 2 µg/L, while *cis*-DCE subsequently decreased to less than 2.0 µg/L. Despite the fact that the data suggest resultant concentrations that are below cleanup criteria, there is very good evidence of contaminant degradation with biostimulation only.

Sub-Plume C, the smallest of the four sub-plumes, has shown decreases in both PCE and TCE with very little increases in *cis*-DCE and VC after the substrate injections. All contaminants have been at or near analytical detection levels of (less than 1 µg/L). Despite total organic carbon (TOC) concentrations of less than 20 µg/L, methane concentrations increased from 0.32 µg/L at the start of injections to consistently above 10,000 µg/L, indicating low redox conditions.

At Sub-Plume D, the prime contaminant of concern, TCE, has decreased since the vegetable oil pilot study was initiated in 2000. Although VOC degradation was slow during the first year, degradation rates have substantially increased since sodium acetate injections began in May 2005. TCE concentrations in MW PESMW3S have decreased from a high of 680 µg/L in August 2005 to 110 µg/L in November 2007. Additionally, the daughter products *cis*-DCE and VC slightly decreased to 120 µg/L and 89 µg/L, respectively, during the November 2007, but remain elevated in comparison to the historical data. Notably, VC concentrations have increased in numerous wells to levels near or exceeding 100 µg/L. As encountered in the other sub-plumes, indications of reducing conditions are evident as DO concentrations range from 0.5 to 1.0 mg/L and elevated total iron concentrations in several locations as high as 5,740 µg/L in one well. November 2007 dissolved hydrogen concentrations were above 1.0 nM in all monitoring wells, with concentrations as high as 8.4 nM. Methane concentrations, which are generally greater than 1,000 µg/L (with the highest value of 16,000 µg/L) indicate that Sub-Plume D is reduced and methanogenic.

Bioaugmentation Sites

5.2.6 Site 59, NAS Cecil Field

A pilot test consisting of in-situ biostimulation and bioaugmentation has been performed from August 2006 through March 2007. The system recirculated groundwater via one extraction well and two injection wells; sodium lactate and sodium bicarbonate were added to the recirculated water. After approximately 7 weeks of sodium lactate and sodium bicarbonate addition, 6 liters of an inoculum containing *Dhc* (TtNUS, 2008) was injected into the injection wells. A comprehensive, 30-week sampling program was performed and samples were collected at approximately monthly intervals. The graphs provided in **Appendix B-6** illustrate the change in concentrations of TCE, *cis*-DCE, and VC over the course of the project. Strong reducing conditions were achieved at an early stage in the project. These data were used to generate the correlations of *Dhc* RDase genes (e.g., *tceA*, *bvcA*, and/or *vcrA*) with daughter to parent compound concentration ratios (e.g., [*cis*-DCE, VC]/TCE; [VC, ethene]/*cis*-DCE) and VOC concentrations (e.g., TCE, *cis*-DCE, VC) in Section 6. Bioaugmentation with lactate resulted in complete dechlorination to ethene, corresponding to increases in *Dhc* cell titers as illustrated in **Appendix B-6**.

5.2.7 MAG-1 Area, Fort Dix

One of the primary goals of this field demonstration (Shaw, 2009), funded by the Environmental Security Technology Certification Program (ESTCP), was to evaluate the amount of bioaugmetation culture needed to effectively remediate a VOC contaminant plume and to determine the effect of inoculum dose on remedial time. The field demonstration involved the construction and operation of 4 groundwater recirculation loops, each inoculated with a different amount of Shaw's SDC-9 dechlorinating culture (**Appendix B-7)**. VOC biotransformation and growth of the added organisms were monitored. In addition, because of the low pH at the site, the ability to increase and maintain a circumneutral pH conducive for reductive dechlorination activity by adding buffers was conducted and evaluated.

5.2.8 OU 24 NAS North Island

The primary groundwater contaminants at the site are *cis*-DCE and VC. Contaminants were detected at 5 and 40 feet bgs, at concentrations as high as 3,700 ppb and 1,500 ppb, respectively. Remedial measures have been conducted at OU 24, including active (forced gradient) EISB in the source area and passive (natural gradient) EISB via biobarriers in the downgradient plume. This configuration is illustrated in **Appendix B-8**. The source area was inoculated with KB-1 dechlorinating culture and dosed with lactate (i.e., soluble electron donor). Post-injection quarterly monitoring was conducted to characterize geochemical, chemical, and microbiological changes. In general, the geochemical conditions at the site trended toward strongly reducing conditions from baseline conditions as the implementation began. Concurrent with this shift were increases in DOC concentration, increases in the abundance of the biomarker genes *vcrA* and *bvcA*, associated with an increase of *Dhc* cell titers and decreases in sulfate concentration in some source area monitoring wells. The average total VOC concentration in the source area

performance monitoring wells decreased as the implementation started. Interestingly, increases in ethene concentration were not observed suggesting that VOC decreases may have been related to the intrinsic variability of total VOC concentrations or rapid turnover of ethene.

5.2.9 Plume B, Bachman Road

Source area PCE concentrations of greater than 100 mg/L have been detected in site groundwater, indicating the likely presence of DNAPL. Baseline investigation data, collected prior to implementation of EISB measures, indicated reductive dechlorination processes were active (evidenced by geochemical parameters and presence of chlorinated ethene daughter products and *Dhc* biomarkers).

Remedial measures were conducted at the Bachman Road site in 2008, including active (forced gradient) EISB in the source area and downgradient plume area. Recirculated groundwater was amended with KB-1 dechlorinating culture and emulsified soybean oil (i.e., electron donor). Post-injection monitoring has been performed utilizing multi-level sampling wells positioned in the source and three downgradient transect areas. Samples were collected for geochemical, chemical, and MBT analyses on a quarterly basis. A discussion of post-injection data relevant to this study is presented in Section 5.7.9.

5.2.10 Milledgeville

Prior to bioaugmentation, baseline TCE concentrations were detected as high as 12,000 µg/L, and *Dhc* cell titers were at 10^3 cells/L. Daughter product concentrations of *cis*-DCE and VC were generally in the range of 300 µg/L and 1 µg/L, respectively. The absence of notable VC and ethene production is indicative of a *cis*-DCE "stall" or "slowdown" and thus incomplete reductive dechlorination. Bioremediation pilot testing was conducted at the Milledgeville site to evaluate the potential for EISB. The fundamental design element for the bioaugmentation process was a series of groundwater recovery, injection, and performance monitoring wells (**Appendix B-10)**. The injection wells are oriented perpendicular to the prevailing direction of groundwater flow. Groundwater was withdrawn from the recovery wells, amended with soluble electron donor (i.e., sodium lactate and very small concentrations of ammonium chloride and phosphate salts), and injected back into the saturated formation to the same depth interval through the injection wells. Once the extracted water was determined to be anaerobic, the extracted groundwater was amended with a single application of the dechlorinating consortium BDI and the electron donor (continual application) until the objectives of the pilot test were satisfied. Groundwater sampling was performed on a routine basis from the performance monitoring wells and recovery wells after startup of the system and analyzed for the various geochemical, chemical, and biological parameters.

5.2.11 Former NAWC Trenton

Prior to bioaugmentation with KB-1, baseline TCE, *cis*-DCE, and VC concentrations were detected as high as 88, 52, and 21 µg/L, respectively. *Dhc* cell titers were at 10^3 cells/L prior to bioaugmentation activities. The feasibility of *in situ* bioaugmentation technology for treatment of TCE groundwater at the site was investigated at the pilot scale, beginning in 2005. Injections

of EOS (the brand name of the emulsified soybean oil selected as the electron donor) and dechlorinating consortium KB-1 were performed at the site in July 2005 (**Appendix B-11)**. Groundwater monitoring events were conducted at regular intervals to assess technology performance. Groundwater samples were characterized for geochemical, chemical, and biological changes. Due to apparent electron donor consumption (evidenced by monitoring data) in the pilot test area, additional emulsified soybean oil was introduced in July 2008. Performance monitoring through groundwater sampling events continued following the second injection.

5.3 TREATABILITY OR LABORATORY STUDY RESULTS

There were no treatability or laboratory confirmation studies conducted as part of this demonstration as the qPCR technology is mature and does not require a "proof of principle" effort. Considerable efforts were expended to compare groundwater sampling and on site versus off site biomass collection procedures. All samples were collected from field sites described above and analyzed in the laboratory utilizing qPCR methodology.

5.4 DESIGN AND LAYOUT OF TECHNOLOGY COMPONENTS

MNA Sites

5.4.1 Operable Unit 1, Anniston Army Depot

Groundwater monitoring has been conducted biannually since 2002 and a natural attenuation assessment has been conducted recently (Tetra Tech, 2009). Anaerobic biological activity, including reductive dechlorination, is evident across all four primary source areas of the SIA. This has been demonstrated by collecting samples from nearly 50 wells at various locations and is shown in figures in **Appendix B-1**. Samples are analyzed for VOCs, including the contaminants of concern (e.g., TCE, *cis*-DCE, VC), hydrogeologic and geochemical parameters (e.g., DO, ORP, ferrous iron, nitrate/nitrite, sulfate/sulfide, hydrogen, etc.), reduced gases (e.g., methane, ethene and ethane, etc.), and microbial biomarker genes (e.g., Dhc 16S rRNA gene and RDase gene targets). Wells were selected to represent background conditions, source, plume (interior and perimeter), and downgradient locations at each of the four source areas. As described in Section 5.2.1 and illustrated in **Appendix B-1**, observation of daughter products as well as geochemistry data suggest moderate to strong reducing conditions exist at the site. Therefore, the data generated at the four Anniston sites are sufficient to evaluate the use of qPCR data as indicators of natural attenuation performance.

5.4.2 SWMU 21, NAS Dallas

Samples collected from this site for this effort were only for the purposes of evaluation of evaluating sample and biomass collection procedures (Phase I), therefore, no discussion was included on the design and layout.

Biostimulation Sites

5.4.3 MLP/VAB, NASA Cape Canaveral

Corrective measures were implemented in 2007. Biostimulation injections were performed to source area impacts, using ethyl lactate as an electron donor. Performance monitoring of source area and resultant plumes was conducted once per month. Performance monitoring locations were selected to provide adequate data for assessment. Samples were analyzed for VOCs, a full suite of geochemical/chemical, volatile fatty acids (VFAs), and *Dhc* biomarker target genes. As described in Section 5.2.3 and illustrated in **Appendix B-3**, observation of daughter products as well as geochemistry data suggest moderate to strong reducing conditions exist. These data suggest that this site meets the criteria and was therefore included in this study as a site to evaluate MBT methodology at a site undergoing biostimulation.

5.4.4 Site 8 & 13-14, Vandenberg AFB

Samples collected from this site for this effort were only for the purposes of evaluating sample and biomass collection procedures (Phase I), therefore, no discussion was included on the design and layout.

5.4.5 AOC A, NSA Mid-South

Samples have been collected at each of the four locations (AOC Plumes A, B, C, and D) where bioremediation followed by MNA has been conducted from 2000 to 2007. Samples have been analyzed for VOCs, including the contaminants of concern (e.g., TCE, *cis*-1,2 DCE, VC), hydrogeologic, a full suite of geochemistry parameters (e.g., DO, ORP, ferrous iron, nitrate/nitrite, sulfate/sulfide, hydrogen, methane, etc), VFAs, and qPCR (e.g., Dhc and RDases) at numerous wells in the source area and the plume. Background source, plume (interior, and perimeter), and downgradient wells were selected. The figures in **Appendix B-5** illustrate the locations of wells sampled. Further, as described in Section 5.2.5, samples were collected in zones where daughter products as well as geochemistry data suggest moderate to strong reducing conditions exist in the presence of injected substrate. As a result, these data indicate conditions sufficient to evaluate the use of MBTs as an indicator of biostimulation remediation performance of chlorinated ethenes at each of these 4 sites.

Bioaugmentation Sites

5.4.6 Site 59, NAS Cecil Field

The in-situ biostimulation and bioaugmentation performed from August 2006 through March 2007 was constructed using a recirculation configuration as shown in **Appendix B-6.** In preparation of the bioaugmentation the site was prepared for inoculation. That is reducing conditions and favorable geochemical conditions were created prior to innoculation to determine if bioaugmentation was needed. A 60 percent sodium lactate solution was added to the aquifer in wells as shown in Appendix B-6. The sodium lactate feed rate was adjusted to maintain reducing conditions in the aquifer. Sodium bicarbonate was added as needed to maintain the pH between

6.5 and 8. The quantity of bicarbonate was estimated from a field test performed as part of the Work Plan preparation. The pH in the injection wells was greater than 6.5 by August 29, 2006 and was consistently greater than 7 after September 26, 2006. The feed rate of the buffer solution was adjusted as needed based on the results of the routine pH measurements. Over the course of the study approximately 370 pounds of sodium lactate and approximately 2,000 pounds of sodium bicarbonate were added. A comprehensive sample program including collection of VOCs, hydrogeologic, a full suite of geochemistry parameters, VFAs, and *Dhc* and RDase biomarker genes were collected in various locations throughout the plume including upgradient, source, and downgradient locations as shown in **Appendix B-6**. As a result of the aquifer preparation procedures described above, the site conditions were conducive for successful bioaugmentation and therefore to evaluate the values of MBTs for bioremediation monitoring at this site.

5.4.7 MAG-1 Area, Fort Dix

The MAG-1 Area was included in this study to evaluate qPCR results as an indication of bioaugmentation as described previously. This site was being evaluated under ESTCP Project ER-0515 (Bioaugmentation for Groundwater Remediation) (Shaw, 2009) and had similar criteria as the ER-0518 demonstration project with concentrations of PCE and/or TCE concentrations ranging between 1 and 30 mg/L with low *cis*-DCE concentrations and no VC or ethene. Therefore, this site met the criteria for evaluation of *Dhc*-targeted MBTs. As for the other bioaugmentation sites, the subsurface conditions were required to be modified to generate conditions allowing survival and growth of the inoculum and resultant degradation of the TCE. Similar to the NAS Cecil Field, this site exhibited a low natural pH (<5). Laboratory studies demonstrated that the SDC-9 bioaugmentation consortium used for the demonstration is inhibited at pH values below 5.1. As a result, the aquifer was buffered (using sodium bicarbonate and/or sodium carbonate powder) prior to inoculum injection. Further, strong anaerobic conditions were achieved through addition of lactate (60% solution) as electron donor prior to bioaugmentation (Shaw, 2009). After repeated buffering and electron donor additions under various circulation schemes (**Appendix B-7**), the site conditions were sufficient for inoculation (Shaw, 2009). A rigorous sampling scheme was implemented at the demonstration site to evaluate changes in chlorinated ethene concentrations, geochemical conditions, electron donor concentrations and consumption rates, and microbial growth and distribution (via qPCR analysis). A total of 12 performance monitoring groundwater sampling events including analyses of VOCs, geochemistry, reduced gases, VFAs, and *Dhc* biomarker genes were conducted in the demonstration area between January 30, 2008 and January 5, 2009 to monitor treatment performance. An extensive analysis of these data has been completed by Shaw (2009).

5.4.8 OU 24 NAS North Island

Remedial measures were conducted at OU 24, including active (forced gradient) enhanced EISB in the source area. Site figures (**Appendix B-7**) illustrate the location of the location of the injection biobarriers and the recirculation wells in proximity to the monitoring wells at the site. The concentrations of VOCs were relatively low at this site as illustrated in **Appendix B-8**. Along the centerline of the plume, VC concentrations were elevated (greater than 1,000 µg/L) but soon after the start of the study, the resultant concentrations decreased to less than 500 µg/L.

As a result of these low TCE concentrations (maximum concentration of 2.5 µg/L), only limited correlation analysis could be performed at this site.

5.4.9 Plume B, Bachman Road

Remedial measures were conducted at Bachman Road in 2008, including active (forced gradient) EISB in the source area and downgradient plume area. Recirculated groundwater was amended with KB-1 dechlorinating culture and emulsified soybean oil (i.e., electron donor). Baseline and post-injection monitoring was performed, utilizing multi-level sampling wells positioned in the source and three downgradient transect areas. Samples were collected for geochemical, chemical, and MBT analyses on a quarterly basis. Baseline and post-injection geochemical data indicated reducing conditions, the presence of *Dhc* biomarkers, and PCE daughter products. As described in Section 5.2.9 and illustrated in **Appendix B-9**, *Dhc* biomarker data, observation of daughter products, and geochemical data suggest evidence of reductive dechlorination. These data suggest that this site meets the criteria and was therefore included in this study as a site to evaluate MBT analysis for a site undergoing bioaugmentation.

5.4.10 Milledgeville

Bioremediation pilot testing was conducted at the Milledgeville site to address TCE-impacted groundwater. Site figures (**Appendix B-10**) illustrate the locations of the recirculation wells in proximity to the monitoring wells at the site. Groundwater sampling was performed on a routine basis from the performance monitor wells and recovery wells after startup of the system and analyzed for the various geochemical, contaminant, and biological parameters. As described in Section 5.2.10 and illustrated in **Appendix B-10**, observation of daughter products as well as geochemical data suggest that reducing conditions exist. These data suggest that this site meets the criteria and was therefore included in this study as a site to evaluate the value of MBT analysis for a site undergoing bioaugmentation.

5.4.11 Former NAWC Trenton

The feasibility of in situ bioaugmentation technology for treatment of TCE groundwater impacts at the site was investigated at the pilot scale, beginning in 2005. Injections of EOS®(the brand name of the emulsified soybean oil selected as the electron donor) and KB-1 (microbial culture) were performed at the Site in July 2005 (**Appendix B-11)**. Groundwater monitoring events were conducted at regular intervals to assess technology performance. Groundwater samples were characterized for geochemical and contaminant concentration changes, and changes in *Dhc* biomarker gene abundances. Due to apparent electron donor consumption (evidenced by monitoring data) in the pilot test area, additional emulsified soybean oil was introduced in July 2008. Performance monitoring through groundwater sampling events continued following the second injection. As described in Section 5.2.11 and illustrated in **Appendix B-11**, observation of daughter products as well as geochemistry data indicate that reducing conditions exist. These data suggest that this site meets the criteria and was therefore included in this study as a site to evaluate *Dhc*-targeted MBT analysis for a site undergoing bioaugmentation.

5.5 FIELD TESTING

This project was conducted in a phased approach. The phases outlined in this section have been designed to meet the objectives as described in Section 1.2. A phased approach was selected to allow information from initial phase(s) to be incorporated and/or utilized for the subsequent phase(s) of the project.

During Phase I, groundwater preservation methods and handling procedures, as well as on site versus off site biomass collection, were evaluated to determine the most cost-efficient and effective methodology/procedure for the remaining sampling planned for this project. In Phase II, the superior methodology/procedure was used to collect additional samples, with which the distribution and abundance of *Dhc* biomarker genes at the select demonstration sites were evaluated. These phases are described in greater detail in the following sections.

5.5.1 Phase I: Sample Preservation and Handling

In the first phase, groundwater sampling, sample preservation and handling methods, as well as on site and off site biomass collection procedures, were evaluated. This initial evaluation of preservation and handling was essential as the results of this phase were used as the methodology/procedure for the remaining sampling for this project.

This evaluation yielded an effective and efficient methodology for groundwater sampling and biomass collection in the field (i.e., on-site). The improved technology combines several advantages over contemporary procedures by (1) minimizing biomarker loss due to sample handling (e.g., produce false negative results), and (2) generating other benefits such as reducing overall sampling and analytical costs. Cost savings by on-site filtration are realized through lower shipping costs and reduced extraction and handling efforts in the laboratory. Two preservation methods were tested: (1) collecting groundwater samples in a container, preserving it at 4 degrees Celsius, and shipping it to the laboratory (i.e., traditional method); and (2) on-site filtering of the same volume of groundwater and shipment of the filter cartridges to the laboratory at 4 degrees Celsius.

In the first method groundwater samples were collected and shipped in a cooler to the Georgia Institute of Technology for biomass collection, nucleic acid extraction and qPCR analysis. Although this approach is routinely applied to quantify microbial biomarkers in groundwater, several confounding issues exist. For example, replicate sampling from multiple wells requires shipping numerous containers and large volumes of contaminated water that must be properly disposed by the analytical laboratory as hazardous waste. Large, heavy, ice-filled coolers delivered by overnight delivery add to the shipping cost, and cross-contamination due to leakage/breakage of bottles is always a concern (Ritalahti et al., 2010). The second method included on-site biomass collection as a new, yet alternative method, which ideally addresses the issues listed above. The intent was to determine the effect of on-site groundwater filtration and biomass collection on the results of *Dhc* biomarker gene analysis by qPCR when compared with the prevailing procedure that requires shipping of groundwater to the analytical laboratory for off-site filtration and biomass collection. The *Dhc* biomarker genes were determined for samples collected with each method in order to compare the alternative methods.

5.5.2 Phase II: Quantification of Biomarkers and Chemical Constituents

In the second phase of the project, qPCR was used to assess the distribution and abundance of *Dhc* biomarker genes at selected sites, which were in various stages of bioremediation treatment (e.g., MNA, biostimulation/bioaugmentation treatment). In addition, contaminants of concern (COCs), including chlorinated ethenes and other VOCs, were analyzed based upon project specific requirements. At each site, standard sampling methods were followed and traditional MNA/geochemistry parameters were collected, as described in Section 5.5 and summarized in **Appendix C**.

5.5.3 Data Compilation

The scope of project ER-0518 was to analyze groundwater samples collected at sites undergoing different bioremediation treatment and to demonstrate the value of quantitative *Dhc* biomarker gene analysis. This project relied on non-ESTCP resources to obtain samples for qPCR analysis. Following sample processing and quantification of *Dhc* biomarker genes, the results were transferred to a central database. Likewise, to the extent practical and available, the information from the site managers, including VOC and sampling/geochemical parameters, were transferred from the site managers to the ER-0518 project team and into the central database. The central database was maintained by Tetra Tech NUS, the prime contractor in this project.

Phase I compared field (on site) and laboratory (off site) sample filtration approaches for MBT analysis. Sites identified by the ESTCP Project Team for Phase I sampling included Site 59 at Naval Air Station (NAS) Cecil Field, FL, OU24 at NAS North Island, Vandenberg AFB Sites 8 and 13/14, and SWMU 21 NAS Dallas. Phase I sampling was initiated in the first quarter of 2007 and completed by the second quarter of 2007.

In Phase II, the methodology/procedure determined in Phase I was used to collect samples to evaluate the distribution and abundance of *Dhc* biomarker genes at the select demonstration sites. Phase II data collection began in the third quarter of 2007 and completed by July 2009. Samples were collected from eight sites, including Site 59 NAS Cecil Field, OU24 NAS North Island, Milledgeville, Anniston Army Depot, Bachman Road site, and Ft Dix. Some data was also collected from Anniston Army Depot and NSA Mid South. These sites were selected to represent sites where:

- Implementation of long-term MNA may be effective.
- Biostimulation may achieve complete dechlorination without DCE/VC “stall”.
- Bioaugmentation is required (early in the design) to initiate dechlorination to ethene to reduce project lengths and remediation times.

Among the sites utilized, Anniston Army Depot represents a site where MNA has been implemented. Vandenberg AFB Site 8 and Site 13/14 were included as biostimulation sites. Site 59 NAS Cecil Field, OU24 NAS North Island, MAG-1 Area of Ft. Dix, the Bachman Road Site, and Milledgeville are all bioaugmentation sites.

Generally, site samples were collected from a well in the source area, two to three wells inside the plume near source and downgradient from the source, a well upgradient of the source/plume,

and/or a well downgradient of the plume. At sites with active bioaugmentation/biostimulation tests occurring, samples were collected from wells inside the test area and at least one well outside of the test area whenever possible. **Appendix B** includes figures, which illustrate where samples were collected from each site. **Table 5-1** summarizes the sampling activity at each demonstration site.

Table 5-1 Sampling Activity Summary

Site Name	Site Type	Sampling Period	Analytical Program and Responsible Party[1]		
			qPCR	MNA Parameter	VOCs
Anniston Army Depot, (4 sites)	MNA	2004-2008	C	C	C
Former NAS Dallas	MNA	2004-2007	AR	C	C
NSA MidSouth (4 sites)	Biostimulation	2004-2009	C	C	C
Site 8, Vanderberg AFB	Biostimulation	May 07 - Dec 07	AR	C	C
Site 13/14, Vanderberg AFB	Biostimulation	Jun 07	AR	C	C
MLP/VAB NASA Cape Canaveral	Biostimulation	2006-2007	C	C	C
Bachman Road	Bioaugmentation	Aug 08 - Jul 09	AR	C	C
Milledgeville	Bioaugmentation	Sept 04 – Nov 05	AR	C	C
MAG-1, Ft. Dix	Bioaugmentation	Jan 08 - Sept 08	AR	C	C
OU 24, NAS North Island	Bioaugmentation	Apr 07 - Oct 08	AR	C	C
Former NAWC Trenton, NJ	Bioaugmentation	2005-2008	C	C	C
Site 59, NAS Ceil Field	Bioaugmentation	Jan 07 - Jul 08	AR	C	C

Notes:
qPCR - Quantitative Real-Time Polymerase Chain Reaction
1. Responsible Party for Collection (C): project team of individual site
Responsible Party for Analyses & Reporting (AR): ESTCP project team

The actual project schedule for the two-phase field testing is illustrated on **Figure 5-1**

Figure 5-1 Field Testing Schedule

Activity	**2007**				**2008**				**2009**			
Phase I Sampling	X	X										
Phase II Sampling			X	X	X	X	X	X	X	X	X	X

5.6 SAMPLING METHODS

Standard low-flow purging and sampling was the selected sampling method for all samples in this study. This procedure followed standard well purging procedures (Puls et al., 1996; Yeskis et al., 2002) outlined and accepted across the environmental industry. These procedures are described in detail in **Appendix C** and summarized below.

Groundwater sampling methods can affect the quantification of *Dhc* biomarker genes (SERDP and ESTCP, 2005; Casey, 2006). Thus, initial efforts focused on standardizing groundwater sampling, sample handling, and laboratory analytical procedures to establish Standard Operating

Procedures (SOPs). To the extent possible, the SOPs in **Appendix C** were followed for all groundwater sampling events.

5.6.1 Sample Collection, Filtration and Handling

Field sample collection, preservation and handling methods were evaluated and validated during this project. Groundwater samples were collected in amber, 1-L glass bottles and shipped on bagged ice in a cooler (i.e., 4°C) to the analytical laboratory where the biomass was collected via vacuum filtration on membrane filters for subsequent DNA extraction and *Dhc* biomarker quantification. In the laboratory, a peristaltic pump and easy load drive head were used with peroxide-cured silicone tubing (L/S 16) for laboratory filtration. These procedures are described in detail in **Appendix C** and in other publications **(**Ritalahti et al., 2010).

The *on-site* field filtration method includes the collection of suspended particles (i.e., microbial cells) from groundwater samples with the use of a single-use sterile filter cartridge. The ready-to-use cartridge consists of a hydrophilic polyethersulfone membrane (0.2-µm pore size) in a 1.7 cm diameter and 6.7 cm length copolyester housing. The filter was affixed to the effluent end of the discharge sample tubing and once a sufficient and quantified amount of groundwater passed through the filter, the filter was removed, packaged and shipped according to standardized procedures. The sterile filter cartridges were connected via leak-proof adapters to tubing. Male and Female adapter plugs were used to seal the inlet and outlet of each filter cartridge. The filter cartridges were designed for removing particles and microbial cells from large volumes of aqueous solutions, but for this study were used to collect biomass from groundwater for subsequent DNA extraction and *Dhc* biomarker gene quantification. These procedures as described in more detail in **Appendix C**.

5.6.2 Analyses

The analytical parameters required for this study include nucleic acid-based qPCR analyses, contaminants of concern (COCs), and geochemical parameters. The nucleic acid-based analyses include *Dhc* biomarker genes including the 16S rRNA gene and the RDase genes *tceA*, *bvcA*, and *vcrA*. The COCs for all sites are VOCs, including but not limited to chlorinated ethenes (e.g., TCE, *cis*-DCE, and VC). The MNA / geochemistry parameters include field measurements (conductivity, dissolved oxygen, oxidation-reduction potential, pH, temperature, alkalinity, carbon dioxide, ferrous iron, total iron, and hydrogen sulfide) and laboratory analyses for VOCs, selected ions, dissolved gases, sulfide, total organic carbon, total and dissolved iron, and volatile fatty acids.

The analyses of these parameters and associated field work were conducted by both the ESTCP project team and non-ESTCP collaborators. Samples collected for nucleic acid analysis were analyzed by the Löffler Lab at Georgia Institute of Technology. Our non-ESTCP collaborators (typically the site's main contractor) at individual demonstration sites were responsible for obtaining other parameters, which were typically obtained by commercial laboratories.

5.7 SAMPLING RESULTS

Data generated in this project include qPCR results for *Dhc* biomarker genes (i.e., the 16S rRNA gene and the RDase genes *tceA*, *bvcA*, and/or *vcrA*) and total bacterial 16S rRNA genes (based on qPCR with a general primer/probe set). These results were generated and/or compiled for every site included in this demonstration. Other data important in this analysis include contaminants of concern and dechlorination daughter products as well as geochemical data. Whenever these data were available and could be transformed into the database format, they were compiled in the project database. In the event that these data were only available in the original reports from other sources, the data are referenced in this report.

Data about the contaminants of concern (PCE, TCE), dechlorination daughter products (*cis*-DCE, VC, ethene, ethane), and geochemical data including dissolved oxygen, oxidation-reduction potential, carbon dioxide, total organic carbon, nitrate/nitrite, sulfate/sulfide, methane, and alkalinity were collected. These data have been retained in both tabular and graphic formats as well as in a project database. Based upon the interpretation of these data, a guidance protocol has been generated for Remedial Project Managers and field practitioners on the application of qPCR for the assessment of *Dhc* biomarker genes.

The data generated for this demonstration, the database and the resultant protocol are described in the following sections.

5.7.1 Sampling Results Generated for this Demonstration

Appendices B-1 through B-11 summarize all of the qPCR results generated for this demonstration. The tables illustrate the samples collected for each well of the sample period defined in Table 5-1, the sample preservation method, DNA concentration, the average *Dhc* 16S rRNA gene abundance per liter, and the associated standard deviations for each analysis for both Phase I and Phase II of this project.

5.7.1.1 MNA Sites

Operable Unit 1, Anniston Army Depot

Semi-annual sample results from 2004 to 2007 were tabulated and evaluated. The target genes, which had not been previously analyzed, were analyzed for all 379 samples collected during this period. qPCR analysis was conducted from preserved DNA by an environmental biotechnology company specializing in the development and application of cutting edge molecular biological tools (MBTs). Based upon the review of this analysis, contaminant data, and geochemical data, reductive dechlorination is occurring to various degrees in each of the four primary source areas of the SIA. The plume emanating from the Trench Area has evidence of TCE degradation but contains stable *cis*-DCE and very low VC, ethene, and ethane concentrations. These observations suggest a *cis*-DCE "stall" or "slowdown" and thus incomplete reductive dechlorination is occurring. The *Dhc* cell titers and RDase gene copy numbers are low and in nearly all wells, the titers were at or near the detection limit of 1×10^3 cells/L.

The plume originating from the Northeast Area and the plume from the Industrial Area are considered one integrated plume (**Appendix B-1**). In this plume, reductive dechlorination is

occurring. Similar to the Trench Area, there appears to be some evidence of *cis*-DCE "stall" or "slowdown" and thus incomplete reductive dechlorination in the source areas of the Industrial and Northeast Areas. Consistently, *Dhc* cell titers were low (less than 6.4×10^1) or below the detection limits. There is insufficient information to determine if the "stall" or "slowdown" is due to the microbial catalysts, the lack of bioavailable carbon/electron donor, or both. However, downgradient from the Industrial area there is some evidence of reductive dechlorination, at locations even where concentrations of TCE are much lower (less than 50 parts per billion).

Conversely, immediately to the south of the Trench Area, the weight-of-evidence (including the decrease in the size of the plume), elevated levels of daughter products (*cis*-DCE, VC, and ethene), and presence of elevated levels of the *Dhc* biomarker genes indicates that reductive dechlorination is evident in various locations in the Landfill Area. In areas where elevated amounts of organic carbon were measured, a *Dhc* community (e.g., at wells SWMU 1201 and 02TEWB01) of up to 2.6×10^7 cells/mL was detected as shown in **Appendix B-1.** The resultant ethene and ethane provide strong evidence that biological degradation processes are occurring in this area. It is not clear why there is more organic carbon at this site as compared to other sites, but a good correlation between organic carbon content and reductive dechlorination was established. This suggests that the "stall" at the other 3 sites at Anniston may be due to a lack of organic carbon/electron donor.

SWMU 21, NAS Dallas

Samples collected from this site for this effort were only for the purposes of evaluation of on site and off site biomass collection procedures (Phase I). Discussion of Phase I data is included in the Vandenberg results below.

5.7.1.2 Biostimulation Sites

MLP/VAB, NASA Cape Canaveral

Biostimulation injections were performed to address source area impacts, using ethyl lactate as an electron donor. Performance monitoring of source area groundwater was conducted by sampling wells in the source area every other month. Samples were analyzed for key geochemical, chemical, and biological parameters. Baseline geochemical analysis indicated that groundwater conditions were anaerobic, although sulfate concentrations were elevated (450 mg/L). Source zone concentrations of TCE in groundwater revealed a maximum of 50,000 µg/L (**Appendix B-3**). Biostimulation with ethyl lactate resulted in complete dechlorination of TCE to ethene. Some increases in *Dhc* cell titers were observed, but source area *Dhc* generally remained in the 10^6 to 10^8 cells/L range. Elevated sulfate concentrations remained relatively unchanged during EISB. Figures illustrating changes in chlorinated solvent concentrations, geochemical parameters, and *Dhc* biomarker data are provided in **Appendix B-3**.

Site 8 & 13-14, Vandenberg AFB

As discussed in Section 5.5.3, fifty nine (59) samples were collected from six chlorinated ethene-contaminated sites to compare on site (field) and off site (laboratory) groundwater filtration and

biomass collection approaches for DNA extraction and qPCR analysis. To determine the percent recovery from the sterile filter cartridge membranes as compared to the vacuum filtration on membrane filters, the average recovery from the sterile filter cartridge membranes was divided by the average value obtained from the vacuum filtration on membrane filters. The standard deviation of the percent recovery was calculated using the standard error propagation method. These results are tabulated in **Appendix B-4** for sites 8 and 13/14. This data showed that wells sampled at Vandenberg AFB contained up to $8.5 \pm 2.3 \times 10^{11}$, $1.8 \pm 4.6 \times 10^{6}$, $2.6 \pm 2.8 \times 10^{7}$ and $5.3 \pm 1.0 \times 10^{6}$ copies per liter of bacterial 16S rRNA, *Dhc* 16S rRNA, *vcrA* and *tceA* gene copies, respectively.

AOC A, NSA Mid-South

Analytical results from over 140 samples from more than 50 wells are tabulated in **Appendix B-5** for each of the four plumes at AOC A. These data show that wells sampled over the 2 year sampling period (2007-2009) contained up to 2.03×10^{7}, 3.52×10^{5}, 5.09×10^{5}, and 1.02×10^{5} copies per liter of bacterial 16S rRNA, *Dhc* 16S rRNA, *bvcA* and *tceA* gene copies, respectively. The highest *Dhc* 16S rRNA, *bvcA* and *tceA* gene copy numbers were detected in wells PESGMW4S05, 007G59LFBJ, and 007G66LF02, respectively. The wells with the highest contaminant concentrations are shown in **Appendix B-5**.

Several wells exhibit sharp reductions of TCE and contaminant increase in VC concentrations (e.g., PESGMW2S and PESGMW4S). Unfortunately, the qPCR data were not obtained during the period when the sharp TCE concentration reductions occurred in 2005. More recent qPCR analysis determined 10^{5} *Dhc* 16S rRNA gene copies per liter, suggesting a moderate *Dhc* population exists; however, VC dechlorination to ethene did not occur at appreciable rates. In other wells (e.g., 007G57LF, 007G64LF, and PESGMW7D) the 2008 and 2009 *Dhc* 16S rRNA gene copy numbers were less than 10^{4} per liter indicating moderate or sub optimal gene copies sufficient for high rates of degradation. TCE was the main contaminant in these wells. In several wells, VC concentrations sustained high levels (i.e., accumulated) or increased with respect to TCE (e.g., TCE detected at 5 ug/L and VC detected at 460 ug/L at PESMW7D) (**Appendix B-5)**. The geochemical data suggest that in some of these cases sufficient levels of organic carbon (greater than 10 mg/L) and moderate reducing conditions (-200mv) were present (e.g., PESMW4S), however, in other cases (e.g., PESMW7D) insufficient organic carbon (less than 2 mg/L) and aerobic conditions were present. Unfortunately, considering this variability and without *vcrA* and *bvcA* results (analysis was not completed) it is not evident why VC stall has occurred at this site.

5.7.1.3 Bioaugmentation Sites

Site 59, NAS Cecil Field

These results from over 50 samples are tabulated in **Appendix B-6** for the Site 59 biostimulation and bioaugmentation project at NAS Cecil Field. These data exhibit the sample results taken over a 2 year period from 2006 to 2008. NAS Cecil Site 59 was biostimulated with sodium lactate and buffered by adding sodium bicarbonate beginning in late August 2006, bioaugmented with the KB-1 consortium in October 2010. Following biostimulation/augmentation treatment,

the site is now in MNA mode. The data collected from this site suggest that *Dhc* are maintained at 10^5 to 10^7 16S rRNA gene copies per liter after the cessation of active treatment, suggesting that active *Dhc* populations persist and that biostimulation/augmentation generated a lasting effect at this site (**Appendix B-6)**.

Based on a review of the graphical display of the data (**Appendix B-6**), it is apparent that the total bacterial 16S rRNA genes have moderately decreased over time after the electron donor injection, the *Dhc* 16S rRNA gene copies and *vcrA* gene copies both followed similar trends over time in wells MW-1A, IW-2A and NG-12D followed by a decrease over time in all wells. The *bvcA* gene was detected at high abundances is well MW1A, but declined to undetected levels over the sampling period in the other wells. While *tceA* gene copies initially increased in wells MW-1A and IW-2A, they remained an order of magnitude less than *vcrA gene copies*. In well NG-12D, *tceA* was not detected at the final sampling in July 2008. Over the sample period TCE, DCE, and VC concentrations measurements (**Appendix B-6**) are consistent with the qPCR analysis, and *tceA* and *vcrA* gene copy numbers were highest at elevated TCE concentrations whereas elevated *vcrA* gene copy numbers were measured throughout *cis*-DCE and VC dechlorination.

MAG-1 Area, Fort Dix

Figures C-1 through C-14 from Shaw (2010) and ER-0515 (**Appendix B-7)** provide chlorinated ethene and ethene concentration trend graphs for demonstration area wells. TCE concentrations in transect performance monitoring wells BMW-1 through BMW-6 in Loops 1 through 3 (test loops) declined significantly during the demonstration. TCE contaminant mass decreased 90 percent or more and in many wells TCE was no longer detected (i.e., TCE was below the detection limit of 5 µg/L). As shown on Figures C-1 through C-6 (**Appendix B-7**), with the exception of well BMW-5, these declines primarily occurred after the second bioaugmentation.

Appendix B-7 illustrates the data of two bioaugmentation treatments. There were several differences between the first and the second injection including the pH moderation and stabilization and the increase *Dhc* cells injected. The *Dhc* cell titer of the inoculum injected first was 2.17×10^{10} cells/liter and 100 liters, 10 liters, and 1 liter of bioaugmentation culture were injected in wells IW-1, IW-2 and IW-3, respectively. The *Dhc* cell titers of the injected culture in the second injection round was measured at 1.45×10^{12} cells/liter (approximately 2 orders of magnitude higher than the first injected culture). The same volumes were injected into the same injection wells (Shaw 2010).

Bacteria injected during the first bioaugmentation (injection wells IW-1, IW-2 and IW-3) did not appear to acclimate and the practitioners (Shaw, 2010) concluded that the inoculation failed, or rendered ineffective, by a high pH spike in the injection wells (pH values >10) (**Appendix B-7**). *Dhc* cell titers in groundwater increased immediately by approximately 6 orders of magnitude culture injection in wells BMW-1, BMW-3 and BMW-5, and 3 orders of magnitude increased *Dhc* cell titers were observed in the downgradient monitoring wells.

As shown in **Appendix B-7,** subsequent decreases in TCE and VC concentrations, along with ethene generation, were consistently detected. Vinyl chloride and ethene were observed when *Dhc* cell titers in groundwater reached approximately 1.0 x 10^7 per liter, or greater. Unlike the relatively consistent *Dhc* 16S rRNA gene copy numbers observed after the first injection, the *Dhc* cell titers after the second injection decreased somewhat over time but remained at elevated levels between 10^5 and 10^7 cells/liter (**Appendix B-7**). In some wells the total bacterial 16S rRNA gene copies increased to very high numbers (between 10^{10} and 10^{11} gene copies/liter) during (May and June 2008) and remained high for subsequent sample rounds in well BMW-4 (July and September 2008). After an initial burst in bacterial growth, the abundance of total 16S rRNA genes declined to 10^8 to 10^9 copies/L.

OU 24 NAS North Island

Post-injection quarterly monitoring was conducted to characterize geochemical, chemical, and microbiological changes. In general, the geochemical conditions at the site trended toward strongly reducing. Concurrent with this redox shift, DOC concentration increased, sulfate concentrations decreased in some source monitoring wells, and in several samples *vcrA* and *bvcA* gene copies increased. As discussed in Section 5.2.8, although average total VOC concentrations decreased in the source area, the absence of a comparable increase in ethene concentrations suggests that this decrease may have been related to the intrinsic variability of total VOC concentrations. Along the centerline of the plume, VC concentrations were elevated (greater than 1,000 µg/L) but soon after the start of the remedial treatment the resultant concentrations decreased to less than 500 µg/L. However, due to low TCE concentrations (maximum concentration of 2.5 µg/L) limited correlations between TCE degradation to VC and VC to ethene could be performed using the available data for this site. Sampling results are shown in **Appendix B-8**.

Plume B, Bachman Road

Post-injection monitoring has been performed, utilizing multi-level sampling wells positioned in the source and three downgradient transect areas. Samples were collected for geochemical, chemical, and microbiological analyses on a quarterly basis. Geochemical data indicated reducing conditions. Following injections of electron donor (i.e., EVO) sulfate concentrations decreased and the redox potential was below -100 mV. PCE concentrations in the source area were reduced from 120,000 µg/L to non-detect levels in the source area, with transient production of *cis*-DCE and complete dechlorination to ethene. *Dehalobacter* cell titers increased to 10^7 cells/L in the source area, while *Dhc* cell titers were variable. *Dhc* cell titers increased to 10^6 cells/L downgradient of the source area. In the downgradient biobarrier, initial PCE concentrations were reduced from 7,500 µg/L to ND levels, with variable concentrations of PCE dechlorination products up to 100 µg/L. Ethene was produced, and *Dhc* cell titers reached 10^8 cells/L. Sampling results are shown in **Appendix B-9**.

Milledgeville

Groundwater sampling was performed on a routine basis from monitoring wells and recovery wells after startup of the system and analyzed for various geochemical, chemical, and

microbiological parameters. Following bioaugmentation, TCE concentrations decreased from 12,000 µg/L to non-detect levels with transient production of *cis*-DCE and VC. Methane and ethane production were also noted concurrent with TCE reduction and increases in daughter product concentrations. *Dhc* 16S rRNA gene and *vcrA* gene titers each increased from 10^3 to 10^8 copies/L. Sampling results are shown in **Appendix B-10**.

Former NAWC Trenton

Following bioaugmentation injections, groundwater monitoring events were conducted at regular intervals to assess technology performance. Groundwater samples were analyzed for various geochemical, chemical, and microbiological parameters. Geochemical parameters routinely indicated reducing conditions in the treatment area. Analytical data showed TCE concentrations have been reduced from 15,800 µg/L to non-detect levels. Following an intermediate period of *cis*-DCE and VC concentration increases, these constituents were then reduced to non-detect levels with concomitant increases in *Dhc* cell titers over 10^8 cells/L. Complete dechlorination to ethene was observed as well as methane production. pH values remained at circumneutral in the treatment area wells. Additional site sampling data are included in **Appendix B-11**.

5.7.2 Database Development

5.7.2.1 Database Development

A central database was developed containing groundwater monitoring, geochemical and microbiological data. The database was designed to allow queries that support the development of correlations between field-observed dechlorination activities, geochemistry, and the presence and abundance of *Dhc* biomarker genes. The database contains a collection of historical data and current data obtained during this study for many of the sites included in this demonstration. Information generated by the ESTCP project team including qPCR data were included in the database.

To the extent possible, database-compatible data available from collaborations with the host site owners and site contractors were included. In the event that these data were only available in the original reports from other sources (**Table 5-1**), these data are included in this report by reference.

Tetra Tech Inc. maintained this central database for the Navy. The database used formats mandated by the NAVFAC and managed currently by Tetra Tech for NAVFAC Southeast. Data from NAS Cecil Field were included via courtesy of NAVFAC Southeast and Tetra Tech. Data from Anniston Army Depot were provided via courtesy of Tetra Tech, USACE Mobile District, and Microbial Insights. Data from NAS North Island were provided via courtesy of NAVFAC SW.

A relational database management and analysis system was utilized for data integration, analysis, and reporting. The database structure was defined to facilitate the compilation of existing site data and new data collected for this project. Laboratory Electronic Data Deliverables (EDDs) or data "entry" conversion submittals adhered to this structure when available. The Relational Database Management System (RDMS) conformed to the Tetra Tech

Information Management System (TIMS) architecture. The database model was modified to accommodate the qPCR data sets. All associated data management formats (including historical, existing site data, as well as third party contractor submissions) were held to the standard of the TIMS database management system. Historical data was also uploaded, and to the extent practical, adhered to these standards.

The TIMS database structure and associated valid value tables, field requirements, data triggers, and primary key constraints were developed to ensure maintenance of data retention efficiency and consistency within the RDMS. The database was preserved with nightly server backups. Data integrity, availability and security were maintained with default settings and password policy enforcement. Data Transformation Services (DTS) were used as the mechanism for efficient transfer of both existing databases, conversion of historical or legacy data, and third party site data.

Lab data involved transposition of electronic data conversion as related to Georgia Institute of Technology and other contractor submissions. Similarly, data "mapping" of data sets were conducted using the DTS. Utilization of the DTS accommodated data mapping and conversion of various source file formats including tab delimited .txt, .csv, or excel files from multiple entities and contractors, which minimized contractor efforts and simplified data preparation.

The SQL Server database was made available to users through Open Database Connectivity (ODBC). A data review process was conducted and included a series of automated quality control procedures implemented during the data formatting process. These quality control procedures ensured relational integrity and accessibility via a web browser.

5.7.2.2 Database Usage

As described above the database is Web accessible. Access to this database can be conducted by following the steps below.

- Go to http://www.ttnus.com/imrg/links.asp
- Click ESTCP TIMS access from Database Links
- The username is estcp and the password is estcp1
- Click Query Data from the Database Access menu on the left side of the page
- Using the drop done menu select the ESTCP database.

5.7.2.3 Database Results

The developed database provided a platform for determining the correlations that provided the basis for evaluating the outcomes of this demonstration. Various queries facilitated data analysis and allowed for the establishment of correlations between site geochemical data, contaminant concentrations and microbiological data. The database was also utilized to aid in determining the primary quantitative criteria for the demonstration. In addition, the database was used to compare and contrast data from various sites. Statistical comparisons such as elementary statistics (e.g., minimum, maximum, mean, range, etc.), data trend analyses, and nonparametric correlations (e.g., Spearman correlation) were made amongst the many sites evaluated to determine general conclusions based upon the data.

Historical data from Anniston Army Depot OU1 (2005-2009), Bachman Road (2005 and 2006), NAS North Island OU24 (2007 and 2008), NAS Cecil Field Site 59 (2004-2008), and Vandenberg AFB (2007 and 2008) are included in the database. As described in Section 5.7 the database contains concentration data for contaminants of concern (e.g., PCE, TCE, DCEs, VC), dechlorination products (DCEs, VC, ethane), and related gases (ethane and methane), geochemical data (listed in Section 5.7) and the qPCR analytical results. The qPCR, contaminants of concern and geochemistry data trends are described in the sections above and in Section 6.

5.7.3 Protocol Development

Based upon the results of this effort, selection criteria were derived to assist in the selection and application of MNA, biostimulation, and/or bioaugmentation at chlorinated solvents sites. Such guidance, developed during the course of the project, is documented under a separate document titled "Guidance Protocol: Application of Nucleic Acid-based Tools for Monitoring MNA, Biostimulation or Bioaugmentation at Chlorinated Solvent Sites".

The purpose of the protocol is to provide guidance to Remedial Project Managers (RPMs) and field practitioners on the application of MBTs, specifically, nucleic-acid based tools for evaluating MNA, biostimulation and bioaugmentation at chlorinated solvent sites. This protocol summarizes the current state of the practice of these tools and is intended to provide a technically sound and practical approach for using these tools. This guidance document provides recommendations regarding sampling approaches and data evaluation criteria for use in remedial decision-making.

The protocol includes background information to provide RPMs with basic understanding of the reductive dechlorination process and the bacteria of interest. A description of qPCR analysis and guidance with data interpretation is included. Most importantly, MBT application to MNA evaluation and decision-making on bioaugmentation are presented in flowcharts. SOPs for groundwater sampling are presented.

6. PERFORMANCE ASSESSMENT

This section provides a summary of data analysis in support of the assessment of performance objectives. The performance criteria were categorized into qualitative and quantitative criteria as shown in **Table 3-1.** These criteria constitute the performance objectives of this demonstration, which were developed from the criteria listed in **Table 3-1**, and have been linked to the objectives of the demonstration defined in Section 1.2. Quantitative metrics have a numerical value or precise determination. Conversely, the qualitative metrics do not have a numerically or otherwise precise result (e.g., a positive correlation of target genes with a dominant TEAP).

The success of the technology demonstration has been evaluated using the performance confirmation methods presented in **Table 3-1**. This evaluation included assessment with qPCR, VOC analysis, and geochemical analyses to provide data for determining the success of the demonstration. As discussed in Section 5.7.2.3, statistical comparisons were calculated using data from the demonstration sites (statistical correlation data are presented in **Appendix D**).
In addition, the Spearman's rank correlation coefficient (a measure of statistical dependence between two variables) was calculated for the data from the following sites: Anniston Army Depot OU1, NASA Cape Canaveral, NAS North Island OU24, Milledgeville, and NAWC Trenton sites. The Spearman correlation is a non-parametric correlation, which was used since the distributions of the data were unknown. If the data for the sites referenced above contained more than six data pairs, an evaluation of whether a statistical correlation existed was performed, but if there were less than six data pairs, only a general evaluation of the correlation could be performed. The general evaluation for the Spearman correlation is based on the following:

- less than or equal to |0.33| indicates a low correlation;
- between |0.34| and |0.66| indicates a medium correlation; and
- greater than or equal to |0.67| indicates a strong correlation.

The results of the statistical analyses have been incorporated in the performance assessment discussions below, as appropriate. Performance assessment results are described in the following subsections; a subsection is provided for each demonstration performance criterion.

6.1 VALIDATION OF RDase TARGET GENES

Correlations of *Dhc* RDase biomarker genes (e.g., *tceA*, *bvcA*, and/or *vcrA*) with daughter product to parent compound concentration ratios (e.g., [*cis*-DCE, VC]/TCE; [VC, ethene]/*cis*-DCE) and combined VOC concentrations (e.g., TCE, *cis*-DCE, VC) were used to evaluate the predictive use of qPCR data on in-situ reductive dechlorination performance. The confirmation metric for this performance objective was the achievement of a Spearman correlation of greater than |0.33|.

MBT data for the sites are presented in Tables 5-2 through 5-12. Correlations between dechlorination product ratios and *tceA* and *bvcA* gene abundances were evaluated in data sets collected from the Milledgeville site, and correlations with the *vcrA* gene were evaluated in data sets collected from NAS North Island OU24, NASA Cape Canaveral, and the Milledgeville site. A summary of the *Dhc* RDase gene evaluations is provided below.

The Spearman correlation between the *tceA* gene and the daughter to parent compound concentration ratio of (*cis*-DCE, VC)/TCE was weak (less than or equal to |0.33|) for the Milledgeville data. Similarly, the Spearman correlation between the *tceA* gene and the individual contaminant concentrations (i.e., TCE and *cis*-DCE) was weak (less than or equal to |0.33|) for Milledgeville site data sets.

The Spearman correlation was greater than |0.33| for *vcrA* to the daughter to parent compound concentration ratio of (VC, ethene)/TCE for NAS North Island, NASA Cape Canaveral and the Milledgeville site. A statistical correlation coefficient (r) greater than $r_{critical}$ was found between the ratio of *vcrA* to the daughter to parent compound concentration ratio of (VC, ethene)/TCE for the data from the NASA Cape Canaveral site. No correlation was observed between *bvcA* and the daughter to parent compound concentration ratio of (VC, ethene)/*cis*-DCE.

For the Milledgeville site data, a Spearman correlation greater than |0.33| was observed between *bvcA* and VC, and a statistical correlation (r greater than $r_{critical}$) was obtained for this correlation. A weak correlation was observed between *bvcA* and *cis*-DCE for the Milledgeville site data. A Spearman correlation of greater than |0.33| was obtained between *vcrA* and *cis*-DCE and VC for NAS North Island OU24, NASA Cape Canaveral, and the Milledgeville site. A statistical correlation (r greater than $r_{critical}$) was obtained between *vcrA* and *cis*-DCE for the Milledgeville site data.

These results suggest that correlations between the *Dhc* RDase genes and ratios of dechlorination product/parent compound and/or the individual contaminant concentrations are inconsistent between sites. Therefore, the selection of an appropriate suite of functional gene target(s) will be governed by site-specific conditions and data objectives.

The *Dhc* 16S rRNA gene, and the *tceA*, *bvcA* and *vcrA* genes were included in the analyses of samples from most sites. With the expected identification of additional biomarker genes for the reductive dechlorination process, the analysis of select biomarker genes that provide the key information for the contaminants of interest at a given site should be envisioned because the analysis of all possible biomarker genes may not yield additional information for decision-making.

6.2 IDENTIFICATION OF MINIMUM NUMBER OF *Dhc* TARGET GENE COPIES

An assessment of *Dhc* 16S rRNA gene and RDase target gene copies was conducted to establish minimum abundances in support of complete reductive dechlorination (e.g., ethene formation). In addition to data compiled from the study sites, information in support of this performance objective was gathered from bioremediation efforts at several additional sites, which was available to the project team.

Dhc 16S rRNA gene and/or RDase gene targets below 10^4 to 10^5 gene copies per liter have typically been associated with sub-optimal conditions to support and sustain effective reductive dechlorination rates and detoxification (Dennis 2010, personal communication and Ritalahti et al., 2010). At sites where *Dhc* 16S rRNA and/or RDase gene targets have been detected at greater than 10^6 to 10^7 gene copies per liter, appreciable dechlorination rates and ethene formation have been reported (this study; Lu, 2006). However, the presence of a certain

abundance of *Dhc* 16S rRNA gene and/or RDase gene targets is not necessarily an indicator of complete reductive dechlorination. A study by van der Zaan et al. (2009) showed that the presence of VC RDase genes did not always relate to VC dechlorination, but an order of magnitude or more increase above baseline values in VC RDase gene abundance in response to treatment (e.g., biostimulation) correlated well with VC dechlorination activity.

Following biostimulation and/or bioaugmentation, *Dhc* 16S rRNA gene targets were detected at or above 10^7 gene copies per liter, and ethene production was noted at the Milledgeville, NASA MLP/VAB, and NAWC Trenton study sites. Samples collected as part of this study were grouped into four categories by *Dhc* cell abundances: greater than 10^6 cells/L, 10^3 to 10^6 cells/L, detected but not quantifiable (DNQ), and not detected (ND) (**Figure 6-1**). All sites had wells with *Dhc* abundances in the 10^3 to 10^6 cells/L range, and three sites each had wells with *Dhc* $>10^6$ cells/L, DNQ and ND. Among the 25 wells where ethene was detected at concentrations up to 75 ppb, 21 had detectable or quantifiable *Dhc*. Six wells had $>10^6$ *Dhc* cells/L, but two of them tested negative for the known VC RDase genes *bvcA* and *vcrA* suggesting that other as yet unidentified genes encode VC RDases. The known *Dhc* are strict organohalide respiring bacteria and presumably strains carrying *vcrA* or *bvcA* are responsible for VC reductive dechlorination to ethene. High abundances of *Dhc* 16S rRNA genes significantly exceeding the number of *Dhc* cells carrying *bvcA* and *vcrA* at sites producing ethene suggest that the unknown VC RDase genes are encoded on *Dhc* genomes. In two wells, *Dhc* 16S rRNA and all three *Dhc* RDase biomarker genes were present at titers exceeding 10^6 cells/L but no ethene was detected. In one of these wells, total chlorinated ethene concentrations were in the low ppb range and ethene concentrations may have been too low for detection. At the other well, temporal concentration measurements suggested polychlorinated ethene reductive dechlorination progressed and VC was consumed but no ethene was detected.

Detoxification of VC without measureable ethene has been reported (Bradley and Chapelle, 2000). A general correlation has been found between the presence of *Dhc* and ethene generation (Hendrickson et al. 2002; Major et al., 2002; Imfeld et al., 2008; Abe et al, 2009; van der Zaan et al., 2009). Frequently, ethene formation serves as a benchmark for successful reductive dechlorination (i.e., detoxification), but recent observations suggest that the lack of ethene formation should be interpreted cautiously because implementation of the anaerobic reductive dechlorination process can achieve cleanup goals without measureable ethene.

Several processes including anaerobic VC and/or ethene oxidation may explain detoxification without ethene formation, and alternative degradation pathways should be explored (Bradley and Chapelle 2000, Gossett 2010). Ethene was observed in just one third of the wells (11 out of 32) with *Dhc* abundances between 10^3 and 10^6 cells/L. Only three of the 11 ethene-producing wells had detectable *tceA*, one had *bvcA*, and *vcrA* was absent, supporting the notion that additional *Dhc* VC RDases exist. Higher ethene concentrations correlated with higher *Dhc* cell titers. The minimum number of *Dhc* cells that predict ethene production is 10^6 cells/L. Supporting this conclusion are the results of a recent study that compared 24 wells at six sites and found that active dechlorination of DCEs and VC occurred with $>10^7$ *Dhc* cells cells/L (Lu et al., 2006). In wells with $<10^4$ or DNQ *Dhc* cells L^{-1}, ethene concentrations were below 2 ppb (6 out of 7 wells) or ethene was not detected at all (18 out of 18 wells). In 11 of the 59 wells evaluated, *Dhc* were not detected, and in seven of those ethene was not detected; however, in four of the wells, ethene was observed in low concentrations (<2 ppb) even though none of the known *Dhc* RDase

biomarker genes were present, and the contaminants, PCE, TCE and *cis*-DCE, were not being reduced to VC.

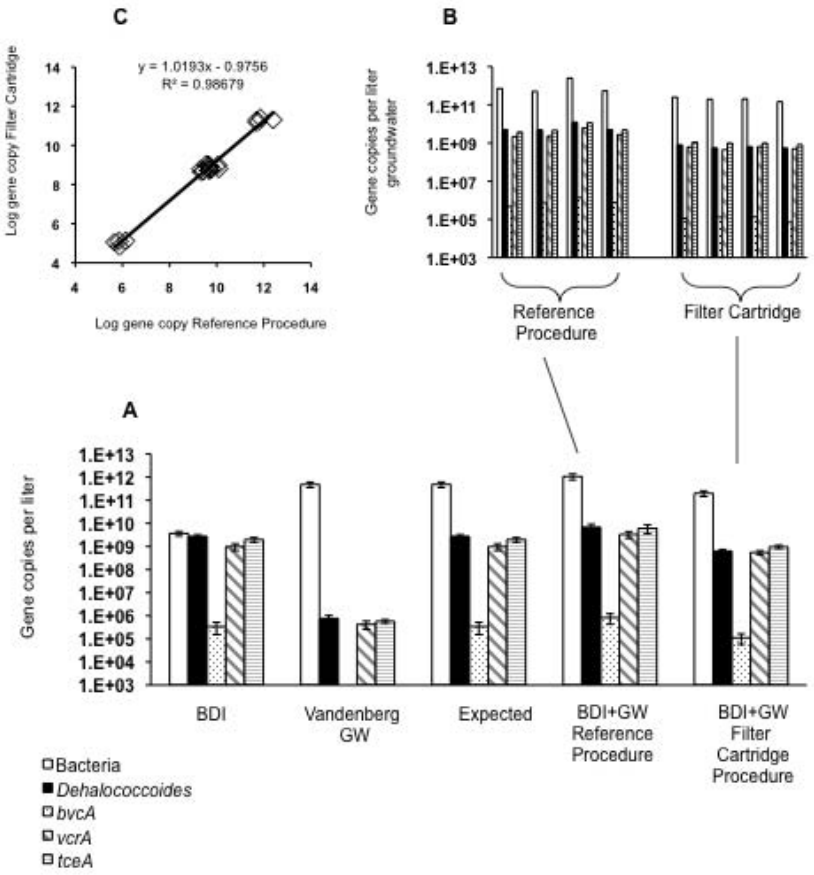


Figure 6.1. The reference procedure using vacuum collection compared with peristaltic pump collection to filter cartridges with 0.2 μm membranes using the same simulated microbial community and the same DNA extraction protocol. Shown in the main (A) graph are total bacterial and *Dehalococcoides* 16S rRNA gene abundances and those of three RDase genes as detrmined using qPCR. The "expected" gene abundance was calculated by adding together the BDI and the Vandenberg GW abundances. Panel B shows the reproducibility of the qPCR results obtained for each replicate DNA extraction. The log gene copies obtained using reference procedure and filter cartridges were linearly correlated, panel C (top left).

Table 6-1 shows ranges of observed *Dhc* cell titers and their associated activity. These results may be used by practitioners as rules of thumb when interpreting *Dhc* data at chlorinated solvent sites.

Table 6-1. Observed *Dhc* and associated dechlorination activity

***Dehalococcoides* 16S rRNA gene copies per L**	**Interpretation**
$<10^4$	Low *Dhc*, efficient dechlorination and ethene production unlikely
$10^4 – 10^6$	Moderate *Dhc*, which may or may not be associated with observable dechlorination and ethene formation
$>10^6$	High *Dhc*, which is often associated with high rates of dechlorination and ethene production

6.3 CORRELATION OF *Dhc* TARGET GENE COPY NUMBERS WITH CONTAMINANT DECHLORINATION RATES

Correlations of average *Dhc* 16S rRNA gene copy numbers and *vcrA* gene copy numbers with TCE, *cis*-DCE or VC dechlorination rates were used to evaluate the predictive use of qPCR data on reductive dechlorination. The performance metric for this performance objective was the achievement of a positive Spearman correlation of greater than |0.33|.

The calculation of dechlorination rates was only performed for the NASA Cape Canaveral site, since this site had data from multiple monitoring wells that were collected frequently (bi-monthly) over several years. Dechlorination rates were calculated assuming first-order reaction kinetics and were evaluated for TCE, *cis*-DCE and VC, utilizing data from three monitoring wells at the site. Since rates could only be calculated from three monitoring wells, the data set contained less than six data points, and thorough statistical analyses were not possible.

Spearman correlations between *Dhc* 16S rRNA gene copies and *vcrA* RDase gene copies with TCE, *cis*-DCE and VC dechlorination rates were found to be greater than |0.33|. The Spearman correlation between the *Dhc* 16S rRNA gene copies and TCE, *cis*-DCE and VC dechlorination rates were all strong (greater than |0.67|), while the Spearman correlation between the *vcrA* gene copies and TCE, *cis*-DCE and VC dechlorination rates were all medium (between |0.34| and |0.66|.

These results suggest a correlation between the 16S rRNA gene copies, the *vcrA* gene copies and the observed dechlorination rates; however, only three wells were included in the analysis, which precluded a robust statistical testing. Further evaluation of the correlation between *Dhc* 16S rRNA gene copies and individual RDase (e.g., *vcrA*) gene copies with dechlorination rates is recommended to establish a metric to evaluate reductive dechlorination.

6.4 CORRELATION OF CONTAMINANT CONCENTRATIONS ON *Dhc* POPULATION SIZE

Correlations of *Dhc* 16S rRNA gene copy abundances with TCE, *cis*-DCE, or VC concentrations and with daughter to parent compound (e.g., [*cis*-DCE, VC]/TCE; [VC, ethene]/*cis*-DCE) concentration ratios were used to evaluate the predictive use of qPCR data on reductive dechlorination activity. The metric used for this performance objective were positive Spearman correlation coefficients of greater than |0.33|.

Spearman correlations between the *Dhc* 16S rRNA gene copy number and a daughter to parent compound concentration ratio of (*cis*-DCE, VC)/TCE were found to be greater than |0.33| for the Anniston and NASA Cape Canaveral sites. This correlation was either not performed or resulted in a weak correlation for the NAS North Island OU24, NAWC Trenton and Milledgeville sites, which was probably due to low concentrations of TCE present at these sites.

Spearman correlations between the *Dhc* 16S rRNA gene copy number and the daughter to parent compound concentration ratio of (VC, ethene)/*cis*-DCE were found to be greater than |0.33| for all sites evaluated. A statistical correlation (r greater than $r_{critical}$) was observed between the *Dhc* 16S rRNA gene copy number and the daughter to parent compound concentration ratio of (VC, ethene)/*cis*-DCE for the data from NAWC Trenton site.

Spearman correlations between the *Dhc* 16S rRNA gene copy number and contaminant concentrations (e.g., TCE, *cis*-DCE, or VC) greater than |0.33| were observed for all sites. A statistical correlation was observed between the *Dhc* 16S rRNA gene and TCE and *cis*-DCE concentrations for data from the NAWC Trenton site.

These results suggest that there is no correlation between the *Dhc* 16S rRNA gene abundance and the contaminant concentrations or the daughter to parent compound (e.g., [*cis*-DCE, VC]/TCE; [VC, ethene]/*cis*-DCE) concentration ratios. The limitation of the Spearman correlation analysis was the low number of data sets included in the analysis; however, further evaluation with additional data sets is warranted. Data should be obtained from a larger number of suitable sites to establish or reject correlations between *Dhc* 16S rRNA gene abundance data, contaminant concentrations and the daughter to parent compound concentration ratios as measures for reductive dechlorination performance. Several environmental biotechnology companies specializing in the development and application of molecular biological tools (MBTs) have compiled larger data sets from their customers' sites. Such data sets could be evaluated using the Spearman approach to corroborate correlations between *Dhc* biomarker gene abundances and dechlorination performance.

6.5 INFLUENCE OF TEAP ON *Dhc* ABUNDANCE

A qualitative evaluation of groundwater geochemistry and its influence on *Dhc* biomarker gene abundances was conducted. Biodegradation of chemical groundwater constituents have been associated with particular geochemical conditions. For example, PCE and TCE are resistant to metabolic degradation under aerobic conditions but can be reductively dechlorinated stepwise to less chlorinated ethenes under reducing conditions in the absence of oxygen. DCEs and VC can be reductively dechlorinated to ethene, and sometimes to ethane, by anaerobic microorganisms,

or they can be mineralized to carbon dioxide and inorganic chloride under aerobic conditions (Coleman et al., 2002; Singh et al., 2004; Gossett, 2010).

The findings of recent studies suggested that *Dhc* strains containing *tceA* are more tolerant of oxidizing conditions, whereas *Dhc* strains containing *vcrA* or *bvcA* are more susceptible to redox fluctuations (van der Zaan et al., 2009, Amos et al., 2008; Fletcher et al., 2011). Studies by van der Zaan et al. (2009) showed a strong negative correlation between the abundance of *Dhc* 16S rRNA genes and the *vcrA* gene to increasing sulfate concentrations, but found a positive correlation between *Dhc* 16S rRNA gene and *vcrA* gene abundances to high methane concentrations. Apparently, sulfate, or the reduced product sulfide, does not favor VC-dechlorinating *Dhc* populations whereas methanogenic conditions support VC reduction.

Field and analytical data collected for this demonstration support the findings of these investigations, and indicate that lower redox conditions representative re generally favorable for reductive dechlorination of chlorinated ethenes. For example, following biostimulation and bioaugmentation at the Bachman Road demonstration site, increases in *Dhc* biomarker gene copies were noted concurrent with reductions in TCE and sulfate concentrations as well as increases in dissolved methane concentrations.

6.6 IDENTIFICATION OF FALSE POSITIVES/NEGATIVE qPCR DATA

A qualitative assessment of false positives and false negative detections of *Dhc* biomarker genes was conducted to evaluate potential impacts of MBT data on the decision making process.

A comparison of different membrane filter materials and DNA extraction methods showed that false negative results can be reduced through consistent and appropriate sample handling and adherence to SOPs. Adopting an *on-site* filtration approach combined with DNA extraction with a commercially available DNA extracting kit reduced false negative results (Ritalahti et al. 2010). A key issue is the volume of groundwater collected for biomass collection. As a rule of thumb, reproducible results were obtained when volumes containing $>10^4$ total *Dhc* target gene copies were collected. The careful design and thorough testing of qPCR parameters and the application of a TaqMan approach (rather than SYBR Green chemistry for monitoring target gene amplification) eliminated false negative results. It is important to note that different qPCR protocols can yield accurate data, but it is crucial that each analytical laboratory establishes rigorous SOPs to avoid false positive and false negative qPCR results.

6.7 IMPLEMENTABILITY OF GROUNDWATER SAMPLING AND BIOMASS COLLECTION

Any MBT analysis can only be as good and representative as the groundwater sample collected from the site. A prerequisite for meaningful MBT data interpretation is the application of a standardized groundwater sampling procedure that captures *Dhc* biomarker genes, protects these biomarkers during sample handling, transport and storage, and allows quantitative extraction of the biomarkers of interest. Contemporary procedures to quantify *Dhc* biomarker genes use a peristaltic pump to collect site groundwater in 1-L glass bottles, which are shipped with overnight carrier to the analytical laboratory. Biomass is then collected on membrane filters by vacuum filtration for subsequent DNA extraction with commercial purification kits. This

standard procedure suffers from a number of issues, including the formation of iron oxide precipitates that impede the biomass collection process due to membrane fouling, or interfere with subsequent DNA extraction procedures and qPCR protocols. Further, the delivery of large volumes may require groundwater storage for days or weeks at 4°C with unknown consequences for biomarker quantification. Sample loss and cross-contamination through leakage or breakage are always of concern when shipping large volumes of groundwater. Shipping large volumes of groundwater and disposal of contaminated groundwater in the analytical laboratory add to the overall costs. To avoid such problems, a straightforward on-site biomass collection approach using sterile filter cartridges was compared to the contemporary procedures using vacuum filtration in the analytical laboratory for biomass collection.

In initial laboratory studies with groundwater amended with known amounts of *Dhc* target cells, the sterile filter cartridges yielded one third of the total DNA and 9-18% of the total *Dhc* biomarker gene copies compared with vacuum filtration. Subsequent method optimization increased DNA yields to 94 ± 38% of those obtained with the vacuum filtration method. A comparative analysis of *on-site* and *off-site* biomass collection procedures, performed with groundwater from 59 wells at nine chlorinated ethene-contaminated sites, corroborated the applicability of the sterile filter cartridge for *Dhc* biomarker quantification in groundwater. *On-site* biomass collection with sterile filter cartridges avoids problems associated with shipping groundwater and has broad applicability for biomarker monitoring in aqueous samples. From most wells included in this demonstration, sterile filter cartridges and groundwater for off-site (i.e., in the analytical laboratory) biomass collection were available for direct comparison of on-site and off-site procedures.

To provide additional evidence that the sterile filter cartridges have advantages over the traditional methodology, two defined laboratory experiments were conducted with the PCE-to-ethene-dechlorinating consortia BDI and KB-1. In separate experiments, groundwater, which did not contain *Dhc* biomarkers, and artificial groundwater samples were augmented with defined amounts of consortium BDI and consortium KB-1, respectively. In the laboratory, the biomass was collected from triplicate, augmented groundwater samples. The data corroborated the observations with the field samples, and it was concluded that the on-site filter cartridge filtration approach is a viable and superior alternative for groundwater sampling and biomass collection for subsequent qPCR analysis (Ritalahti et al. 2010).

The detailed findings of the method development and application of the on-site biomass collection approach using commercial sterile filter cartridges have been published in the peer-reviewed literature (Ritalahti et al. 2009, Ritalahti et al. 2010, Petrovskis et al. 2011).

6.8 ANALYTICAL SENSITIVITY

The sensitivity of the PCR method for quantification of *Dhc* biomarker genes was evaluated. Like all analytical procedures, qPCR has a detection limit and a minimum number of target gene copies (i.e., template DNA) is required in the qPCR reaction tube to generate measurable fluorescence increase during the light cycler run. For detection, >5 biomarker gene copies must be distributed into each of the three replicate reaction tubes. For reliable quantification, >20 *Dhc* biomarker gene copies should be present in the reaction tube. In other words, with a 100 mL groundwater sample, the qPCR assays can enumerate *Dhc* biomarker genes at abundances >2 x

10^4 L^{-1}, even in samples with high bacterial background (e.g., bacterial 16S rRNA gene copy abundances of 10^{12} per L, or a seven orders of magnitude difference). Quantification uses standard curves prepared with dilutions of known amounts of plasmid DNA that contains the target gene(s). The dynamic range spans concentrations over several orders of magnitude and linear standard curves over 8 orders of magnitude are utilized for environmental monitoring (Ritalahti et al. 2006). The quantification limits for individual genes with the TaqMan approach vary somewhat with the primers and probe combinations used but accurate quantification is typically achieved when > 100 target gene copies are present per reaction tube.

The presence of PCR inhibitors can affect *Dhc* biomarker gene detection and quantification. Consequences of the presence of inhibitors are false negative results (i.e., *Dhc* biomarker genes are present but were not detected or accurately quantified). The presence of PCR inhibitor is always a concern and substantial efforts have been devoted to remove such inhibitors during DNA extraction. Unfortunately, additional purification steps that efficiently remove inhibitors lead to reduced DNA and biomarker gene recoveries. Even more troublesome is the fact that no universal purification procedure for removal of inhibitors from all sample types exists and DNA extraction procedures must be optimized for different samples, or the compromise is accepted, with the understanding that additional analysis may be warranted for some site materials. To recognize PCR inhibition, undiluted, 1:10 and 1:100 diluted template DNA samples were assayed with qPCR. Non-exponential fluorescence signal increase, or other than a 10-fold difference in target enumeration in the dilutions of template DNA, indicated inhibition, and those samples were not included in this analysis. This procedure adds to total number of qPCR assays (and hence increases cost); however, assaying template DNA dilutions reliably detected PCR inhibition and also helped identifying tubes that yielded erroneous results due to pipetting errors. Further, the results from the dilution tubes added robustness to statistical analyses and increased confidence in the qPCR data.

6.9 ANALYTICAL SAMPLE REPRODUCIBILITY

An assessment of data reproducibility was conducted to evaluate potential impacts on the outcome of the MBT results and their interpretations. A thorough comparative analysis using defined laboratory samples and site groundwater demonstrated that the sterile filter cartridge approach is suitable for reproducible collection of microbial biomass. When combined with commercially available DNA extraction kits, the DNA preparations yielded highly reproducible qPCR data for the *Dhc* biomarker genes. Analysis of replicate samples comparing on-site filter cartridge filtration with off-site filter cartridge filtration methods demonstrated that cartridge handling, shipping and storage did not affect qPCR enumeration of *Dhc* biomarker genes. Differences between replicate samples analyzed in terms of DNA yields and biomarker gene quantification using the same biomass collection method were less than two-fold. All qPCR data were generated with at least two replicate DNA extractions, each analyzed for at least two dilutions in triplicate qPCR runs.

6.10 RECOMMENDATIONS FOR THE APPLICATION OF MBTs AT VINYL CHLORIDE-CONTAMINATED SITES

With the currently available knowledge about *Dhc* and *Dhc* RDase genes involved in VC reductive dechlorination, the following conclusions can be drawn.

- *vcrA* and the *bvcA* encode for RDases that dechlorinate VC to ethene. Both genes, *vcrA* and *bvcA*, have only been found on the genomes of *Dhc*, and no other microbes harboring these genes are known. Therefore, the presence and abundance of *Dhc* carrying *vcrA* or *bvcA* are linked to VC-to-ethene dechlorination.

- At some sites with VC as the major chlorinated ethene, the total number of *Dhc* cells exceeds the sum of *Dhc* cells carrying *vcrA* and *bvcA*. This finding indicates that additional, not yet identified VC RDase genes harbored on *Dhc* genomes exist. Nevertheless, in the vast majority of wells where VC dechlorination to ethene occurs, *Dhc* carrying the *vcrA* or *bvcA* genes are present.

- Data from very few sites suggest that VC-to-ethene dechlorination occurs in the presence of *Dhc* but where neither *vcrA* or *bvcA* were detected. These are exceptions and research teams would be very interested to receive samples from such sites.

- If *Dhc* are abundant (i.e., $>10^5$ cells per liter) at sites where chlorinated ethenes are the predominant contaminants, it is very likely that these *Dhc* strains are using one or more chlorinated ethenes as electron acceptors.

- If VC is the predominant contaminant, and qPCR data suggest a high abundance of *Dhc* 16S rRNA genes, it is very likely that these *Dhc* strains respire VC. Correlating the abundance of *Dhc* 16S rRNA genes with the abundances of the *vcrA* and *bvcA* genes provides additional confidence that VC-to-ethene dechlorination is occurring.

- The argument can be made that the presence of the *Dhc* 16S rRNA gene alone is sufficient to infer that *Dhc* strains are responsible for VC reductive dechlorination at VC-contaminated sites, and additional analyses targeting individual RDase genes will not provide additional information. This conclusion is based on the assumption that *Dhc* require a halogenated compound (e.g., VC) for growth. However, this assumption is only valid if VC is the only halogenated compound from which *Dhc* can derive energy. At most sites, higher chlorinated ethenes and other chlorinated compounds (i.e., co-contaminants) are present that may support a sizable *Dhc* population. Therefore, *Dhc* 16S rRNA genes, *vcrA* and *bvcA* should be quantitatively monitored.

- For site assessment and to predict if indigenous *Dhc* strains will be able to respire VC, the *Dhc* 16S rRNA genes and both the *vcrA* and the *bvcA* should be enumerated.

- Following bioaugmentation with the consortia currently in use, *bvcA* will not be abundant at most sites; however, site monitoring, especially following the initial phase of PCE/TCE dechlorination, should quantify the *Dhc* 16S rRNA genes and both the *vcrA* and the *bvcA* genes.

- Currently, only three *Dhc* biomarker genes are available for monitoring chlorinated ethenes reductive dechlorination. For the analytical laboratory, the efforts to analyze two or three target genes are not significantly different. While experts may be able to guide practitioners

to reduce the number of samples tested for all three target genes, the cost savings will be marginal. Customized qPCR assays can be envisioned that target only those RDase genes that provide information that influences decision-making. However, such customized assays will only make sense when a larger number of biomarker genes that inform about the process of interest are available.

7. COST ASSESSMENT

7.1 SAMPLE COLLECTION AND ANALYSIS COST ASSESSMENT

The costs for a typical chlorinated solvent site involving bioremediation usually include capital costs and the subsequent monitoring costs. Use of the MBTs will result in incremental additional costs (i.e., the costs for qPCR analysis) that are small in comparison to the total project costs; however, the return on investment is significant, as reflected by the improvement in site assessment and remediation performance. These benefits could lead to shortened remediation timeframes (early site closures) and reductions in the associated overall remediation costs (see Section 7.2).

Costs for the use of qPCR were tracked throughout the demonstration using a management information system, which allows detailed tracking of material, labor, travel, and subcontractor costs by major project milestones. A summary of the cost break down is presented in **Table 7-1**. The cost items in the table include groundwater sampling and laboratory analyses for *Dhc* biomarker genes. As shown in **Table 7-1,** the additional cost for qPCR analysis is currently $400 to $485 per sample.

Table 7-1 Cost Summary

Cost Category	Sub Category	Details
Start-Up Costs		Not Applicable
Capital Costs		Not Applicable
Operating Costs	Consumables, supplies (membrane filter, tubing, shipping)	Approximately $15 per sample(1)
	Operator Labor	Approximately $75 per sample
	Equipment maintenance and calibration	Approximately $10 per sample depending on procedures(1)
	Purge water disposal	Approximately $0 to $10 per sample depending on procedures(1)
	Laboratory analysis	$300 to $375 per sample(2)
Indirect Environmental Costs		Not Applicable
Demobilization		Not Applicable

Note:

(1) These costs are already incurred with traditional groundwater sampling.

(2) Costs of on-site field filtering are included in the cost of sample analysis.

The Phase I sampling results suggest that cost reductions can be achieved due to savings associated with on-site biomass collection using the sterile filter cartridges. The major cost component for use of qPCR is for laboratory analysis at the current cost of $300 to $375 per sample. However, these costs are expected to decrease due to technological advances and the increasing demand for nucleic acid-based analyses (i.e., more vendors will offer these services). Nevertheless, the greatest cost savings realized by this technology are through improved decision making in remedial design and implementation of pilot test and full-scale remedies of MNA, biostimulation and bioaugmentation.

7.2 COST MODEL

To estimate the reduction of project costs that could result from MBT use, a cost model was developed to allow estimation and comparison of the costs associated with three remediation scenarios, which achieve project objectives under different conditions. A summary of this cost model is included in **Table 7-2** and a detailed version of it is included in (**Appendix E**):

- With only MNA selected as the remedial alternative, monitoring the site for a longer term (e.g., 20 years) to achieve remedial action goals.
- With biostimulation selected as the remedial alternative, conducting several rounds of EVO injections (e.g., two rounds of oil injection), and monitoring the site for a shorter timeframe (e.g., 5 years) to achieve remedial action goals.
- With bioaugmentation selected as the remedial alternative, conducting EVO and KB-1 injection (e.g., one round), and monitoring the site for a shorter timeframe (e.g., 2 years) to achieve remedial action goals.

The items in the cost model can be adjusted for specific site conditions (including monitoring duration, number of injections, and the types of biostimulation substrate) to obtain site-specific cost estimates. A cost estimate for the three scenarios described above was conducted to provide an order-of-magnitude estimate for cost savings that could result from the application of MBT tools.

The cost estimation was based on modified bioaugmentation implementation costs for Site 59 at NAS Cecil Field. The following assumptions were made for the cost estimate:

- Thirty monitoring wells will be installed at the site for monitoring purposes for each bioremediation scenario.
- For the scenario with MNA only, the site will be monitored for 20 years. Capital costs for this scenario include regulatory submittals, monitoring, well installation, and baseline sampling and analytical analyses. Monitoring will be conducted quarterly for the first year, semi-annually for the second the third years, and annually from the 4^{th} year forward. Annual operating costs include site visits and documentation, sampling, analytical work (VOCs and other geochemical parameters, and qPCR), and reporting. The costs for 5-year reviews are also included.

- For the biostimulation scenario, two rounds of emulsified vegetable oil (EVO) injections will be conducted in 5 years with the second injection conducted in the third year. The site will be monitored for 5 years. Capital costs for the first injection include regulatory submittals, monitoring well installation, baseline sampling and analytical analyses, and EVO injection via direct push technology. Capital costs for the second injection are assumed to be 10% of that for the first injection. Monitoring will be conducted quarterly during the first year, semi-annually in the second and the third years, and annually from the 4th year on forward. Annual operating costs include site visit and documentation, sampling, analytical work (VOCs and geochemical parameters, and qPCR), and reporting. The costs for one 5-year review are also included.

- For the bioaugmentation scenario, one round of EVO and injection of a suitable consortium (e.g., KB-1) will be conducted. The site will be monitored for 2 years. Capital costs include regulatory submittals, monitoring well installation, baseline sampling and chemical analyses, qPCR analysis, and EVO and KB-1 injection via direct push technology. Monitoring will be conducted quarterly during the first year and semi-annually during the second year. The annual operating costs include site visit and documentation, sampling, analytical work (VOCs, geochemical parameters, and qPCR), and reporting.

- MBT analysis indicates that bioaugmentation is required.

A summary of the cost comparisons for the three scenarios is shown in **Table 7-2**. Results of the described estimates suggest that the costs for the MNA scenario are the highest and the costs for implementing bioaugmentation treatment with the use of MBTs are the lowest. The qPCR results assisted bioaugmentation can save approximately 15% of the costs in comparison to MNA. Greater cost savings are possible depending on specific site conditions. More benefits of using MBTs are realized through much shorter site longevity and the associated liability issues because early site closures can likely be realized. The developed cost model can assist site managers and other users in decision making processes.

Table 7-2 Summary of Project Cost Comparison for Three Remediation Scenarios

Scenario	Estimated Remediation Timeframe (years)	Remediation Specifics	Capital Costs	Long-term Monitoring and Management Costs	Total Projects Costs
MNA Only	20	No active remediation	$414,067	$509,150	$923,217
Biostimulation Only	5	Two rounds of EVO injections	$567,339	$327,879	$895,219
Bioaugmentation	2	One round of EVO and KB-1 injection	$609,793	$177,404	$787,197

8. IMPLEMENTATION ISSUES

As a result of this work, the performers have published a peer-reviewed manuscript, three book chapters and a guidance protocol that will aid the future implementation of MBTs at chlorinated solvent sites. Publications associated with this work are listed in **Appendix F**. In addition, this work has been presented at DoD training sessions and scientific conferences to inform end users of this technology.

No specific regulations pertain to the use of MBTs at chlorinated solvent sites. However, as members of the Interstate Technology & Regulatory Council (ITRC) Environmental Molecular Diagnostics Committee, project team members are drafting guidance and developing training materials to support the use of MBTs.

Sampling supplies are available commercially. MBT analyses are available from commercial laboratories. Use of sterile filter cartridges eliminates the need for packaging and shipping groundwater. Investigation-derived wastes must be properly disposed, as for all sampling activities at impacted sites. Therefore, avoiding the shipment of groundwater is a major benefit of on-site biomass collection with the sterile filter cartridges.

A Guidance Protocol entitled "*Use of Nucleic Acid-Based Tools for Site Assessment and Monitoring Bioremediation at Chlorinated Solvent Sites*" has been drafted as a result of this project. Site RPMs and contractors across DoD will be able to use the Guidance Protocol for implementing engineered bioremediation and to support decision making regarding MNA and enhanced bioremediation. With the increased knowledge and understanding of the reductive dechlorination process, along with improved and rigorously tested assessment and monitoring tools, as well as appropriate guidance documents, site managers and regulators will have the means to convincingly argue that MNA and/or enhanced treatment are viable, cost-effective approaches for source zone remediation and plume control to achieve long-lasting risk reduction.

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APPENDICES

Appendix A: Points of Contact

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Appendix B: Supplemental Site information

Appendix B-1: Anniston Army Depot

NORTHEAST AREA

TRENCH AREA

INDUSTRIAL AREA

LANDFILL AREA

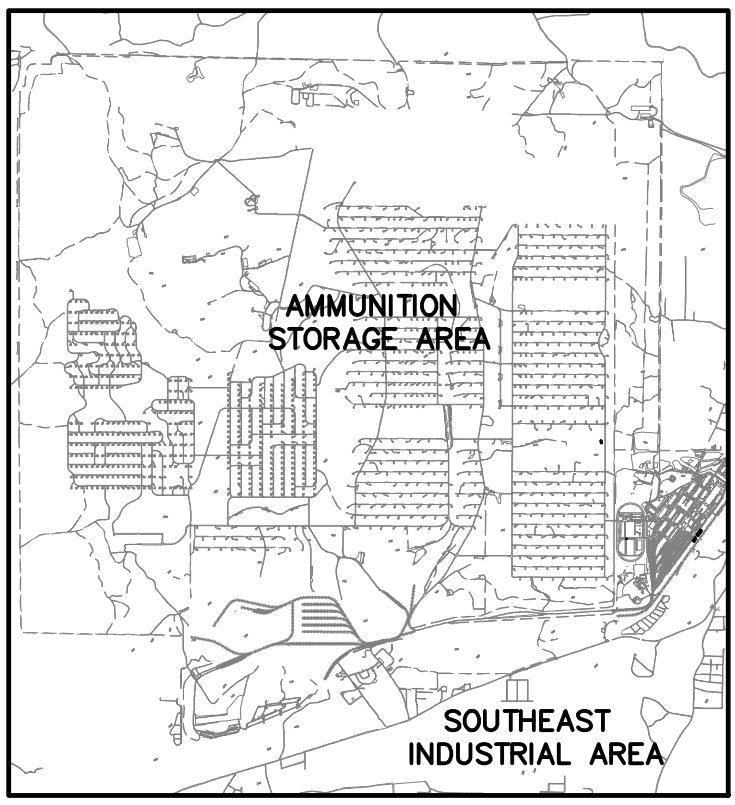
SWMU 1
SWMU 2
SWMU 7
SWMU 21
SWMU 23
SWMU 28
SWMU 29
SWMU 30
SWMU 32
SWMU 33
SWMU 3,4
SWMU 38
SWMU 39
SWMU 41
SWMU 25
SWMU 42
SWMU 38
SWMU 41
SWMU 41
SWMU 40
SWMU 38
BUILDING 114
SWMU 31
SWMU 43
DRY CREEK
SWMU 38
SWMU 44
SWMU 22
SWMU 24
SWMU 9
SWMU 12
SWMU 19, 20
SWMU 13
SWMU 6

700

ANNISTON ARMY DEPOT, AMMUNITION STORAGE AREA AND SOUTHEAST INDUSTRIAL AREA SITE MAP

0 1.5 MILES 3 MILES

SCALE: 1" = 1.5 MILES



LEGEND:

- BUILDINGS
- PRIMARY ROADS
- SECONDARY ROADS
- CHANNEL, TRIBUTARIES, & CREEK
- RAILROAD TRACKS
- FENCE
- SOUTHEAST INDUSTRIAL AREA SOURCE AREA BOUNDARIES
- SWMU BOUNDARIES

NOTE:

1.) PRIMARY CONTAMINANT SOURCE AREAS DESIGNATED IN SIA PHASE II RI (SAIC 1998a)

SOURCE: COMPREHENSIVE GROUNDWATER FEASIBILITY STUDY FOR OPERABLE UNIT 1 (FINAL), ANNISTON ARMY DEPOT, ANNISTON, ALABAMA, APRIL 2008 PREPARED BY: SAIC

TETRA TECH

ANNISTON ARMY DEPOT
ANNISTON, ALABAMA

LOCATION OF SWMU'S WITHIN FOUR CONTAINMENT SOURCE AREAS

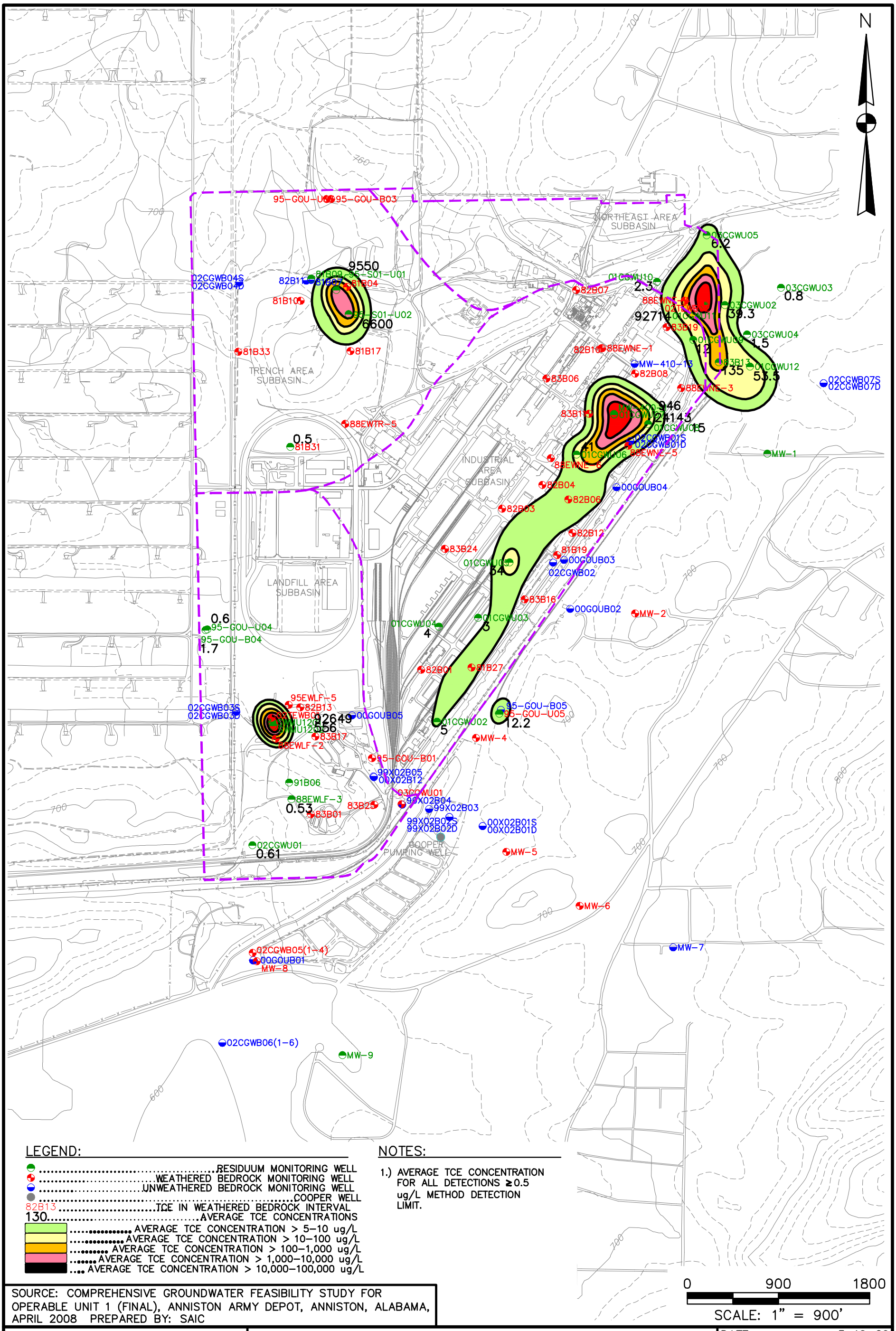
SCALE: AS NOTED

DATE:	3-19-09
PROJECT NO.:	112IG01862
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DRAWN BY:	BH
CHECKED BY:	BH
SHEET: 15 OF ---	

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FIGURE 1-2

R:\1862\Civil\1862C015.dwg PIT BEN.HOPPE 3/6/2009 12:16:41 PM



LEGEND:

- RESIDUUM MONITORING WELL
- WEATHERED BEDROCK MONITORING WELL
- UNWEATHERED BEDROCK MONITORING WELL
- COOPER WELL
- 82B13 — TCE IN WEATHERED BEDROCK INTERVAL
- 130 — AVERAGE TCE CONCENTRATIONS
- AVERAGE TCE CONCENTRATION > 5–10 ug/L
- AVERAGE TCE CONCENTRATION > 10–100 ug/L
- AVERAGE TCE CONCENTRATION > 100–1,000 ug/L
- AVERAGE TCE CONCENTRATION > 1,000–10,000 ug/L
- AVERAGE TCE CONCENTRATION > 10,000–100,000 ug/L

NOTES:

1.) AVERAGE TCE CONCENTRATION FOR ALL DETECTIONS ≥ 0.5 ug/L METHOD DETECTION LIMIT.

SOURCE: COMPREHENSIVE GROUNDWATER FEASIBILITY STUDY FOR OPERABLE UNIT 1 (FINAL), ANNISTON ARMY DEPOT, ANNISTON, ALABAMA, APRIL 2008 PREPARED BY: SAIC

0 900 1800

SCALE: 1" = 900'

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ANNISTON ARMY DEPOT
ANNISTON, ALABAMA

AVERAGE TRICHLOROETHENE CONCENTRATION MAP FOR RESIDUUM LAYER

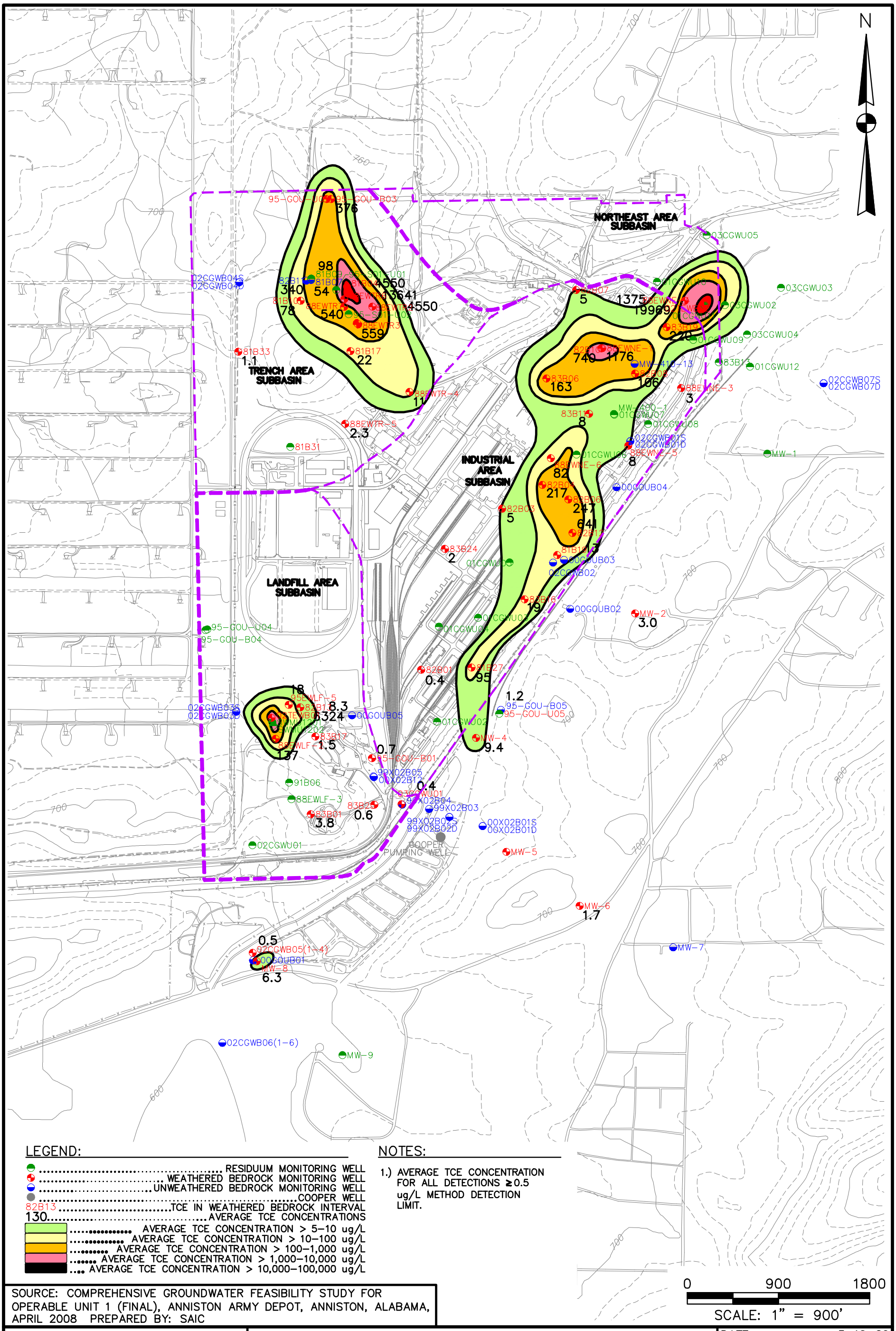
SCALE: 1" = 900'

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PROJECT NO.:	112IG01862
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FIGURE 2-5

R:\1862\Civil\1862C020.dwg PIT CARLY.KRAMER 3/9/2009 2:34:28 PM



LEGEND:

- RESIDUUM MONITORING WELL
- WEATHERED BEDROCK MONITORING WELL
- UNWEATHERED BEDROCK MONITORING WELL
- COOPER WELL
- 82B13 ..... TCE IN WEATHERED BEDROCK INTERVAL
- 130 ..... AVERAGE TCE CONCENTRATIONS
- AVERAGE TCE CONCENTRATION > 5–10 ug/L
- AVERAGE TCE CONCENTRATION > 10–100 ug/L
- AVERAGE TCE CONCENTRATION > 100–1,000 ug/L
- AVERAGE TCE CONCENTRATION > 1,000–10,000 ug/L
- AVERAGE TCE CONCENTRATION > 10,000–100,000 ug/L

NOTES:

1.) AVERAGE TCE CONCENTRATION FOR ALL DETECTIONS ≥ 0.5 ug/L METHOD DETECTION LIMIT.

SOURCE: COMPREHENSIVE GROUNDWATER FEASIBILITY STUDY FOR OPERABLE UNIT 1 (FINAL), ANNISTON ARMY DEPOT, ANNISTON, ALABAMA, APRIL 2008 PREPARED BY: SAIC

0 900 1800

SCALE: 1" = 900'

TETRA TECH

ANNISTON ARMY DEPOT
ANNISTON, ALABAMA

AVERAGE TRICHLOROETHENE CONCENTRATION MAP FOR WEATHERED BEDROCK LAYER

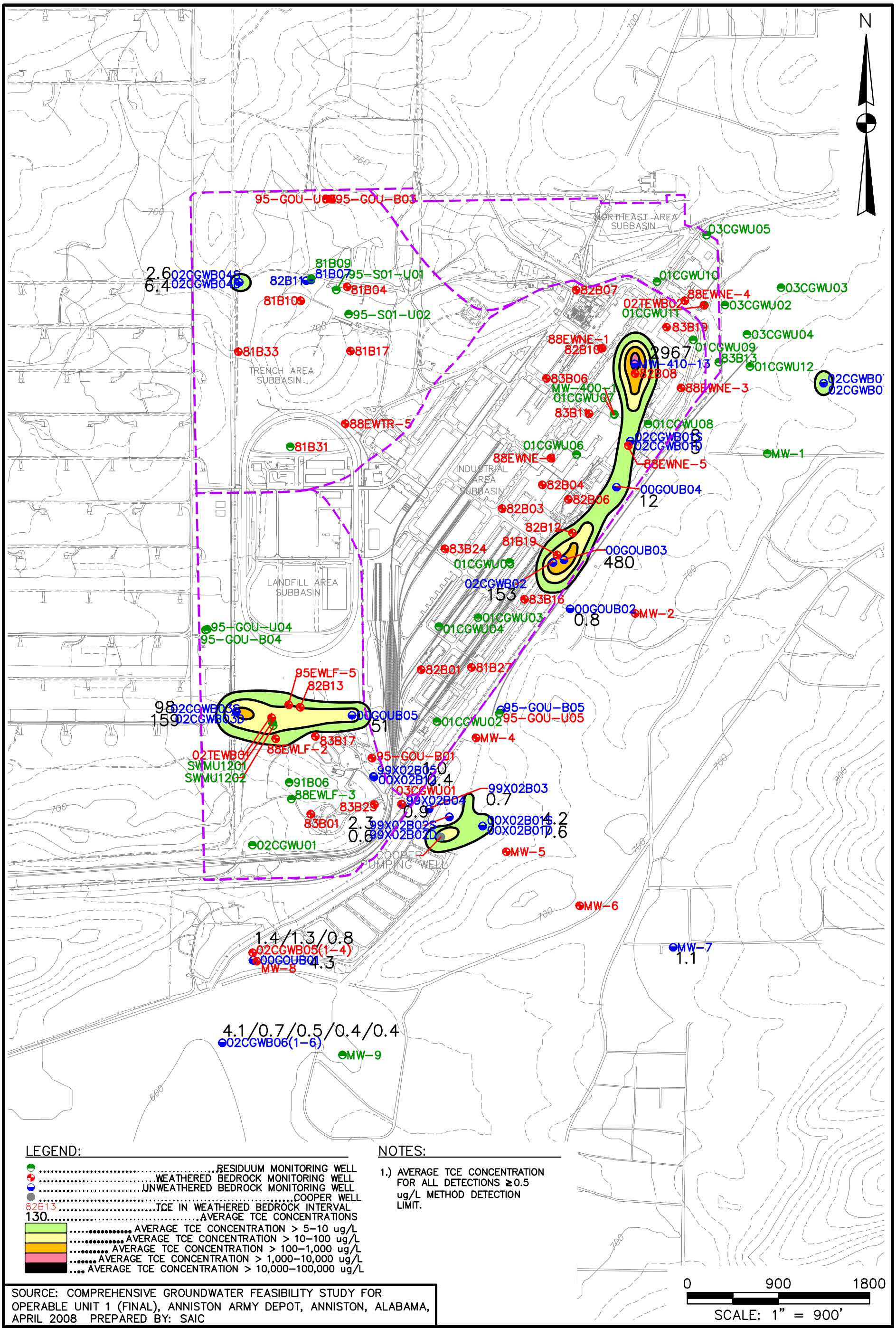
SCALE: 1" = 900'

DATE:	3–19–09
PROJECT NO.:	112IG01862
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FIGURE 2–6

R:\1862\Civil\1862C021.dwg PIT BEN.HOPPE 3/6/2009 12:31:37 PM



LEGEND:

- RESIDUUM MONITORING WELL
- WEATHERED BEDROCK MONITORING WELL
- UNWEATHERED BEDROCK MONITORING WELL
- COOPER WELL
- 82B13 — TCE IN WEATHERED BEDROCK INTERVAL
- 130 — AVERAGE TCE CONCENTRATIONS
- AVERAGE TCE CONCENTRATION > 5–10 ug/L
- AVERAGE TCE CONCENTRATION > 10–100 ug/L
- AVERAGE TCE CONCENTRATION > 100–1,000 ug/L
- AVERAGE TCE CONCENTRATION > 1,000–10,000 ug/L
- AVERAGE TCE CONCENTRATION > 10,000–100,000 ug/L

NOTES:

1.) AVERAGE TCE CONCENTRATION FOR ALL DETECTIONS ≥ 0.5 ug/L METHOD DETECTION LIMIT.

SOURCE: COMPREHENSIVE GROUNDWATER FEASIBILITY STUDY FOR OPERABLE UNIT 1 (FINAL), ANNISTON ARMY DEPOT, ANNISTON, ALABAMA, APRIL 2008 PREPARED BY: SAIC

TETRA TECH

ANNISTON ARMY DEPOT
ANNISTON, ALABAMA

AVERAGE TRICHLOROETHENE CONCENTRATION MAP FOR UNWEATHERED BEDROCK LAYER

SCALE: 1" = 900'

DATE:	3-19-09
PROJECT NO.:	112IG01862
DESIGNED BY:	BH
DRAWN BY:	BH
CHECKED BY:	BH
SHEET:	22 OF ---

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FIGURE 2-7

R:\1862\Civil\1862C022.dwg PIT CARLY.KRAMER 3/9/2009 2:35:37 PM

N 1,144,200

E 632,500

NORTHEAST AREA SUBBASIN

TRENCH AREA SUBBASIN

INDUSTRIAL AREA SUBBASIN

LANDFILL AREA SUBBASIN

95-GOU-U03 95-GOU-B03 19

03CGWU05 1.6

1.4 02CGWB04S 02CGWB04D 2

11.5 76 81B09 95-S01-U01 82B11 81B07 81B04 41.5 4.6 14

81B10 315 95-S01-U02 3800

82B07 1.3

01CGWU10 0 88EWNE-4 02TEWB02 13000 36732 01CGWU11 03CGWU03 03CGWU02 1.5

83B19 51 01CGWU09 03CGWU04 0.5

81B33 81B17 45

82B10 88EWNE-1 628 2388 110 MW-410-13 83B13 01CGWU12 6.3

83B06 102 82B08 32 88EWNE-3 2.3 02CGWB07S 7.2 02CGWB07D 1.0

1000 MW-400-1 01CGWU07 3960 01CGWU08 2.4

83B11 53 88EWTR-5 3.2

0.6 81B31

35 02CGWB01S 02CGWB01D 2.2 01CGWU06 88EWNE-5 1.7 MW-1

88EWNE-6 55 82B04 60 00GOUB04 6.3 82B06 58 82B03 2.6 90 82B12

83B24 1.4 01CGWU05 10.4 81B19 1.1 00GOUB03 22 02CGWB02 33

83B16 2.2 00GOUB02 0.8 MW-2 0.4 01CGWU03 1.2 01CGWU04 1.6

3.2 95-GOU-U04 95-GOU-B04 0.4

82B01 81B27 6.6

1059 95EWLF-5 82B13 105 185 02CGWB03S 367 02CGWB03D 02TEWB01 00GOUB05 411763 2574 SWMU12C SWMU12D 80840 83B17 0.54 88EWLF-2 1246

1.5 95-GOU-B05 95-GOU-U05 1.6 01CGWU02 1.7 MW-4 1.8

95-GOU-B01 99X02B01 00X02B12 1.3 1.5 91B06

21 030GWU01 99X02B04 1.2 99X02B03 88EWLF-3 0.64 83B21 83B01 1.4 1.7 99X02B02S 99X02B02D 0.6 00X02B01S 00X02B01D 6.1 11

0.32 02CGWU01 COOPER PUMPING WELL 55 MW-5

MW-6

MW-7

02CGWB05(1-4) 00GOUB01 MW-8 16 1.8

02CGWB06(1-6) MW-9

MW-10 1.6 MW-11 2.3 MW-12 2.2 MW-13

24,700

700 800 900 600

LEGEND:

- RESIDUUM MONITORING WELL
- WEATHERED BEDROCK MONITORING WELL
- UNWEATHERED BEDROCK MONITORING WELL
- SPRING LOCATION
- COOPER WELL
- 82B13 1 DETECTION > 70 ug/L
- 82B09 AVERAGE FROM 2 DETECTIONS > 70 ug/L
- 130 AVERAGE 1,2-DCE CONCENTRATIONS
- 1,2-DCE CONCENTRATION >70-100 ug/L
- 1,2-DCE CONCENTRATION >100-1,000 ug/L
- 1,2-DCE CONCENTRATION >1,000-10,000 ug/L
- 1,2-DCE CONCENTRATION >10,000-100,000 ug/L

NOTES:

1.) AVERAGE 1,2-DCE CONCENTRATION FOR ALL DETECTIONS ≥MCL (70 µg/L).

AL STATE PLANE NAD 83 (EAST)

0 300 600 900

SCALE: 1" = 900'

SOURCE: COMPREHENSIVE GROUNDWATER FEASIBILITY STUDY FOR OPERABLE UNIT 1 (FINAL), ANNISTON ARMY DEPOT, ANNISTON, ALABAMA, APRIL 2008 PREPARED BY: SAIC

TETRA TECH

ANNISTON ARMY DEPOT
ANNISTON, ALABAMA

AVERAGE 1,2 DICHLOROETHENE ISOCONCENTRATION MAP

SCALE: 1" = 900'

DATE:	3-19-09
PROJECT NO.:	112IG01862
DESIGNED BY:	BH
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SHEET:	23 OF ---

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FIGURE 2-8

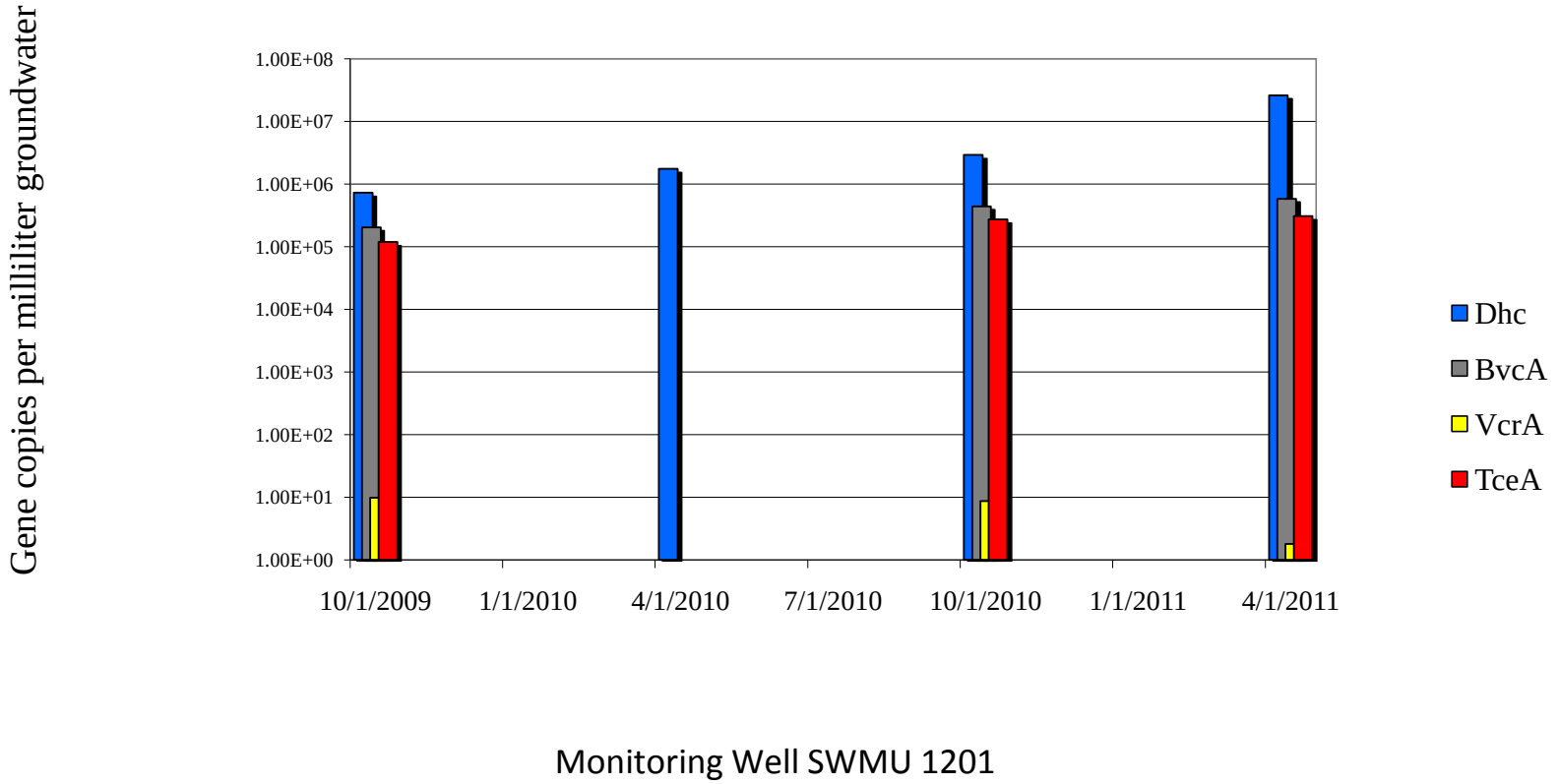
R:\1862\Civil\1862C023.dwg PIT BEN.HOPPE 3/19/2009 12:28:25 PM

Table 5-13
PCR Results[1, 2]
Annniston Army Depot (4 sites)

MI ID	Sample Name	Date Sampled	DHC	EBAC	TCE	BVC	VCR
085GC1	00-GOU-B05 SAIC 15	3/31/2013	6.86E+04	1.95E+06	6.38E+04	2.23E+04	4.48E+04
085GC13	02-TEW-B01 SAIC10	4/2/2013	2.32E+04	7.58E+05	4.67E+02	7.73E+03	2.55E+04
085GC14	88-EWNE-1 SAIC16	4/2/2013	4.98E+03	5.14E+06	6.49E+02	1.10E+04	2.50E+04
040FI23	SWMU-1201 SAIC 11	10/1/2012	3.49E+05		5.10E+04	8.38E+03	<5.00E-02
040FI30	00-GOU-B05 SAIC 14	10/3/2012	2.97E+04		2.73E+03	9.88E+02	5.39E+02
040FI33	02-CGW-B03S SAIC 14	10/7/2012	2.99E+04		2.18E+02	1.27E+03	1.23E+03
014FD2	SWMW-1201	4/9/2012	8.42E+05		1.08E+05	1.11E+05	2.50E+00
014FD3	SWMW-1201 D	4/9/2012	1.27E+06		2.13E+05	1.99E+05	2.70E+00
014FD25	02CGWB03S	4/12/2012	9.64E+03		1.44E+02	5.78E+03	1.59E+04
014FD26	02CGWB03D	4/12/2012	1.04E+04		4.30E+02	3.32E+03	1.35E+04
014FD27	02TEWB01	4/12/2012	3.68E+04		4.40E+03	2.09E+04	6.14E+04
014FD30	88EWNE-1	4/15/2012	5.02E+03		1.59E+01	1.12E+03	6.95E+02
045EJ2	00GOUB05 SAIC 12	10/17/2011	9.74E+04		<5.00E-02	<5.00E-02	2.00E-01 (J)
045EJ3	00GOUB05 SAIC 12D	10/17/2011	6.47E+04		<5.00E-02	<5.00E-02	8.00E-01
045EJ14	02TEWB01 SAIC08	10/19/2011	4.29E+04		1.52E+02	2.36E+03	5.97E+03
045EJ15	02TEWB01 SAIC08D	10/19/2011	7.35E+04		2.16E+02	3.53E+03	6.52E+03
045EJ26	02CGWB03S SAIC 12	10/21/2011	1.27E+05		2.90E+01	2.51E+03	2.10E+04
045EJ27	02CGWB03D SAIC 12	10/21/2011	8.49E+04		2.21E+02	5.36E+03	9.31E+03
023ED3	SWMU 1201	4/11/2011	2.61E+07		3.08E+05	5.81E+05	1.80E+00
023ED4	00-GOU-B05	4/11/2011	8.76E+04		1.15E+02	3.49E+03	1.10E+03
023ED5	00-GOU-BO1	4/11/2011	4.84E+04		1.66E+02	7.47E+02	2.50E+00
023ED6	02TEWB01	4/12/2011	4.65E+05		6.15E+02	1.09E+04	1.15E+04
023ED7	88EWNE1	4/12/2011	5.40E+03		6.00E-01	4.93E+01	1.90E+00
023ED10	02CGWB03S	4/13/2011	8.52E+04		2.57E+01	3.51E+03	1.60E+04
08DJ2	02TEWB-01	10/4/2010	3.64E+04		9.50E+02	9.46E+04	5.69E+04
08DJ3	88EWNE-1	10/4/2010	1.00E+04		1.54E+03	3.61E+03	7.86E+01
08DJ17	SWMU 1201	10/6/2010	2.93E+06		2.73E+05	4.39E+05	8.70E+00
029DD7	02TEWB01	4/20/2010	1.19E+06		1.45E+04	6.04E+05	5.27E+05
029DD29	00GOUB05	4/25/2010	4.20E+04		4.75E+01	7.80E+03	2.80E+03
029DD42	SWMU1201	4/27/2010	1.75E+06		<5.00E-02	<5.00E-02	<5.00E-02
007CJ25	01CGWU09	10/11/2009	5.06E+05		<5.00E-02	<5.00E-02	<5.00E-02
007CJ30	SWMU1201	10/12/2009	7.30E+05		1.19E+05	2.04E+05	9.80E+00
007CJ31	02TEWB01	10/12/2009	3.91E+04		7.82E+01	4.49E+03	4.41E+03
017CE6	02-CGW-B03S	5/11/2009	6.05E+03		2.39E+02	2.60E+03	4.62E+03
017CE7	02-CGW-B03D	5/11/2009	9.49E+03		<5.00E-02	4.00E+00	<5.00E-02
017CE35	88-EWLF-2	5/19/2009	3.27E+04		<5.00E-02	6.92E+03	1.62E+04
017CE38	02-TEW-B01	5/20/2009	1.00E+06		<5.00E-02	<5.00E-02	<5.00E-02
017CE39	88-EWNE-1	5/20/2009	1.68E+04		3.18E+02	2.35E+04	1.62E+03

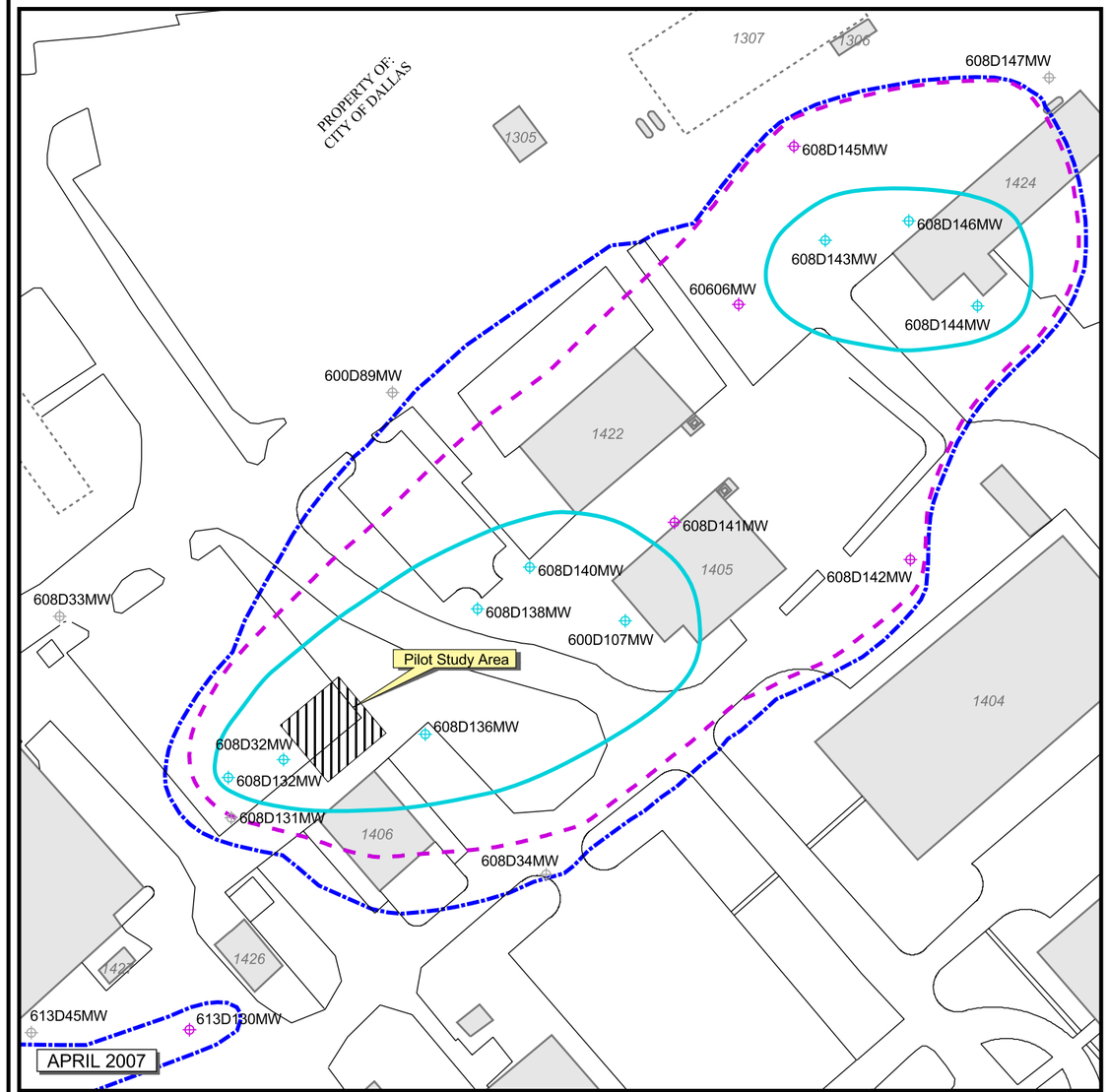
Notes
1 analytical results supplied by Microbial Insights.
2 Results are in cells/mL

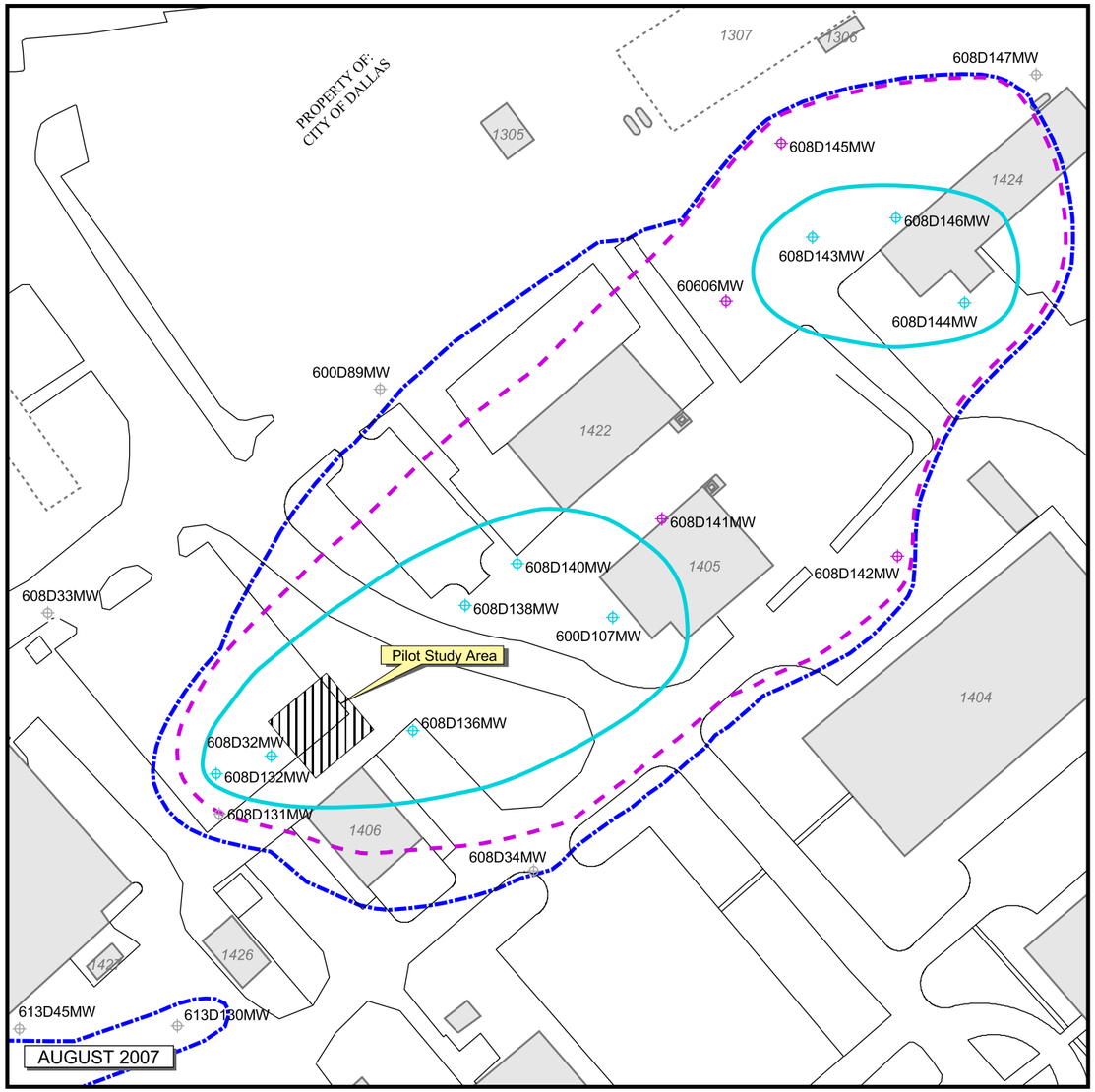
Figure 5-12
PCR Results
Anniston Army Depot



Appendix B-2: Former NAS Dallas

P:\GIS\NAS_DALLAS_DS\APR\SWMU21_TM.APR 12-12-07 JFF





N

NOTE: This Figure should not be used separately from the original report.

100 0 100 Feet

LEGEND

- Road
- Shore Line
- Railroad
- Gross PCLE Zone Outline
- Property Ownership Boundary
- Demolished Building
- Existing Structure

TCE Concentration (mg/L)
Detected at Each Sample Location:

- Not Sampled
- ND - 0.005
- > 0.005 - 0.05
- > 0.05 - 0.5
- > 0.5 - 5

0.005 mg/L
0.05 mg/L
0.5 mg/L

Note: The PCL for TCE = 0.005 mg/L.

DRAWN BY	DATE
J. FLESCH	09-13-07
CHECKED BY	DATE
G. POPE	09-13-07
REVISED BY	DATE
----	----
SCALE	
AS NOTED	

TRICHLOROETHENE
ISOCONCENTRATION CONTOURS
SWMU 21 PLUME
APRIL 2007 AND AUGUST 2007
TECHNICAL MEMORANDUM
SWMU 21 EMNA PILOT STUDY

NAS DALLAS, TEXAS

CONTRACT NO.	
3986	
OWNER NO.	
CTO 0163	
APPROVED BY	DATE
DRAWING NO.	REV.
FIGURE 3-1	0

Appendix B-3: NASA Cape Canaveral

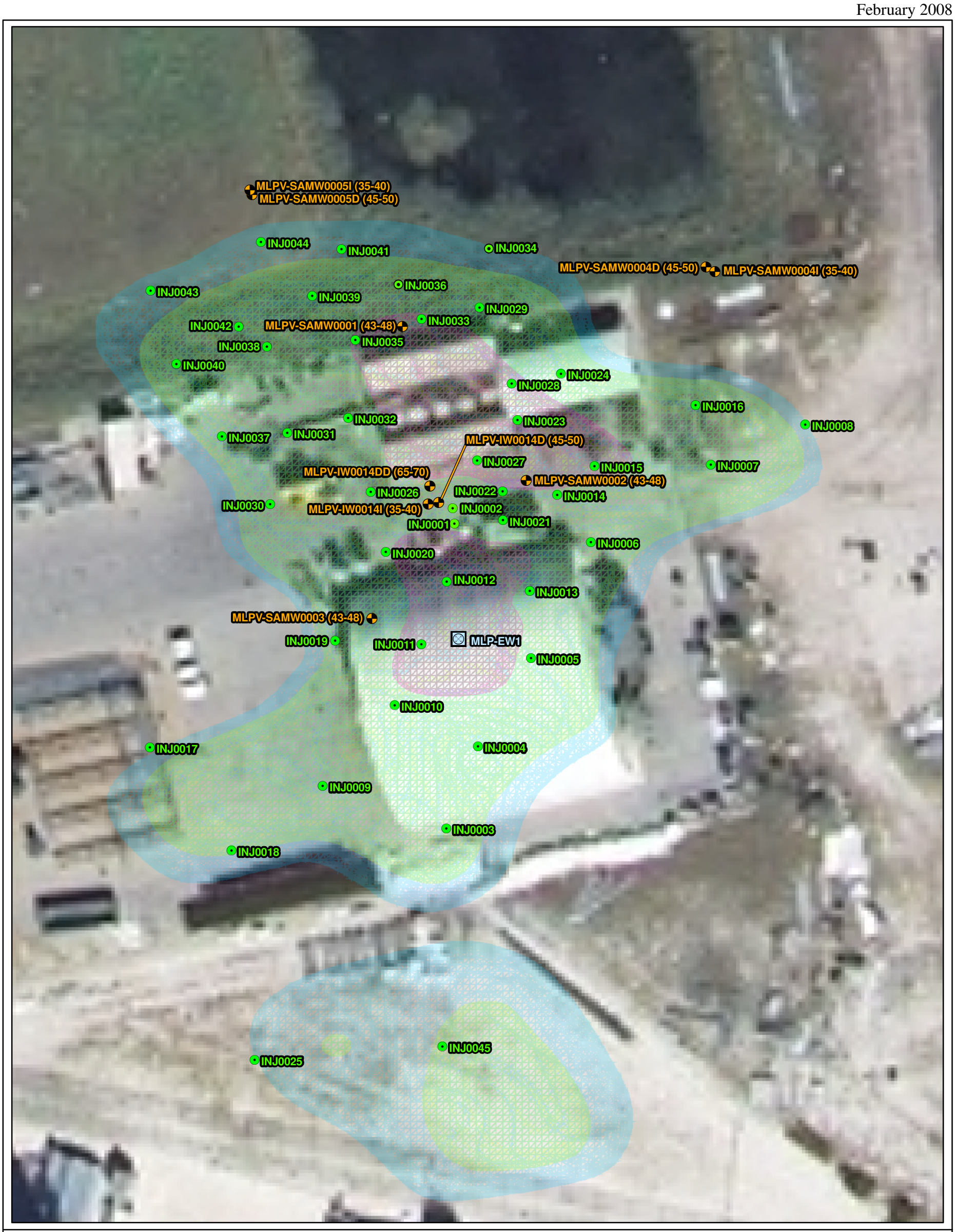


Figure 2-1
Well Locations

Legend

- Injection Well Location
- Extraction Well Location
- Monitoring Well Location showing screen interval (ft, BLS)
- 30,000 µg/L TCE Isopleth
- 3,000 µg/L TCE Isopleth
- 1,000 µg/L TCE Isopleth

Note;
ft, BLS indicates feet, below land surface.

N

30 15 0 30
Feet

Titusville-01\Data\0GIS\FR0579\MXD\Injection_MW_Well_locs_July2007.mxd



MLPV-SAMW0005I

DATE	TCE	cDCE	tDCE	VC
8/10/2006	1.00 U	**84.1**	4.60	**183**
11/21/2006	0.50 U	55.4	5.10	**162**
1/30/2007	0.50 U	46.9	4.10	**101**
3/29/2007	1.0 U	52	3.9	**161**
5/29/2007	0.78	**79**	5.9	**166**
7/25/2007	0.19	49.4	4.9	**189**
9/27/2007	0.19	20.8	0.1	**136**

MLPV-SAMW0005D

DATE	TCE	cDCE	tDCE	VC
8/10/2006	**30.1**	**3,070**	41.3	**974**
11/21/2006	25.0 U	**1,680**	26.7	**761**
1/30/2007	25.0 U	**1,980**	32.2	**457**
3/29/2007	10 U	**1,600**	39.1	**533**
5/29/2007	10 U	**1,450**	26.8	**544**
7/25/2007	1.9	**546**	33.5	**392**
9/27/2007	0.19	**185**	19.7	**168**

MLPV-SAMW0001

DATE	TCE	cDCE	tDCE	VC
8/10/2006	**4,170**	**8,080**	**357**	**384**
11/21/2006	**5,530**	**9,770**	**484**	**654**
1/30/2007	**3,690**	**7,620**	**346**	**277**
3/29/2007	**4,210**	**6,840**	**342**	**453**
5/29/2007	**4,530**	**9,070**	**491**	**488**
7/25/2007	**6,830**	**11,500**	**670**	**939**
9/27/2007	**5,200**	**8,950**	**450**	**1,930**

MLPV-SAMW0004I

DATE	TCE	cDCE	tDCE	VC
8/10/2006	1.00 U	**326**	36.1	**4,580**
11/21/2006	2.50 U	**330**	36.5	**5,530**
1/30/2007	5.00 U	**292**	35.6	**3,780**
3/29/2007	50 U	**289**	50 U	**6,230**
5/29/2007	2.5 U	**281**	37.8	**4,930**
7/25/2007	**9.5**	**108**	26	**2,750**
9/27/2007	0.95	**275**	29.9	**3,280**

MLPV-SAMW0004D

DATE	TCE	cDCE	tDCE	VC
8/10/2006	**988**	**5,590**	**284**	**917**
11/21/2006	**460**	**4,540**	**262**	**1,200**
1/30/2007	**337**	**3,530**	**175**	**500**
3/29/2007	**365**	**4,610**	**224**	**972**
5/29/2007	**372**	**5,660**	**302**	**1,310**
7/25/2007	**362**	**5,990**	**335**	**1,160**
9/27/2007	**295**	**6,850**	10	**1,480**

MLPV-IW0014DD

DATE	TCE	cDCE	tDCE	VC
8/10/2006	1.00 U	1.00 U	1.00 U	1.00 U
11/10/2006	0.53	0.50 U	0.50 U	0.50 U
11/21/2006	0.50 U	0.50 U	0.50 U	0.50 U
1/30/2007	0.50 U	0.50 U	0.50 U	0.50 U
3/29/2007	0.50 U	0.50 U	0.50 U	0.50 U
5/17/2007	0.50 U	0.50 U	0.50 U	**1.1**
7/25/2007	0.19	0.14	0.1	0.17
9/27/2007	0.19	0.14	0.1	0.17

MLPV-IW0014I

DATE	TCE	cDCE	tDCE	VC
8/10/2006	1.00 U	14.40	4.30	**173**
11/10/2006	2.50 U	11.50	2.90	**39.9**
11/21/2006	0.66	3.80	3.00	**52.1**
1/30/2007	0.50 U	0.59	0.50 U	**7.40**
3/29/2007	0.50 U	4.4	0.50 U	**27.9**
5/17/2007	1.1	0.71 I	0.50 U	**10**
7/25/2007	1.8	1.5	0.1	**14.5**
9/27/2007	1.8	2.2	0.1	**38.8**

MLPV-IW0014D

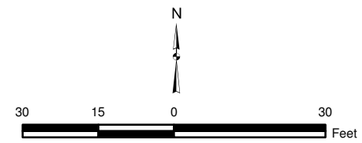
DATE	TCE	cDCE	tDCE	VC
8/10/2006	**24,900**	**11,200**	**405**	**1,100**
11/10/2006	**24,400**	**14,300**	**459**	**2,130**
11/21/2006	**2,220**	**12,200**	**289**	**4,670**
1/30/2007	**819**	**1,910**	**154**	**4,080**
3/29/2007	25 U	25 U	62.3	**4,900**
5/17/2007	2.6	4.5	51.4	**1,500**
7/25/2007	1.9	26.5	37.7	**1,430**
9/27/2007	**11.3**	25.9	7.4	**519**

MLPV-SAMW0002

DATE	TCE	cDCE	tDCE	VC
8/10/2006	**12,700**	**10,700**	**421**	**1,710**
11/21/2006	**10,700**	**9,910**	**405**	**1,900**
1/30/2007	**7,940**	**7,810**	**267**	**1,210**
3/29/2007	**7,010**	**7,670**	**293**	**1,260**
5/29/2007	**3,790**	**4,020**	**247**	**2,540**
7/25/2007	**3,680**	**4,980**	**422**	**5,680**
9/27/2007	0.2	0.6	67.4	**5,100**

MLPV-SAMW0003

DATE	TCE	cDCE	tDCE	VC
8/10/2006	**18,900**	**21,000**	**324**	**917**
11/21/2006	**6,210**	**9,220**	**422**	**1,310**
1/30/2007	**5,580**	**8,750**	**360**	**694**
3/29/2007	**4,410**	**8,110**	**347**	**1,170**
5/29/2007	**2,970**	**6,270**	**299**	**1,170**
7/25/2007	**2,480**	**6,410**	**342**	**1,440**
9/27/2007	**1,440**	**4,200**	**200**	**1,360**



Legend

- Monitoring Well Location showing screen interval (ft BLS)
- Monitoring Well Location showing screen interval (ft BLS)

Screening Criteria			
Parameter	Abbreviation	GCTLs	NADC
Trichloroethene	TCE	3	300
cis-1,2-Dichloroethene	cDCE	70	700
trans-1,2-Dichloroethene	tDCE	100	1000
Vinyl Chloride	VC	1	100

Notes:
1. ft BLS indicates feet below land surface.
2. Results are shown in micrograms per liter (µg/L).
3. Bold text indicates exceedance of FDEP Groundwater Cleanup Target Level (GCTLs).
4. Bold, yellow shaded text indicates exceedance of Natural Attenuation Default Criteria (NADC).
5. U = not detected.
6. The 8/10/2006 data is from the baseline sampling event.

Titusville-01\data\0GIS\FR0579\MXD\VOCs_July2007_Sept2007.mxd

Figure 3-1
VOC Groundwater Sampling Results



MLPV-SAMW0005I

DATE	METHANE	ETHANE	ETHENE	SULFATE
8/10/2006	1,510	1.00 U	1.60	NA
11/21/2006	1,600	0.60 U	1.40	NA
1/30/2007	1,810	0.60 U	1.30	NA
3/29/2007	8,180	0.60 U	4.84	NA
5/29/2007	8,910	NA	NA	NA
7/25/2007	11,200	0.16	15.5	NA
9/27/2007	9,760	0.36	3.1	NA

MLPV-SAMW0005D

DATE	METHANE	ETHANE	ETHENE	SULFATE
8/10/2006	469	1.00 U	10.8	NA
11/21/2006	350	0.60 U	12.5	NA
1/30/2007	417	0.60 U	10.5	NA
3/29/2007	316	0.60 U	912	NA
5/29/2007	559	0.60 U	24.1	NA
7/25/2007	6,330	14.8	242	NA
9/27/2007	7,870	60.9	138	NA

MLPV-SAMW0001

DATE	METHANE	ETHANE	ETHENE	SULFATE	DHC	VCR
8/10/2006	575	1.00 U	13.6	579	2×10^7	2×10^6
11/21/2006	526	0.60 U	13.3	502	NA	NA
1/30/2007	462	0.60 U	11.1	509	5×10^6	NA
3/29/2007	438	0.60 U	8.85	512	NA	NA
5/29/2007	405	1.0 U	8.02	567	NA	NA
7/25/2007	1,170	0.36	15.5	547	NA	NA
9/27/2007	1,300	0.16	23.6	436	NA	NA

MLPV-SAMW0004I

DATE	METHANE	ETHANE	ETHENE	SULFATE
8/10/2006	2,400	0.91	210	NA
11/21/2006	3,460	0.88 J	211	NA
1/30/2007	3,970	0.99	258	43.6
3/29/2007	3,290	3.0 U	285	47.6
5/29/2007	2,520	NA	NA	45.6
7/25/2007	4,520	0.88	343	39.8
9/27/2007	2,980	0.16	452	40.6

MLPV-SAMW0004D

DATE	METHANE	ETHANE	ETHENE	SULFATE
8/10/2006	817	1.00 U	36.0	431
11/21/2006	585	0.60 U	22.2	NA
1/30/2007	479	0.60 U	15.5	NA
3/29/2007	504	0.60 U	16.9	405
5/29/2007	604	0.60 U	20.7	415
7/25/2007	867	0.16	33	469
9/27/2007	893	0.16	37.2	453

MLPV-IW0014DD

DATE	METHANE	ETHANE	ETHENE	SULFATE
8/10/2006	1,150	1.20	1.00 U	NA
11/10/2006	1,380	2.29	0.80 U	11.8
11/21/2006	1,310	1.50	0.80 U	1.00 U
1/30/2007	1,310	1.10	0.80 U	NA
3/29/2007	1,010	3.9	0.80 U	NA
5/17/2007	1,120	1.2 U	1.6 U	NA
7/25/2007	1,040	1.39	0.22	NA
9/27/2007	1,580	1.24	0.22	NA

MLPV-IW0014I

DATE	METHANE	ETHANE	ETHENE	SULFATE	DHC	VCR
8/10/2006	2,360	5.00 U	5.00 U	46.4	2×10^6	1×10^6
11/10/2006	2,350	0.60 U	6.65	70.7	NA	NA
11/21/2006	2,180	0.60 U	2.90	51.1	NA	NA
1/30/2007	3,160	0.60 U	2.90	50.1	2×10^6	NA
3/29/2007	4,690	0.60 U	0.80 U	NA	NA	NA
5/17/2007	7,290	0.60 U	26.7	NA	NA	NA
7/25/2007	8,990	1.35	3.48	NA	NA	NA
9/27/2007	9,900	0.94	0.56	NA	NA	NA

MLPV-SAMW0002

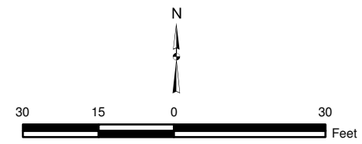
DATE	METHANE	ETHANE	ETHENE	SULFATE	DHC	VCR
8/10/2006	886	1.00 U	54.3	505	NA	NA
11/21/2006	810	0.60 U	47.5	466	NA	NA
1/30/2007	678	0.60 U	40.8	426	NA	NA
3/29/2007	746	0.60 U	42.7	455	2×10^7	3×10^5
5/29/2007	2,870	1.0	75.3	216	7×10^7	1×10^6
7/25/2007	7,860	0.65	164	150	7×10^8	7×10^6
9/27/2007	4,520	0.16	445	0.5	3×10^7	4×10^6

MLPV-IW0014D

DATE	METHANE	ETHANE	ETHENE	SULFATE	DHC	VCR
8/10/2006	721	1.00 U	23.2	457	3×10^6	1×10^4
11/10/2006	964	0.60 U	39.7	533	NA	NA
11/21/2006	2,420	0.60 U	60.5	281	NA	NA
1/30/2007	3,250	1.50	88.2	8.70	5×10^6	NA
3/29/2007	5,460	0.60 U	90.4	9.6	6×10^6	9×10^4
5/17/2007	2,370	6.0 U	19.4	NA	NA	NA
5/29/2007	NA	NA	NA	11.2	2×10^6	4×10^4
7/25/2007	5,730	0.16	38.6	11.7	7×10^6	4×10^5
9/27/2007	3,220	0.16	19.9	0.5	7×10^4	5×10^4

MLPV-SAMW0003

DATE	METHANE	ETHANE	ETHENE	SULFATE	DHC	VCR
8/10/2006	774	2.51	32.2	451	NA	NA
11/21/2006	754	0.60 U	20.7	412	NA	NA
1/30/2007	882	0.60 U	27.4	452	NA	NA
3/29/2007	901	0.60 U	31.3	476	3×10^7	1×10^7
5/29/2007	684	1.0 U	19.8	479	1×10^7	2×10^6
7/25/2007	3,040	0.46	256	348	4×10^8	3×10^8
9/27/2007	5,460	0.28	376	283	6×10^7	2×10^7



Legend

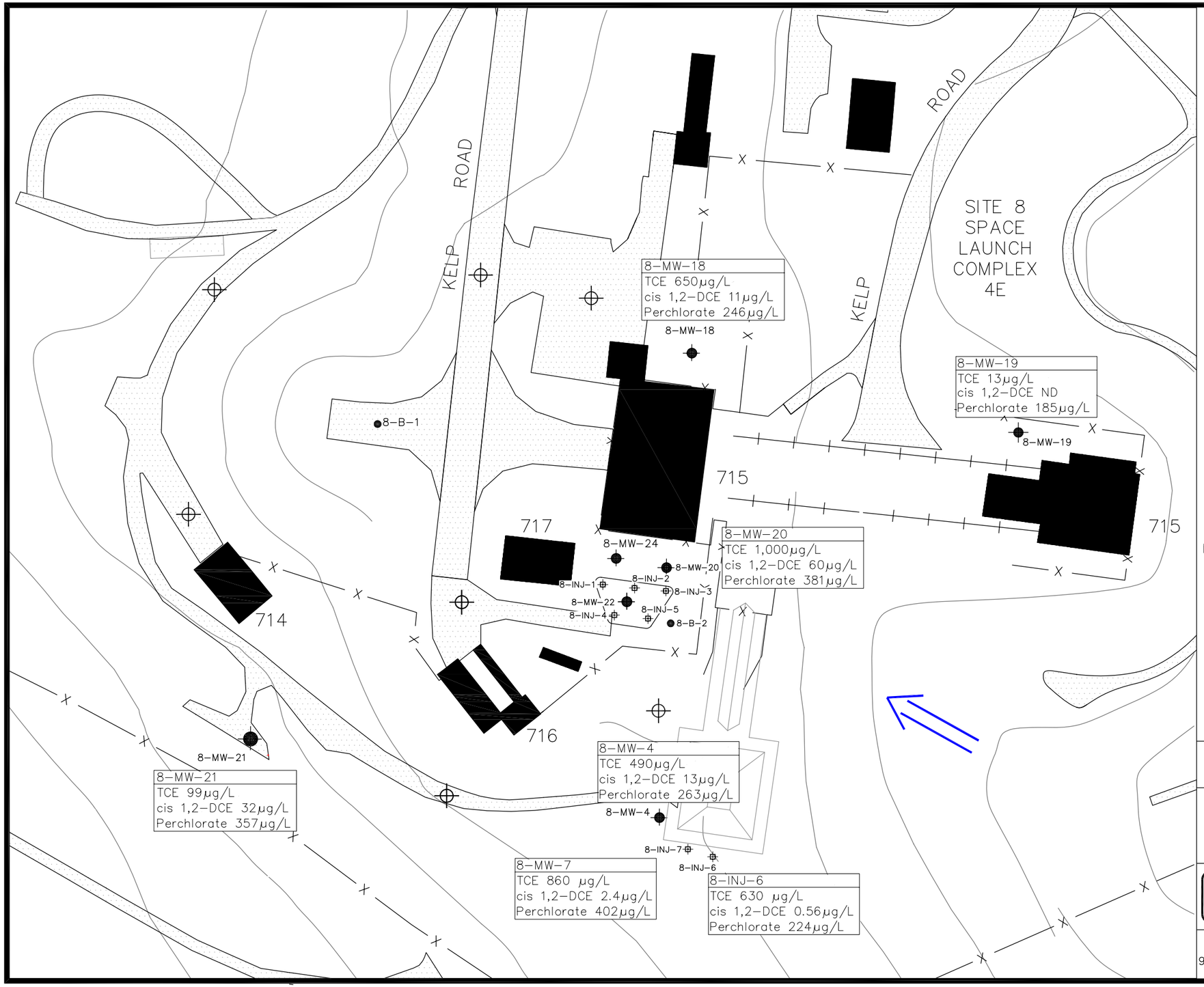
- Monitoring Well Location showing screen interval (ft BLS)
- Monitoring Well Location showing screen interval (ft BLS)

Notes:
1. ft BLS indicates feet below land surface.
2. Sulfate results are shown in milligrams per liter (mg/L); all other results are shown in micrograms per liter (µg/L).
3. NA = not analyzed.
4. DHC = *Dehalococcoides* 16S rRNA Gene Copies (gene copies per liter).
5. VCR = Vinyl Chloride reductase (gene copies per liter).
6. U = not detected.
7. The 8/10/2006 data is from the baseline sampling event.

Titusville-01\data\0GIS\FR0579\MXD\diss_gas_July2007_Sept2007.mxd

Figure 3-2
Groundwater Results (Dissolved Gases, Sulfate and DHC)

Appendix B-4: Vandenberg AFB



LEGEND

- 200 — CONTOUR OF GROUND SURFACE ELEVATIONS IN FEET ABOVE MSL (25-FOOT INTERVALS) (NAVD 1988)
- x — FENCE
- PAVED ROAD OR STREET
- DIRT ROAD
- 727 — BUILDING
- CONCRETE OR PAVED AREAS
- EXISTING INJECTION WELL
- SOIL BORING LOCATION
- GROUNDWATER MONITORING WELL
- 8-MW-11 — PROPOSED GROUNDWATER MONITORING WELL
- APPROXIMATE GROUNDWATER FLOW DIRECTION

NOTE(S): TRICHLOROETHENE (TCE)
cis 1,2 DICHLOROETHENE (cis1,2-DCE)
ND: NOT DETECTED ABOVE LABORATORY REPORTING LIMITS
MICROGRAMS/LITER (µg/L)

N

0 50' 100' 150'
SCALE

UNITED STATES AIR FORCE
VANDENBERG AIR FORCE BASE

SITE 8 CLUSTER
KEY VOCs IN GROUNDWATER
NEAR SOURCE AREA

TETRA TECH, INC.
4213 State Street, Suite 100
Santa Barbara, CA 93110-2847

TC	DATE	DRAWN BY	MADE FROM	DWG NO.	Figure
97002-09	2/9/07	RANDALL	TAB 31,32 34,35	6104	

14–MW–16(s&d)
14–B–3
125
14–MW–13(s&d)
14–MW–10
14–MW–21
14–MW–9
14–MW–3
14–INJ–5/6
14–INJ–3/4
14–INJ–1/2
14–INJ–12(s&d)
14–INJ–11(s&d)
14–INJ–10(s&d)
14–MW–19
14–MW–12(s&d)
14–B–9
14–INJ–14
14–MW–22
14–INJ–13
14–INJ–9(s&d)
14–MW–11(s&d)
14–INJ–8(s&d)
14–MW–20
WATT ROAD
14–INJ–7(s&d)
14–MW–17
14–MW–18
14–B–8
125
150

LEGEND

- 125 — CONTOUR LINE, 5–FOOT INTERVALS (FEET ABOVE MSL, NAVD 1988)
- PAVED ROAD OR STREET
- INFERRED GROUNDWATER FLOW DIRECTION
- 14–MW–3 GROUNDWATER MONITORING WELL
- 14–INJ–8 ESO INJECTION WELL
- 14–B–8 SOIL BORING
- s = SCREENED IN SHALLOW AQUIFER
- d = SCREENED IN DEEP AQUIFER

PHASE II IRA WELLS AND SOIL BORINGS

N

0 15’ 30’ 45’

SCALE

UNITED STATES AIR FORCE
VANDENBERG AIR FORCE BASE

SITE 13 CLUSTER
IRA WATT ROAD AREA

TETRA TECH, INC.
4213 State Street, Suite 100
Santa Barbara, CA 93110-2847

TC NO.	DATE	DRAWN BY	MADE FROM	DWG NO.	FIGURE
99046–09	9/19/07	PRICHARD	TAB14	6367	3

Table 5-9
PCR Results
Site 13/14 Vandedberg AFB

Well ID	Description	*DNA conc (ng/ml GW)*	*stdev*	*BAC 16S rRNA*	*stdev*	*DHC*	*stdev*	*BVCA*	*stdev*	*VCRA*	*stdev*	*TCEA*	*stdev*	*date*	*vol. (ml)*
V14MW 11D A	MoBio-Water Kit	4.77	0.17	2.97E+08	1.27E+08	**1.06E+07**	**6.75E+05**	**3.14E+03**	**2.80E+03**	**2.58E+07**	**2.82E+06**	**5.67E+04**	**1.05E+04**	1-Jun-07	940
V14MW 11D B	MoBio-Water Kit	3.86	0.33	2.19E+08	1.08E+08	**6.66E+06**	**2.80E+05**	**1.05E+03**	**1.50E+03**	**1.65E+07**	**5.66E+05**	**3.89E+04**	**1.07E+04**	1-Jun-07	940
V14MW 12D A	MoBio-Water Kit	105.43	0.32	**3.07E+08**	**4.03E+07**	3.62E+05	4.60E+05							1-Jun-07	100
V14MW 12D B	MoBio-Water Kit	50.78	0.60	**7.77E+07**	**1.12E+07**	**2.45E+04**	**4.24E+04**					**3.46E+05**	**3.55E+05**	1-Jun-07	100
V14MW 13D A	MoBio-Water Kit	20.15	0.05	1.58E+09	4.64E+08	**2.41E+04**	**6.06E+03**							1-Jun-07	500
V14MW 13D B	MoBio-Water Kit	19.57	1.25	1.91E+09	7.61E+08	**4.38E+04**	**8.16E+02**							1-Jun-07	500
V14MW 13S A	MoBio-Water Kit	8.76	0.59	**7.56E+09**	**3.80E+08**	**4.68E+05**	**9.41E+04**							1-Jun-07	350
V14MW 13S B	MoBio-Water Kit	8.26	0.17	**6.16E+09**	**1.19E+09**	**4.40E+05**	**4.87E+04**							1-Jun-07	350
V14MW 11D C (nd)														nd	nd
V14MW 11D D (nd)														nd	nd
V14MW 12D C	Sterivex(Lab)-Water Kit	477.50	11.31	**6.24E+08**	**7.49E+07**	**5.59E+07**	**5.73E+06**	**4.02E+05**	**8.25E+04**	**1.55E+06**	**7.78E+05**	**2.68E+08**	**5.10E+07**	1-Jun-07	10
V14MW 12D D	Sterivex(Lab)-Water Kit	266.44	3.94	**1.67E+09**	**8.48E+07**	**6.31E+07**	**5.47E+07**	**1.35E+06**	**1.12E+06**	**4.14E+05**	**5.98E+05**	**2.85E+08**	**2.65E+08**	1-Jun-07	26
V14MW 13D C	Sterivex(Lab)-Water Kit	39.75	0.94	1.41E+09	3.62E+08	**7.74E+04**	**3.30E+04**					**2.28E+05**	**1.60E+05**	1-Jun-07	390
V14MW 13D D	Sterivex(Lab)-Water Kit	35.61	0.35	1.63E+09	7.04E+08	**3.74E+04**	**2.43E+04**					**3.90E+04**	**4.30E+04**	1-Jun-07	420
V14MW 13S C	Sterivex(Lab)-Water Kit	6.36	0.65	**7.57E+08**	**1.14E+07**	**1.78E+06**	**4.63E+05**	**1.56E+04**	**4.53E+03**	**3.83E+04**	**4.23E+04**	**5.34E+06**	**1.27E+06**	1-Jun-07	60
V14MW 13S D	Sterivex(Lab)-Water Kit	10.80	0.72	**1.28E+09**	**1.78E+08**	**4.20E+05**	**9.93E+04**					**3.74E+05**	**1.16E+05**	1-Jun-07	70
V14MW 11D E	Sterivex(Field)-Water Kit	46.25	1.84	**2.91E+07**	**4.18E+06**	**5.15E+05**	**2.49E+04**			**1.10E+06**	**8.54E+04**			1-Jun-07	100
V14MW 11D F	Sterivex(Field)-Water Kit	46.73	1.87	**1.72E+07**	**2.84E+06**	**2.38E+05**	**5.55E+04**			**5.37E+05**	**4.40E+04**			1-Jun-07	100
V14MW 12D E	Sterivex(Field)-Water Kit	32.93	0.60	**1.74E+09**	**2.01E+08**	**1.49E+08**	**3.23E+06**	**4.76E+05**	**5.98E+04**	**3.55E+06**	**5.37E+05**	**8.83E+08**	**4.54E+07**	1-Jun-07	100
V14MW 12D F	Sterivex(Field)-Water Kit	23.03	1.94	**3.20E+09**	**1.90E+08**	**2.54E+08**	**2.13E+07**	**2.95E+06**	**9.00E+05**	**6.36E+06**	**5.36E+05**	**1.79E+09**	**5.35E+07**	1-Jun-07	100
V14MW 13D E	Sterivex(Field)-Water Kit	12.66	0.83	**3.23E+08**	**5.23E+07**									1-Jun-07	450
V14MW 13D F	Sterivex(Field)-Water Kit	40.52	2.80	**1.04E+09**	**1.17E+08**									1-Jun-07	260
V14MW 13S E	Sterivex(Field)-Water Kit	6.70	0.39	**1.80E+09**	**1.53E+08**	**1.43E+05**	**5.91E+04**							1-Jun-07	340
V14MW 13S F	Sterivex(Field)-Water Kit	13.44	1.58	**3.03E+09**	**7.32E+08**	**1.11E+05**	**1.24E+05**							1-Jun-07	150

Positive and quantifiable in both dilutions
Positive and quantifiable in one or both dilutions, one used for ave.
Only sporadic samples tested positive one or two PCR positive
PCR done, no samples tested positive

Figure 5-9
PCR Results
Site 13/14 Vandenberg AFB:

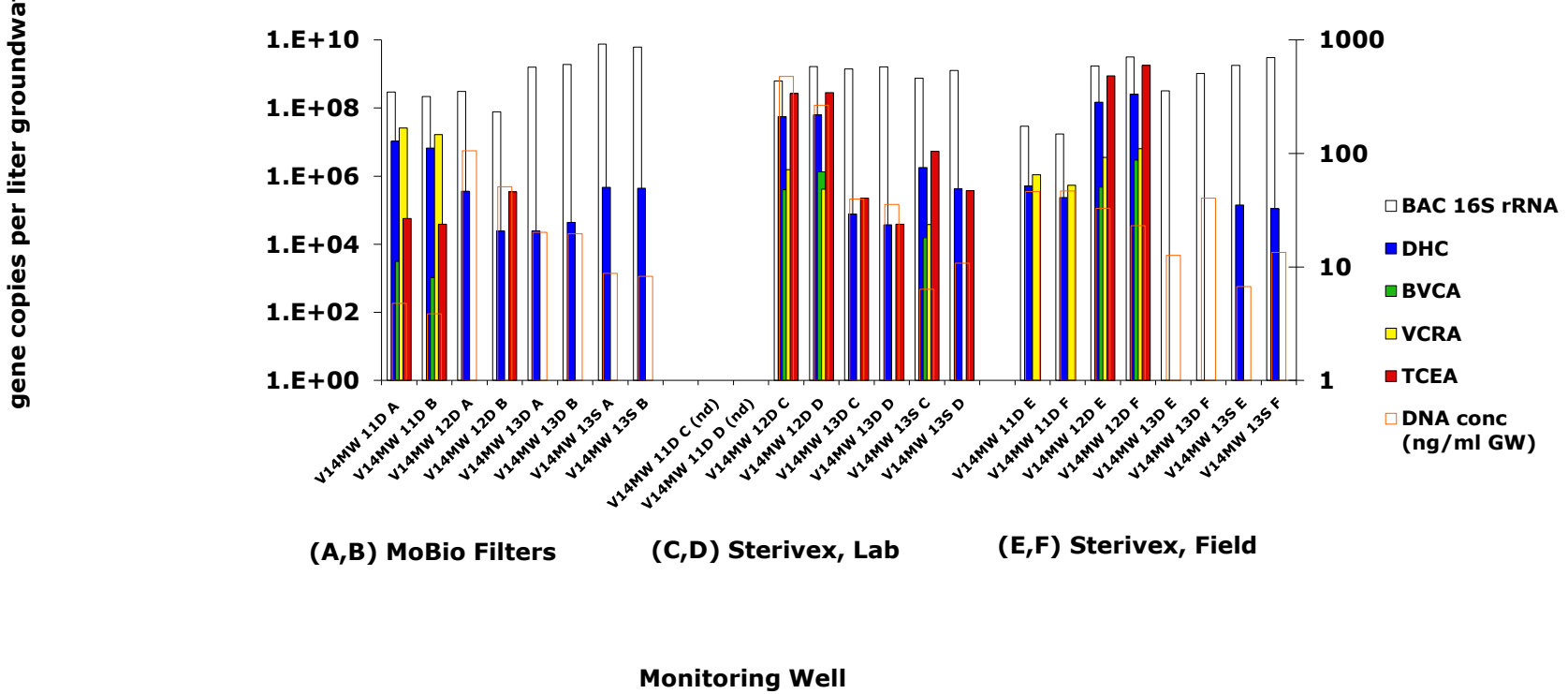


Table 5-10
PCR Results (May '07)
Site 8 Vandenberg AFB

Average two extractions	Description	*DNA conc (ng/ul)*	*stdev*	GENE COPIES PER LITER *BAC 16S rRNA*		*DHC*	*stdev*	*BVCA*	*stdev*	*VCRA*	*stdev*	*TCEA*	*stdev*
V8 Inj 4 (A,B) May9	MoBio Filter-Water Kit	83	17	1.55E+10	2.63E+09	2.56E+04	6.44E+03			3.41E+01	5.91E+01	1.62E+02	5.70E+01
Inj 7 (A,B) May9	MoBio Filter-Water Kit	209	9	2.83E+12	3.07E+11	3.84E+04	3.65E+04			1.08E+02	9.02E+03	2.51E+02	4.35E+02
MW 22 (A,B) May9	MoBio Filter-Water Kit	182	16	2.63E+12	1.21E+12	1.13E+05	8.11E+04			2.55E+02	4.41E+02	8.52E+02	1.48E+03
V8 Inj 4 (C) May9	Sterivex(Lab)-Water Kit	94	2	1.39E+09	8.60E+07	1.43E+04	3.86E+03						
Inj 7 (C,D) May9	Sterivex(Lab)-Water Kit	91	5	8.53E+10	2.31E+10								
MW 22 (C,D) May9	Sterivex(Lab)-Water Kit	94	28	2.08E+11	1.13E+11	1.35E+05	3.61E+04					3.85E+03	6.99E+03
V8 Inj 4 (E,F) May9	Sterivex(Field)-Water Kit	43	2	4.80E+08	3.75E+08	2.75E+04	1.43E+04						
Inj 7 (E,F) May9	Sterivex(Field)-Water Kit	177	10	1.55E+11	1.91E+10					4.66E+02	5.38E+02		
MW 22 (E,F) May9	Sterivex(Field)-Water Kit	76	20	1.39E+11	3.32E+10	6.14E+04	3.88E+04			7.07E+02	6.15E+02	1.68E+04	1.05E+04
										only in 2/3 "E" direct		3/3 in E direct	
												2/6 in C,D direct	

Positive in 1:10 dilutions, both extractions
Positive in one extraction, generally 2/3 (or 4/6) positive
PCR done, no samples tested positive
Only sporadic samples tested positive >2/6 one or two extractions

Figure 5-10
PCR Results (May 07)
Site 8 Vandenberg AFB

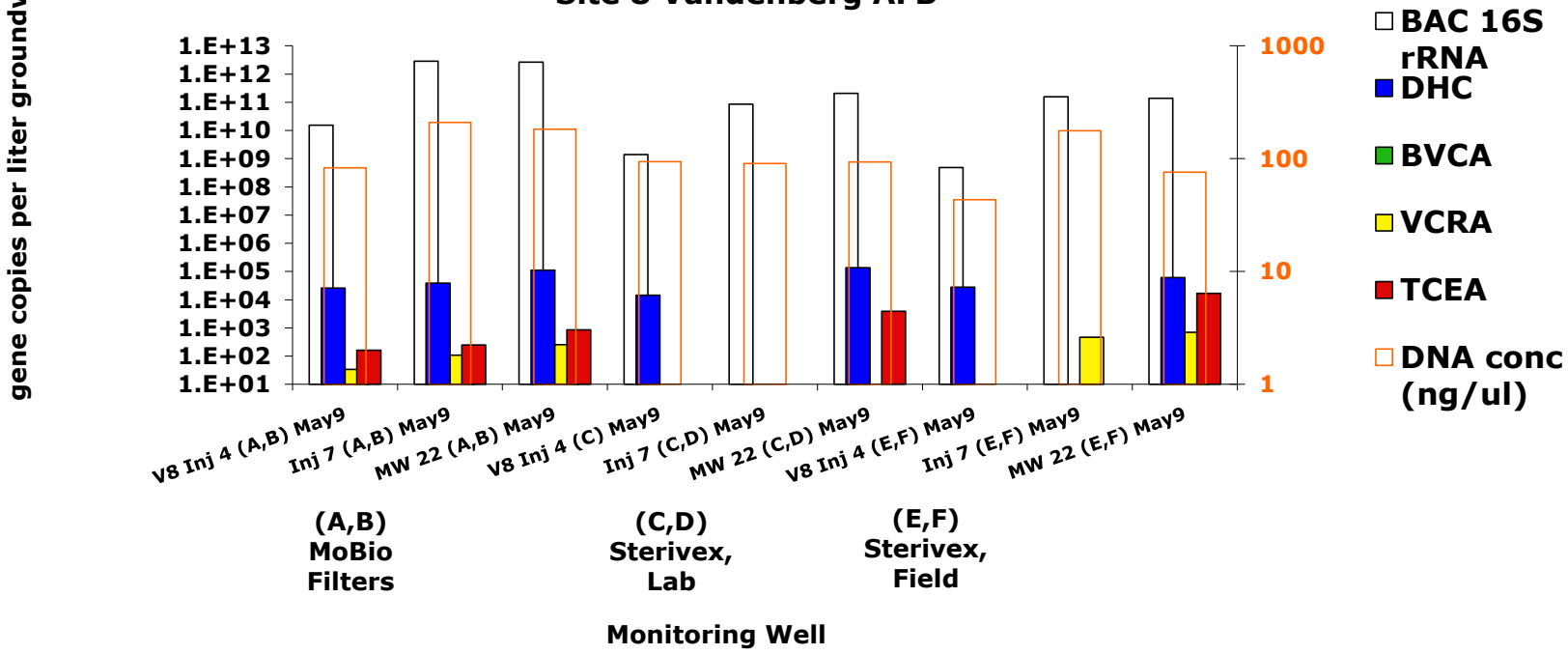


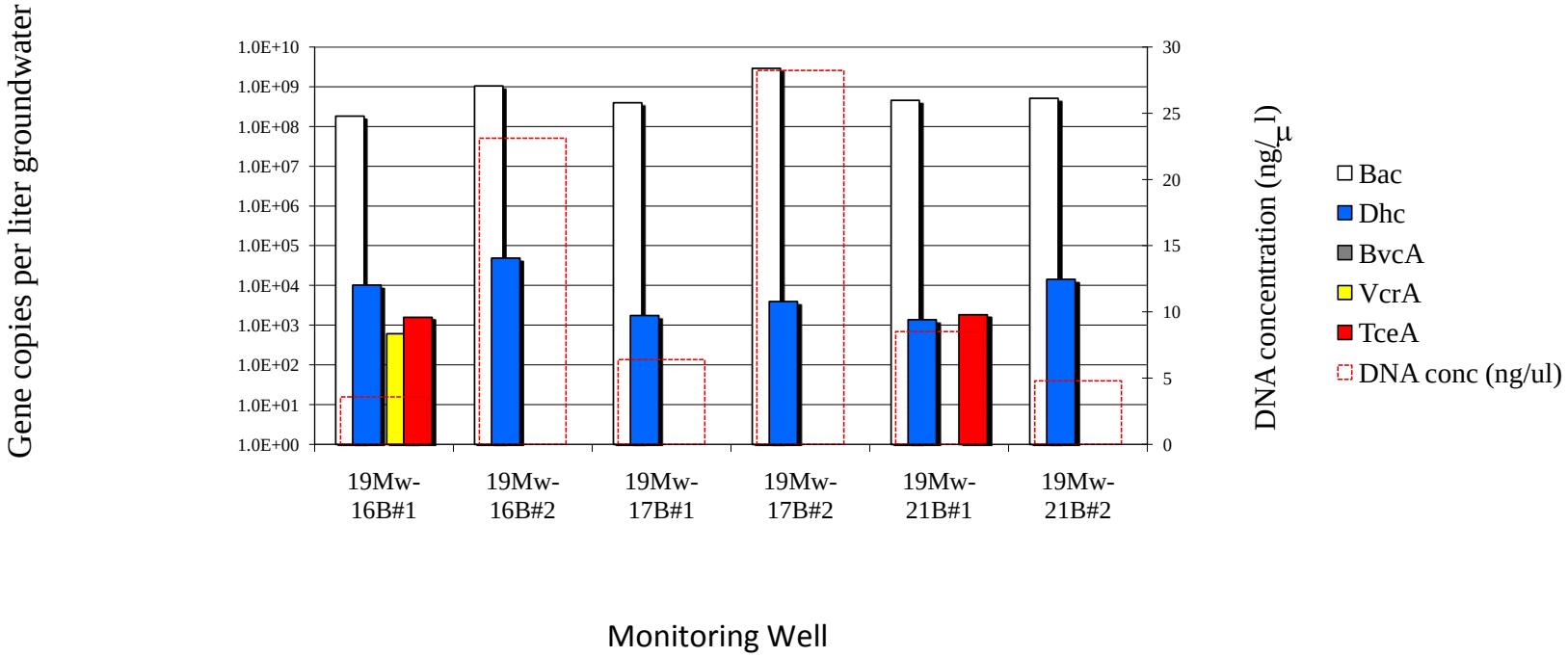
Table 5-11
PCR Results (Dec '07)
Site 8 Vandenberg AFB

	Well ID	Description	*Bac*		*Dhc*		*BvcA*		*VcrA*		*TceA*		DNA conc (ng/ul)
			average/L	*stdev/L*	*average/L*	*stdev/L*	*average/L*	*stdev/L*	*average/L*	*stdev/L*	*average/L*	*stdev/L*	
DNA	19Mw-16B#1	Sterivex-Water Kit	1.8E+08	4.9E+07	1.0E+04	2.6E+03			6.1E+02	1.1E+02	1.6E+03	1.3E+03	3.6
	19Mw-16B#2	Sterivex-Fast DNA Spin Kit	1.0E+09	6.4E+08	4.9E+04	2.7E+04							23.1
	19Mw-17B#1	Sterivex-Water Kit	3.9E+08	1.6E+08	1.8E+03	2.5E+02							6.4
	19Mw-17B#2	Sterivex-Fast DNA Spin Kit	2.9E+09	6.2E+08	3.9E+03	2.2E+03							28.2
	19Mw-21B#1	Sterivex-Water Kit	4.5E+08	8.8E+07	1.4E+03	1.1E+03					1.8E+03	1.8E+03	8.5
	19Mw-21B#2	Sterivex-Fast DNA Spin Kit	5.1E+08	6.3E+07	1.4E+04	5.0E+03							4.8
													cDNA conc (ng/ul)
RNA	19Mw-16B#1 cDNA	Sterivex-Trizol	1.1E+09	4.0E+08	1.4E+06	1.0E+05							14.1
	19Mw-16B#2 cDNA	Sterivex-Trizol	3.1E+09	6.6E+08	4.2E+06	1.0E+05			2.6E+03	1.0E+03			5.9
	19Mw-17B#1 cDNA	Sterivex-Trizol	2.3E+08	3.5E+07	1.8E+04	1.9E+03							16.9
	19Mw-17B#2 cDNA	Sterivex-Trizol	4.4E+07	4.7E+06	1.1E+04	5.2E+02					1.3E+04	2.6E+03	30.7
	19Mw-21B#1 cDNA	Sterivex-Trizol	2.2E+10	1.9E+09	1.5E+06	3.6E+04							10.2
	19Mw-21B#2 cDNA	Sterivex-Trizol	6.0E+09	1.6E+09	6.9E+05	5.0E+04							14.4
	If values are averaged for both types of DNA preps												
DNA	19Mw-16B DNA	Sterivex-(average water-soil	1.0E+09	6.4E+08	1.8E+04	1.4E+04			6.1E+02	1.1E+02	1.6E+03	1.3E+03	
	19Mw-17B DNA	Sterivex-(average water-soil	2.9E+09	6.2E+08	1.7E+03	1.1E+03							
	19Mw-21B DNA	Sterivex-(average water-soil	4.8E+08	7.8E+07	5.0E+03	3.8E+03					1.8E+03	1.8E+03	
RNA	19Mw-16B cDNA	Sterivex-Trizol	2.1E+09	1.2E+09	2.8E+06	1.5E+06			2.6E+03	1.0E+03			
	19Mw-17B cDNA	Sterivex-Trizol	1.4E+08	1.0E+08	1.5E+04	4.0E+03					1.3E+04	2.6E+03	
	19Mw-21B cDNA	Sterivex-Trizol	1.5E+10	8.4E+09	1.1E+06	4.3E+05							

Note: For RDase gene copies and cDNAs we get only sporadic amplification on the low end of our standard curve.
When we got a triplet at either one or the other prep those numbers are shown.

For all DNA extraction methods used (both MoBio water prep and FastDNA prep)

Figure 5-11
PCR Results (Dec 07)
Site 8 Vandenberg AFB



Appendix B-5: NSA Mid-South

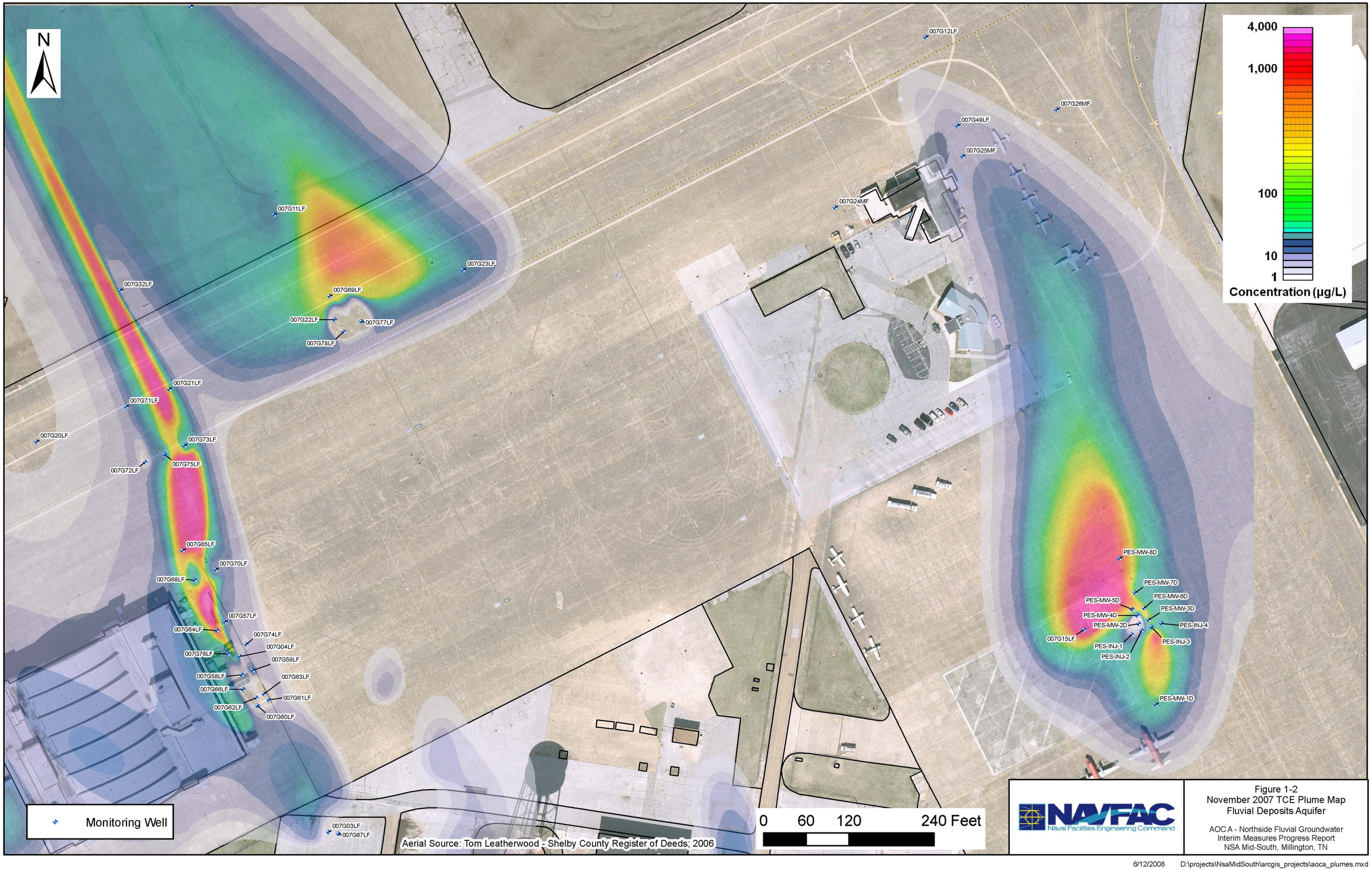


Figure 1-2
November 2007 TCE Plume Map
Fluvial Deposits Aquifer

AOC A - Northside Fluvial Groundwater
Interim Measures Progress Report
NSA Mid-South, Millington, TN

6/12/2008 D:\projects\NsaMidSouth\arcgis_projects\aoca_plumes.mxd

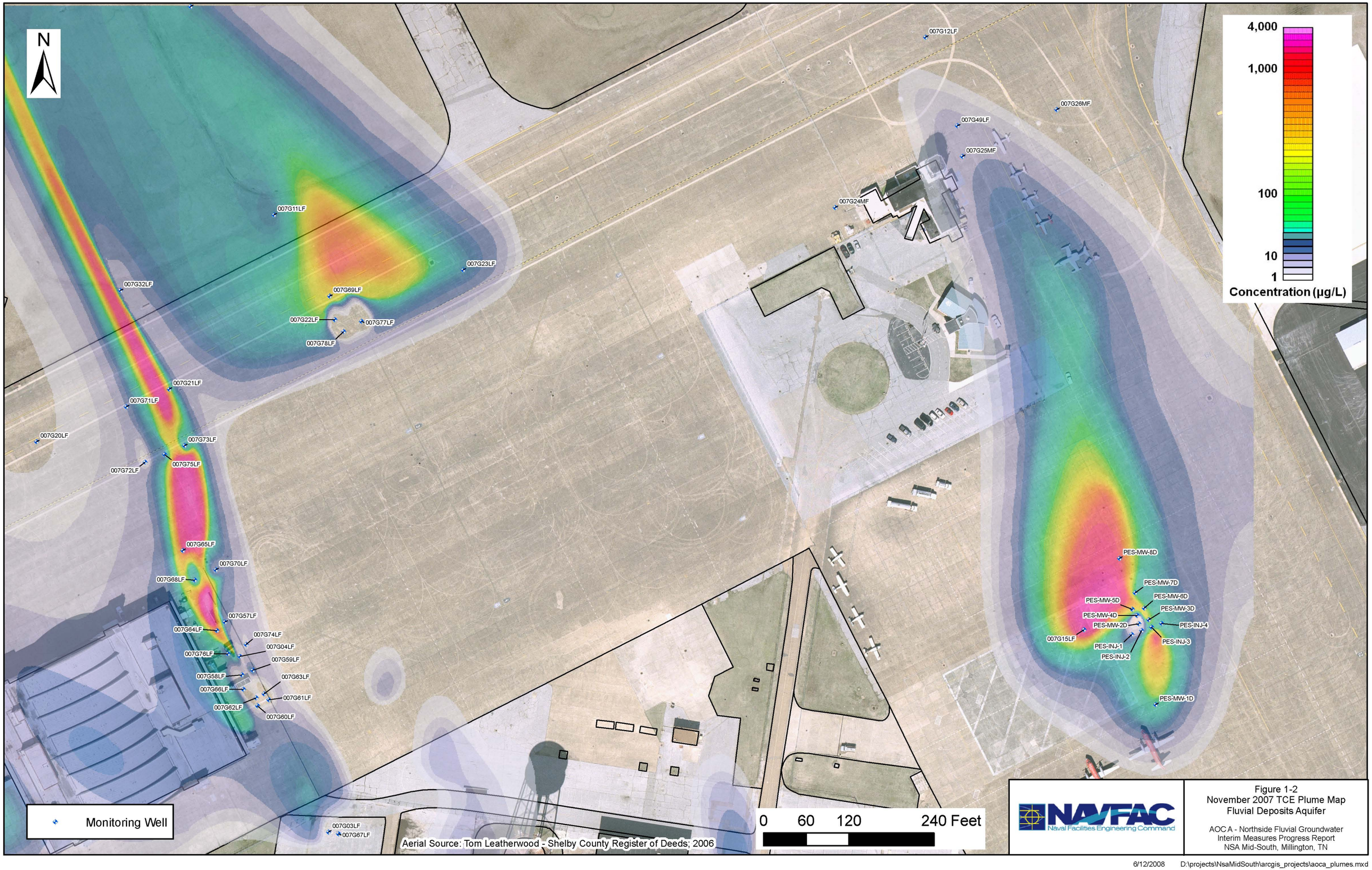
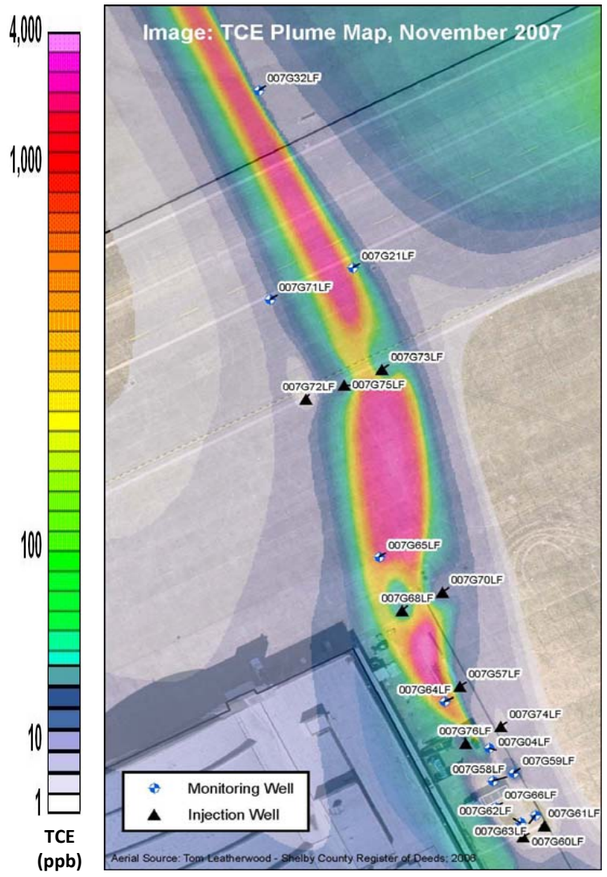


Figure 1-2
November 2007 TCE Plume Map
Fluvial Deposits Aquifer

AOC A - Northside Fluvial Groundwater
Interim Measures Progress Report
NSA Mid-South, Millington, TN

6/12/2008 D:\projects\NsaMidSouth\arcgis_projects\aoca_plumes.mxd



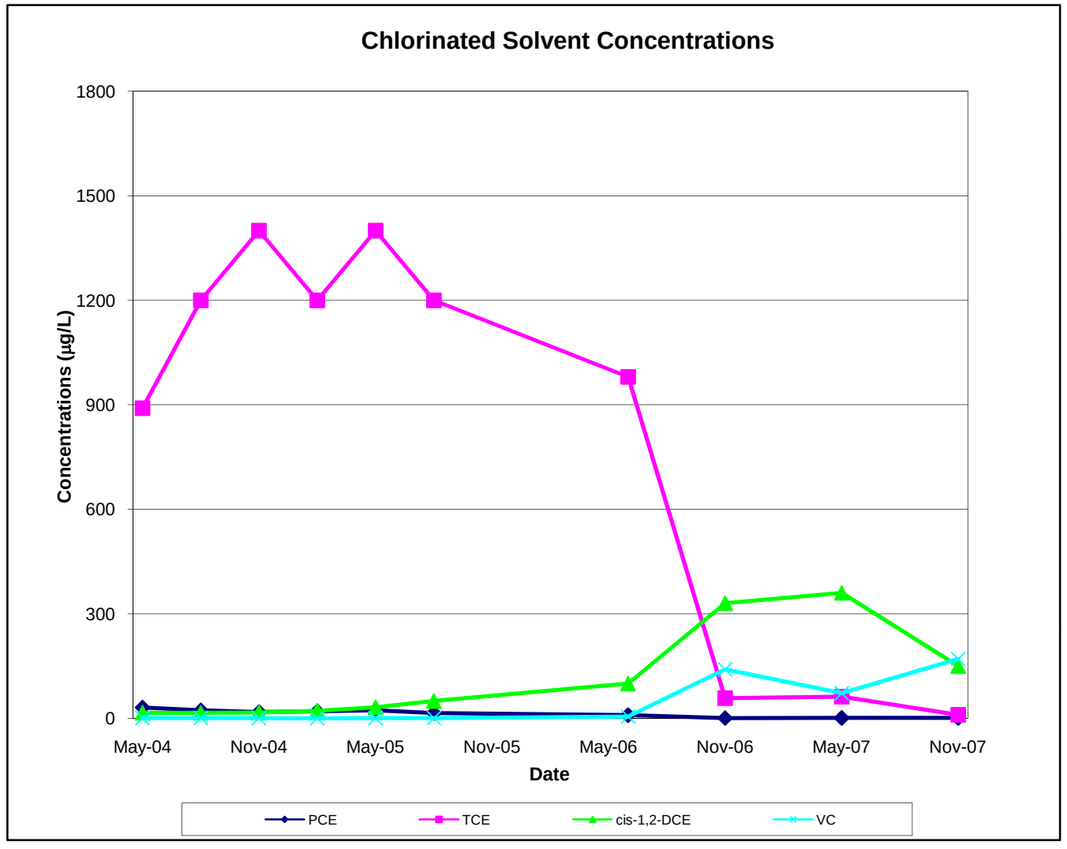
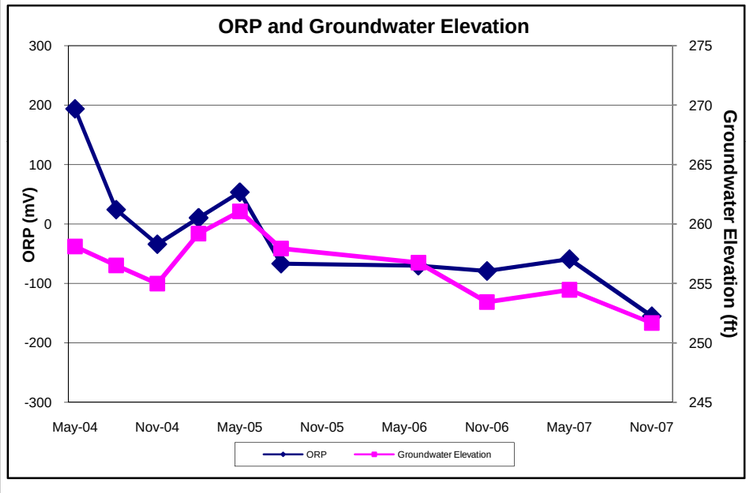


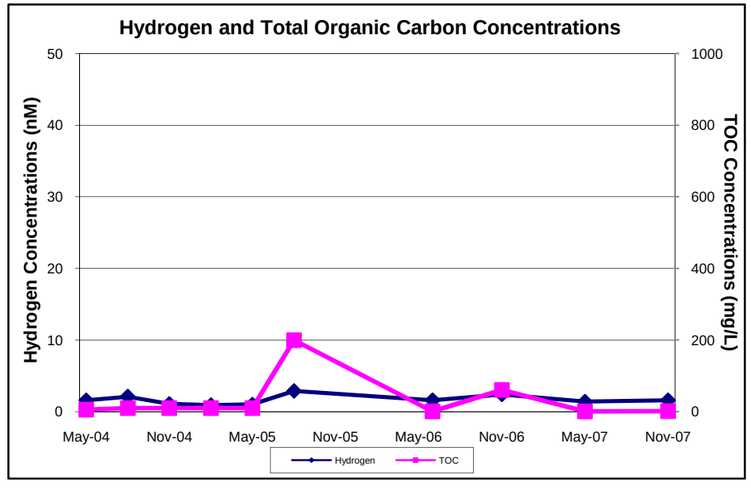
Figure C-7
Sub-Plume A:
MW 007G57LF

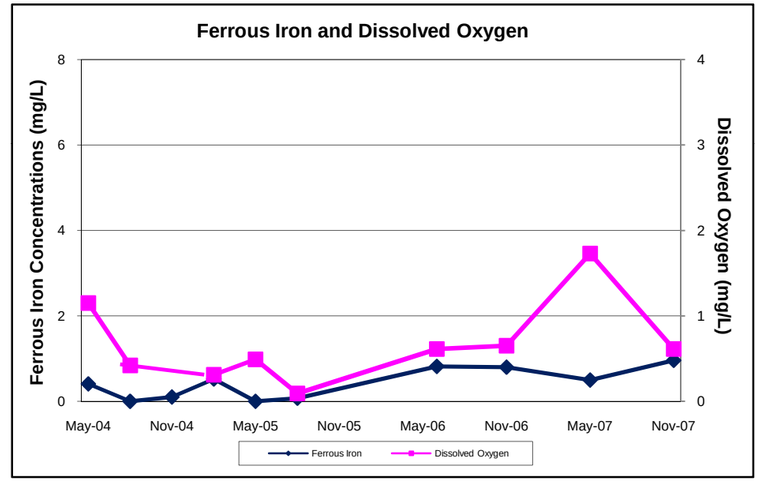
Date	Contaminants (µg/L)			
	PCE	TCE	cis-1,2-DCE	VC
May-04	31	890	16	<0.5
Aug-04	23	1,200	16	<0.5
Nov-04	18	1,400	14	<0.5
Feb-05	20	1,200	22	<10
May-05	23	1,400	32	0.44 J
Aug-05	15	1,200	50	0.6
Jun-06	9	980	100	5
Nov-06	<1	58	330 J	140
May-07	1 U	62	360	72
Nov-07	1 U	10	150	170

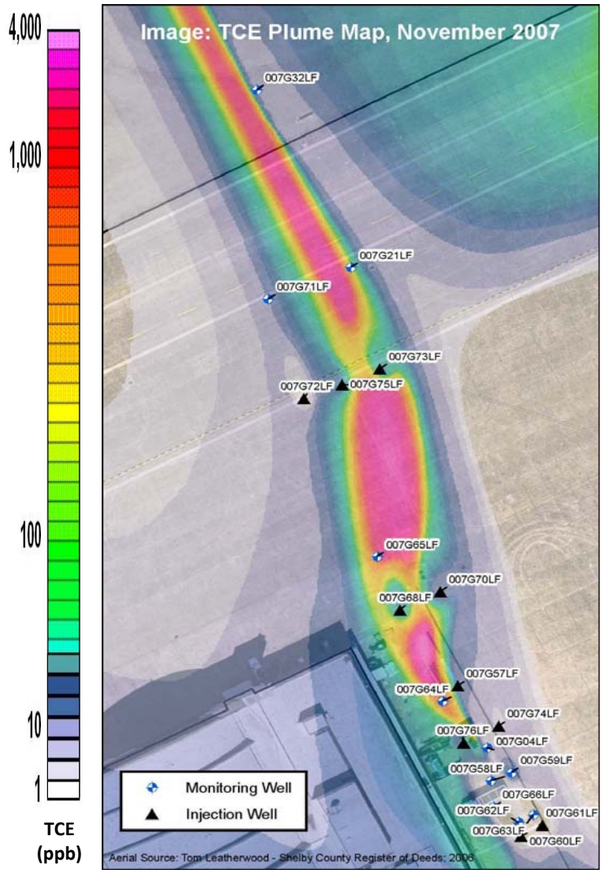
Notes:

µg/L	micrograms per liter
PCE	tetrachloroethene
TCE	trichloroethene
cis-1,2-DCE	cis-1,2-dichloroethene
VC	vinyl chloride
J	Estimated
<	Undetected at the limit indicated









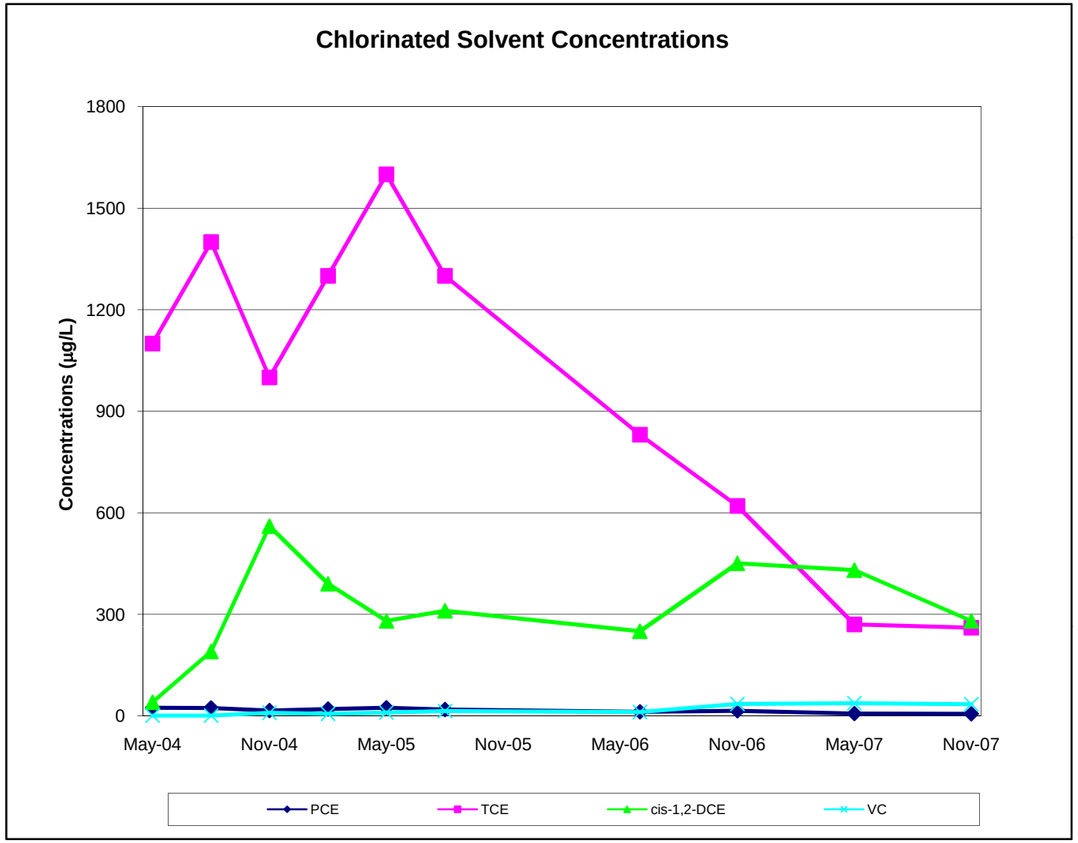
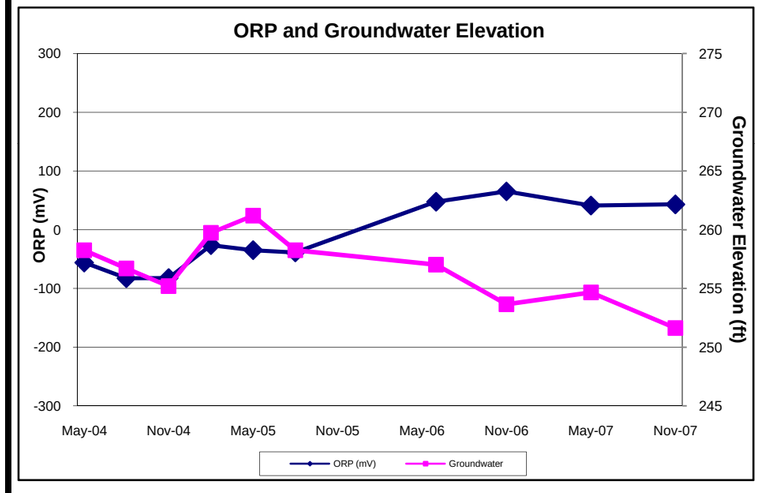


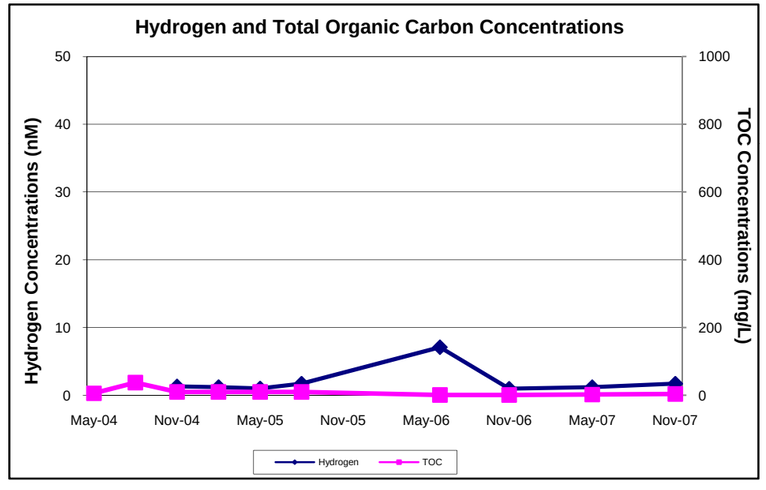
Figure C-8
Sub-Plume A:
MW 007G64LF

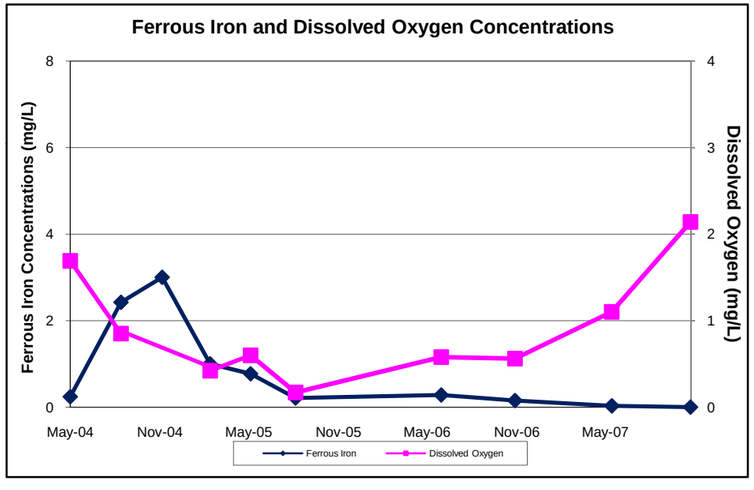
Date	Contaminants (µg/L)			
	PCE	TCE	cis-1,2-DCE	VC
May-04	24 J	1,100	40	<0.5
Aug-04	23	1,400	190	0.6
Nov-04	16	1,000	560	9
Feb-05	20	1,300	390	6
May-05	24	1,600	280	9.6
Aug-05	19	1,300	310	14
Jun-06	12	830	250	11
Nov-06	15	620	450 J	35
May-07	7	270	430	37
Nov-07	6	260	280	34

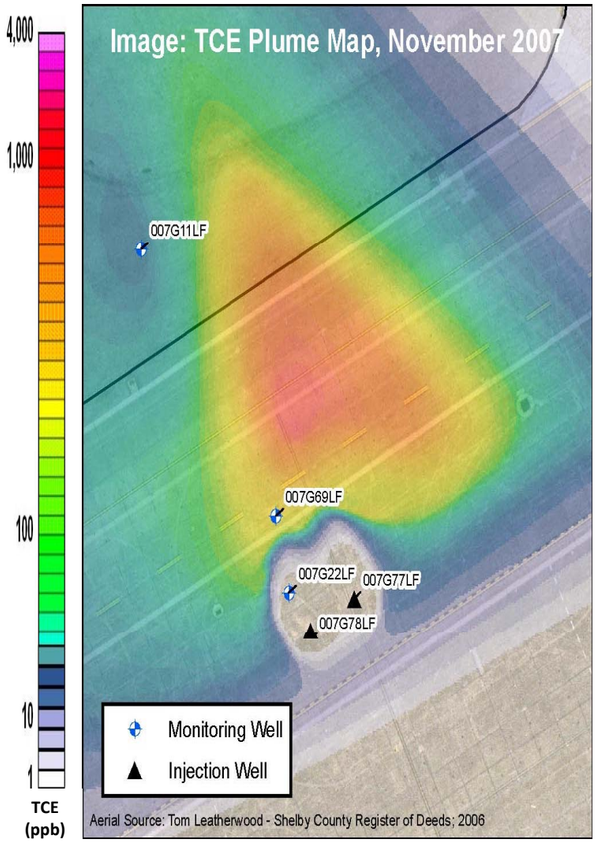
Notes:

µg/L	micrograms per liter
PCE	tetrachloroethene
TCE	trichloroethene
cis-1,2-DCE	cis-1,2-dichloroethene
VC	vinyl chloride
J	Estimated
<	Undetected at the limit indicated









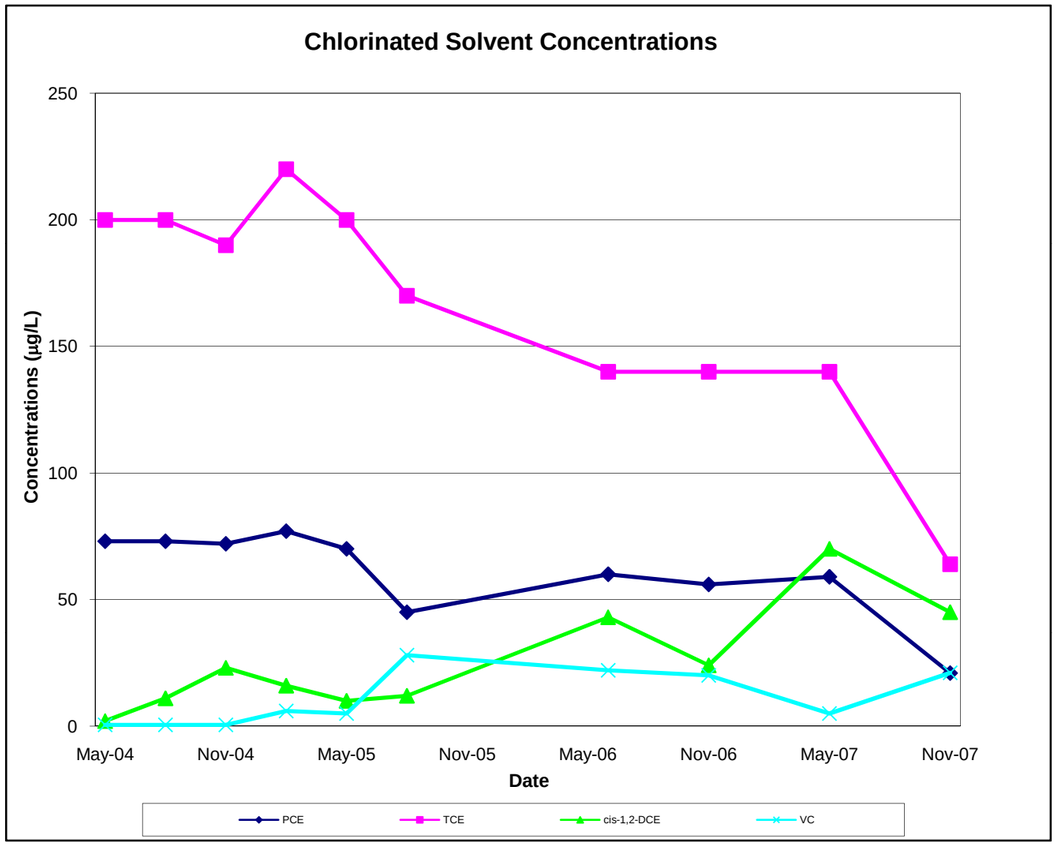
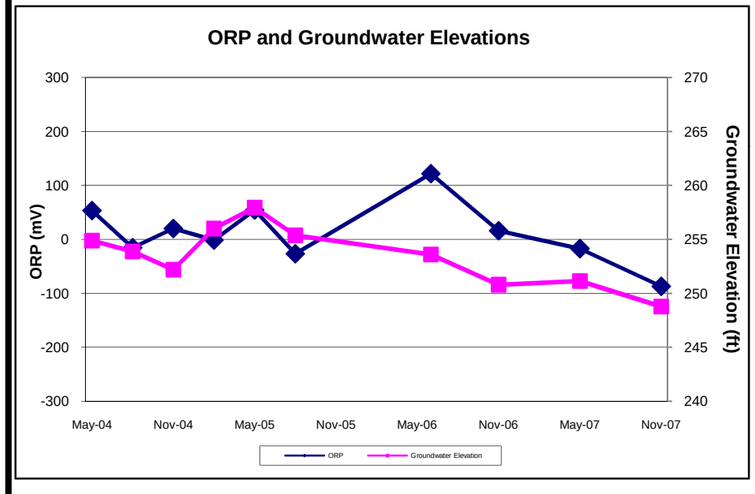


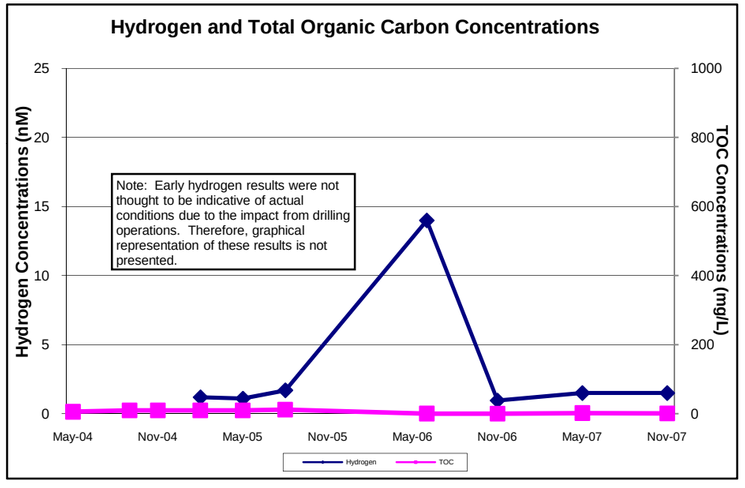
Figure C-13
Sub-Plume B:
MW 007G69LF

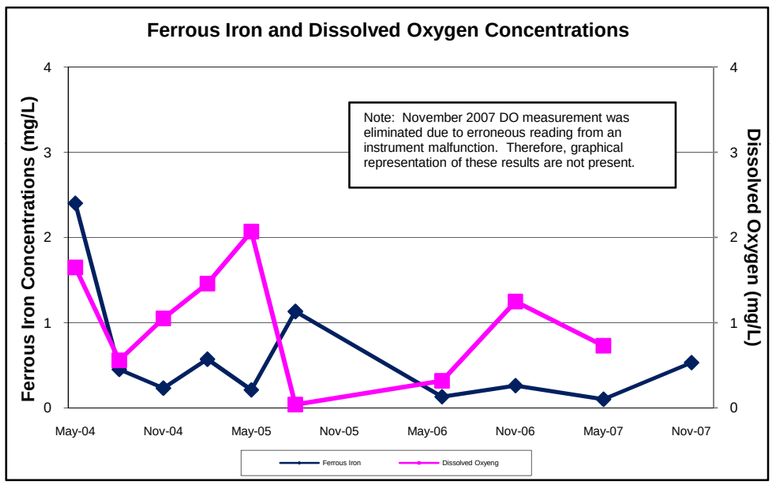
Date	Contaminants (µg/L)			
	PCE	TCE	cis-1,2-DCE	VC
May-04	73	200	2	<0.5
Sep-04	73	200	11	<0.5
Nov-04	72	190	23	<0.5
Feb-05	77	220	16	6
May-05	70	200	10	5
Aug-05	45	170	12 J	28 J
Jun-06	60	140	43	22
Nov-06	56	140	24	20
May-07	59	140	70	5
Nov-07	21	64	45	21

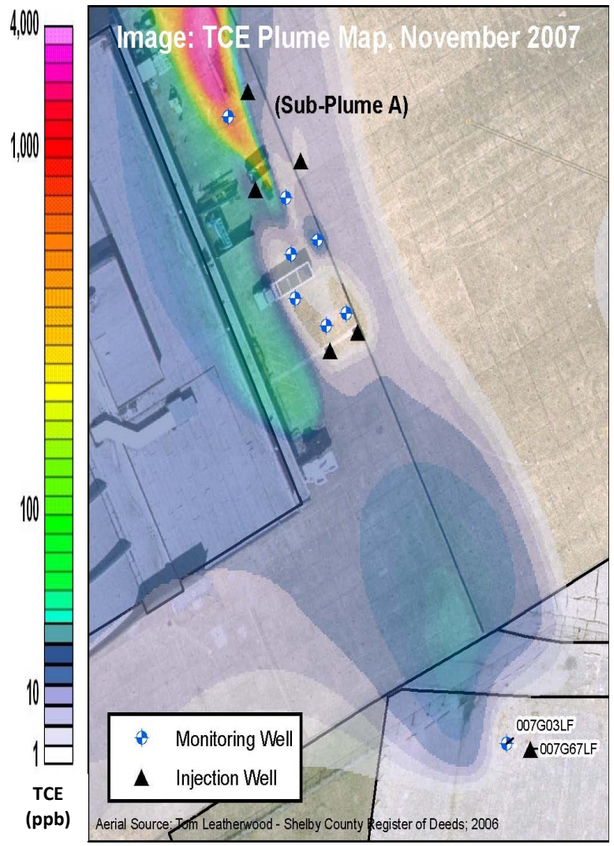
Notes:

µg/L	micrograms per liter
PCE	tetrachloroethene
TCE	trichloroethene
cis-1,2-DCE	cis-1,2-dichloroethene
VC	vinyl chloride
J	Estimated
<	Undetected at the limit indicated









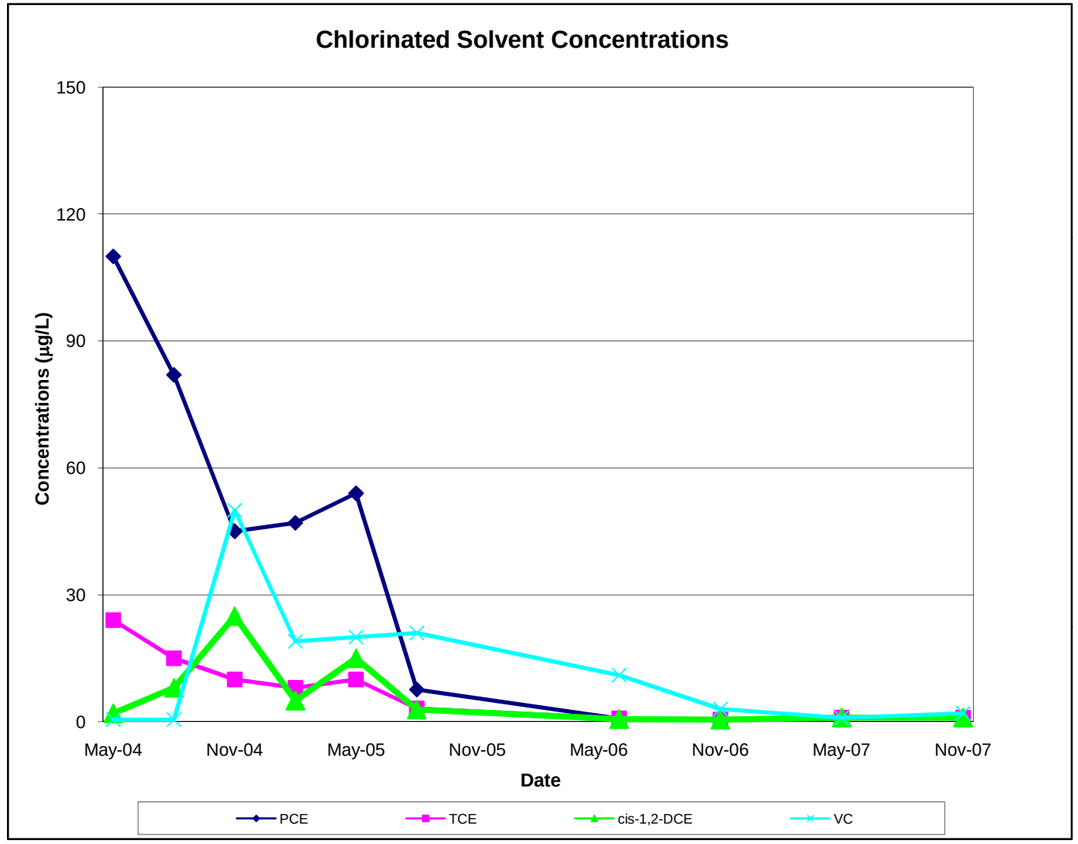
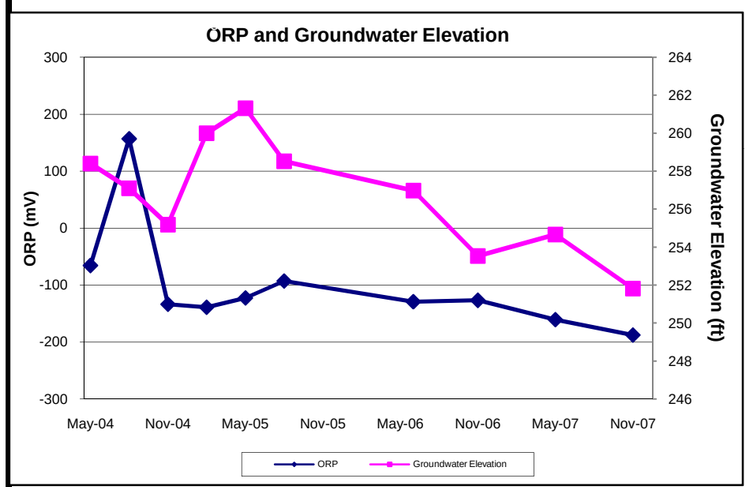


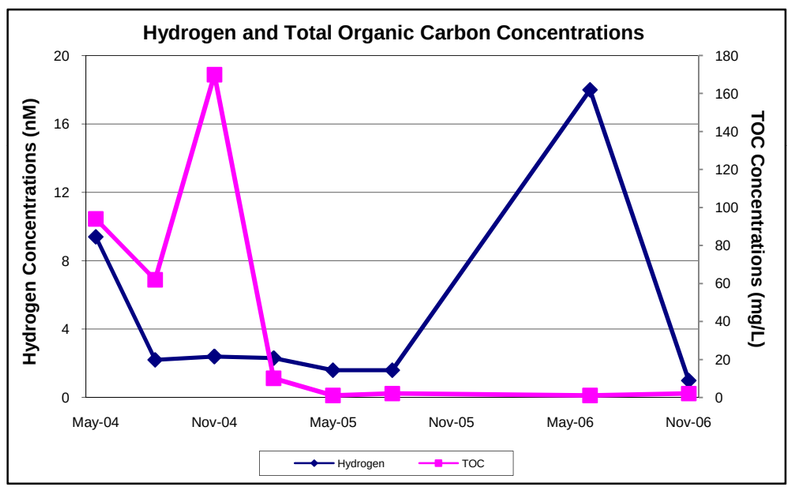
Figure C-14
Sub-Plume C:
MW 007G03LF

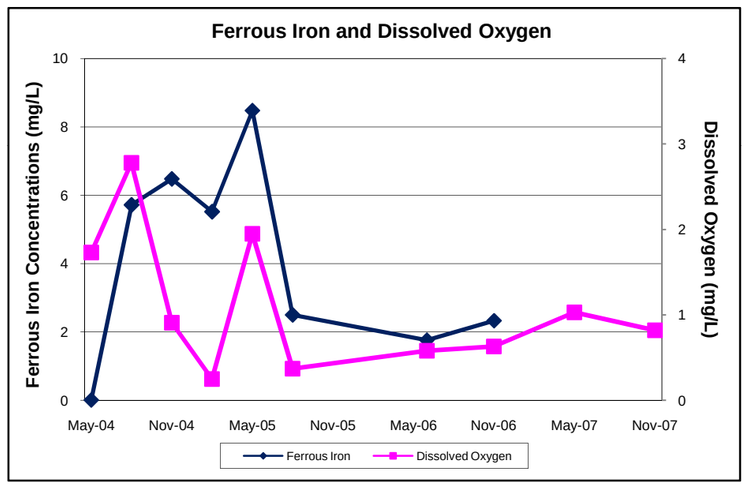
Date	Contaminants (µg/L)			
	PCE	TCE	cis-1,2-DCE	VC
May-04	110	24	2	<0.5
Aug-04	82	15	8	<0.5
Nov-04	44	10	25	46
Feb-05	47	7	5	19
May-05	54	10	15	20
Aug-05	7.6	3.1	2.9	21
Jun-06	0.7 J	0.8 J	0.6 J	11
Nov-06	<1	0.5 J	<1	3
May-07	<1	1	<1	0.9 J
Nov-07	<1	1	<1	<2

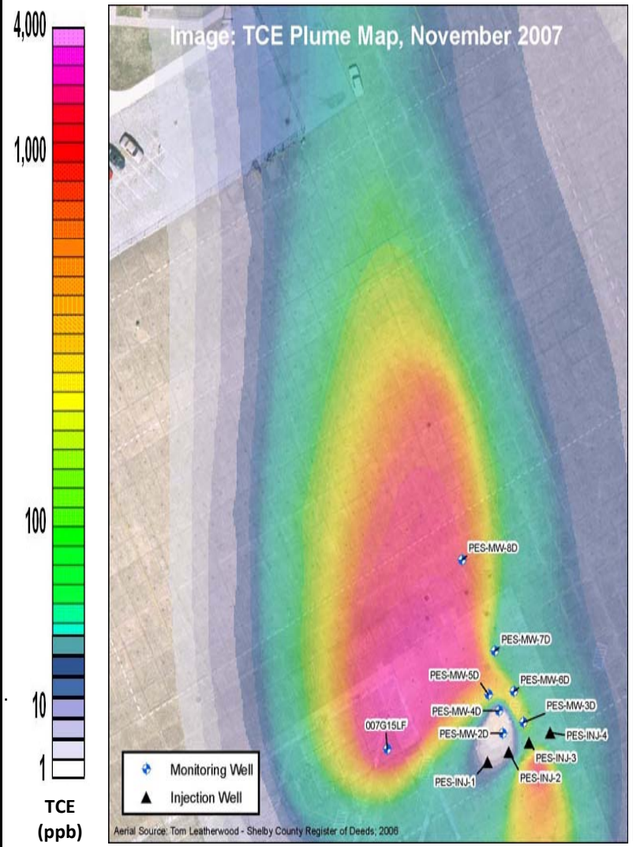
Notes:

µg/L	micrograms per liter
PCE	tetrachloroethene
TCE	trichloroethene
cis-1,2-DCE	cis-1,2-dichloroethene
VC	vinyl chloride
<	Undetected at the limit indicated
J	Estimated









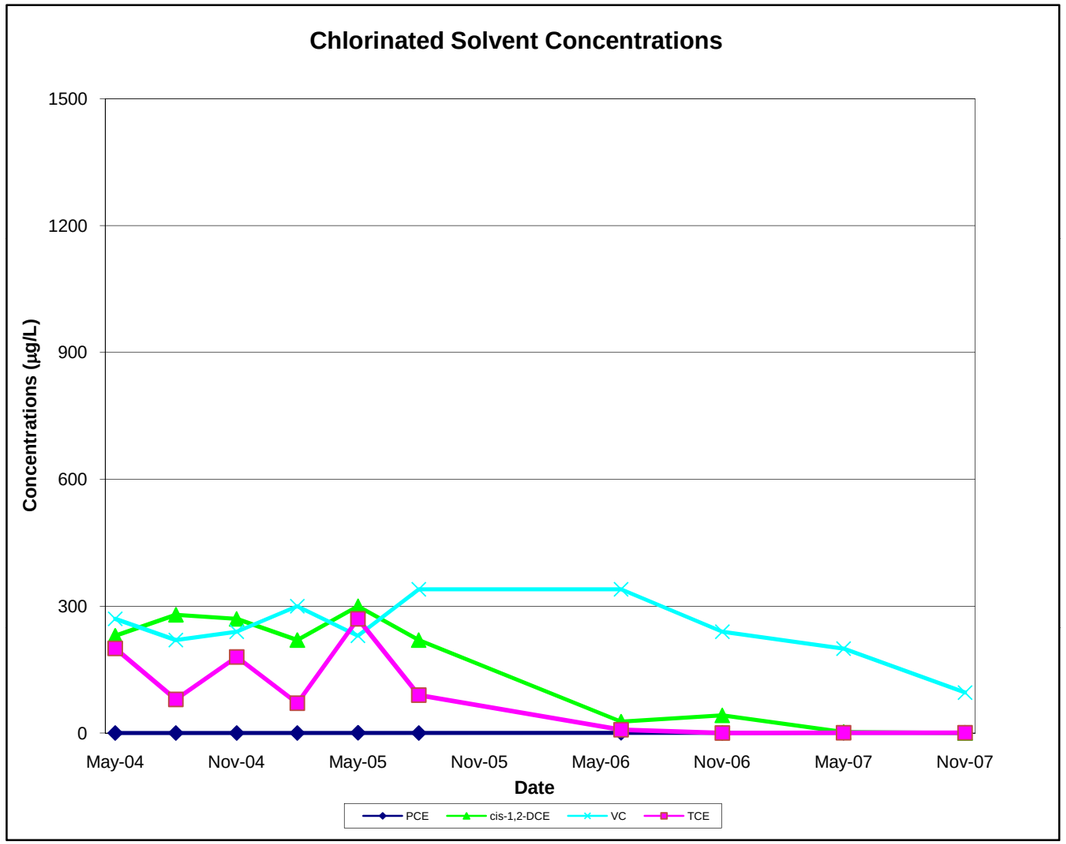
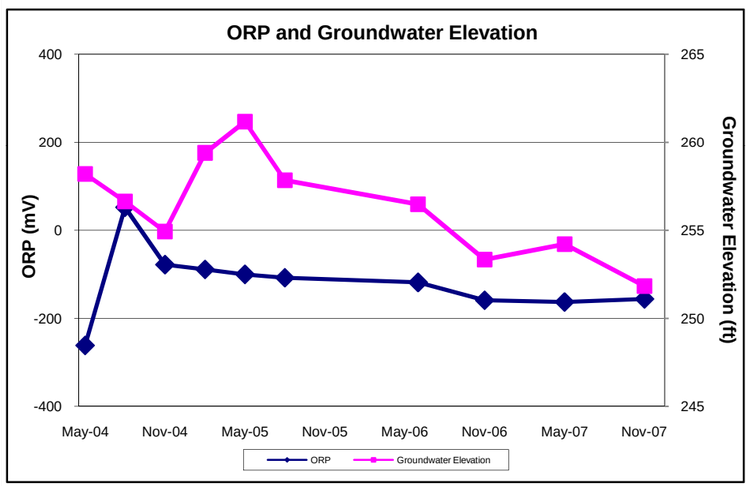


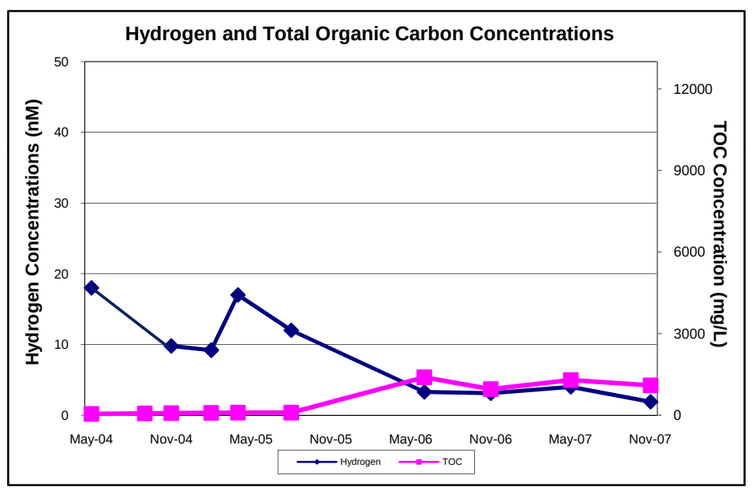
Figure C-15
Sub-Plume D:
MW PESMW2S

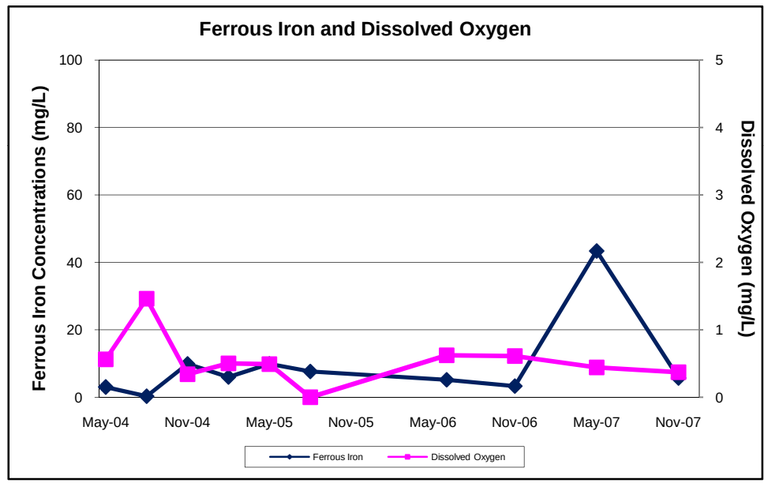
Date	Constituents (µg/L)			
	PCE	TCE	cis-1,2-DCE	VC
May-04	<0.5	200	230	270
Aug-04	<0.5	80	280	220
Nov-04	<0.5	180	270	240
Feb-05	<0.5	71	220	300
May-05	1	270	300	230
Aug-05	<0.5	90	220	340
Jun-06	<1	8	27	340
Nov-06	<1	<1	42	240
May-07	<1	<1	3	200
Nov-07	<1	<1	0.4 J	96

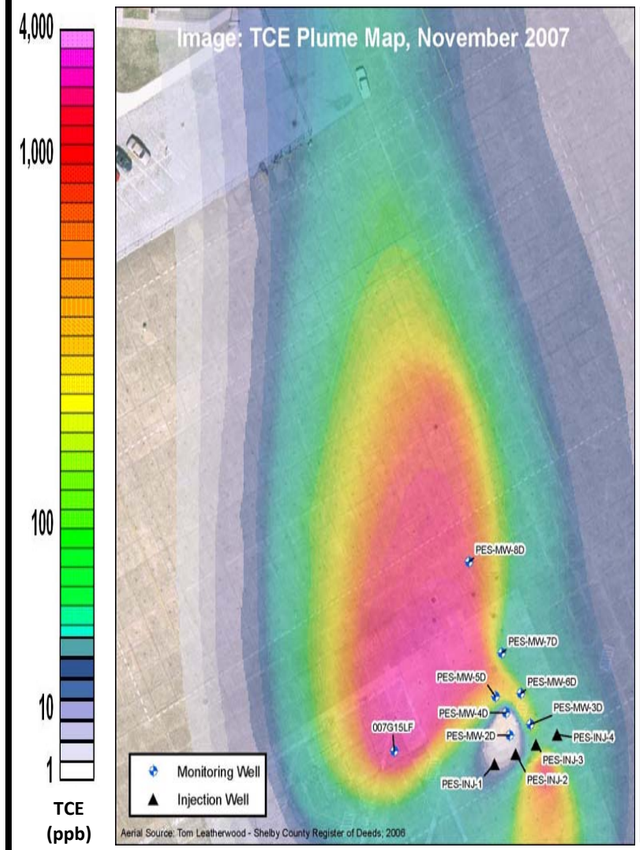
Notes:

µg/L	micrograms per liter
PCE	tetrachloroethene
TCE	trichloroethene
cis-1,2-DCE	cis-1,2-dichloroethene
VC	vinyl chloride
<	Undetected at the limit indicated









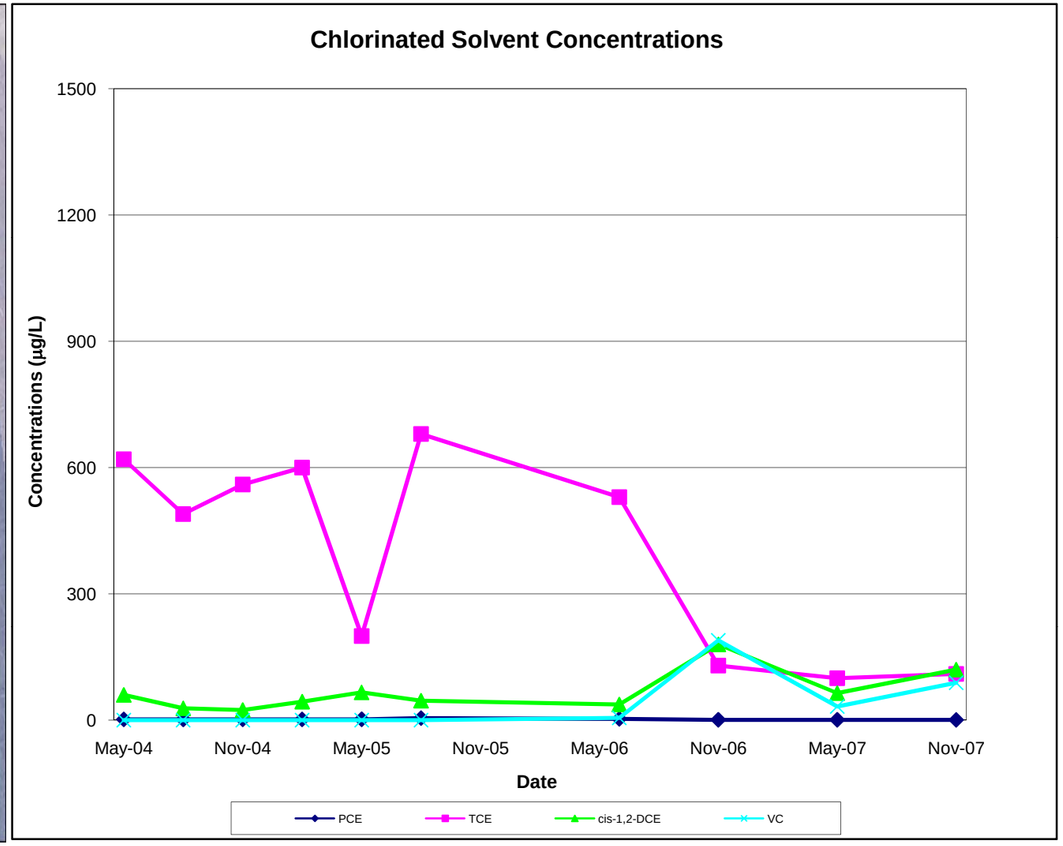
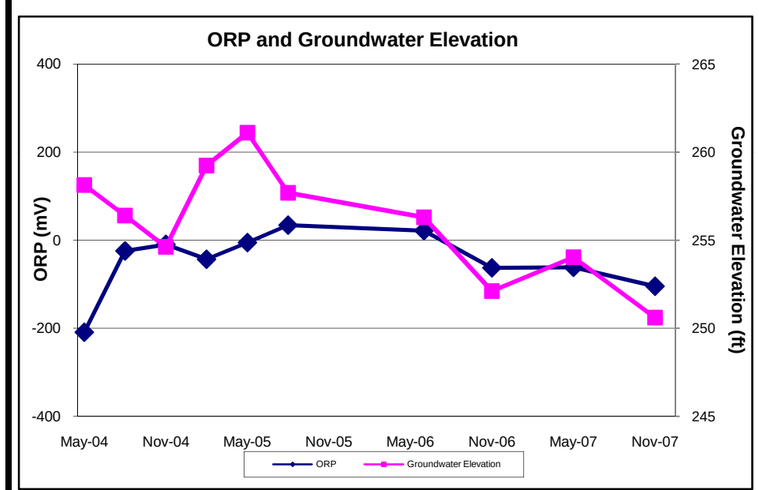


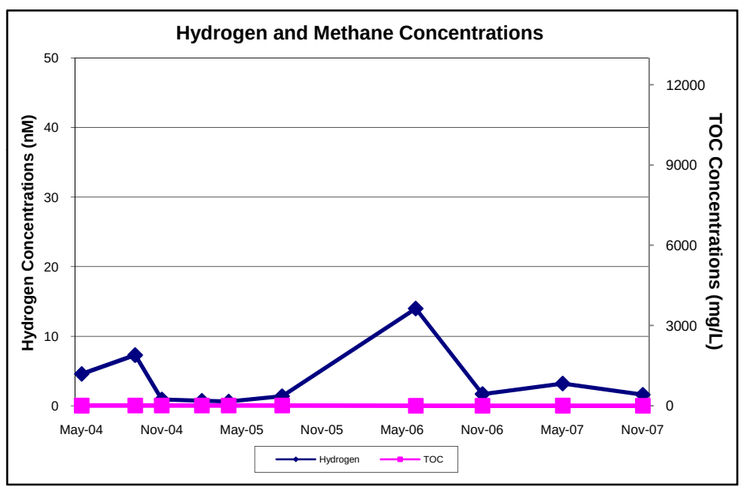
Figure C-17
Sub-Plume D:
MW PESMW3S

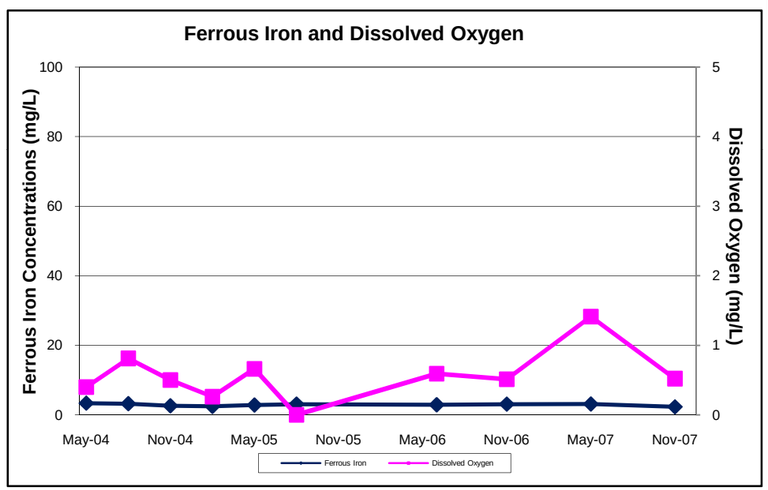
Date	Constituents (µg/L)			
	PCE	TCE	cis-1,2-DCE	VC
May-04	2	620	60	<0.5
Aug-04	2	490	28 J	<0.5
Nov-04	2 J	560	24 J	0.4 J
Feb-05	2	600	44	<0.5
May-05	2.2	200	66	0.66
Aug-05	4.9 J	680	46	0.66 J
Jun-06	3	530	37	7
Nov-06	1	130	180	190
May-07	<1	100	64	33
Nov-07	0.8 J	110	120	89

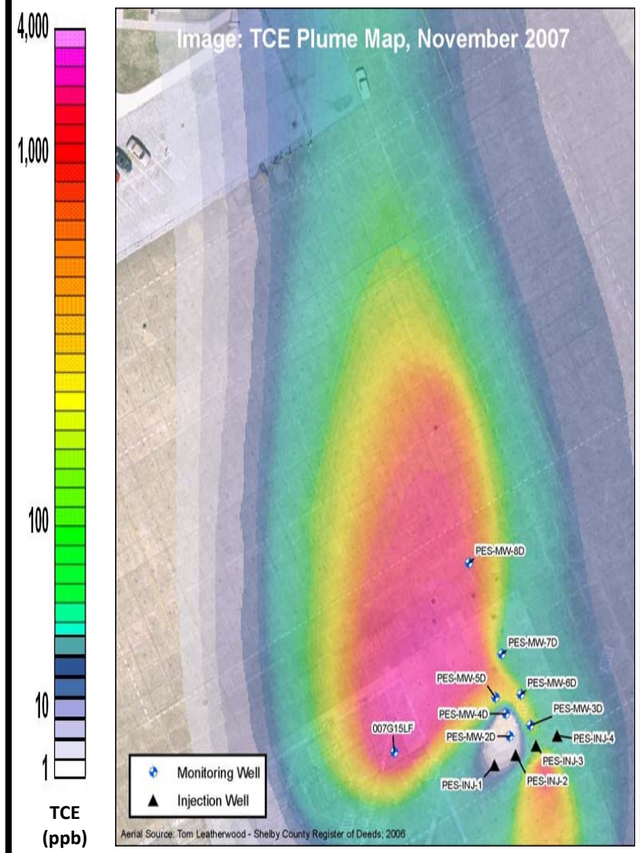
Notes:

µg/L	micrograms per liter
PCE	tetrachloroethene
TCE	trichloroethene
cis-1,2-DCE	cis-1,2-dichloroethene
VC	vinyl chloride
J	Estimated
<	Undetected at the limit indicated









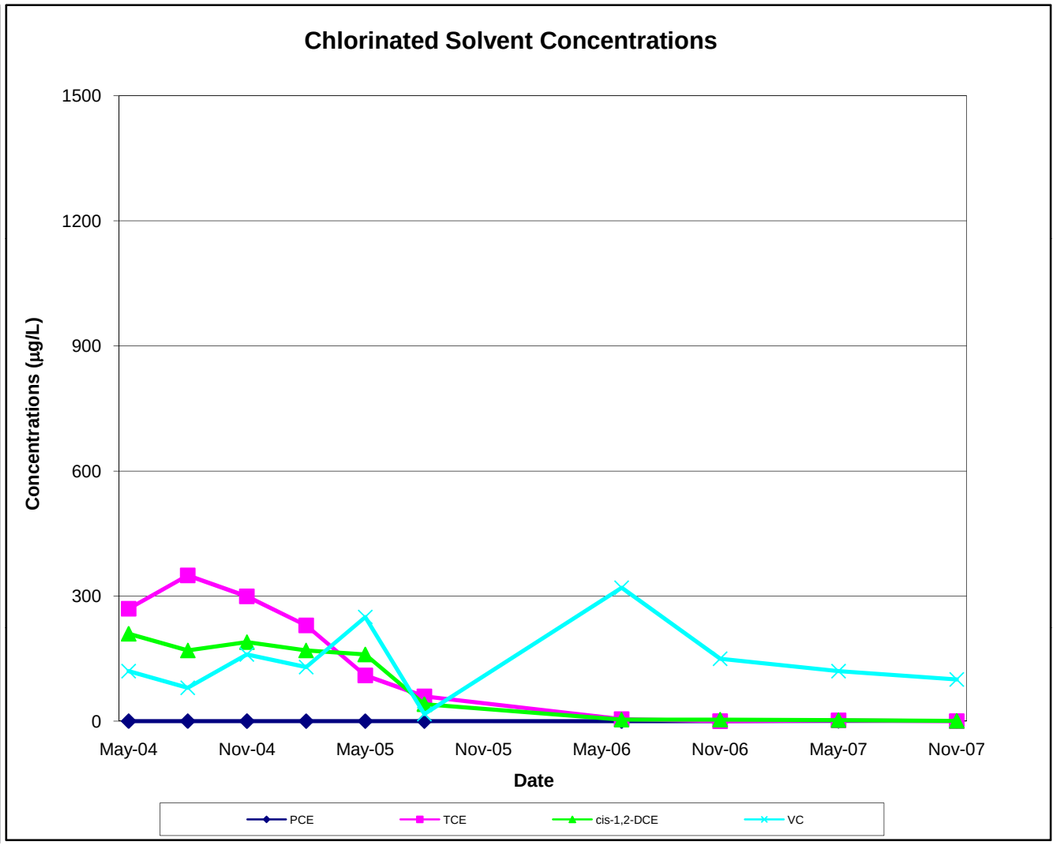
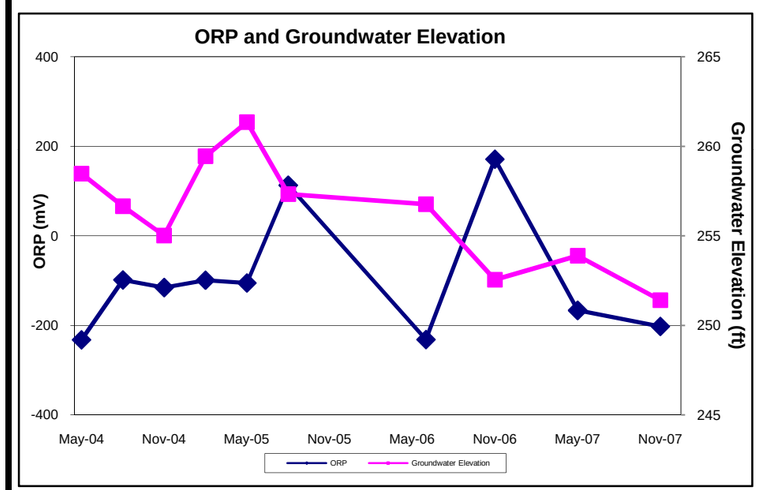


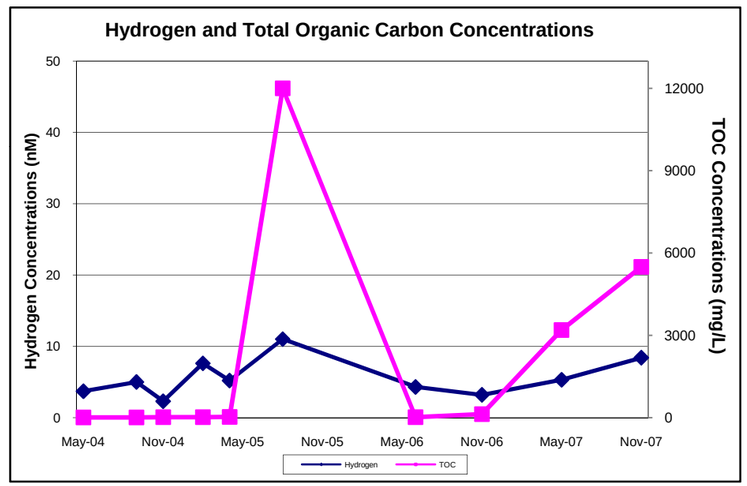
Figure C-19
Sub-Plume D:
MW PESMW4S

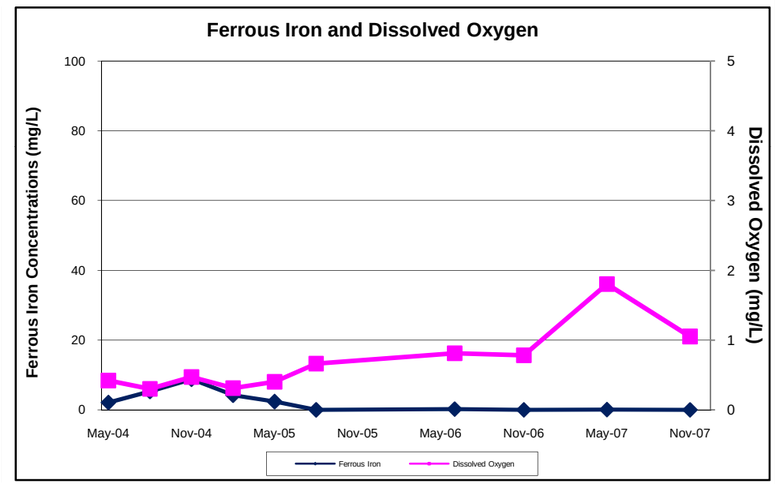
Date	Constituents (µg/L)			
	PCE	TCE	cis-1,2-DCE	VC
May-04	0.6	270	210	120
Aug-04	0.6	350	170	80
Nov-04	0.4 J	300	190	160
Feb-05	<0.5	230	170	130
May-05	<0.5	110	160	250
Aug-05	<0.5	60	41	17
Jun-06	<1	5	4	320
Nov-06	<1	<1	4	150
May-07	<1	2	3	120
Nov-07	<1	0.6 J	0.9 J	100

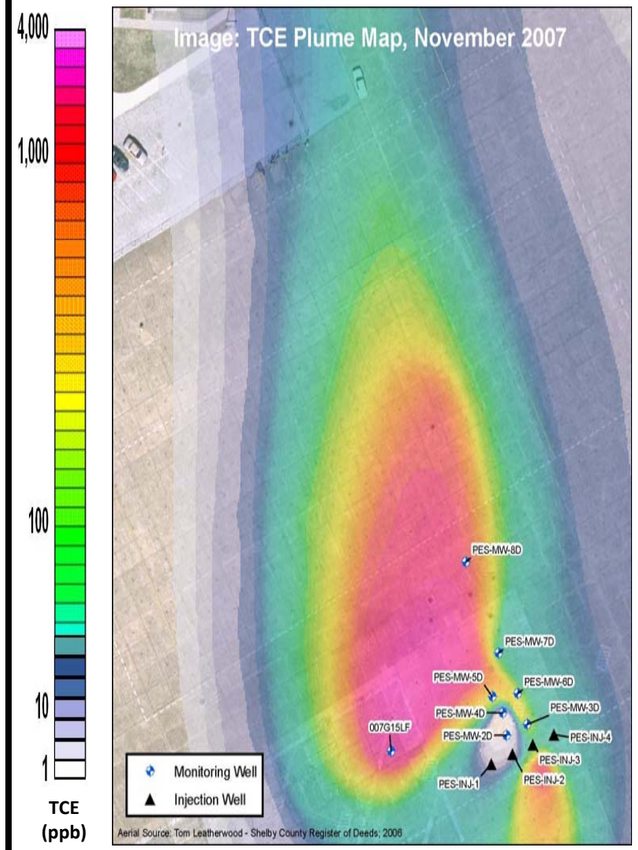
Notes:

µg/L	micrograms per liter
PCE	tetrachloroethene
TCE	trichloroethene
cis-1,2-DCE	cis-1,2-dichloroethene
VC	vinyl chloride
J	Estimated
<	Undetected at the limit indicated









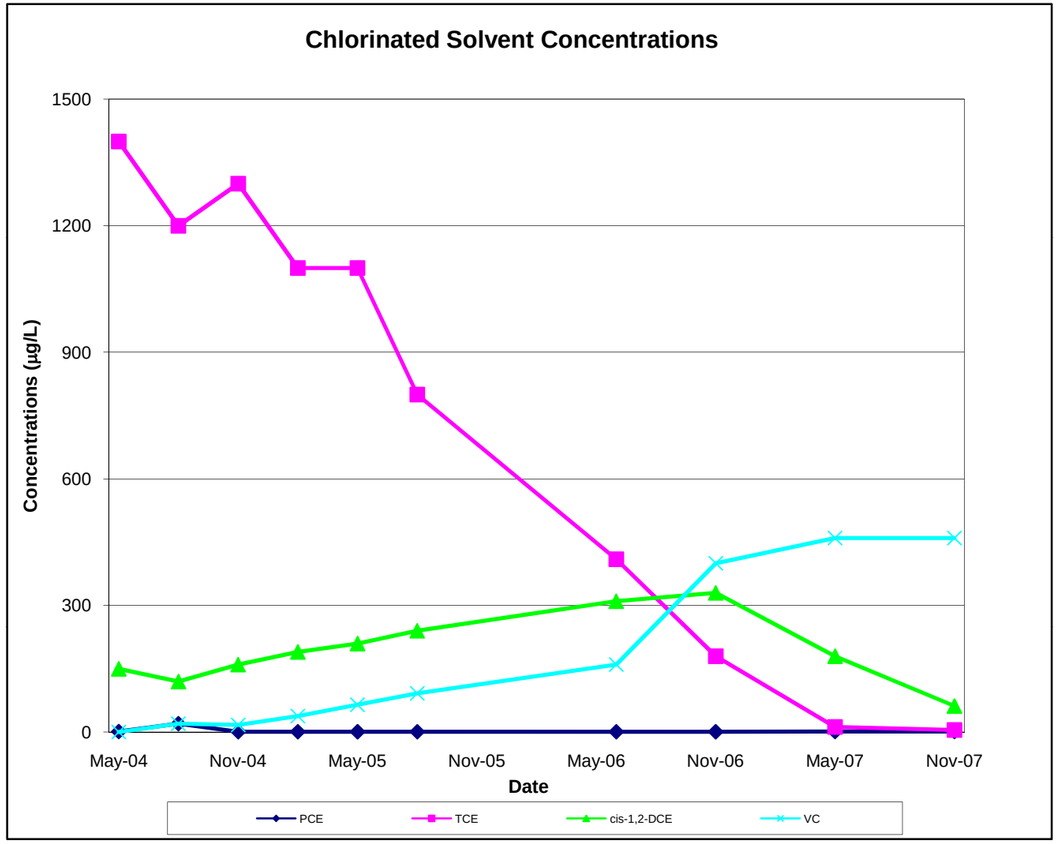
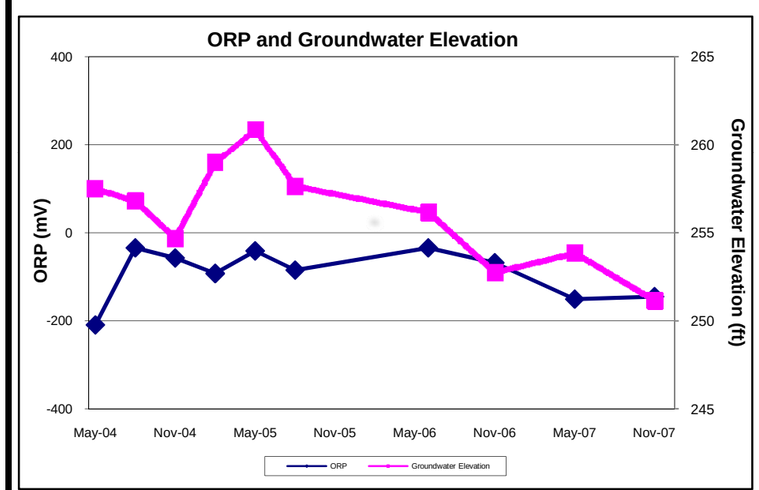


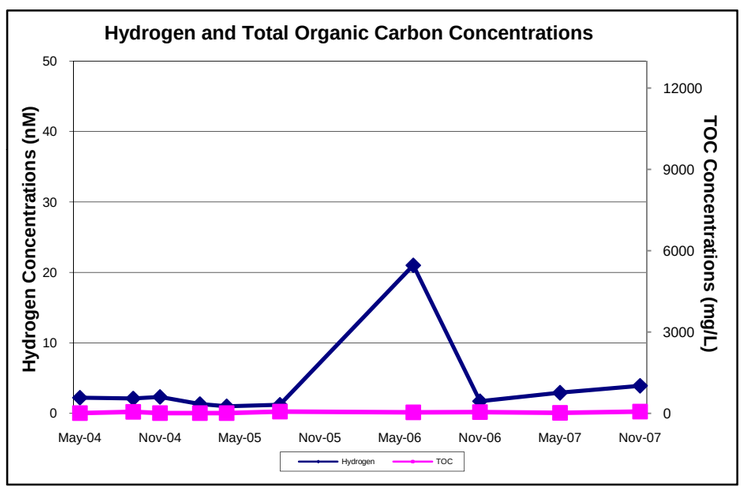
Figure C-22
Sub-Plume D:
MW PESMW7D

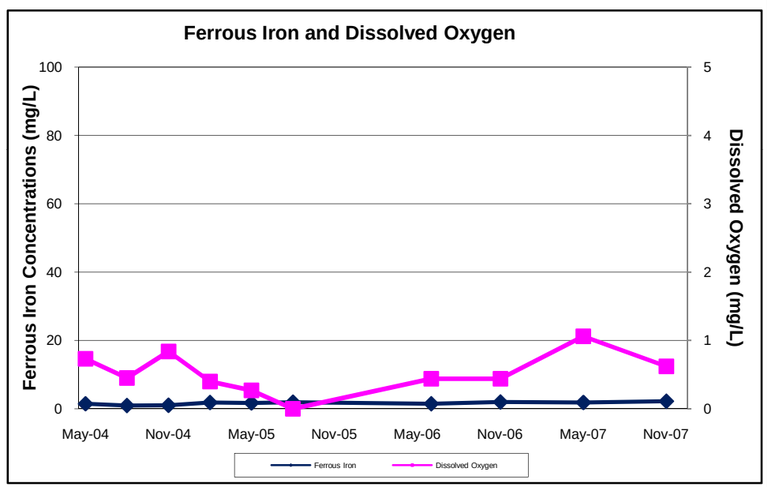
Date	Constituents (µg/L)			
	PCE	TCE	cis-1,2-DCE	VC
May-04	1	1,400	150	<0.5
Aug-04	<20	1,200	120	<0.5
Nov-04	0.9	1,300	160	17
Feb-05	0.9	1,100	190	38
May-05	0.74	1,100	210	65
Aug-05	0.85	800	240	92
Jun-06	<1	410	310	160
Nov-06	<1	180	330	400
May-07	<1	12	180	460
Nov-07	<1	5	62	460

Notes:

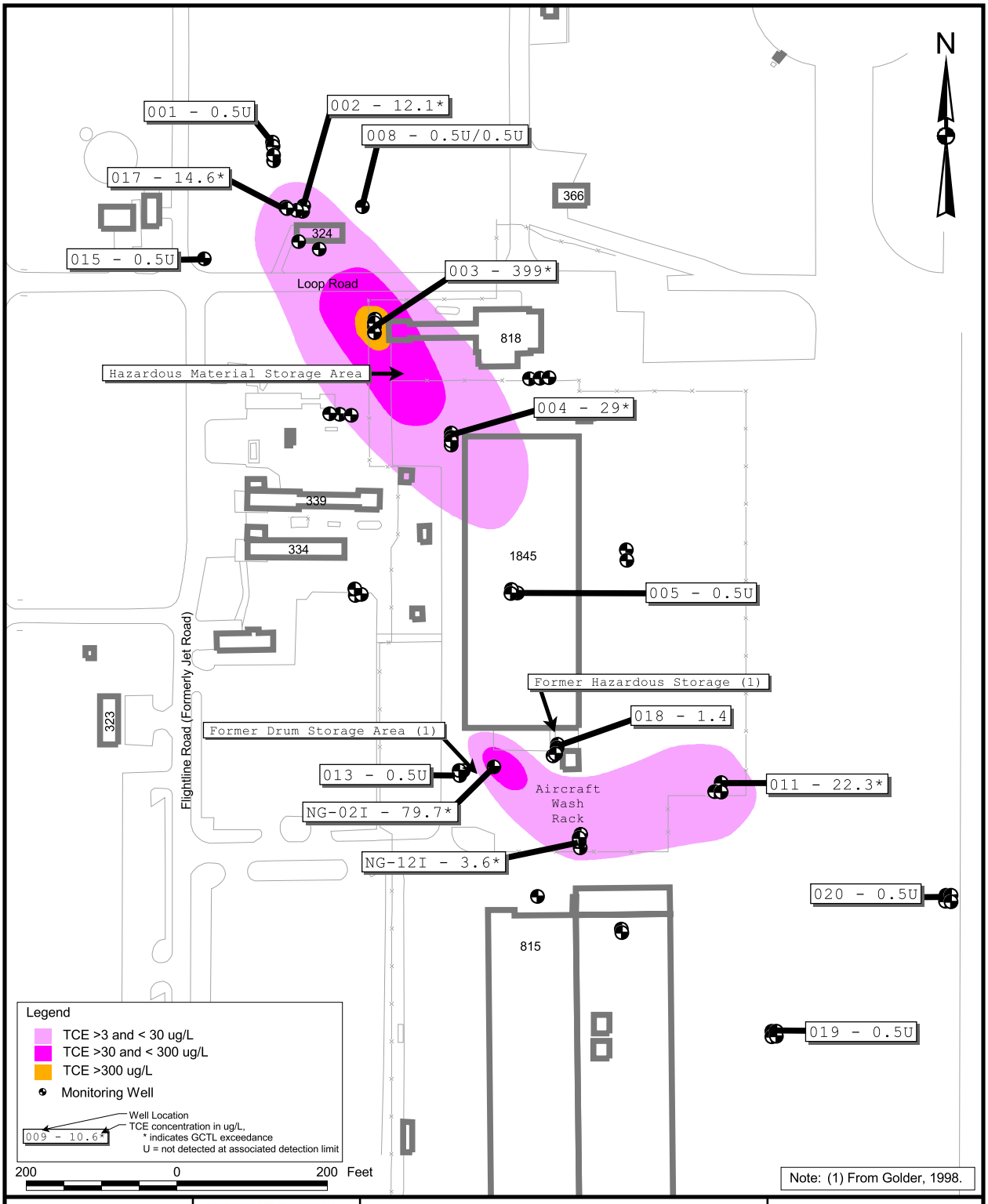
µg/L	micrograms per liter
PCE	tetrachloroethene
TCE	trichloroethene
cis-1,2-DCE	cis-1,2-dichloroethene
VC	vinyl chloride
<	Undetected at the limit indicated





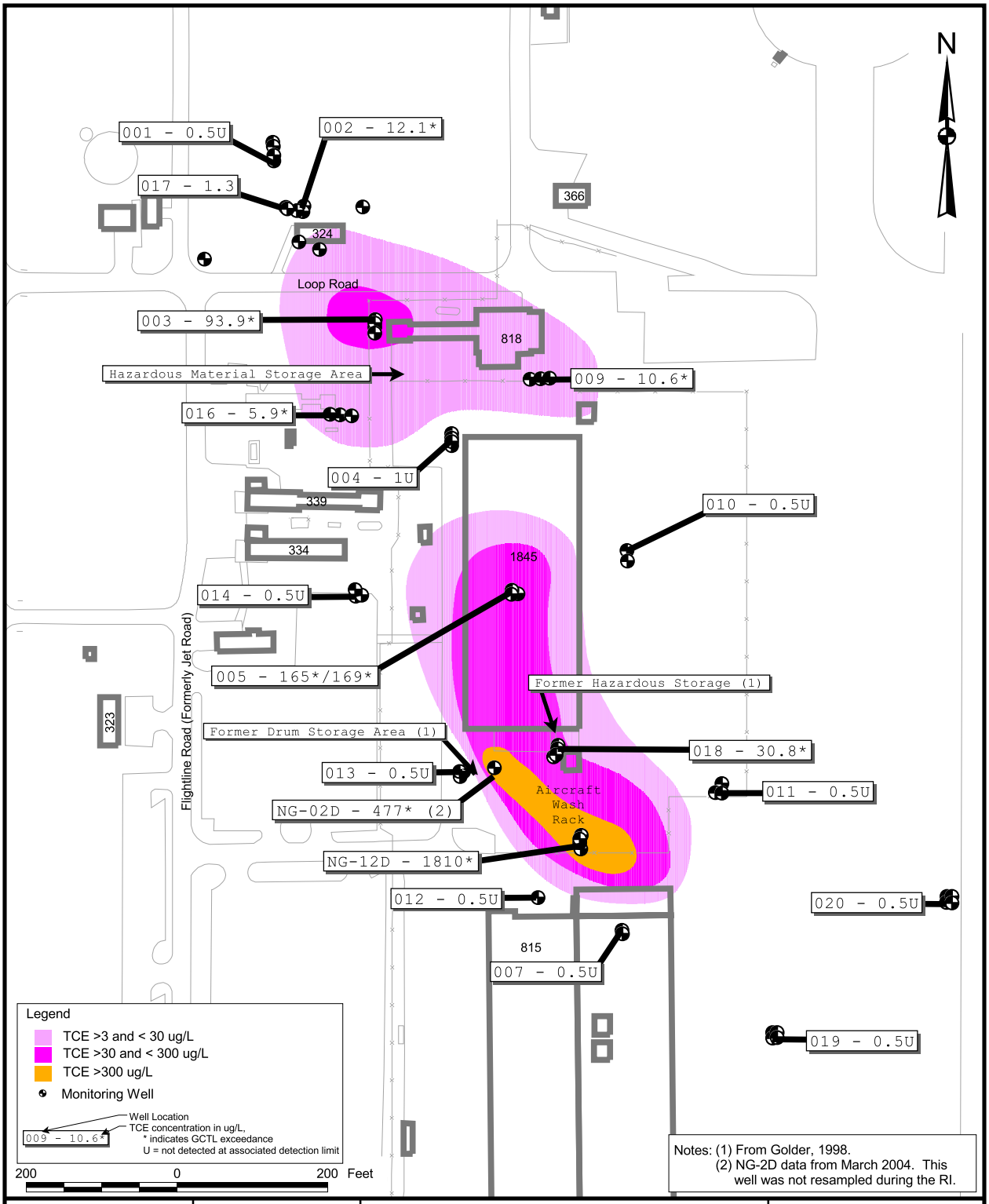


Appendix B-6: Former NAS Cecil Field



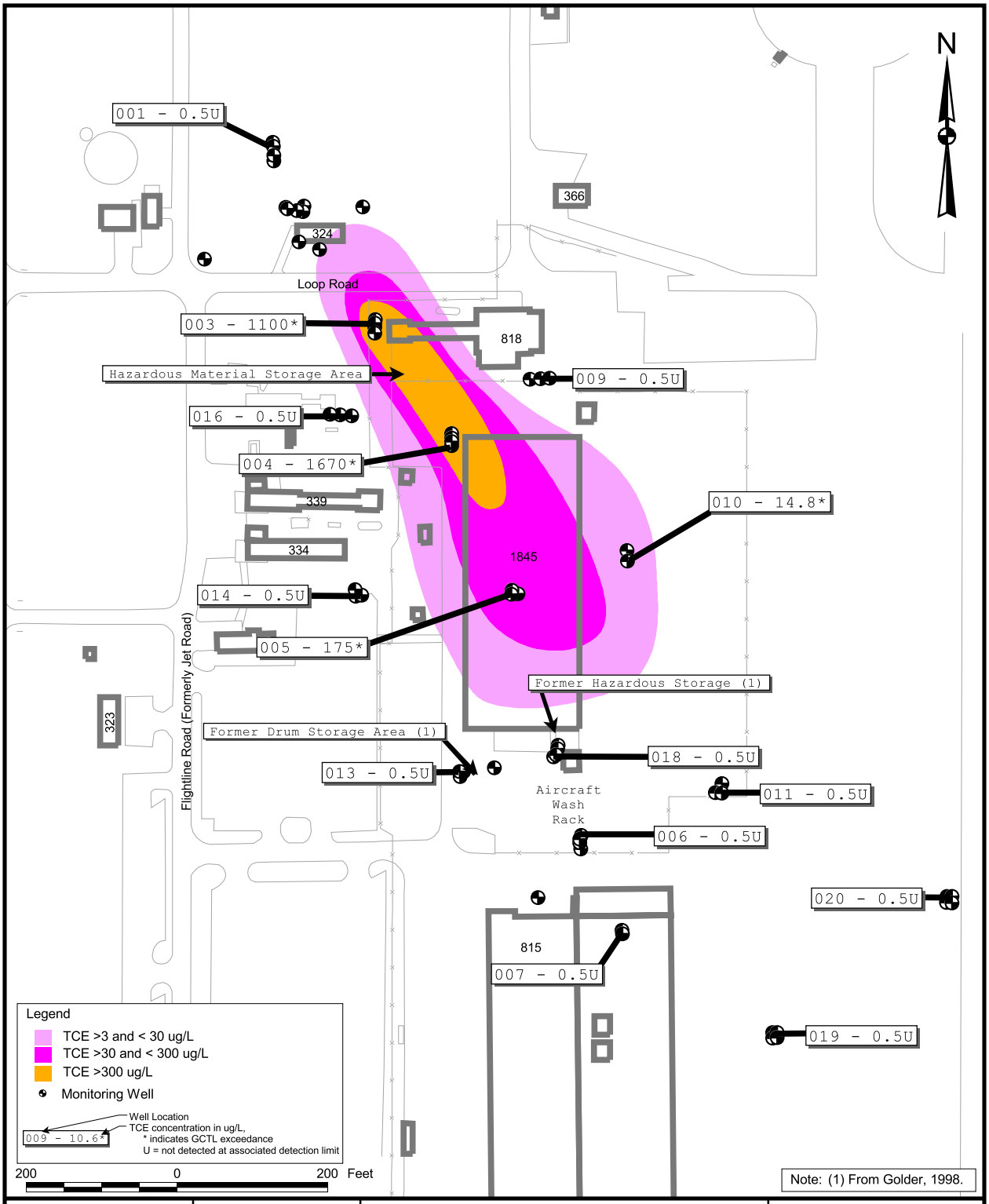
DRAWN BY MJJ	DATE 17Jan06	VOCs IN GROUNDWATER, RI DATA 30-FOOT ZONE OU 9, SITE 59 REMEDIAL INVESTIGATION REPORT NAVAL AIR STATION CECIL FIELD JACKSONVILLE, FLORIDA	CONTRACT NUMBER 8925
CHECKED BY	DATE		APPROVED BY DATE
COST/SCHEDULE-AREA			APPROVED BY DATE
SCALE AS NOTED			DRAWING NO. FIGURE 5-1 / REV 0

P:\GIS\NAS_CecilField\Site-59_RI.apr 25Feb06 MJJ Layout041



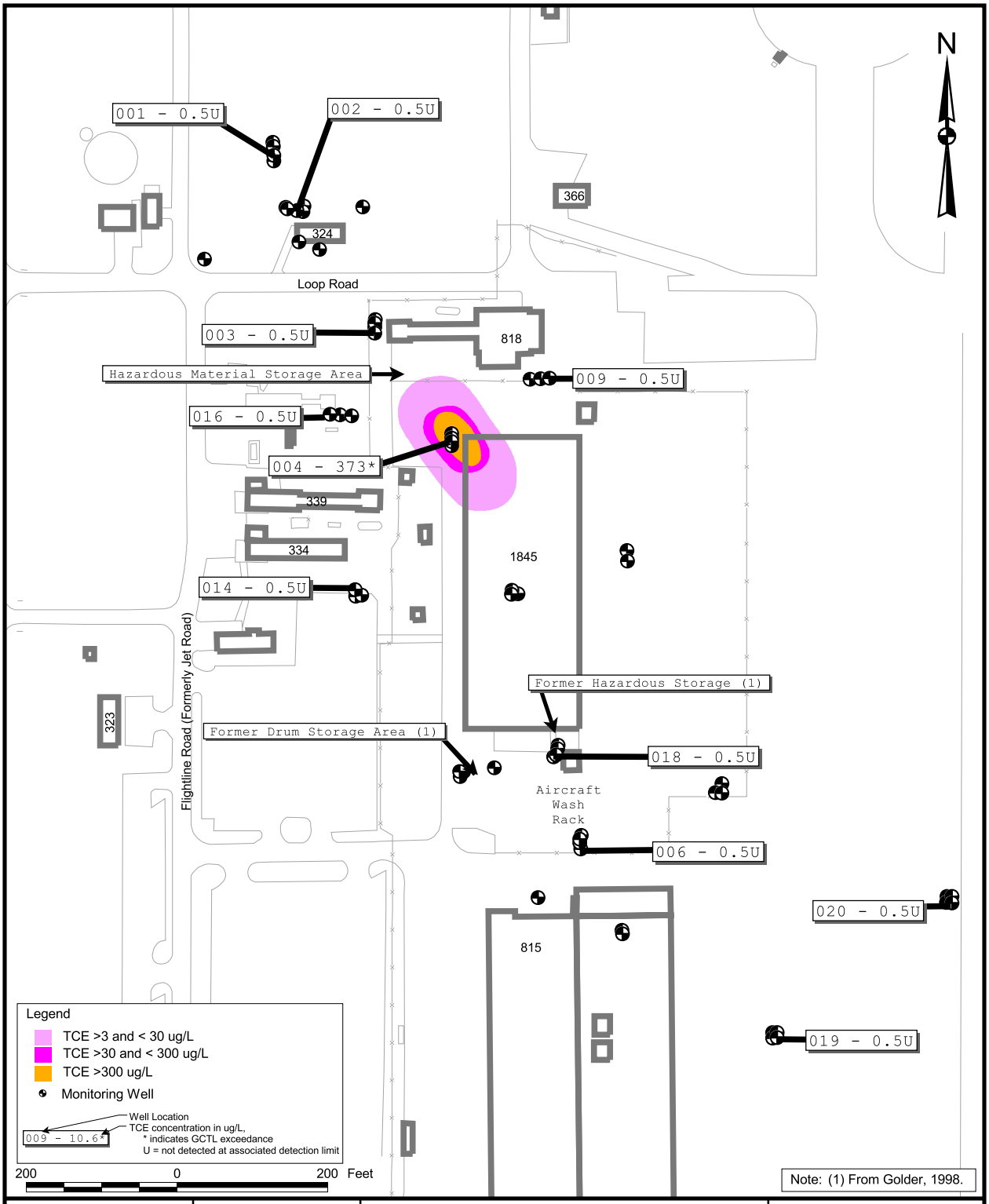
DRAWN BY MJJ	DATE 17Jan06	VOCs IN GROUNDWATER, RI DATA	CONTRACT NUMBER 8925
CHECKED BY	DATE	50-FOOT ZONE	APPROVED BY DATE
COST/SCHEDULE-AREA		OU 9, SITE 59	APPROVED BY DATE
SCALE AS NOTED		REMEDIAL INVESTIGATION REPORT NAVAL AIR STATION CECIL FIELD JACKSONVILLE, FLORIDA	DRAWING NO. FIGURE 5-2 / REV 0

P:\GIS\NAS_CecilField\Site-59_RI.apr 25Feb06 MJJ Layout051



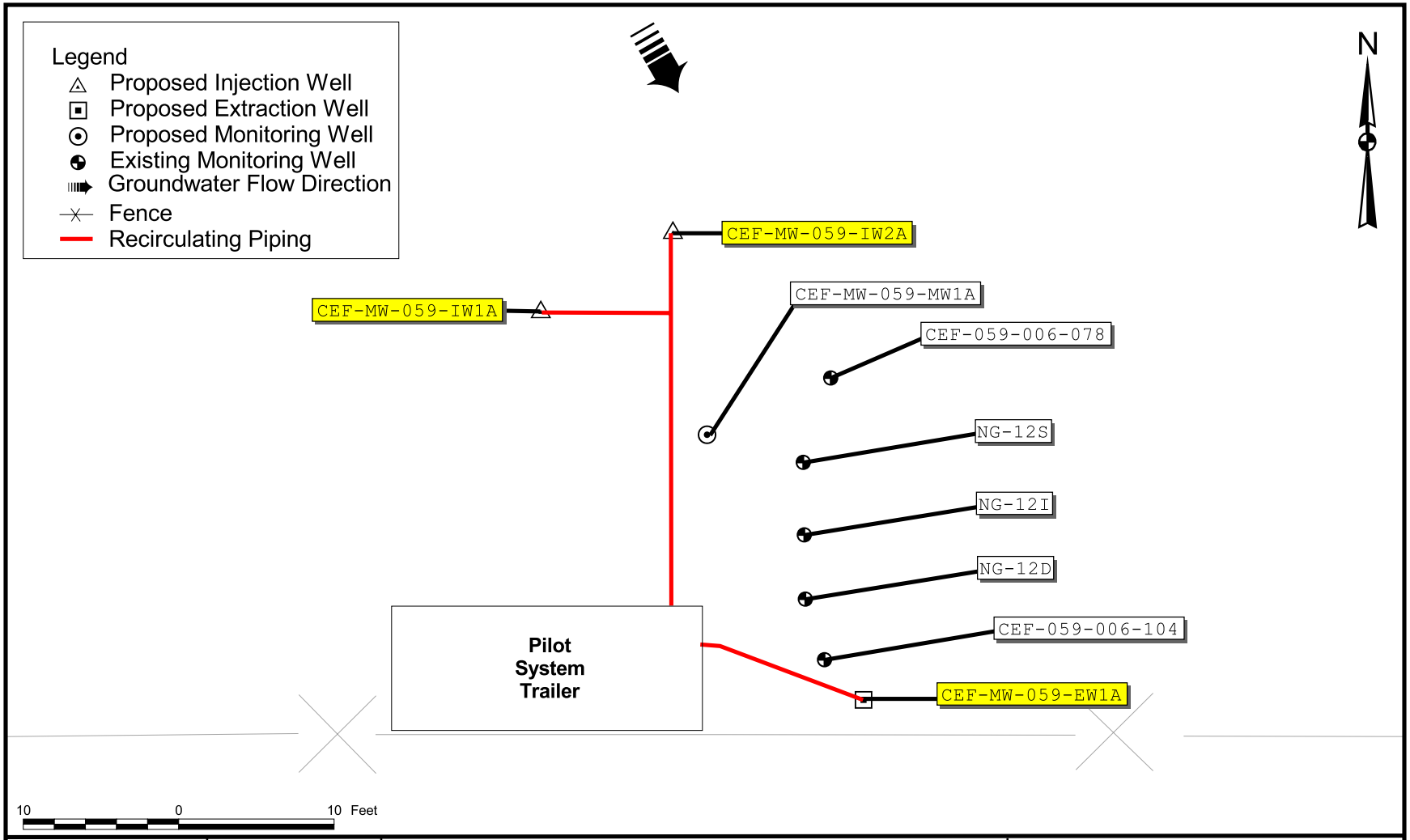
DRAWN BY MJJ	DATE 17Jan06	VOCs IN GROUNDWATER, RI DATA 70- TO 80-FOOT ZONE OU 9, SITE 59 REMEDIAL INVESTIGATION REPORT NAVAL AIR STATION CECIL FIELD JACKSONVILLE, FLORIDA	CONTRACT NUMBER 8925
CHECKED BY	DATE		APPROVED BY DATE
COST/SCHEDULE-AREA			APPROVED BY DATE
SCALE AS NOTED			DRAWING NO. FIGURE 5-3 / REV 0

P:\GIS\NAS_CecilField\Site-59_RI.apr 25Feb06 MJJ Layout061



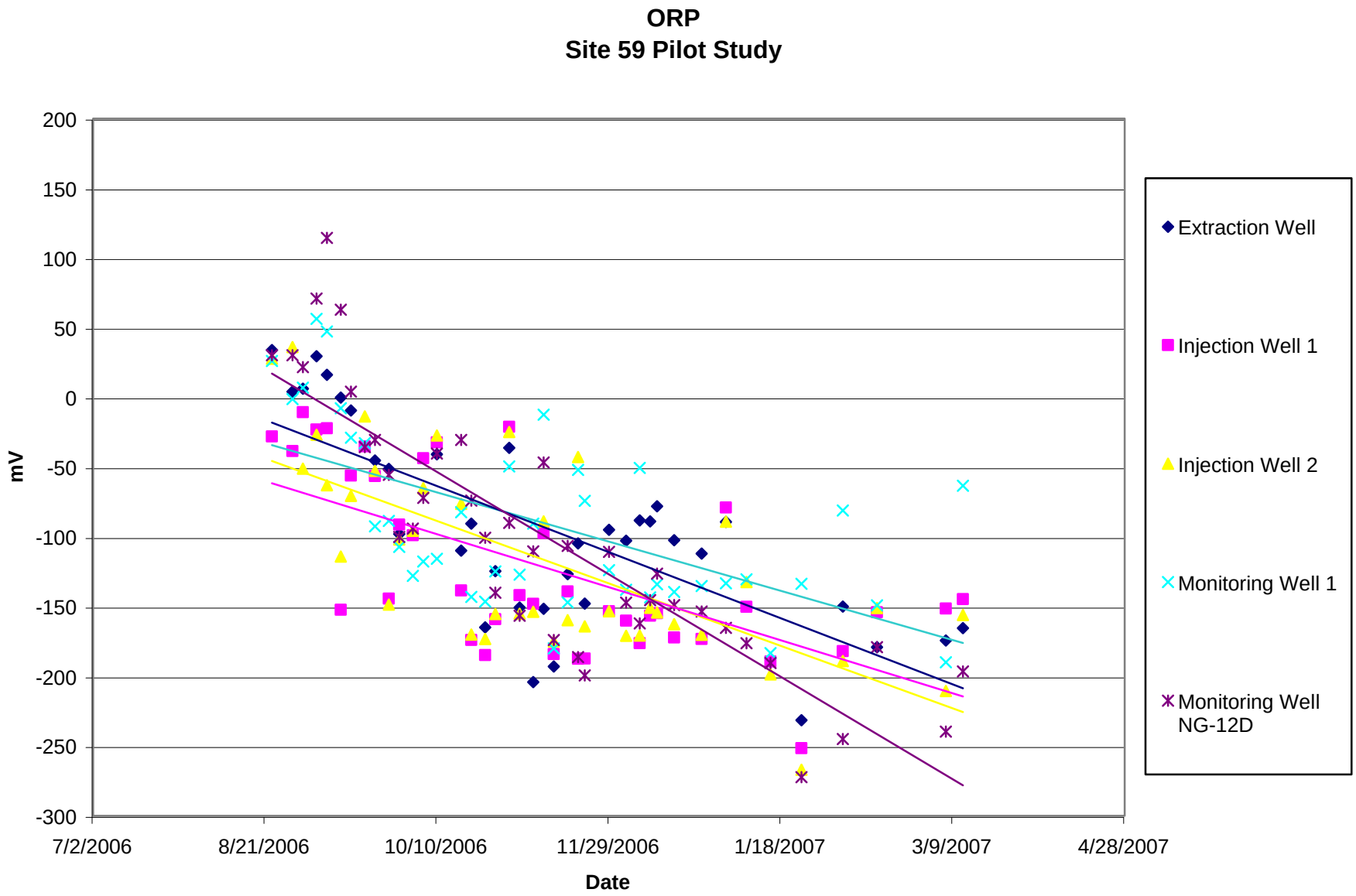
DRAWN BY MJJ	DATE 17Jan06	VOCs IN GROUNDWATER, RI DATA TOP OF ROCK ZONE OU 9, SITE 59 REMEDIAL INVESTIGATION REPORT NAVAL AIR STATION CECIL FIELD JACKSONVILLE, FLORIDA	CONTRACT NUMBER 8925
CHECKED BY	DATE		APPROVED BY DATE
COST/SCHEDULE-AREA			APPROVED BY DATE
SCALE AS NOTED			DRAWING NO. FIGURE 5-4 / REV 0

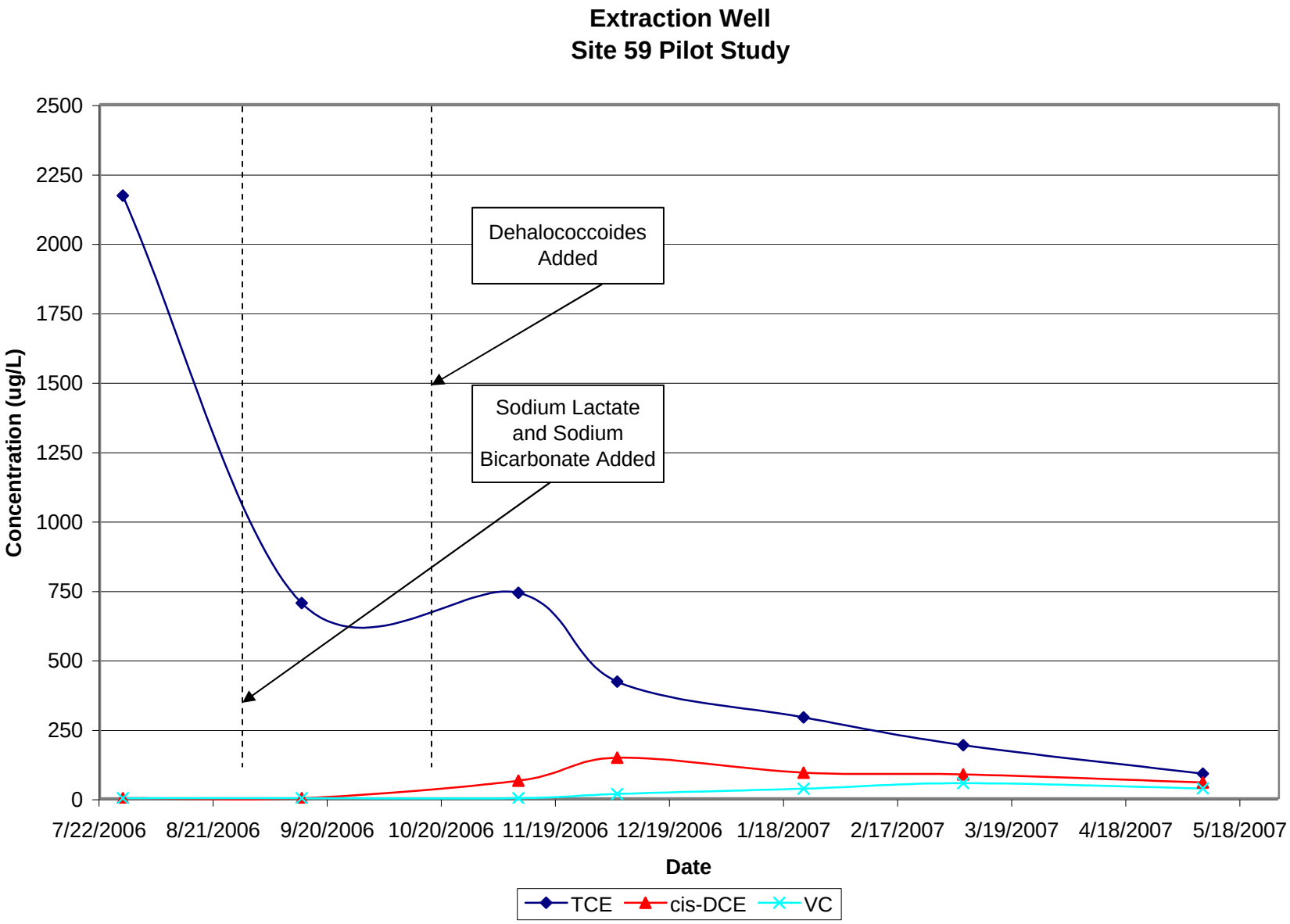
P:\GIS\NAS_CecilField\Site-59_RI.apr 25Feb06 MJJ Layout071

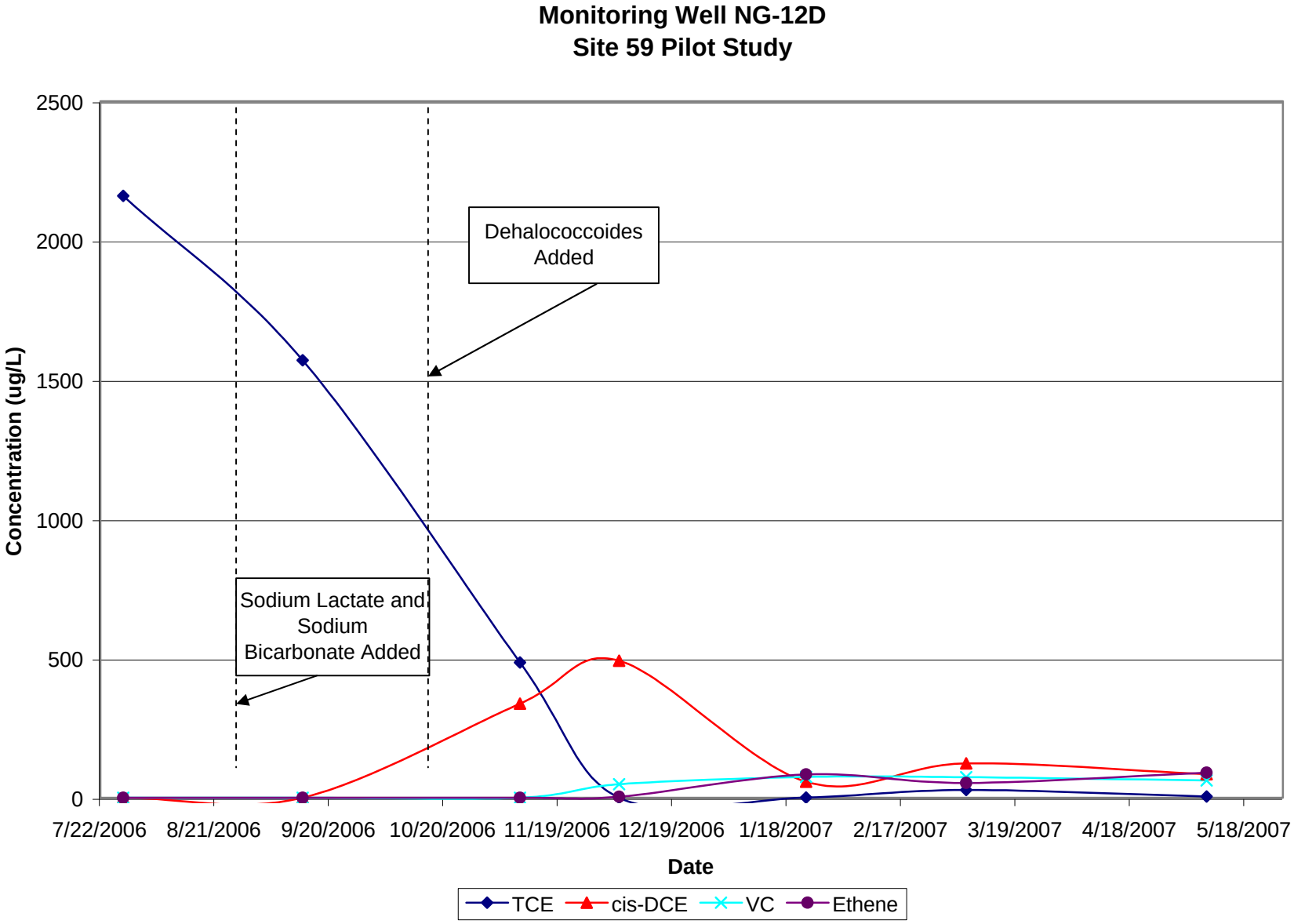


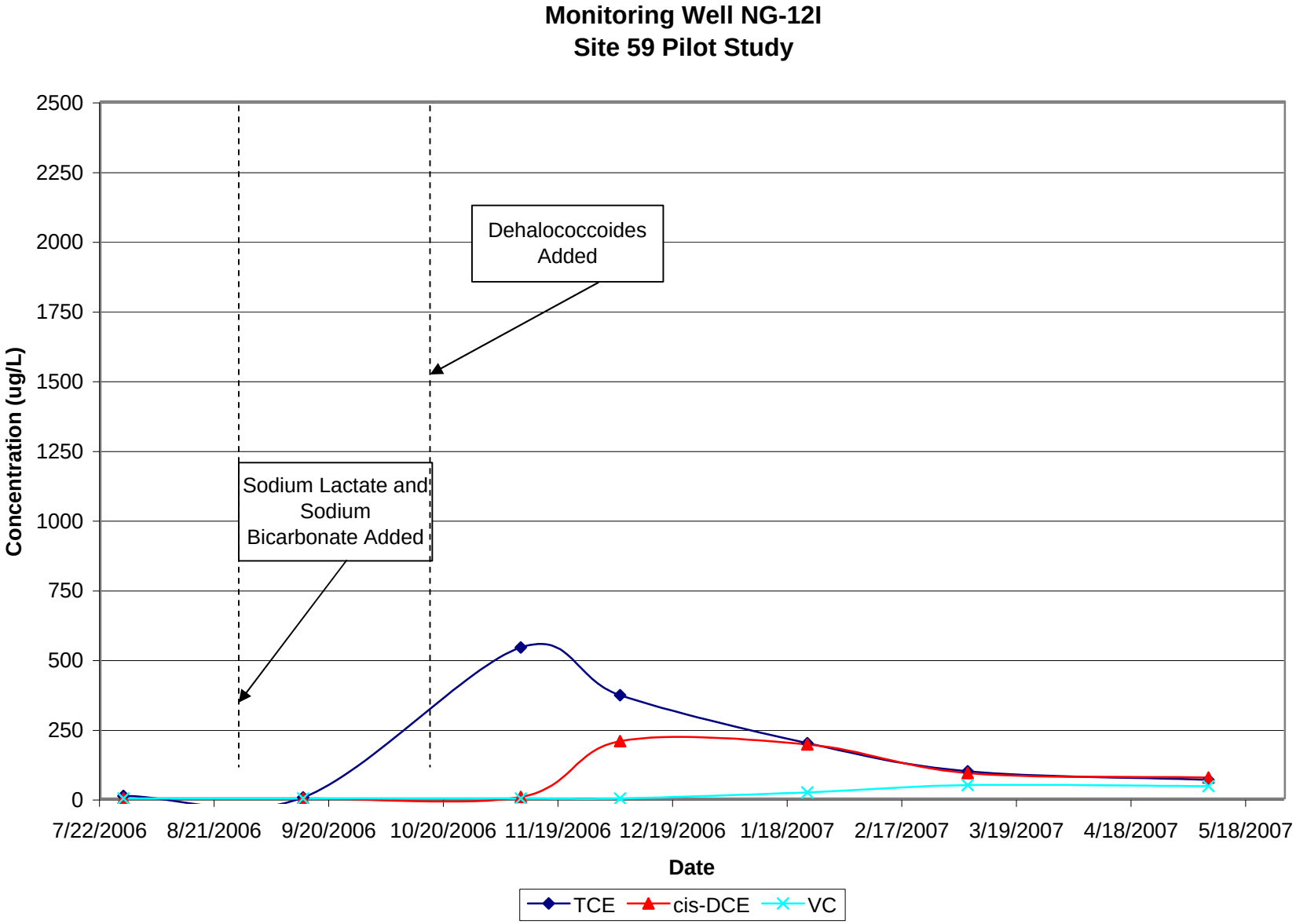
DRAWN BY: MJJ — DATE: 16Jun04		PILOT SYSTEM SITE PLAN PILOT STUDY WORK PLAN No. 2 OPERABLE UNIT 9, SITE 59 NAVAL AIR STATION CECIL FIELD JACKSONVILLE, FLORIDA	CONTRACT NUMBER: 0039
CHECKED BY: J. LOGAN — DATE: 10/26/05			APPROVED BY — DATE
COST/SCHEDULE-AREA			APPROVED BY — DATE
SCALE: AS NOTED	NAVFAC		DRAWING NO.: FIGURE 4-1 — REV: 0

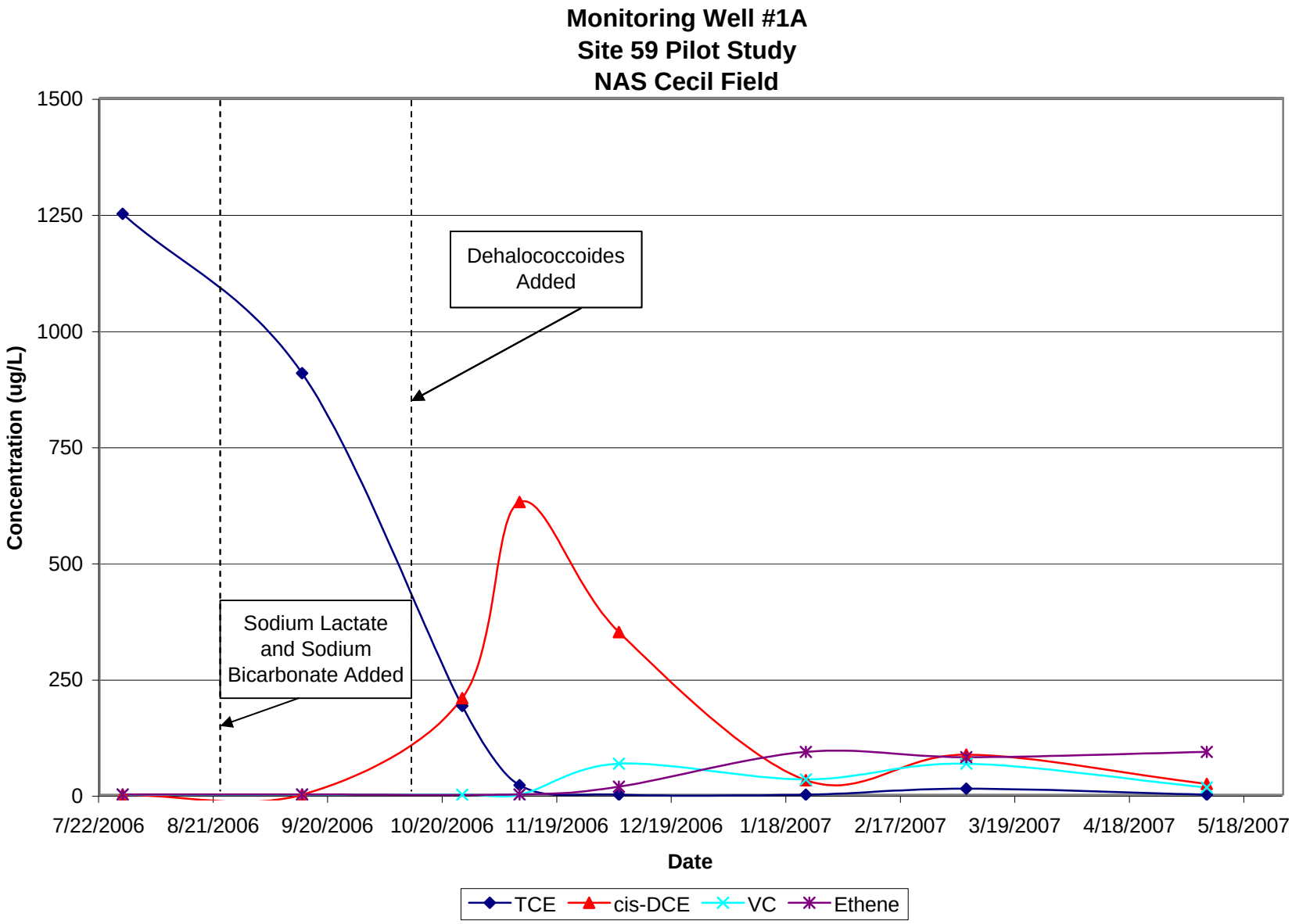
P:\GIS\NAS_CecilField\Site-59_20050616.apr 19Jun06 MJJ Layout1


ORP
Site 59 Pilot Study
200
150
100
50
0
-50
-100
-150
-200
-250
-300
mV
7/2/2006
8/21/2006
10/10/2006
11/29/2006
1/18/2007
3/9/2007
4/28/2007
Date
Extraction Well
Injection Well 1
Injection Well 2
Monitoring Well 1
Monitoring Well NG-12D


Extraction Well
Site 59 Pilot Study
Concentration (ug/L)
2500
2250
2000
1750
1500
1250
1000
750
500
250
0
Dehalococcoides Added
Sodium Lactate and Sodium Bicarbonate Added
7/22/2006
8/21/2006
9/20/2006
10/20/2006
11/19/2006
12/19/2006
1/18/2007
2/17/2007
3/19/2007
4/18/2007
5/18/2007
Date
TCE
cis-DCE
VC


Monitoring Well NG-12D
Site 59 Pilot Study
Concentration (ug/L)
2500
2000
1500
1000
500
0
Dehalococcoides Added
Sodium Lactate and Sodium Bicarbonate Added
7/22/2006
8/21/2006
9/20/2006
10/20/2006
11/19/2006
12/19/2006
1/18/2007
2/17/2007
3/19/2007
4/18/2007
5/18/2007
Date
TCE
cis-DCE
VC
Ethene


Monitoring Well NG-12I
Site 59 Pilot Study
Concentration (ug/L)
2500
2250
2000
1750
1500
1250
1000
750
500
250
0
Dehalococcoides Added
Sodium Lactate and Sodium Bicarbonate Added
7/22/2006
8/21/2006
9/20/2006
10/20/2006
11/19/2006
12/19/2006
1/18/2007
2/17/2007
3/19/2007
4/18/2007
5/18/2007
Date
TCE
cis-DCE
VC


Monitoring Well #1A
Site 59 Pilot Study
NAS Cecil Field
1500
1250
1000
750
500
250
0
Concentration (ug/L)
Dehalococcoides Added
Sodium Lactate and Sodium Bicarbonate Added
7/22/2006
8/21/2006
9/20/2006
10/20/2006
11/19/2006
12/19/2006
1/18/2007
2/17/2007
3/19/2007
4/18/2007
5/18/2007
Date
TCE
cis-DCE
VC
Ethene

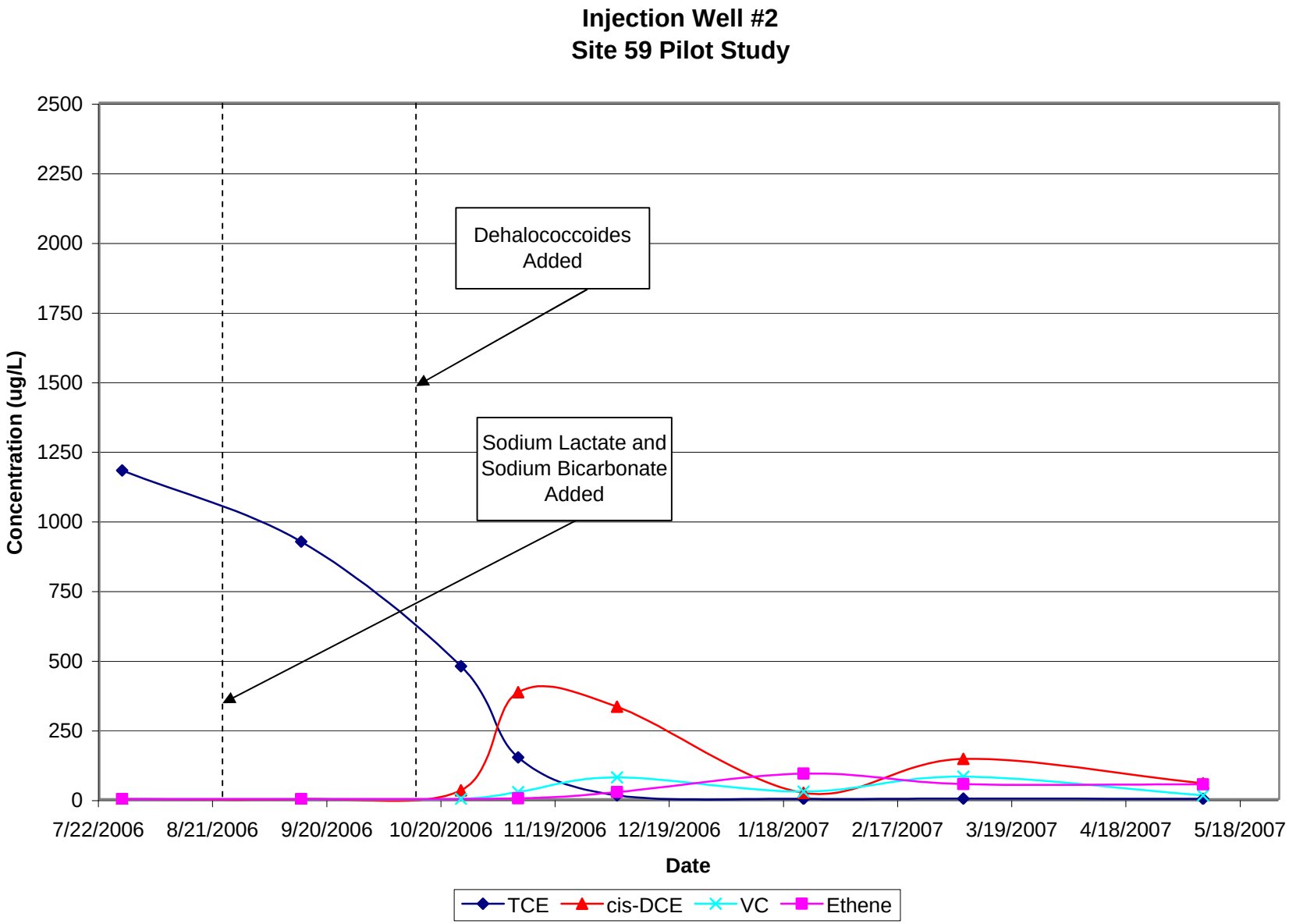

Injection Well #2
Site 59 Pilot Study
Concentration (ug/L)
2500
2250
2000
1750
1500
1250
1000
750
500
250
0
Dehalococcoides Added
Sodium Lactate and Sodium Bicarbonate Added
7/22/2006
8/21/2006
9/20/2006
10/20/2006
11/19/2006
12/19/2006
1/18/2007
2/17/2007
3/19/2007
4/18/2007
5/18/2007
Date
TCE
cis-DCE
VC
Ethene

Table 5-3
PCR Results, Site 59
NAS Cecil Field

Well ID	Description	from sterivex	filters only	GENE COPIES PER LITER											vol
		DNA conc (ng/ul)	*stdev*	*BAC 16S rRNA*	*stdev*	*Dhc 16S rRNA*	*stdev*	*bvcA*	*stdev*	*vcrA*	*stdev*	*tceA*	*stdev*	*date*	*(ml)*
MW1A-01B Jul06	Unknown (from database)													28-Jul-06	
MW1A-07B Dec06	Unknown (from database)					7.15E+04		1.68E+02		1.83E+05				5-Dec-06	
MW1A-08A-MB Jan07	MoBio Water Filter-Water Kit	27.8	2.26	2.93E+07	1.52E+07	2.79E+06	1.20E+05	3.48E+05	3.67E+04	4.03E+06	3.89E+05	1.62E+03	1.57E+03	23-Jan-07	500
MW1A-08B-MB Jan07	MoBio Water Filter-Water Kit	23.1	4.14	2.67E+08	1.67E+08	3.53E+07	1.24E+06	4.66E+06	2.28E+05	5.29E+07	5.95E+06	9.38E+03	2.66E+03	23-Jan-07	500
MW1A-08-SX Jan07	Sterivex- Water Kit	20.0	1.66	5.87E+06	4.57E+06	1.22E+06	4.08E+04	1.74E+04	1.10E+03	9.28E+05	1.15E+05	5.28E+03	3.22E+03	23-Jan-07	2000
MW1A-10A-MB Mar 07	MoBio Water Filter-Water Kit	13.4	11.77	4.77E+09	5.69E+08	1.35E+08	1.82E+07	4.81E+07	1.50E+06	1.91E+08	7.44E+06	8.28E+06	1.08E+06	7-Mar-07	475
MW1A-10B-MB Mar 07	MoBio Water Filter-Water Kit	5.3	0.95	6.63E+08	2.10E+08	1.30E+08	4.21E+07	6.56E+06	1.19E+06	1.70E+08	7.81E+06	1.69E+06	9.14E+04	7-Mar-07	475
MW1A-10-SX Mar 07	Sterivex- Water Kit			1.74E+06	1.66E+05	1.68E+05	2.47E+04	8.48E+03	1.82E+03	1.70E+05	8.45E+03	1.19E+03	4.98E+02	7-Mar-07	3000
Mw1A-08 - SX Jul 08	Sterivex-PowerSoil Kit	**6.5**		**1.21E+08**	**5.98E+06**	**7.21E+06**	**5.09E+05**	***2.29E+05***	***6.01E+03***	**9.32E+06**	**3.98E+05**	**2.72E+06**	**1.08E+05**	**9-Jul-08**	**400**
IW2A Jul06	Unknown (from database)													28-Jul-06	
IW2A-08A-MB Jan07	MoBio Water Filter-Water Kit	22.4	4.07	5.31E+09	1.97E+09	3.09E+08	8.20E+07	2.34E+04	1.05E+04	4.82E+08	4.87E+07	1.31E+03	1.16E+03	23-Jan-07	250
IW2A-08B-MB Jan07	MoBio Water Filter-Water Kit	17.1	3.78	1.46E+10	5.37E+09	7.12E+08	4.00E+07	6.29E+04	2.03E+04	2.11E+09	1.76E+08	7.93E+02	1.37E+03	23-Jan-07	250
IW2A A-08A-SX Jan 07	Sterivex- Water Kit	26.1	4.45	3.32E+09	1.12E+09	2.06E+08	3.43E+07	8.53E+02	9.47E+02	3.82E+08	2.10E+08	1.29E+04	8.88E+03	23-Jan-07	250
IW2A B-08B-SX Jan 07	Sterivex- Water Kit	10.1	0.64	3.95E+09	1.91E+09	2.28E+08	9.23E+07	2.20E+02	2.09E+02	2.19E+08	3.37E+07	5.89E+05	5.01E+04	23-Jan-07	250
IW2A-10A-MB Mar 07	Sterivex- Water Kit			2.67E+07	1.29E+07	1.84E+06	2.18E+05			1.28E+06	4.38E+04	1.31E+03	1.16E+03	7-Mar-07	200
IW2A-10B-MB Mar 07	Sterivex- Water Kit			1.66E+07	9.32E+06	1.42E+06	6.08E+04			6.16E+02	5.85E+02	7.93E+02	1.37E+03	7-Mar-07	250
IW2A-10B - SX Mar 07	Sterivex- Water Kit			2.43E+05	1.76E+04	1.18E+05	1.78E+04			1.77E+05	1.48E+04	4.63E+01	3.30E+01	7-Mar-07	3000
Iw2A-08.1 SX Jul 08	Sterivex-PowerSoil Kit	**14.6**		**3.03E+08**	**9.07E+07**	**8.62E+06**	**3.38E+06**			**1.24E+07**	**1.27E+06**	**3.87E+05**	**2.80E+05**	**9-Jul-08**	**50**
Iw2A-08.2 SX Jul 08	Sterivex-PowerSoil Kit	**6.9**		**4.41E+08**	**9.66E+07**	**2.58E+07**	**1.42E+06**			**3.52E+07**	**5.47E+05**	**7.39E+05**	**6.55E+04**	**9-Jul-08**	**50**
NG-12D Jul06	Unknown (from database)					1.34E+04								31-Jul-08	
NG-12D-08A-MB Jan07	MoBio Water Filter-Water Kit	11.9	1.84	1.76E+08	6.01E+07	5.24E+04	7.69E+03	8.45E+02	7.35E+02	2.32E+05	3.13E+04			23-Jan-07	350
NG-12D-08B-MB Jan07	MoBio Water Filter-Water Kit	20.4	0.42	3.18E+10	3.10E+10	2.00E+07	6.21E+06	3.93E+03	4.65E+03	1.89E+07	2.60E+05	2.87E+03	4.65E+02	23-Jan-07	350
NG-12D-08-SX Jan 07	Sterivex- Water Kit	6.6	1.03	9.78E+07	4.52E+07	9.13E+06	1.11E+05	7.73E+01	8.30E+01	7.66E+06	9.46E+05	2.98E+05	1.79E+04	23-Jan-07	1000
NG-12D-10A-MB Mar 07	Sterivex- Water Kit			9.72E+07	7.82E+06	3.88E+06	1.01E+06	2.93E+03	1.08E+03	5.00E+06	3.57E+05	1.10E+03	4.55E+02	7-Mar-07	460
NG-12D-10B-MB Mar 07	Sterivex- Water Kit			6.79E+07	8.08E+06	1.41E+06	4.60E+04	1.77E+03	1.53E+03	2.22E+06	2.80E+05	8.82E+02	2.47E+02	7-Mar-07	350
NG-12D-10- SX Mar 07	Sterivex- Water Kit			6.8E+05	1.6E+05	2.73E+04	3.13E+03			6.00E+04	6.12E+03	8.1E+01	4.8E+01	7-Mar-07	3000
NG-12D SX Jul 08	Sterivex-PowerSoil Kit	**7.0**		**1.65E+06**	**7.29E+05**	**1.54E+05**	**1.26E+04**			**1.67E+05**	**1.61E+04**			**9-Jul-08**	**250**
NG-12I Nov04	Unknown (from database)													16-Nov-04	
EW1A-07B Jul 06	Unknown (from database)					7.03E+07		1.32E+05		2.13E+05					
CEF59-002-28 Nov 04	Unknown (from database)													16-Nov-04	
CEF59-003-53 Nov 04	Unknown (from database)													16-Nov-04	
CEF59-004-73 Nov 04	Unknown (from database)													16-Nov-04	
CEF59-004-73 Jul 08	Sterivex-PowerSoil Kit	**6.0**		**1.54E+05**	**5.00E+04**									**8-Jul-08**	**750**
Iw-31-1 Jul 08	Sterivex-PowerSoil Kit	**6.3**		**2.24E+06**	**8.35E+05**									**8-Jul-08**	**1000**
Iw-31-2 Jul 08	Sterivex-PowerSoil Kit	**11.7**		**2.17E+08**	**2.98E+07**									**8-Jul-08**	**1000**
Mw-3-1 Jul 08	Sterivex-PowerSoil Kit	**4.7**		**1.22E+07**	**2.08E+06**	***1.45E+03***	***1.22E+03***							**8-Jul-08**	**300**
Mw-3-3 Jul 08	Sterivex-PowerSoil Kit	**5.2**		**5.35E+06**	**3.44E+06**									**8-Jul-08**	**150**

edge of detection

= Undetected

= DNQ

= did not assay

gene copies per Liter GW

new data in red

prelim data in blue -from database

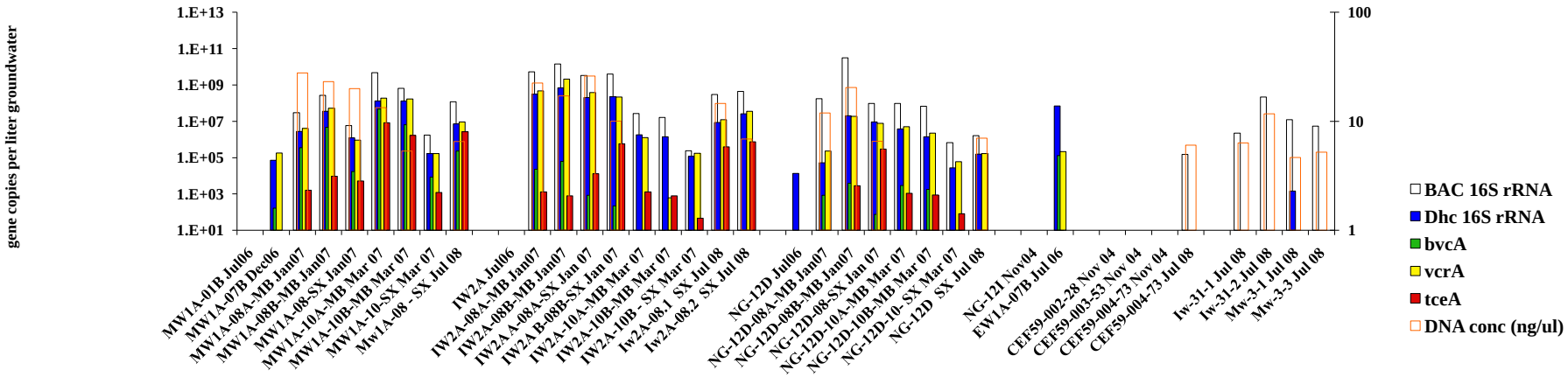
Bac values are from 1:10 dilution which gave higher numbers for all except Mw-3-1

Dhc values are from undiluted samples except for Iw2A-08.1

BvcA values are from undiluted samples for qPCR on 9-24-08

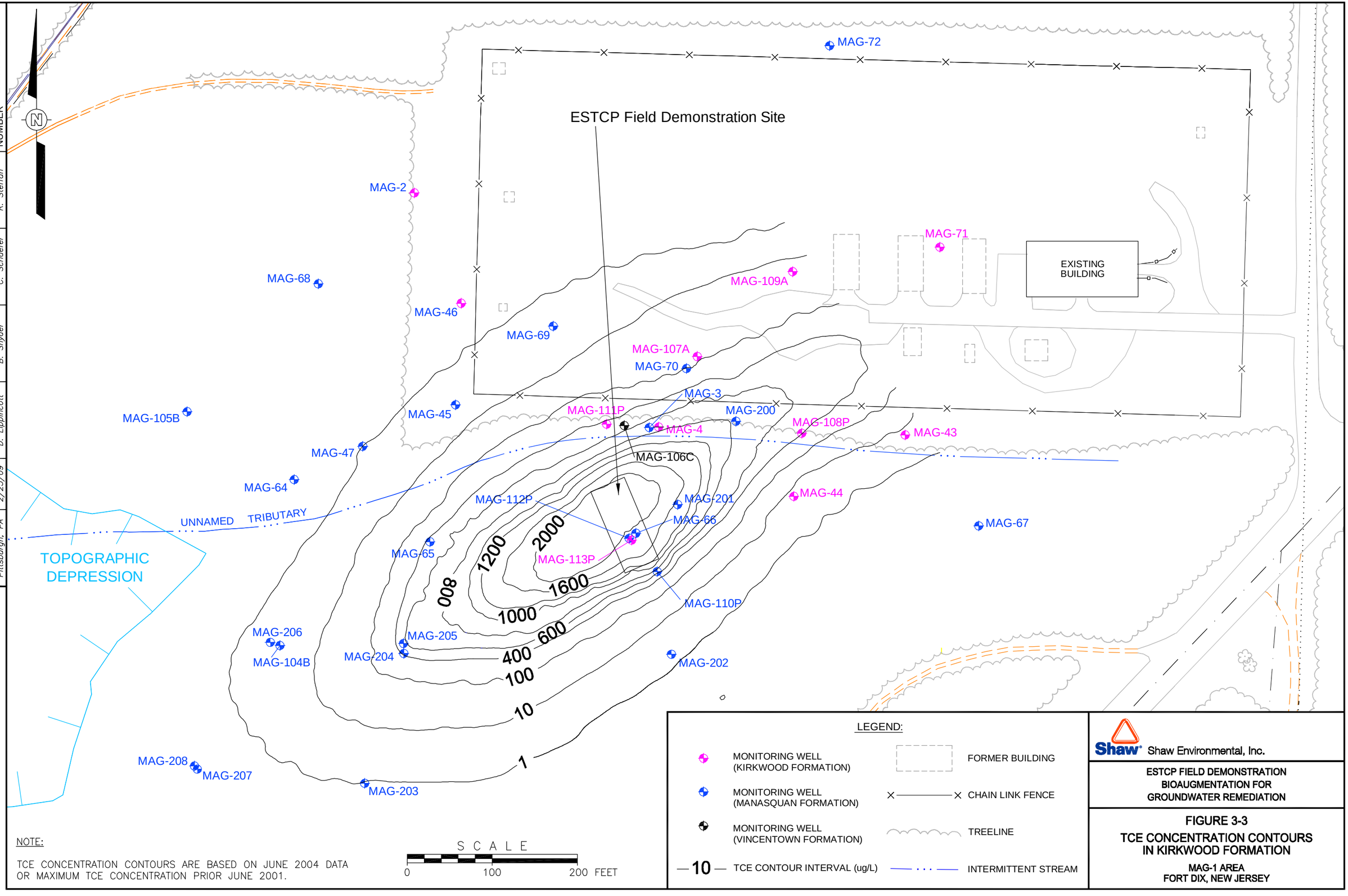
VcrA and *TceA* values are all from undiluted samples

Figure 5-3
PCR Results
Site 59, NAS Cecil Field



Appendix B-7: Fort Dix

DRAWING NUMBER	APPROVED BY	CHECKED BY	DRAWN BY	DESIGNED BY	DATE	OFFICE
115893-B4	R. Steffan	C. Schaefer	B. Snyder	D. Lippincott	2/23/09	Pittsburgh, PA



NOTE:

TCE CONCENTRATION CONTOURS ARE BASED ON JUNE 2004 DATA OR MAXIMUM TCE CONCENTRATION PRIOR JUNE 2001.

SCALE

0 100 200 FEET

LEGEND:

- MONITORING WELL (KIRKWOOD FORMATION)
- MONITORING WELL (MANASQUAN FORMATION)
- MONITORING WELL (VINCENTOWN FORMATION)
- 10 — TCE CONTOUR INTERVAL (ug/L)
- FORMER BUILDING
- CHAIN LINK FENCE
- TREELINE
- INTERMITTENT STREAM

Shaw Environmental, Inc.

ESTCP FIELD DEMONSTRATION BIOAUGMENTATION FOR GROUNDWATER REMEDIATION

FIGURE 3-3

TCE CONCENTRATION CONTOURS IN KIRKWOOD FORMATION

MAG-1 AREA
FORT DIX, NEW JERSEY

Xref: XREF1
Image:

File: O:\Project\AFCEE\Fort Dix\115869B4.dwg
Plot Date/Time: Mar 09, 2009 - 1:27pm
Plotted By: william.snyder

DRAWING NUMBER	APPROVED BY	CHECKED BY	DRAWN BY	DESIGNED BY	DATE	OFFICE
115893-B5	R. Steffan	C. Schaefer	B. Snyder	D. Lippincott	2/23/09	Pittsburgh, PA

ESTCP Field Demonstration Site

MAG-72
MAG-2
MAG-68
MAG-46
MAG-69
MAG-71
MAG-109A
EXISTING BUILDING
MAG-107A
MAG-70
MAG-3
MAG-105B
MAG-45
MAG-111P
MAG-200
MAG-108P
MAG-4
MAG-43
MAG-106C
MAG-47
MAG-64
MAG-112P
1200
MAG-201
MAG-66
MAG-44
MAG-67
UNNAMED TRIBUTARY
MAG-65
MAG-113P
1000
700
TOPOGRAPHIC DEPRESSION
MAG-110P
500
300
MAG-206
MAG-205
MAG-104B
MAG-204
100
MAG-202
10
1
MAG-208
MAG-207
MAG-203

NOTE:

TCE CONCENTRATION CONTOURS ARE BASED ON JUNE 2004 DATA OR MAXIMUM TCE CONCENTRATION PRIOR JUNE 2001.

SCALE
0 100 200 FEET

LEGEND:

- MONITORING WELL (KIRKWOOD FORMATION)
- MONITORING WELL (MANASQUAN FORMATION)
- MONITORING WELL (VINCENTOWN FORMATION)
- —10— CIS-1,2 CONTOUR INTERVAL (ug/L)
- FORMER BUILDING
- CHAIN LINK FENCE
- TREELINE
- INTERMITTENT STREAM

Shaw Environmental, Inc.

ESTCP FIELD DEMONSTRATION
BIOAUGMENTATION FOR
GROUNDWATER REMEDIATION

FIGURE 3-4

CIS-1,2 DCE CONCENTRATION CONTOURS IN KIRKWOOD FORMATION

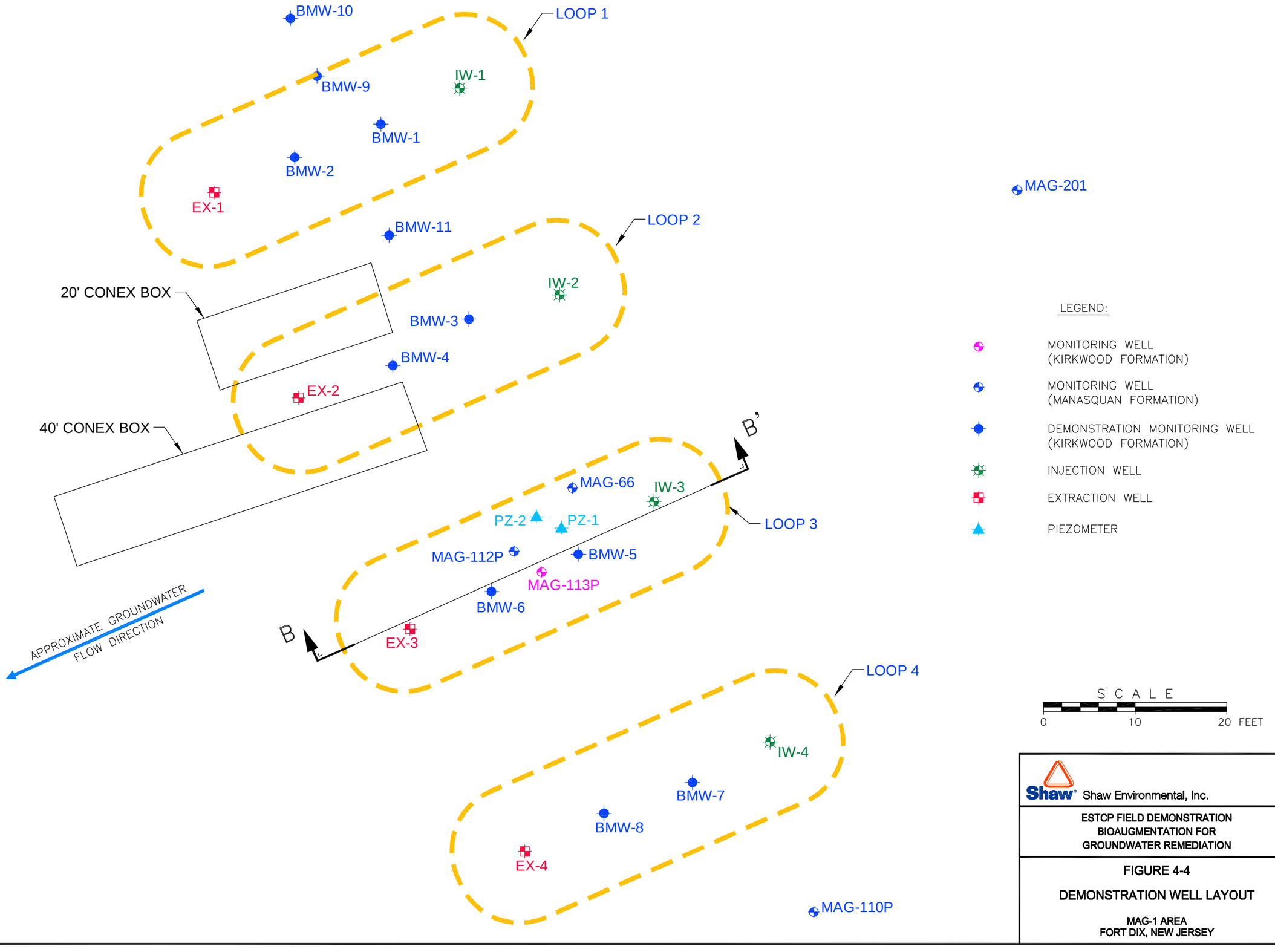
MAG-1 AREA
FORT DIX, NEW JERSEY

Xref : XREF1
Image :

File: O:\Project\AFCEE\Fort Dix\115869B5.dwg
Plot Date/Time: Mar 09, 2009 - 1:29pm
Plotted By: william.snyder

DESIGNED BY	DRAWN BY	CHECKED BY	APPROVED BY	DRAWING NUMBER
D. Lippincott	B. Snyder	C. Schaefer	R. Steffan	115869-B8

OFFICE	DATE
Pittsburgh, PA	2/26/09



Shaw Shaw Environmental, Inc.

ESTCP FIELD DEMONSTRATION
BIOAUGMENTATION FOR
GROUNDWATER REMEDIATION

FIGURE 4-4

DEMONSTRATION WELL LAYOUT

MAG-1 AREA
FORT DIX, NEW JERSEY

Xref : XREF1
Image :

File: O:\Project\AFCEE\Fort Dix\115869B8.dwg
Plot Date/Time: Mar 10, 2009 - 7:09am
Plotted By: william.snyder

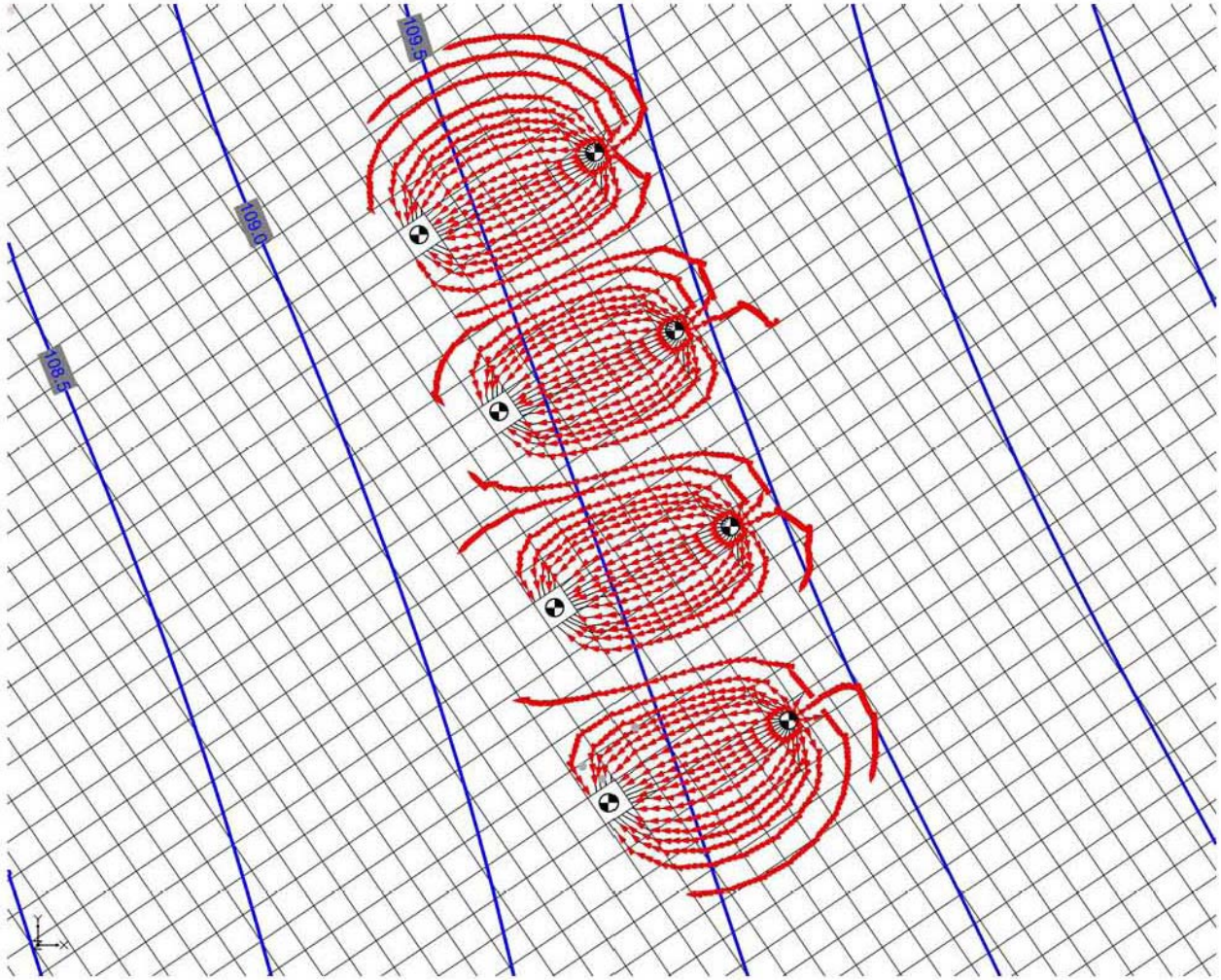


Figure 4-6. Particle tracking simulation. At a flow of 0.5 gpm per recirculation loop, and with 25 feet spacing between loops, cross flow between the loops is expected to be negligible.

DRAWING NUMBER	APPROVED BY	CHECKED BY	DRAWN BY	DESIGNED BY
115869-B9	R. Steffan	C. Schaefer	B. Snyder	D. Lippincott

OFFICE	DATE
Pittsburgh, PA	3/5/09



BMW-10
250/62/<5

LOOP 1

BMW-9
110/130/<5

IW-1
620/49/<100

BMW-1
140/210/<5

BMW-2
30/190/<5

EX-1
150/220/<10

MAG-201

BMW-11
580/37/<100

LOOP 2

20' CONEX BOX

IW-2
970/62/<100

BMW-3
98/470/<100

BMW-4
680/1300/<100

EX-2
17/210/<10

40' CONEX BOX

LOOP 3

MAG-66

PZ-2
1000/130/<100

PZ-1
490/500/<100

IW-3
1600/260/<100

MAG-112P

BMW-5
1500/410/<5

MAG-113P
1400/270/<100

BMW-6
950/480/<100

EX-3
180/430/<10

APPROXIMATE GROUNDWATER FLOW DIRECTION

LOOP 4

IW-4
78/50/<100

BMW-7
190/100/<5

BMW-8
260/140/<100

EX-4
26/77/<10

MAG-110P

LEGEND:

- MONITORING WELL (KIRKWOOD FORMATION)
- MONITORING WELL (MANASQUAN FORMATION)
- DEMONSTRATION MONITORING WELL (KIRKWOOD FORMATION)
- INJECTION WELL
- EXTRACTION WELL
- PIEZOMETER
- 110/130/<5 — TCE/DCE/VINYL CHLORIDE CONCENTRATIONS ($\mu g/L$)

SCALE
0 10 20 FEET

Shaw Environmental, Inc.

ESTCP FIELD DEMONSTRATION
BIOAUGMENTATION FOR
GROUNDWATER REMEDIATION

FIGURE 6-3

BASELINE CHLORINATED ETHENE CONCENTRATIONS

MAG-1 AREA
FORT DIX, NEW JERSEY

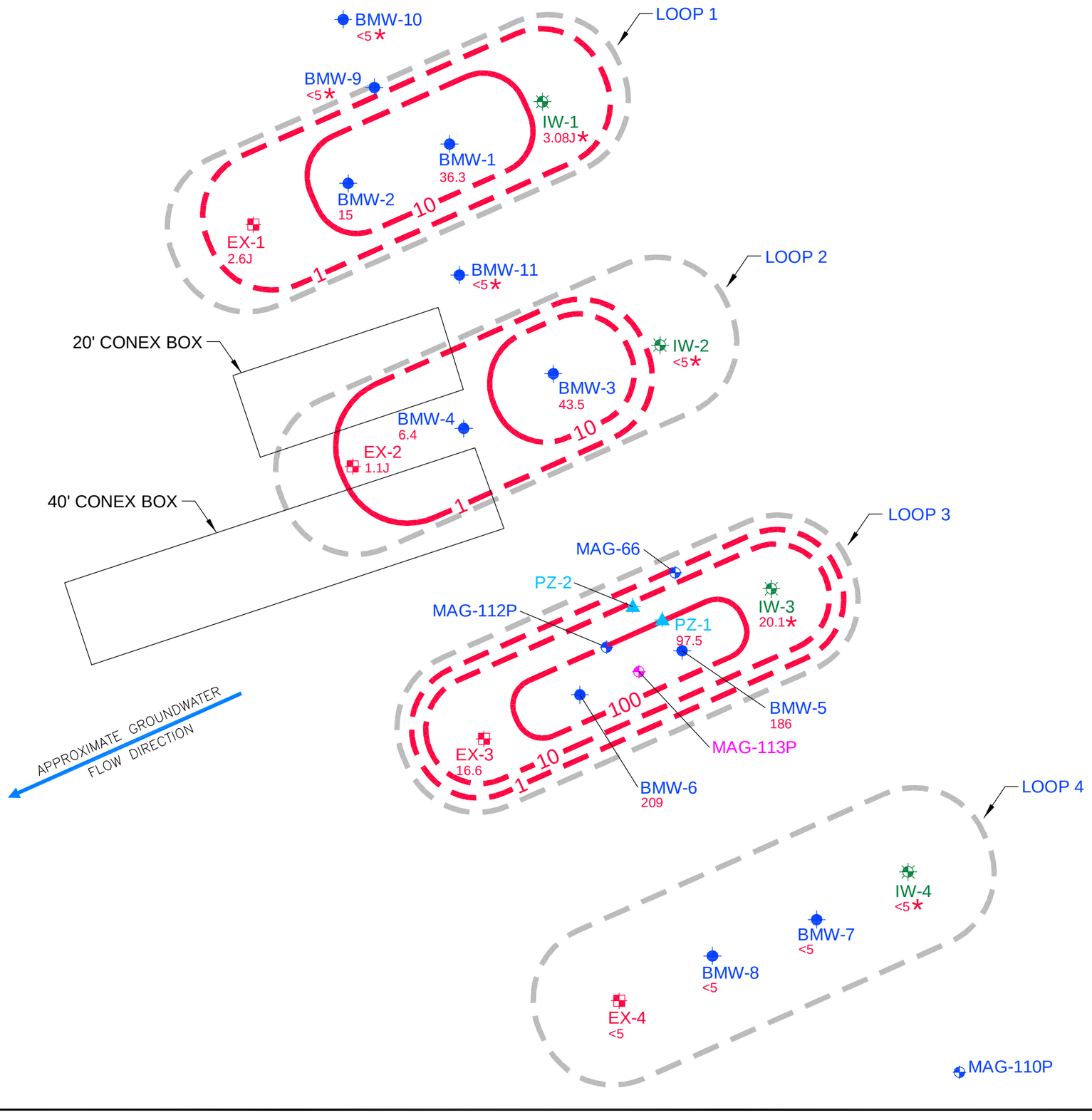
File: O:\Project\AFCEE\Fort Dix\115869B9.dwg
Plot Date/Time: Mar 30, 2009 - 8:24am
Plotted By: william.snyder

Xref: XREF1
Image:

DESIGNED BY	DRAWN BY	CHECKED BY	APPROVED BY	DRAWING NUMBER
D. Lippincott	B. Snyder	C. Schaefer	R. Steffan	115869-B12

OFFICE	DATE
Pittsburgh, PA	3/5/09





MAG-201

LEGEND:

- MONITORING WELL (KIRKWOOD FORMATION)
- MONITORING WELL (MANASQUAN FORMATION)
- DEMONSTRATION MONITORING WELL (KIRKWOOD FORMATION)
- INJECTION WELL
- EXTRACTION WELL
- PIEZOMETER
- 110/130/<5 — ETHENE CONCENTRATION ($\mu g/L$)
- 10 — ETHENE CONCENTRATION CONTOUR ($\mu g/L$) DASHED WHERE INFERRED

NOTE:

* DATA FROM 11/11/08 SAMPLING EVENT. WELL NOT SAMPLED ON 1/5/09.

SCALE

0 10 20 FEET

Shaw Environmental, Inc.

ESTCP FIELD DEMONSTRATION
BIOAUGMENTATION FOR
GROUNDWATER REMEDIATION

FIGURE 6-7

ETHENE CONCENTRATIONS
JANUARY 5, 2009

MAG-1 AREA
FORT DIX, NEW JERSEY

File: O:\Project\AFCEE\Fort Dix\115869B12.dwg
Plot Date/Time: Mar 18, 2009 - 8:40am
Plotted By: william.snyder

Xref : XREF1
Image :

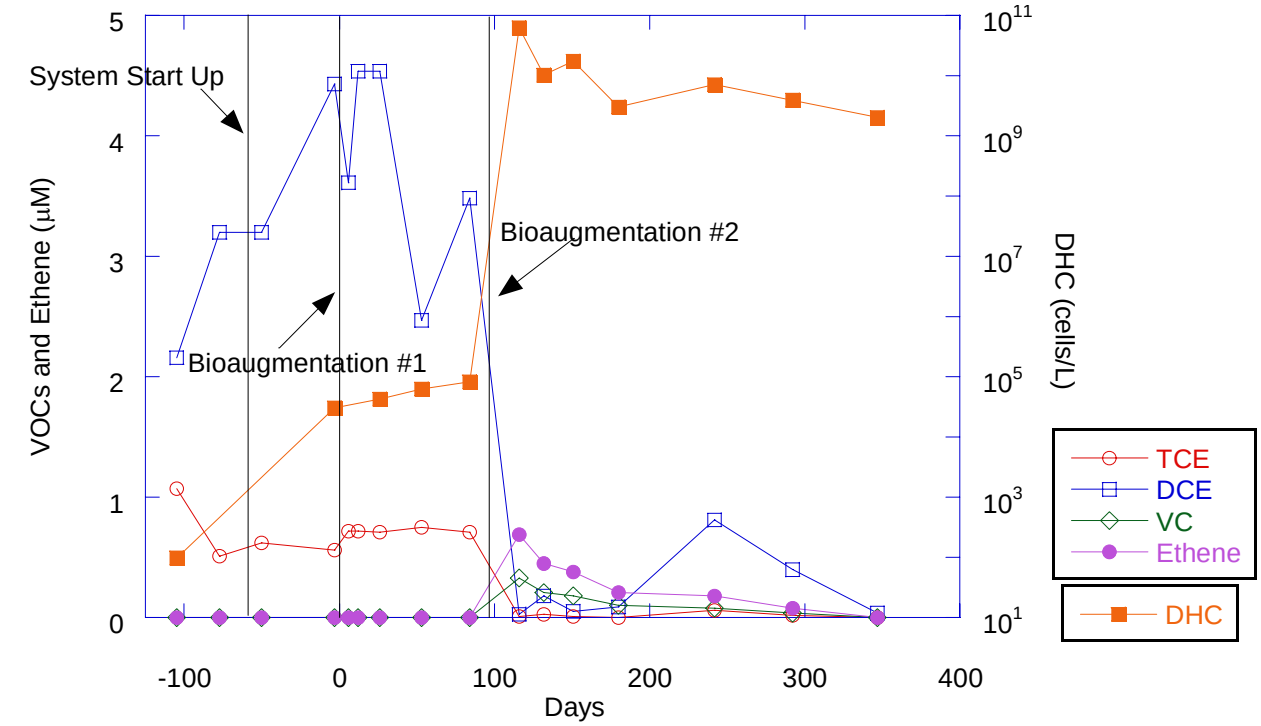


Figure C-1. Chlorinated Ethenes, Ethene, and DHC: Monitoring Well BMW-1 (Loop 1)

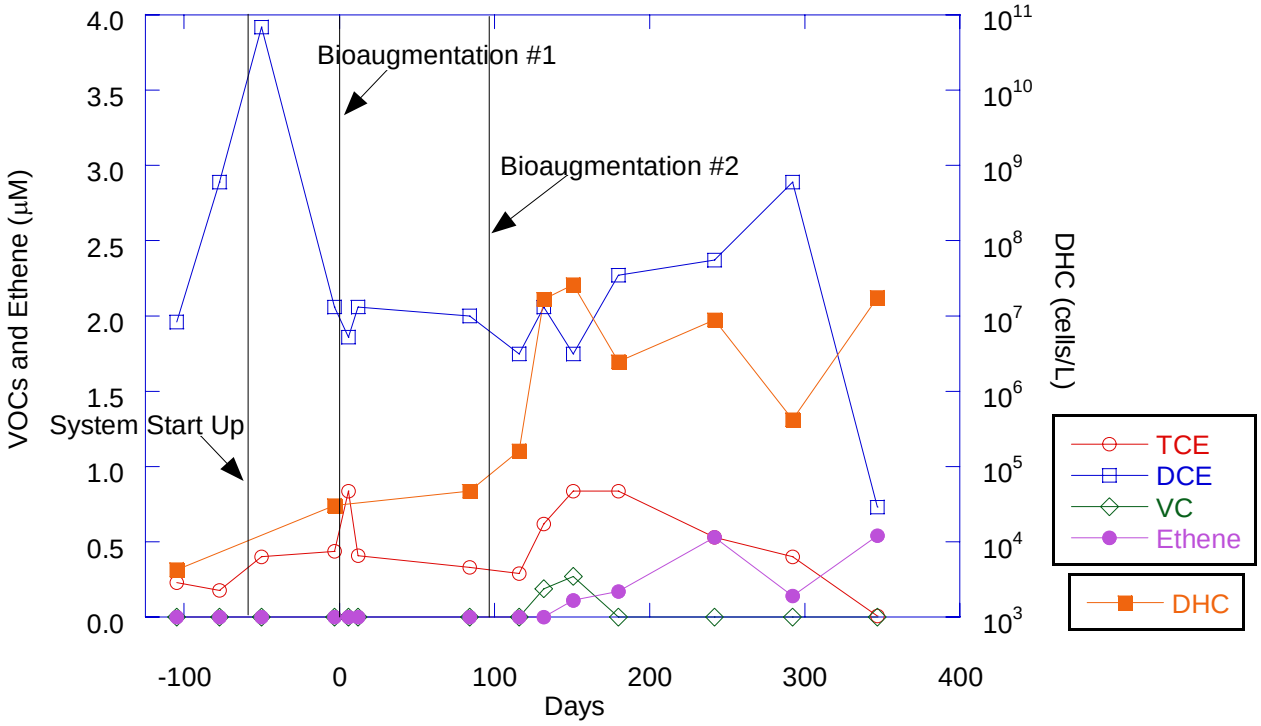


Figure C-2. Chlorinated Ethenes, Ethene, and DHC: Monitoring Well BMW-2 (Loop 1)

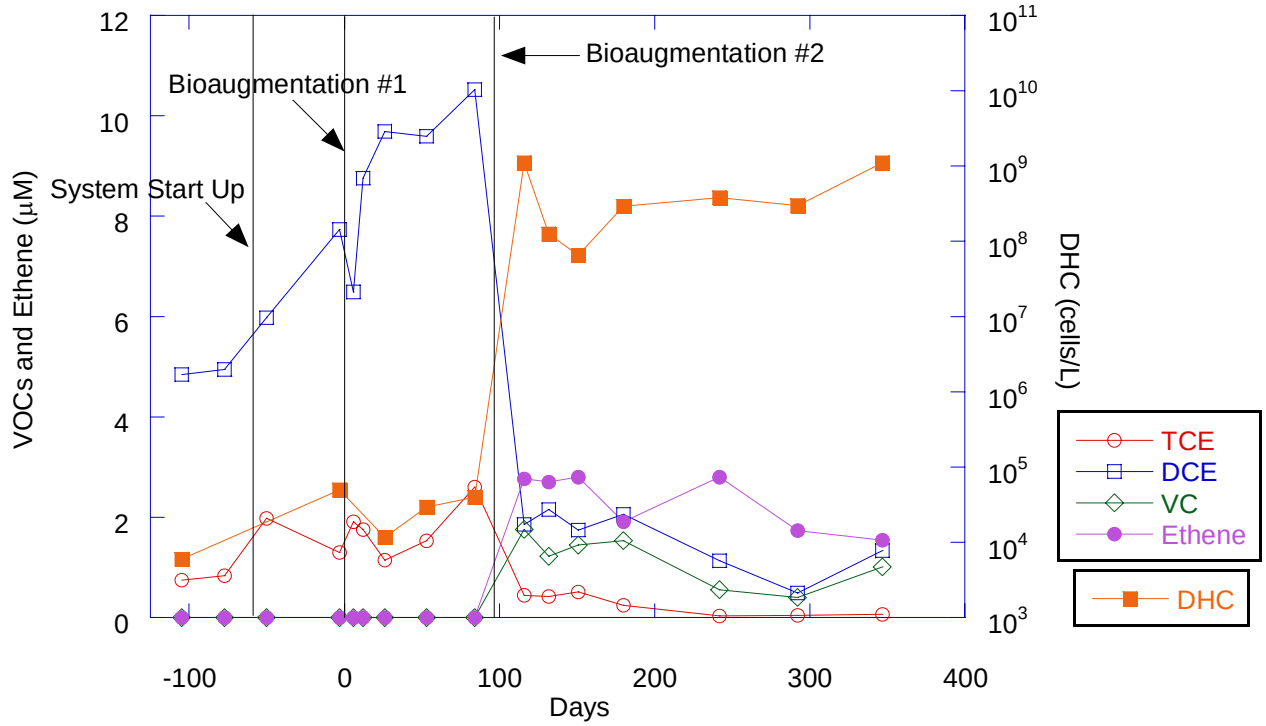


Figure C-3. Chlorinated Ethenes, Ethene, and DHC: Monitoring Well BMW-3 (Loop 2)

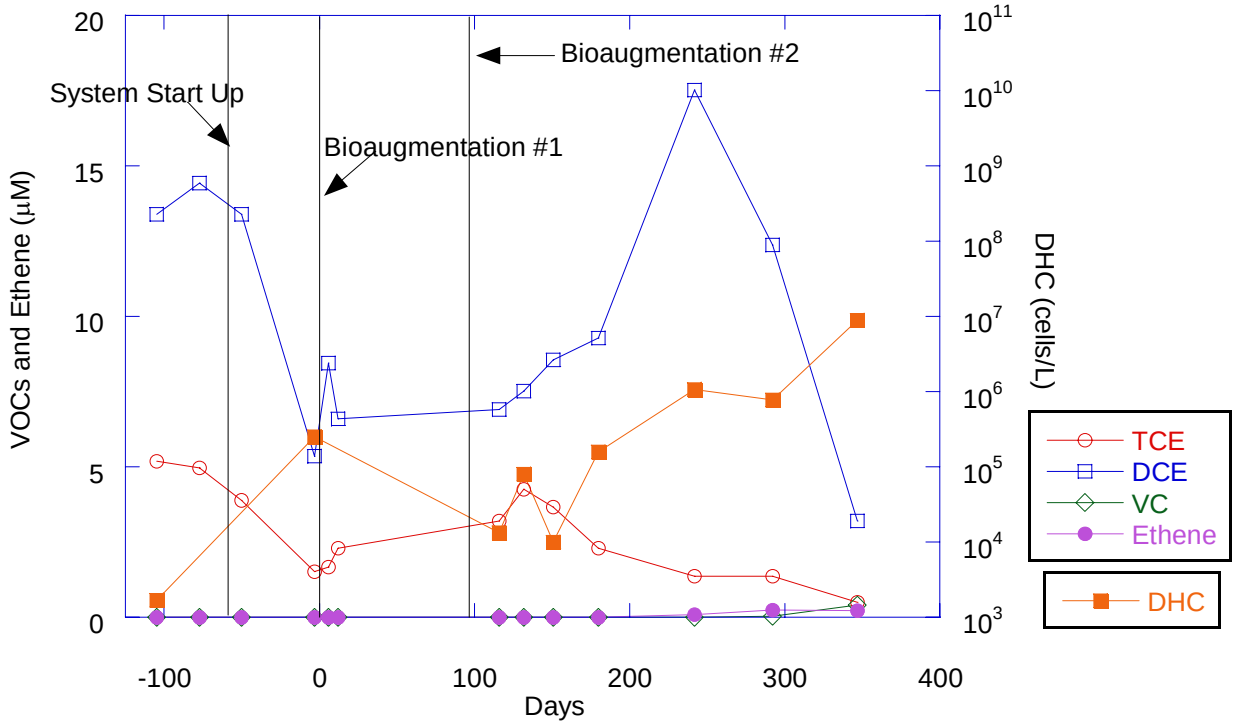


Figure C-4. Chlorinated Ethenes, Ethene, and DHC: Monitoring Well BMW-4 (Loop 2)

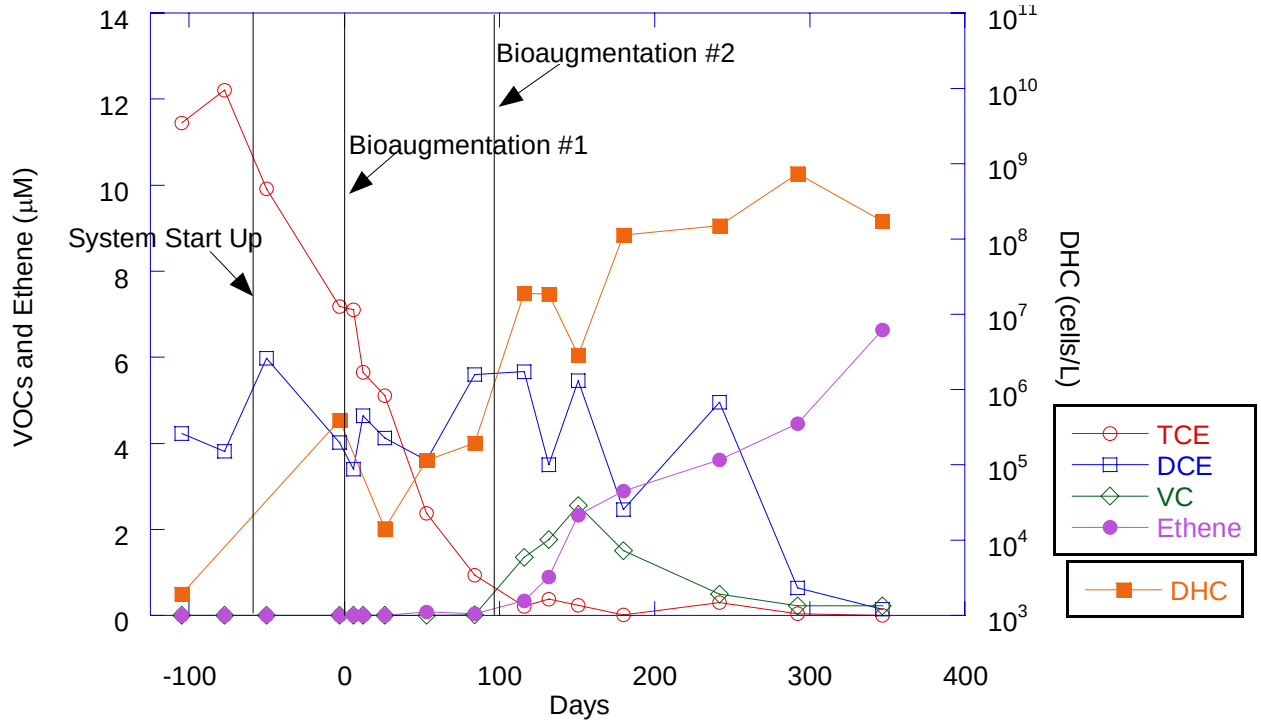


Figure C-5. Chlorinated Ethenes, Ethene, and DHC: Monitoring Well BMW-5 (Loop 3)

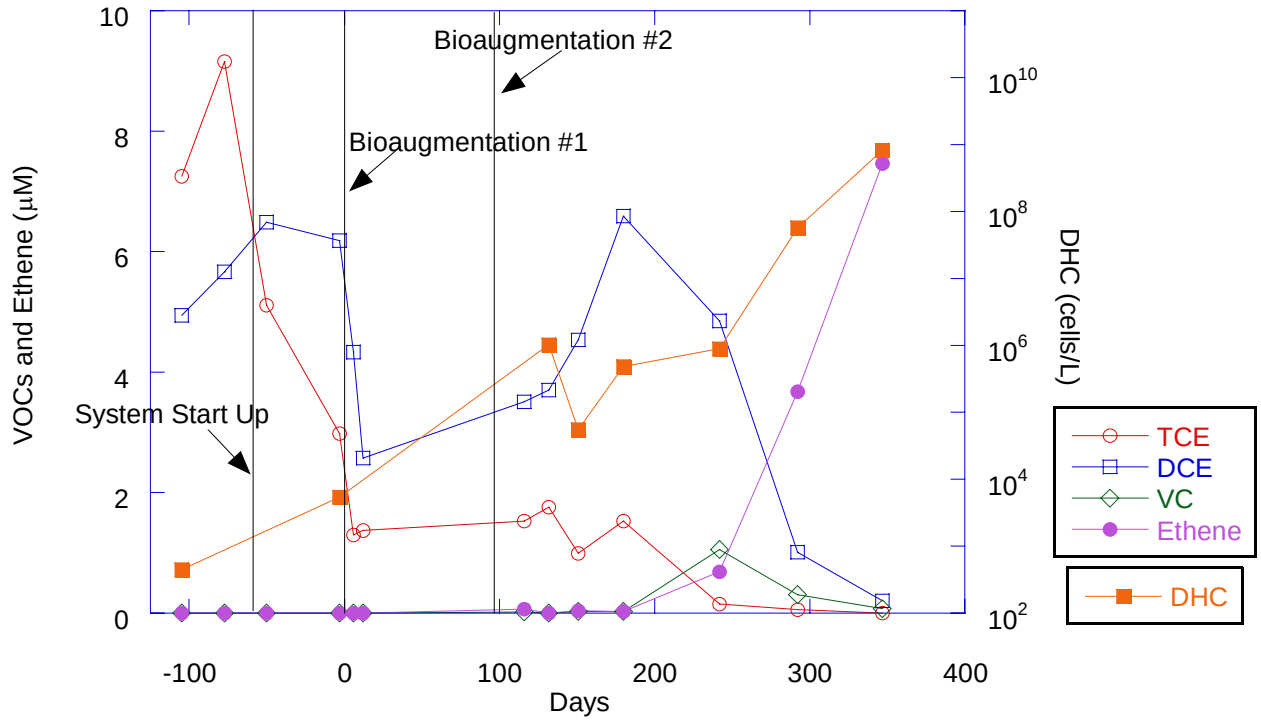


Figure C-6. Chlorinated Ethenes, Ethene, and DHC: Monitoring Well BMW-6 (Loop 3)

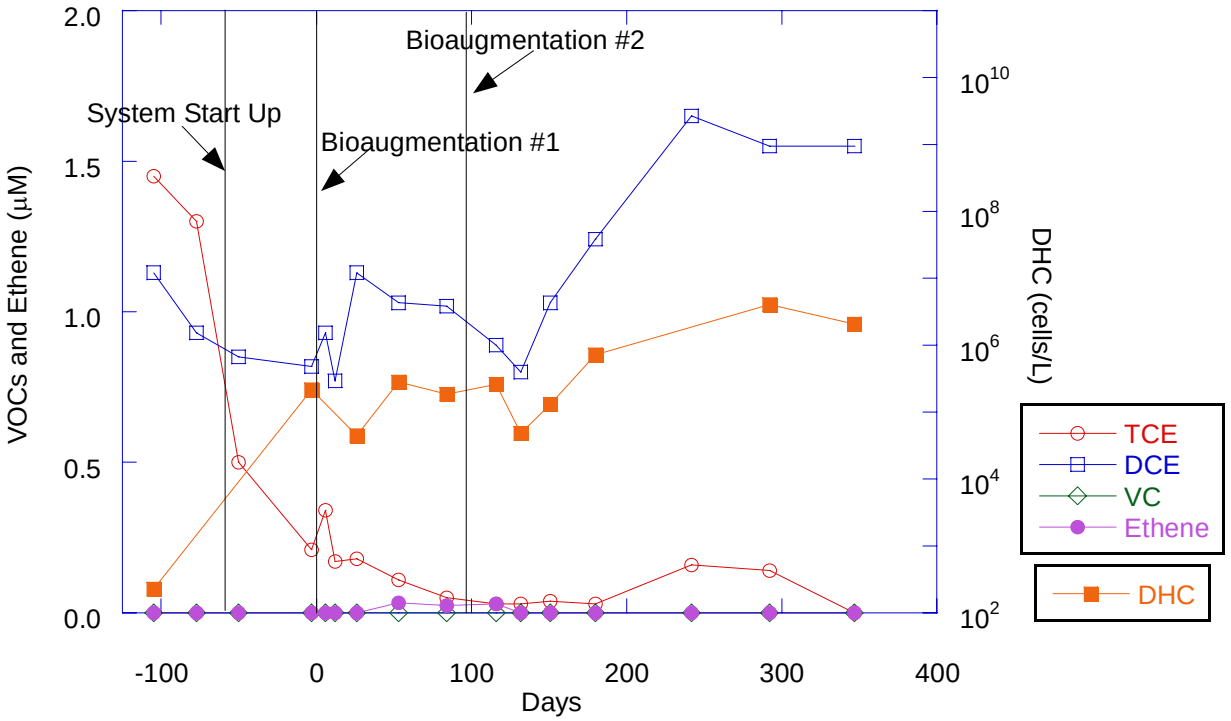


Figure C-7. Chlorinated Ethenes, Ethene, and DHC: Monitoring Well BMW-7 (Loop 4)

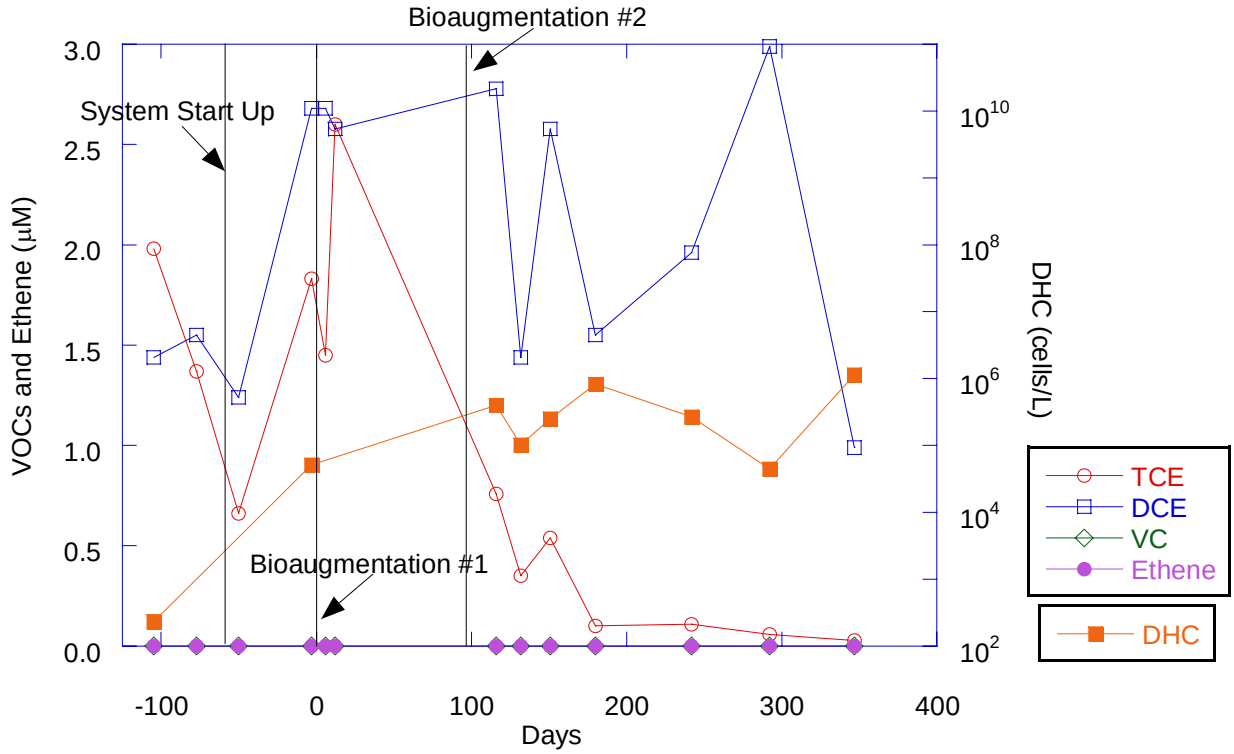


Figure C-8. Chlorinated Ethenes, Ethene, and DHC: Monitoring Well BMW-8 (Loop 4)

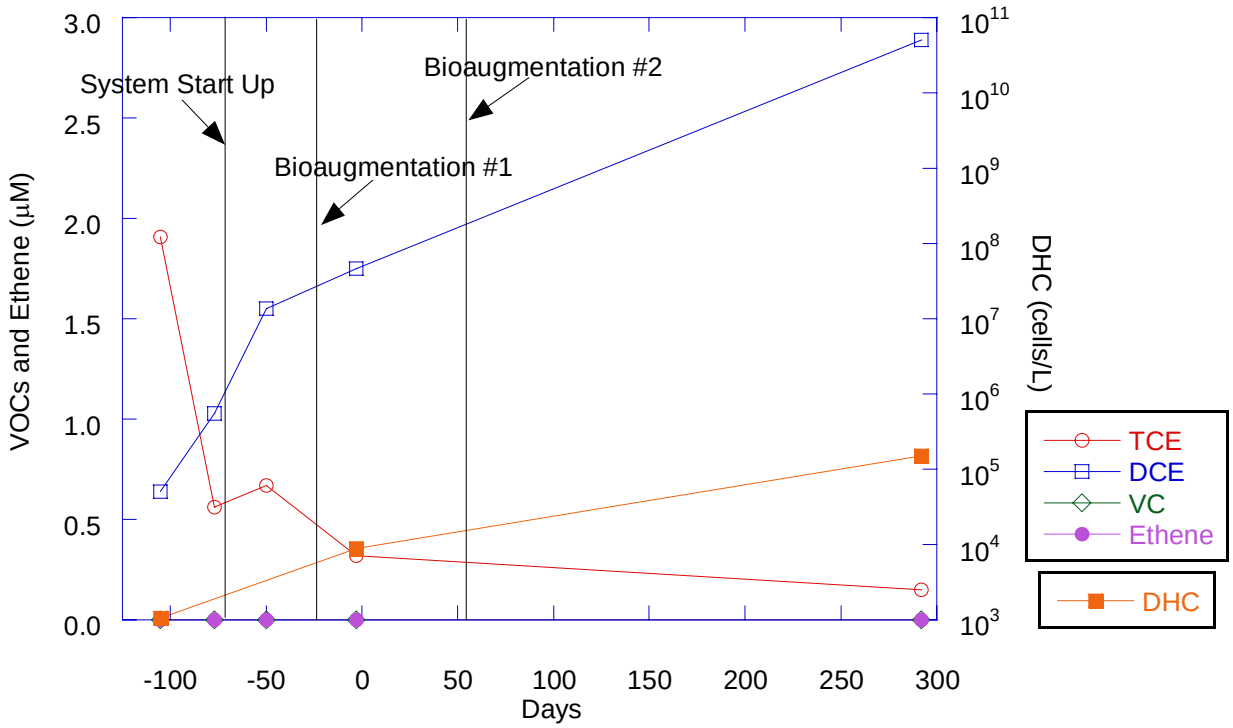


Figure C-9. Chlorinated Ethenes, Ethene, and DHC: Monitoring Well BMW-9 (Loop 1)

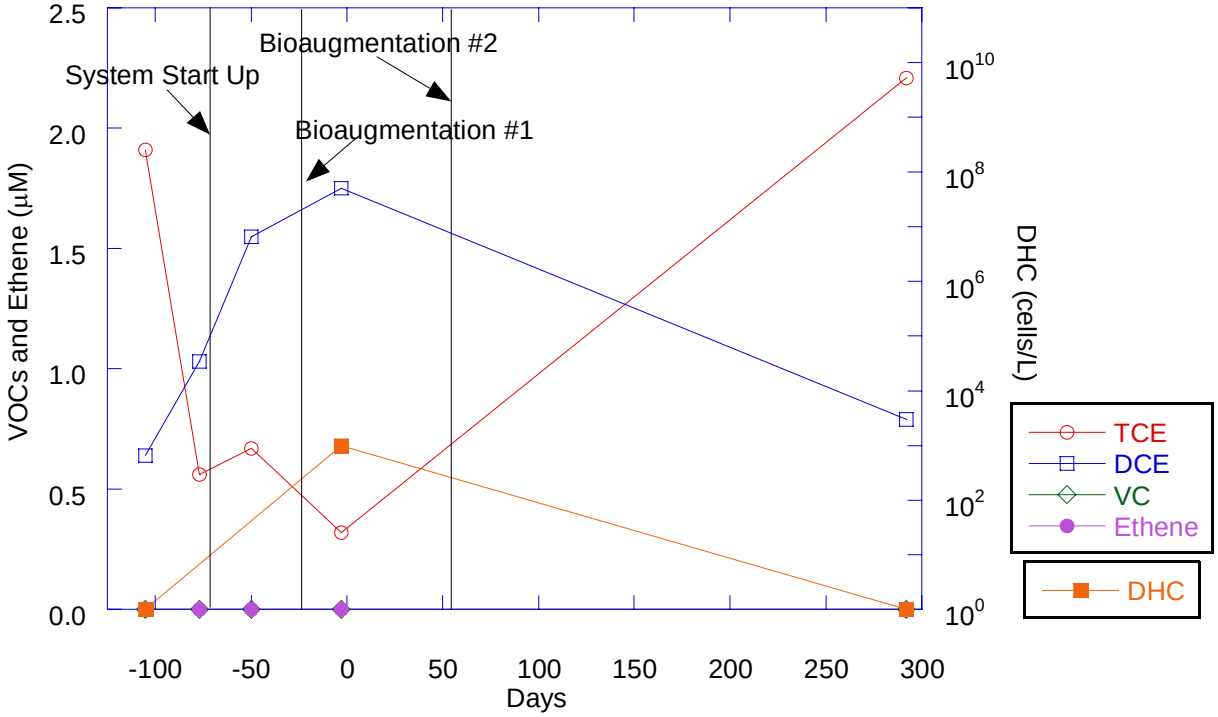


Figure C-10. Chlorinated Ethenes, Ethene, and DHC: Monitoring Well BMW-10 (Loop 1)

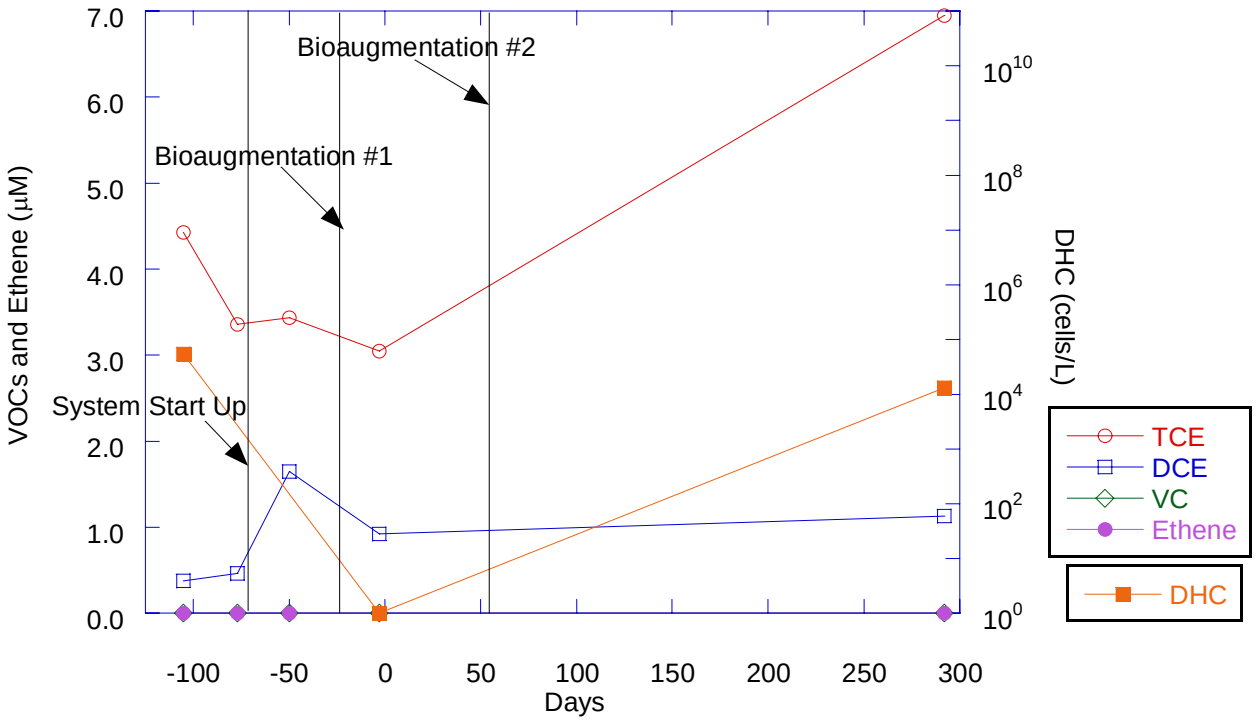


Figure C-11. Chlorinated Ethenes, Ethene, and DHC: Monitoring Well BMW-11 (Loops 1 & 2)

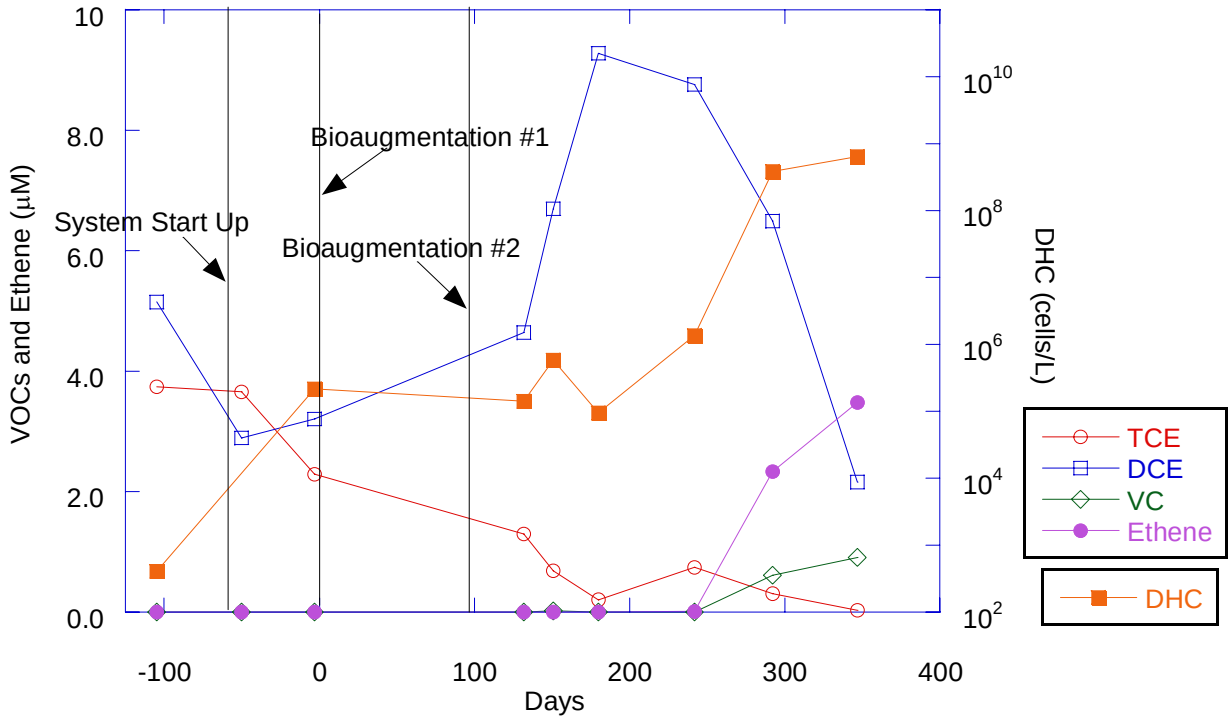


Figure C-12. Chlorinated Ethenes, Ethene, and DHC: Monitoring Well PZ-1 (Loop 3)

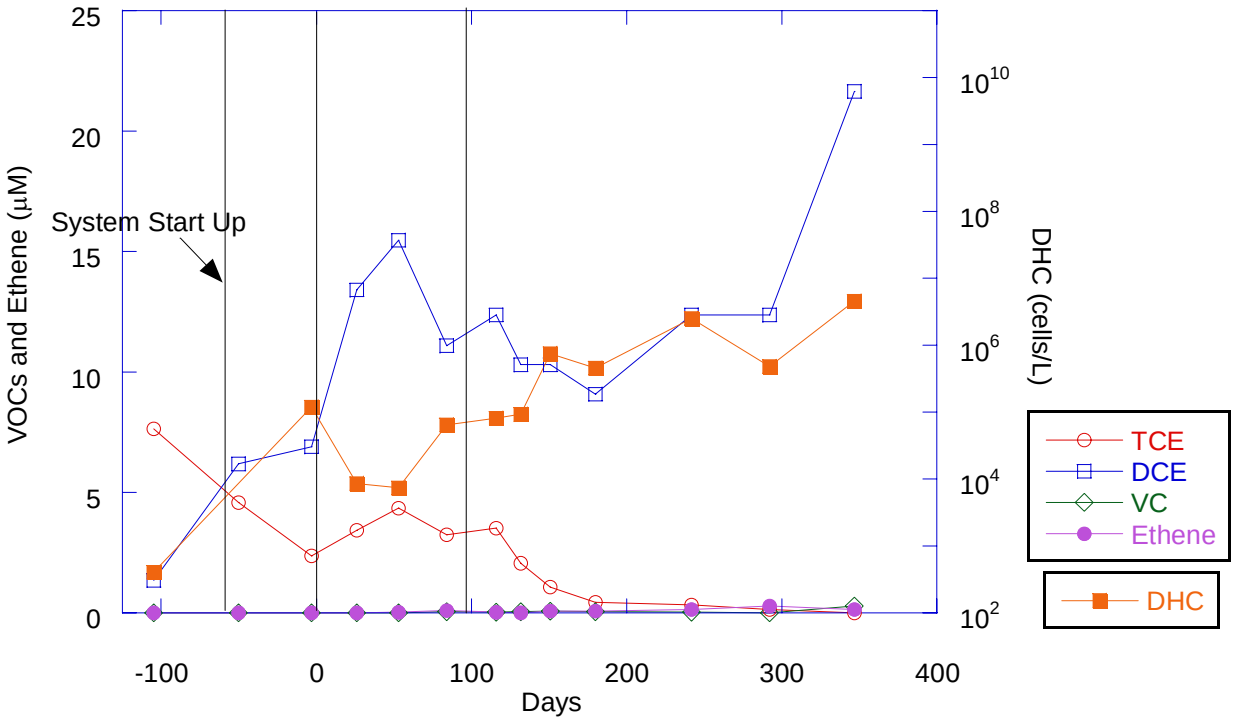


Figure C-13. Chlorinated Ethenes, Ethene, and DHC: Monitoring Well PZ-2 (Loop 3)

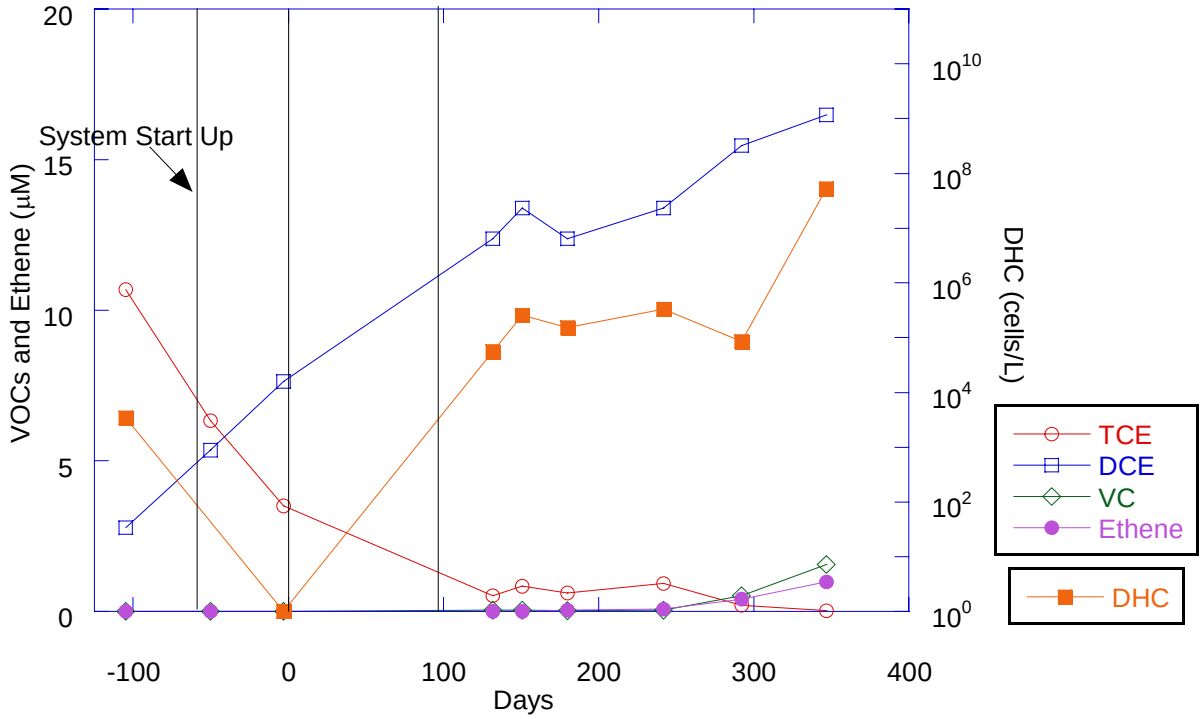


Figure C-14. Chlorinated Ethenes and Ethene: Monitoring Well MAG-113P (Loop 3)

Table 5-4
PCR Results through April 2008, Fort Dix

Date	Well ID	Description	*Bac*		*Dhc*		*BvcA*		*VcrA*		*TceA*		DNA conc (ng/µl)
			average/L	*stdev/L*	*average/L*	*stdev/L*	*average/L*	*stdev/L*	*average/L*	*stdev/L*	*average/L*	*stdev/L*	
21-Jan & 22-Jan	BMW-1	Sterivex-PowerSoil Kit	2.68E+09	3.56E+08	6.32E+03	3.37E+03			2.98E+03	1.24E+03	3.81E+03	2.98E+03	4.9
samples	BMW-3	Sterivex-PowerSoil Kit	6.27E+08	8.31E+07	2.13E+04	3.66E+03			9.66E+03	3.54E+03	1.90E+04	2.97E+03	4.5
	BMW-5	Sterivex-PowerSoil Kit	9.52E+09	1.72E+09	1.73E+05	8.54E+04			3.73E+04	2.47E+03	1.15E+05	6.87E+04	4.9
	BMW-5X	Sterivex-PowerSoil Kit	6.74E+09	6.31E+08	1.13E+05	3.52E+04			6.56E+04	1.05E+04	1.06E+05	2.17E+04	5.0
	MAG4	Sterivex-PowerSoil Kit	1.52E+09	1.17E+08	6.60E+02	1.77E+02			4.22E+02	2.80E+01	1.25E+03	6.42E+02	10.7
30-Jan & 31-Jan	BMW-1	Sterivex-PowerSoil Kit	4.38E+09	4.16E+08	2.06E+04	3.42E+03			9.69E+03	6.18E+02	1.48E+04	5.28E+03	4.3
samples	BMW-3	Sterivex-PowerSoil Kit	5.30E+08	3.39E+07	2.91E+04	7.86E+03			5.44E+03	2.45E+02	7.46E+03	4.04E+03	6.1
	BMW-5	Sterivex-PowerSoil Kit	1.91E+09	2.79E+08	7.37E+04	1.13E+04			2.66E+04	1.08E+04	4.56E+04	1.38E+04	4.9
	BMW-5X	Sterivex-PowerSoil Kit	1.43E+09	7.05E+07	1.24E+05	8.12E+03			2.61E+04	3.70E+03	4.60E+04	1.27E+04	5.0
	BMW-7	Sterivex-PowerSoil Kit	4.70E+08	5.29E+07	4.90E+04	9.25E+03			1.22E+04	4.42E+03	2.99E+04	5.19E+03	5.4
Feb 08	BMW-1	Sterivex-PowerSoil Kit	5.44E+08	9.20E+07	***8.33E+04***	***1.07E+04***			***1.30E+05***	***1.26E+04***	***1.22E+05***	***1.72E+04***	5.9
	BMW-3	Sterivex-PowerSoil Kit	1.03E+08	2.75E+07		1.23E+03							3.5
	BMW-5	Sterivex-PowerSoil Kit	6.61E+08	5.87E+08		9.68E+02							4.2
	BMW-5X	Sterivex-PowerSoil Kit	1.36E+09	3.44E+08		5.19E+03							2.6
	BMW-7	Sterivex-PowerSoil Kit	5.91E+08	2.57E+08		3.95E+03							6.6
Mar 08	BMW-1	Sterivex-PowerSoil Kit	1.39E+09	3.96E+08	***8.89E+03***	***1.09E+03***			***1.63E+04***	***4.21E+03***	***2.91E+04***	***1.56E+03***	9.9
	BMW-3	Sterivex-PowerSoil Kit	1.35E+08	4.59E+07									3.6
	BMW-5	Sterivex-PowerSoil Kit	2.08E+09	7.75E+08									4.3
	BMW-5X	Sterivex-PowerSoil Kit	1.54E+09	1.93E+08			*9.35E+03*	*2.73E+03*					5.3
	BMW-7	Sterivex-PowerSoil Kit	1.04E+09	2.23E+08									4.6
	IW-1	Sterivex-PowerSoil Kit	1.40E+09	8.62E+07	8.42E+03	1.32E+03			1.74E+04	1.73E+03	9.50E+03	1.70E+03	9.5
	IW-2	Sterivex-PowerSoil Kit	2.78E+09	6.38E+08	5.50E+04	9.12E+03			1.27E+05	1.69E+04	7.10E+04	1.51E+04	14.0
	IW-3	Sterivex-PowerSoil Kit	2.91E+09	5.08E+08	***3.51E+03***	***2.99E+02***			1.01E+04	3.54E+03	5.37E+03	1.22E+03	7.9
	IW-4	Sterivex-PowerSoil Kit	1.34E+09	1.48E+08									11.1
Apr 08	BMW-1	Sterivex-PowerSoil Kit	5.00E+08	2.26E+08	***1.64E+04***	***1.57E+03***			***1.39E+04***	***3.20E+03***	***3.18E+04***	***8.37E+03***	5.5
	BMW-3	Sterivex-PowerSoil Kit	3.02E+08	1.78E+08	***9.36E+03***	***2.35E+03***			***8.81E+03***	***1.34E+03***	***9.70E+03***	***9.67E+01***	5.0
	BMW-5	Sterivex-PowerSoil Kit	3.46E+08	1.25E+08									4.4
	BMW-5X	Sterivex-PowerSoil Kit	7.53E+08	2.01E+08									5.2
	BMW-7	Sterivex-PowerSoil Kit	2.75E+08	1.55E+08									4.8
	IW-1	Sterivex-PowerSoil Kit	2.52E+07	7.40E+06	2.24E+04	3.84E+03			1.52E+04	6.06E+03	1.41E+04	5.27E+03	4.6
	IW-2	Sterivex-PowerSoil Kit	1.42E+09	5.05E+08	1.56E+04	4.28E+03			1.37E+04	1.29E+03	9.91E+03	3.63E+03	24.5
	IW-3	Sterivex-PowerSoil Kit	1.06E+08	2.35E+07	***1.53E+03***	***9.69E+01***			1.26E+03	1.66E+02	***1.49E+03***	***4.41E+02***	8.8
	IW-4	Sterivex-PowerSoil Kit	4.39E+09	9.87E+08									29.4

= Undetected
= DNQ
gene copies = only detectable in one dilution otherwise DNQ
Edge of detection

Figure 5-4
PCR Results Through April 2008
Fort Dix

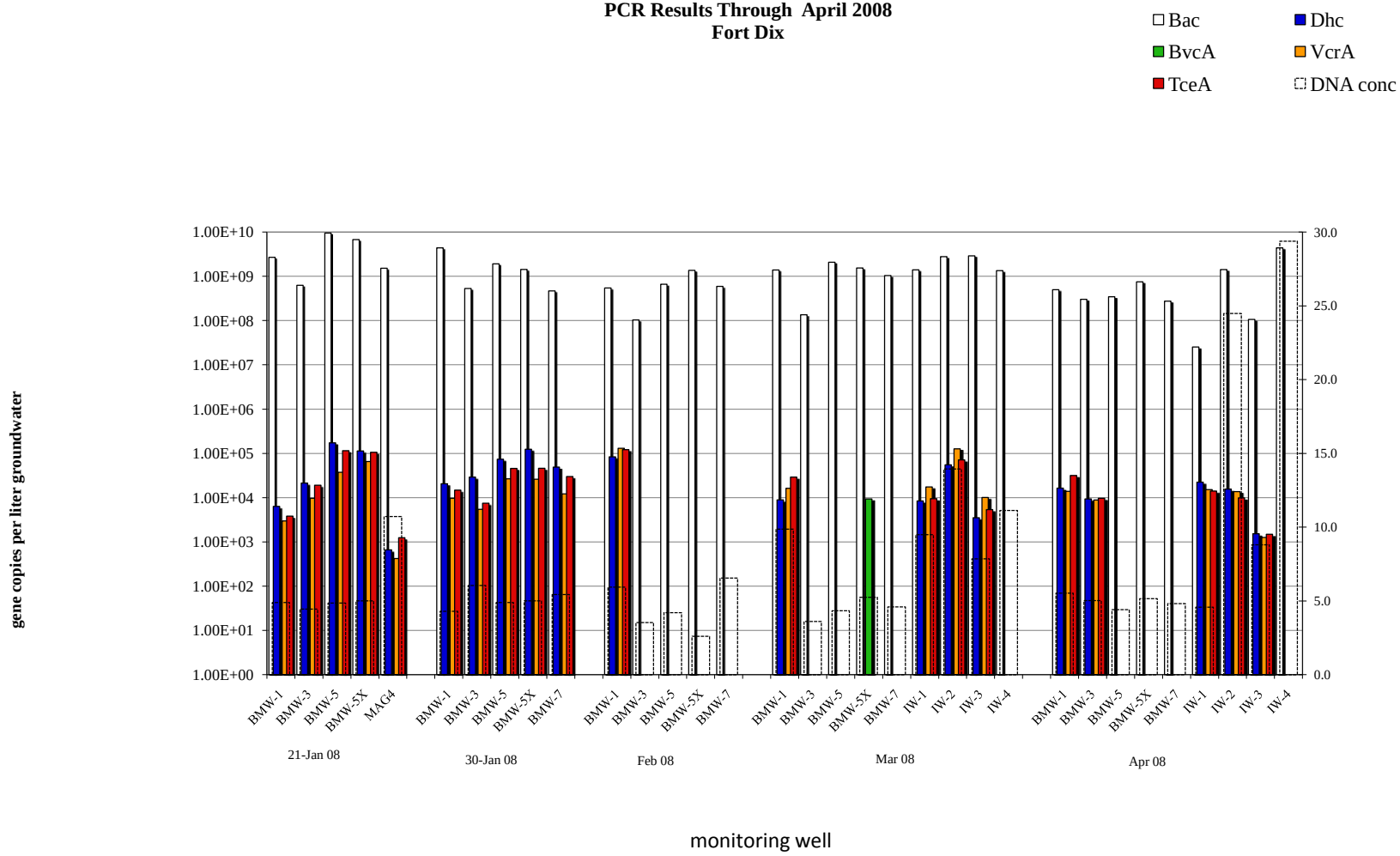


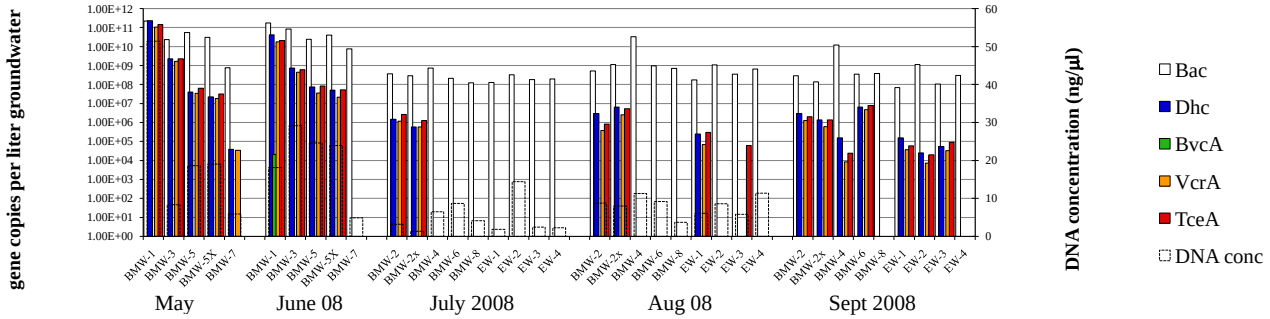
Table 5-5
PCR Results Since May 2008 (2nd Round of bioaugmentatuon)
Fort Dix

Description	*Bac*		*Dhc*		*BvcA*		*VcrA*		*TceA*		
	average/L	*stdev/L*	*average/L*	*stdev/L*	*average/L*	*stdev/L*	*average/L*	*stdev/L*	*average/L*	*stdev/L*	DNA conc (ng/µl)
Sterivex-PowerSoil Kit	2.26E+11	5.68E+10	2.27E+11	3.09E+10			1.08E+11	2.76E+10	1.46E+11	2.05E+10	51.5
Sterivex-PowerSoil Kit	2.36E+10	7.12E+09	2.29E+09	1.49E+08			1.69E+09	1.80E+08	2.30E+09	1.49E+08	8.3
Sterivex-PowerSoil Kit	5.37E+10	3.89E+09	3.98E+07	7.47E+06			3.37E+07	1.81E+06	6.38E+07	1.26E+07	18.6
Sterivex-PowerSoil Kit	3.03E+10	2.76E+10	2.24E+07	1.04E+06			1.85E+07	2.13E+06	3.15E+07	4.02E+06	19.0
Sterivex-PowerSoil Kit	7.64E+08	4.59E+07	***3.79E+04***	***1.13E+04***			***3.41E+04***	***1.29E+04***			5.9
Sterivex-PowerSoil Kit	1.74E+11	4.42E+10	4.11E+10	1.90E+09	***2.09E+04***	***6.51E+03***	1.78E+10	3.51E+09	2.08E+10	6.42E+09	18.2
Sterivex-PowerSoil Kit	8.43E+10	1.06E+10	7.32E+08	7.79E+07			4.48E+08	5.60E+07	6.15E+08	1.24E+08	29.2
Sterivex-PowerSoil Kit	2.38E+10	2.10E+09	7.50E+07	3.92E+06			3.56E+07	2.10E+06	8.42E+07	5.71E+06	24.6
Sterivex-PowerSoil Kit	3.98E+10	6.91E+09	5.10E+07	4.12E+06			2.19E+07	1.23E+07	5.24E+07	1.57E+07	23.9
Sterivex-PowerSoil Kit	7.44E+09	1.21E+09									4.8
Sterivex-PowerSoil Kit	3.67E+08	6.38E+07	1.47E+06	1.96E+04			1.19E+06	4.25E+04	2.61E+06	7.83E+04	3.2
Sterivex-PowerSoil Kit	2.87E+08	4.99E+07	5.84E+05	3.84E+04			5.73E+05	3.09E+04	1.25E+06	1.27E+05	1.3
Sterivex-PowerSoil Kit	7.22E+08	6.77E+07									6.5
Sterivex-PowerSoil Kit	2.11E+08	2.52E+07									8.7
Sterivex-PowerSoil Kit	1.23E+08	1.18E+07									4.1
Sterivex-PowerSoil Kit	1.28E+08	2.97E+07									1.8
Sterivex-PowerSoil Kit	3.24E+08	2.33E+07									14.4
Sterivex-PowerSoil Kit	1.85E+08	1.83E+07									2.4
Sterivex-PowerSoil Kit	1.98E+08	2.24E+07									2.3
Sterivex-PowerSoil Kit	5.19E+08	2.41E+07	*3.00E+06*	*3.47E+05*			3.85E+05	7.14E+04	8.19E+05	3.69E+05	8.8
Sterivex-PowerSoil Kit	1.13E+09	7.23E+07	*6.35E+06*	*1.46E+05*			2.50E+06	1.27E+05	5.32E+06	2.65E+05	8.0
Sterivex-PowerSoil Kit	3.26E+10	2.00E+09									11.3
Sterivex-PowerSoil Kit	9.75E+08	2.00E+07									9.2
Sterivex-PowerSoil Kit	7.08E+08	8.62E+07									3.7
Sterivex-PowerSoil Kit	1.73E+08	4.89E+06	2.49E+05	9.87E+04			6.89E+04	7.78E+03	2.99E+05	4.42E+04	6.0
Sterivex-PowerSoil Kit	1.09E+09	1.05E+08									8.6
Sterivex-PowerSoil Kit	3.52E+08	1.28E+07							6.11E+04	1.67E+04	5.8
Sterivex-PowerSoil Kit	6.64E+08	6.97E+07									11.4
Sterivex-PowerSoil Kit	*2.85E+08*	*2.34E+07*	2.92E+06	2.49E+05			*1.26E+06*	*3.82E+05*	1.99E+06	1.09E+06	4.5
Sterivex-PowerSoil Kit	*1.40E+08*	*1.99E+07*	*1.36E+06*	*2.12E+05*			*6.10E+05*	*7.21E+04*	1.35E+06	6.16E+05	3.6
Sterivex-PowerSoil Kit	1.21E+10	1.35E+09	*1.56E+05*	*2.76E+04*			***8.56E+03***	***5.19E+03***	***2.44E+04***	***2.84E+03***	5.9
Sterivex-PowerSoil Kit	3.54E+08	2.73E+07	6.47E+06	6.09E+05			4.74E+06	7.38E+05	7.93E+06	7.91E+05	3.3
Sterivex-PowerSoil Kit	3.85E+08	2.68E+07									2.1
Sterivex-PowerSoil Kit	*6.76E+07*	*1.61E+07*	*1.54E+05*	*5.16E+04*			3.63E+04	6.26E+03	5.92E+04	1.93E+04	5.9
Sterivex-PowerSoil Kit	1.11E+09	1.25E+08	***2.49E+04***	***6.45E+03***			7.22E+03	1.64E+03	***2.01E+04***	***1.77E+03***	4.4
Sterivex-PowerSoil Kit	1.04E+08	3.46E+07	5.52E+04	2.06E+04			***3.29E+04***	***2.56E+03***	8.88E+04	2.10E+04	5.2
Sterivex-PowerSoil Kit	2.98E+08	3.90E+07									2.8

Note: Don't usually see inhibition in Bac samples because of using 1:10 and 1:100 dilutions. This time I assayed undil and 1:10.
Note: New wells sampled from June 23, 2008

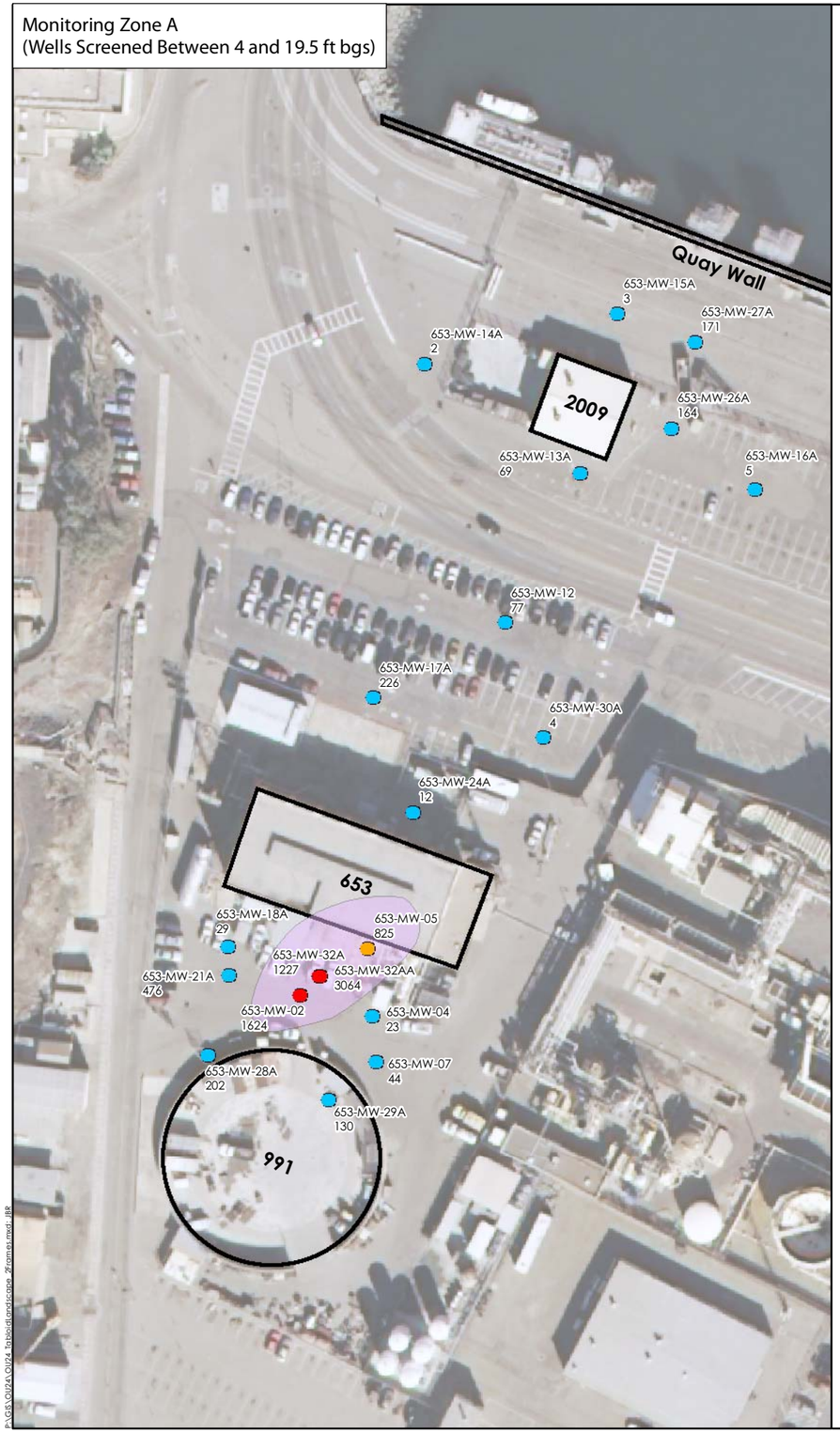
= Undetected
= DNQ
= Did not assay
gene copies = only detectable in one dilution

Figure 5-5
PCR Results Since May 2008
Fort Dix

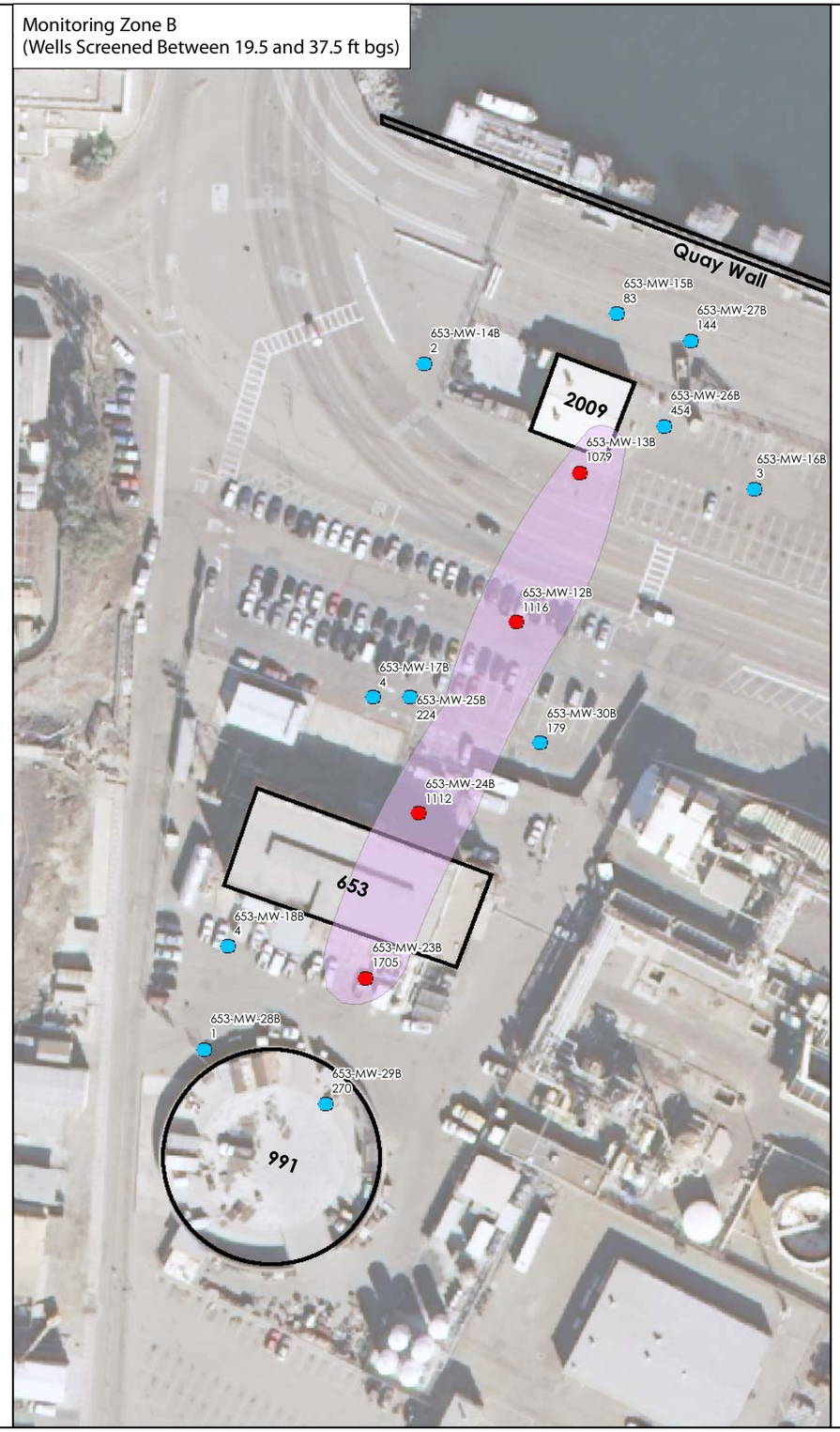


Appendix B-8: NAS North Island

Monitoring Zone A
(Wells Screened Between 4 and 19.5 ft bgs)



Monitoring Zone B
(Wells Screened Between 19.5 and 37.5 ft bgs)



N

Total CVOC Concentration as VC Equivalents

- >1,050
- >787.5 - 1,050
- >525 - 787.5
- ≤525
- Total CVOC concentration as VC equivalents greater than 525 µg/L

Notes:
ft bgs - feet below ground surface
All concentrations in micrograms per liter (µg/L)
All samples collected between March 26 and April 4, 2007
CVOC - chlorinated volatile organic compounds
(sum of the only detected chloroethenes, cis-1,2-dichloroethene and vinyl chloride, expressed as the equivalent concentration of VC). For purposes of calculation, non-detect results are assumed to be equal to the quantitation limit.
VC - vinyl chloride
Aerial photograph taken November 2003, courtesy of the USGS

100 50 0 100 Feet

Distribution of Total Chlorinated Volatile Organic Compounds in Groundwater - Baseline (March 26 - April 4, 2007)

Operable Unit 24, Naval Air Station North Island
Coronado, California

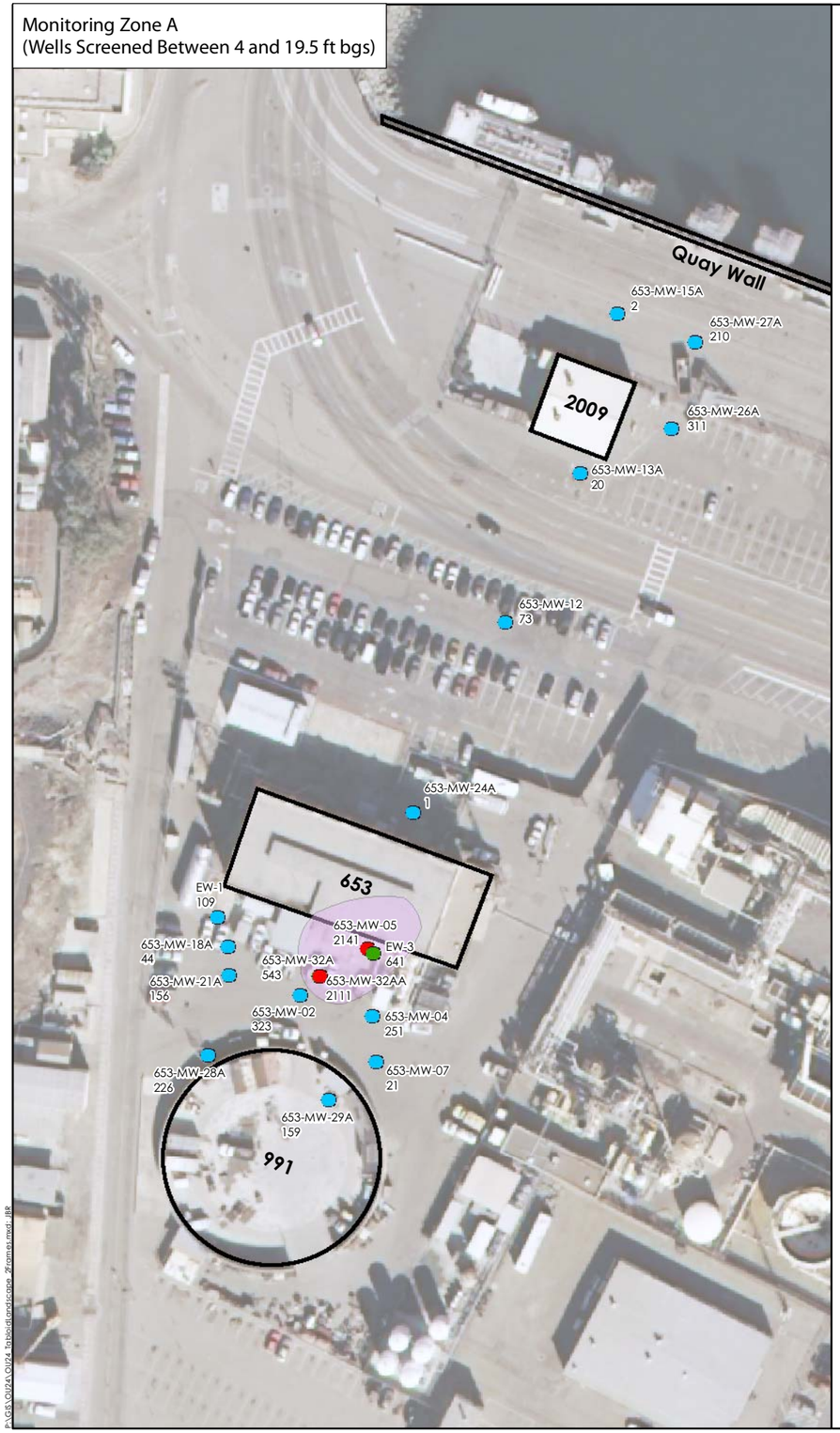
Geosyntec consultants

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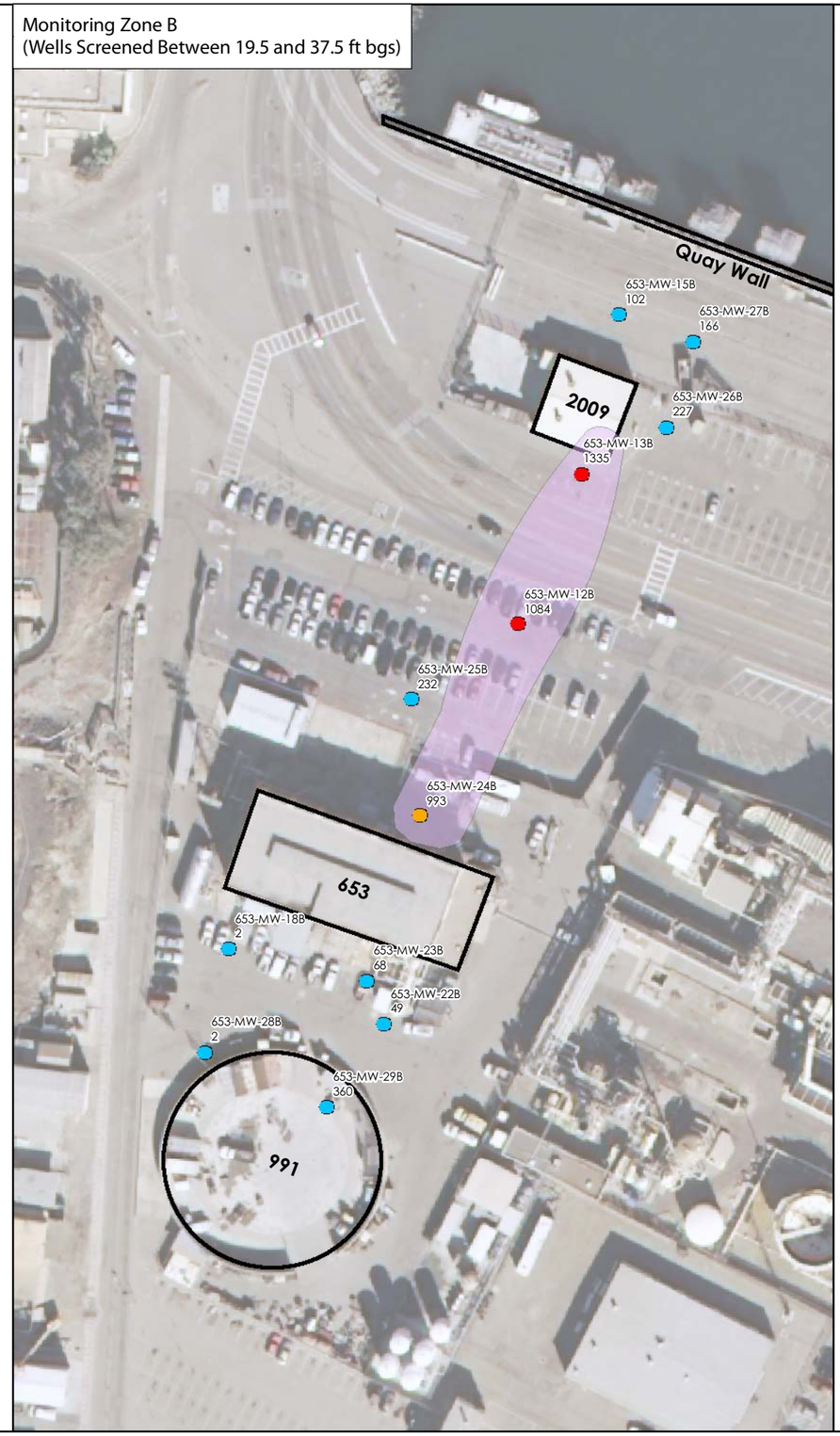
Figure **3a**

P:\GIS\OU24\OU24_tablet\Landscape_2frames.mxd - JBR

Monitoring Zone A
(Wells Screened Between 4 and 19.5 ft bgs)



Monitoring Zone B
(Wells Screened Between 19.5 and 37.5 ft bgs)



N

Total CVOC Concentration as VC Equivalents

- >1050
- >787.5 - 1050
- >525 - 787.5
- ≤525
- Total CVOC concentration as VC equivalents greater than 525 µg/L

Notes:
ft bgs - feet below ground surface
All concentrations in micrograms per liter (µg/L)
All samples collected between July 31 and August 7, 2007
CVOC - chlorinated volatile organic compounds
(sum of the only detected chloroethenes, cis-1,2-dichloroethene and vinyl chloride, expressed as the equivalent concentration of VC). For purposes of calculation, non-detect results are assumed to be equal to the quantitation limit.
VC - vinyl chloride
Aerial photograph taken November 2003, courtesy of the USGS

100 50 0 100 Feet

Distribution of Total Chlorinated Volatile Organic Compounds in Groundwater - Q1 (July 31 - August 7, 2007)

Operable Unit 24, Naval Air Station North Island
Coronado, California

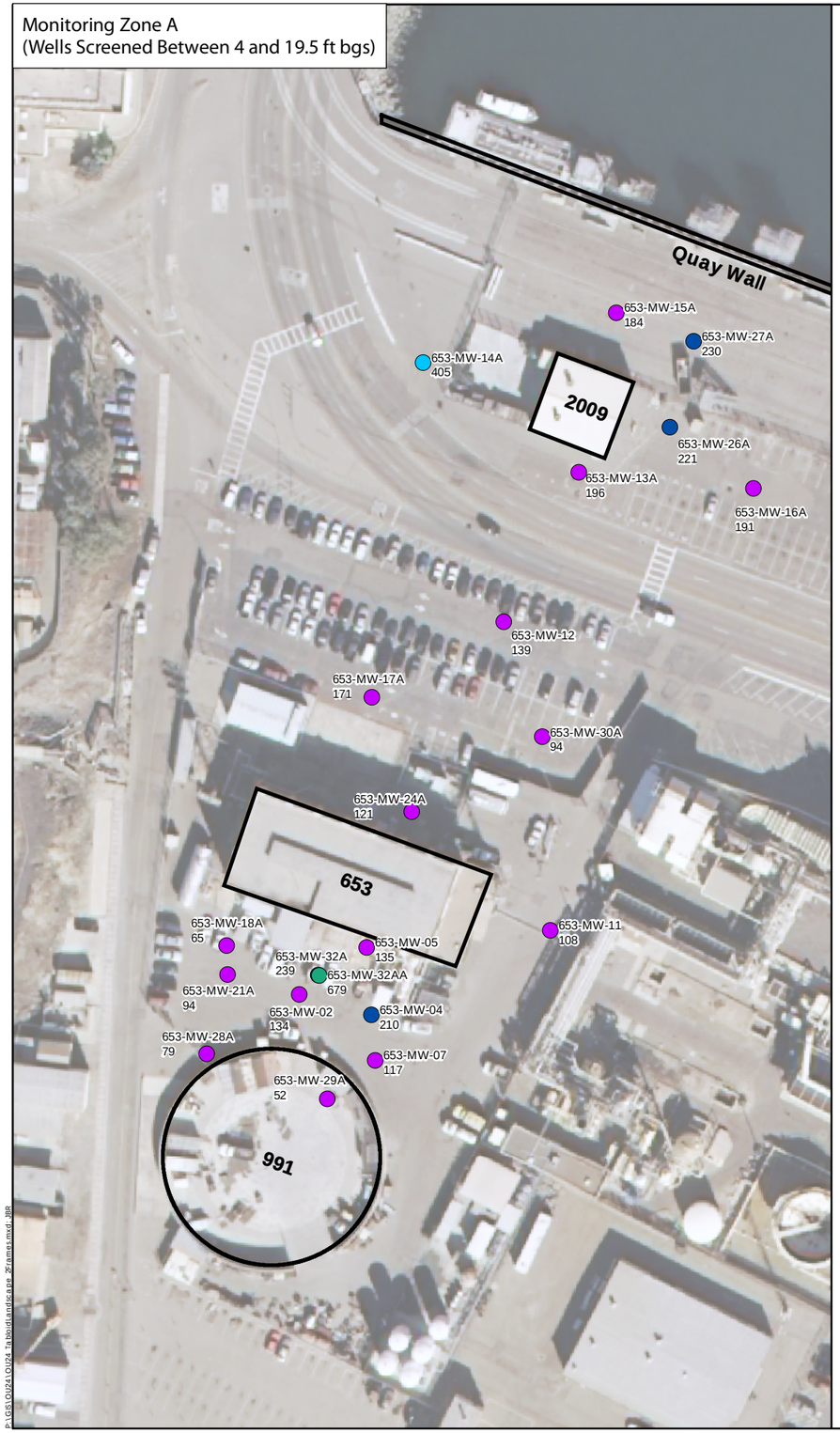
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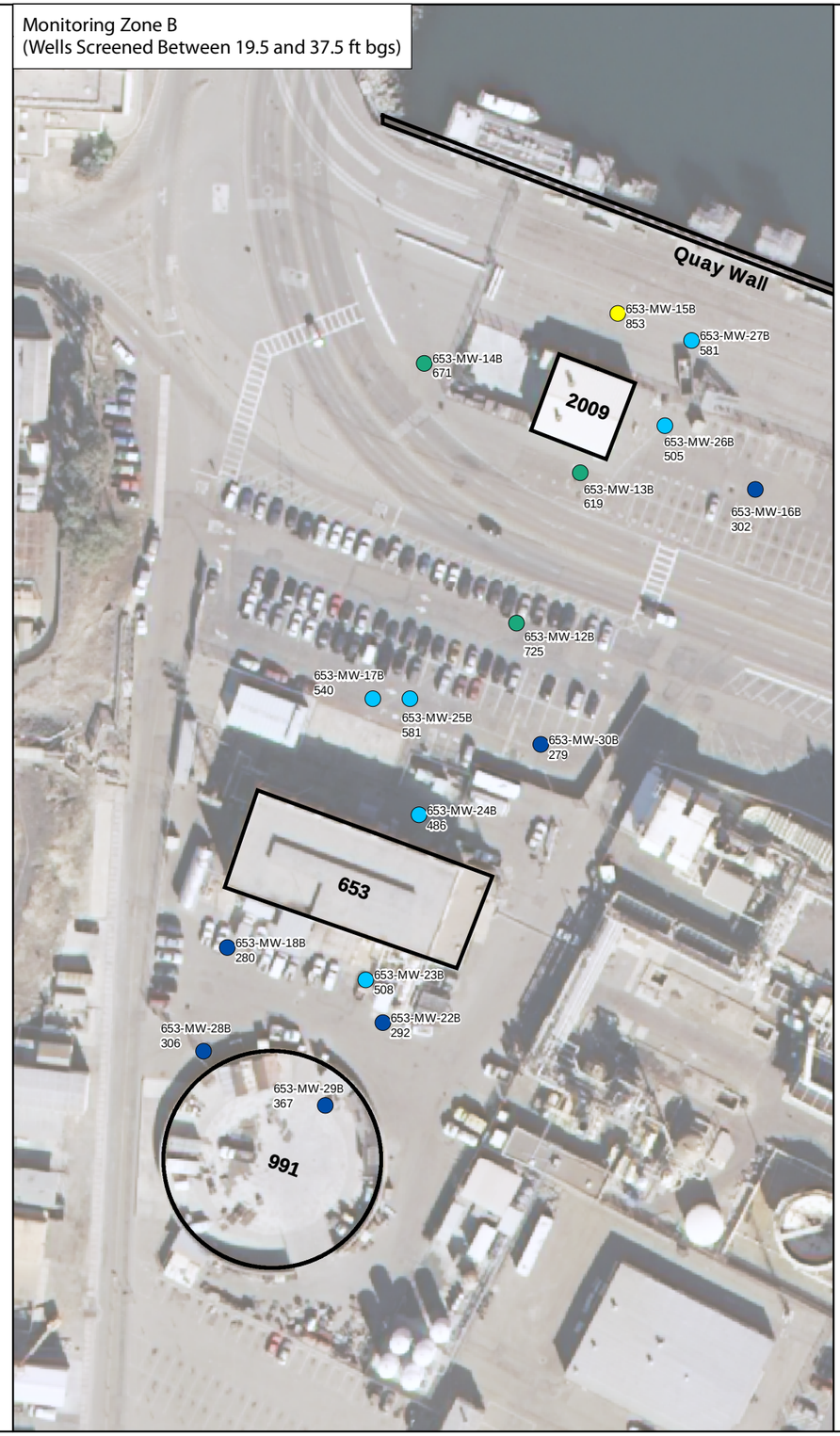
Figure **3b**

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Monitoring Zone A
(Wells Screened Between 4 and 19.5 ft bgs)



Monitoring Zone B
(Wells Screened Between 19.5 and 37.5 ft bgs)



N

Sulfate Concentration (mg/L)

- >800 - 1,000
- >600 - 800
- >400 - 600
- >200 - 400
- ≤200

Notes:
mg/L - milligrams per Liter
ft bgs - feet below ground surface
All samples collected between March 26 and April 4, 2007
Aerial photograph taken November 2003, courtesy of the USGS

100 50 0 100 Feet

Distribution of Sulfate in Groundwater - Baseline (March 26 - April 4, 2007)

Operable Unit 24, Naval Air Station North Island
Coronado, California

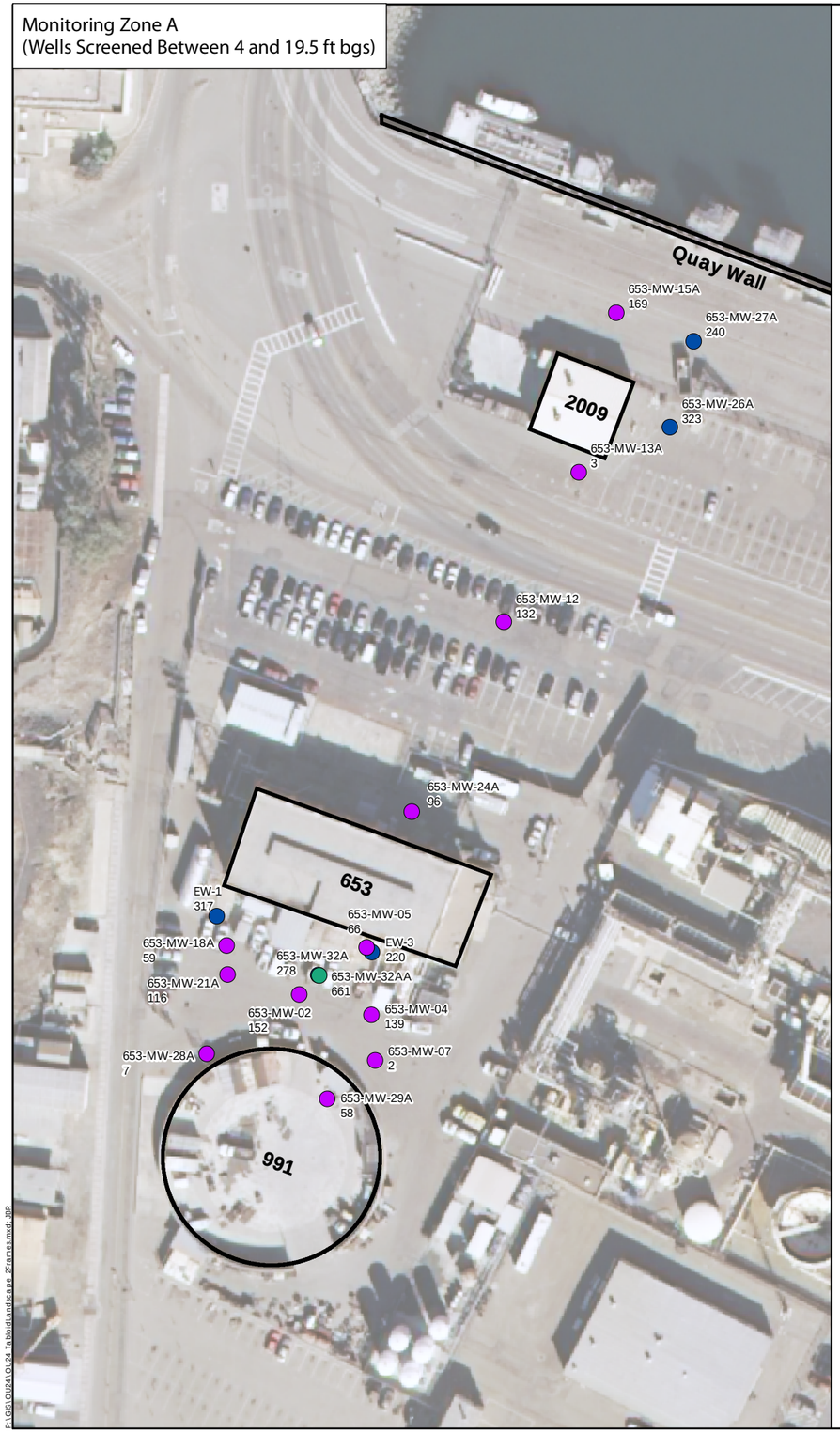
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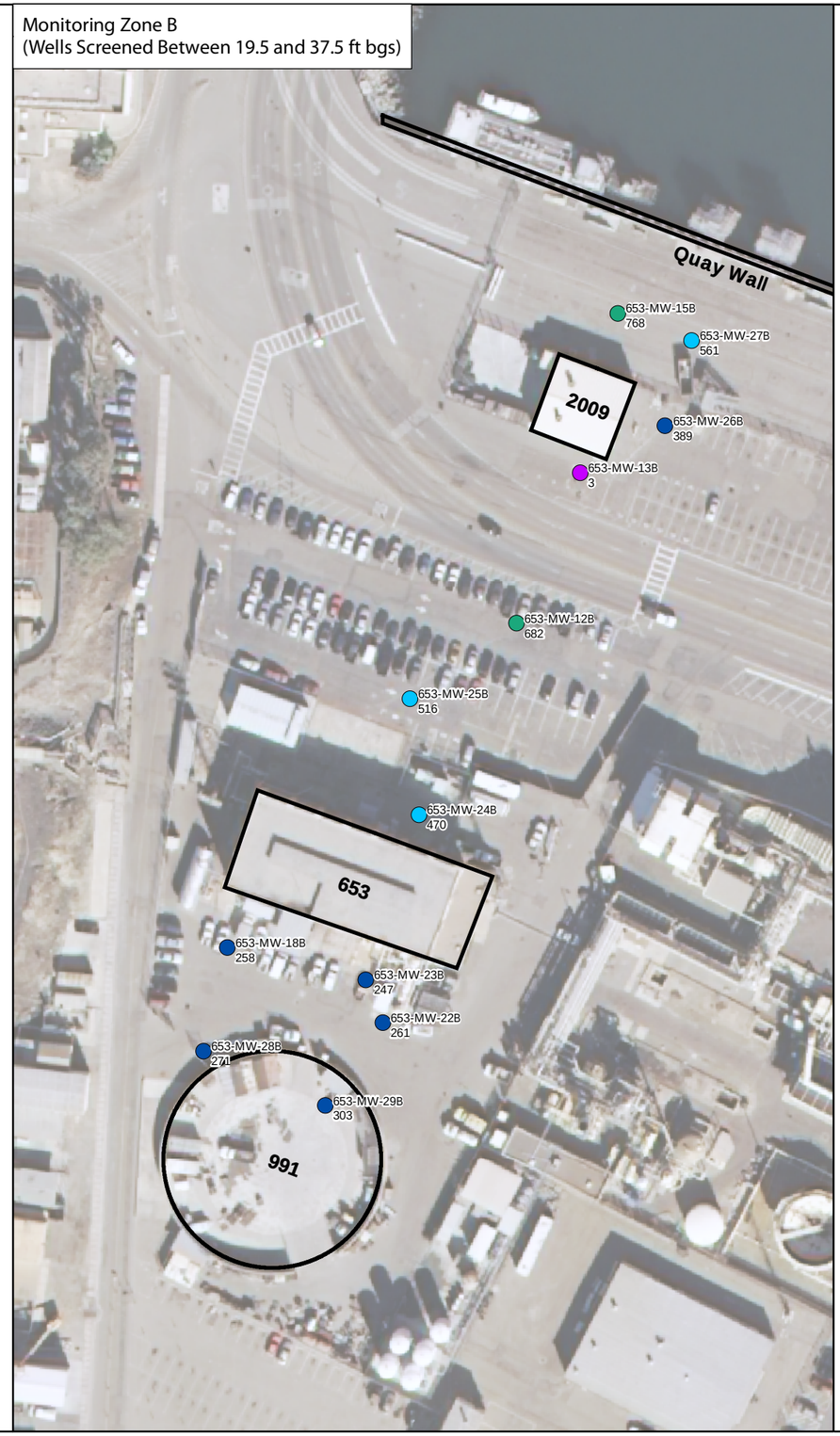
Figure **4a**

P:\GIS\OU24\OU24_Tabloidlandscape_2frames.mxd: JBR

Monitoring Zone A
(Wells Screened Between 4 and 19.5 ft bgs)



Monitoring Zone B
(Wells Screened Between 19.5 and 37.5 ft bgs)



N

Sulfate Concentration (mg/L)

- >800 - 1,000
- >600 - 800
- >400 - 600
- >200 - 400
- ≤200

Notes:
mg/L - milligrams per Liter
ft bgs - feet below ground surface
All samples collected between July 31 and August 7, 2007
Aerial photograph taken November 2003, courtesy of the USGS

100 50 0 100 Feet

Distribution of Sulfate in Groundwater - Q1 (July 31 - August 7, 2007)

Operable Unit 24, Naval Air Station North Island
Coronado, California

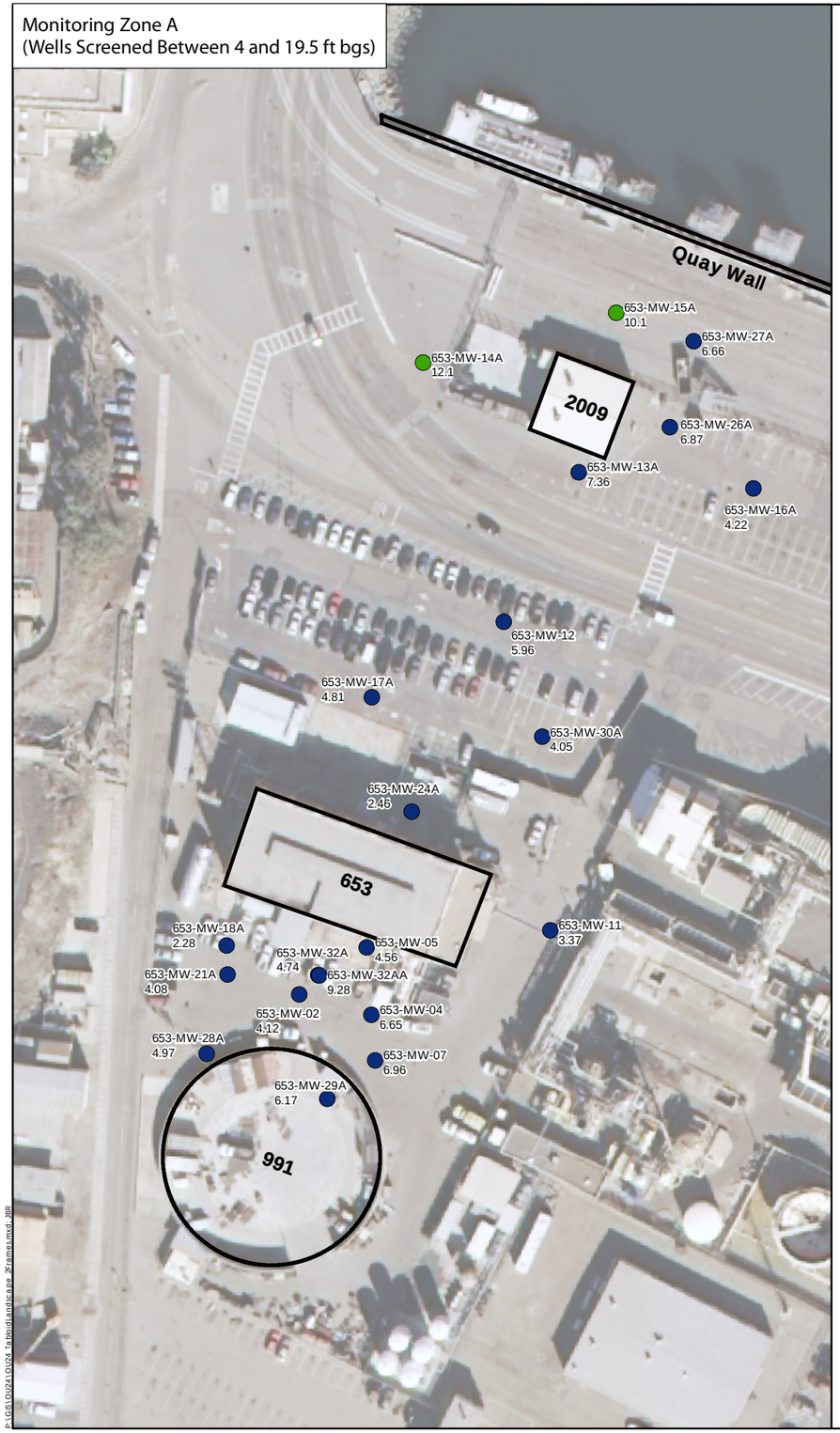
Geosyntec consultants

Guelph, Ontario | 15-Oct-2007

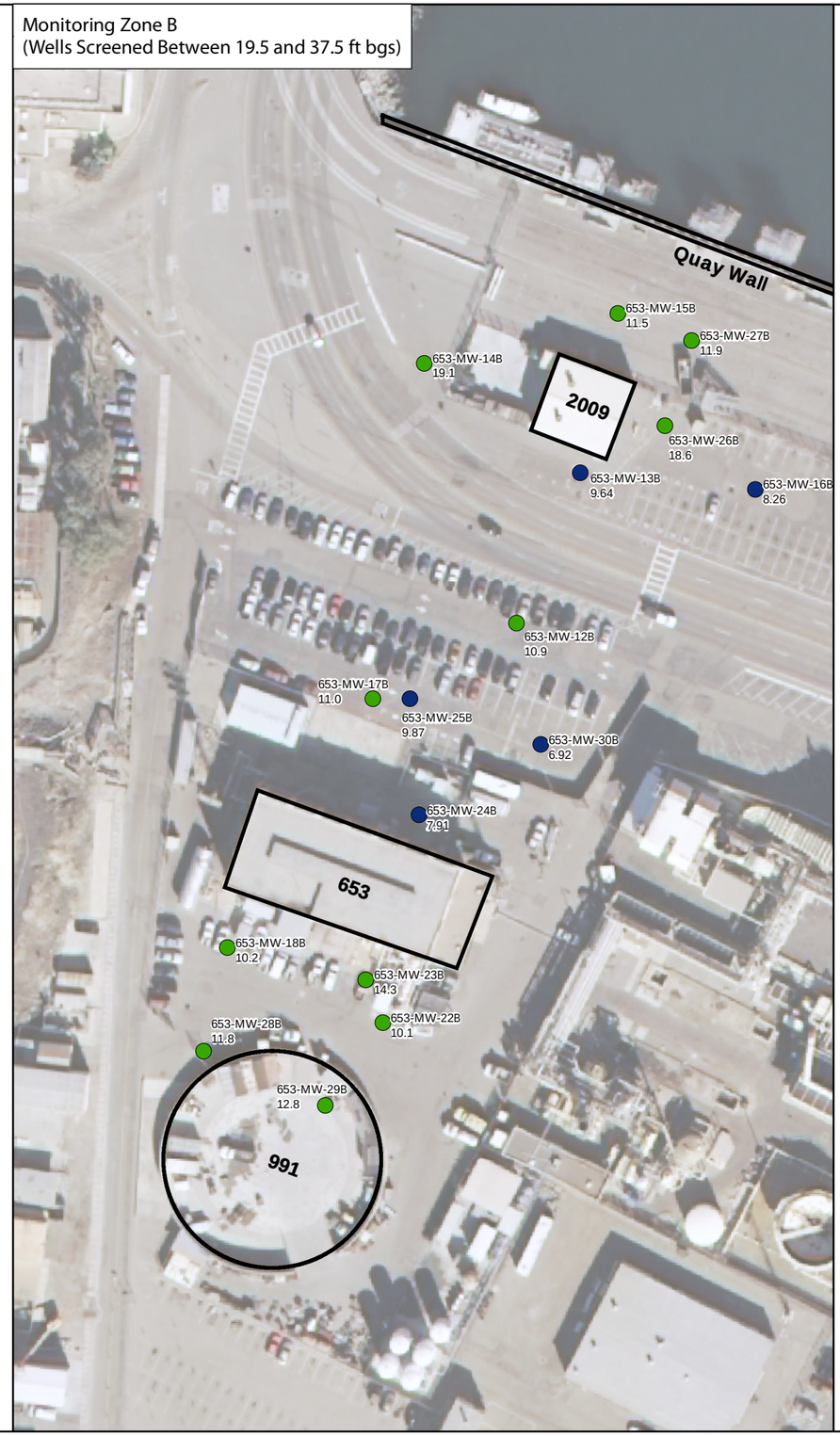
Figure **4b**

P:\GIS\OU24\OU24_Tabloidlandscape_2Frames.mxd JBR

Monitoring Zone A
(Wells Screened Between 4 and 19.5 ft bgs)



Monitoring Zone B
(Wells Screened Between 19.5 and 37.5 ft bgs)



N

Dissolved Organic Carbon Concentration (mg/L)

- >10 - 100
- ≤10

Notes:
mg/L - milligrams per Liter
ft bgs - feet below ground surface
All samples collected between March 26 and April 4, 2007
Aerial photograph taken November 2003, courtesy of the USGS

100 50 0 100 Feet

Distribution of Dissolved Organic Carbon in Groundwater - Baseline (March 26 - April 4, 2007)

Operable Unit 24, Naval Air Station North Island
Coronado, California

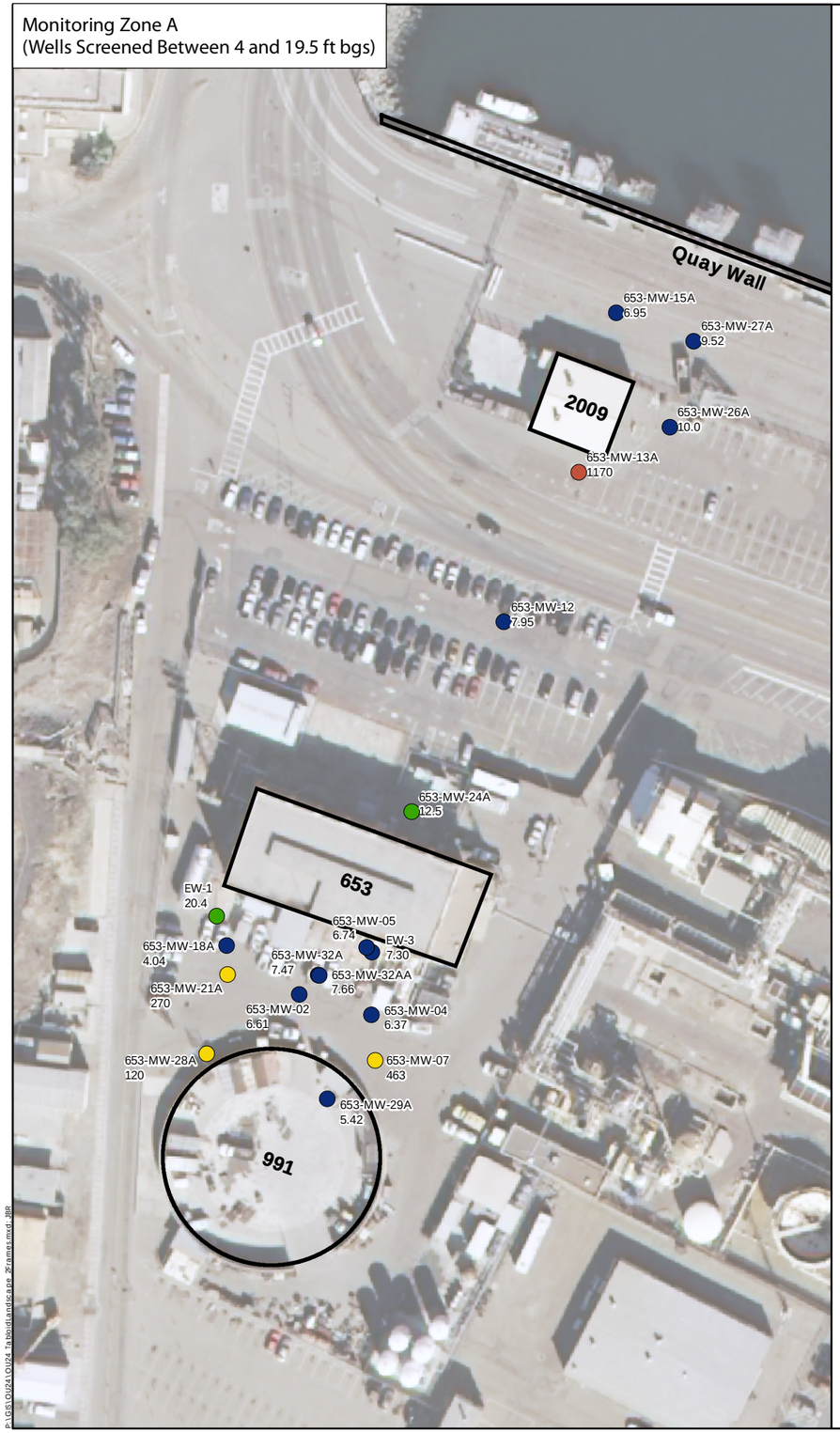
Geosyntec consultants

Guelph, Ontario | 15-Oct-2007

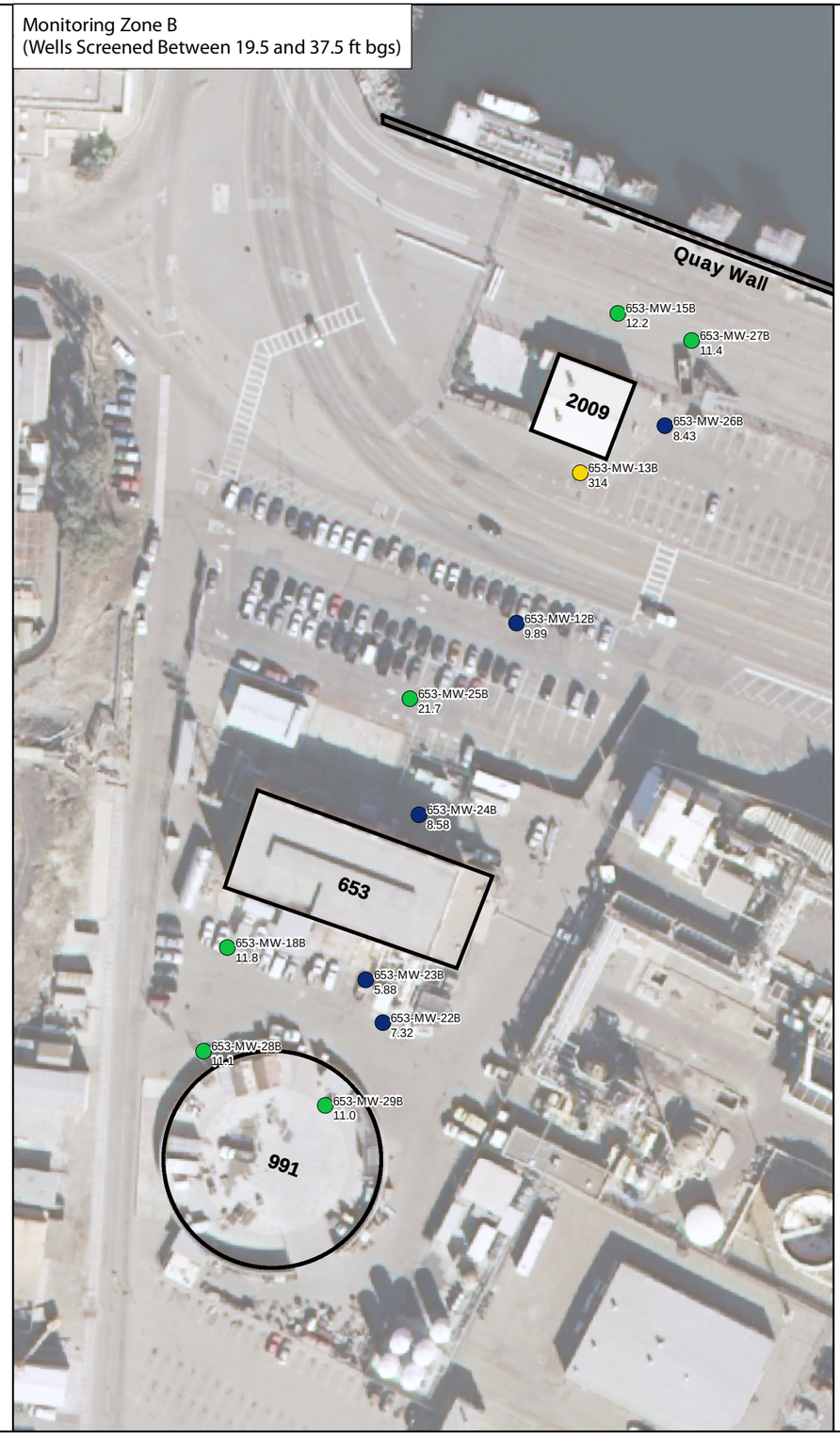
Figure **5a**

P:\GIS\OU24\OU24_Tablet\landscape_2frames.mxd: JBR

Monitoring Zone A
(Wells Screened Between 4 and 19.5 ft bgs)



Monitoring Zone B
(Wells Screened Between 19.5 and 37.5 ft bgs)



N

Dissolved Organic Carbon Concentration (mg/L)

- >1,000-10,000
- >100-1,000
- >10-100
- ≤10

Notes:
mg/L - milligrams per Liter
ft bgs - feet below ground surface
All samples collected between July 31 and August 7, 2007
Aerial photograph taken November 2003, courtesy of the USGS

100 50 0 100 Feet

Distribution of Dissolved Organic Carbon in Groundwater - Q1 (July 31 - August 7, 2007)

Operable Unit 24, Naval Air Station North Island
Coronado, California

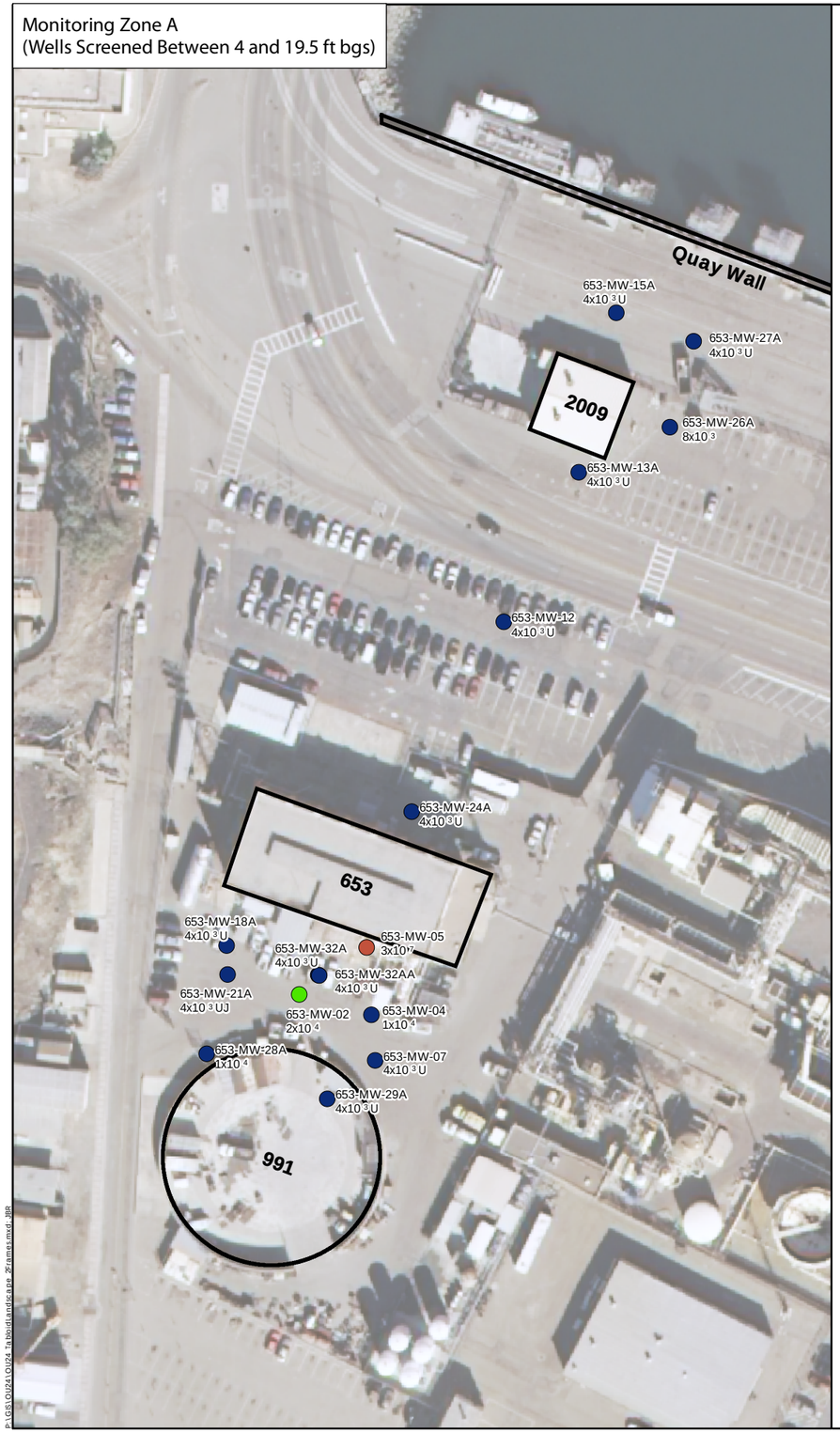
Geosyntec consultants

Guelph, Ontario | 15-Oct-2007

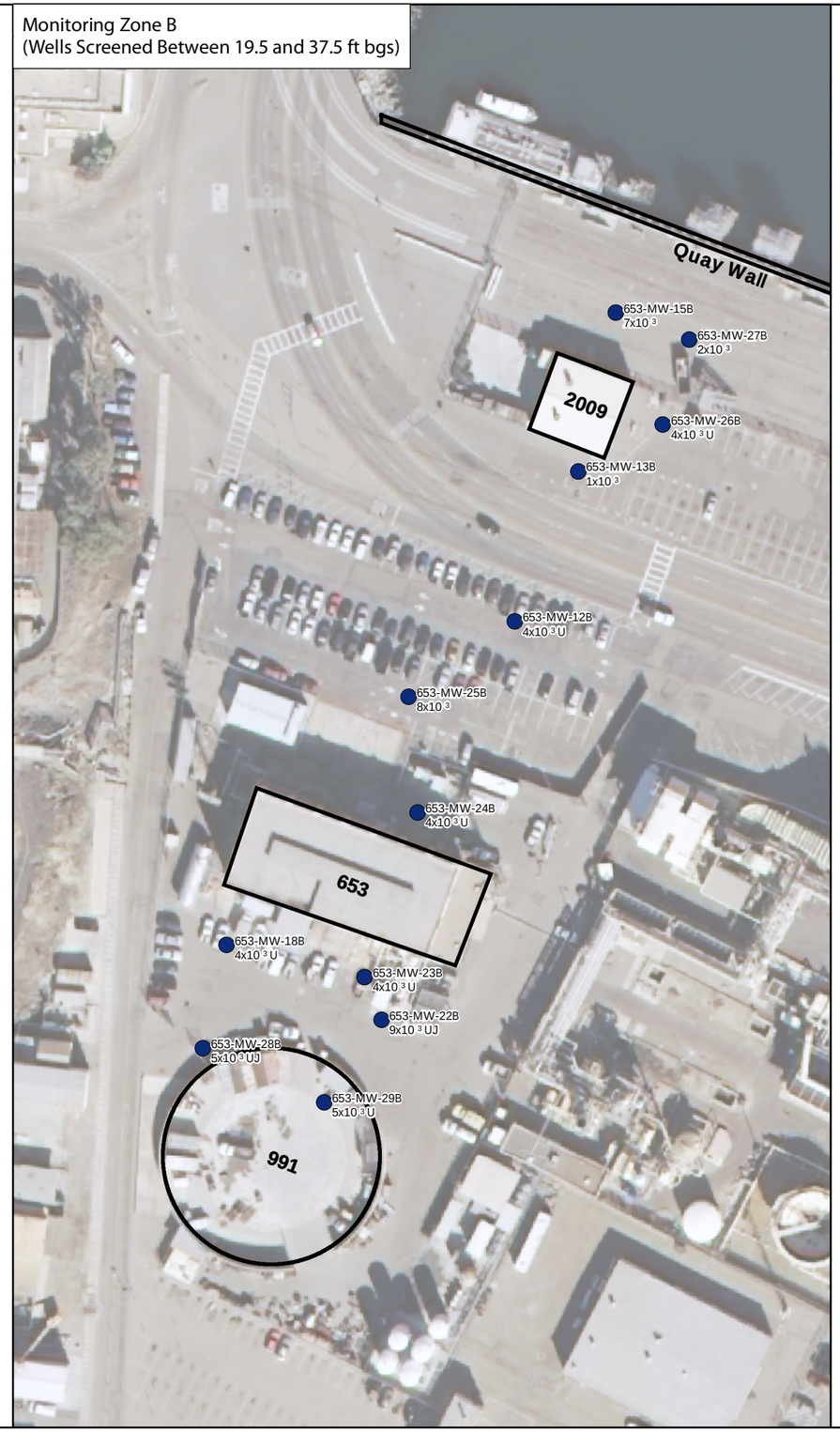
Figure **5b**

P:\GIS\OU24\OU24 Tabloidlandscape 2Frames.mxd JBR

Monitoring Zone A
(Wells Screened Between 4 and 19.5 ft bgs)



Monitoring Zone B
(Wells Screened Between 19.5 and 37.5 ft bgs)



N

GeneTrac-VC (gene copies/L)

- $>10^7$
- $>10^5 - 10^7$
- $>10^5 - 10^6$
- $>10^4 - 10^5$
- $\leq 10^4$

Notes:
U - The analyte was analyzed for but not detected at or above the Reporting Limit.
J - The reported result is an estimated value.
UJ - The analyte was analyzed but not detected. The sample quantitation limit is an estimated value.
ft bgs - feet below ground surface
All samples collected between March 26 to April 4, 2007
Aerial photograph taken November 2003, courtesy of the USGS

100 50 0 100 Feet

Distribution of Dehalococcoides Vinyl Chloride Reductase Genes - Baseline (March 26 - April 4, 2007)

Operable Unit 24, Naval Air Station North Island
Coronado, California

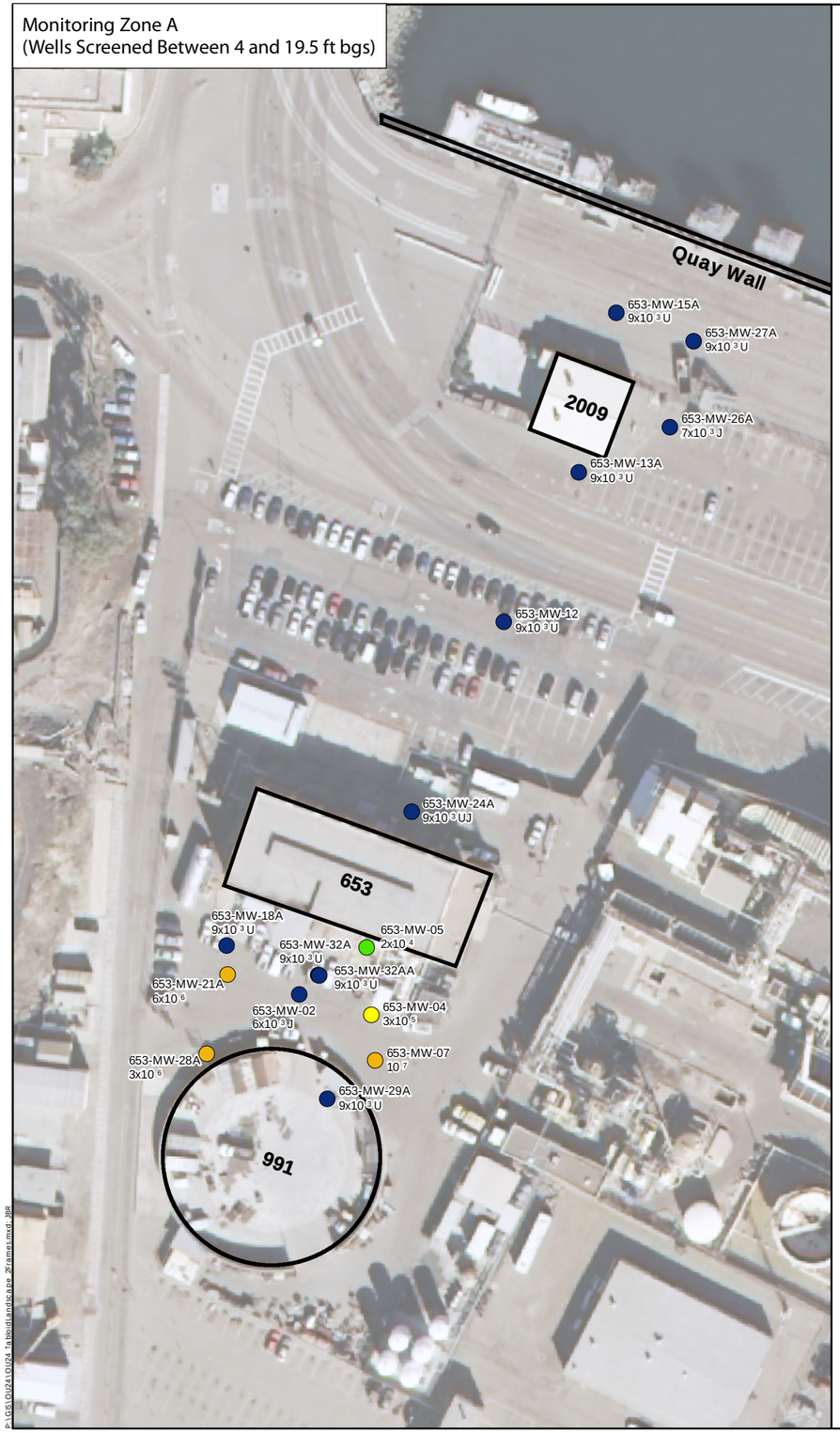
Geosyntec consultants

Guelph, Ontario | 15-Oct-2007

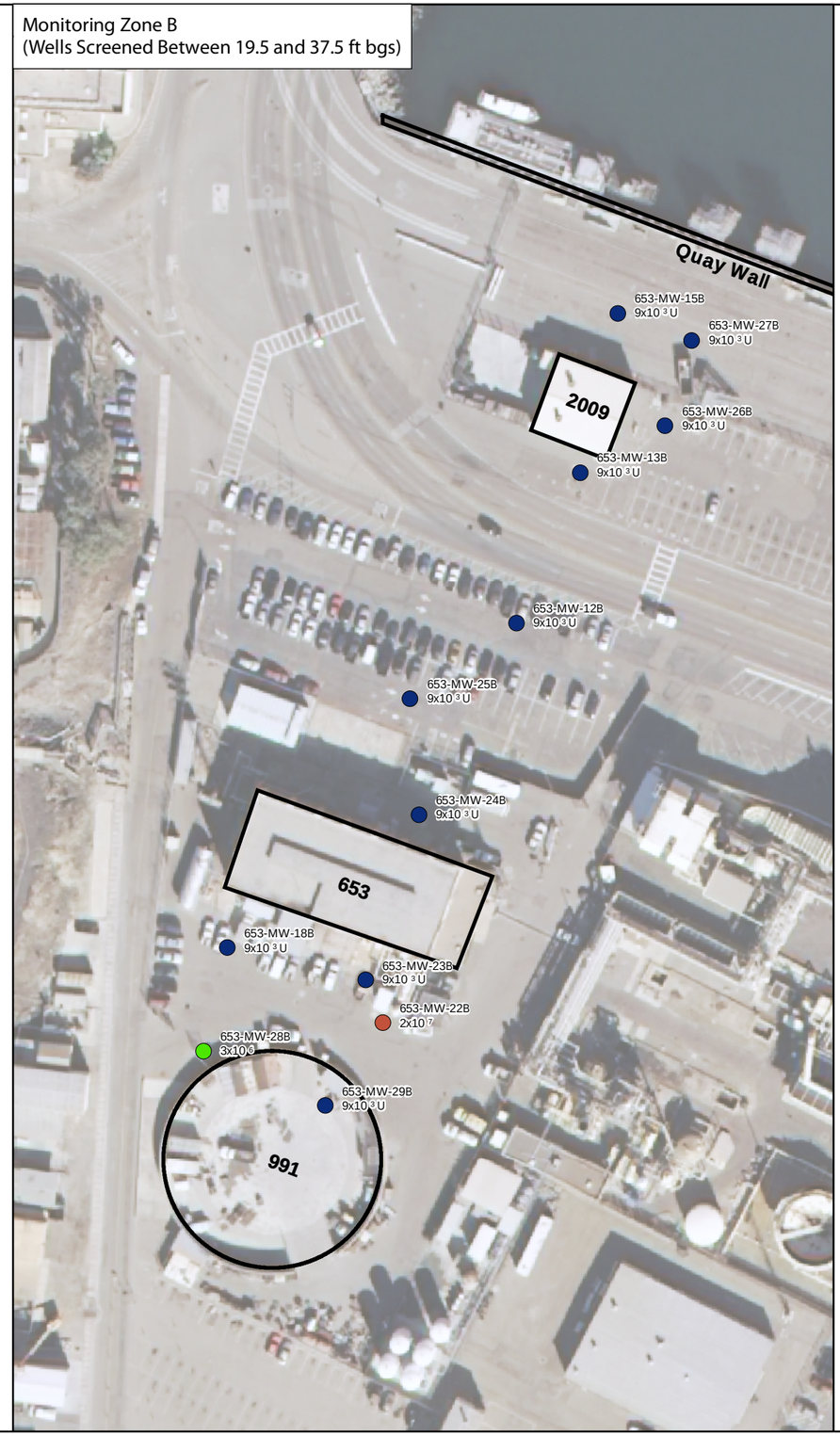
Figure **6a**

P:\GIS\OU24\OU24_Tabloidlandscape_2Frames.mxd JBR

Monitoring Zone A
(Wells Screened Between 4 and 19.5 ft bgs)



Monitoring Zone B
(Wells Screened Between 19.5 and 37.5 ft bgs)



N

GeneTrac-VC (gene copies/L)

- $>10^7$
- $>10^5 - 10^7$
- $>10^5 - 10^6$
- $>10^4 - 10^5$
- $\leq 10^4$

Notes:
U - The analyte was analyzed for but not detected at or above the Reporting Limit.
J - The reported result is an estimated value.
UJ - The analyte was analyzed but not detected. The sample quantitation limit is an estimated value.
ft bgs - feet below ground surface
All samples collected between July 31 to August 7, 2007
Aerial photograph taken November 2003, courtesy of the USGS

100 50 0 100 Feet

Distribution of Dehalococcoides Vinyl Chloride Reductase Genes - Q1 (July 31 - August 7, 2007)

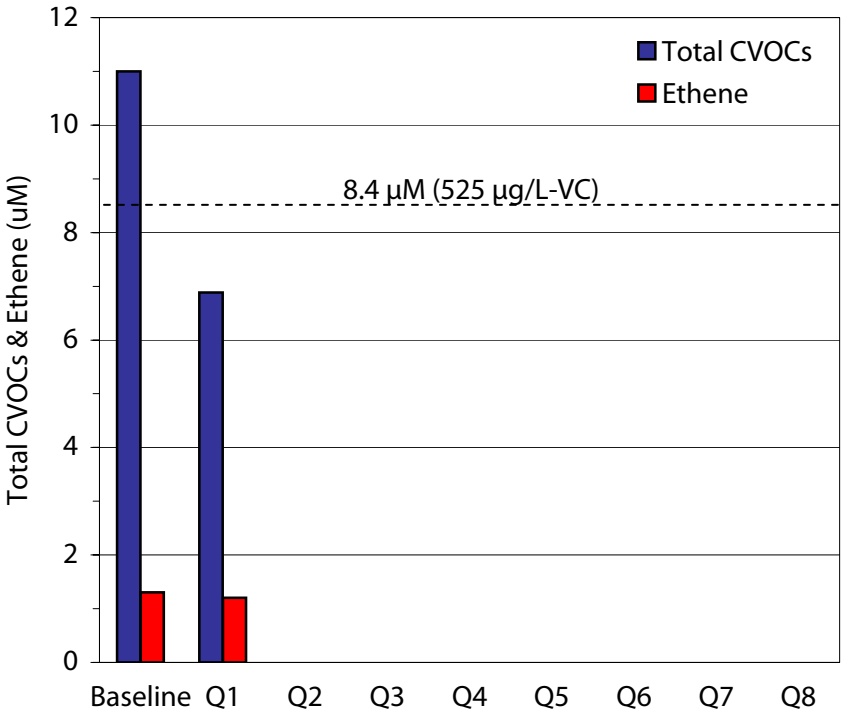
Operable Unit 24, Naval Air Station North Island
Coronado, California

Geosyntec consultants

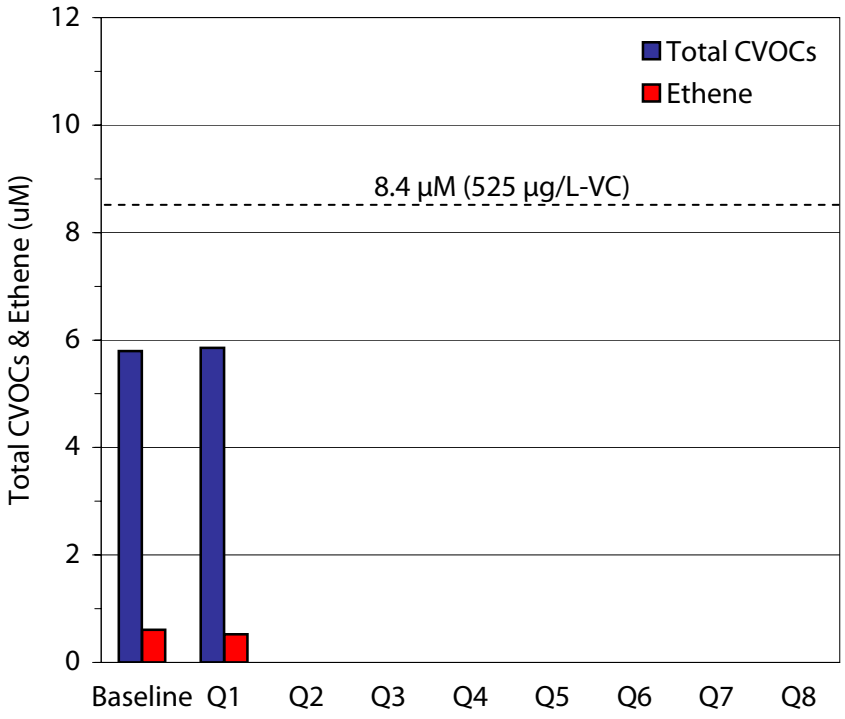
Guelph, Ontario | 15-Oct-2007

Figure **6b**

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Source Area Performance Monitoring Wells



Downgradient Area Performance Monitoring Wells

Notes

1) Concentrations reported are the average concentrations of these parameters in all performance monitoring wells in the source and downgradient areas

2) Total CVOCs - Total chlorinated volatile organic compounds (the sum of cis-1,2-dichloroethene and vinyl chloride, the only chloroethenes of significance). For purposes of calculation, non-detect results are assumed to be equal to the sample-specific quantitation limit.

Average Concentrations of Total Chlorinated Volatile Organic Compounds (as VC) and Ethene

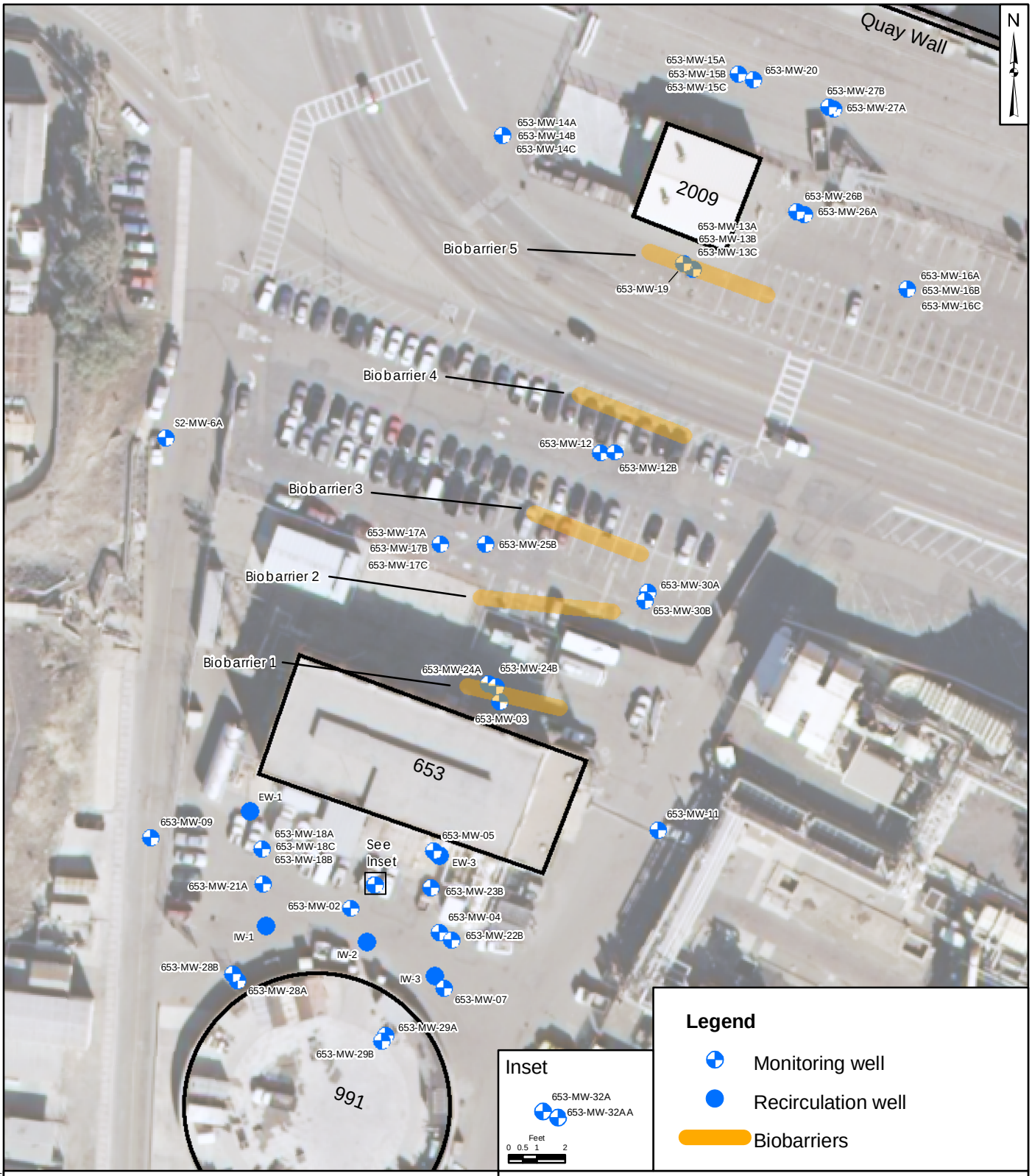
Operable Unit 24, Naval Air Station North Island, Coronado, California

Geosyntec consultants

Guelph | 27-Sept-2007

Figure **7**

P:\PRJ\Projects\MR0437.NAS North Island\Phase I & II Implementation\Quarterly Reporting\EF7 Summary of CVOC & ethene concentrations.xls[Figure 7 summary figure



Notes:
Aerial photograph taken November 2003, courtesy of the USGS
EW - Extraction well
IW - Injection well
MW - Monitoring Well

Well Locations

Operable Unit 24, Naval Air Station North Island
Coronado, California

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Figure 2

P:\GIS\OU24\OU24_LP_MonitoringWells.mxd; Rob.Roff; 28-Sep-07

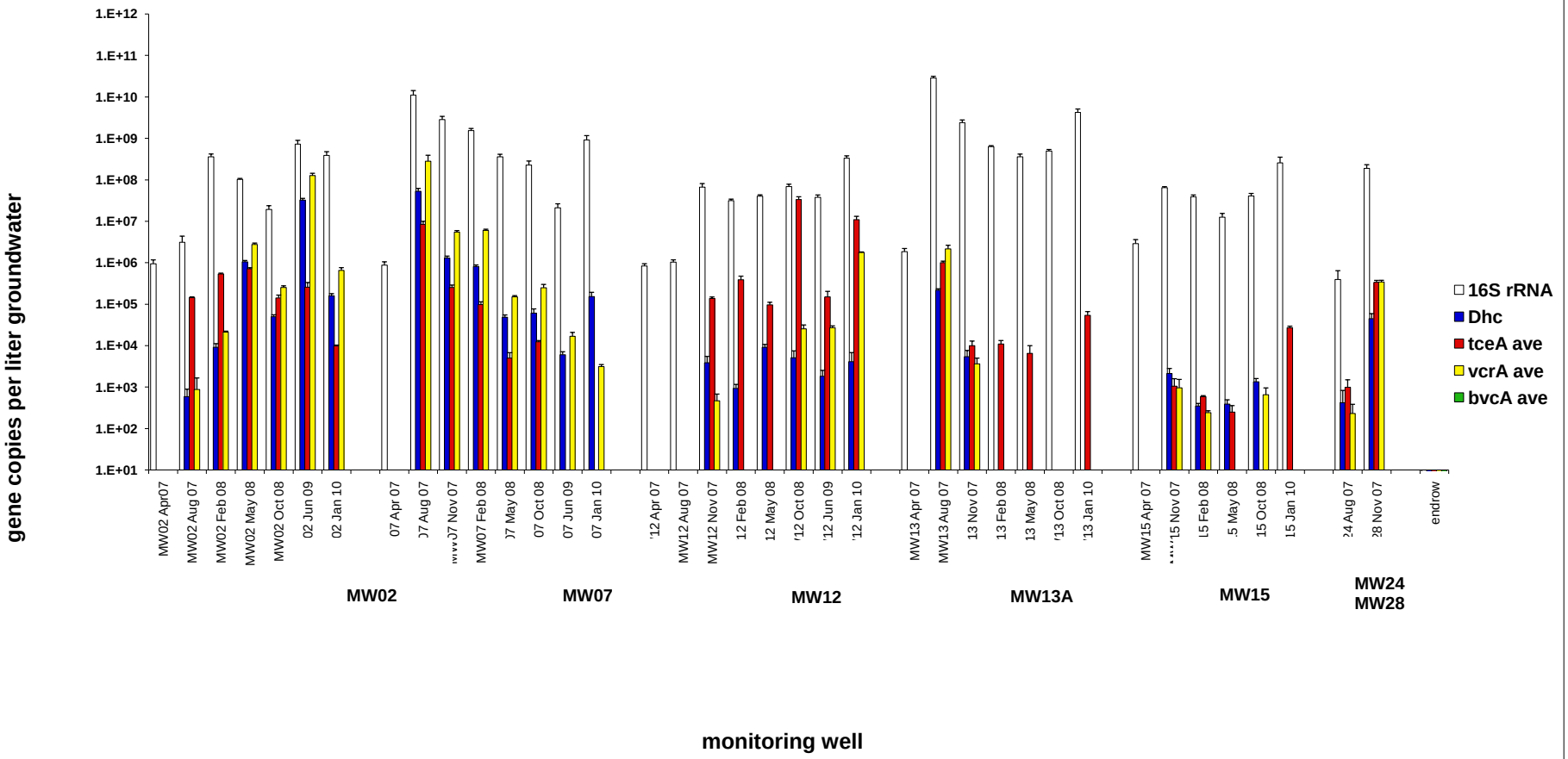
Table 5-8
PCR Results, NAS North Island

Sample	Description	ng/µl DNA	stdev	16S rRNA	Stdev	Dhc	Stdev	bvcA ave	Stdev	vcrA ave	Stdev	tceA ave	Stdev	Date	ug/L PCE	TCE	cis-DCE	VC	Ethene	Methane	Ethane
MW02 Apr07	Sterivex-Water Kit	38.4	5.69	9.4E+05	2.3E+05									3-Apr-07			1200.00	850.00	24.00	74.00	
MW02 Aug 07	Sterivex-Water Kit	6.6	0.32	3.12E+06	1.28E+06	5.91E+02	3.03E+02			8.82E+02	7.78E+02	1.43E+05	7.11E+03	8-Aug-07		0.27	160.00	220.00	6.50	140.00	
MW02 Feb 08	Sterivex-PowerSoil Ki	18.2		3.61E+08	5.83E+07	9.12E+03	2.02E+03			2.13E+04	1.06E+03	5.38E+05	2.34E+04	1-Feb-08			110.00	270.00	27.00	10000.00	
MW02 May 08	Sterivex-PowerSoil Ki	5.4		1.03E+08	5.39E+06	1.05E+06	8.81E+04			2.77E+06	2.12E+05	7.19E+05	3.76E+04	1-May-08			32.00	380.00	21.00	13000.00	
MW02 Oct 08	Sterivex-PowerSoil Ki	3.3		1.93E+07	4.48E+06	4.98E+04	5.72E+03			2.54E+05	2.24E+04	1.42E+05	2.40E+04	1-Oct-08			0.00	8.70	7.20	11000.00	4.7
MW02 Jun 09	Sterivex-PowerSoil Ki	12.6	0.74	7.21E+08	1.72E+08	3.22E+07	3.49E+06			1.26E+08	1.74E+07	2.57E+05	7.51E+04	9-Jun-09							
MW02 Jan 10	Sterivex-PowerSoil Ki	23.4	0.14	3.85E+08	9.26E+07	1.59E+05	2.03E+04			6.54E+05	1.07E+05	***9.88E+03***	***4.45E+02***	28-Jan-10							
															PCE	**TCE**	**cis-DCE**	**VC**	**Ethene**	**Methane**	***Ethane***
MW07 Apr 07	Sterivex-Water Kit	12.1	3.64	8.7E+05	1.8E+05									3-Apr-07		2.50	66.00	1.00		2.50	
MW07 Aug 07	Sterivex-Water Kit	114.0	0.49	1.10E+10	3.21E+09	5.30E+07	9.13E+06			2.82E+08	1.10E+08	8.45E+06	1.51E+06	7-Aug-07			3.60	19.00	57.00	2500.00	
MW07 Nov 07	Sterivex-PowerSoil Ki	10.4	0.35	2.82E+09	5.78E+08	1.30E+06	1.46E+05			5.50E+06	4.69E+05	2.51E+05	3.73E+04	13-Nov-07			0.41	3.40	8.40	7300.00	
MW07 Feb 08	Sterivex-PowerSoil Ki	69.9		1.55E+09	1.90E+08	8.14E+05	7.18E+04			6.05E+06	3.96E+05	9.78E+04	1.66E+04	1-Feb-08				1.40	12.00	14,000	
MW07 May 08	Sterivex-PowerSoil Ki	18.9		3.58E+08	5.59E+07	4.87E+04	6.35E+03			1.50E+05	1.09E+04	5.00E+03	1.77E+03	1-May-08				1.60	8.50	9,600	
MW07 Oct 08	Sterivex-PowerSoil Ki	14.8		2.25E+08	5.82E+07	6.07E+04	1.62E+04			2.51E+05	4.97E+04	***1.23E+04***	***9.52E+02***	1-Oct-08							
MW07 Jun 09	Sterivex-PowerSoil Ki	4.2	0.67	2.12E+07	5.23E+06	6.05E+03	1.09E+03			***1.68E+04***	***4.07E+03***			9-Jun-09							
MW07 Jan 10	Sterivex-PowerSoil Ki	16.5	0.18	9.12E+08	2.54E+08	1.52E+05	4.10E+04			3.12E+03	3.82E+02			28-Jan-10							
															PCE	**TCE**	**cis-DCE**	**VC**	**Ethene**	**Methane**	***Ethane***
MW12 Apr 07	Sterivex-Water Kit	8.8	1.31	8.4E+05	1.1E+05									3-Apr-07			7.50	72.00	0.67	190.00	
MW12 Aug 07	Sterivex-Water Kit	10.0	0.71	*1.0E+06*	*1.4E+05*									2-Aug-07			0.21	73.00	0.88	270.00	
MW12 Nov 07	Sterivex-PowerSoil Ki	1.1	0.46	6.6E+07	1.5E+07	3.87E+03	1.63E+03			4.66E+02	2.12E+02	1.37E+05	1.20E+04	8-Nov-07			1.30	64.00		250.00	
MW12 Feb 08	Sterivex-PowerSoil Ki	8.4		3.14E+07	2.82E+06	9.39E+02	2.24E+02					3.86E+05	8.82E+04	1-Feb-08			6.30	16.00		67.00	
MW12 May 08	Sterivex-PowerSoil Ki	0.5		4.04E+07	3.03E+06	9.14E+03	1.56E+03					9.63E+04	1.48E+04	1-May-08			0.29	34.00		730.00	
MW12 Oct 08	Sterivex-PowerSoil Ki	5.3		6.84E+07	9.96E+06	5.14E+03	2.36E+03			2.54E+04	6.20E+03	3.33E+07	5.77E+06	1-Oct-08							
MW12 Jun 09	Sterivex-PowerSoil Ki	2.8	0.21	3.80E+07	5.16E+06	***1.82E+03***	***7.07E+02***			2.73E+04	2.75E+03	1.50E+05	5.42E+04	9-Jun-09							
MW12 Jan 10	Sterivex-PowerSoil Ki	14.5	0.35	3.35E+08	4.29E+07	4.09E+03	2.70E+03			1.76E+06	6.42E+04	1.09E+07	2.29E+06	28-Jan-10			65.00	500.00	7.50	4400.00	0.68
															PCE	**TCE**	**cis-DCE**	**VC**	**Ethene**	**Methane**	**Ethane**
MW13 Apr 07	Sterivex-Water Kit	16.8	4.07	1.9E+06	3.5E+05									4-Apr-07			56.00	33.00	0.60	640.00	
MW13 Aug 07	Sterivex-Water Kit	58.2	0.18	2.87E+10	2.79E+09	2.18E+05	1.78E+04			2.15E+06	4.93E+05	1.00E+06	9.24E+04	2-Aug-07			22.00	5.70		3100.00	
MW13 Nov 07	Sterivex-PowerSoil Ki	5.5		2.39E+09	3.87E+08	5.39E+03	2.27E+03			3.63E+03	1.36E+03	9.99E+03	2.89E+03	7-Nov-07			25.00	6.30		8700.00	
MW13 Feb 08	Sterivex-PowerSoil Ki	4.9		6.31E+08	3.60E+07							***1.09E+04***	2.38E+03	1-Feb-08			22.00	12.00		12000.00	
MW13 May 08	Sterivex-PowerSoil Ki	9.2		3.61E+08	5.65E+07							6.49E+03	3.52E+03	1-May-08			19.00	18.00		12000.00	
MW13 Oct 08	Sterivex-PowerSoil Ki	7.8		4.97E+08	4.19E+07									1-Oct-08							
MW13 Jan 10	Sterivex-PowerSoil Ki	12.2	0.04	4.24E+09	8.76E+08							***5.42E+04***	***1.21E+04***	28-Jan-10			3.20	860.00	33.00	3900.00	0.71
															PCE	**TCE**	**cis-DCE**	**VC**	**Ethene**	**Methane**	
MW15 Apr 07	Sterivex-Water Kit	10.9	2.30	2.9E+06	7.3E+05									4-Apr-07			2.00	1.40		100.00	
MW15 Nov 07	Sterivex-PowerSoil Ki	2.6		6.47E+07	3.63E+06	2.14E+03	6.70E+02			9.67E+02	5.65E+02	1.06E+03	5.37E+02	6-Nov-07			1.60	2.80		110.00	
MW15 Feb 08	Sterivex-PowerSoil Ki	9.5		3.88E+07	4.27E+06	***3.51E+02***	5.38E+01			***2.44E+02***	2.43E+01	***5.90E+02***	3.24E+01	1-Feb-08				0.25		30.00	
MW15 May 08	Sterivex-PowerSoil Ki	0.9		1.25E+07	2.94E+06	***3.87E+02***	***1.08E+02***					***2.48E+02***	***1.09E+02***	1-May-08						29.00	
MW15 Oct 08	Sterivex-PowerSoil Ki	4.0		4.09E+07	5.99E+06	***1.35E+03***	***2.55E+02***			***6.58E+02***	***2.98E+02***			1-Oct-08							
MW 15 Jan 10	Sterivex-PowerSoil Ki	4.5	1.13	2.56E+08	9.61E+07							***2.72E+04***	***2.30E+03***	28-Jan-10			3.20	860.00	33.00	3900.00	0.71
MW24 Aug 07	Sterivex-Water Kit	6.0	0.53	3.92E+05	2.51E+05	4.23E+02	4.10E+02			2.31E+02	1.54E+02	9.94E+02	5.06E+02	3-Aug-07							
MW28 Nov 07	Sterivex-PowerSoil Ki	2.2	0.07	1.89E+08	4.30E+07	4.50E+04	1.43E+04			3.42E+05	3.53E+04	3.41E+05	3.48E+04	13-Nov-07							
endrow				**1.0E+00**	**1.0E+00**	**1.0E+00**	**1.0E+00**	**1.0E+00**	**1.0E+00**	**1.0E+00**	**1.0E+00**	**1.0E+00**	**1.0E+00**								

All samples are extracted from Sterivex filter cartridges
RED= Added Apr 2010

Tested, but not detected
Not Tested (e.g. Dhc negative, no bvcA present)
Detected not quantified (DNQ)
Values in bold = very near detection limit (not detected in 1:10 dil)

Figure 5-8
PCR Results
NAS North Island OU24



Appendix B-9: Bachman Road

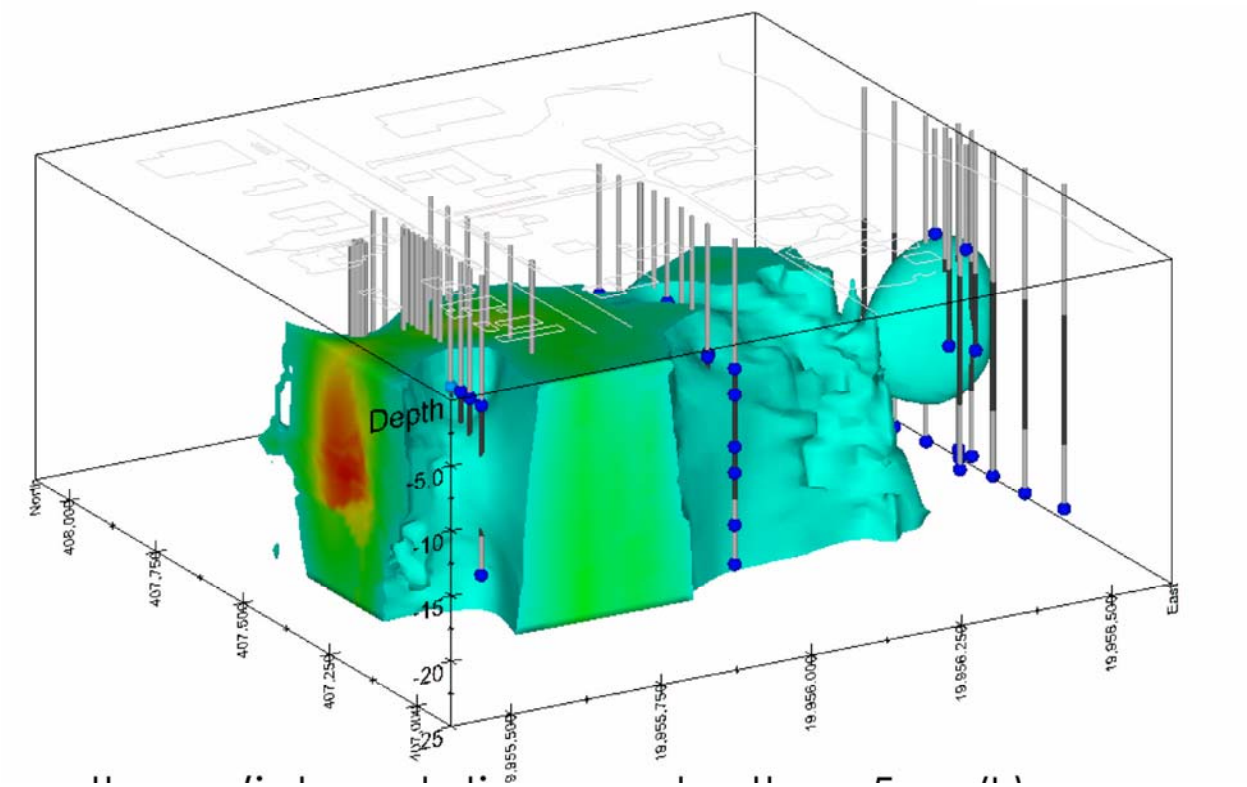


Figure C-7 – 3: TCE Concentrations visualization

Prior to Bioremediation Injections

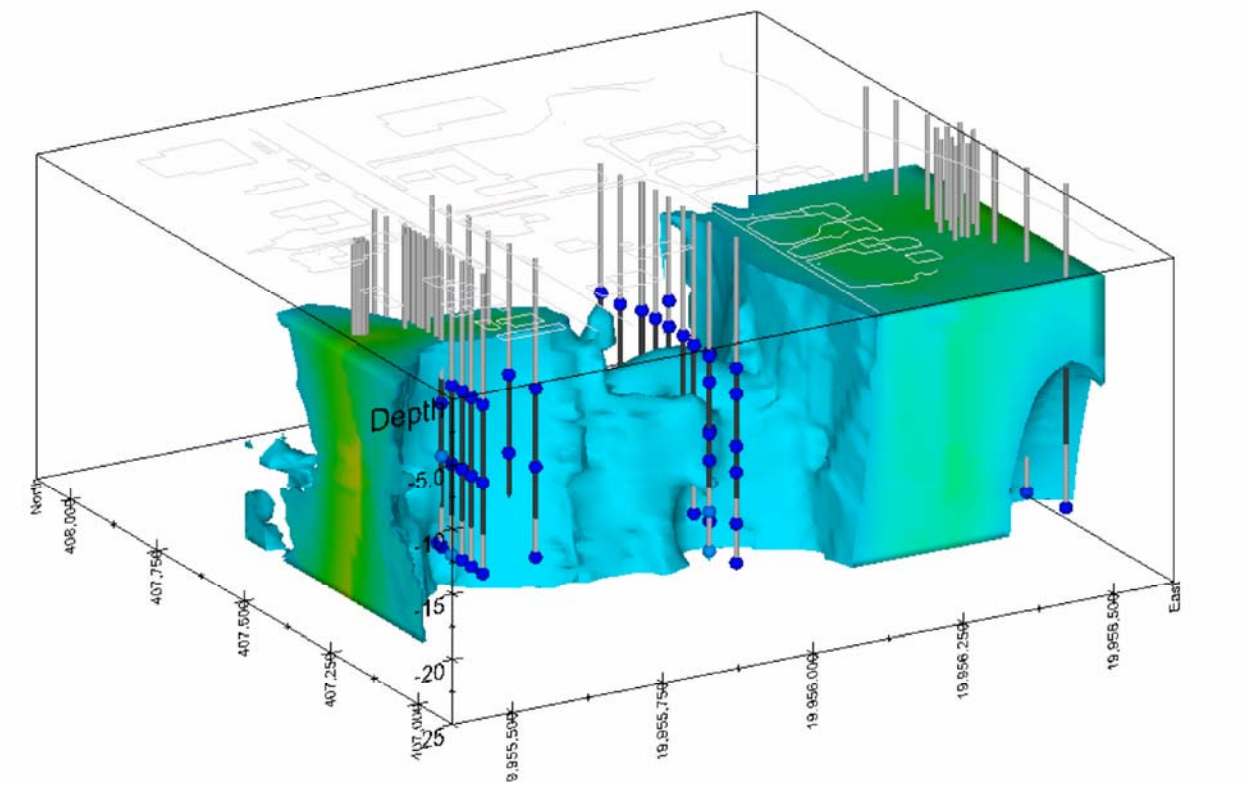


Figure C-7 – 4: DCE Concentrations visualization

Prior to Bioremediation Injections

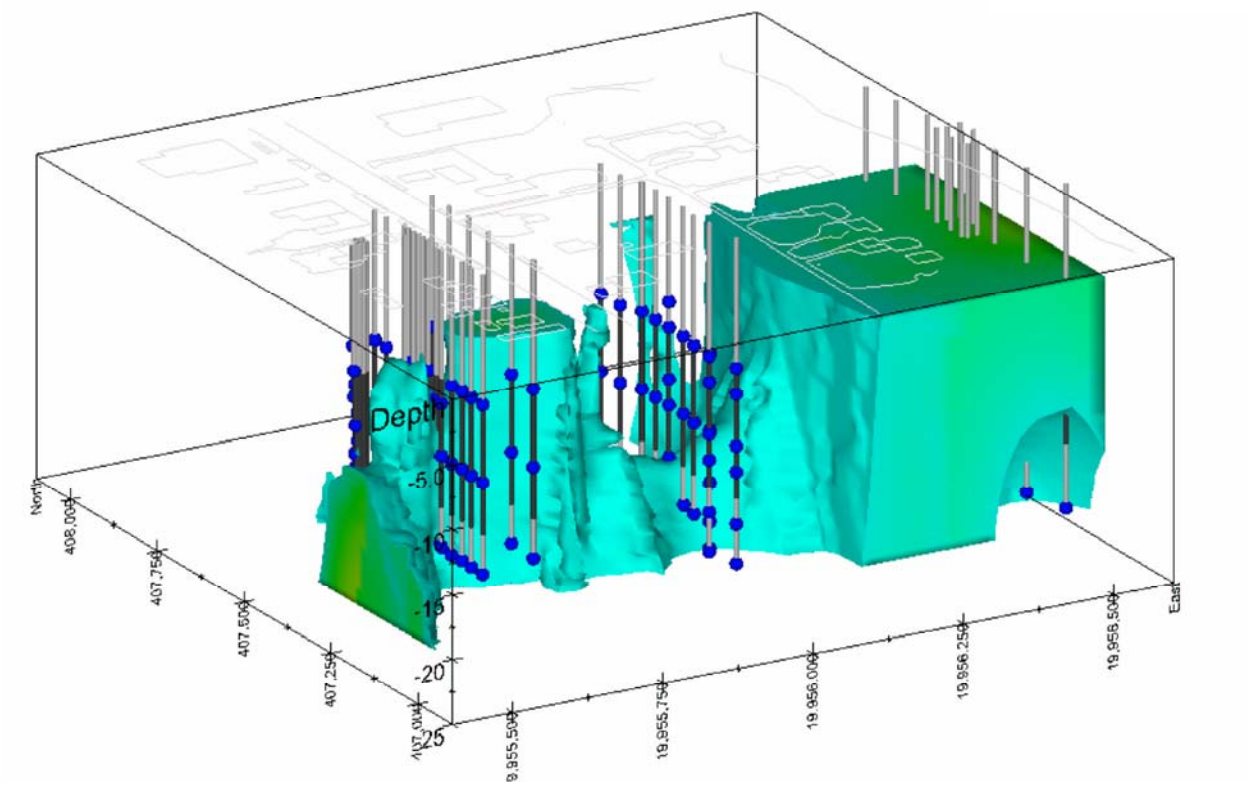


Figure C-7 – 5: VC Concentrations visualization
Prior to Bioremediation Injections

DRAFT

N

A

A'

B

B'

C

C'

ML-17
ML-16
ML-13
ML-6
ML-7
ML-8
ML-9
ML-10
ML-11
ML-12
ML-14
ML-15
ML-18
ML-19

ML-35
ML-34
ML-33
MW-2A
MW-2
ML-32
ML-30
ML-31

ML-40
ML-41
ML-42
ML-43
ML-44
ML-45
ML-46
ML-47
ML-50
ML-49

ML-4
ML-5
ML-3
ML-2
ML-1
SB-2

SIW-1
SIW-2
SIW-3
SIW-4
SIW-5
SIW-6
SIW-7
SIW-8
SEW-1
SEW-2

IJ-9
IJ-10
IJ-11
IJ-12
IJ-13
IJ-14
IJ-15
IJ-16
IJ-17
IJ-18
IJ-19
IJ-20
IJ-21
IJ-22
IJ-23
IJ-24
IJ-25
IJ-26
IJ-27
IJ-28

EW-5
EW-6
EW-7
EW-8
EW-9
EW-10
EW-11
EW-12
EW-13
EW-14

Former ASP Building
(5147 US-23)

Legend

- Source Area Wells
- Extraction Well
- Injection Well
- Sample Location
- Cross Section
- Source Area
- Biobarrier 2

Notes:

40 20 0 40
Feet

Site Map Including Bioremediation Injection and Extraction Well Locations
Bachman Road Site
Oscoda, MI

Geosyntec consultants

Ann Arbor | 26-Jan-09

Figure
1

J:\GIS\Projects\CHA\B160\BachmanRoad\GIS\BachmanRd_SiteMap.mxd; Chicago-JD

15	25
Nov-08	Nov-08
ND	12,000
3,400	12,000
120	1,300
ND	11,000
2.8	5,100
ND	ND
240,000	1,600
29,000	8,800
ND	2,700

15	23
Nov-08	Nov-08
320	79
13,000	330
2,200	14
ND	110
58	530
ND	ND
120,000	140,000
10,000	12,000
ND	1,500

16	23
Nov-08	Nov-08
1,200	1,800
5,100	5,100
620	290
ND	150
190	390
1.3	ND
31,000	16,000
4,000	4,000
ND	1.5

14.1	22.3
Nov-08	Nov-08
22,000	73,000
2,600	2,300
2,000	ND
ND	26,000
1,500	1,100
2.6	2.4
ND	ND
910,000	340,000
110	5,100

Former ASP Building (5147 US-23)

22.3
Nov-08
6,400
ND
160
1,200
2,000
4
ND
1,200,000
510

24
Nov-08
20
1,500
44
ND
NA
NA
11,000
1,500
NA

12
Nov-08
6.2
970
36
ND
NA
NA
8,400
1,300
NA

23
Nov-08
1,800
5,900
590
540
NA
NA
14,000
4,800
NA

16
Nov-08
3,300
ND
ND
ND
220
ND
ND
370,000
4.2

10-25
Nov-08
1,600
ND
ND
ND
74
ND
ND
480,000
1.6

ML-35 ML-40 ML-17 ML-16 ML-34 ML-41 ML-13 ML-6 ML-33 ML-42 ML-5 ML-4 ML-7 ML-3 ML-1 SB-1 SB-2 ML-2 ML-8 ML-43 ML-9 MW-2A MW-2 ML-10 ML-32 ML-44 EW-8 ML-11 ML-12 ML-45 EW-10 ML-30 ML-14 ML-46 ML-15 EW-12 ML-31 ML-18 ML-19 ML-47

N

Legend

- Sample Location
- Source Area Treatment Zone
- Biobarrier 2

Depth (ft bgs)
Month-Year Sampled
cis-1,2-Dichloroethene
Tetrachloroethene
Trichloroethene
Vinyl Chloride
Methane
Ethane
Sulfate
Total Organic Carbon (TOC)
Ethene

Notes:
1. ND - Non-detect
2. Shaded values indicate exceedance of Michigan Part 201 Residential & Commercial Drinking Water Criteria.
3. All results in µg/L.
4. NA - Not Analyzed

Michigan Part 201 Residential & Commercial GSI Criteria
Cis-1,2-Dichloroethene = 620 µg/L
Tetrachloroethene = 45 µg/L
Trichloroethene = 200 µg/L
Vinyl Chloride = 15 µg/L
*Criteria not available for remaining parameters.

40 20 0 40 Feet

Plume B
1st Post-Injection Monitoring Event Data
Bachman Road

Geosyntec consultants

Ann Arbor | 26-Jan-09

Figure 2

H:\GSProjects\CHA8161-BachmanRoad\GIS\BachmanRd_DataPosting_PlumeB_1st Sampling.mxd; Chicago-JD

10	12	14	16	18	20	22
Nov-08	Nov-08	Nov-08	Nov-08	Nov-08	Nov-08	Nov-08
ND	ND	ND	ND	ND	ND	11
98	230	580	130	110	95	380
ND	ND	ND	ND	ND	ND	ND
ND	ND	ND	ND	ND	ND	ND

12	16	23
Nov-08	Nov-08	Nov-08
ND	ND	ND
35	42	180
ND	ND	1.4
ND	ND	ND

11	13	17	19	21	23
Nov-08	Nov-08	Nov-08	Nov-08	Nov-08	Nov-08
ND	ND	ND	ND	ND	3,200
330	990	310	200	130	150
ND	17	ND	ND	ND	180
ND	ND	ND	ND	ND	2,600

11	13	15	17	19	21	23	25
Nov-08	Nov-08	Nov-08	Nov-08	Nov-08	Nov-08	Nov-08	Nov-08
11	ND	ND	20	9.6	18	5,500	12,000
1,200	2,000	3,400	1,700	570	220	2,500	12,000
15	21	120	110	27	14	470	1,300
ND	ND	ND	ND	ND	6.8	7,400	11,000

11	13	15	17	19	21	23
Nov-08	Nov-08	Nov-08	Nov-08	Nov-08	Nov-08	Nov-08
860	360	320	750	430	220	79
6,400	20,000	13000	1,100	730	450	330
560	670	2200	1,200	290	67	14
ND	ND	ND	ND	ND	ND	110

10	12	14	16	18	20	22
Nov-08	Nov-08	Nov-08	Nov-08	Nov-08	Nov-08	Nov-08
540	1,100	820	1,000	900	550	1,800
1,700	4,100	24,000	46,000	3,600	4,600	3,300
130	370	760	2,200	1,200	990	210
ND	ND	ND	ND	ND	ND	1,200

12	16	23
Nov-08	Nov-08	Nov-08
12	120	ND
100	170	5,500
9.4	21	ND
ND	ND	ND

12	16	23
Nov-08	Nov-08	Nov-08
ND	7.5	17
26	130	670
ND	6.7	10
ND	ND	ND

ML-1
ML-2
ML-3
ML-4
ML-5
ML-6
ML-7
ML-8
ML-9
ML-10
ML-11
ML-12
ML-13
ML-14
ML-32
ML-33
SB-2
MW-2A
MW-2

N

Legend

- Sample Location
- Source Area

Depth (ft bgs)
Month-Year Sampled
cis-1,2-Dichloroethene
Tetrachloroethene
Trichloroethene
Vinyl Chloride

Notes:
1. ND - Non-detect
2. Shaded values indicate exceedance of Michigan Part 201 Residential & Commercial Drinking Water Criteria.
3. All results in µg/L.

Michigan Part 201 Residential & Commercial Drinking Water Criteria
Cis-1,2-Dichloroethene = 620 µg/L
Tetrachloroethene = 45 µg/L
Trichloroethene = 200 µg/L
Vinyl Chloride = 15 µg/L

20 10 0 20 Feet

Plume B - Line A
1st Post Injection Monitoring Event Data
Bachman Road

Geosyntec consultants

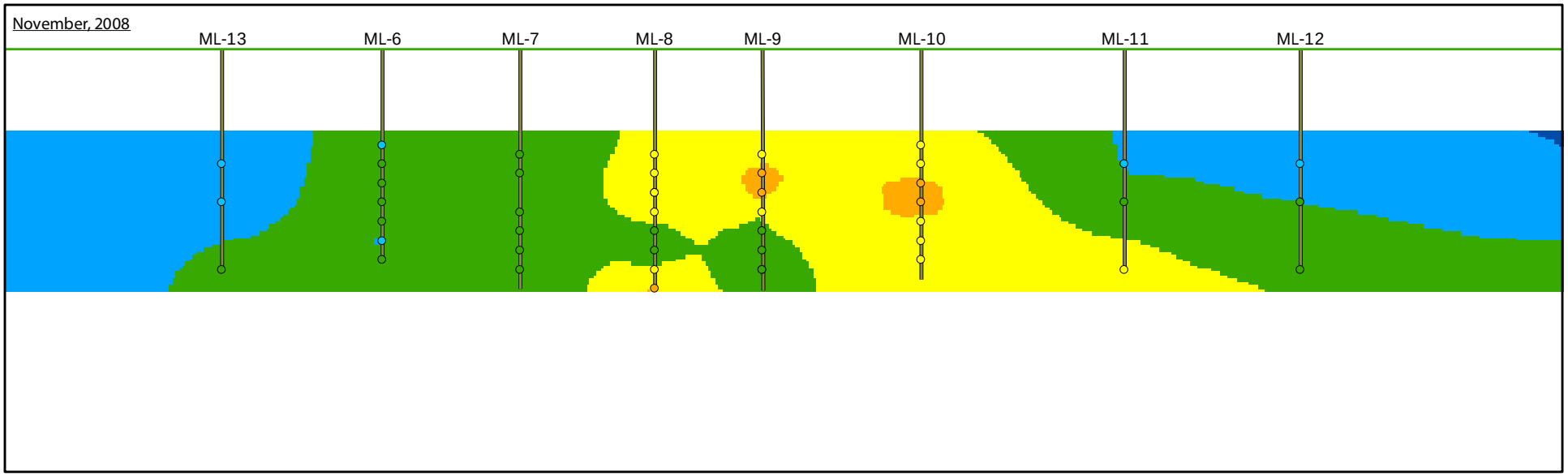
Ann Arbor | 26-Jan-09

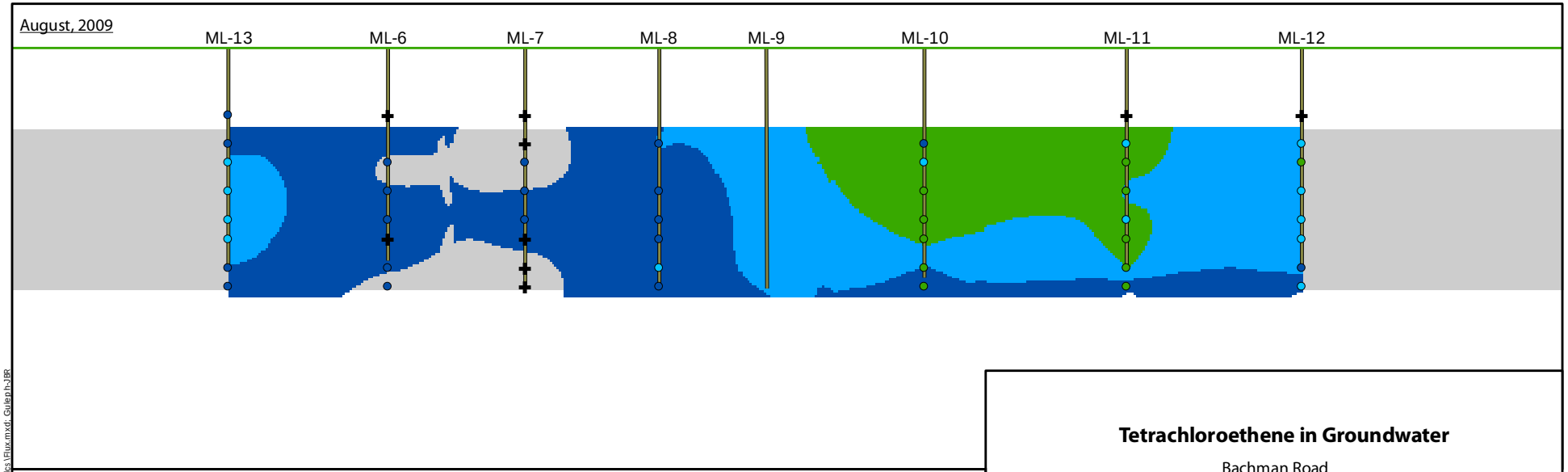
Figure 3

H:\GSProjects\CHA5161-BachmanRoad\GIS\BachmanRd_DataPosting PlumeB_Line A.mxd Chicago-JD


N
ML-35
ML-40
ML-34
ML-41
ML-33
ML-42
ML-43
MW-2
MW-2A
ML-32
ML-44
EW-8
ML-45
EW-10
ML-30
ML-46
EW-12
ML-47
ML-50
EW-14
ML-49
16 | 23
Nov-08 | Nov-08
200 | 500
320 | 2,100
2,100 | 2,400
ND | ND
16 | 23
Nov-08 | Nov-08
1,200 | 1,800
5,100 | 5,100
620 | 290
ND | 150
10-25
Nov-08
1,600
ND
ND
ND
10-25
Nov-08
8100
ND
ND
150
16 | 20 | 23
Nov-08 | Nov-08 | Nov-08
3,300 | 2,100 | 3,500
ND | ND | 100
ND | ND | ND
ND | ND | 82
10-25
Nov-08
6500
ND
ND
100
10-25
Nov-08
200
170
7.4
ND
Legend
Sample Location
Source Area
Biobarrier 2
Depth (ft bgs)
Month-Year Sampled
cis-1,2-Dichloroethene
Tetrachloroethene
Trichloroethene
Vinyl Chloride
Notes:
1. ND - Non-detect
2. Shaded values indicate exceedance of Michigan Part 201 Residential & Commercial Drinking Water Criteria.
3. All results in µg/L.
Michigan Part 201 Residential & Commercial Drinking Water Criteria
Cis-1,2-Dichloroethene = 620 µg/L
Tetrachloroethene = 45 µg/L
Trichloroethene = 200 µg/L
Vinyl Chloride = 15 µg/L
40 20 0 40 Feet
Plume B - Line C
1st Post Injection Monitoring Event Data
Bachman Road
Geosyntec consultants
Ann Arbor
26-Jan-09
Figure
4
H:\GISProjects\CHA8161-BachmanRoad\GIS\BachmanRd_DataPosting_PlumeB_Line C.mxd, Chicago-JD

DRAFT





Measured Concentration (µg/L)

- Non-Detect
- ≤10
- >10 - 10
- >100 - 1,000
- >1,000 - 10,000
- >10,000

Interpolated Concentration (µg/L)

- Non-Detect
- ≤10
- >10 - 100
- >100 - 1,000
- >1,000 - 10,000
- >10,000

No Vertical Exaggeration

10 5 0 10 Feet

Multi-Level Probe

Note:
µg/L - micrograms per liter

Tetrachloroethene in Groundwater

Bachman Road

Geosyntec consultants

Ann Arbor | 27-Aug-2008

Figure 1

P:\GIS\Misc\Bachman Road\Flux\FluxCales\Flux.mxd Gulen1-JBR

Table 5-2
PCR Results, Bachman Road
Data Summary, August 2008

Well ID		Description	*Bac*		*Dhc*		*BvcA*		*VcrA*		*TceA*		DNA conc (ng/µl)	
			average/L	*stdev/L*	*average/L*	*stdev/L*	*average/L*	*stdev/L*	*average/L*	*stdev/L*	*average/L*	*stdev/L*		
ML-1E 24' Wp	4 Wp	Sterivex-Water Kit	7.75E+07	1.56E+07	8.02E+04	1.80E+04					2.06E+04	3.74E+03	3.0	0.14
ML-1E 24' Sp	3 Sp	Sterivex-PowerSoil Kit	3.94E+08	7.42E+07									5.5	0.28
ML-3B 1		Sterivex-PowerSoil Kit	4.79E+08	4.52E+07			8.24E+02	2.12E+02	5.92E+02	3.83E+02			0.8	0.21
ML-4E 1		Sterivex-PowerSoil Kit	1.41E+07	1.27E+06									0.1	0.04
ML-8 15' 1		Sterivex-PowerSoil Kit	3.49E+07	1.43E+06									0.4	0.18
ML-8 25' Wp	4 Wp	Sterivex-Water Kit	2.75E+06	5.59E+05	*6.85E+05*	*4.57E+04*					*6.21E+04*	*1.56E+04*	2.9	0.25
ML-8 25' Sp	3 Sp	Sterivex-PowerSoil Kit	4.72E+07	3.67E+06									4.9	0.11
ML-9 15' 1		Sterivex-PowerSoil Kit	9.62E+07	5.21E+06	4.10E+04	1.51E+04							1.2	0.18
ML-9 23' Wp	4 Wp	Sterivex-Water Kit	1.08E+07	1.00E+06	*1.65E+06*	*1.66E+05*			*2.87E+05*	*7.51E+03*	*3.02E+06*	*5.22E+05*	1.4	0.28
ML-9 23' Sp	3 Sp	Sterivex-PowerSoil Kit	1.59E+08	2.32E+07									6.5	0.59
ML-32 23' Wp	4 Wp	Sterivex-Water Kit	2.04E+06	6.14E+05	1.64E+05	2.53E+04			9.67E+03	1.62E+03	1.03E+05	1.29E+04	1.7	0.04
ML-32 23' Sp	3 Sp	Sterivex-PowerSoil Kit	1.73E+07	2.05E+06									2.6	0.11
ML-44 16' 1		Sterivex-PowerSoil Kit	2.31E+07	1.54E+06	*1.42E+05*	*6.51E+04*							0.9	0.25
MW-2 1		Sterivex-PowerSoil Kit	7.98E+07	9.94E+06									1.0	0.28
EW-8 16' Wp	4 Wp	Sterivex-Water Kit	6.56E+09	8.89E+08	*1.81E+06*	*9.13E+04*			*2.93E+04*	*1.08E+04*	*1.22E+05*	*2.91E+04*	47.6	0.11
EW-8 16' Sp	3 Sp	Sterivex-PowerSoil Kit	4.74E+09	3.06E+08									18.9	0.42
ML-1E		Sterivex-PowerSoil Kit	3.33E+09	3.75E+08									21.8	0.21
ML-3B		Sterivex-PowerSoil Kit	5.65E+09	6.84E+08									37.5	0.25
ML-3E		Sterivex-PowerSoil Kit	3.51E+08	5.35E+07									10.0	0.60
ML-8 15'		Sterivex-PowerSoil Kit	1.66E+08	1.18E+07	3.40E+02	9.69E+01							7.5	0.53
ML-8 25'		Sterivex-PowerSoil Kit	2.13E+08	1.83E+07									8.6	0.25
ML-9 15'		Sterivex-PowerSoil Kit	3.78E+08	1.18E+07	3.06E+04	3.04E+03							12.8	0.04
ML-9 23'		Sterivex-PowerSoil Kit	7.29E+07	7.69E+06	3.93E+02	2.08E+02							4.2	0.04
ML-44 16'		Sterivex-PowerSoil Kit	1.32E+09	3.27E+08									17.1	0.46
ML-44 23'		Sterivex-PowerSoil Kit	1.92E+08	1.34E+07									7.9	0.49
ML-46 16'		Sterivex-PowerSoil Kit	5.69E+09	4.30E+08	1.23E+04	4.22E+03			1.31E+04	1.56E+03			31.7	0.25
Ew-8		Sterivex-PowerSoil Kit	2.53E+09	2.77E+08	8.68E+03	1.26E+03			1.05E+04	1.36E+03			29.1	0.92
ML-1E		Sterivex-PowerSoil Kit	6.30E+08	1.33E+08										
ML-3B		Sterivex-PowerSoil Kit	1.05E+09	3.49E+08										
ML-3E		Sterivex-PowerSoil Kit	8.05E+07	2.74E+07										
ML-8 15'		Sterivex-PowerSoil Kit	2.72E+08	5.45E+07	***7.88E+02***	***6.51E+01***								
ML-8 25'		Sterivex-PowerSoil Kit	1.84E+08	6.19E+07					***7.53E+01***	***9.91E+00***				
ML-9 15'		Sterivex-PowerSoil Kit	2.35E+08	6.60E+07	3.15E+04	1.28E+04								
ML-9 23'		Sterivex-PowerSoil Kit	9.35E+07	2.51E+07	7.49E+02	8.20E+01								
ML-44 16'		Sterivex-PowerSoil Kit	1.08E+09	1.09E+09										
ML-44 23'		Sterivex-PowerSoil Kit	6.24E+07	1.82E+07										
ML-46 16'		Sterivex-PowerSoil Kit	1.05E+08	2.41E+07	4.53E+03	6.50E+02			3.63E+03	7.69E+02				
Ew-8		Sterivex-PowerSoil Kit	2.11E+09	3.71E+08	6.00E+06	2.01E+05			6.05E+06	3.83E+05				

= Undetected
= DNQ (Detected Not Quantifiable)

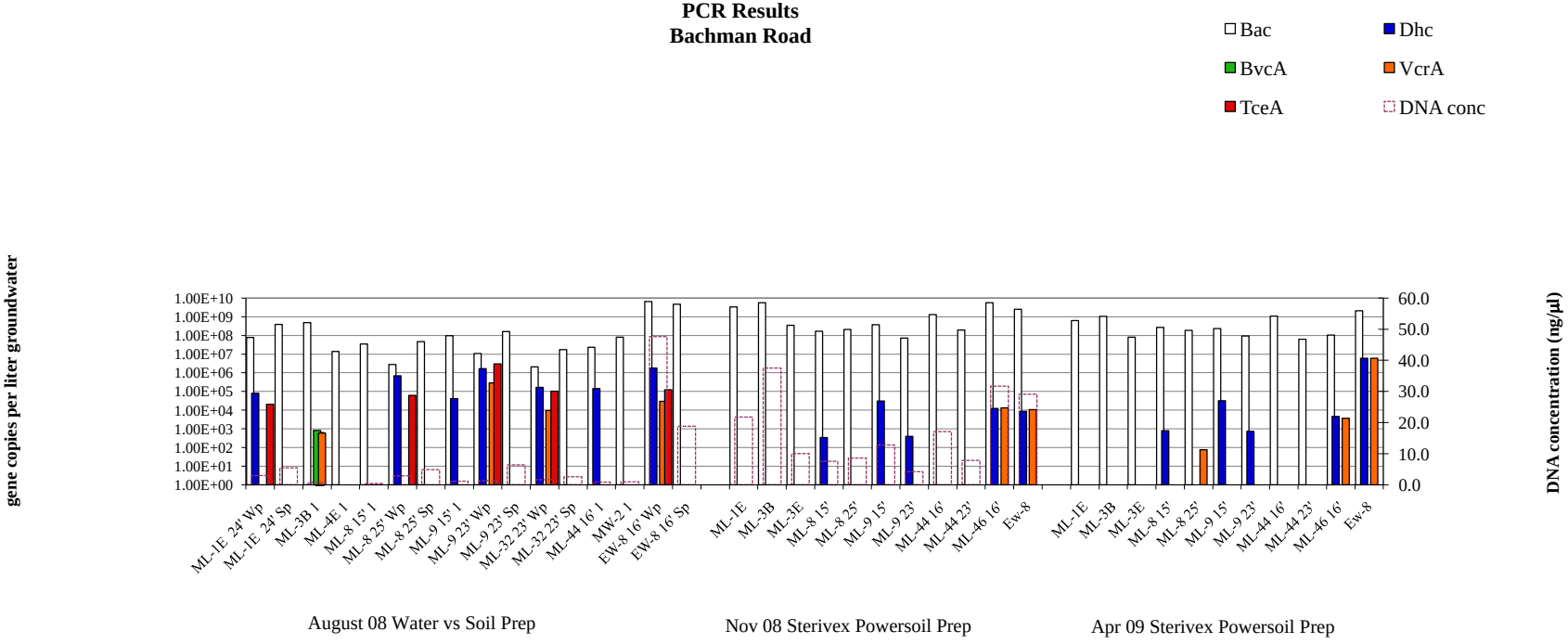
inhibition in undiluted

BOLD = Filters were split into 2 tubes because so much soil/sediment was in the GW. The samples were recombined before elution.

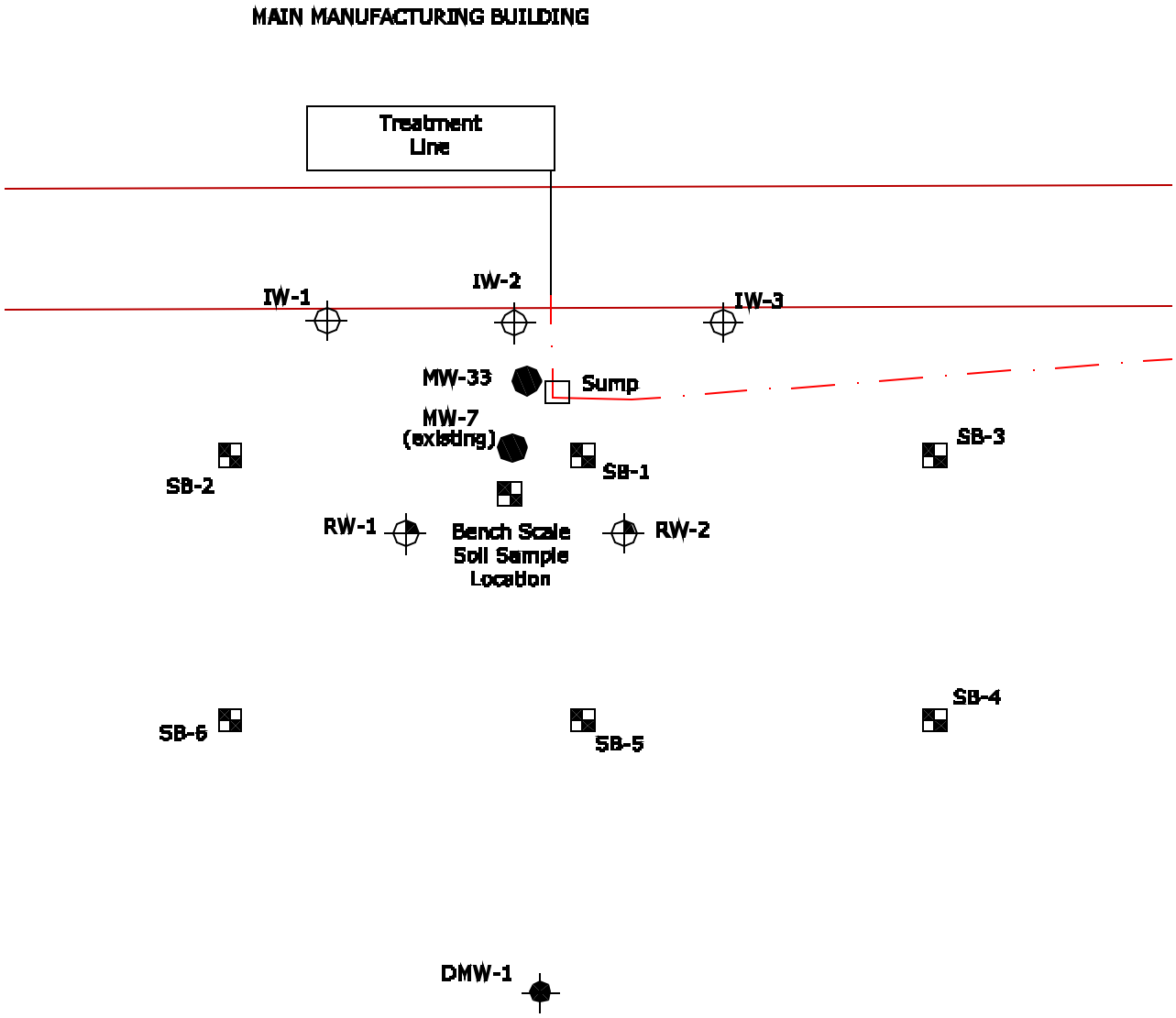
italics = On the edge of detection

Note: For the Aug 08 set, if not labeled Wp or Sp for Water or Soil prep respectively then was prepared using Powersoil prep.
For the Nov 08 vs Apr 09 samples, *vcrA* was not assayed for the ML-8 25' for the Nov 08 set.

Figure 5-2
PCR Results
Bachman Road



Appendix B-10: Milledgeville



EXPLANATION

- MONITOR WELL LOCATION
- DEEP MONITOR WELL LOCATION
- SOIL BORINGS
- RECOVERY WELL
- INJECTION WELL

0 10 20

FEET

FIGURE 2
BIOAUGMENTATION
PILOT TEST
SYSTEM LAYOUT

HSW

Dehalococcoides and Chloroethenes

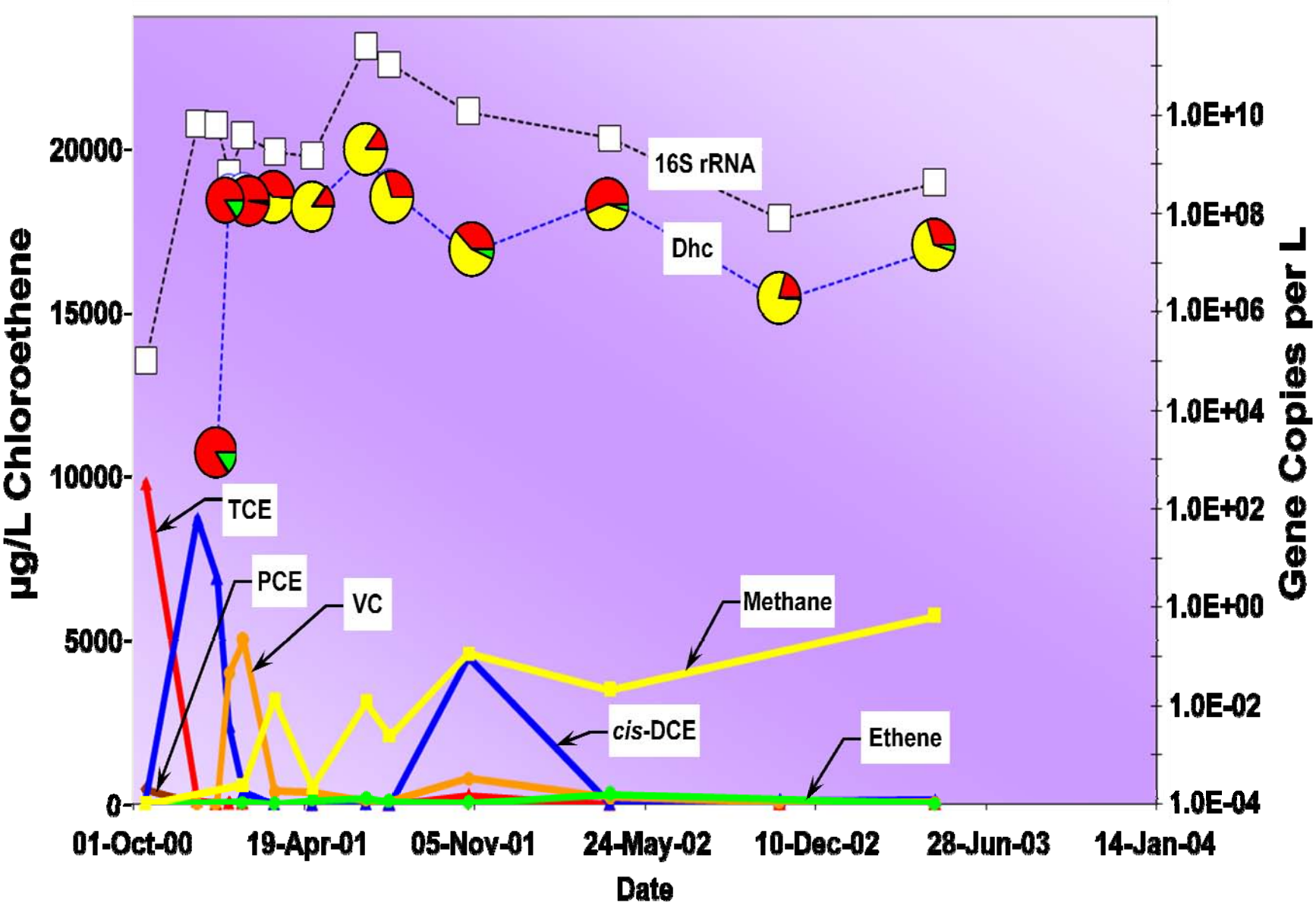


Table 5-6
PCR Results (complete data set)
Milledgeville

Sample	Description	ng/µl DNA	stdev	16S rRNA	Stdev	non-Dhc 16S	Dhc	Stdev	bvcA ave	Stdev	vcrA ave	Stdev	tceA ave	Stdev	Date	ug/L PCE	TCE	cis-DCE	VC	Ethene	Methane
MW7 (Oct04)	MoBio Water Filter-Water Kit	**11**		**9.8E+04**	**4.6E+04**	**9.6E+04**	**2.1E+03**	**6.3E+02**	**1.6E+03**	**1.0E+03**	**4.1E+02**	**1.4E+02**			**15-Oct-04**	**460**	**9800**	**120**	**0.9**	**3.4**	**1.3**
MW7															**20-Oct-04**	**770**	**12000**	**310**	**50**		
MW7															**12-Nov-04**	**430**	**7800**	**2000**	**50**		
MW7															**14-Nov-04**	**540**	**7300**	**5700**	**50**		
MW7															**2-Dec-04**	**310**	**610**	**9600**	**25**		
MW7 (Dec04)	MoBio Water Filter-Water Kit	**551**	**138**	**6.4E+09**	**5.0E+09**	**6.4E+09**									**15-Dec-04**	**34**	**96**	**8700**	**25**		
MW7															**22-Dec-04**	**34**	**28**	**6800**	**50**	**0.33**	**110**
MW7 Jan05)	MoBio Water Filter-Water Kit	**218**	**87**	**6.2E+09**	**3.3E+09**	**6.2E+09**	**1.5E+03**	**2.4E+03**	**3.7E+03**	**2.0E+03**	**2.4E+02**	**2.5E+02**	**2.2E+04**	**1.8E+04**	**7-Jan-05**	**17**	**14**	**6900**	**25**		
MW7 (Jan05-2)	MoBio Water Filter-Water Kit	70	2	**6.5E+08**	**3.4E+08**	**4.0E+08**	**2.5E+08**	**2.3E+07**	**6.0E+05**	**9.4E+04**	**4.6E+04**	**3.0E+04**	**3.8E+08**	**5.5E+07**	**21-Jan-05**	**34**	**28**	**2400**	**4000**		
MW7 (Feb05)	MoBio Water Filter-Water Kit	188	12	**3.8E+09**	**4.3E+08**	**3.5E+09**	**2.7E+08**	**5.8E+07**	**2.3E+06**	**1.2E+06**	**7.1E+06**	**1.3E+06**	**3.7E+08**	**1.6E+08**	**7-Feb-05**	**17**	**29**	**370**	**5000**	**48**	**590**
MW7 (bio-5)		25	6												**16-Feb-05**	**17**	**14**	**45**	**2900**	**110**	**1100**
MW7 (bio-6)		23	3												**2-Mar-05**	**8.5**	**7**	**16**	**2600**	**0.33**	**2500**
MW7 (Mar05)	MoBio Water Filter-Water Kit	**70**	**1**	**1.8E+09**	**5.4E+08**	**1.5E+09**	**2.7E+08**	**3.9E+07**	**1.5E+06**	**1.6E+05**	**1.3E+08**	**8.1E+06**	**8.7E+07**	**6.3E+06**	**15-Mar-05**						
MW7															**29-Mar-05**	**1.7**	**4.6**	**4.2**	**400**	**0.33**	**3200**
MW7 (Apr05)	MoBio Water Filter-Water Kit	106	11	**2.6E+09**	**6.4E+08**	**2.4E+09**	**2.7E+08**	**5.4E+07**	**2.9E+05**	**7.5E+04**	**1.3E+08**	**2.0E+07**	**1.6E+08**	**1.9E+07**	**5-Apr-05**						
MW7 (Apr05-2)	MoBio Water Filter-Water Kit	**82**	**13**	**1.4E+09**	**6.9E+07**	**1.3E+09**	**1.4E+08**	**1.3E+07**	**3.1E+05**	**7.2E+04**	**1.3E+08**	**1.4E+07**	**2.1E+07**	**1.0E+07**	**29-Apr-05**	**1.4**	**1.1**	**2.6**	**360**	**120**	**530**
MW7															**31-May-05**	**2.7**	**13**	**12**	**59**		
MW7															**1-Jul-05**	**1.7**	**9**	**12**	**110**	**200**	**3100**
MW7 (Jul05)	MoBio Water Filter-Water Kit	84	1	**2.5E+11**	**1.4E+10**	**2.5E+11**	**1.6E+09**	**4.0E+08**	**4.9E+05**	**1.3E+05**	**8.9E+08**	**1.4E+08**	**1.5E+08**	**2.8E+07**	**1-Jul-05**						
MW7 (Jul05-2)	MoBio Water Filter-Water Kit	183	24	**1.1E+11**	**2.6E+10**	**1.1E+11**	**3.0E+08**	**8.2E+07**	**1.4E+05**	**3.7E+04**	**5.8E+07**	**5.1E+07**	**2.5E+07**	**5.9E+06**	**29-Jul-05**	**0.34**	**3.1**	**3.9**	**94**	**74**	**2100**
MW7 (Oct05)	MoBio Water Filter-Water Kit	104		**1.2E+10**	**5.4E+09**	**1.2E+10**	**1.6E+07**	**4.4E+06**	**1.2E+06**	**1.6E+05**	**1.1E+07**	**1.0E+06**	**7.7E+06**	**1.4E+04**	**29-Oct-05**	**6.8**	**260**	**4500**	**790**	**36**	**4600**
															14-Dec-05	**28**	**16**	**4200**	**480**		
															26-Jan-05	**17**	**47**	**3400**	**690**	**32**	
MW7 (Feb06)	MoBio Water Filter-Water Kit	91	1	4.3E+09	2.7E+08	4.1E+09	1.9E+08	1.2E+08	1.2E+08	2.7E+07	2.6E+07	1.3E+07	2.6E+07	1.3E+06	28-Feb-06						
MW7 (Apr06)	MoBio Water Filter-Water Kit	77	19	3.5E+09	1.2E+09	3.3E+09	2.0E+08	9.3E+07	4.9E+06	2.3E+06	4.4E+07	1.4E+07	6.1E+07	2.0E+07	13-Apr-06	0.34	2.2	18	220	299	3480
MW7 (Jun06)	MoBio Water Filter-Water Kit	91	13	4.7E+09	2.9E+09	4.8E+08	9.5E+07	2.0E+07	3.6E+05	1.2E+05	2.8E+07	8.6E+06	1.8E+07	3.0E+06							
MW7 (Jul06)	MoBio Water Filter-Water Kit	78	2	4.8E+09	1.0E+09	4.6E+09	6.5E+07	2.2E+07	1.5E+05	8.3E+04	3.5E+07	8.6E+06	5.3E+06	4.6E+06	5-Jul-06	**0.5**	**9.7**	**97**	**77**	**38.6**	**2040**
MW7 (Oct06)	MoBio Water Filter-Water Kit	7	2	7.8E+07	2.8E+07	7.7E+07	1.7E+06	6.5E+05	7.0E+04	3.3E+04	5.9E+06	1.5E+06	1.5E+06	6.3E+05	30-Oct-06	0.5	5.6	110	60		
MW7 (Jan07)	MoBio Water Filter-Water Kit	4	3	5.6E+08	1.5E+08	5.5E+08	9.9E+06	5.1E+06	3.4E+05	1.4E+05	9.2E+06	2.8E+06	2.1E+06	8.4E+05	29-Jan-07	2	24	300	67	45.28	12560
MW7 (Apr07)	MoBio Water Filter-Water Kit	12	2	4.2E+08	2.2E+08	4.0E+08	1.8E+07	2.3E+07	2.0E+05	1.5E+05	3.1E+06	1.9E+06	1.4E+06	1.1E+06	**30-Apr-07**	0.5	**5.3**	**140**	**54**	**10.9**	**5730**
MW7 (Jul07)	MoBio Water Filter-Water Kit	**25**	**7**	7.2E+09	3.0E+09	7.1E+09	6.6E+07	1.3E+07	8.3E+05	1.1E+05	8.5E+06	3.3E+06	4.2E+06	1.7E+06	**5-Jul-07**	0.5	**4.3**	**110**	**43**	**3.61**	**3230**
MW7 (Oct07)	MoBio Water Filter-Water Kit	**28**	**10**	5.8E+08	2.4E+08	5.7E+08	1.4E+07	4.6E+06	9.4E+05	4.2E+05	4.0E+06	1.2E+06	1.4E+06	2.2E+04	**18-Oct-07**						
MW7 (Feb08)	MoBio Water Filter-Water Kit	9.9	3.9	3.4E+08	1.4E+08	3.4E+08	1.6E+06	1.1E+05	1.2E+06	1.3E+05	3.1E+06	5.4E+05	6.6E+05	2.0E+05	**19-Feb-08**						
																PCE	**TCE**	**cis-DCE**	VC	Ethene	Methane
MW33 (Oct04)	MoBio Water Filter-Water Kit	**21**		**7.1E+07**	**2.2E+07**	**7.1E+07**	**6.8E+02**	**5.6E+02**	**4.0E+03**	**8.9E+02**	**2.8E+02**				**15-Oct-04**	**430**	**8600**	**200**	**1**	**0.33**	**1.8**
															20-Oct-04	**560**	**11000**	**270**	**84**		
															17-Nov-04	**700**	**10000**	**1200**	**100**		
MW33 (Dec04)	MoBio Water Filter-Water Kit	**260**	**22**	**4.3E+09**	**1.6E+09**	**4.3E+09**									**15-Dec-04**	**34**	**150**	**10000**	**50**		
MW33															**22-Dec-04**	**34**	**140**	**7700**	**50**	**1.8**	**42**
MW33 (Jan05-2)	MoBio Water Filter-Water Kit	58	2	**2.4E+08**	**9.9E+07**	**1.4E+08**	**1.0E+08**	**1.8E+07**	**3.3E+02**	**2.1E+02**	**7.2E+07**	**6.3E+06**	**1.1E+08**	**3.1E+07**	**21-Jan-05**	**34**	**35**	**5100**	**1500**		
MW33 (Feb05)	MoBio Water Filter-Water Kit	113	3	**1.4E+09**	**1.3E+09**	**1.1E+09**	**3.0E+08**	**1.5E+08**	**1.2E+04**	**1.6E+03**	**1.6E+08**	**1.2E+07**	**4.1E+08**	**9.4E+07**	**7-Feb-05**	**17**	**32**	**2300**	**2600**	**130**	**130**
MW33 (bio-5)		14	1												**16-Feb-05**	**8.5**	**10**	**540**	**2100**	**340**	**180**
MW33 (bio-6)		47	8												**2-Mar-05**	**6.8**	**5.6**	**160**	**2300**	**0.33**	**1080**
MW33 (Mar05)	MoBio Water Filter-Water Kit	**58**	**2**	**1.5E+09**	**1.9E+08**	**1.2E+09**	**3.2E+08**	**2.5E+07**	**6.0E+03**	**3.9E+02**	**1.4E+08**	**2.5E+07**	**2.5E+08**	**2.6E+07**	**15-Mar-05**						
MW33 (Apr05)	MoBio Water Filter-Water Kit	115	24	**2.1E+09**	**8.9E+08**	**1.9E+09**	**2.3E+08**	**1.1E+08**	**1.2E+05**	**3.6E+04**	**1.2E+08**	**1.7E+07**	**2.3E+08**	**4.3E+07**	**5-Apr-05**	**3.4**	**2.8**	**120**	**1000**	**0.33**	**970**
MW33															**28-Apr-05**	**1.4**	**1.1**	**55**	**310**		
MW33 (Jul05)	MoBio Water Filter-Water Kit	67	14	**2.0E+10**	**5.2E+09**	**1.9E+10**	**2.7E+08**	**1.2E+08**	**8.3E+02**	**5.0E+02**	**7.7E+07**	**3.2E+07**	**1.2E+08**	**3.7E+07**	**29-Jul-05**	**1.4**	**3.3**	**110**	**550**	**3.9**	**62**
MW33 (Oct05)	MoBio Water Filter-Water Kit	22	13	**1.2E+09**	**9.6E+08**	**1.2E+09**	**2.3E+07**	**1.1E+07**	**1.4E+04**	**7.4E+03**	**1.3E+07**	**5.8E+06**	**2.2E+07**	**1.1E+07**	**29-Oct-05**	**17**	**87**	**3100**	**1100**	**71**	**4600**
															14-Dec-05	**17**	**200**	**3300**	**980**		
															26-Jan-06	**17**	**97**	**2100**	**940**	**130**	**2000**
MW33 (Apr06)	MoBio Water Filter-Water Kit	21	3	5.0E+08	1.4E+08	4.8E+08	1.4E+07	2.9E+06	7.0E+05	3.7E+05	1.3E+06	4.0E+05	6.4E+06	2.2E+06	13-Apr-06	**0.34**	**2.2**	**18**	**220**	**299**	**3480**
MW33 (Jul06)	MoBio Water Filter-Water Kit	25	10	1.1E+09	5.1E+08	4.6E+09	6.5E+06	3.6E+06	8.7E+04	4.4E+04	9.3E+05	6.9E+05	1.7E+06	1.8E+06	5-Jul-06	13	1000	2700	2600	64.4	994
MW33 (Oct 06)	MoBio Water Filter-Water Kit	13	1	2.7E+08	4.0E+07	2.6E+08	7.3E+06	2.6E+06	7.6E+05	2.3E+05	3.4E+06	2.7E+05	7.3E+06	1.8E+06	30-Oct-06	10	5400	6700	1900		
MW33 (Jan07)	MoBio Water Filter-Water Kit	3	8	5.0E+08	1.9E+08	4.9E+08	6.7E+06	4.0E+06	5.1E+05	3.2E+05	1.6E+06	1.4E+06	2.8E+06	1.6E+06	29-Jan-07	25	4200	3600	1300	138.14	10620
MW33 (Apr07)	MoBio Water Filter-Water Kit	30	2	1.7E+09	5.4E+08	1.7E+09	2.2E+07	5.8E+06	3.8E+06	2.5E+06	1.9E+06	4.4E+05	3.5E+06	1.3E+06	**30-Apr-07**	25	6300	2300	880	96	1570
MW33 (Jul07)	MoBio Water Filter-Water Kit	**33**	**12**	1.2E+10	3.0E+09	1.2E+10	9.2E+07	2.0E+07	4.3E+06	1.3E+06	4.4E+06	1.1E+06	1.1E+07	4.1E+06	**5-Jul-07**	25	2100	540	140	15.65	1322
MW33 (Oct07)	MoBio Water Filter-Water Kit	**39**	**20**	6.6E+08	3.0E+08	6.6E+08	8.4E+05	1.4E+05	2.8E+04	7.3E+03	1.4E+05	3.7E+04	8.8E+04	3.5E+04	**18-Oct-07**						
MW33 (Feb08)	MoBio Water Filter-Water Kit	12.2	2.3	3.92E+08	1.01E+08	3.9E+08	2.24E+05	2.59E+04	6.05E+04	2.21E+04	2.30E+05	1.26E+05	4.21E+05	1.99E+05	**19-Feb-08**						
																PCE	**TCE**	**cis-DCE**	VC	Ethene	Methane
R-1/R-2- (O)ct04)	MoBio Water Filter-Water Kit	**18**		**2.5E+08**	**3.2E+07**	**2.5E+08**	**3.7E+03**	**7.3E+02**	**1.5E+03**	**6.4E+02**	**2.6E+03**	**2.2E+03**	**1.7E+03**	**5.4E+02**	**15-Oct-04**	**670**	**12000**	**120**	**1.4**	**0.3**	**1.7**
RW1 (May05)	MoBio Water Filter-Water Kit	44	1	**5.1E+09**	**1.5E+09**	**5.1E+09**	**4.6E+06**	**4.0E+06**	**2.5E+01**	**9.5E+00**	**2.0E+06**	**1.6E+06**	**1.6E+05**	**1.2E+05**	**31-May-05**	**400**	**600**	**450**	**32**		
RW1 (Jan07)	MoBio Water Filter-Water Kit	31	8	**3.3E+10**	**1.1E+10**	**3.3E+10**	**6.4E+08**	**2.2E+08**	**1.2E+07**	**3.9E+06**	**1.1E+08**	**5.4E+07**	**4.7E+08**	**1.3E+08**	**29-Jan-07**	**140**	**190**	**82**	**280**	**13.2**	**2865**
RW1 (Apr07)	MoBio Water Filter-Water Kit	59	3	**3.4E+10**	**2.9E+10**	**3.4E+10**	**4.3E+08**	**1.3E+08**	**1.1E+06**	**3.6E+05**	**2.3E+06**	**9.1E+05**	**4.5E+08**	**9.9E+07**	**30-Apr-07**	**250**	**270**	**96**	**110**	**3.6**	**324**
RW1 (Jul07)	MoBio Water Filter-Water Kit	**28**	**9**	**6.8E+09**	**4.8E+09**	**6.6E+09**	**2.1E+08**	**7.7E+07**	**1.4E+05**	**4.4E+04**	**7.5E+05**	**2.8E+05**	**4.2E+07**	**1.9E+07**	**5-Jul-07**	**260**	**340**	**370**	**120**	**0.8**	**575**
RW1 (Oct07)	MoBio Water Filter-Water Kit	**31**	**7**	1.39E+09	1.97E+08	1.34E+09	5.15E+07	1.32E+07	1.33E+06	3.75E+05	6.45E+06	2.53E+06	7.42E+06	2.51E+06	**18-Oct-07**						
RW1 (Feb08)	MoBio Water Filter-Water Kit	43.1	24.7	3.57E+09	3.07E+09	3.5E+09	1.04E+08	3.19E+07	4.18E+05	1.59E+05	5.26E+07	2.60E+07	3.19E+07	1.61E+07	**19-Feb-08**						
																PCE	**TCE**	**cis-DCE**	VC	Ethene	Methane
R-1/R-2- (O)ct04)	MoBio Water Filter-Water Kit	**18**		**2.5E+08**	**3.2E+07**	**2.5E+08**	**3.7E+03**	**7.3E+02**	**1.5E+03**	**6.4E+02**	**2.6E+03**	**2.2E+03**	**1.7E+03**	**5.4E+02**	**15-Oct-04**	**670**	**12000**	**120**	**1.4**	**0.3**	**1.7**
RW2 (May05)	MoBio Water Filter-Water Kit	202	1	**1.6E+10**	**8.4E+09**	**1.6E+10**	**5.6E+08**	**6.7E+08**	**9.7E+02**	**1.2E+03**	**3.5E+07**	**3.7E+07**	**6.1E+07**	**3.7E+07**	**31-May-05**	**170**	**2200**	**4300**	**2400**		
RW2															**31-May-05**	**63**	**1300**	**9700**	**5400**	**0.3**	**720**
RW2															**1-Jul-05**	**80**	**660**	**2000**	**1600**	**53**	**850**
RW2 (Jul05)	MoBio Water Filter-Water Kit	31	13	**4.6E+09**	**1.1E+09**	**4.5E+09**	**8.3E+07**	**3.3E+07**	**1.2E+02**	**7.3E+01**	**1.3E+07**	**6.6E+06**	**3.2E+07**	**9.6E+06**	**29-Jul-05**	**28**	**260**	**5300**	**7200**	**3.6**	**10**

Table 5-6
PCR Results (complete data set)
Milledgeville

RW2 (Oct05)	MoBio Water Filter-Water Kit	28	6	**9.6E+08**	**2.7E+08**	**9.5E+08**	**1.5E+07**	**1.9E+06**	**5.1E+01**	**6.8E+01**	**1.6E+07**	**3.5E+06**	**7.3E+06**	**1.2E+06**	**29-Oct-05**	**9**	**280**	**1200**	**1800**	**370.0**	**2700**
															14-Dec-05	**4**	**180**	**600**	**140**		
RW2 (Feb06)	MoBio Water Filter-Water Kit	27	9	1.8E+09	2.0E+08	1.8E+09	1.3E+06	3.3E+05	4.8E+03	3.1E+03	1.4E+06	3.3E+05	1.2E+06	4.8E+05	28-Feb-06						
RW2 (Jul06)	MoBio Water Filter-Water Kit	131	9	1.0E+10	1.6E+09	3.3E+09	1.8E+08	1.0E+08	4.0E+05	2.5E+05	4.9E+07	3.6E+07	2.7E+07	2.6E+07	5-Jul-06	1	3400	13000	5500	366.0	1520
RW2 (Oct06)	MoBio Water Filter-Water Kit	57	3	1.9E+09	4.9E+08	1.6E+09	3.0E+08	1.4E+08	3.1E+06	7.9E+05	1.5E+08	3.6E+07	9.6E+08	2.7E+08	30-Oct-06	50	16000	11000	4400		
RW2 (Jan07)	MoBio Water Filter-Water Kit	40	10	**6.0E+10**	**1.1E+10**	**6.0E+10**	**4.6E+08**	**2.0E+08**	**3.6E+06**	**1.4E+06**	**1.4E+08**	**4.2E+07**	**3.9E+08**	**1.5E+08**	**29-Jan-07**	**10**	**2100**	**1300**	**870**	**173.6**	**9610**
RW2 (Apr07)	MoBio Water Filter-Water Kit	36	4	**4.2E+10**	**4.0E+10**	**-1.8E+09**	**1.8E+09**	**8.4E+08**	**7.3E+04**	**2.3E+04**	**1.5E+08**	**3.9E+07**	**1.5E+09**	**4.3E+08**	**30-Apr-07**	**25**	**11000**	**2300**	**860**	**294**	**4030**
RW2 (Jul07)	MoBio Water Filter-Water Kit	**56**	**13**	**6.2E+10**	**2.1E+10**	**5.2E+10**	**9.8E+09**	**2.8E+09**	**6.3E+05**	**1.7E+05**	**7.0E+08**	**1.1E+08**	**1.0E+09**	**2.1E+08**	**5-Jul-07**	**25**	**9300**	**8500**	**2800**	**132**	**1271**
RW2 (Oct07)	MoBio Water Filter-Water Kit	**36**	**7**	1.92E+09	5.67E+08	1.48E+09	4.42E+08	1.44E+08	4.34E+04	1.26E+04	1.30E+08	2.84E+07	5.55E+07	1.46E+07	**18-Oct-07**						
RW2 (Feb08)	MoBio Water Filter-Water Kit	17.0	0.7	1.41E+09	7.98E+08	1.4E+09	2.63E+07	2.62E+06	1.09E+04	2.34E+03	8.23E+06	9.61E+05	1.92E+07	3.80E+06	**19-Feb-08**						
MW34 (Oct05)	MoBio Water Filter-Water Kit	51	5	**2.5E+09**	**1.1E+09**	**2.5E+09**	**3.6E+04**	**1.7E+04**			**9.5E+02**	**6.6E+02**	**1.5E+04**	**1.9E+03**	**29-Oct-05**	**70**	**390**	**1200**	**1900**	**1200.0**	**1900**
															14-Dec-05	**17**	**1300**	**2800**	**1100**		
															26-Jan-06	**68**	**3100**	**3400**	**590**		
															11-Apr-06	**210**	**960**	**1800**	**310**		
IW1 (Feb08)	MoBio Water Filter-Water Kit	83.5	18.3	6.4E+09	2.2E+09	6.4E+09	2.6E+05	6.5E+04			5.9E+05	3.7E+05	3.7E+05	1.3E+05	**19-Feb-08**						
IW2 (Feb08)	MoBio Water Filter-Water Kit	40.1	4.2	2.0E+09	8.2E+08	2.0E+09	1.7E+05	2.7E+04			1.2E+04	2.5E+03	5.6E+05	1.3E+05	**19-Feb-08**						
IW3 (Feb08)	MoBio Water Filter-Water Kit	30.1	20.3	3.1E+09	2.0E+09	3.1E+09	1.1E+06	4.9E+05	*2.1E+04*	*4.1E+03*	3.3E+05	2.3E+05	3.0E+06	1.6E+06	**19-Feb-08**						
endrow				**1.0E+00**	**1.0E+00**	**1.0E+00**	**1.0E+00**	**1.0E+00**	**1.0E+00**	**1.0E+00**	**1.0E+00**	**1.0E+00**	**1.0E+00**	**1.0E+00**							

Figure 5-6
PCR Results MoBio Results (complete data set)
Milledgeville

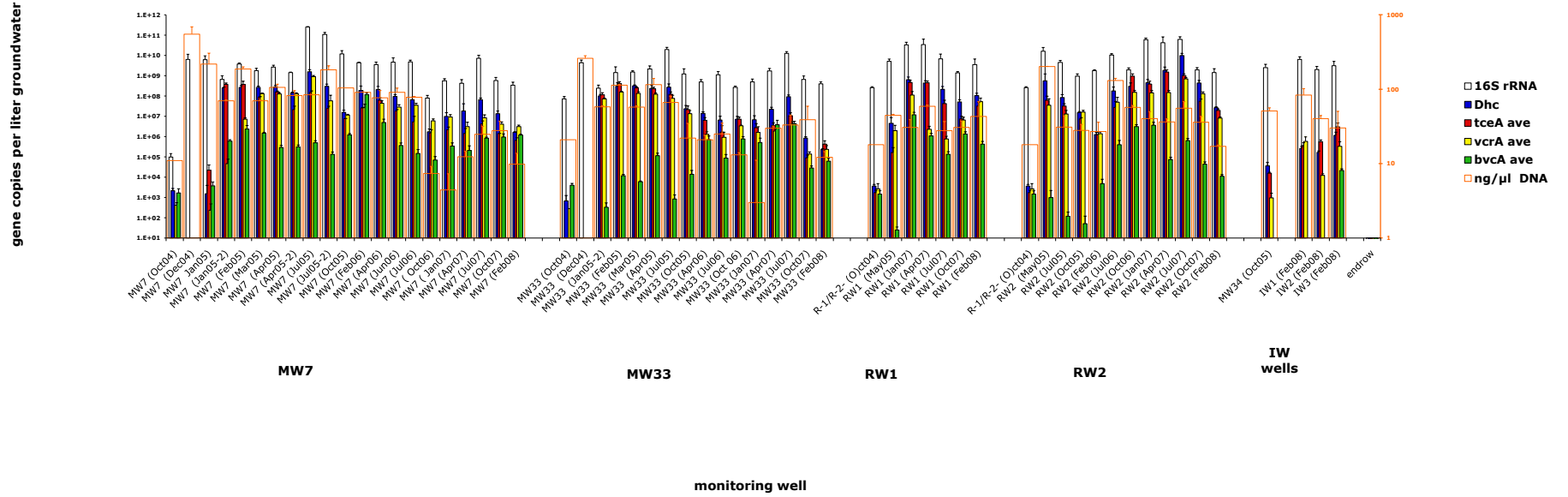
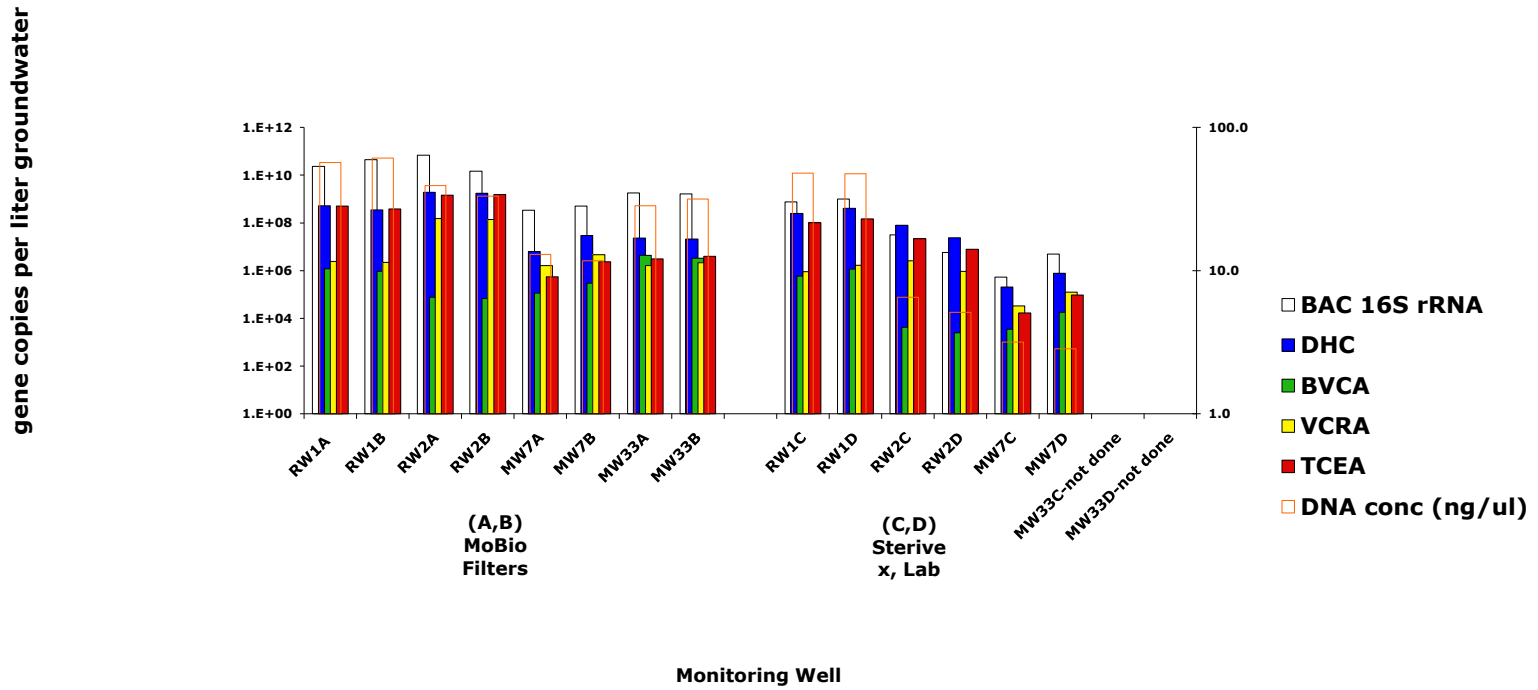


Table 5-7
PCR Results (method comparison data set)
Milledgeville

Sample ID		DNA conc (ng/ul)	stdev	BAC 16S rRNA	stdev	DHC	stdev	BVCA	stdev	VCRA	stdev	TCEA	stdev	date	vol. (ml)
RW1A	MoBio Filter-Water Kit	**56.8**	**0.28**	2.33E+10	5.04E+09	5.22E+08	1.06E+08	1.19E+06	3.86E+05	2.39E+06	1.33E+06	5.09E+08	8.46E+07	**20-Apr-07**	**500**
RW1B	MoBio Filter-Water Kit	**61.1**	**0.25**	4.52E+10	3.94E+10	3.47E+08	8.97E+07	9.28E+05	3.09E+05	2.19E+06	1.59E+05	3.86E+08	7.39E+07	**20-Apr-07**	**500**
RW2A	MoBio Filter-Water Kit	**39.3**	**0.07**	6.94E+10	4.10E+10	1.89E+09	1.22E+09	7.73E+04	2.84E+04	1.54E+08	4.39E+07	1.46E+09	4.02E+08	**20-Apr-07**	**250**
RW2B	MoBio Filter-Water Kit	**33.2**	**1.27**	1.45E+10	3.78E+09	1.76E+09	2.44E+08	6.85E+04	1.67E+04	1.38E+08	3.66E+07	1.54E+09	4.97E+08	**20-Apr-07**	**250**
MW7A	MoBio Filter-Water Kit	**13.0**	**3.68**	3.37E+08	2.30E+08	6.33E+06	2.48E+05	1.14E+05	1.45E+05	1.61E+06	4.56E+05	5.57E+05	2.82E+05	**20-Apr-07**	**1000**
MW7B	MoBio Filter-Water Kit	**11.7**	**0.99**	5.00E+08	2.00E+08	2.98E+07	2.94E+07	2.95E+05	7.53E+04	4.66E+06	1.60E+06	2.32E+06	6.97E+05	**20-Apr-07**	**1000**
MW33A	MoBio Filter-Water Kit	**28.3**	**0.53**	1.80E+09	5.50E+08	2.28E+07	7.64E+06	4.37E+06	3.16E+06	1.63E+06	3.73E+05	3.07E+06	1.38E+06	**20-Apr-07**	**1000**
MW33B	MoBio Filter-Water Kit	**31.7**	**0.04**	1.66E+09	5.77E+08	2.12E+07	3.81E+06	3.28E+06	1.80E+06	2.17E+06	3.31E+05	3.92E+06	1.07E+06	**20-Apr-07**	**1000**
RW1C	Sterivex Filter-Water Kit	**48.1**	**1.66**	7.67E+08	2.59E+08	2.52E+08	7.78E+07	5.83E+05	6.07E+04	9.00E+05	2.59E+05	1.00E+08	2.05E+07	**20-Apr-07**	**520**
RW1D	Sterivex Filter-Water Kit	**47.4**	**0.92**	9.88E+08	2.14E+08	4.02E+08	3.00E+07	1.14E+06	3.40E+05	1.69E+06	6.54E+05	1.47E+08	2.59E+07	**20-Apr-07**	**500**
RW2C	Sterivex Filter-Water Kit	**6.5**	**0.28**	3.16E+07	6.59E+06	7.87E+07	1.76E+07	4.19E+03	2.50E+03	2.54E+06	5.04E+05	2.18E+07	1.10E+07	**20-Apr-07**	**150**
RW2D	Sterivex Filter-Water Kit	**5.1**	**0.32**	5.78E+06	1.82E+06	2.39E+07	4.92E+06	2.45E+03	4.68E+03	9.29E+05	1.71E+05	7.80E+06	4.68E+06	**20-Apr-07**	**150**
MW7C	Sterivex Filter-Water Kit	**3.2**	**0.25**	5.32E+05	5.42E+04	2.04E+05	3.74E+04	3.52E+03	2.69E+03	3.32E+04	2.32E+03	1.70E+04	8.75E+03	**20-Apr-07**	**500**
MW7D	Sterivex Filter-Water Kit	**2.9**	**0.14**	4.95E+06	8.39E+06	7.67E+05	5.42E+04	1.80E+04	8.50E+03	1.25E+05	1.91E+04	9.51E+04	5.23E+04	**20-Apr-07**	**500**
MW33C-not done														**20-Apr-07**	
MW33D-not done														**20-Apr-07**	

Figure 5-7
PCR Results Mob Bio versus Sterivex (comparison data set)
Milledgeville



Appendix B-11: Former NAWC Trenton



Figure 3. Locations of wells used in aquifer tests and tracer tests, 2003, and line of section A-A' at the Naval Air Warfare Center, West Trenton, New Jersey.

N

MW-64BR
MW-40BR
MW-60BR
TREATMENT BUILDING
MW-45BR
WEST DITCH WELL
SITE 1
(FORMER BRINE HANDLING AREA AND WEST END DITCH)
MW-61BR
MW-43S
MW-4S
MW-36BR
MW-20BR
MW-26BR
MW-
55
BRP-2
MW-5BR
MW-29BR
MW-1S
BUILDING 41
MW -8BR
MW-15S
MW-9BR
68
69
MW-55BR
MW-21BR
MW-24S
35-MW-2
MW-26S
MW-25S
67
66
35-MW-1
31
TANK FARM
MW-20S
MW-18S
30
38
COOLING TOWERS
MW-19S
S-7
98
29
60
36
59
MW-46BR
BLDG. 28
PARKWAY AVENUE
BLDG. 40
43
MW-68BR
MW-38BR
BUILDING 42
MW-63BR
MW-27BR
BLDG. 62
MW -4BR
MW-47BR
BRP-1
MW-65BR
MW-30BR
MW -23BR
MW-16S
MW-22BR
MW-52BR
MW-44S
MW-17BR
O.H. PIPELINE
MW-17S
MW-41BR
MW-16BR
MW-28BR
BUILDING 21

LEGEND

- RECOVERY WELL
- OVERBURDEN MONITORING WELL
- BEDROCK MONITORING WELL
- FORMER SOURCE AREA
- BIOAUGMENTATION PLOT BOUNDARY
- BIOAUGMENTATION INJECTION WELL
- BIOAUGMENTATION MONITORING WELL

SITE PLAN
AREA OF CONCERN

FORMER NAVAL AIR WARFARE CENTER (NAWC)
WEST TRENTON, NEW JERSEY

ECOR Solutions
1075 Andrew Drive, Suite I, West Chester, PA 19380

SCALE IN FEET	DATE	FIGURE
0 250	06-11-07	1

ECOR Solutions, Inc.

Figure 2A: Methane Concentrations

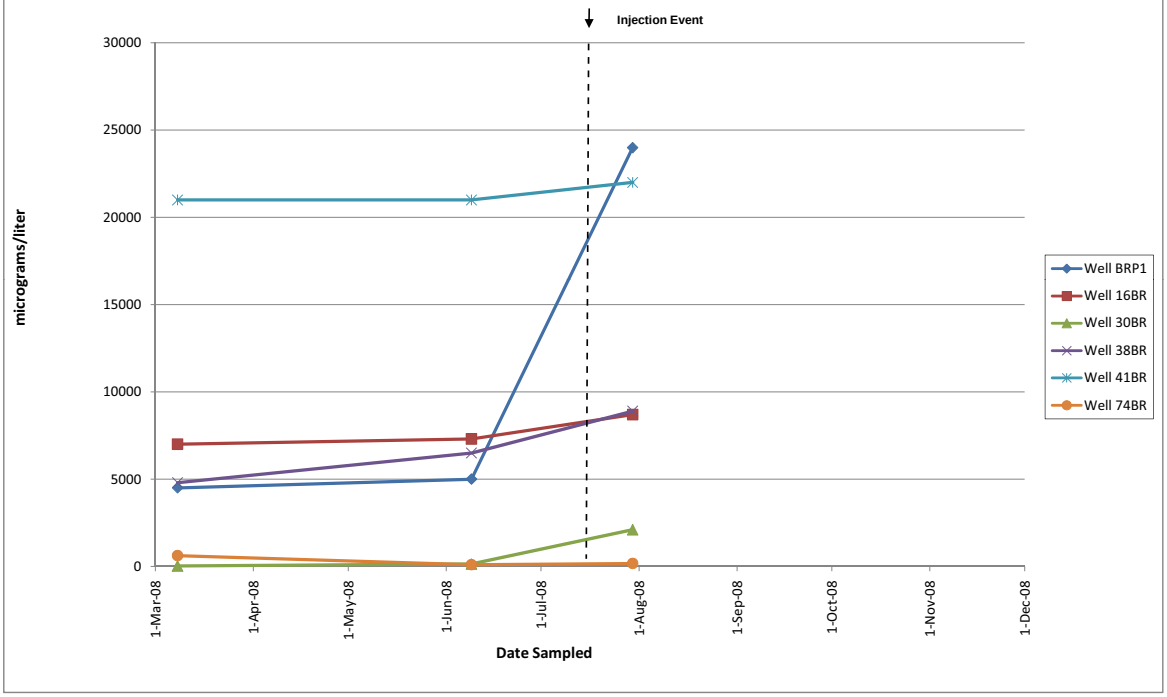


Figure 2B: Methane Concentrations at 68BR

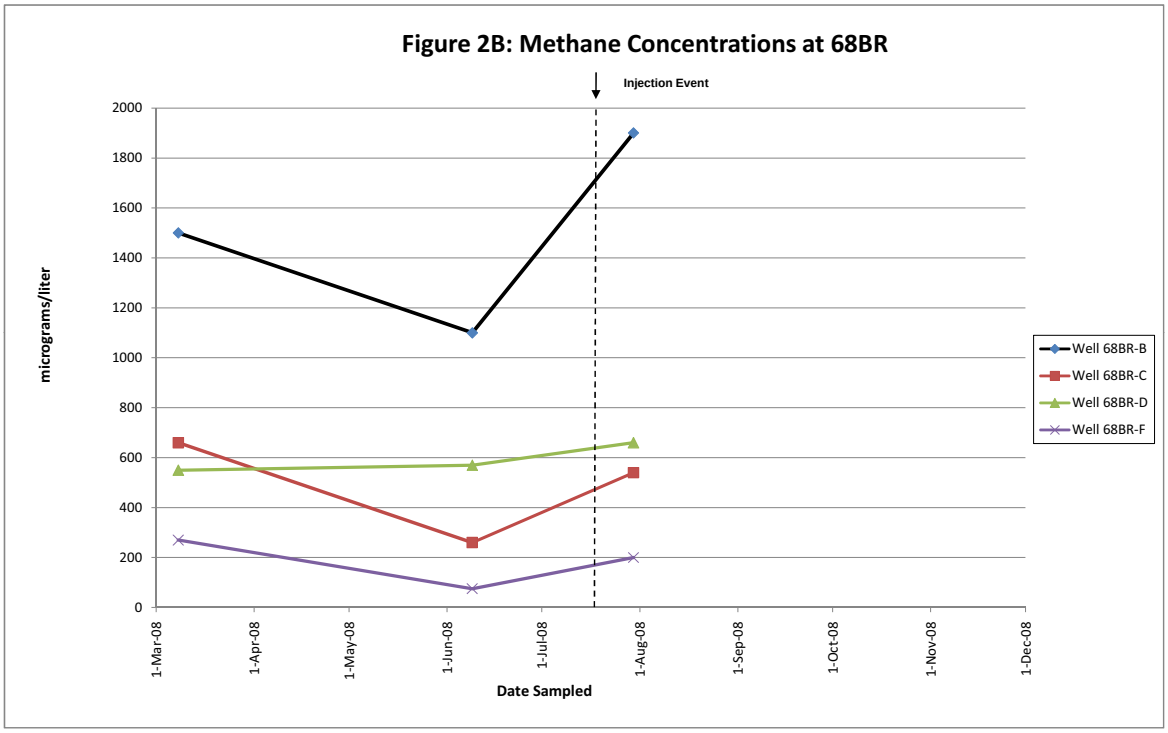


Figure 3A: Well 16BR CVOC Molar Concentrations

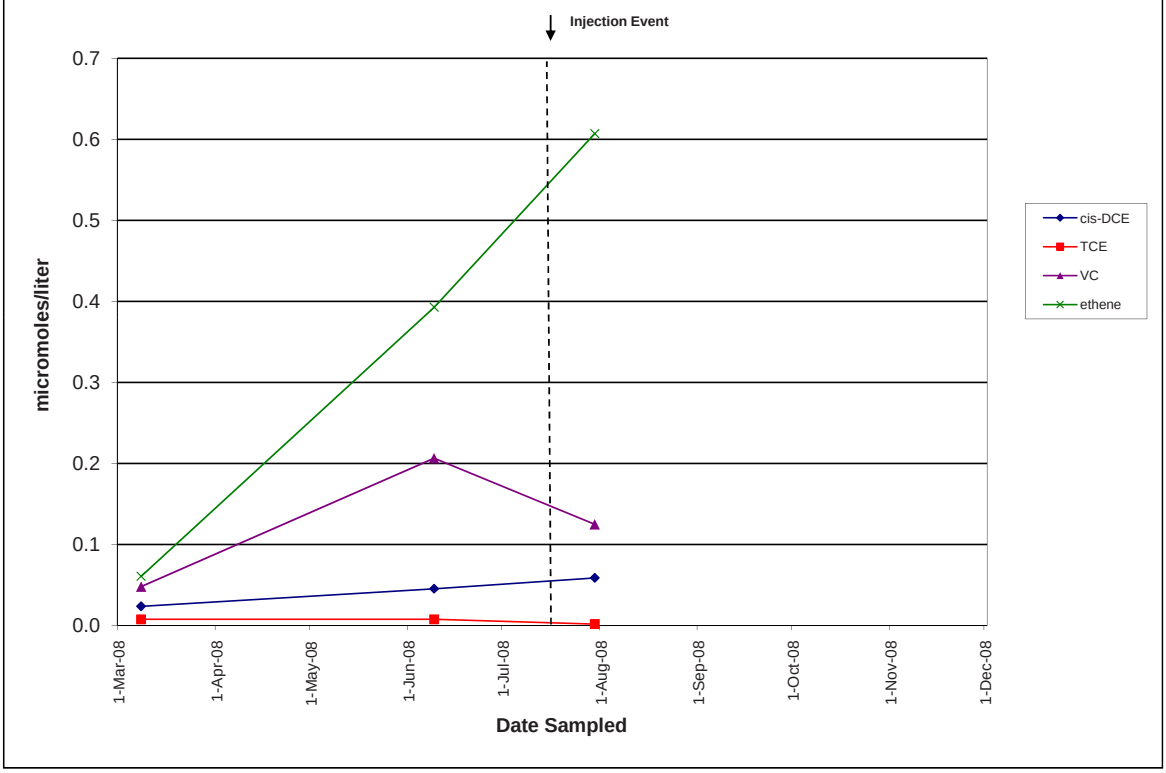


Figure 3B: Well 16BR CVOC Concentrations

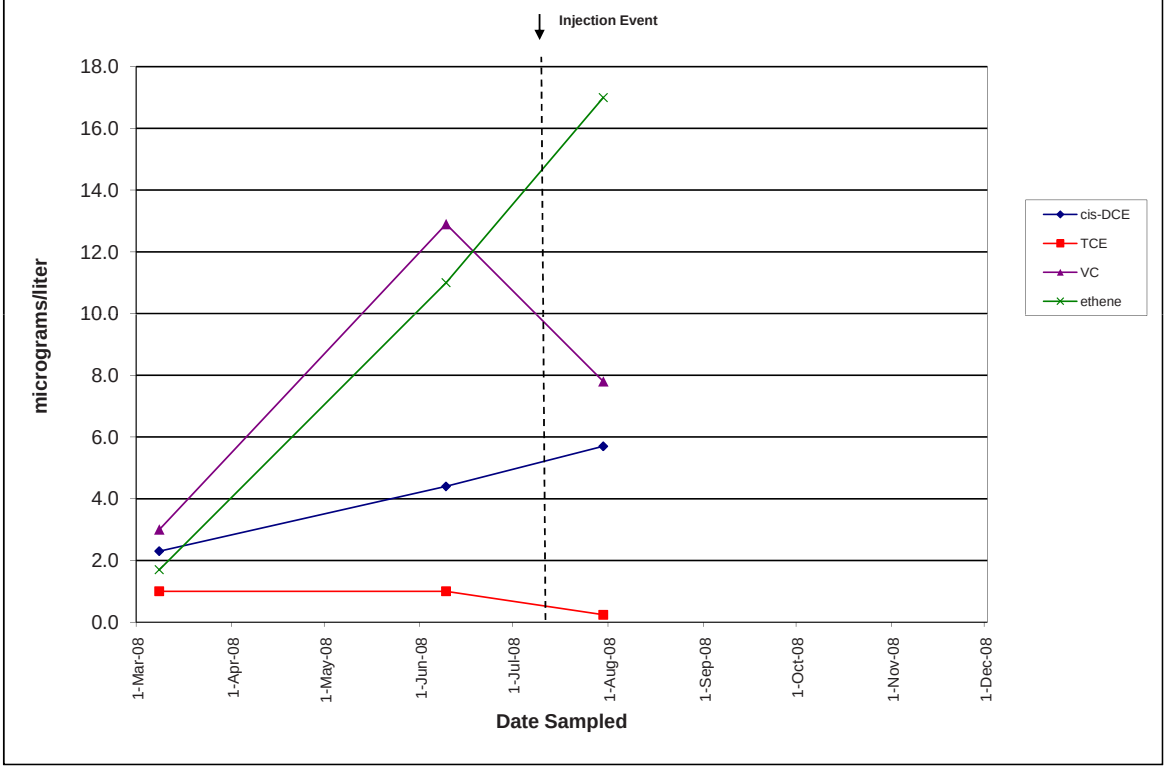


Figure 4A: Well 41BR CVOC Molar Concentrations

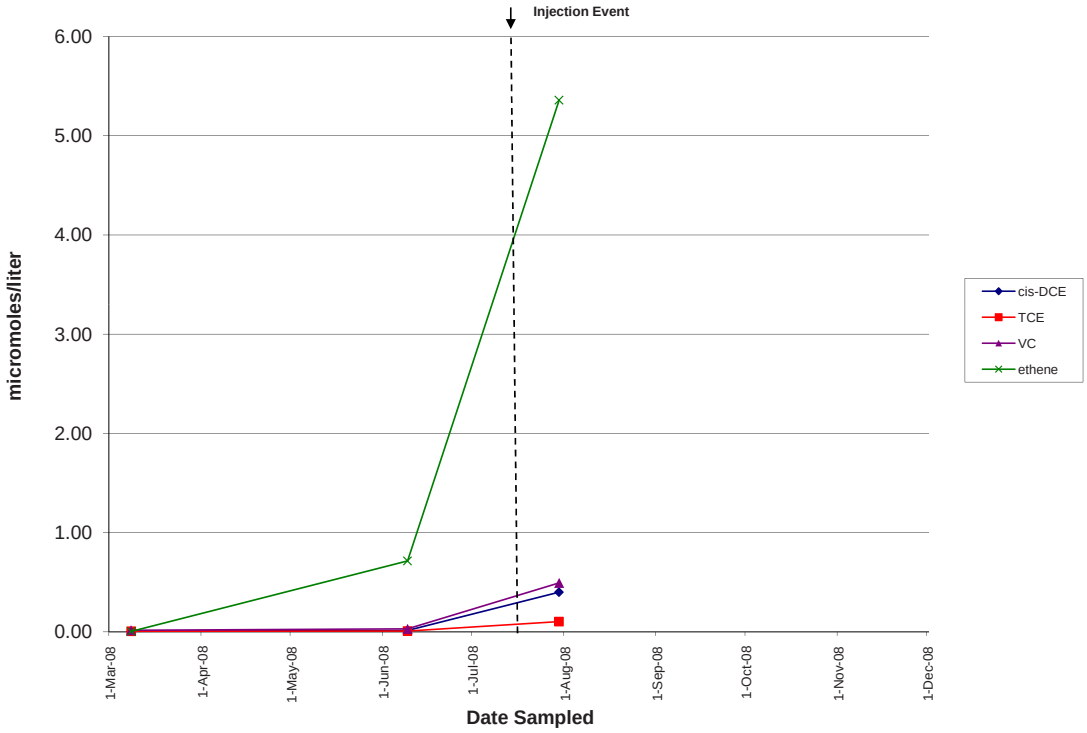


Figure 4B: Well 41BR CVOC Concentrations

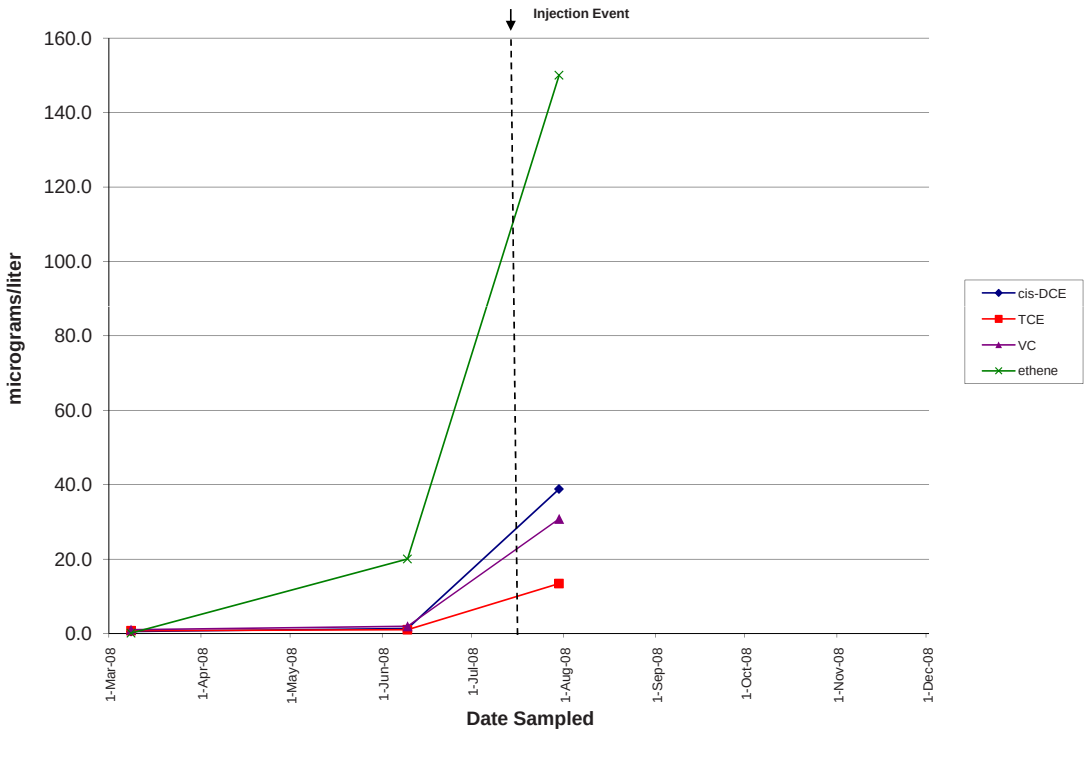


Figure 5A: Well BRP1 CVOC Molar Concentrations

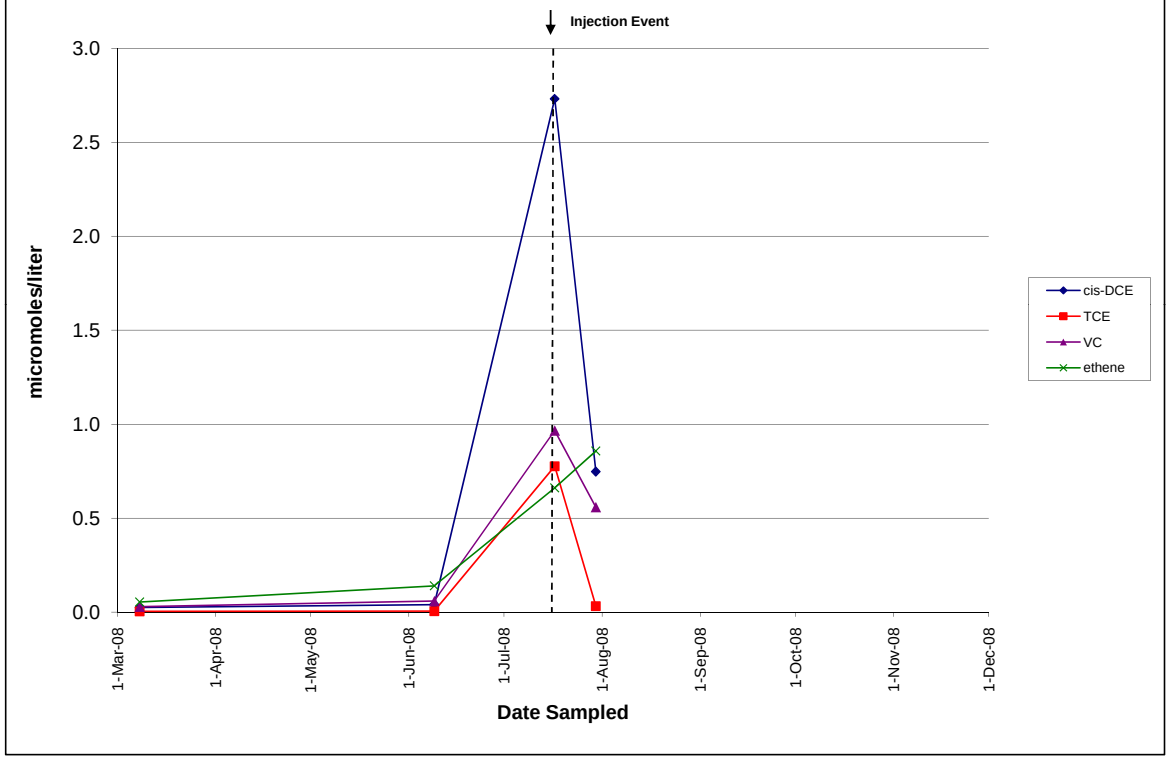


Figure 5B: Well BRP1 Concentrations of CVOCs

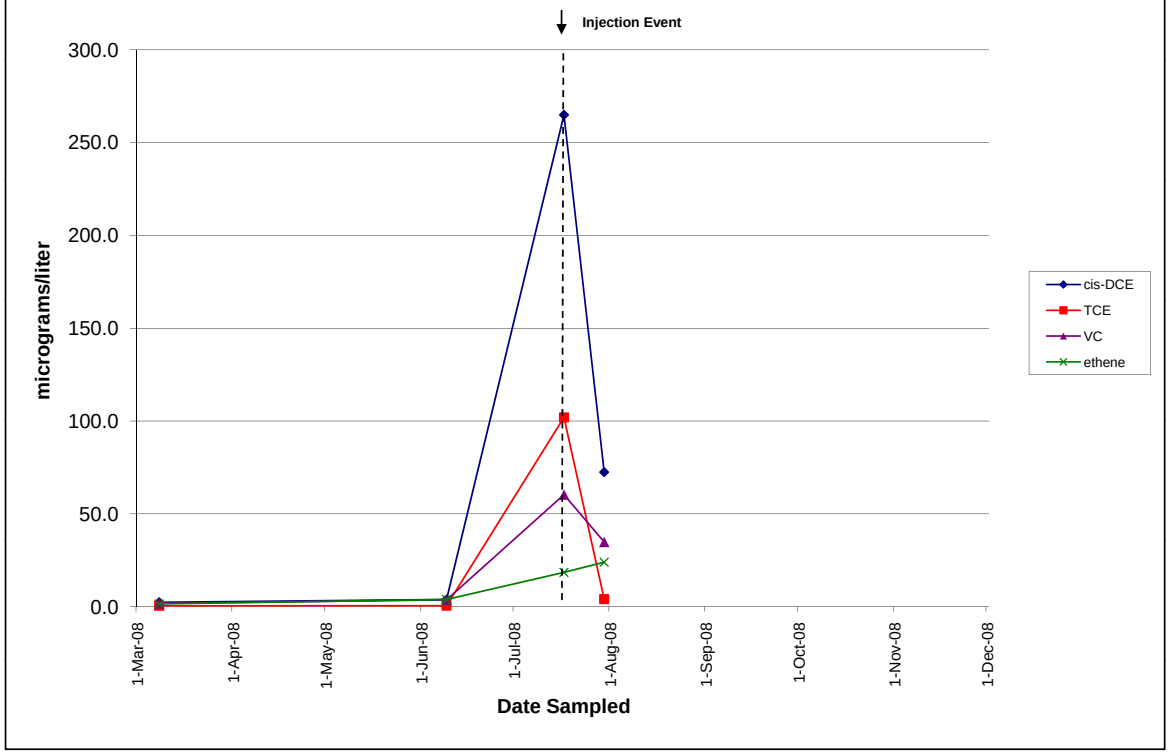


Figure 6A: Well 38BR CVOC Molar Concentrations

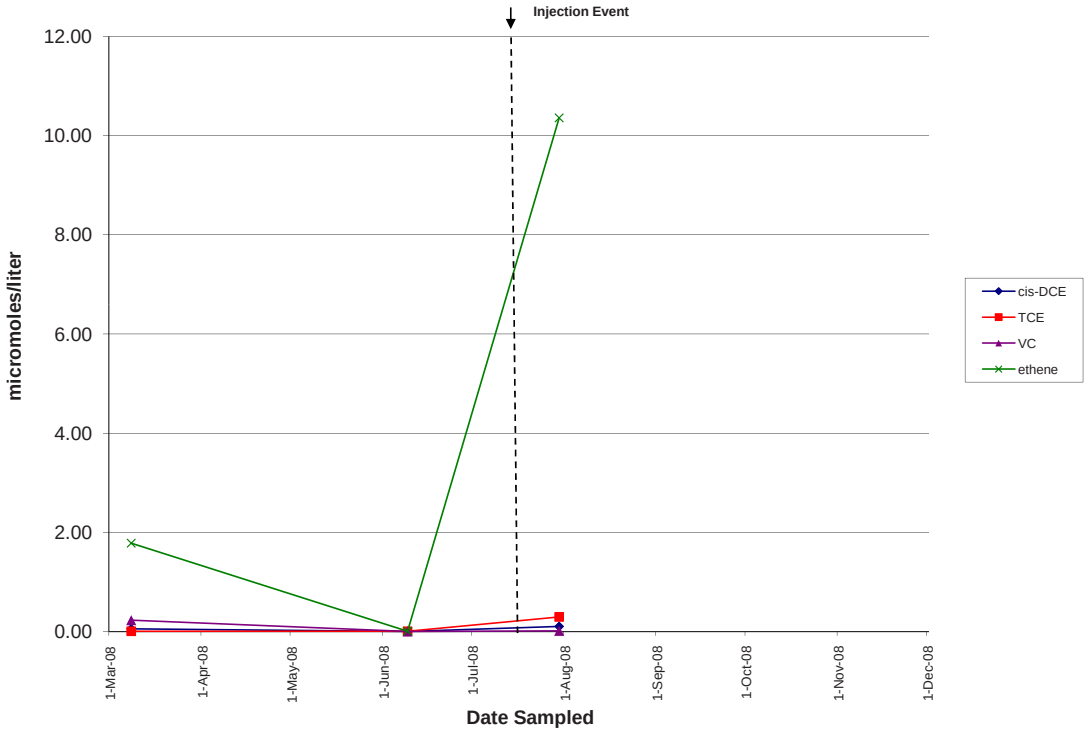


Figure 6B: Well 38BR CVOC Concentrations

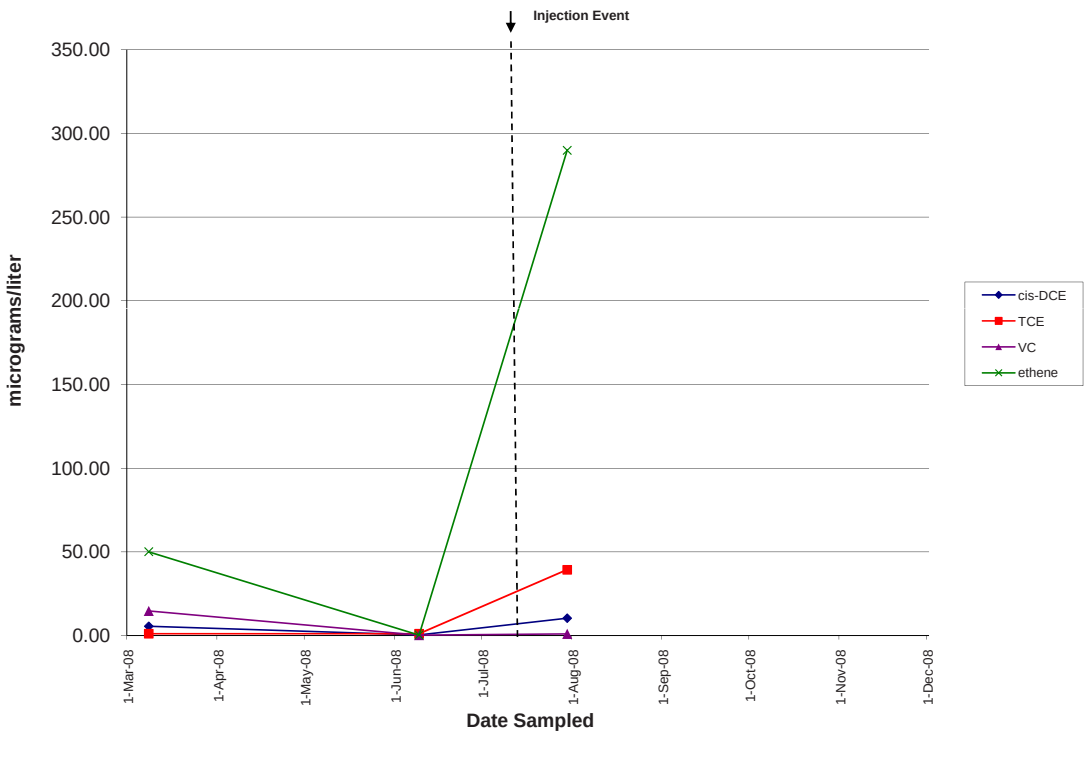


Figure 7A: Well 30BR CVOC Molar Concentrations

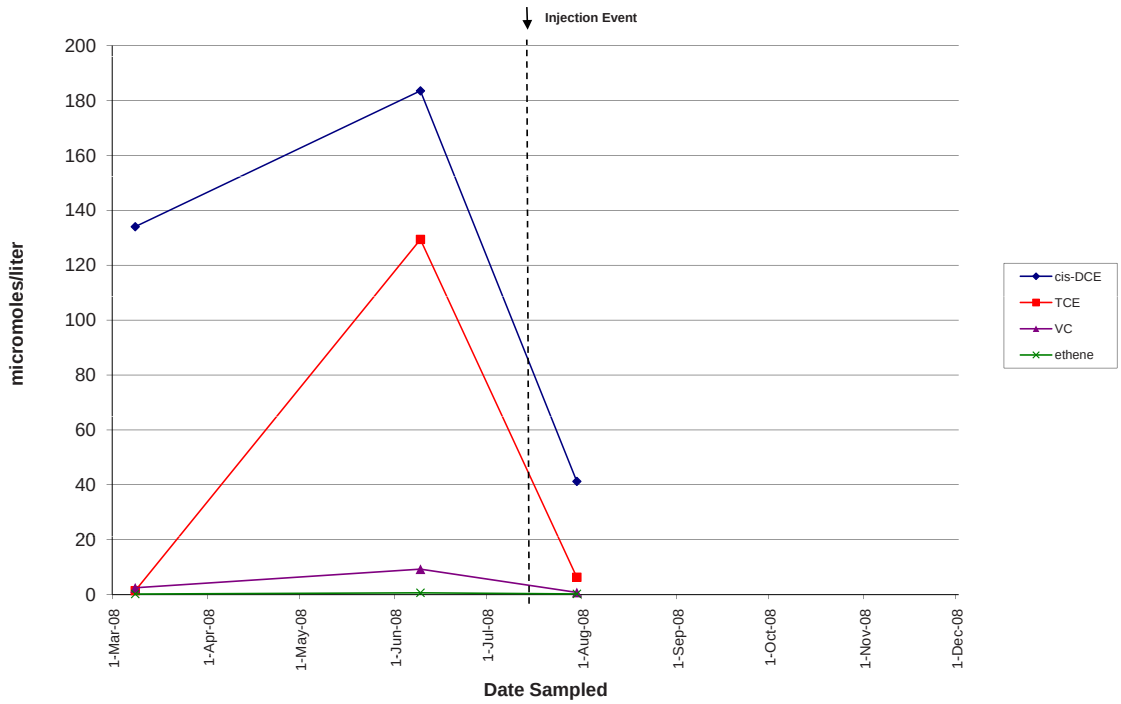


Figure 7B: Well 30BR CVOC Concentrations

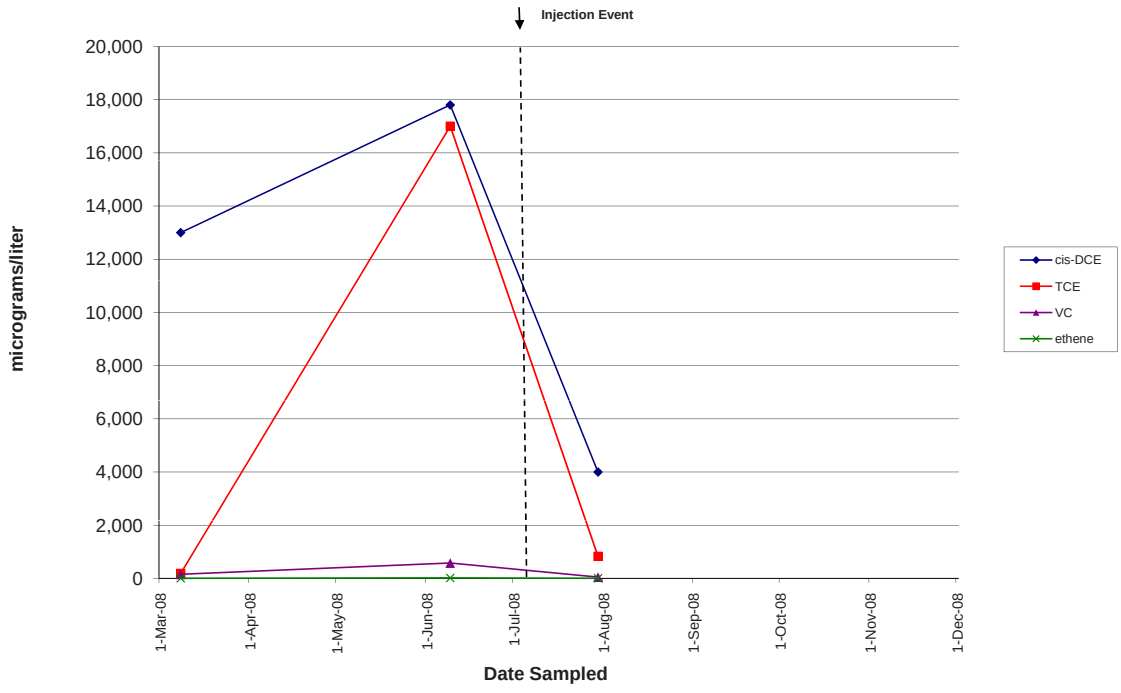


Figure 8A: Well 68BR-B CVOC Molar Concentrations

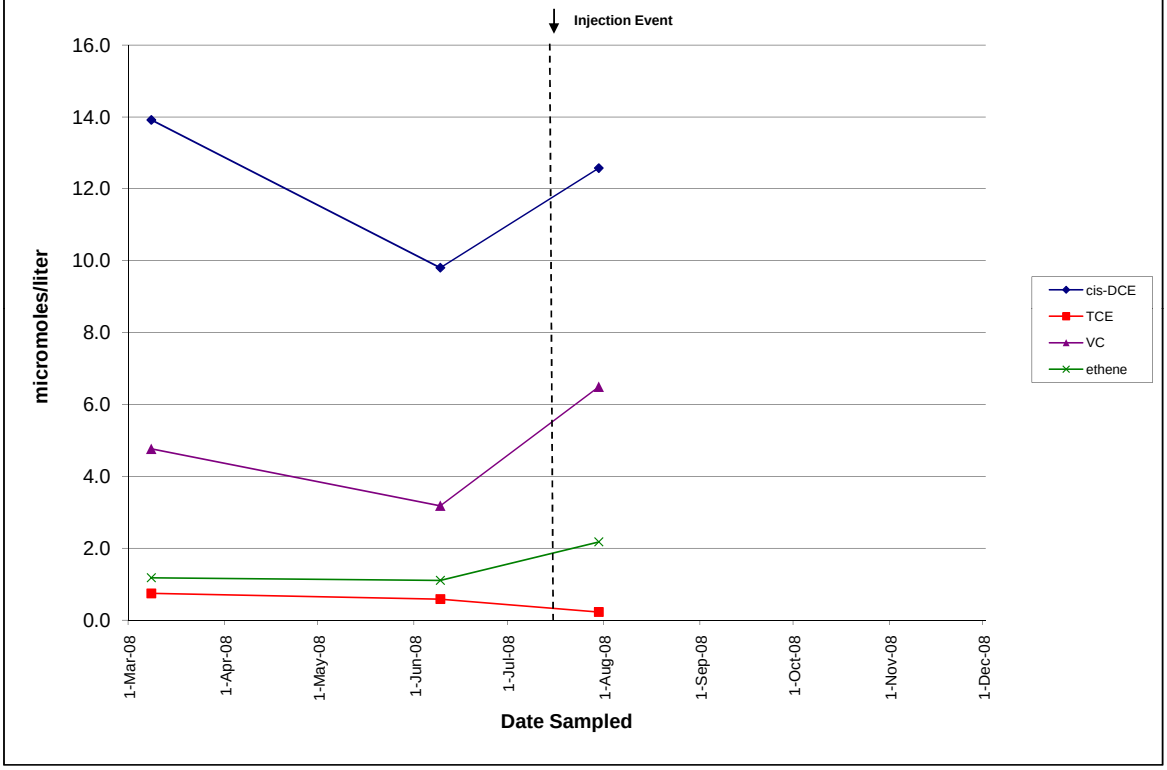


Figure 8B: Well 68BR-B CVOC Concentrations

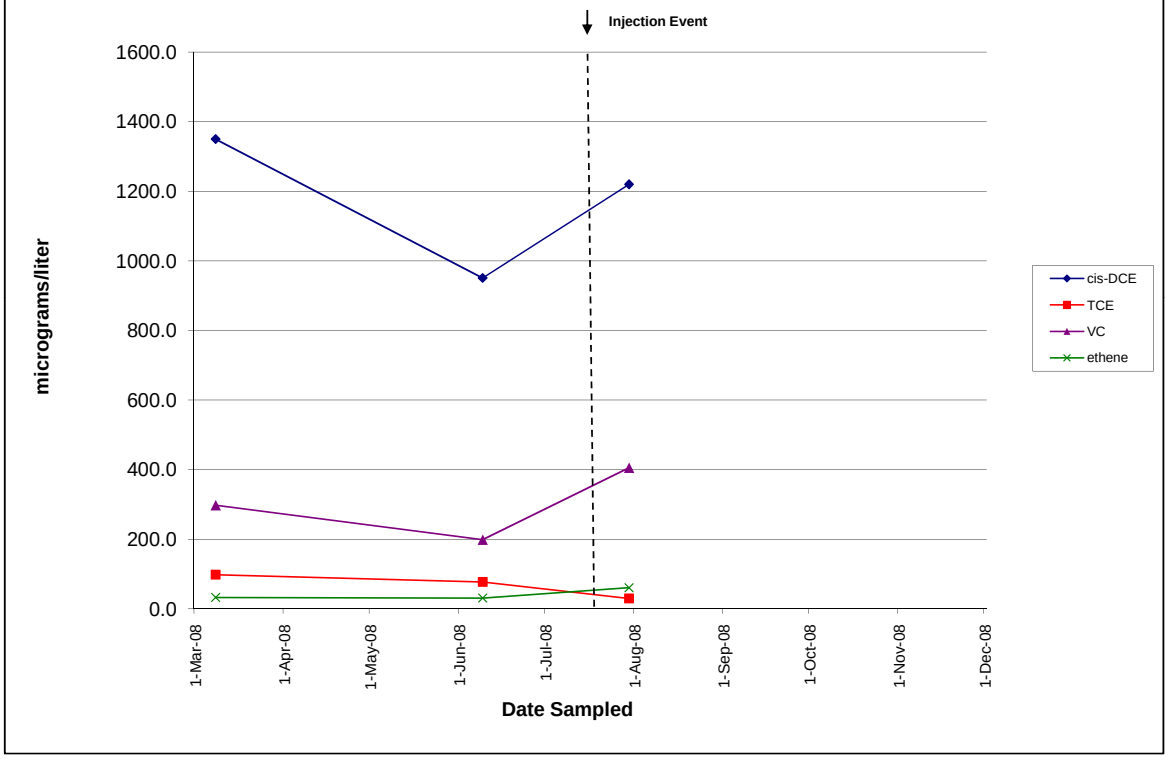


Figure 9A: Well 68BR-C CVOC Molar Concentrations

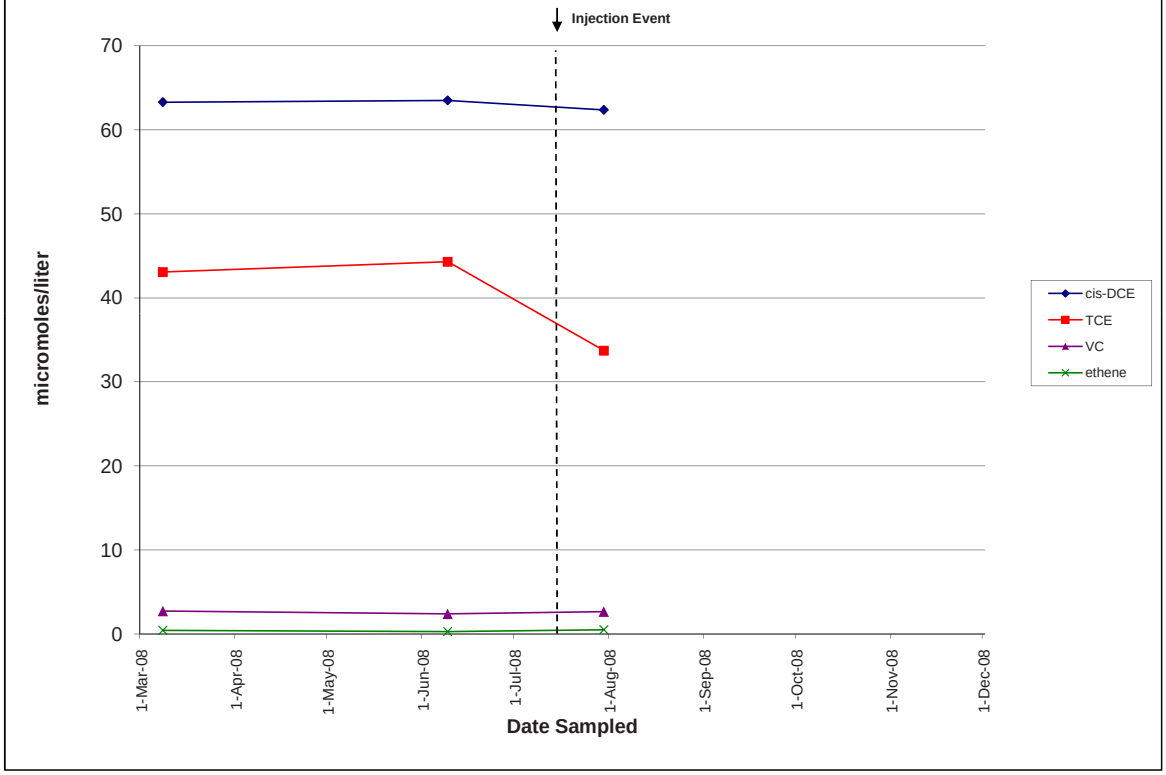


Figure 9B: Well 68BR-C CVOC Concentrations

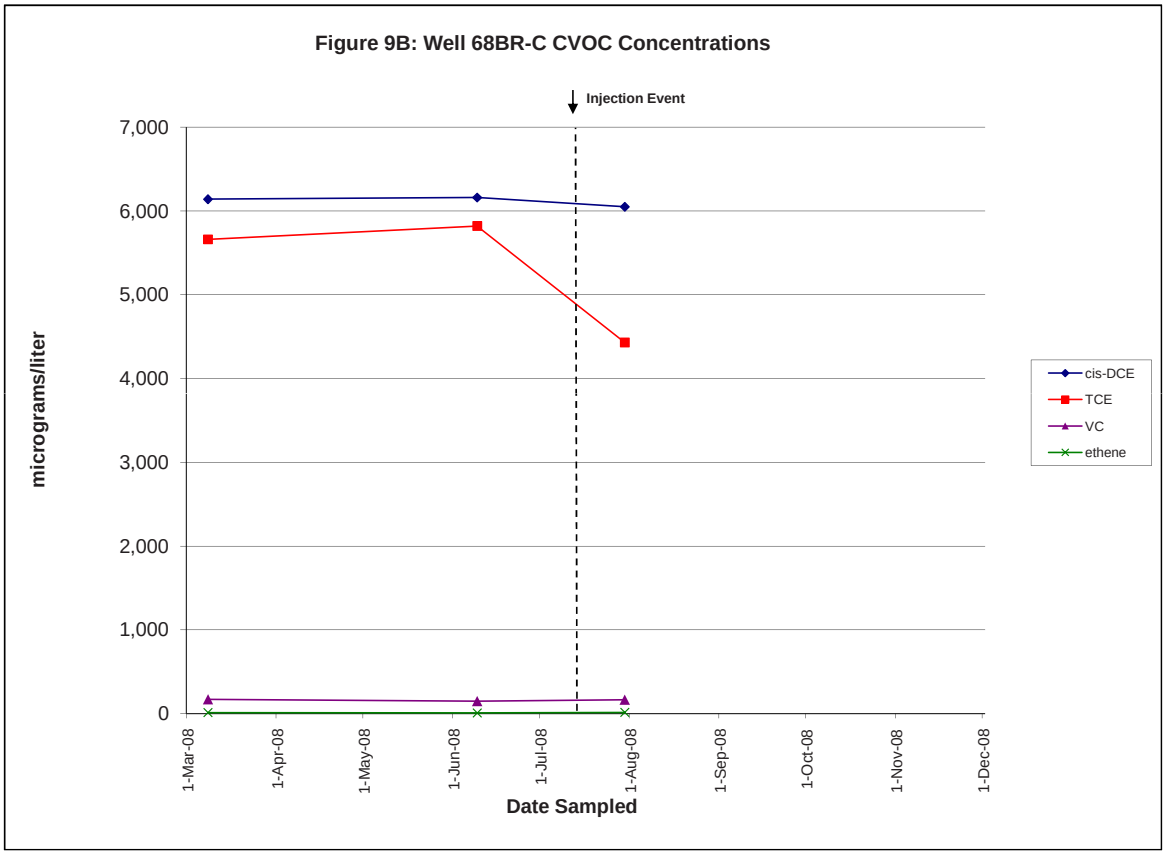


Figure 10A: Well 68BR-D CVOC Molar Concentrations

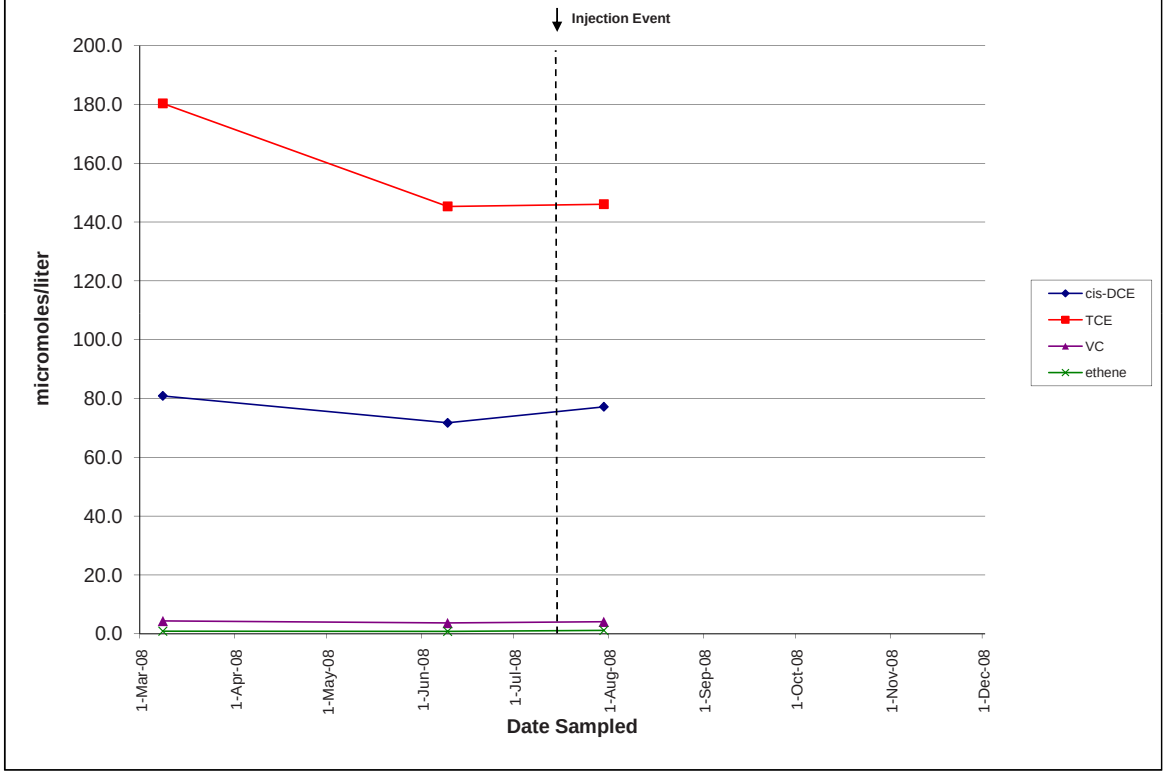


Figure 10B: Well 68BR-D CVOC Concentrations

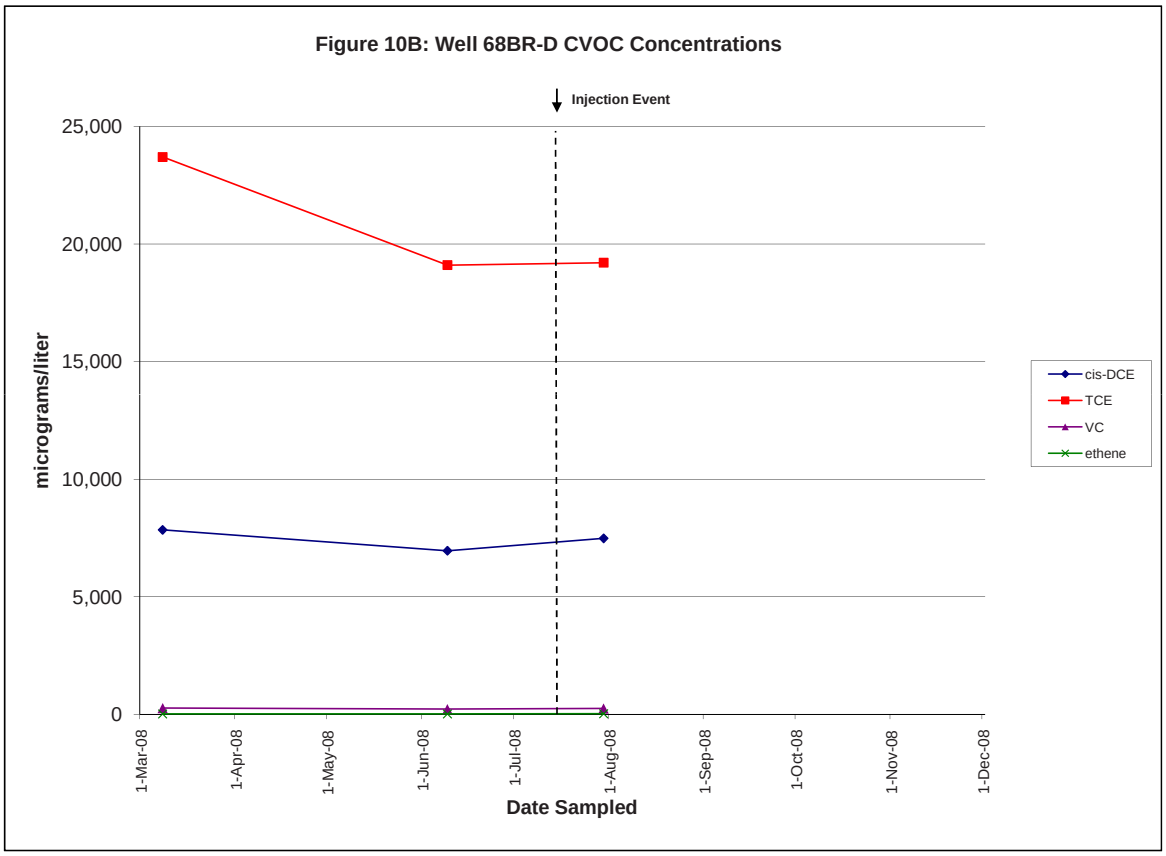


Figure 11A: Well 68BR-F CVOC Molar Concentrations

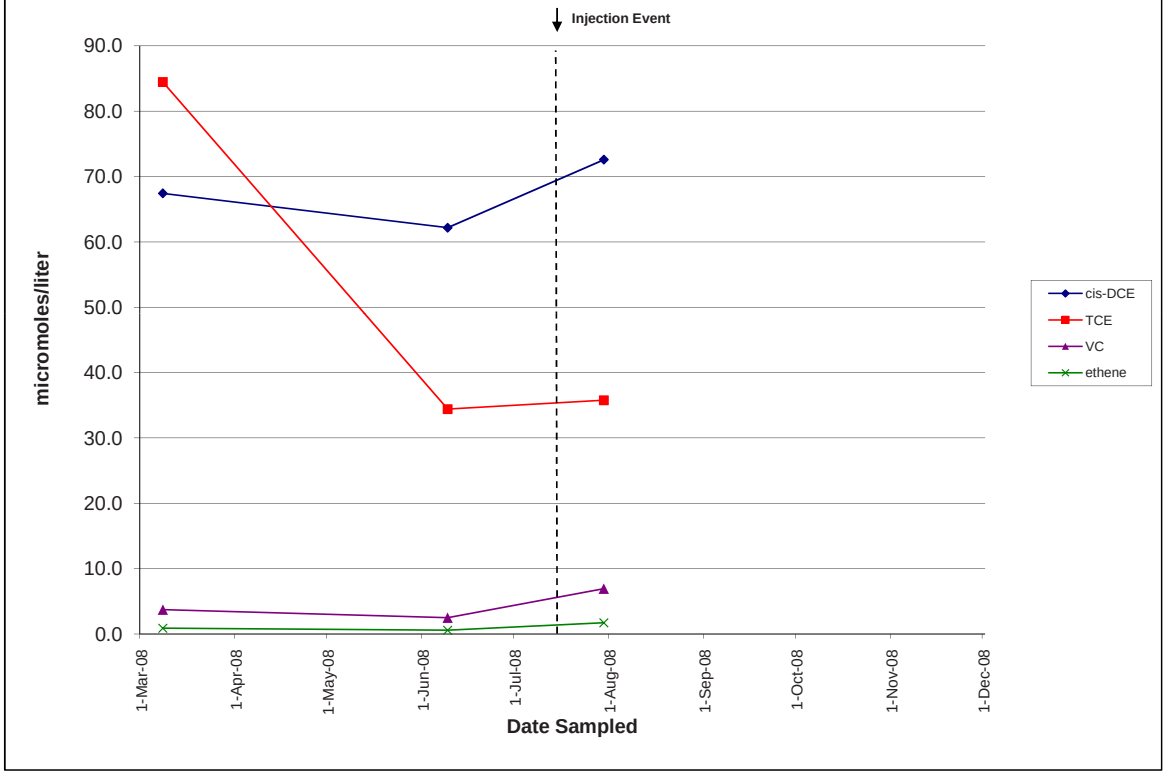
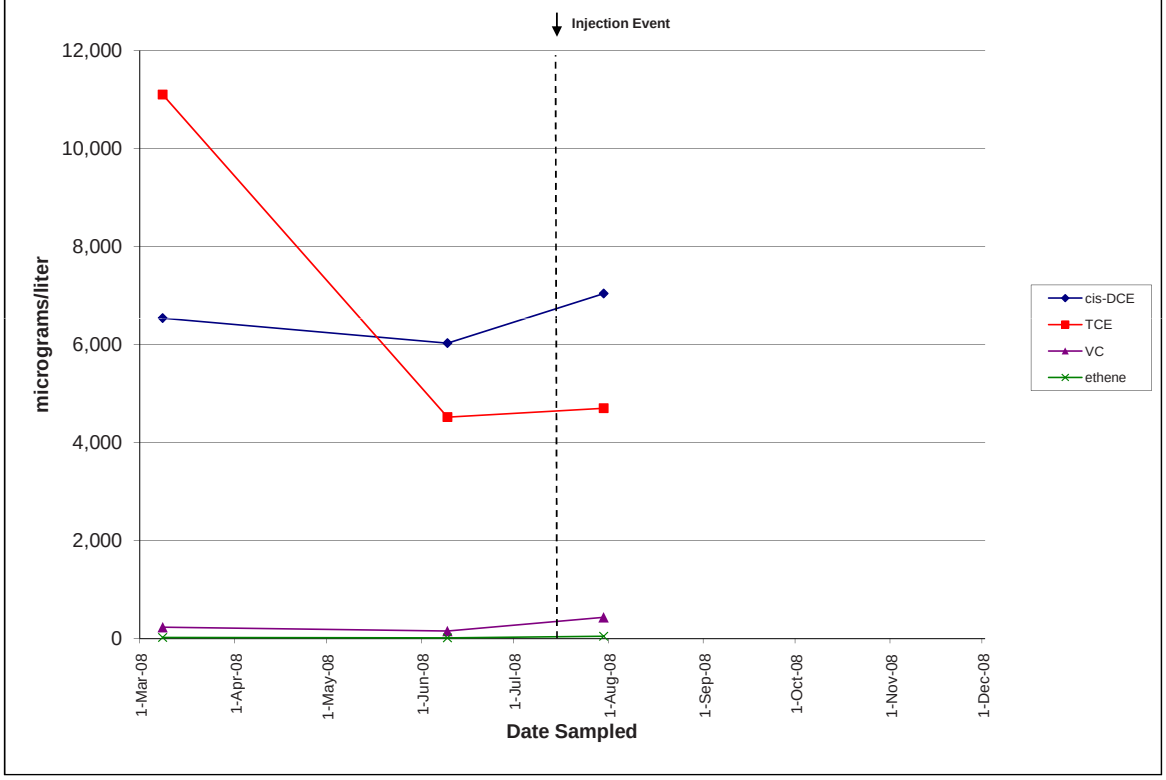
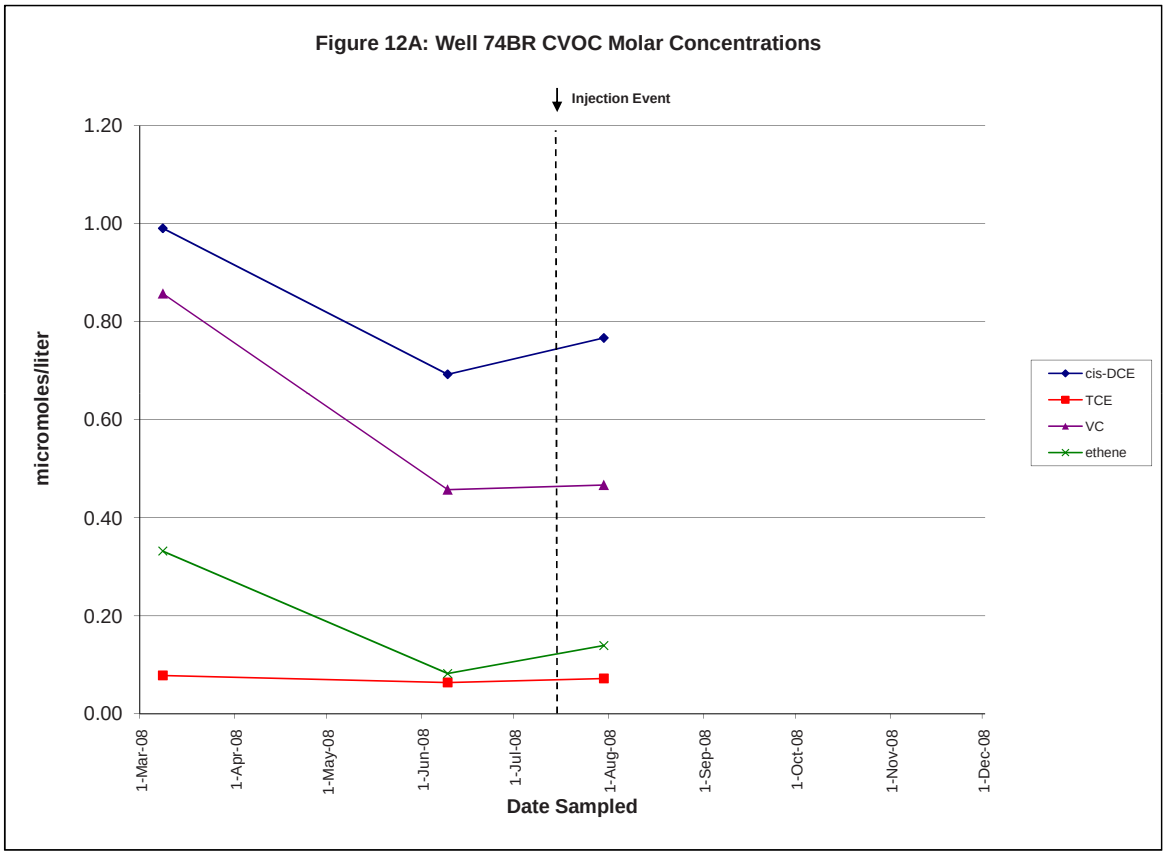


Figure 11B: Well 68BR-F CVOC Concentrations




Figure 12A: Well 74BR CVOC Molar Concentrations
Injection Event
micromoles/liter
1.20
1.00
0.80
0.60
0.40
0.20
0.00
1-Mar-08
1-Apr-08
1-May-08
1-Jun-08
1-Jul-08
1-Aug-08
1-Sep-08
1-Oct-08
1-Nov-08
1-Dec-08
Date Sampled
cis-DCE
TCE
VC
ethene

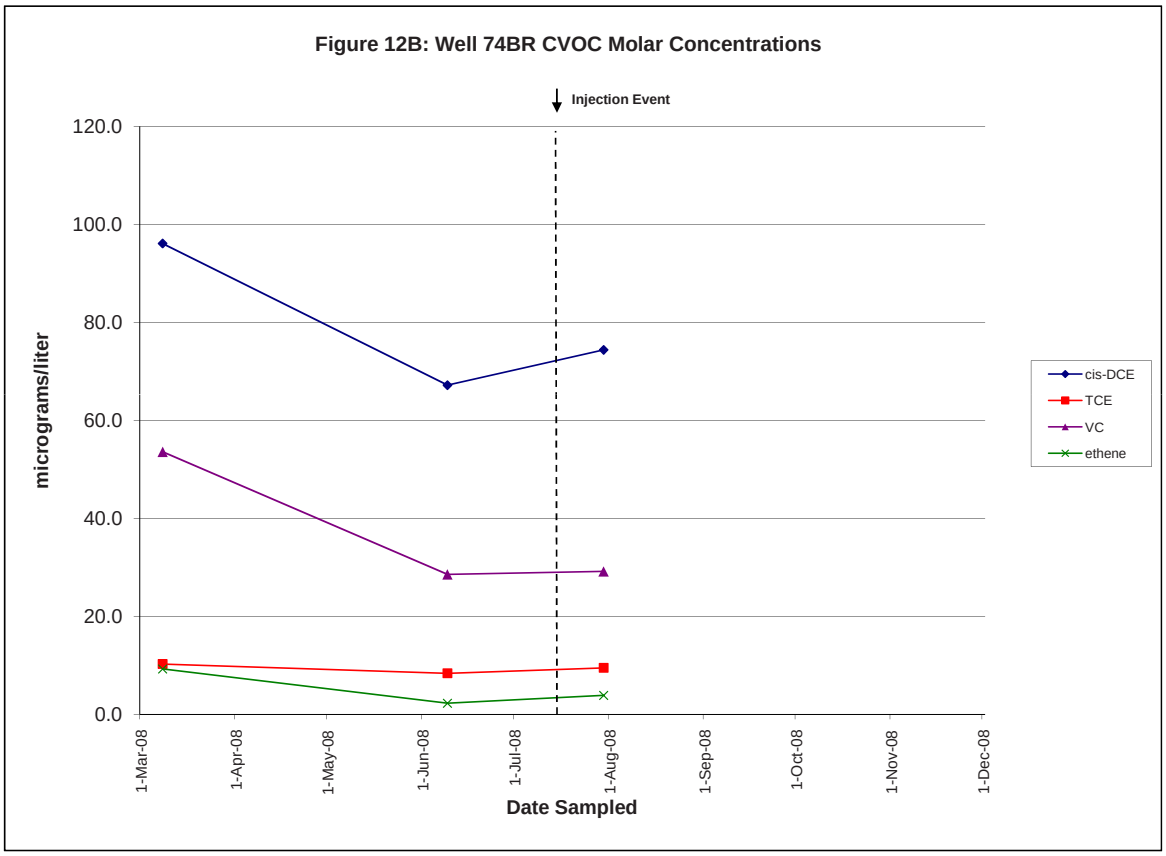

Figure 12B: Well 74BR CVOC Molar Concentrations
Injection Event
micrograms/liter
120.0
100.0
80.0
60.0
40.0
20.0
0.0
1-Mar-08
1-Apr-08
1-May-08
1-Jun-08
1-Jul-08
1-Aug-08
1-Sep-08
1-Oct-08
1-Nov-08
1-Dec-08
Date Sampled
cis-DCE
TCE
VC
ethene

Figure 13A: Molar Concentrations of cVOCs at MW41 During Injection

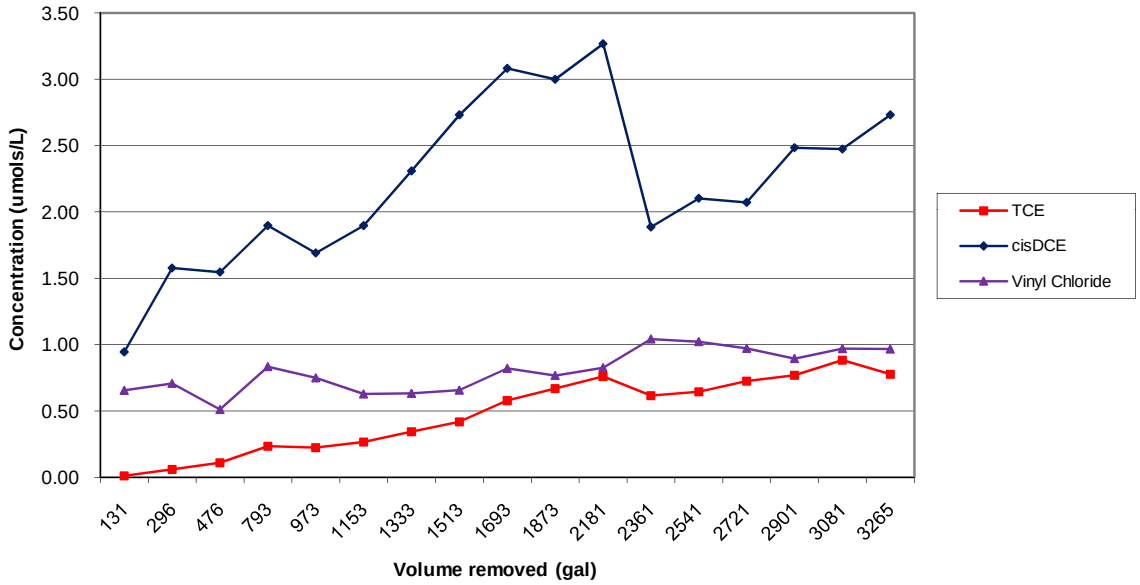
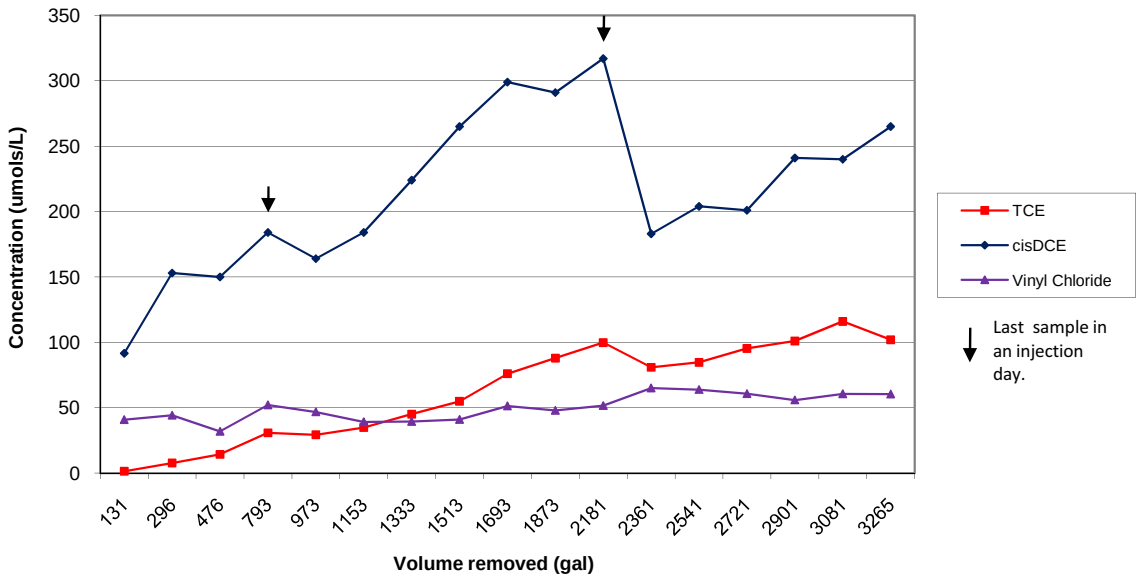


Figure 13B: Concentrations of cVOCs at MW41 During Injection



Appendix C: Sampling Procedures

Appendix C-1: Low-Flow Groundwater Purging and Sampling

APPENDIX C-1

STANDARD OPERATING PROCEDURE

LOW-FLOW GROUNDWATER PURGING AND SAMPLING

1.0 INTRODUCTION

1.1 Purpose

This Standard Operating Procedure (SOP) establishes the procedure for well purging and sampling utilizing low-flow techniques in support of the ESTCP – Nucleic Acid-Based Tools demonstration.

1.2 General Procedure

The low-flow techniques discussed herein are in accordance with regulatory guidance for low flow sampling as defined by Puls and Barcelona (1996). Natural attenuation, geochemistry and related sampling is also conducted in accordance with Wiedemeier et. al (1998) and Parsons, et. al. (2004). This guidance is summarized below. Groundwater sampling shall also be conducted in compliance with all site-specific work plans and local, state, and federal guidelines as appropriate to the project.

2.0 SUPPLIES, EQUIPMENT, AND ANALYSES

This SOP requires that all supplies and equipment normally utilized for low-flow purging and sampling are available. In addition, one 1.5" polyethylene disposable bailer is required for each well sampled. The bailer will be utilized to gently surge the well prior to sampling for molecular biological analyses.

It is recommended that the list of fixed-based and field analysis kits listed in Table A be conducted on groundwater collected. Example field log sheets are attached to this SOP.

3.0 PROCEDURES FOR WELL PURGING

3.1 Obtain a static water level measurement of the well to be purged. Leave the water level meter suspended inside the well riser.

3.2 A peristaltic or submersible bladder pump shall be used for purging and sampling. The intake should be placed within the screen interval as defined in the project-specific work plan. (Regardless of where the intake is placed it should be consistently located at the same location for all sampling events).

3.3 Start with the initial pump rate set at approximately 0.1 to 0.2 liters/minute. Adjust pumping rates as necessary to prevent drawdown from exceeding 0.3 feet during purging. If no drawdown is noted, the pump rate may be increased (to a max of 0.4 liters/minute) to expedite the purging and sampling event.

3.4 Utilize a water quality meter with flow through cell to measure pH, dissolved oxygen, oxidation-reduction potential (ORP), temperature, and specific conductance. It is recommended that a separate meter be used to measure turbidity (e.g., LeMotte turbidity meter).

3.5 Every five to ten minutes, record the depth to water and water quality parameters (pH, specific conductance, temperature, turbidity, oxidation-reduction potential, and dissolved oxygen).

3.6 Stabilization is achieved and sampling can begin when a minimum of one casing volume has been removed *and* three consecutive readings, taken at 5 to 10 minute intervals, are within the following limits:

pH ± 0.1 standard units
Specific conductance ± 3%
Temperature ± 1.0 ºC
Turbidity less than 10 NTUs

If the above conditions have still not been met after the well has been purged for two hours, purging will be considered complete and sampling can begin. Record the final well stabilization parameters. Note: Depending on local, state, or federal guidelines more than one casing may need to be purged prior to sample collection.

4.0 GROUNDWATER SAMPLING PROCEDURES

Note: Items 4.1 through 4.4 address collection of the samples for volatile organic compound (VOC) and natural attenuation parameter analysis. Collection of samples for molecular biological analysis is addressed in items 4.4 through 4.9. The VOC and natural attenuation samples will be sent to the project laboratory for analysis. The molecular biological samples will be forwarded to Dr. Kirsti Ritalahti, at Georgia Tech (address below).

4.1 Ground water sampling may be initiated when the monitoring well has been purged and stabilized (Item 3.6).

4.2 Record the sample start time and the field measurements for pH, ORP, specific conductance, temperature, dissolved oxygen, and turbidity.

4.3 Disconnect the flow-through cell and fill the VOC, natural attenuation, and any other project required parameter sample containers. At each location, the VOC samples shall be collected first. See Table A for recommended VOC and natural attenuation analyses.

Samples for fixed-based analysis will be sent to the project-specific laboratory (not ESTCP project laboratory) for analysis.

4.4 Follow manufacturers' instructions and complete field analyses for dissolved oxygen, alkalinity, carbon dioxide, ferrous iron, and hydrogen sulfide using the field test kits. Record this information on the field log sheet.

After collection of all natural attenuation parameter samples, allow the pump to continue to run. At this time samples for molecular biological analysis can be collected. Based upon site specific planning documents, knowledge of historical turbidly levels, and communication with the ESTCP project team well surging may or may not be required prior to molecular biological sample collection. If surging is not required proceed to step 4.7. If surging is required proceed to Step 4.5

4.5 At the present time, insufficient information is available to determine if surging will increase sediment from the formation in the well versus sediment from the well sump. After the factors noted above are considered, if well surging is appropriate to increase turbidity in a given well follow Step 4.5 below.

Lower a disposable bailer into the well at the midpoint of the screen and move the bailer up and down in the water column. The purpose of this is to surge the well and increase the groundwater turbidity for molecular biological sample collection (only). It is important to agitate at the midpoint of the well screen as it this step is not intended to stir up sediment in the sump and/or the bottom of the well.

4.6 While continuing to surge the well with the bailer, re-connect the flow-through cell and record the field measurements for pH, ORP, specific conductance, temperature, dissolved oxygen, and turbidity. Continuing to surge the well with the bailer through step 4.7 and disconnect the flow-through cell.

4.7 If sample filtration is **not** planned (SOP A-2), fill the appropriate sample containers directly from the effluent end of the pump for molecular biology samples. Sample containers include two 1 liter plastic, unpreserved sample containers shall be filled for each well. Headspace in these containers must be minimized. If sample filtration is planned proceed to SOP A-2.

4.8 **These samples, collected for molecular biological analysis, shall be placed in a cooler (on ice to 4^{o}C) separate from the VOC and natural attenuation samples and shipped under separate chain-of-custody. That cooler should be shipped via overnight carrier to:**

Frank Loeffler, Ph.D.
Environmental Engineering
Georgia Institute of Technology
311 Ferst Drive, ES&T, Room 3228
Atlanta, GA 30332-0512
Phone: (404) 894-0279
Email: frank.loeffler@ce.gatech.edu

4.9 **Collect and ship all field log-sheets and chain of custody forms to the name and address listed below at the completion of the field-work:**

Chris Pike
Tetra Tech NUS, Inc.
661 Anderson Drive
Pittsburgh, PA 15220
Phone: 412-921-8861
Email: chris.pike@ttnus.com

5.0 ATTACHMENTS

Table A – Recommended VOC and Natural Attenuation Analytical Parameters
Attachment 1 – Example Low-Flow Purge Data Sheet
Attachment 2 – Example Groundwater Sample Log Sheet

6.0 REFERENCES

Parsons, et. al. 2004. Principles and Practices of Enhanced Anaerobic Bioremediation of Chlorinated Solvents, Prepared for Air Force Center for Environmental Excellence, Naval Facilities Engineering Service Center, and Environmental Security Technology Certification Program.

Puls, R.W. and M.J. Barcelona. 1996. Low-Flow (Minimal Drawdown) Ground-Water Sampling Procedures. EPA/540/S-95/504.

Wiedemeier, T.H., et al. 1998. Technical Protocol For Evaluating Natural Attenuation Of Chlorinated Solvents In Groundwater. EPA/600/R-98/128.

Table A
Recommended VOC and Natural Attenuation Analytical Parameter List[1]

Parameter	Laboratory/Field Analysis	Analytical or Field Screening Method
VOCs[2]	Laboratory[5]	SW846 8260B
Selected Ions[3]	Laboratory[5]	EPA 300
Dissolved gases[4]	Laboratory[5]	RSK SOP 147 & 175
Sulfide	Laboratory[5]	EPA 376.1
Total Organic Carbon	Laboratory[5]	EPA 415.1
Iron (total and dissolved)	Laboratory[5]	SW846 6010B
Volatile fatty acids	Laboratory[5,6]	lab specific SOP (e.g., AM23G)
Dissolved Oxygen	Field[7]	CHEMetrics: K-7501 and/or K-7512
Alkalinity	Field[7]	CHEMetrics: K-9810, K-9815, and/or K-9820
Carbon dioxide	Field[7]	CHEMetrics: K-1910, K-1920, and/or K-1925
Ferrous iron	Field[7]	HACH IR-18C or equivalent
Hydrogen sulfide	Field[7]	HACH-HS-C or equivalent
Conductivity, DO, ORP, pH, and temperature	Field[7]	Field meter (flow through cell)

NOTES:

1 – Samples should be collected using low flow sampling techniques as defined by Puls and Barcelona (1996) and in accordance with Weidermeier et al. (1998) and Parsons et al. (2004). Additional parameters including hydrogen (RSK SOP 147 & 175) and compound specific stable isotopes, etc. would also be helpful but are not necessary. Please provide all results to the ESTCP project team in electronic format (e.g., Microsoft Excel or database format) at the address listed below upon completion of analysis:

Mr. Chris Pike
Tetra Tech NUS, Inc.
661 Anderson Drive
Pittsburgh, PA 15220
Phone: 412-921-8146
Email: chris.pike@ttnus.com

2 – It is preferred that VOCs under SW846 8260B are reported however, at a minimum, PCE, TCE, cis-1,2-DCE, trans-1,2-DCE, 1,1-DCE, and VC are required.
3 – Selected ions include chloride, nitrate, nitrite, phosphate and sulfate.
4 – Dissolved gases include methane, ethene, and ethane.
5 – Sample containers for laboratory analyses are to be determined by the project laboratory utilized.
6 – Volatile fatty acids are only necessary for biostimulation and bioaugmentation projects (not monitoring natural attenuation projects).
7 – Tests should be conducted at the well head immediately after groundwater is extracted from the well. Follow manufacturers' instructions for exact field test procedures.

Tetra Tech NUS, Inc.

LOW FLOW PURGE DATA SHEET

PROJECT : _____ **WELL ID.:** _____
PROJECT NUMBER: _____ **DATE:** _____
PROJECT SITE NAME: _____ **WEATHER:** _____

Time (Hrs.)	Water Level (Ft. below TOC)	Volume (gal/L)	Flowrate (mL/min)	pH (S.U.)	Cond. (mS/cm)	Turb. (NTU)	DO (mg/L)	Temp. (Celsius)	ORP (mV)	Comments

Water Quality Meter (S/N) _____
Control Box Type (S/N) _____
Turbidity Meter (S/N) _____

Pump Intake Depth _____

SIGNATURE(S): _____

PAGE___OF___

ATTACHMENT 1
EXAMPLE LOW-FLOW PURGE DATA SHEET

ATTACHMENT 2

EXAMPLE GROUND WATER SAMPLE LOG SHEET

GROUND WATER SAMPLE LOG SHEET

Page___ of ___

Project Site Name: _____
Project No.: _____

[] Domestic Well Data
[] Monitoring Well Data
[] Other Well Type: _____
[] QA Sample Type: _____

Sample ID No.: _____
Sample Location: _____
Sampled By: _____
C.O.C. No.: _____
Type of Sample:
[] Low Concentration
[] High Concentration

SAMPLING DATA:

Date: / Time:	Color Visual	pH Standard	S.C. mS/cm	Temp. ^0C	Turbidity NTU	DO mg/l	TBD	TBD
Method:								

PURGE DATA:

Date:	Volume	pH	S.C.	Temp. (C)	Turbidity	DO	TBD	TBD
Method:								
Monitor Reading (ppm):								
Well Casing Diameter & Material Type:								
Total Well Depth (TD):								
Static Water Level (WL):								
One Casing Volume(gal/L):								
Start Purge (hrs):								
End Purge (hrs):								
Total Purge Time (min):								
Total Vol. Purged (gal/L):								

SAMPLE COLLECTION INFORMATION:

Analysis	Preservative	Container Requirements	Collected

OBSERVATIONS / NOTES:

Circle if Applicable:		Signature(s):
MS/MSD	Duplicate ID No.:	

TBD: To Be Determined

Appendix C-2: Molecular Biological Sample Collection Using Field Filters

APPENDIX C-2

STANDARD OPERATING PROCEDURE

MOLECULAR BIOLOGICAL SAMPLE COLLECTION USING FIELD FILTERS

1.0 INTRODUCTION

1.1 Purpose

This Standard Operating Procedure (SOP) establishes the procedure for microbial sampling using a filter to capture the well sediment in support of the ESTCP – Nucleic Acid-Based Tools demonstration.

1.2 General Procedure

The sampling technique discussed herein is used as an alternative to collection of water in a jar for molecular biological analyses as defined in groundwater purging and sampling SOP in Appendix A-1. This procedure should be used after completion of step 4.4 in the SOP in Appendix A-1.

2.0 REQUIRED SUPPLIES AND EQUIPMENT

This SOP requires that all supplies and equipment utilized for purging and sampling are available (see the SOP in Appendix A-1). In addition, several other items are required:

- A graduated cylinder to accurately measure total flow through the filter (minimum 1000 mL).
- In-line sampling filter. Specifically, the Sterivex™ sample filter units (www.millipore.com) is strongly preferred. This filter is also available from Microbial Insights (www.microbe.com) as the as Bio-Flo samplers. Typically, one filter will be required for each well sampled; however, additional filters should be taken to the site to accommodate unexpected problems.

3.0 PROCEDURES FOR SAMPLE FILTRATATION

3.1 Follow the procedures for purging and sampling. If low flow sampling is identified in the project specific planning documents then SOP Appendix A-1 should be sought.

3.2 After completion of sample collection for other parameters and the surging described in the SOP in Appendix A-1 (through step 4.4), attach the biological sampling filter to the end of the discharge sample tubing using a luer-lock adaptor. Place the graduated cylinder downstream of the filter to measure groundwater flow through the filter.

3.3 Begin pumping water through the filter. Allow the water to pass through the filter until it is clogged (i.e., until there is little or no flow through the filter). The volume required to clog the filter is highly dependent on the turbidity of the water, but is approximately 1 liter or less for relatively turbid water. Record the volume of water passed through the filter prior to clogging.

3.4 If the filter does not clog, pass a maximum of 3 liters of water through the filter. Record the volume of water passed through the filter.

3.5 Following completion of the sample collection, remove the filter from the tubing. Place the filter in a labeled falcon tube. Record the date and time the sample was collected, the volume of water filtered, sample identification, site name and installation on the falcon tube. Place the falcon tube with the filter in a sealed freezer Ziplock® or equivalent bag. The sealed bag should then be placed on ice.

3.6 A chain of custody associated with the samples should contain the same information as that on the falcon tube as well as clarifying comments. Record this information on the field logsheet along with other biogeochemical parameters as noted in SOP A-1.

3.7 Once the water that has passed through the filter has been recorded, the water can be discarded in accordance with project-specific requirements.

3.8 **These samples shall be placed in a cooler (on ice to 4^{o}C) and shipped with the chain of custody, via overnight mail to**:

Frank Loeffler, Ph.D.
Environmental Engineering
Georgia Institute of Technology
311 Ferst Drive, ES&T, Room 3228
Atlanta, GA 30332-0512
Phone: (404) 894-0279
Email: frank.loeffler@ce.gatech.edu

3.9 **Collect and ship all field logsheets and chain of custody forms to the name and address listed below at the completion of the field work:**

Chris Pike
Tetra Tech NUS, Inc.
661 Anderson Drive
Pittsburgh, PA 15220
Phone: 412`-921-8861
Email: chris.pike@ttnus.com

Appendix D: Statistical Summary

Operable Unit 1, Anniston Army Depot

Site: Anniston
Monitoring well: 01CGW07

sample date	TCE µg/L	cDCE µg/L	tDCE µg/L	VC µg/L	Ethene µg/L	Ethane µg/L	DHC gene copies/L
5/20/2005	57000	11000	97	160			
10/7/2005	14000	2200	16	20	1	1	1.98E+03
4/23/2006	5700	1300	5.1	11	1	1	5.78E+02
10/4/2006	35000	8400	34	94	1	3	4.03E+04
4/20/2007	26000	5000	10	72	1	1	1.17E+03
10/18/2007	460	240	10	20	19	23	7.50E+05

sample date	daughter Products (DCE+VC+eth)	daughter products to TCE Ratio	Daughter Products (VC+eth)	VC and ETH to DCE Ratio	ratio VC to DCE	daughter prod (DCE+VC)	ratio to TCE
5/20/2005	11257	0.197	160	0.014	0.014	11257	0.20
10/7/2005	2237	0.160	21	0.009	0.009	2236	0.16
4/23/2006	1317.1	0.231	12	0.009	0.008	1316.1	0.23
10/4/2006	8529	0.244	95	0.011	0.011	8528	0.24
4/20/2007	5083	0.196	73	0.015	0.014	5082	0.20
10/18/2007	289	0.628	39	0.156	0.080	270	0.59

ratio daughter products to TCE (DHC)

Rank Ratio	Rank DHC	D (Ratio-DHC)	D^2 (ratio - DHC)	Spearman correlation	correlation results
1	3	-2	4	0.60	Medium
3	1	2	4		
4	4	0	0		
2	2	0	0		
5	5	0	0		
		sum D^2	8		

ratio VC and ethene to DCE (DHC)

Ratio Rank	Rank DHC	D (Ratio-DHC)	D^2 (ratio - DHC)	Spearman correlation	correlation results
2	3	-1	1	0.70	Strong
1	1	0	0		
3	4	-1	1		
4	2	2	4		
5	5	0	0		
		sum D^2	6		

ratio VC to DCE (DHC)

Ratio Rank	Rank DHC	D (Ratio-DHC)	D^2 (ratio - DHC)	Spearman correlation	correlation results
2	3	-1	1	0.70	Strong
1	1	0	0		
3	4	-1	1		
4	2	2	4		
5	5	0	0		
		sum D^2	6		

ratio daughter prod (DCE+VC)

Rank Ratio	Rank DHC	D (Ratio-DHC)	D^2 (ratio - DHC)	Spearman correlation	correlation results
1	3	-2	4	0.60	Medium
3	1	2	4		
4	4	0	0		
2	2	0	0		
5	5	0	0		
		sum D^2	8		

Site: Anniston
Monitoring well: 82B06

sample date	TCE µg/L	cDCE µg/L	tDCE µg/L	VC µg/L	Ethene µg/L	Ethane µg/L	DHC gene copies/L
5/13/2005	360	68		0.5	0	0	
10/12/2005	220	55	5	10	1	1	1.39E+01
4/25/2006	250	52	2.5	5	1	1	1.07E+02
10/5/2006	280	850	5.6	4.8	1	1	1.82E+06
4/18/2007	310	63	7.9	0.5	1	1	5.21E+02
10/24/2007	200	400	4.9	0.5	1	1	2.34E+04

		daughter products to TCE		VC and ETH to DCE			ratio to TCE
sample date	daughter Products (DCE+VC+eth)	Ratio	Daughter Products (VC+eth)	Ratio	ratio VC to DCE	daughter prod (DCE+VC)	
5/13/2005	68.5	0.190	0.5	0.007	0.007	68.5	0.19
10/12/2005	71	0.323	11	0.183	0.167	70	0.32
4/25/2006	60.5	0.242	6	0.110	0.092	59.5	0.24
10/5/2006	861.4	3.076	5.8	0.007	0.006	860.4	3.07
4/18/2007	72.4	0.234	1.5	0.021	0.007	71.4	0.23
10/24/2007	406.4	2.032	1.5	0.004	0.001	405.4	2.03

ratio daughter products to TCE (DHC)

Rank Ratio	Rank DHC	D (Ratio-DHC)	D^2 (ratio - DHC)	Spearman correlation	correlation results
3	1	2	4	0.60	Medium
2	2	0	0		
5	5	0	0		
1	3	-2	4		
4	4	0	0		
		sum D^2	8		

ratio VC and ethene to DCE (DHC)

Ratio Rank	Rank DHC	D (Ratio-DHC)	D^2 (ratio - DHC)	Spearman correlation	correlation results
5	1	4	16	-0.45	Medium
4	2	2	4		
2	5	-3	9		
3	3	0	0		
1	4	-3	9		
		sum D^2	29		

ratio VC to DCE (DHC)

Ratio Rank	Rank DHC	D (Ratio-DHC)	D^2 (ratio - DHC)	Spearman correlation	correlation results
5	1	4	16	-0.45	Medium
4	2	2	4		
2	5	-3	9		
3	3	0	0		
1	4	-3	9		
		sum D^2	29		

ratio daughter prod (DCE+VC)

Rank Ratio	Rank DHC	D (Ratio-DHC)	D^2 (ratio - DHC)	Spearman correlation	correlation results
3	1	2	4	0.60	Medium
2	2	0	0		
5	5	0	0		
1	3	-2	4		
4	4	0	0		
		sum D^2	8		

Site: Anniston
Monitoring well: 81B027

sample date	TCE µg/L	cDCE µg/L	tDCE µg/L	VC µg/L	Ethene µg/L	Ethane µg/L	DHC gene copies/L
5/12/2005	75	11	5	0.5	0	5	
10/12/2005	50	9	2.5	10	1	1	7.67E+02
4/25/2006	89	16	5	2.5	1	1	2.46E+01
10/11/2006	170	2.2	0.5	1	1	1	3.02E+05
4/17/2007	89	7.1	0.5	0.5	1	1	7.76E+05
10/17/2007	250	3.6	0.5	0.5	1	1	2.31E+04

sample date	daughter products to TCE			VC and ETH to DCE			ratio to TCE
5/12/2005	daughter Products (DCE+VC+eth)	Ratio	Daughter Products (VC+eth)	Ratio	ratio VC to DCE	daughter prod (DCE+VC)	
10/12/2005	16.5	0.220	0.5	0.031	0.031	16.5	0.22
4/25/2006	22.5	0.450	11	0.957	0.870	21.5	0.43
10/11/2006	24.5	0.275	3.5	0.167	0.119	23.5	0.26
4/17/2007	4.7	0.028	2	0.741	0.370	3.7	0.02
10/17/2007	9.1	0.102	1.5	0.197	0.066	8.1	0.09
	5.6	0.022	1.5	0.366	0.122	4.6	0.02

ratio daughter products (DCE+VC+eth) to TCE (DHC)

Rank Ratio	Rank DHC	D (Ratio-DHC)	D^2 (ratio - DHC)	Spearman correlation	correlation results
5	2	3	9	-0.50	Medium
4	1	3	9		
2	4	-2	4		
3	5	-2	4		
1	3	-2	4		
		sum D^2	30		

ratio VC and ethene to DCE (DHC)

Ratio Rank	Rank DHC	D (Ratio-DHC)	D^2 (ratio - DHC)	Spearman correlation	correlation results
5	2	3	9	0.10	Weak
1	1	0	0		
4	4	0	0		
2	5	-3	9		
3	3	0	0		
		sum D^2	18		

ratio VC to DCE (DHC)

Ratio Rank	Rank DHC	D (Ratio-DHC)	D^2 (ratio - DHC)	Spearman correlation	correlation results
5	2	3	9	-0.30	Weak
2	1	1	1		
4	4	0	0		
1	5	-4	16		
3	3	0	0		
		sum D^2	26		

ratio daughter prod (DCE+VC)

Rank Ratio	Rank DHC	D (Ratio-DHC)	D^2 (ratio - DHC)	Spearman correlation	correlation results
5	2	3	9	-0.50	Medium
4	1	3	9		
2	4	-2	4		
3	5	-2	4		
1	3	-2	4		
		sum D^2	30		

NASA Cape Canaveral

Site: NASA Cape Canaveral
Monitoring well: SAMW02

SAMW02	TCE µg/L	cDCE µg/L	VC µg/L	Ethene µg/L	DHC gene copies/L	vcrA gene copies/L
8/10/2006	12700	10700	1710	54.3		
11/21/2006	10700	9910	1900	47.5		
1/30/2007	7940	7810	1210	40.8		
3/29/2007	7010	7670	1260	42.7	2.00E+07	3.00E+06
5/29/2007	3790	4020	2540	75.3	7.00E+07	1.00E+06
7/25/2007	3680	4980	5680	164	7.00E+08	7.00E+06
9/27/2007	0.19	0.63	5100	445	3.00E+07	4.00E+06
5/28/2008	0.46	0.1	69	116	8.00E+07	2.00E+07
7/24/2008	0.5	0.1	63.5	173	1.00E+07	6.00E+06
9/22/2008	0.16	0.1	71.1	66.4	5.00E+07	1.00E+06

SAMW02	sum cDCE, VC, ethene	ratio to TCE	m VC and ethe	ratio to cDCE	ratio VC to ethene
8/10/2006	-	-	-	-	-
11/21/2006	-	-	-	-	-
1/30/2007	-	-	-	-	-
3/29/2007	8972.7	1.3	1302.7	0.17	0.03
5/29/2007	6635.3	1.8	2615.3	0.65	0.03
7/25/2007	10824	2.9	5844	1.17	0.03
9/27/2007	5545.63	29187.5	5545	8801.59	0.09
5/28/2008	185.1	402.4	185	1850.00	1.68
7/24/2008	236.6	473.2	236.5	2365.00	2.72
9/22/2008	137.6	860.0	137.5	1375.00	0.93

TCE comparison

Rank TCE	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=6)
7	2	5	25	-0.04	Weak	0.886
6	5	1	1			no proof of correlation
5	7	-2	4			
2	3	-1	1			
3	6	-3	9			
4	1	3	9			
1	4	-3	9			
		sum D^2	58			

cDCE comparison

Rank cDCE	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=6)
7	2	5	25	-0.09	Weak	0.886
5	5	0	0			no proof of correlation
6	7	-1	1			
4	3	1	1			
1	6	-5	25			
1	1	0	0			
1	4	-3	9			
		sum D^2	61			

Site: NASA Cape Canaveral
Monitoring well: SAMW02

VC comparison

Rank VC	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=6)
4	2	2	4	0.46	Medium	0.886
5	5	0	0			no proof of correlation
7	7	0	0			
6	3	3	9			
2	6	-4	16			
1	1	0	0			
3	4	-1	1			
		sum D^2	30			

Ethene comparison

Rank Ethene	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=6)
1	2	-1	1	-0.04	Weak	0.886
3	5	-2	4			no proof of correlation
5	7	-2	4			
7	3	4	16			
4	6	-2	4			
6	1	5	25			
2	4	-2	4			
		sum D^2	58			

cDCE comparison

Rank cDCE	Rank vcrA	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=6)
7	3	4	16	-0.50	Medium	0.886
5	1	4	16			no proof of correlation
6	6	0	0			
4	4	0	0			
1	7	-6	36			
1	5	-4	16			
1	1	0	0			
		sum D^2	84			

VC comparison

Rank VC	Rank vcrA	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=6)
4	3	1	1	-0.20	Weak	0.886
5	1	4	16			no proof of correlation
7	6	1	1			
6	4	2	4			
2	7	-5	25			
1	5	-4	16			
3	1	2	4			
		sum D^2	67			

Site: NASA Cape Canaveral
Monitoring well: SAMW02
Ethene comparison

Rank Ethene	Rank vcrA	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=6)
1	3	-2	4	0.48	Medium	0.886
3	1	2	4			no proof of correlation
5	6	-1	1			
7	4	3	9			
4	7	-3	9			
6	5	1	1			
2	1	1	1			
		sum D^2	29			

Ratio of daughter products to TCE

Rank Ratio	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=6)
1	2	-1	1	-0.18	Weak	0.886
2	5	-3	9			no proof of correlation
3	7	-4	16			
7	3	4	16			
4	6	-2	4			
5	1	4	16			
6	4	2	4			
		sum D^2	66			

Ratio of daughter products to cDCE

Rank Ratio	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=6)
1	2	-1	1	-0.21	Weak	0.886
2	5	-3	9			no proof of correlation
3	7	-4	16			
7	3	4	16			
5	6	-1	1			
6	1	5	25			
4	4	0	0			
		sum D^2	68			

Ratio of ethene to VC

Rank Ratio	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=6)
3	2	1	1	-0.50	Medium	0.886
2	5	-3	9			no proof of correlation
1	7	-6	36			
4	3	1	1			
6	6	0	0			
7	1	6	36			
5	4	1	1			
		sum D^2	84			

Site: NASA Cape Canaveral

Monitoring well: SAMW02

Ratio of daughter products to cDCE

Rank Ratio	Rank vcrA	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=6)
1	3	-2	4	0.34	Medium	0.886
2	1	1	1			no proof of correlation
3	6	-3	9			
7	4	3	9			
5	7	-2	4			
6	5	1	1			
4	1	3	9			
		sum D^2	37			

Ratio of ethene to VC

Rank Ratio	Rank vcrA	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=6)
3	3	0	0	0.16	Weak	0.886
2	1	1	1			no proof of correlation
1	6	-5	25			
4	4	0	0			
6	7	-1	1			
7	5	2	4			
5	1	4	16			
		sum D^2	47			

Site: NASA Cape Canaveral
Monitoring well: SAMW03

SAMW03	TCE µg/L	cDCE µg/L	VC µg/L	Ethene µg/L	DHC gene copies/L	vcrA gene copies/L
8/10/2006	18900	21000	917	32.2		
11/21/2006	6210	9220	1310	20.7		
1/30/2007	5580	8750	694	27.4		
3/29/2007	4410	8110	1170	31.3	3.00E+07	1.00E+07
5/29/2007	2970	6270	1170	19.8	1.00E+07	2.00E+06
7/25/2007	2480	6410	1440	256	4.00E+08	3.00E+08
9/27/2007	1440	4200	1360	376	6.00E+07	2.00E+07
5/28/2008	0.16	0.25	67.7	477	7.00E+08	4.00E+08
7/24/2008	0.16	0.1	86.9	437	7.00E+07	9.00E+07
9/23/2008	0.16	0.1	83.9	129	5.00E+07	8.00E+06

SAMW03	sum cDCE, VC, ethene	ratio to TCE	sum VC and ethene	ratio to cDCE	ratio VC to ethene
8/10/2006	-	-	-	-	-
11/21/2006	-	-	-	-	-
1/30/2007	-	-	-	-	-
3/29/2007	9311.3	2.1	1201.3	0.15	0.03
5/29/2007	7459.8	2.5	1189.8	0.19	0.02
7/25/2007	8106	3.3	1696	0.26	0.18
9/27/2007	5936	4.1	1736	0.41	0.28
5/28/2008	544.95	3405.9	544.7	2178.80	7.05
7/24/2008	524	3275.0	523.9	5239.00	5.03
9/23/2008	213	1331.3	212.9	2129.00	1.54

TCE comparison

Rank TCE	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=6)
7	2	5	25	-0.911	Strong	0.886
6	1	5	25			correlation
5	6	-1	1			
4	4	0	0			
1	7	-6	36			
1	5	-4	16			
1	3	-2	4			
		sum D^2	107			

cDCE comparison

Rank cDCE	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=6)
7	2	5	25	-0.38	Medium	0.886
5	1	4	16			no proof of correlation
6	6	0	0			
4	4	0	0			
3	7	-4	16			
1	5	-4	16			
1	3	-2	4			
		sum D^2	77			

Site: NASA Cape Canaveral
Monitoring well: SAMW03

VC comparison

Rank VC	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=6)
4	2	2	4	-0.05	Weak	0.886
4	1	3	9			no proof of correlation
7	6	1	1			
6	4	2	4			
1	7	-6	36			
3	5	-2	4			
2	3	-1	1			
		sum D^2	59			

Ethene comparison

Rank Ethene	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=6)
2	2	0	0	0.893	Strong	0.886
1	1	0	0			correlation
4	6	-2	4			
5	4	1	1			
7	7	0	0			
6	5	1	1			
3	3	0	0			
		sum D^2	6			

cDCE comparison

Rank cDCE	Rank vcrA	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=6)
7	3	4	16	-0.16	Weak	0.886
5	1	4	16			no proof of correlation
6	6	0	0			
4	4	0	0			
3	7	-4	16			
1	5	-4	16			
1	2	-1	1			
		sum D^2	65			

VC comparison

Rank VC	Rank vcrA	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=6)
4	3	1	1	0.02	Weak	0.886
4	1	3	9			no proof of correlation
7	6	1	1			
6	4	2	4			
1	7	-6	36			
3	5	-2	4			
2	2	0	0			
		sum D^2	55			

Site: NASA Cape Canaveral
Monitoring well: SAMW03

Ethene comparison

Rank Ethene	Rank vcrA	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=6)
2	3	-1	1	0.857	Strong	0.886
1	1	0	0			no proof of correlation
4	6	-2	4			
5	4	1	1			
7	7	0	0			
6	5	1	1			
3	2	1	1			
		sum D^2	8			

Ratio of daughter products to TCE

Rank Ratio	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=6)
1	2	-1	1	0.714	Strong	0.886
2	1	1	1			no proof of correlation
3	6	-3	9			
4	4	0	0			
7	7	0	0			
6	5	1	1			
5	3	2	4			
		sum D^2	16			

Ratio of daughter products to cDCE

Rank Ratio	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=6)
1	2	-1	1	0.64	Medium	0.886
2	1	1	1			no proof of correlation
3	6	-3	9			
4	4	0	0			
6	7	-1	1			
7	5	2	4			
5	3	2	4			
		sum D^2	20			

Ratio of ethene to VC

Rank Ratio	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=6)
2	2	0	0	0.750	Strong	0.886
1	1	0	0			no proof of correlation
3	6	-3	9			
4	4	0	0			
7	7	0	0			
6	5	1	1			
5	3	2	4			
		sum D^2	14			

Site: NASA Cape Canaveral
Monitoring well: SAMW03

Ratio of daughter products to cDCE

Rank Ratio	Rank vcrA	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=6)
1	3	-2	4	0.50	Medium	0.886
2	1	1	1			no proof of correlation
3	6	-3	9			
4	4	0	0			
6	7	-1	1			
7	5	2	4			
5	2	3	9			
		sum D^2	28			

Ratio of ethene to VC

Rank Ratio	Rank vcrA	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=6)
2	3	-1	1	0.64	Medium	0.886
1	1	0	0			no proof of correlation
3	6	-3	9			
4	4	0	0			
7	7	0	0			
6	5	1	1			
5	2	3	9			
		sum D^2	20			

Site: NASA Cape Canaveral
Monitoring well: SAMW01

SAMW01	TCE µg/L	cDCE µg/L	VC µg/L	Ethene µg/L	Ethane µg/L	DHC gene copies/L	vcrA gene copies/L
8/10/2006	4170	8080	384	13.6		2.00E+07	2.00E+07
11/21/2006	5530	9770	654	13.3			
1/30/2007	3690	7620	277	11.1		5.00E+06	
3/29/2007	4210	6840	453	8.85			
5/29/2007	4530	9070	488	8.02			
7/25/2007	6830	11500	939	15.5			
9/27/2007	5200	8950	1930	23.6			
5/28/2008	1350	1960	1250	518		1.00E+09	2.00E+08
7/24/2008	2270	2980	1950	615		7.00E+08	8.00E+07
9/23/2008	3890	3730	2070	225		3.00E+07	1.00E+06

SAMW01	sum cDCE, VC, ethene	ratio to TCE	sum VC and ethene	ratio to cDCE	ratio VC to ethene
8/10/2006	8477.6	2.0	397.6	0.05	0.04
11/21/2006	10437.3	1.9	667.3	0.07	0.02
1/30/2007	7908.1	2.1	288.1	0.04	0.04
3/29/2007	7301.85	1.7	461.85	0.07	0.02
5/29/2007	9566.02	2.1	496.02	0.05	0.02
7/25/2007	12454.5	1.8	954.5	0.08	0.02
9/27/2007	10903.6	2.1	1953.6	0.22	0.01
5/28/2008	3728	2.8	1768	0.90	0.41
7/24/2008	5545	2.4	2565	0.86	0.32
9/23/2008	6025	1.5	2295	0.62	0.11

TCE comparison

Rank TCE	Rank DHC	D	D^2	Spearman Correlation	correlation results
5	2	3	9	-0.700	Strong
3	1	2	4		
1	5	-4	16		
2	4	-2	4		
4	3	1	1		
		sum D^2	34		

cDCE comparison

Rank cDCE	Rank DHC	D	D^2	Spearman Correlation	correlation results
5	2	3	9	-0.900	Strong
4	1	3	9		
1	5	-4	16		
2	4	-2	4		
3	3	0	0		
		sum D^2	38		

Site: NASA Cape Canaveral
Monitoring well: SAMW01

VC comparison

Rank VC	Rank DHC	D	D^2	Spearman Correlation	correlation results
2	2	0	0	0.60	Medium
1	1	0	0		
3	5	-2	4		
4	4	0	0		
5	3	2	4		
		sum D^2	8		

Ethene comparison

Rank Ethene	Rank DHC	D	D^2	Spearman Correlation	correlation results
2	2	0	0	0.60	Medium
1	1	0	0		
3	5	-2	4		
4	4	0	0		
5	3	2	4		
		sum D^2	8		

cDCE comparison

Rank cDCE	Rank vcrA	D	D^2	Spearman Correlation	correlation results
4	2	2	4	-0.800	Strong
1	4	-3	9		
2	3	-1	1		
3	1	2	4		
		sum D^2	18		

VC comparison

Rank VC	Rank vcrA	D	D^2	Spearman Correlation	correlation results
1	2	-1	1	-0.40	Medium
2	4	-2	4		
3	3	0	0		
4	1	3	9		
		sum D^2	14		

Ethene comparison

Rank Ethene	Rank vcrA	D	D^2	Spearman Correlation	correlation results
1	2	-1	1	0.60	Medium
3	4	-1	1		
4	3	1	1		
2	1	1	1		
		sum D^2	4		

Site: NASA Cape Canaveral
Monitoring well: SAMW01

Ratio of daughter products to cDCE

Rank Ratio	Rank DHC		D	D^2		Spearman Correlation	correlation results
2		2	0		0	1.000	Strong
1		1	0		0		
5		5	0		0		
4		4	0		0		
3		3	0		0		
			sum D^2		0		

Ratio of daughter products to TCE

Rank Ratio	Rank DHC		D	D^2		Spearman Correlation	correlation results
2		2	0		0	0.60	Medium
3		1	2		4		
5		5	0		0		
4		4	0		0		
1		3	-2		4		
			sum D^2		8		

Ratio of ethene to VC

Rank Ratio	Rank DHC		D	D^2		Spearman Correlation	correlation results
1		2	-1		1	0.900	Strong
2		1	1		1		
5		5	0		0		
4		4	0		0		
3		3	0		0		
			sum D^2		2		

Ratio of daughter products to cDCE

Rank Ratio	Rank DHC		D	D^2		Spearman Correlation	correlation results
1		2	-1		1	0.800	Strong
4		4	0		0		
3		3	0		0		
2		1	1		1		
			sum D^2		2		

Ratio of ethene to VC

Rank Ratio	Rank DHC		D	D^2		Spearman Correlation	correlation results
1		1	0		0	1.000	Strong
4		4	0		0		
3		3	0		0		
2		2	0		0		
			sum D^2		0		

Site: NASA Cape Canaveral

Monitoring well: Multiple: comparison to rate data

all data		Concentration (µg/L)					gene copies per liter			
Well	**date**	**TCE**	**cDCE**	**tDCE**	**VC**	**Ethene**	**DHC**	**VC Rdase**	**avg DHC**	**avg vcrA**
IW-14I	8/10/2006	1	14.4	4.3	173	4	2.0E+06	1.0E+06		
IW-14I	1/30/2007	0.5	0.59	0.5	7.4	2.9	2.0E+06			
SAMW01	8/10/2006	4170	8080	357	384	13.6	2.0E+07	2.0E+07		
SAMW01	1/30/2007	3690	7620	346	277	11.1	5.00E+06			
IW-14D	8/10/2006	24900	11200	405	1100	23.2	3.0E+06	1.0E+04	3.8E+06	1.2E+05
IW-14D	1/30/2007	819	1910	154	4080	88.2	5.0E+06			
IW-14D	3/29/2007	12.5	12.5	62.3	4900	90.4	6.0E+06	9.0E+04		
IW-14D	5/17/2007	2.6	4.5	51.4	1500	19.4	2.0E+06	4.0E+04		
IW-14D	7/25/2007	1.9	26.5	37.7	1430	38.6	7.0E+06	4.0E+05		
IW-14D	9/27/2007	11.3	25.9	7.4	519	19.9	7.0E+04	5.0E+04		
SAMW02	3/29/2007	7010	7670	293	1260	42.7	2.00E+07	3.00E+06	2.1E+08	3.8E+06
SAMW02	5/29/2007	3790	4020	247	2540	75.3	7.00E+07	1.00E+06		
SAMW02	7/25/2007	3680	4980	422	5680	164	7.00E+08	7.00E+06		
SAMW02	9/27/2007	0.19	0.63	67.4	5100	445	3.00E+07	4.00E+06		
SAMW03	3/29/2007	4410	8110	347	1170	31.3	3.00E+07	1.00E+07	1.25E+08	8.30E+07
SAMW03	5/29/2007	2970	6270	299	1170	19.8	1.00E+07	2.00E+06		
SAMW03	7/25/2007	2480	6410	342	1440	256	4.00E+08	3.00E+08		
SAMW03	9/27/2007	1440	4200	200	1360	376	6.00E+07	2.00E+07		

	First Order Rate Constants		
Well	**TCE rate**	**DCE rate**	**VC rate**
SAMW02	-0.3632	-0.2319	0.3016
SAMW03	-0.5776	-0.264	0.1265
IW14D	-3.85	-2.395	-0.8094

Well	**DHC**	**TCE rate**	**DHC rank**	**TCE rank**	**D**	**D^2**	**Spearman correlation**	**correlation results**
SAMW03	1.25E+08	-0.5776	2	2	0	0	1.00	Strong
SAMW02	2.1E+08	-0.3632	3	3	0	0		
IW14D	3.8E+06	-3.85	1	1	0	0		
					sum D^2	0		

Well	**DHC**	**DCE rate**	**DHC rank**	**DCE rank**	**D**	**D^2**	**Spearman correlation**	**correlation results**
SAMW03	1.25E+08	-0.264	2	2	0	0	1.00	Strong
SAMW02	2.1E+08	-0.2319	3	3	0	0		
IW14D	3.8E+06	-2.395	1	1	0	0		
					sum D^2	0		

Site: NASA Cape Canaveral

Monitoring well: Multiple: comparison to rate data

Well	vcrA	DCE	DHC rank	VC rank	D	D^2	Spearman correlation	correlation results
SAMW03	8.30E+07	-0.264	3	2	1	1	0.50	Medium
SAMW02	3.8E+06	-0.2319	2	3	-1	1		
IW14D	1.2E+05	-2.395	1	1	0	0		
					sum D^2	2		

Well	DHC	VC rate	DHC rank	VC rank	D	D^2	Spearman correlation	correlation results
SAMW03	1.25E+08	0.1265	2	2	0	0	1.00	Strong
SAMW02	2.1E+08	0.3016	3	3	0	0		
IW14D	3.8E+06	-0.8094	1	1	0	0		
					sum D^2	0		

Well	vcrA	VC rate	DHC rank	VC rank	D	D^2	Spearman correlation	correlation results
SAMW03	8.30E+07	0.1265	3	2	1	1	0.50	Medium
SAMW02	3.8E+06	0.3016	2	3	-1	1		
IW14D	1.2E+05	-0.8094	1	1	0	0		
					sum D^2	2		

Well	DHC	overall rate	DHC rank	rate rank	D	D^2	Spearman correlation	correlation results
SAMW03	1.25E+08	-0.2339	2	2	0	0	1.00	Strong
SAMW02	2.1E+08	-0.1587	3	3	0	0		
IW14D	3.8E+06	-0.8835	1	1	0	0		
					sum D^2	0		

Site: NASA Cape Canaveral

Monitoring well: All site wells - ratio calculations

all data

		Concentration (µg/L)				
Well	**date**	**TCE**	**cDCE**	**tDCE**	**VC**	**Ethene**
IW-14I	8/10/2006	1	14.4	4.3	173	4
IW-14I	1/30/2007	0.5	0.59	0.5	7.4	2.9
SAMW01	8/10/2006	4170	8080	357	384	13.6
SAMW01	1/30/2007	3690	7620	346	277	11.1
IW-14D	3/29/2007	12.5	12.5	62.3	4900	90.4
IW-14D	5/17/2007	2.6	4.5	51.4	1500	19.4
IW-14D	7/25/2007	1.9	26.5	37.7	1430	38.6
IW-14D	9/27/2007	11.3	25.9	7.4	519	19.9
SAMW02	3/29/2007	7010	7670	293	1260	42.7
SAMW02	5/29/2007	3790	4020	247	2540	75.3
SAMW02	7/25/2007	3680	4980	422	5680	164
SAMW03	3/29/2007	4410	8110	347	1170	31.3
SAMW03	5/29/2007	2970	6270	299	1170	19.8
SAMW03	7/25/2007	2480	6410	342	1440	256
SAMW03	9/27/2007	1440	4200	200	1360	376

			daughter products to TCE		VC and ETH to DCE	gene copies per liter		
Well	**date**	**daughter Products (DCE+VC+eth)**	**Ratio**	**Daughter Products (VC+eth)**	**Ratio**	**DHC**	**VC Rdase**	**% DHC**
IW-14I	8/10/2006	195.7	195.70	177	9.5	2.0E+06	1.0E+06	
IW-14I	1/30/2007	11.39	22.78	10.3	9.4	2.0E+06		
SAMW01	8/10/2006	8834.6	2.12	397.6	0.0	2.0E+07	2.0E+07	17
SAMW01	1/30/2007	8254.1	2.24	288.1	0.0	5.0E+06		27
IW-14D	3/29/2007	5065.2	405.22	4990.4	66.7	6.0E+06	9.0E+04	2
IW-14D	5/17/2007	1575.3	605.88	1519.4	27.2	2.0E+06	4.0E+04	0.2
IW-14D	7/25/2007	1532.8	806.74	1468.6	22.9	7.0E+06	4.0E+05	0.2
IW-14D	9/27/2007	572.2	50.64	538.9	16.2	7.0E+04	5.0E+04	0.015
SAMW02	3/29/2007	9265.7	1.32	1302.7	0.2	2.0E+07	3.0E+06	30
SAMW02	5/29/2007	6882.3	1.82	2615.3	0.6	7.0E+07	1.0E+06	29.5
SAMW02	7/25/2007	11246	3.06	5844	1.1	7.0E+08	7.0E+06	28
SAMW03	3/29/2007	9658.3	2.19	1201.3	0.1	3.0E+07	1.0E+07	48.5
SAMW03	5/29/2007	7758.8	2.61	1189.8	0.2	1.0E+07	2.0E+06	28
SAMW03	7/25/2007	8448	3.41	1696	0.3	4.0E+08	3.0E+08	55
SAMW03	9/27/2007	6136	4.26	1736	0.4	6.0E+07	2.0E+07	100

SAMW02 only

ratio daughter products to TCE (DHC)

Rank Ratio	**Rank DHC**	**D (Ratio-DHC)**	**D^2 (ratio - DHC)**	**Spearman correlation**	**correlation results**
1	1	0	0	1.0	Strong
2	2	0	0		
3	3	0	0		
		sum D^2	0		

SAMW02 only

ratio VC and ethene to DCE (vcrA)

Ratio Rank	**Rank VC Rdase**	**D (ratio - VC Rdase)**	**D^2 (ratio - VC Rdase)**	**Spearman correlation**	**correlation results**
1	2	-1	1	0.50	Medium
2	1	1	1		
3	3	0	0		
		sum D^2	2		

Site: NASA Cape Canaveral

Monitoring well: All site wells - ratio calculations

SAMW02 only

ratio VC and ethene to DCE (DHC)

Ratio Rank	Rank DHC	D (Ratio-DHC)	D^2 (ratio - DHC)	Spearman correlation	correlation results
1	1	0	0	1.0	Strong
2	2	0	0		
3	3	0	0		
		sum D^2	0		

14D Only

ratio daughter products to TCE (DHC)

Rank Ratio	Rank DHC	D (Ratio-DHC)	D^2 (ratio - DHC)	Spearman correlation	correlation results
2	3	-1	1	0.8	Strong
3	2	1	1		
4	4	0	0		
1	1	0	0		
		sum D^2	2		

14D Only

Rank Ratio	Rank %DHC	D (Ratio-DHC)	D^2 (ratio - DHC)	Spearman correlation	correlation results
2	4	-2	4	0.1	Weak
3	2	1	1		
4	2	2	4		
1	1	0	0		
		sum D^2	9		

14D Only

ratio VC and ethene to DCE (vcrA)

Ratio Rank	Rank VC Rdase	D (ratio - VC Rdase)	D^2 (ratio - VC Rdase)	Spearman correlation	correlation results
4	3	1	1	0.40	Medium
3	2	1	1		
2	4	-2	4		
1	1	0	0		
		sum D^2	6		

14D Only

ratio VC and ethene to DCE (DHC)

Ratio Rank	Rank DHC	D (Ratio-DHC)	D^2 (ratio - DHC)	Spearman correlation	correlation results
4	3	1	1	0.40	Medium
3	2	1	1		
2	4	-2	4		
1	1	0	0		
		sum D^2	6		

14D Only

Ratio Rank	Rank %DHC	D (Ratio-DHC)	D^2 (ratio - DHC)	Spearman correlation	correlation results
4	4	0	0	0.9	Strong
3	2	1	1		
2	2	0	0		
1	1	0	0		
		sum D^2	1		

Site: NASA Cape Canaveral

Monitoring well: All site wells - ratio calculations

SAMW03, SAMW02, and 14D
ratio daughter products to TCE (DHC)

Rank Ratio	Rank DHC	D (Ratio-DHC)	D^2 (ratio - DHC)	Spearman correlation	correlation results	r critical for p = 0.05 (n=10)
9	3	6	36	-0.55	Medium	0.648
10	2	8	64			no proof of correlation
11	4	7	49			
8	1	7	49			
1	6	-5	25			
2	9	-7	49			
5	11	-6	36			
3	7	-4	16			
4	5	-1	1			
6	10	-4	16			
7	8	-1	1			
		sum D^2	**342**			

SAMW03, SAMW02, and 14D

Rank Ratio	Rank %DHC	D (Ratio-DHC)	D^2 (ratio - DHC)	Spearman correlation	correlation results	r critical for p = 0.05 (n=10)
9	4	5	25	-0.645	Medium	0.648
10	2	8	64			no proof of correlation
11	2	9	81			
8	1	7	49			
1	8	-7	49			
2	7	-5	25			
5	5	0	0			
3	9	-6	36			
4	5	-1	1			
6	10	-4	16			
7	11	-4	16			
		sum D^2	362			

SAMW03, SAMW02, and 14D
ratio VC and ethene to DCE (vcrA)

Rank Ratio	Rank VC Rdase	D (ratio - VC Rdase)	D^2 (ratio - VC Rdase)	Spearman correlation	correlation results	r critical for p = 0.05 (n=10)
11	3	8	64	-0.7	Strong	0.648
10	1	9	81			correlation
9	4	5	25			
8	2	6	36			
2	7	-5	25			
6	5	1	1			
7	8	-1	1			
1	9	-8	64			
3	6	-3	9			
4	11	-7	49			
5	10	-5	25			
		sum D^2	**380**			

Site: NASA Cape Canaveral

Monitoring well: All site wells - ratio calculations

SAMW03, SAMW02, and 14D

ratio VC and ethene to DCE (DHC)

Rank Ratio	Rank DHC	D (Ratio-DHC)	D^2 (ratio - DHC)	Spearman correlation	correlation results	r critical for p = 0.05 (n=10)
11	3	8	64	-0.49	Medium	0.648
10	2	8	64			no proof of correlation
9	4	5	25			
8	1	7	49			
2	6	-4	16			
6	9	-3	9			
7	11	-4	16			
1	7	-6	36			
3	5	-2	4			
4	10	-6	36			
5	8	-3	9			
		sum D^2	**328**			

SAMW03, SAMW02, and 14D

Rank Ratio	Rank %DHC	D (Ratio-DHC)	D^2 (ratio - DHC)	Spearman correlation	correlation results	r critical for p = 0.05 (n=10)
11	4	7	49	-0.78	Strong	0.65
10	2	8	64			correlation
9	2	7	49			
8	1	7	49			
2	8	-6	36			
6	7	-1	1			
7	5	2	4			
1	9	-8	64			
3	5	-2	4			
4	10	-6	36			
5	11	-6	36			
		sum D^2	392			

SAMW01,02,03, IW14D

ratio daughter products to TCE (DHC)

Rank Ratio	Rank DHC	D (Ratio-DHC)	D^2 (ratio - DHC)	Spearman correlation	correlation results	r critical for p = 0.05 (n=12)
3	7	-4	16	-0.40	Medium	0.591
5	3	2	4			no proof of correlation
11	4	7	49			
12	2	10	100			
13	5	8	64			
10	1	9	81			
1	7	-6	36			
2	11	-9	81			
7	13	-6	36			
4	9	-5	25			
6	6	0	0			
8	12	-4	16			
9	10	-1	1			
		sum D^2	**509**			

Site: NASA Cape Canaveral

Monitoring well: All site wells - ratio calculations

SAMW01,02,03, IW14D

Rank Ratio	Rank %DHC	D (Ratio-DHC)	D^2 (ratio - DHC)	Spearman correlation	correlation results	r critical for p = 0.05 (n=12)
3	5	-2	4	-0.56	Medium	0.591
5	6	-1	1			no proof of correlation
11	4	7	49			
12	2	10	100			
13	2	11	121			
10	1	9	81			
1	10	-9	81			
2	9	-7	49			
7	7	0	0			
4	11	-7	49			
6	7	-1	1			
8	12	-4	16			
9	13	-4	16			
		sum D^2	568			

SAMW01,02,03, IW14D
ratio VC and ethene to DCE (vcrA)

Rank Ratio	Rank VC Rdase	D (ratio - VC Rdase)	D^2 (ratio - VC Rdase)	Spearman correlation	correlation results	r critical for p = 0.05 (n=12)
1	10	-9	81	-0.7	Strong	0.591
12	3	9	81			correlation
11	1	10	100			
10	4	6	36			
9	2	7	49			
3	7	-4	16			
7	5	2	4			
8	8	0	0			
2	9	-7	49			
4	6	-2	4			
5	12	-7	49			
6	10	-4	16			
		sum D^2	**485**			

SAMW01,02,03, IW14D
ratio VC and ethene to DCE (DHC)

Rank Ratio	Rank DHC	D (Ratio-DHC)	D^2 (ratio - DHC)	Spearman correlation	correlation results	r critical for p = 0.05 (n=12)
2	7	-5	25	-0.2	Weak	0.591
1	3	-2	4			no proof of correlation
13	4	9	81			
12	2	10	100			
11	5	6	36			
10	1	9	81			
4	7	-3	9			
8	11	-3	9			
9	13	-4	16			
3	9	-6	36			
5	6	-1	1			
6	12	-6	36			
7	10	-3	9			
		sum D^2	**443**			

Site: NASA Cape Canaveral

Monitoring well: All site wells - ratio calculations

SAMW01,02,03, IW14D

Rank Ratio	Rank %DHC	D (Ratio-DHC)	D^2 (ratio - DHC)	Spearman correlation	correlation results	r critical for p = 0.05 (n=12)
2	5	-3	9	-0.53	Medium	0.591
1	6	-5	25			no proof of correlation
13	4	9	81			
12	2	10	100			
11	2	9	81			
10	1	9	81			
4	10	-6	36			
8	9	-1	1			
9	7	2	4			
3	11	-8	64			
5	7	-2	4			
6	12	-6	36			
7	13	-6	36			
		sum D^2	558			

Site: NASA Cape Canaveral

Monitoring well: All site wells - individual VOC comparison

all data — Concentration (µg/L)

Well	date	TCE	cDCE	tDCE	DCE total	VC	Ethene	VC and ethene	DHC	VC Rdase	% DHC
IW-14I	8/10/2006	1	14.4	4.3	18.7	173	4	177	2.0E+06	1.0E+06	
IW-14I	1/30/2007	0.5	0.59	0.5	1.09	7.4	2.9	10.3	2.0E+06		
SAMW01	8/10/2006	4170	8080	357	8437	384	13.6	397.6	2.0E+07	2.0E+07	17
SAMW01	1/30/2007	3690	7620	346	7966	277	11.1	288.1	5.0E+06		27
IW-14D	3/29/2007	12.5	12.5	62.3	74.8	4900	90.4	4990.4	6.0E+06	9.0E+04	2
IW-14D	5/17/2007	2.6	4.5	51.4	55.9	1500	19.4	1519.4	2.0E+06	4.0E+04	0.2
IW-14D	7/25/2007	1.9	26.5	37.7	64.2	1430	38.6	1468.6	7.0E+06	4.0E+05	0.2
IW-14D	9/27/2007	11.3	25.9	7.4	33.3	519	19.9	538.9	7.0E+04	5.0E+04	0.015
SAMW02	3/29/2007	7010	7670	293	7963	1260	42.7	1302.7	2.0E+07	3.0E+06	30
SAMW02	5/29/2007	3790	4020	247	4267	2540	75.3	2615.3	7.0E+07	1.0E+06	29.5
SAMW02	7/25/2007	3680	4980	422	5402	5680	164	5844	7.0E+08	7.0E+06	28
SAMW03	3/29/2007	4410	8110	347	8457	1170	31.3	1201.3	3.0E+07	1.0E+07	48.5
SAMW03	5/29/2007	2970	6270	299	6569	1170	19.8	1189.8	1.0E+07	2.0E+06	28
SAMW03	7/25/2007	2480	6410	342	6752	1440	256	1696	4.0E+08	3.0E+08	55
SAMW03	9/27/2007	1440	4200	200	4400	1360	376	1736	6.0E+07	2.0E+07	100

IW14D
TCE comparison

Rank TCE	Rank DHC	D	D^2	Spearman Correlation	correlation results
4	3	1	1	-0.40	Medium
2	2	0	0		
1	4	-3	9		
3	1	2	4		
		sum D^2	14		

IW14D
cDCE comparison

Rank cDCE	Rank DHC	D	D^2	Spearman Correlation	correlation results
2	3	-1	1	0.40	Medium
1	2	-1	1		
4	4	0	0		
3	1	2	4		
		sum D^2	6		

IW14D
total DCE comparison

Rank total DCE	Rank DHC	D	D^2	Spearman Correlation	correlation results
4	3	1	1	0.80	Strong
2	2	0	0		
3	4	-1	1		
1	1	0	0		
		sum D^2	2		

IW14D
VC only comparison

Rank VC	Rank DHC	D	D^2	Spearman Correlation	correlation results
4	3	1	1	0.40	Medium
3	2	1	1		
2	4	-2	4		
1	1	0	0		
		sum D^2	6		

Site: NASA Cape Canaveral
Monitoring well: All site wells - individual VOC comparison

IW14D
VC only comparison

Rank VC	Rank vcrA	D	D^2	Spearman Correlation	correlation results
4	3	1	1	0.00	Weak
3	1	2	4		
2	4	-2	4		
1	2	-1	1		
		sum D^2	10		

IW14D
Eth only comparison

Rank Eth	Rank DHC	D	D^2	Spearman Correlation	correlation results
4	3	1	1	0.60	Medium
1	2	-1	1		
3	4	-1	1		
2	1	1	1		
		sum D^2	4		

IW14D
Eth only comparison

Rank Eth	Rank vcrA	D	D^2	Spearman Correlation	correlation results
4	3	1	1	0.80	Strong
1	1	0	0		
3	4	-1	1		
2	2	0	0		
		sum D^2	2		

IW14D
VC and ethene comparison

Rank VC and ETH	Rank DHC	D	D^2	Spearman Correlation	correlation results
4	3	1	1	0.40	Medium
3	2	1	1		
2	4	-2	4		
1	1	0	0		
		sum D^2	6		

IW14D
VC and ethene comparison

Rank VC and ETH	Rank vcrA	D	D^2	Spearman Correlation	correlation results
4	3	1	1	0.00	Weak
3	1	2	4		
2	4	-2	4		
1	2	-1	1		
		sum D^2	10		

Site: NASA Cape Canaveral

Monitoring well: All site wells - individual VOC comparison

SAMW02

TCE comparison

Rank TCE	Rank DHC	D	D^2	Spearman Correlation	correlation results
3	1	2	4	-1.00	Strong
2	2	0	0		
1	3	-2	4		
		sum D^2	8		

SAMW02

cDCE comparison

Rank cDCE	Rank DHC	D	D^2	Spearman Correlation	correlation results
3	1	2	4	-0.50	Medium
1	2	-1	1		
2	3	-1	1		
		sum D^2	6		

SAMW02

total DCE comparison

Rank total DCE	Rank DHC	D	D^2	Spearman Correlation	correlation results
3	1	2	4	-0.50	Medium
1	2	-1	1		
2	3	-1	1		
		sum D^2	6		

SAMW02

VC comparison

Rank VC	Rank DHC	D	D^2	Spearman Correlation	correlation results
1	1	0	0	1.00	Strong
2	2	0	0		
3	3	0	0		
		sum D^2	0		

SAMW02

VC comparison

Rank VC	Rank vcrA	D	D^2	Spearman Correlation	correlation results
1	2	-1	1	0.50	Medium
2	1	1	1		
3	3	0	0		
		sum D^2	2		

SAMW02

Eth comparison

Rank ETH	Rank DHC	D	D^2	Spearman Correlation	correlation results
1	1	0	0	1.00	Strong
2	2	0	0		
3	3	0	0		
		sum D^2	0		

Site: NASA Cape Canaveral

Monitoring well: All site wells - individual VOC comparison

SAMW02

Eth comparison

Rank ETH	Rank vcrA	D	D^2	Spearman Correlation	correlation results
1	2	-1	1	0.50	Medium
2	1	1	1		
3	3	0	0		
		sum D^2	2		

SAMW02

VC and ethene comparison

Rank VC and ETH	Rank DHC	D	D^2	Spearman Correlation	correlation results
1	1	0	0	1.00	Strong
2	2	0	0		
3	3	0	0		
		sum D^2	0		

SAMW02

VC and ethene comparison

Rank VC and ETH	Rank vcrA	D	D^2	Spearman Correlation	correlation results
1	2	-1	1	0.50	Medium
2	1	1	1		
3	3	0	0		
		sum D^2	2		

SAMW03, SAMW02, and 14D

TCE comparison

Rank TCE	Rank DHC	D	D^2	Spearman Correlation	correlation results	r for p = 0.05 (n=10)	Rank DHC %	D	D^2	Spearman Correlation	correlation results	r for p = 0.05 (n=10)
4	3	1	1	0.61	Medium	0.648	4	0	0	0.62	Medium	0.648
2	2	0	0			no proof of correlation	2	0	0			no proof of correlation
1	4	-3	9				2	-1	1			
3	1	2	4				1	2	4			
11	6	5	25				8	3	9			
9	9	0	0				7	2	4			
8	11	-3	9				5	3	9			
10	7	3	9				9	1	1			
7	5	2	4				5	2	4			
6	10	-4	16				10	-4	16			
5	8	-3	9				11	-6	36			
		sum D^2	86					**sum D^2**	84			

Site: NASA Cape Canaveral

Monitoring well: All site wells - individual VOC comparison

SAMW03, SAMW02, and 14D
cDCE comparison

Rank cDCE	Rank DHC	D	D^2	Spearman Correlation	correlation results	r for p = 0.05 (n=10)	Rank DHC %	D	D^2	Spearman Correlation	correlation results	r for p = 0.05 (n=10)
2	3	-1	1	0.62	Medium	0.648	4	-2	4	0.71	Strong	0.648
1	2	-1	1			no proof of correlation	2	-1	1			correlation
4	4	0	0				2	2	4			
3	1	2	4				1	2	4			
10	6	4	16				8	2	4			
5	9	-4	16				7	-2	4			
7	11	-4	16				5	2	4			
11	7	4	16				9	2	4			
8	5	3	9				5	3	9			
9	10	-1	1				10	-1	1			
6	8	-2	4				11	-5	25			
		sum D^2	84					**sum D^2**	64			

SAMW03, SAMW02, and 14D
total DCE comparison

Rank total DCE	Rank DHC	D	D^2	Spearman Correlation	correlation results	r for p = 0.05 (n=10)	Rank DHC %	D	D^2	Spearman Correlation	correlation results	r for p = 0.05 (n=10)
4	3	1	1	0.64	Medium	0.648	4	0	0	0.76	Strong	0.648
2	2	0	0			no proof of correlation	2	0	0			correlation
3	4	-1	1				2	1	1			
1	1	0	0				1	0	0			
10	6	4	16				8	2	4			
5	9	-4	16				7	-2	4			
7	11	-4	16				5	2	4			
11	7	4	16				9	2	4			
8	5	3	9				5	3	9			
9	10	-1	1				10	-1	1			
6	8	-2	4				11	-5	25			
		sum D^2	80					**sum D^2**	52			

SAMW03, SAMW02, and 14D
VC comparison

Rank VC	Rank DHC	D	D^2	Spearman Correlation	correlation results	r for p = 0.05 (n=10)	Rank DHC %	D	D^2	Spearman Correlation	correlation results	r for p = 0.05 (n=10)
10	3	7	49	0.34	Medium	0.648	4	6	36	-0.12	Weak	0.648
8	2	6	36			no proof of correlation	2	6	36			no proof of correlation
6	4	2	4				2	4	16			
1	1	0	0				1	0	0			
4	6	-2	4				8	-4	16			
9	9	0	0				7	2	4			
11	11	0	0				5	6	36			
2	7	-5	25				9	-7	49			
2	5	-3	9				5	-3	9			
7	10	-3	9				10	-3	9			
5	8	-3	9				11	-6	36			
		sum D^2	145					**sum D^2**	247			

Site: NASA Cape Canaveral

Monitoring well: All site wells - individual VOC comparison

SAMW03, SAMW02, and 14D
VC comparison

Rank VC	Rank vcrA	D	D^2	Spearman Correlation	correlation results	r for p = 0.05 (n=10)
10	3	7	49	-0.10	Weak	0.648
8	1	7	49			no proof of correlation
6	4	2	4			
1	2	-1	1			
4	7	-3	9			
9	5	4	16			
11	8	3	9			
2	9	-7	49			
2	6	-4	16			
7	11	-4	16			
5	10	-5	25			
		sum D^2	243			

SAMW03, SAMW02, and 14D
ETH comparison

Rank ETH	Rank DHC	D	D^2	Spearman Correlation	correlation results	r for p = 0.05 (n=10)	Rank DHC %	D	D^2	Spearman Correlation	correlation results	r for p = 0.05 (n=10)
8	3	5	25	0.70	Strong	0.648	4	4	16	0.62	Medium	0.648
1	2	-1	1			correlation	2	-1	1			no proof of correlation
5	4	1	1				2	3	9			
3	1	2	4				1	2	4			
6	6	0	0				8	-2	4			
7	9	-2	4				7	0	0			
9	11	-2	4				5	4	16			
4	7	-3	9				9	-5	25			
2	5	-3	9				5	-3	9			
10	10	0	0				10	0	0			
11	8	3	9				11	0	0			
		sum D^2	66					**sum D^2**	84			

SAMW03, SAMW02, and 14D
ETH comparison

Rank ETH	Rank vcrA	D	D^2	Spearman Correlation	correlation results	r for p = 0.05 (n=10)
8	3	5	25	0.65	Medium	0.648
1	1	0	0			no proof of correlation
5	4	1	1			
3	2	1	1			
6	7	-1	1			
7	5	2	4			
9	8	1	1			
4	9	-5	25			
2	6	-4	16			
10	11	-1	1			
11	10	1	1			
		sum D^2	76			

Site: NASA Cape Canaveral
Monitoring well: All site wells - individual VOC comparison

SAMW03, SAMW02, and 14D
VC and ethene comparison

Rank VC and ETH	Rank DHC	D	D^2	Spearman Correlation	correlation results	r for p = 0.05 (n=10)	Rank DHC %	D	D^2	Spearman Correlation	correlation results	r for p = 0.05 (n=10)
10	3	7	49	0.53	Medium	0.648	4	6	36	0.18	Weak	0.648
6	2	4	16			no proof of correlation	2	4	16			no proof of correlation
5	4	1	1				2	3	9			
1	1	0	0				1	0	0			
4	6	-2	4				8	-4	16			
9	9	0	0				7	2	4			
11	11	0	0				5	6	36			
3	7	-4	16				9	-6	36			
2	5	-3	9				5	-3	9			
7	10	-3	9				10	-3	9			
8	8	0	0				11	-3	9			
		sum D^2	104					**sum D^2**	180			

SAMW03, SAMW02, and 14D
VC and ethene comparison

Rank VC and ETH	Rank vcrA	D	D^2	Spearman Correlation	correlation results	r for p = 0.05 (n=10)
10	3	7	49	0.17	Weak	0.648
6	1	5	25			no proof of correlation
5	4	1	1			
1	2	-1	1			
4	7	-3	9			
9	5	4	16			
11	8	3	9			
3	9	-6	36			
2	6	-4	16			
7	11	-4	16			
8	10	-2	4			
		sum D^2	182			

SAMW01, SAMW03, SAMW02, and 14D
TCE comparison

Rank TCE	Rank DHC	D	D^2	Spearman Correlation	correlation results	r for p = 0.05 (n=12)	Rank DHC %	D	D^2	Spearman Correlation	correlation results	r for p = 0.05 (n=12)
11	7	4	16	0.44	Medium	0.591	5	6	36	0.55	Medium	0.591
9	3	6	36			no proof of correlation	6	3	9			no proof of correlation
4	4	0	0				4	0	0			
2	2	0	0				2	0	0			
1	5	-4	16				2	-1	1			
3	1	2	4				1	2	4			
13	7	6	36				10	3	9			
10	11	-1	1				9	1	1			
8	13	-5	25				7	1	1			
12	9	3	9				11	1	1			
7	6	1	1				7	0	0			
6	12	-6	36				12	-6	36			
5	10	-5	25				13	-8	64			
		sum D^2	205					**sum D^2**	162			

Site: NASA Cape Canaveral
Monitoring well: All site wells - individual VOC comparison

SAMW01, SAMW03, SAMW02, and 14D
cDCE comparison

Rank cDCE	Rank DHC	D	D^2	Spearman Correlation	correlation results	r for p = 0.05 (n=12)	Rank DHC %	D	D^2	Spearman Correlation	correlation results	r for p = 0.05 (n=12)
12	7	5	25	0.40	Medium	0.591	5	7	49	0.57	Medium	0.591
10	3	7	49			no proof of correlation	6	4	16			no proof of correlation
2	4	-2	4				4	-2	4			
1	2	-1	1				2	-1	1			
4	5	-1	1				2	2	4			
3	1	2	4				1	2	4			
11	7	4	16				10	1	1			
5	11	-6	36				9	-4	16			
7	13	-6	36				7	0	0			
13	9	4	16				11	2	4			
8	6	2	4				7	1	1			
9	12	-3	9				12	-3	9			
6	10	-4	16				13	-7	49			
		sum D^2	217					**sum D^2**	158			

SAMW01, SAMW03, SAMW02, and 14D
total DCE comparison

Rank total DCE	Rank DHC	D	D^2	Spearman Correlation	correlation results	r for p = 0.05 (n=12)	Rank DHC %	D	D^2	Spearman Correlation	correlation results	r for p = 0.05 (n=12)
12	7	5	25	0.40	Medium	0.591	5	7	49	0.58	Medium	0.591
11	3	8	64			no proof of correlation	6	5	25			no proof of correlation
4	4	0	0				4	0	0			
2	2	0	0				2	0	0			
3	5	-2	4				2	1	1			
1	1	0	0				1	0	0			
10	7	3	9				10	0	0			
5	11	-6	36				9	-4	16			
7	13	-6	36				7	0	0			
13	9	4	16				11	2	4			
8	6	2	4				7	1	1			
9	12	-3	9				12	-3	9			
6	10	-4	16				13	-7	49			
		sum D^2	219					**sum D^2**	154			

SAMW01, SAMW03, SAMW02, and 14D
VC comparison

Rank VC	Rank DHC	D	D^2	Spearman Correlation	correlation results	r for p = 0.05 (n=12)	Rank DHC %	D	D^2	Spearman Correlation	correlation results	r for p = 0.05 (n=12)
2	7	-5	25	0.40	Medium	0.591	5	-3	9	0.01	Weak	0.591
1	3	-2	4			no proof of correlation	6	-5	25			no proof of correlation
12	4	8	64				4	8	64			
10	2	8	64				2	8	64			
8	5	3	9				2	6	36			
3	1	2	4				1	2	4			
6	7	-1	1				10	-4	16			
11	11	0	0				9	2	4			
13	13	0	0				7	6	36			
4	9	-5	25				11	-7	49			
4	6	-2	4				7	-3	9			
9	12	-3	9				12	-3	9			
7	10	-3	9				13	-6	36			
		sum D^2	218					**sum D^2**	361			

Site: NASA Cape Canaveral
Monitoring well: All site wells - individual VOC comparison

SAMW01, SAMW03, SAMW02, and 14D
VC comparison

Rank VC	Rank vcrA	D	D^2	Spearman Correlation	correlation results	r for p = 0.05 (n=12)
1	10	-9	81	-0.19	Weak	0.591
11	3	8	64			no proof of correlation
9	1	8	64			
7	4	3	9			
2	2	0	0			
5	7	-2	4			
10	5	5	25			
12	8	4	16			
3	9	-6	36			
3	6	-3	9			
8	12	-4	16			
6	10	-4	16			
		sum D^2	340			

SAMW01, SAMW03, SAMW02, and 14D
ETH comparison

Rank ETH	Rank DHC	D	D^2	Spearman Correlation	correlation results	r for p = 0.05 (n=12)	Rank DHC %	D	D^2	Spearman Correlation	correlation results	r for p = 0.05 (n=12)
2	7	-5	25	0.68	Strong	0.591	5	-3	9	0.54	Medium	0.591
1	3	-2	4			correlation	6	-5	25			no proof of correlation
10	4	6	36				4	6	36			
3	2	1	1				2	1	1			
7	5	2	4				2	5	25			
5	1	4	16				1	4	16			
8	7	1	1				10	-2	4			
9	11	-2	4				9	0	0			
11	13	-2	4				7	4	16			
6	9	-3	9				11	-5	25			
4	6	-2	4				7	-3	9			
12	12	0	0				12	0	0			
13	10	3	9				13	0	0			
		sum D^2	117					**sum D^2**	166			

SAMW01, SAMW03, SAMW02, and 14D
ETH comparison

Rank ETH	Rank vcrA	D	D^2	Spearman Correlation	correlation results	r for p = 0.05 (n=12)
1	10	-9	81	0.41	Medium	0.591
9	3	6	36			no proof of correlation
2	1	1	1			
6	4	2	4			
4	2	2	4			
7	7	0	0			
8	5	3	9			
10	8	2	4			
5	9	-4	16			
3	6	-3	9			
11	12	-1	1			
12	10	2	4			
		sum D^2	169			

Site: NASA Cape Canaveral
Monitoring well: All site wells - individual VOC comparison

SAMW01, SAMW03, SAMW02, and 14D
VC and ethene comparison

Rank VC and ETH	Rank DHC	D	D^2	Spearman Correlation	correlation results	r for p = 0.05 (n=12)
2	7	-5	25	0.54	Medium	0.591
1	3	-2	4			no proof of correlation
12	4	8	64			
8	2	6	36			
7	5	2	4			
3	1	2	4			
6	7	-1	1			
11	11	0	0			
13	13	0	0			
5	9	-4	16			
4	6	-2	4			
9	12	-3	9			
10	10	0	0			
		sum D^2	167			

Rank DHC %	D	D^2	Spearman Correlation	correlation results	r for p = 0.05 (n=12)
5	-3	9	0.23	Weak	0.591
6	-5	25			no proof of correlation
4	8	64			
2	6	36			
2	5	25			
1	2	4			
10	-4	16			
9	2	4			
7	6	36			
11	-6	36			
7	-3	9			
12	-3	9			
13	-3	9			
	sum D^2	282			

SAMW01, SAMW03, SAMW02, and 14D
VC and ethene comparison

Rank VC and ETH	Rank vcrA	D	D^2	Spearman Correlation	correlation results	r for p = 0.05 (n=12)
1	10	-9	81	0.02	Weak	0.591
11	3	8	64			no proof of correlation
7	1	6	36			
6	4	2	4			
2	2	0	0			
5	7	-2	4			
10	5	5	25			
12	8	4	16			
4	9	-5	25			
3	6	-3	9			
8	12	-4	16			
9	10	-1	1			
		sum D^2	281			

OU 24 NAS North Island, Coronado, CA

Site: OU24 NAS North Island

Monitoring well: 653-MW-02

non detect

653-MW-02	TCE µg/L	cDCE µg/L	VC µg/L	Ethene µg/L	Ethane µg/L	DHC gene copies/L	vcrA gene copies/L	tceA gene copies/L
3-Apr-07	2.5	1,200	850	24	1	2.00E+03	NA	NA
7-Aug-07	0.27	160	220	6.5	1	5.91E+02	8.82E+02	8.82E+02
18-Feb-08	0.5	110	270	27	8.6	9.12E+03	2.13E+04	2.13E+04
8-May-08	0.5	32	380	21	7.3	1.05E+06	2.77E+06	2.77E+06

653-MW-02	n VC and eth	ratio to cDCE	ratio to ethene to VC
3-Apr-07			
7-Aug-07	874	0.7	0.028
18-Feb-08	226.5	1.4	0.030
8-May-08	297	2.7	0.100
	401	12.5	0.055

cDCE comparison

Rank cDCE	Rank DHC	D	D^2	Spearman Correlation	correlation results
4	2	2	4	-0.80	Strong
3	1	2	4		
2	3	-1	1		
1	4	-3	9		
		sum D^2	18		

VC comparison

Rank VC	Rank DHC	D	D^2	Spearman Correlation	correlation results
4	2	2	4	0.40	Medium
1	1	0	0		
2	3	-1	1		
3	4	-1	1		
		sum D^2	6		

Ethene comparison

Rank Ethene	Rank DHC	D	D^2	Spearman Correlation	correlation results
3	2	1	1	0.40	Medium
1	1	0	0		
4	3	1	1		
2	4	-2	4		
		sum D^2	6		

Site: OU24 NAS North Island
Monitoring well: 653-MW-02

cDCE comparison

Rank cDCE	Rank vcrA	D	D^2	Spearman Correlation	correlation results
3	1	2	4	-1.00	Strong
2	2	0	0		
1	3	-2	4		
		sum D^2	8		

VC comparison

Rank VC	Rank vcrA	D	D^2	Spearman Correlation	correlation results
1	1	0	0	1.00	Strong
2	2	0	0		
3	3	0	0		
		sum D^2	0		

Ethene comparison

Rank Ethene	Rank vcrA	D	D^2	Spearman Correlation	correlation results
1	1	0	0	0.50	Medium
3	2	1	1		
2	3	-1	1		
		sum D^2	2		

Ratio of Ethene to VC

Rank Ratio	Rank DHC	D	D^2	Spearman Correlation	correlation results
1	2	-1	1	0.60	Medium
2	1	1	1		
4	3	1	1		
3	4	-1	1		
		sum D^2	4		

Ratio of daughter products to cDCE

Rank Ratio	Rank DHC	D	D^2	Spearman Correlation	correlation results
1	2	-1	1	0.80	Strong
2	1	1	1		
3	3	0	0		
4	4	0	0		
		sum D^2	2		

Site: OU24 NAS North Island
Monitoring well: 653-MW-02

Ratio of Ethene to VC

Rank Ratio	Rank vcrA	D	D^2	Spearman Correlation	correlation results
1	1	0	0	0.50	Medium
3	2	1	1		
2	3	-1	1		
		sum D^2	2		

Ratio of daughter products to cDCE

Rank Ratio	Rank vcrA	D	D^2	Spearman Correlation	correlation results
1	1	0	0	1.00	Strong
2	2	0	0		
3	3	0	0		
		sum D^2	0		

Site: OU24 NAS North Island
Monitoring well: 653-MW-12

non detect

653-MW-12	TCE µg/L	cDCE µg/L	VC µg/L	Ethene µg/L	Ethane µg/L	DHC gene copies/L
3-Apr-07	7.5	72	77	0.67	1	2.00E+03
1-Aug-07	0.21	73	74	0.88	1	2.00E+03
28-Nov-07	0.47	110	111	1.3	1	3.87E+03
13-Feb-08	6.3	16	21	1	1	9.39E+02
6-May-08	0.29	34	35	1	1	9.14E+03

653-MW-12	sum VC and ethene	ratio to cDCE	ratio to ethene to VC
3-Apr-07			
1-Aug-07	77.67	1.08	0.009
28-Nov-07	74.88	1.0	0.012
13-Feb-08	112.3	1.0	0.012
6-May-08	22	1.4	0.048
	36	1.1	0.029

cDCE comparison

Rank cDCE	Rank DHC	D	D^2	Spearman Correlation	correlation results
3	2	1	1	0.25	Weak
4	2	2	4		
5	4	1	1		
1	1	0	0		
2	5	-3	9		
		sum D^2	15		

VC comparison

Rank VC	Rank DHC	D	D^2	Spearman Correlation	correlation results
4	2	2	4	0.25	Weak
3	2	1	1		
5	4	1	1		
1	1	0	0		
2	5	-3	9		
		sum D^2	15		

Ratio of Ethene to VC

Rank Ratio	Rank DHC	D	D^2	Spearman Correlation	correlation results
1	2	-1	1	-0.15	Weak
3	2	1	1		
2	4	-2	4		
5	1	4	16		
4	5	-1	1		
		sum D^2	23		

Ratio of daughter products to cDCE

Rank Ratio	Rank DHC	D	D^2	Spearman Correlation	correlation results
4	2	2	4	-0.65	Medium
2	2	0	0		
1	4	-3	9		
5	1	4	16		
3	5	-2	4		
		sum D^2	33		

Milledgeville

Site: Milledgeville
Monitoring well: MW07

Date	PCE µg/L	TCE µg/L	cDCE µg/L	VC µg/L	Ethene µg/L	Dhc gene copies per liter	Stdev	bvcA gene copies per liter	Stdev	vcrA gene copies per liter	Stdev	tceA gene copies per liter	Stdev
7-Jan-05	17	14	6900	25		1.5E+03	2.4E+03	3.7E+03	2.0E+03	2.4E+02	2.5E+02	2.2E+04	1.8E+04
21-Jan-05	34	28	2400	4000		2.5E+08	2.3E+07	6.0E+05	9.4E+04	4.6E+04	3.0E+04	3.8E+08	5.5E+07
7-Feb-05	17	29	370	5000	48	2.7E+08	5.8E+07	2.3E+06	1.2E+06	7.1E+06	1.3E+06	3.7E+08	1.6E+08
15-Mar-05	1.7	4.6	4.2	400	0.33	2.7E+08	3.9E+07	1.5E+06	1.6E+05	1.3E+08	8.1E+06	8.7E+07	6.3E+06
29-Apr-05	1.4	1.1	2.6	360	120	1.4E+08	1.3E+07	3.1E+05	7.2E+04	1.3E+08	1.4E+07	2.1E+07	1.0E+07
1-Jul-05	1.7	9	12	110	200	1.6E+09	4.0E+08	4.9E+05	1.3E+05	8.9E+08	1.4E+08	1.5E+08	2.8E+07
29-Jul-05	0.34	3.1	3.9	94	74	3.0E+08	8.2E+07	1.4E+05	3.7E+04	5.8E+07	5.1E+07	2.5E+07	5.9E+06
29-Oct-05	6.8	260	4500	790	36	1.6E+07	4.4E+06	1.2E+06	1.6E+05	1.1E+07	1.0E+06	7.7E+06	1.4E+04
13-Apr-06	0.34	2.2	18	220	299	2.0E+08	9.3E+07	4.9E+06	2.3E+06	4.4E+07	1.4E+07	6.1E+07	2.0E+07
30-Oct-06	0.5	5.6	110	60		1.7E+06	6.5E+05	7.0E+04	3.3E+04	5.9E+06	1.5E+06	1.5E+06	6.3E+05
30-Apr-07	0.5	5.3	140	54	10.9	1.8E+07	2.3E+07	2.0E+05	1.5E+05	3.1E+06	1.9E+06	1.4E+06	1.1E+06

PCE, TCE, cDCE, VC, ethene to DHC
PCE comparison

Rank PCE	Rank DHC	D	D^2	Spearman correlation	correlation results	critical value n=10 (p=0.05)
9	1	8	64	-0.10	Weak	0.648
11	7	4	16			no proof of correlation
9	9	0	0			
6	8	-2	4			
5	5	0	0			
6	11	-5	25			
1	10	-9	81			
8	3	5	25			
1	6	-5	25			
3	2	1	1			
3	4	-1	1			
	sum D2		242			

TCE comparison

Rank TCE	Rank DHC	D	D^2	Spearman correlation	correlation results	critical value n=10 (p=0.05)
8	1	7	49	-0.13	Weak	0.648
9	7	2	4			no proof of correlation
10	9	1	1			
4	8	-4	16			
1	5	-4	16			
7	11	-4	16			
3	10	-7	49			
11	3	8	64			
2	6	-4	16			
6	2	4	16			
5	4	1	1			
	sum D^2		248			

cDCE, VC, ethene to bvcA
cDCE comparison

Rank cDCE	Rank bvcA	D	D^2	Spearman correlation	correlation results	critical value n=10 (p=0.05)
11	1	10	100	-0.05	Weak	0.648
9	7	2	4			no proof of correlation
8	10	-2	4			
3	9	-6	36			
1	5	-4	16			
4	6	-2	4			
2	3	-1	1			
10	8	2	4			
5	11	-6	36			
6	2	4	16			
7	4	3	9			
	sum D^2		230			

VC comparison

Rank VC	Rank bvcA	D	D^2	Spearman correlation	correlation results	critical value n=10 (p=0.05)
1	1	0	0	0.78	Strong	0.648
10	7	3	9			**correlation**
11	10	1	1			
8	9	-1	1			
7	5	2	4			
5	6	-1	1			
4	3	1	1			
9	8	1	1			
6	11	-5	25			
3	2	1	1			
2	4	-2	4			
	sum D^2		48			

Site: Milledgeville
Monitoring well: MW07

cDCE comparison

Rank cDCE	Rank DHC	D	D^2	Spearman correlation	correlation results	critical value n=10 (p=0.05)
11	1	10	100	-0.52	Medium	0.648
9	7	2	4			no proof of correlation
8	9	-1	1			
3	8	-5	25			
1	5	-4	16			
4	11	-7	49			
2	10	-8	64			
10	3	7	49			
5	6	-1	1			
6	2	4	16			
7	4	3	9			
		sum D^2	334			

VC comparison

Rank VC	Rank DHC	D	D^2	Spearman correlation	correlation results	critical value n=10 (p=0.05)
1	1	0	0	0.41	Medium	0.648
10	7	3	9			no proof of correlation
11	9	2	4			
8	8	0	0			
7	5	2	4			
5	11	-6	36			
4	10	-6	36			
9	3	6	36			
6	6	0	0			
3	2	1	1			
2	4	-2	4			
		sum D^2	130			

Ethene comparison

Rank Ethene	Rank DHC	D	D^2	Spearman correlation	correlation results	critical value n=8 (p=0.05)
4	6	-2	4	0.36	Medium	0.738
1	5	-4	16			no proof of correlation
6	3	3	9			
7	8	-1	1			
5	7	-2	4			
3	1	2	4			
8	4	4	16			
2	2	0	0			
		sum D^2	54			

Ethene comparison

Rank Ethene	Rank bvcA	D	D^2	Spearman correlation	correlation results	critical value n=8 (p=0.05)
4	7	-3	9	0.14	Weak	0.738
1	6	-5	25			no proof of correlation
6	3	3	9			
7	4	3	9			
5	1	4	16			
3	5	-2	4			
8	8	0	0			
2	2	0	0			
		sum D^2	72			

cDCE, VC, ethene to vcrA

cDCE comparison

Rank cDCE	Rank vcrA	D	D^2	Spearman correlation	correlation results	critical value n=10 (p=0.05)
11	1	10	100	-0.80	Strong	0.648
9	2	7	49			**correlation**
8	5	3	9			
3	10	-7	49			
1	9	-8	64			
4	11	-7	49			
2	8	-6	36			
10	6	4	16			
5	7	-2	4			
6	4	2	4			
7	3	4	16			
		sum D^2	396			

VC comparison

Rank VC	Rank vcrA	D	D^2	Spearman correlation	correlation results	critical value n=10 (p=0.05)
1	1	0	0	0.22	Weak	0.648
10	2	8	64			no proof of correlation
11	5	6	36			
8	10	-2	4			
7	9	-2	4			
5	11	-6	36			
4	8	-4	16			
9	6	3	9			
6	7	-1	1			
3	4	-1	1			
2	3	-1	1			
		sum D^2	172			

Site: Milledgeville
Monitoring well: MW07
PCE, TCE, cDCE, VC, ethene to tceA

TCE comparison

Rank TCE	Rank tceA	D	D^2	Spearman correlation	correlation results	critical value n=10 (p=0.05)
8	1	7	49	0.14	Weak	0.648
9	11	-2	4			no proof of correlation
10	10	0	0			
4	8	-4	16			
1	5	-4	16			
7	9	-2	4			
3	6	-3	9			
11	4	7	49			
2	7	-5	25			
6	3	3	9			
5	2	3	9			
		sum D^2	190			

cDCE comparison

Rank cDCE	Rank tceA	D	D^2	Spearman correlation	correlation results	critical value n=10 (p=0.05)
11	1	10	100	-0.20	Weak	0.648
9	11	-2	4			no proof of correlation
8	10	-2	4			
3	8	-5	25			
1	5	-4	16			
4	9	-5	25			
2	6	-4	16			
10	4	6	36			
5	7	-2	4			
6	3	3	9			
7	2	5	25			
		sum D^2	264			

VC comparison

Rank VC	Rank tceA	D	D^2	Spearman correlation	correlation results	critical value n=10 (p=0.05)
1	1	0	0	0.76	Strong	0.648
10	11	-1	1			**correlation**
11	10	1	1			
8	8	0	0			
7	5	2	4			
5	9	-4	16			
4	6	-2	4			
9	4	5	25			
6	7	-1	1			
3	3	0	0			
2	2	0	0			
		sum D^2	52			

Ethene comparison

Rank Ethene	Rank vcrA	D	D^2	Spearman correlation	correlation results	critical value n=8 (p=0.05)
4	2	2	4	0.31	Weak	0.738
1	7	-6	36			no proof of correlation
6	6	0	0			
7	8	-1	1			
5	5	0	0			
3	3	0	0			
8	4	4	16			
2	1	1	1			
	sum D^2		58			

Site: Milledgeville
Monitoring well: MW07

Date	PCE µg/L	TCE µg/L	cDCE µg/L	VC µg/L	Ethene µg/L	Dhc gene copies per liter	Stdev	bvcA gene copies per liter	Stdev	vcrA copies pe	Stdev	tceA gene copies per liter	Stdev
7-Jan-05	17	14	6900	25		1.5E+03	2.4E+03	3.7E+03	2.0E+03	2.4E+02	2.5E+02	2.2E+04	1.8E+04
21-Jan-05	34	28	2400	4000		2.5E+08	2.3E+07	6.0E+05	9.4E+04	4.6E+04	3.0E+04	3.8E+08	5.5E+07
7-Feb-05	17	29	370	5000	48	2.7E+08	5.8E+07	2.3E+06	1.2E+06	7.1E+06	1.3E+06	3.7E+08	1.6E+08
15-Mar-05	1.7	4.6	4.2	400	0.33	2.7E+08	3.9E+07	1.5E+06	1.6E+05	1.3E+08	8.1E+06	8.7E+07	6.3E+06
29-Apr-05	1.4	1.1	2.6	360	120	1.4E+08	1.3E+07	3.1E+05	7.2E+04	1.3E+08	1.4E+07	2.1E+07	1.0E+07
1-Jul-05	1.7	9	12	110	200	1.6E+09	4.0E+08	4.9E+05	1.3E+05	8.9E+08	1.4E+08	1.5E+08	2.8E+07
29-Jul-05	0.34	3.1	3.9	94	74	3.0E+08	8.2E+07	1.4E+05	3.7E+04	5.8E+07	5.1E+07	2.5E+07	5.9E+06
29-Oct-05	6.8	260	4500	790	36	1.6E+07	4.4E+06	1.2E+06	1.6E+05	1.1E+07	1.0E+06	7.7E+06	1.4E+04
13-Apr-06	0.34	2.2	18	220	299	2.0E+08	9.3E+07	4.9E+06	2.3E+06	4.4E+07	1.4E+07	6.1E+07	2.0E+07
30-Oct-06	0.5	5.6	110	60		1.7E+06	6.5E+05	7.0E+04	3.3E+04	5.9E+06	1.5E+06	1.5E+06	6.3E+05
30-Apr-07	0.5	5.3	140	54	10.9	1.8E+07	2.3E+07	2.0E+05	1.5E+05	3.1E+06	1.9E+06	1.4E+06	1.1E+06

ratios

TCE+cDCE+VC+ethene to PCE	cDCE+VC+ethene to TCE	VC+ethene to DCE	cDCE:TCE	VC:cDCE
			492.9	0.00
			85.7	1.67
320.4	186.8	13.6	12.8	13.51
240.7	87.9	95.3	0.9	95.24
345.5	438.7	184.6	2.4	138.46
194.7	35.8	25.8	1.3	9.17
514.7	55.5	43.1	1.3	24.10
821.5	20.5	0.2	17.3	0.18
1585.9	244.1	28.8	8.2	12.22
			19.6	0.55
420.4	38.7	0.5	26.4	0.39

TCE+cDCE+VC+ethene:PCE to DHC

Rank daughter prod:PCE	Rank DHC	D	D^2	Spearman correlation	correlation results	critical value n=8 (p=0.05)
3	6	-3	9	-0.55	Medium	0.738
2	5	-3	9			no proof of correlation
4	3	1	1			
1	8	-7	49			
6	7	-1	1			
7	1	6	36			
8	4	4	16			
5	2	3	9			
	sum D^2		130			

cDCE+VC+ethene:TCE to DHC

Rank daughter prod:TCE	Rank DHC	D	D^2	Spearman correlation	correlation results	critical value n=8 (p=0.05)
6	6	0	0	0.05	Weak	0.738
5	5	0	0			no proof of correlation
8	3	5	25			
2	8	-6	36			
4	7	-3	9			
1	1	0	0			
7	4	3	9			
3	2	1	1			
	sum D^2		80			

VC+ethene:cDCE to DHC

Rank daughter prod:cDCE	Rank DHC	D	D^2	Spearman correlation	correlation results	critical value n=8 (p=0.05)
3	6	-3	9	0.33	Medium	0.738
7	5	2	4			no proof of correlation
8	3	5	25			
4	8	-4	16			
6	7	-1	1			
1	1	0	0			
5	4	1	1			
2	2	0	0			
	sum D^2		56			

TCE+cDCE+VC+ethene:PCE to bvcA

Rank daughter prod:PCE	Rank bvcA	D	D^2	Spearman correlation	correlation results	critical value n=8 (p=0.05)
3	7	-4	16	0.05	Weak	0.738
2	6	-4	16			no proof of correlation
4	3	1	1			
1	4	-3	9			
6	1	5	25			
7	5	2	4			
8	8	0	0			
5	2	3	9			
	sum D^2		80			

cDCE+VC+ethene:TCE to DHC

Rank daughter prod:TCE	Rank bvcA	D	D^2	Spearman correlation	correlation results	critical value n=8 (p=0.05)
6	7	-1	1	0.31	Weak	0.738
5	6	-1	1			no proof of correlation
8	3	5	25			
2	4	-2	4			
4	1	3	9			
1	5	-4	16			
7	8	-1	1			
3	2	1	1			
	sum D^2		58			

VC+ethene:cDCE to DHC

Rank daughter prod:cDCE	Rank bvcA	D	D^2	Spearman correlation	correlation results	critical value n=8 (p=0.05)
#N/A	7	#N/A	#N/A	#N/A	#N/A	0.738
#N/A	6	#N/A	#N/A			no proof of correlation
#N/A	3	#N/A	#N/A			
#N/A	4	#N/A	#N/A			
#N/A	1	#N/A	#N/A			
#N/A	5	#N/A	#N/A			
#N/A	8	#N/A	#N/A			
2	2	0	0			
	sum D^2		#N/A			

Site: Milledgeville
Monitoring well: MW07

TCE+cDCE+VC+ethene:PCE to tceA

Rank daughter prod:PCE	Rank tceA	D	D^2	Spearman correlation	correlation results	critical value n=8 (p=0.05)
3	8	-5	25	-0.57	Medium	0.738
2	6	-4	16			no proof of correlation
4	3	1	1			
1	7	-6	36			
6	4	2	4			
7	2	5	25			
8	5	3	9			
5	1	4	16			
	sum D2		132			

TCE+cDCE+VC+ethene:PCE to vcrA

Rank daughter prod:PCE	Rank vcrA	D	D^2	Spearman correlation	correlation results	critical value n=8 (p=0.05)
3	2	1	1	-0.52	Medium	0.738
2	7	-5	25			no proof of correlation
4	6	-2	4			
1	8	-7	49			
6	5	1	1			
7	3	4	16			
8	4	4	16			
5	1	4	16			
		sum D^2	128			

cDCE+VC+ethene:TCE to tceA

Rank daughter prod:TCE	Rank tceA	D	D^2	Spearman correlation	correlation results	critical value n=8 (p=0.05)
6	8	-2	4	0.24	Weak	0.738
5	6	-1	1			no proof of correlation
8	3	5	25			
2	7	-5	25			
4	4	0	0			
1	2	-1	1			
7	5	2	4			
3	1	2	4			
	sum D2		64			

cDCE+VC+ethene:TCE to vcrA

Rank daughter prod:TCE	Rank vcrA	D	D^2	Spearman correlation	correlation results	critical value n=8 (p=0.05)
6	2	4	16	0.07	Weak	0.738
5	7	-2	4			no proof of correlation
8	6	2	4			
2	8	-6	36			
4	5	-1	1			
1	3	-2	4			
7	4	3	9			
3	1	2	4			
		sum D^2	78			

VC+ethene:cDCE to tceA

Rank daughter prod:cDCE	Rank tceA	D	D^2	Spearman correlation	correlation results	critical value n=8 (p=0.05)
#N/A	8	#N/A	#N/A	#N/A	#N/A	0.738
#N/A	6	#N/A	#N/A			no proof of correlation
#N/A	3	#N/A	#N/A			
#N/A	7	#N/A	#N/A			
#N/A	4	#N/A	#N/A			
#N/A	2	#N/A	#N/A			
#N/A	5	#N/A	#N/A			
1	1	0	0			
	sum D2		#N/A			

VC+ethene:cDCE to vcrA

Rank daughter prod:cDCE	Rank vcrA	D	D^2	Spearman correlation	correlation results	critical value n=8 (p=0.05)
#N/A	2	#N/A	#N/A	#N/A	#N/A	0.738
#N/A	7	#N/A	#N/A			no proof of correlation
#N/A	6	#N/A	#N/A			
#N/A	8	#N/A	#N/A			
#N/A	5	#N/A	#N/A			
#N/A	3	#N/A	#N/A			
#N/A	4	#N/A	#N/A			
1	1	0	0			
		sum D^2	#N/A			

NAWC Trenton

Site: NAWC Trenton

Monitoring well: MWBRP1

Well BRP1	TCE µg/L	cDCE µg/L	VC µg/L	Ethene µg/L	Ethane µg/L	DHC gene copies/L
05/31/05	206.0	174.0	6.4	1.5	2.0	NS
08/08/05	0.5	6.9	1.2	1.5	2.0	NS
08/22/05	11.1	60.5	4.9	1.5	2.0	NS
10/10/05	14.0	100.0	10.0	1.5	2.0	2.00E+04
12/19/05	1.4	85.0	32.0	3.0	2.0	1.00E+06
03/06/06	0.4	5.3	1.5	1.5	2.0	1.00E+06
06/07/06	0.3	2.0	1.2	1.5	8.8	7.00E+04
08/28/06	0.6	106.0	57.1	1.5	37.2	1.00E+07
11/13/06	1.0	104.0	125.0	110.0	130.0	2.00E+07
02/19/07	1.0	19.7	12.2	3.7	42.0	4.00E+06
05/21/07	1.0	3.1	2.9	4.0	27.0	3.00E+06
03/05/08	0.5	2.4	1.8	1.5	6.2	3.00E+07
06/09/08	0.6	3.9	3.7	3.9	5.6	1.00E+05
7/30/2008	4.1	72.5	34.9	24.0	63.0	3.00E+06

Well BRP1	sum cDCE, VC, ethene	ratio to TCE	sum VC and ethene	ratio to cDCE
05/31/05				
08/08/05	181.9	0.88	7.90	0.05
08/22/05	9.6	17.78	2.70	0.39
10/10/05	66.9	6.03	6.40	0.11
12/19/05	111.5	7.96	11.50	0.12
03/06/06	120	88.89	35.00	0.41
06/07/06	8.3	23.71	3.00	0.57
08/28/06	4.7	13.82	2.70	1.35
11/13/06	164.6	265.48	58.60	0.55
02/19/07	339.0	339.00	235.00	2.26
05/21/07	35.6	35.60	15.90	0.81
03/05/08	10	10.00	6.90	2.23
06/09/08	5.7	11.40	3.30	1.38
7/30/2008	11.5	19.17	7.60	1.95
	131.4	32.05	58.9	0.81

Site: NAWC Trenton
Monitoring well: MWBRP1

TCE comparison (no TCE data - ignore)

Rank TCE	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=10)
11	1	10	100	-0.12	Weak	0.648
9	4	5	25			no proof of correlation
2	4	-2	4			
1	2	-1	1			
5	9	-4	16			
6	10	-4	16			
6	8	-2	4			
6	6	0	0			
3	11	-8	64			
4	3	1	1			
10	6	4	16			
		sum D^2	247			

cDCE comparison

Rank cDCE	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=10)
9	1	8	64	0.17	Weak	0.648
8	4	4	16			no proof of correlation
5	4	1	1			
1	2	-1	1			
11	9	2	4			
10	10	0	0			
6	8	-2	4			
3	6	-3	9			
2	11	-9	81			
4	3	1	1			
7	6	1	1			
		sum D^2	182			

VC comparison

Rank VC	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=10)
6	1	5	25	0.41	Medium	0.648
8	4	4	16			no proof of correlation
2	4	-2	4			
1	2	-1	1			
10	9	1	1			
11	10	1	1			
7	8	-1	1			
4	6	-2	4			
3	11	-8	64			
5	3	2	4			
9	6	3	9			
		sum D^2	130			

Site: NAWC Trenton
Monitoring well: MWBRP1

Ethene comparison

Rank Ethene	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=10)
1	1	0	0	-0.05	Weak	0.648
6	4	2	4			no proof of correlation
1	4	-3	9			
1	2	-1	1			
1	9	-8	64			
11	10	1	1			
7	8	-1	1			
9	6	3	9			
1	11	-10	100			
8	3	5	25			
10	6	4	16			
		sum D^2	230			

Ratio of daughter products to TCE

Rank Ratio	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=10)
1	1	0	0	0.45	Medium	0.648
9	4	5	25			no proof of correlation
6	4	2	4			
4	2	2	4			
10	9	1	1			
11	10	1	1			
8	8	0	0			
2	6	-4	16			
3	11	-8	64			
5	3	2	4			
7	6	1	1			
		sum D^2	120			

Ratio of daughter products to cDCE

Rank Ratio	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=10)
1	1	0	0	0.38	Medium	0.648
2	4	-2	4			no proof of correlation
4	4	0	0			
7	2	5	25			
3	9	-6	36			
11	10	1	1			
5	8	-3	9			
10	6	4	16			
8	11	-3	9			
9	3	6	36			
6	6	0	0			
		sum D^2	136			

Site: NAWC Trenton

Monitoring well: MW16BR

Well 16BR	TCE µg/L	cDCE µg/L	VC µg/L	Ethene µg/L	Ethane µg/L	DHC gene copies/L
5/31/05	288	242	8.3	1.5	2	5.80E+03
8/8/05	0.83	788	27.3	1.5	2	NS
8/22/05	2.2	689	38.7	1.5	2	NS
10/10/05	1.5	1.1	1.1	30	2	4.00E+07
12/19/05	0.52	1.7	1.3	1.5	33	2.00E+07
3/6/06	0.67	1.5	0.78	1.5	8.2	6.00E+06
6/5/2006	1.0	26.7	8.2	1.5	58.5	2.00E+05
8/28/2006	1	1.7	4.5	1.5	92.5	2.00E+06
11/13/2006	1	2.7	4.1	1	110	2.00E+07
2/19/07	1	2.1	1.8	0.28	100	9.00E+06
5/21/07	0.24	3.10	5.60	7.9	88	2.00E+06
3/5/08	1.00	2.30	3.00	1.7	46	NS

Well 16BR	sum cDCE, VC, ethene	ratio to TCE	sum VC and ethene	ratio to cDCE
5/31/05	251.8	0.87	9.80	0.04
8/8/05	816.8	984.10	28.80	0.04
8/22/05	729.2	331.45	40.20	0.06
10/10/05	32.2	21.47	31.10	28.27
12/19/05	4.5	8.65	2.80	1.65
3/6/06	3.78	5.64	2.28	1.52
6/5/2006	36.4	36.40	9.70	0.36
8/28/2006	7.7	7.70	6.00	3.53
11/13/2006	7.8	7.80	5.10	1.89
2/19/07	4.18	4.18	2.08	0.99
5/21/07	16.6	69.17	13.50	4.35
3/5/08	7	7.00	4.70	2.04

TCE comparison

Rank TCE	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=9)
9	1	8	64	0.03	Weak	0.683
8	9	-1	1			no proof of correlation
2	7	-5	25			
3	5	-2	4			
4	2	2	4			
4	3	1	1			
4	7	-3	9			
4	6	-2	4			
1	3	-2	4			
		sum D^2	116			

Site: NAWC Trenton
Monitoring well: MW16BR

cDCE comparison

Rank cDCE	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=9)
9	1	8	64	-0.725	Strong	0.68
1	9	-8	64			correlation
3	7	-4	16			
2	5	-3	9			
8	2	6	36			
3	3	0	0			
6	7	-1	1			
5	6	-1	1			
7	3	4	16			
		sum D^2	207			

VC comparison

Rank VC	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=9)
9	1	8	64	-0.78	Strong	0.68
2	9	-7	49			correlation
3	7	-4	16			
1	5	-4	16			
8	2	6	36			
6	3	3	9			
5	7	-2	4			
4	6	-2	4			
7	3	4	16			
		sum D^2	214			

Ethene comparison

Rank Ethene	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=9)
3	1	2	4	0.17	Weak	0.683
9	9	0	0			no proof of correlation
3	7	-4	16			
3	5	-2	4			
3	2	1	1			
3	3	0	0			
2	7	-5	25			
1	6	-5	25			
8	3	5	25			
		sum D^2	100			

Site: NAWC Trenton
Monitoring well: MW16BR

Ratio of daughter products to TCE

Rank Ratio	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=9)
1	1	0	0	0.15	Weak	0.683
7	9	-2	4			no proof of correlation
6	7	-1	1			
3	5	-2	4			
8	2	6	36			
4	3	1	1			
5	7	-2	4			
2	6	-4	16			
9	3	6	36			
		sum D^2	102			

Ratio of daughter products to cDCE

Rank Ratio	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=9)
1	1	0	0	0.53	Medium	0.683
9	9	0	0			no proof of correlation
5	7	-2	4			
4	5	-1	1			
2	2	0	0			
7	3	4	16			
6	7	-1	1			
3	6	-3	9			
8	3	5	25			
		sum D^2	56			

Site: NAWC Trenton

Monitoring well: MW38BR

Well 38BR	TCE µg/L	cDCE µg/L	VC µg/L	Ethene µg/L	Ethane µg/L	DHC gene copies/L
05/31/05	15800	3580	4300	1.5	2	NS
08/08/05	89.2	6380	735	5.3	2	9.20E+04
08/22/05	294	6290	610	1.5	2	NS
10/10/05	7.5	5200	360	7.4	2	2.00E+06
12/19/05	8.1	2100	2500	140	2	6.00E+07
03/06/06	0.69	17	9	130	2	7.00E+07
06/07/06	5	6.1	5	43.2	1.5	6.00E+07
08/28/06	0.47	182	185	170	22.3	2.00E+08
11/13/06	2.7	90.8	61.9	43	96	1.00E+07
02/19/07	1	1.9	1.8	1.2	97	2.00E+07
5/21/07	1	1	1	140	210	1.00E+08
3/5/08	1	5.5	14.6	50	240	NS

Well 38BR	sum cDCE, VC, ethene	ratio to TCE	sum VC and ethene	ratio to cDCE
05/31/05	7881.5	0.50	4301.50	1.20
08/08/05	7120.3	79.82	740.30	0.12
08/22/05	6901.5	23.47	611.50	0.10
10/10/05	5567.4	742.32	367.40	0.07
12/19/05	4740	585.19	2640.00	1.26
03/06/06	156	226.09	139.00	8.18
06/07/06	54.3	10.86	48.20	7.90
08/28/06	537	1142.55	355.00	1.95
11/13/06	195.7	72.48	104.90	1.16
02/19/07	4.9	4.90	3.00	1.58
5/21/07	142	142.00	141.00	141.00
3/5/08	70.1	70.10	64.60	11.75

TCE comparison

Rank TCE	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.02 (n=9)
9	1	8	64	-0.817	Strong	0.738
7	2	5	25			correlation
8	5	3	9			
2	7	-5	25			
6	5	1	1			
1	9	-8	64			
5	3	2	4			
3	4	-1	1			
3	8	-5	25			
		sum D^2	218			

Site: NAWC Trenton
Monitoring well: MW38BR

cDCE comparison

Rank cDCE	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=9)
9	1	8	64	-0.53	Medium	0.683
8	2	6	36			no proof of correlation
7	5	2	4			
4	7	-3	9			
3	5	-2	4			
6	9	-3	9			
5	3	2	4			
2	4	-2	4			
1	8	-7	49			
		sum D^2	183			

VC comparison

Rank VC	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=9)
8	1	7	49	-0.41	Medium	0.683
7	2	5	25			no proof of correlation
9	5	4	16			
4	7	-3	9			
3	5	-2	4			
6	9	-3	9			
5	3	2	4			
2	4	-2	4			
1	8	-7	49			
		sum D^2	169			

Ethene comparison

Rank Ethene	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.01 (n=9)
2	1	1	1	0.850	Strong	0.833
3	2	1	1			correlation
7	5	2	4			
6	7	-1	1			
5	5	0	0			
9	9	0	0			
4	3	1	1			
1	4	-3	9			
7	8	-1	1			
		sum D^2	18			

Site: NAWC Trenton
Monitoring well: MW38BR

Ratio of daughter products to TCE

Rank Ratio	Rank DHC		D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=9)
4	1		3	9	0.36	Medium	0.683
8	2		6	36			no proof of correlation
7	5		2	4			
6	7		-1	1			
2	5		-3	9			
9	9		0	0			
3	3		0	0			
1	4		-3	9			
5	8		-3	9			
		sum D^2		77			

Ratio of daughter products to cDCE

Rank Ratio	Rank DHC		D	D^2	Spearman Correlation	correlation results	r critical for p = 0.01 (n=9)
2	1		1	1	0.842	Strong	0.833
1	2		-1	1			correlation
4	5		-1	1			
8	7		1	1			
7	5		2	4			
6	9		-3	9			
3	3		0	0			
5	4		1	1			
9	8		1	1			
		sum D^2		19			

Site: NAWC Trenton
Monitoring well: MW41BR

Well 41BR	TCE µg/L	cDCE µg/L	VC µg/L	Ethene µg/L	Ethane µg/L	DHC gene copies/L
05/31/05	674	394	5.4	1.5	2	1.08E+03
08/08/05	2.4	12.6	1.7	1.5	2	2.15E+03
08/22/05	1.9	15.4	2.2	1.5	2	1.10E+04
10/10/05	6.6	18	2.0	1.5	2	2.00E+03
12/19/05	1.0	1.3	0.98	17	2	3.00E+07
03/06/06	1.0	0.55	0.5	1.5	5.9	2.00E+06
06/07/06	1.0	0.37	0.54	1.5	54.8	4.00E+06
08/28/06	1	0.28	0.26	1.5	70.8	3.00E+07
11/13/06	2.7	0.55	0.58	2.9	79	1.00E+07
02/19/07	1	0.27	0.44	0.17	150	2.00E+07
05/21/07	1	0.29	1	4.1	170	2.00E+07
03/05/08	0.74	0.49	1	0.085	140	1.00E+08
06/09/08	1.00	1.3	1.9	20	170	1.00E+07
7/30/2008	13.4	38.8	30.8	150	120	4.00E+07

Well 41BR	sum cDCE, VC, ethene	ratio to TCE	sum VC and ethene	ratio to cDCE
05/31/05	400.9	0.59	6.90	0.02
08/08/05	15.8	6.58	3.20	0.25
08/22/05	19.1	10.05	3.70	0.24
10/10/05	21.5	3.26	3.50	0.19
12/19/05	19.28	19.28	17.98	13.83
03/06/06	2.55	2.55	2.00	3.64
06/07/06	2.41	2.41	2.04	5.51
08/28/06	2.04	2.04	1.76	6.29
11/13/06	4.0	1.49	3.48	6.33
02/19/07	0.88	0.88	0.61	2.26
05/21/07	5.39	5.39	5.10	17.59
03/05/08	1.575	2.13	1.09	2.21
06/09/08	23.2	23.20	21.90	16.85
7/30/2008	219.6	16.39	180.8	4.66

Site: NAWC Trenton
Monitoring well: MW41BR
TCE comparison

Rank TCE	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.01 (n=14)
14	1	13	169	-0.84	Strong	0.715
10	3	7	49			correlation
9	4	5	25			
12	2	10	100			
2	11	-9	81			
2	5	-3	9			
2	6	-4	16			
2	11	-9	81			
11	7	4	16			
2	9	-7	49			
2	9	-7	49			
1	14	-13	169			
2	7	-5	25			
13	13	0	0			
		sum D^2	838			

cDCE comparison

Rank cDCE	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=14)
14	1	13	169	-0.418	Medium	0.544
10	3	7	49			no proof of correlation
11	4	7	49			
12	2	10	100			
8	11	-3	9			
6	5	1	1			
4	6	-2	4			
2	11	-9	81			
6	7	-1	1			
1	9	-8	64			
3	9	-6	36			
5	14	-9	81			
8	7	1	1			
13	13	0	0			
		sum D^2	645			

Site: NAWC Trenton
Monitoring well: MW41BR
VC comparison

Rank VC	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=14)
13	1	12	144	-0.26	Weak	0.544
9	3	6	36			no proof of correlation
12	4	8	64			
11	2	9	81			
6	11	-5	25			
3	5	-2	4			
4	6	-2	4			
1	11	-10	100			
5	7	-2	4			
2	9	-7	49			
7	9	-2	4			
7	14	-7	49			
10	7	3	9			
14	13	1	1			
		sum D^2	574			

Ethene comparison

Rank Ethene	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=14)
3	1	2	4	0.23	Weak	0.544
3	3	0	0			no proof of correlation
3	4	-1	1			
3	2	1	1			
12	11	1	1			
3	5	-2	4			
3	6	-3	9			
3	11	-8	64			
10	7	3	9			
2	9	-7	49			
11	9	2	4			
1	14	-13	169			
13	7	6	36			
14	13	1	1			
		sum D^2	352			

Site: NAWC Trenton
Monitoring well: MW41BR
Ratio of daughter products to TCE

Rank Ratio	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=14)
1	1	0	0	0.15	Weak	0.544
10	3	7	49			no proof of correlation
11	4	7	49			
8	2	6	36			
13	11	2	4			
7	5	2	4			
6	6	0	0			
4	11	-7	49			
3	7	-4	16			
2	9	-7	49			
9	9	0	0			
5	14	-9	81			
14	7	7	49			
12	13	-1	1			
		sum D^2	387			

Ratio of daughter products to cDCE

Rank Ratio	Rank DHC	D	D^2	Spearman Correlation	correlation results	r critical for p = 0.05 (n=14)
1	1	0	0	0.541	Medium	0.54
4	3	1	1			correlation
3	4	-1	1			
2	2	0	0			
12	11	1	1			
7	5	2	4			
9	6	3	9			
10	11	-1	1			
11	7	4	16			
6	9	-3	9			
14	9	5	25			
5	14	-9	81			
13	7	6	36			
8	13	-5	25			
		sum D^2	209			

Appendix E: Cost Summary

Capital Cost With Bioaugmentation

Item	Quantity	Unit	Unit Cost Subcontract	Unit Cost Material	Unit Cost Labor	Unit Cost Equipment	Extended Cost Subcontract	Extended Cost Material	Extended Cost Labor	Extended Cost Equipment	Subtotal
1 PROJECT PLANNING & DOCUMENTS											
1.1 Prepare LUC Documents	200	hr			$35.00		$0	$0	$7,000	$0	$7,000
1.2 Prepare Documents & Plans including Permit	250	hr			$35.00		$0	$0	$8,750	$0	$8,750
1.3 Prepare Groundwater Monitoring Plar	120	hr			$35.00		$0	$0	$4,200	$0	$4,200
1.4 Completion Repor	100	hr			$35.00		$0	$0	$3,500	$0	$3,500
2 MOBILIZATION AND DEMOBILIZATION											
2.1 Preconstruction Meeting	30	hr			$55.00		$0	$0	$1,650	$0	$1,650
2.2 Site Support Facilities (trailers, phone, electric, etc.	1	ls		$1,000.00		$3,500.00	$0	$1,000	$0	$3,500	$4,500
2.3 Equipment Mobilization/Demobilizatio	3	ea			$158.00	$384.00	$0	$0	$474	$1,152	$1,626
2.4 Well Equipment Mobilization/Demobilizatio	1	ea	$2,000.00				$2,000	$0	$0	$0	$2,000
3 FIELD SUPPORT											
3.1 Site Support Facilities (trailers, phone, electric, etc.	3	mo		$210.00	$350.00		$0	$630	$1,050	$0	$1,680
3.2 Survey Suppor	15	day	$935.00				$14,025	$0	$0	$0	$14,025
3.3 Site Superintenden	11	week			$1,234.20		$0	$0	$13,576	$0	$13,576
3.4 Site Health & Safety and QA/QC	11	week			$701.20		$0	$0	$7,713	$0	$7,713
3.5 Decontamination Services	3	mo		$210.00		$315.00	$0	$630	$0	$945	$1,575
4 SITE PREPARATION AND MBT SAMPLING											
4.1 Underground Utility Clearance	1	ls	$7,500.00				$7,500	$0	$0	$0	$7,500
4.2 Equipment Decon Pac	1	ls		$1,850.00			$0	$1,850	$0	$0	$1,850
4.2 Consumerables and Supplies (filter, tubing, shipping	30	per sample		$15.00			$0	$450	$0	$0	$450
4.3 Operator Labor	30	per sample			$75.00		$0	$0	$2,250	$0	$2,250
4.4 Equipment Maintenance and Calibratio	30	per sample				$10.00	0	$0	$0	$300	$300
4.5 Purge Water Disposal	30	per sample	$10.00				$300	$0	$0	$0	$300
4.6 Laboratory Analysis (qPCR)	8	per sample	$350.00				$2,800	$0	$0	$0	$2,800
4.7 Laboratory Analysis (non-qPCR)	1	ls	$8,800.00				$8,800	$0	$0	$0	$8,800
7 MONITORING WELL INSTALLATION SITES											
7.1 Well Installation & Development (30 wells	750	lf	$80.00				$60,000	$0	$0	$0	$60,000
7.2 Technician	13	day			$273.00		0	$0	$3,549	$0	$3,549
7.3 Protective Well Casing & Apror	20	ea	$750.00				$15,000	$0	$0	$0	$15,000
7.4 Flush Well Casing & Apror	10	ea	$550.00				$5,500	$0	$0	$0	$5,500
7.5 IDW Transport & Disposal, solid non-haz	25	drum	$185.00				$4,625	$0	$0	$0	$4,625
7.6 IDW Transport & Disposal, liquid non-haz	8	drum	$175.00				$1,400	$0	$0	$0	$1,400
8 BIO-ENHANCED INJECTIONS											
8.1 DPT Mobilization/Demobilizatior	1	ea	$2,000.00				$2,000	$0	$0	$0	$2,000
8.2 Technician	5	day			$273.00		0	$0	$1,365	$0	$1,365
8.3 DPT Rig	14	day	$3,000.00				$42,000	$0	$0	$0	$42,000
8.4 Injection Point Supplies	1,536	lf	$4.00				$6,144	$0	$0	$0	$6,144
8.5 EVO	1	ls		$20,000.00			$0	$20,000	$0	$0	$20,000
8.6 Drill Asphalt & Repai	50	ea	$95.00				$4,750	$0	$0	$0	$4,750
8.7 KB-1	1	ls		$2,000.00			0	$2,000	$0	$0	$2,000
9 SITE RESTORATION											
9.1 Pavement Replacemen	150	sy	$46.00				$6,900	$0	$0	$0	$6,900
9.2 Top Dress Soil	250	cy	$30.00				$7,500	$0	$0	$0	$7,500
9.3 Site Restoration, seed, fertilization, mulch	15	msf	$71.00				$1,065	$0	$0	$0	$1,065
Subtotal							$192,309	$26,560	$55,077	$5,897	$279,843
Overhead on Labor Cost @ 30%									$16,523		$16,523
G & A on Labor Cost @ 10%									$5,508		$5,508
G & A on Material Cost @ 10%								$2,656			$2,656
G & A on Equipment Cost @ 10%										$590	$590
G & A on Subcontract Cost @ 10%							$19,231				$19,231
Tax on Materials and Equipment Cost @ 5%								$1,328		$295	$1,623
Total Direct Cost							$211,540	$30,544	$77,108	$6,782	$325,974
Indirects on Total Direct Cost @ 30%											$97,792
Profit on Total Direct Cost @ 10%											$32,597
Subtotal											$456,363
Health & Safety Monitoring @ 2%											$9,127
Total Field Cost											$465,491
Contingency on Total Field Costs @ 25%											$116,373
Engineering on Total Field Cost @ 6%											$27,929
TOTAL CAPITAL COST											**$609,793**

Capital Cost Without Bioaugmentation

Item	Quantity	Unit	Unit Cost Subcontract	Material	Labor	Equipment	Extended Cost Subcontract	Material	Labor	Equipment	Subtotal
1 PROJECT PLANNING & DOCUMENTS											
1.1 Prepare LUC Documents	200	hr			$35.00		$0	$0	$7,000	$0	$7,000
1.2 Prepare Documents & Plans including Permit	250	hr			$35.00		$0	$0	$8,750	$0	$8,750
1.3 Prepare Groundwater Monitoring Plar	120	hr			$35.00		$0	$0	$4,200	$0	$4,200
1.4 Completion Repor	100	hr			$35.00		$0	$0	$3,500	$0	$3,500
2 MOBILIZATION AND DEMOBILIZATION											
2.1 Preconstruction Meeting	30	hr			$55.00		$0	$0	$1,650	$0	$1,650
2.2 Site Support Facilities (trailers, phone, electric, etc.	1	ls		$1,000.00		$3,500.00	$0	$1,000	$0	$3,500	$4,500
2.3 Equipment Mobilization/Demobilizatio	3	ea			$158.00	$384.00	$0	$0	$474	$1,152	$1,626
2.4 Well Equipment Mobilization/Demobilizatio	1	ea	$2,000.00				$2,000	$0	$0	$0	$2,000
3 FIELD SUPPORT											
3.1 Site Support Facilities (trailers, phone, electric, etc.	2	mo		$210.00	$350.00		$0	$420	$700	$0	$1,120
3.2 Survey Suppor	8	day	$935.00				$7,480	$0	$0	$0	$7,480
3.3 Site Superintenden	9	week			$1,234.20		$0	$0	$11,108	$0	$11,108
3.4 Site Health & Safety and QA/QC	9	week			$701.20		$0	$0	$6,311	$0	$6,311
3.5 Decontamination Services	2	mo		$210.00		$315.00	$0	$420	$0	$630	$1,050
4 SITE PREPARATION AND MBT SAMPLING											
4.1 Underground Utility Clearance	1	ls	$7,500.00				$7,500	$0	$0	$0	$7,500
4.2 Equipment Decon Pac	1	ls		$1,850.00			$0	$1,850	$0	$0	$1,850
4.2 Consumerables and Supplies (filter, tubing, shipping	30	per sample		$15.00			$0	$450	$0	$0	$450
4.3 Operator Labor	30	per sample			$75.00		$0	$0	$2,250	$0	$2,250
4.4 Equipment Maintenance and Calibratio	30	per sample				$10.00	0	$0	$0	$300	$300
4.5 Purge Water Disposal	30	per sample	$10.00				$300	$0	$0	$0	$300
4.6 Laboratory Analysis (qPCR)	0	per sample	$350.00				$0	$0	$0	$0	$0
4.7 Laboratory Analysis (non-qPCR)	1	ls	$8,800.00				$8,800	$0	$0	$0	$8,800
7 MONITORING WELL INSTALLATION SITES											
7.1 Well Installation & Development (30 wells	750	lf	$80.00				$60,000	$0	$0	$0	$60,000
7.2 Technician	13	day			$273.00		0	$0	$3,549	$0	$3,549
7.3 Protective Well Casing & Apror	20	ea	$750.00				$15,000	$0	$0	$0	$15,000
7.4 Flush Well Casing & Apror	10	ea	$550.00				$5,500	$0	$0	$0	$5,500
7.5 IDW Transport & Disposal, solid non-haz	25	drum	$185.00				$4,625	$0	$0	$0	$4,625
7.6 IDW Transport & Disposal, liquid non-haz	8	drum	$175.00				$1,400	$0	$0	$0	$1,400
8 BIO-ENHANCED INJECTIONS											
8.1 DPT Mobilization/Demobilizatior	0	ea	$2,000.00				$0	$0	$0	$0	$0
8.2 Technician	0	day			$273.00		0	$0	$0	$0	$0
8.3 DPT Rig	0	day	$3,000.00				$0	$0	$0	$0	$0
8.4 Injection Point Supplies	0	lf	$4.00				$0	$0	$0	$0	$0
8.5 EVO	0	per wel		$2,000.00			$0	$0	$0	$0	$0
8.6 Drill Asphalt & Repai	0	ea	$95.00				$0	$0	$0	$0	$0
8.7 KB-1	0	per wel		$570.00			0	$0	$0	$0	$0
9 SITE RESTORATION											
9.1 Pavement Replacemen	150	sy	$46.00				$6,900	$0	$0	$0	$6,900
9.2 Top Dress Soil	250	cy	$30.00				$7,500	$0	$0	$0	$7,500
9.3 Site Restoration, seed, fertilization, mulch	15	msf	$71.00				$1,065	$0	$0	$0	$1,065
Subtotal							$128,070	$4,140	$49,492	$5,582	$187,284
Overhead on Labor Cost @ 30%									$14,847		$14,847
G & A on Labor Cost @ 10%									$4,949		$4,949
G & A on Material Cost @ 10%								$414			$414
G & A on Equipment Cost @ 10%										$558	$558
G & A on Subcontract Cost @ 10%							$12,807				$12,807
Tax on Materials and Equipment Cost @ 5%								$207		$279	$486
Total Direct Cost							$140,877	$4,761	$69,288	$6,419	$221,346
Indirects on Total Direct Cost @ 30%											$66,404
Profit on Total Direct Cost @ 10%											$22,135
Subtotal											$309,884
Health & Safety Monitoring @ 2%											$6,198
Total Field Cost											$316,081
Contingency on Total Field Costs @ 25%											$79,020
Engineering on Total Field Cost @ 6%											$18,965
TOTAL CAPITAL COST											**$414,067**

Capital Cost With Bioaugmentation

Item	Quantity	Unit	Unit Cost Subcontract	Unit Cost Material	Unit Cost Labor	Unit Cost Equipment	Extended Cost Subcontract	Extended Cost Material	Extended Cost Labor	Extended Cost Equipment	Subtotal
1 PROJECT PLANNING & DOCUMENTS											
1.1 Prepare LUC Documents	200	hr			$35.00		$0	$0	$7,000	$0	$7,000
1.2 Prepare Documents & Plans including Permit	250	hr			$35.00		$0	$0	$8,750	$0	$8,750
1.3 Prepare Groundwater Monitoring Plar	120	hr			$35.00		$0	$0	$4,200	$0	$4,200
1.4 Completion Repor	100	hr			$35.00		$0	$0	$3,500	$0	$3,500
2 MOBILIZATION AND DEMOBILIZATION											
2.1 Preconstruction Meeting	30	hr			$55.00		$0	$0	$1,650	$0	$1,650
2.2 Site Support Facilities (trailers, phone, electric, etc.	1	ls		$1,000.00		$3,500.00	$0	$1,000	$0	$3,500	$4,500
2.3 Equipment Mobilization/Demobilizatio	3	ea			$158.00	$384.00	$0	$0	$474	$1,152	$1,626
2.4 Well Equipment Mobilization/Demobilizatio	1	ea	$2,000.00				$2,000	$0	$0	$0	$2,000
3 FIELD SUPPORT											
3.1 Site Support Facilities (trailers, phone, electric, etc.	3	mo		$210.00	$350.00		$0	$630	$1,050	$0	$1,680
3.2 Survey Suppor	11	day	$935.00				$10,285	$0	$0	$0	$10,285
3.3 Site Superintenden	11	week			$1,234.20		$0	$0	$13,576	$0	$13,576
3.4 Site Health & Safety and QA/QC	11	week			$701.20		$0	$0	$7,713	$0	$7,713
3.5 Decontamination Services	3	mo		$210.00		$315.00	$0	$630	$0	$945	$1,575
4 SITE PREPARATION AND MBT SAMPLING											
4.1 Underground Utility Clearance	1	ls	$7,500.00				$7,500	$0	$0	$0	$7,500
4.2 Equipment Decon Pac	1	ls		$1,850.00			$0	$1,850	$0	$0	$1,850
4.2 Consumerables and Supplies (filter, tubing, shipping	30	per sample		$15.00			$0	$450	$0	$0	$450
4.3 Operator Labor	30	per sample			$75.00		$0	$0	$2,250	$0	$2,250
4.4 Equipment Maintenance and Calibratio	30	per sample				$10.00	0	$0	$0	$300	$300
4.5 Purge Water Disposal	30	per sample	$10.00				$300	$0	$0	$0	$300
4.6 Laboratory Analysis (qPCR)	0	per sample	$350.00				$0	$0	$0	$0	$0
4.7 Laboratory Analysis (non-qPCR)	1	ls	$8,800.00				$8,800	$0	$0	$0	$8,800
7 MONITORING WELL INSTALLATION SITES											
7.1 Well Installation & Development (30 wells	750	lf	$80.00				$60,000	$0	$0	$0	$60,000
7.2 Technician	13	day			$273.00		0	$0	$3,549	$0	$3,549
7.3 Protective Well Casing & Apror	20	ea	$750.00				$15,000	$0	$0	$0	$15,000
7.4 Flush Well Casing & Apror	10	ea	$550.00				$5,500	$0	$0	$0	$5,500
7.5 IDW Transport & Disposal, solid non-haz	25	drum	$185.00				$4,625	$0	$0	$0	$4,625
7.6 IDW Transport & Disposal, liquid non-haz	8	drum	$175.00				$1,400	$0	$0	$0	$1,400
8 BIO-ENHANCED INJECTIONS											
8.1 DPT Mobilization/Demobilizatior	1	ea	$2,000.00				$2,000	$0	$0	$0	$2,000
8.2 Technician	5	day			$273.00		0	$0	$1,365	$0	$1,365
8.3 DPT Rig	12	day	$2,500.00				$30,000	$0	$0	$0	$30,000
8.4 Injection Point Supplies	1,536	lf	$4.00				$6,144	$0	$0	$0	$6,144
8.5 EVO	1	ls		$20,000.00			$0	$20,000	$0	$0	$20,000
8.6 Drill Asphalt & Repai	50	ea	$95.00				$4,750	$0	$0	$0	$4,750
8.7 KB-1	0	ls		$2,000.00			0	$0	$0	$0	$0
9 SITE RESTORATION											
9.1 Pavement Replacemen	150	sy	$46.00				$6,900	$0	$0	$0	$6,900
9.2 Top Dress Soil	250	cy	$30.00				$7,500	$0	$0	$0	$7,500
9.3 Site Restoration, seed, fertilization, mulch	15	msf	$71.00				$1,065	$0	$0	$0	$1,065
Subtotal							$173,769	$24,560	$55,077	$5,897	$259,303
Overhead on Labor Cost @ 30%									$16,523		$16,523
G & A on Labor Cost @ 10%									$5,508		$5,508
G & A on Material Cost @ 10%								$2,456			$2,456
G & A on Equipment Cost @ 10%										$590	$590
G & A on Subcontract Cost @ 10%							$17,377				$17,377
Tax on Materials and Equipment Cost @ 5%								$1,228		$295	$1,523
Total Direct Cost							$191,146	$28,244	$77,108	$6,782	$303,280
Indirects on Total Direct Cost @ 30%											$90,984
Profit on Total Direct Cost @ 10%											$30,328
Subtotal											$424,592
Health & Safety Monitoring @ 2%											$8,492
Total Field Cost											$433,084
Contingency on Total Field Costs @ 25%											$108,271
Engineering on Total Field Cost @ 6%											$25,985
TOTAL CAPITAL COST											**$567,339**

Item	Item Cost year 1	Item Cost year 2	Item Cost year 3	Item Cost years 4-20	Item Cost every 5 years	Notes
Site Visit	$3,500	$3,500	$3,500	$3,500		Labor and supplies to visit site once a year to inspect Land Use Control
Work Plan Memo	$1,400	$1,400	$1,400	$1,400		Document site visit & Work Plan memo
Sampling	$60,000	$30,000	$30,000	$15,000		Labor and supplies to collect samples from 30 wells using a crew of two, quarterly year 1, semi-annual years 2-3, annual years 4-20.
Analysis/Water for VOCs	$19,200	$9,600	$9,600	$4,800		Analyze groundwater samples for VOCs from 30 wells including QA/QC cost. ($110 for VOC + QA/QC)
Analysis/Water for Natural Attenuation Parameters	$32,000	$16,000	$8,000	$4,000		Analyze groundwater samples for natural attenuation parameters from 17 wells including QA/QC cost.
Report	$8,000	$4,000	$4,000	$2,000		Document sampling events and results
Site Review					$25,000	Five-Year Site Reviews
TOTAL	**$124,100**	**$64,500**	**$56,500**	**$30,700**	**$25,000**	

Present Worth Analysis for site without bioremediation - 20 years

Year	Capital Cost	Annual Cost	Total Year Cost	Annual Discount Rate at 7%	Present Worth
0	$414,067		$414,067	1.000	$414,067
1		$124,100	$124,100	0.935	$116,034
2		$64,500	$64,500	0.873	$56,309
3		$56,500	$56,500	0.816	$46,104
4		$30,700	$30,700	0.763	$23,424
5		$55,700	$55,700	0.713	$39,714
6		$30,700	$30,700	0.666	$20,446
7		$30,700	$30,700	0.623	$19,126
8		$30,700	$30,700	0.582	$17,867
9		$30,700	$30,700	0.544	$16,701
10		$55,700	$55,700	0.508	$28,296
11		$30,700	$30,700	0.475	$14,583
12		$30,700	$30,700	0.444	$13,631
13		$30,700	$30,700	0.415	$12,741
14		$30,700	$30,700	0.388	$11,912
15		$55,700	$55,700	0.362	$20,163
16		$30,700	$30,700	0.339	$10,407
17		$30,700	$30,700	0.317	$9,732
18		$30,700	$30,700	0.296	$9,087
19		$30,700	$30,700	0.277	$8,504
20		$55,700	$55,700	0.258	$14,371
				TOTAL PRESENT WORTH	**$923,217**

Present Worth Analysis for site with biostimulation - 5 years

Year	Capital Cost	Annual Cost	Total Year Cost	Annual Discount Rate at 7%	Present Worth
0	$567,339		$567,339	1.000	$567,339
1		$124,100	$124,100	0.935	$116,034
2		$64,500	$64,500	0.873	$56,309
3	$56,734	$56,500	$113,234	0.816	$92,399
4		$30,700	$30,700	0.763	$23,424
5		$55,700	$55,700	0.713	$39,714
				TOTAL PRESENT WORTH	**$895,219**

Present Worth Analysis for site with bioaugmentation

Year	Capital Cost	Annual (1) Cost	Total Year Cost	Annual Discount Rate at 7%	Present Worth
0	$609,793		$609,793	1.000	$609,793
1		$126,900	$126,900	0.935	$118,652
2		$67,300	$67,300	0.873	$58,753
				TOTAL PRESENT WORTH	**$787,197**

Notes:
1. qPCR analysis added for long term monitoring sampling for bioaugmentation scenario.

Appendix F: Publications & Presentations Generated on this Project

Lebrón, C., E. Petrovskis, F. Löffler, C. Casey, K. Henn, 2005, Application of Nucleic Acid-Based Tools for Monitoring MNA, Biostimulation, and Bioaugmentation at Chlorinated Solvent Sites, 2005 SERDP & ESTCP Partners in Environmental Technology Technical Symposium & Workshop, November 29-December 1, 2005.

Lebrón, C., E. Petrovskis, F. Löffler, C. Casey, K. Henn, 2006, Interim Guidance Protocol, Application of Nucleic Acid-Based Tools for Monitoring MNA, Biostimulation, and Bioaugmentation at Chlorinated Solvent Sites, DoD Environmental Security Technology Certification Program (ESTCP); Project # ER-0518.

Henn, K.W. 2006, Using Molecular Biological Tools (MBTs) to Optimize MNA and Bioremediation, Tetra Tech Technology Transfer (T4) Webcast Series, Internal presentation to Tetra Tech, Inc.

Lebrón, C., E. Petrovskis, F. Löffler, K. Henn, C. Casey, 2006, Application of Nucleic Acid-Based Tools for Monitoring MNA, Biostimulation and Bioaugmentation at Chlorinated Solvent Sites, 2006 SERDP & ESTCP Partners in Environmental Technology Technical Symposium & Workshop, November 28-30, 2006.

Petrovskis, E., C. Lebron, F. Loeffler, K. Henn, K. and C. Casey, 2007, "Application of Nucleic Acid-Based Tools, for Monitoring, Chlorinated Solvent Site Bioremediation," Ninth In Situ and On-Site Bioremediation Symposium, Battelle, Baltimore, MD; May 7-10, 2007.

Lebrón, C., K. Henn, C., E. Petrovskis, K. Ritalahti, 2007, Protocol for Use of Nucleic Acid-Based Tools for Montioring, 2007 SERDP & ESTCP Partners in Environmental Technology Technical Symposium & Workshop, December 4-6, 2007

Singletary, M., June 2007, "Use of Bioaugmentation at NAVFAC SE Environmental Restoration (ER) Sites" Navy ARTT Meeting, Charleston, SC
Mike Singletary, Feb 2008, "Lessons Learned Evaluating the Need for Bioaugmentation at NAVFAC Southeast Chlorinated Solvent Sites, Navy IR Conference, Port Hueneme

Lebrón, C., E. Petrovskis, F.E. Löffler, K. Ritalahti, and K. Henn. 2008. Application of nucleic acid-based tools for monitoring bioremediation at chlorinated solvent sites. 6th International Conference on Remediation of Chlorinated and Recalcitrant Compounds, Monterey, CA, May 19-22, 2008.

Petrovskis, E., Application of Molecular Biological Tools for Site Remediation, Naval Facilities Alternative Restoration Technology Team Workgroup, October 29, 2008.

Lebrón, C., E. Petrovskis, F.E. Löffler, K. Ritalahti, and K. Henn. 2008. Application of Nucleic Acid-Based Tools for Monitoring MNA, Biostimulation, and Bioaugmentation at Chlorinated Solvent Sites. SERDP & ESTCP Partners in Environmental Technology Technical Symposium & Workshop, December 2-4, 2008

Petrovskis, E., Application of Molecular Biological Tools for Site Remediation, Naval Facilities Alternative Restoration Technology Team Workgroup, October 29, 2008.

Ritalahti, K.M, J. Hatt, K.W. Henn, E. Petrovskis, C.Lebrón, and F.E.Löffler, 2009. Standardization and comparison of sampling and DNA extraction procedures for analysis of biomarkers in groundwater, Remediation Technologies Symposium 2009

Petrovskis, E., R. Daprato, C. Lebrón, F. Löffler, K. Ritalahti, and K. Henn. 2009. Nucleic Acid-Based Tools for Monitoring Bioremediation at Chlorinated Solvent Sites, Environment, Energy, and Sustainability Symposium & Exhibition, May 4-7, 2008, Denver, Colorado.

Petrovskis, E., R. Daprato, C. Lebrón, F. Löffler, K. Ritalahti, and K. Henn. 2009. Protocol for Use of Nucleic Acid-Based Tools for Monitoring Bioremediation at Chlorinated Solvent Sites, In Situ and On-Site Bioremediation The Tenth International Symposium Baltimore, May 2009

Petrovskis, E., Application of Molecular Biological Tools for Site Remediation, Navy Remediation Innovative Technology Seminar, Spring 2009.

Löffler, F. E., K. M. Ritalahti and S. H. Zinder. 2010. *Dehalococcoides* and reductive dechlorination. SERDP and ESTCP Remediation Technology Monograph Series. Volume 4: Bioaugmentation for Groundwater Remediation. In Press.

Ritalahti, K. M., C. Cruz-García, E. Padilla-Crespo, J. K. Hatt, and F. E. Löffler. 2010. RNA extraction and cDNA analysis for quantitative assessment of biomarker transcripts in groundwater. In K. N. Timmis (ed.), Handbook of Hydrocarbon and Lipid Microbiology. Springer Berlin Heidelberg, Part 32, 10.1007/978-3-540-77587-4_289, pages 3671-3685.

Ritalahti, K. M., J. K. Hatt, E. Petrovskis, and F. E. Löffler. 2010. Groundwater sampling for nucleic acid biomarker analysis. In K. N. Timmis (ed.), Handbook of Hydrocarbon and Lipid Microbiology. Springer Berlin Heidelberg, Part 31, 10.1007/978-3-540-77587-4_265, pages 3407-3418.

Ritalahti, K.M, J. Hatt, J., V. Lugmayr, K.W. Henn, E. Petrovskis, D. Ogles, G. Davis, C.Lebrón, and F.E. Löffler. 2010. Comparing On-Site to Off-Site Biomass Collection for Dehalococcoides Biomarker Gene Quantification to Predict In Situ Chlorinated Ethene Detoxification Potential, Environmental Science and Technology, In press.

Petrovskis E.A., W. Amber, C. Walker. 2011. Microbial monitoring during bioaugmentation with *Dehalococcoides*. SERDP and ESTCP Remediation Technology Monograph Series. Volume 4: Bioaugmentation for Groundwater Remediation. In Press.

Ritalahti, K.M, J. Hatt, J., V. Lugmayr, K.W. Henn, E. Petrovskis, D. Ogles, G. Davis, C.Lebrón, and F.E. Löffler. 2011. Spatial and Temporal Biochemical and Molecular Biomarker Correlations at Chlorinated Ethene Sites. Environmental Science and Technology, In preparation.