WSRC-STI-2006-00340, Rev. 0

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## (A Research Study of the Monitored Natural Attenuation/Enhanced Attenuation for Chlorinated Solvents Technology Alternative Project)

## January 2, 2007

Washington Savannah River Company
Savannah River Site
Aiken, SC 29808

**Prepared for the U.S. Department of Energy Under Contract Number DEAC09-96-SR18500**

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SAVANNAH RIVER NATIONAL LABORATORY

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**Executive Summary**

Various attenuation mechanisms control the destruction, stabilization, and/or removal of contaminants from contaminated subsurface systems. Measuring the rates of the controlling attenuation mechanisms is a key to employing mass balance as a means to evaluate and monitor the expansion, stability and subsequent shrinkage of a contaminant plume. A team of researchers investigated the use of push-pull tests for measuring reductive dechlorination rates *in situ* at sites with low chlorinated solvent concentrations (<1 ppm). The field research also examined the synergistic use of a suite of geochemical and microbial assays. Previous push-pull tests applied to environmental remediation objectives focused on general hydrological characterization or on designing bioremediation systems by examining the response of the subsurface to stimulation. In this research, the push-pull technique was tested to determine its "low-range" sensitivity and uncertainty. Can these tests quantify relatively low attenuation rates representative of natural attenuation? The results of this research indicate that push-pull testing will be useful for measurement of *in situ* reductive dechlorination rates for chlorinated solvents at "Monitored Natural Attenuation" (MNA) sites. Further, using principal component analysis and other techniques, the research confirmed the usefulness of multiple lines of evidence in site characterization and in up-scaling measurements made in individual wells – especially for sites where there is a geochemical gradient or varying geochemical regimes within the contaminant plume.

**Introduction**

Over the past three decades, much progress has been made in the remediation of soil and groundwater contaminated by chlorinated solvents. Yet these pervasive contaminants continue to present a significant challenge to the U.S. Department of Energy (DOE), other federal agencies, and other public and private organizations. The physical and chemical properties of chlorinated solvents make it difficult to rapidly reach the low concentrations typically set as regulatory limits. These technical challenges often result in high costs and long remediation time frames. In 2003, the DOE through the Office of Environmental Management funded a science-based technical project that uses the U.S. Environmental Protection Agency's technical protocol (EPA, 1998) and directives (EPA, 1999) on Monitored Natural Attenuation (MNA) as the foundation on which to introduce supporting concepts and new scientific developments that will support remediation of chlorinated solvents based on natural attenuation processes. This project supports the direction in which many site owners want to move to complete the remediation of their site(s), that being to complete the active treatment portion of the remedial effort and transition into MNA.

The overarching objective of the effort was to examine environmental remedies that are based on natural processes – remedies such as Monitored Natural Attenuation (MNA) or Enhanced Attenuation (EA). The research program did identify several specific opportunities for advances based on: 1) mass balance as the central framework for attenuation based remedies, 2) scientific advancements and achievements during the past ten years, 3) regulatory and policy development and real-world experience using MNA, and 4) exploration of various ideas for integrating attenuation

remedies into a systematic set of "combined remedies" for contaminated sites. These opportunities are summarized herein and are addressed in more detail in referenced project documents and journal articles, as well as in the technical and regulatory documents being developed within the Interstate Technology and Regulatory Council.

Three topic areas were identified to facilitate development during this project. Each of these topic areas, 1) mass balance, 2) enhanced attenuation (EA), and 3) innovative characterization and monitoring, was explored in terms of policy, basic and applied research, and the results integrated into a technical approach. Each of these topics is documented in stand alone reports, WSRC-STI-2006-00082, WSRC-STI-2006-00083, and WSRC-STI-2006-00084, respectively. In brief, the mass balance efforts are examining methods and tools to allow a site to be evaluated in terms of a system where the inputs, or loading, are compared to the attenuation and destruction mechanisms and outputs from the system to assess if a plume is growing, stable or shrinking. A key in the mass balance is accounting for the key attenuation processes in the system and determining their rates. EA is an emerging concept that is recognized as a transition step between traditional treatments and MNA. EA facilitates and enables natural attenuation processes to occur in a sustainable manner to allow transition from the primary treatment to MNA. EA technologies are designed to either boost the level of the natural attenuation processes or decrease the loading of contaminants to the system for a period of time sufficient to allow the remedial goals to be met over the long-term. For characterization and monitoring, a phased approach based on documenting the site specific mass balance was developed. Tools and techniques to support the approach included direct measures of the biological processes and various tools to support cost-effective long-term monitoring of systems where the natural attenuation processes are the main treatment remedies. The effort revealed opportunities for integrating attenuation mechanisms into a systematic set of "combined remedies" for contaminated sites.

An important portion of this project was a suite of 14 research studies that supported the development of the three topic areas. A research study could support one or more of these three topic areas, with one area identified as the primary target. The following report documents the results of the evaluation of applying push-pull tests as a field method to measure *in situ* rates of reductive dechlorination for sites with low concentrations of chlorinated solvents (<1 ppm) that are either being considered for or in a state of MNA. This effort was led by Aaron Peacock of Microbial Insights and Jack Istok of Oregon State. This study supports the topic area(s) of characterization and monitoring and mass balance. The research challenge was to develop and field test technologies to determine microbial processes in situ using a method that will integrate over distance and volume. The objective of this research project was to expand the role of push-pull tests in characterizing contaminated groundwater to meet the above described research challenge. Previous push-pull tests that were applied to environmental remediation objectives used geochemical measurements to estimate hydrologic properties and/or used amendment solutions during the push phase to help design bioremediation. The bioremediation design work has been for both organic contaminants such as chlorinated solvents and for metals/radionuclides, such as uranium and technetium. In the DOE MNA/EA project the researchers expanded the measured parameters to include

microbial assays and focused on unamended systems. The test results were evaluated for the ability to use the field data to determine natural attenuation rates within the system. The results of the combined tests were used to assess the ongoing and potential microbial processes occurring in situ and direct field-scale correlations between microbial dynamics and degradation rates and potential.

The results of the study indicated that push-pull tests using contaminant surrogates provided information from a relatively large subsurface volume (circa 0.1 to 0.3 $m^3$) under field conditions and provided sufficient sensitivity to estimate natural attenuation rates in most wells. Zero-order transformation rates of trichlorofluoroethene (a surrogate for trichloroethene) ranged from < 0.05 to 1.00 nM/hr. The data correlated with geochemical measurements and principal component analysis to provide practical and useful input to mass balance modeling efforts – two major geochemical and microbial regimes were identified. These regimes, using appropriate field-measured contaminant transformation rates for each, can be incorporated into future contaminant fate and transport models. This study demonstrated for the first time that push-pull tests with reactive tracers can be used to detect and quantify reductive dechlorination of chlorinated ethenes and ethanes under monitored natural attenuation conditions.

As the MNA/EA project's Technical Working Group (TWG) evaluated the importance of mass balance in determining if an attenuation-based remedy would be appropriate for a site, they identified the need to measure the rates of the various attenuation mechanisms. For the most part these rates are estimated based on combining various data sets. A low-cost characterization method that would result in the measure of the *in situ* rates of attenuation would provide valuable input data for mass balance calculations.

The TWG was supportive of this project in that it provides a method to measure in situ rates of attenuation in a cost efficient manner over a large spatial area and the results can be inputted into site groundwater fate and transport models and evaluations/projections of future conditions/sustainability. TWG members found the use of the principal components analysis an innovative approach for ordination and grouping of wells and defining zones of a plume with distinct characteristics. They also noted the researchers effectively integrated chlorinated solvent and daughter products data, geochemical data and data from the use of molecular biological tools with the push-pull test data to provide supporting evidence for their results. Because this is the first time push-pull tests were used for this application it was noted by the TWG that additional tests of this application should be conducted at other locations to validate its use.

**References for Introduction**

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# Final Report: Detecting and quantifying reductive dechlorination during monitored natural attenuation at the Savannah River CBRP site

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**ABSTRACT**

Field sampling and testing were used to investigate the relationship between baseline geochemical and microbial community data and *in situ* reductive dechlorination rates at a site contaminated with trichloroethene (TCE) and carbon tetrachloride (CTET). Ten monitoring wells were selected to represent conditions along a groundwater flow path from the contaminant source zone to a wetlands groundwater discharge zone. Background ground water samples were analyzed for a suite of geochemical and microbial parameters, and push-pull tests with fluorinated reactive tracers were used to measure *in situ* reductive dechlorination rates. Geochemical data provided some evidence that reductive dechlorination was occurring at the site, and microbial data confirmed the presence of known dechlorinating organisms as well as sulfate reducers, iron reducers, and methanogens. A principal component analysis identified three groups of wells with similar geochemical and microbial characteristics. Push-pull tests were conducted using trichlorofluoroethene (TCFE) as a reactive tracer for trichloroethene (TCE) and trichlorofluoromethane (TCFM) as a reactive tracer for tetrachloromethane (carbon tetrachloride or CTET). Injected TCFE was transformed *in situ* to *cis*- and *trans*-dichlorofluoroethene and chlorofluoroethene. In one test, TCFE was completely dechlorinated to fluoroethene. Injected TCFM was transformed *in situ* to dichlorofluoromethane and chlorofluoromethane. Zero-order TCFE transformation rates ranged from < 0.05 to 1.00 nM/hr (< 0.44 to 8.76 μM/yr). TCFE reduction rates were different among the three groups of wells identified by principal component analysis, providing preliminary evidence that geochemical, microbiological, and *in situ* reductive dechlorination rates may provide complimentary and, perhaps similar, information. A

single TCFM transformation rate was estimated as < 0.05 nM/hr (0.44 μM/yr). This study demonstrated for the first time that push-pull tests with reactive tracers can be used to detect and quantify reductive dechlorination of chlorinated ethenes and ethanes under monitored natural attenuation conditions.

## INTRODUCTION

Trichloroethene (TCE), tetrachloromethane (carbon tetrachloride, CTET), and related chlorinated ethenes and ethanes are among the most-frequently detected organic contaminants in groundwater. Under anaerobic conditions, TCE and CTET are transformed via reductive dechlorination pathways to produce less-chlorinated products (Ballapragada et al., 1997; de Best, 1997, 1999; Newell et al., 2000; Adamson and Parkin, 2001; Major et al., 2002). However, the rate and extent of reductive dechlorination and the distribution of transformation products are known to vary widely. For example, complete dechlorination of TCE to ethene has been observed at some sites, whereas incomplete transformation has resulted in the accumulation of dichloroethene (DCE) isomers or vinyl chloride (VC) with no ethene production at other sites (Middeldorp et al., 1999).

Reliable information on the occurrence, extent, and rate of reductive dechlorination is particularly important for monitored natural attenuation (MNA) assessments. Currently, environmental assessments utilize a range of techniques to estimate the potential of MNA. These methods include long-term contaminant data supplemented with synoptic geochemical and microbial sampling and numerical modeling to estimate the likelihood that MNA will be successful (see e.g., Wiedemier et al., 1996). Geochemical and microbial indicator data are commonly used to assess whether groundwater conditions are favorable for reductive dechlorination. For example, measurements of electron acceptors (e.g., $O_2$, $NO_3^-$, and $SO_4^{2-}$) or reduced metabolic products (e.g., $HS^-$, $Fe^{2+}$, $Mn^{2+}$) can be used to detect the activity of anaerobic microorganisms, whereas quantitative polymerase chain reaction (qPCR) can demonstrate the presence of

organisms that are known to partially or completely dehalogenate chlorinated ethenes (Ritalahti et al., 2006).

Critical factors in MNA assessments include demonstrating that a particular contaminant transformation reaction is occurring, and determining the reaction rate. Geochemical and microbial indicator data are often used to provide indirect 'lines of evidence' of transformation processes, but can yield inconclusive or conflicting results. Contaminant transformation rates are often estimated by either fitting transport models to monitoring well data using the transformation rate as an adjustable parameter or by conducting laboratory microcosm experiments. However, the applicability of these estimates to field-scale predictions of contaminant transport is unknown. For example, transformation rates obtained by fitting monitoring well data to the models have a high level of uncertainty because the diagnostic transformation products may be present as co-contaminants. The observed changes in contaminant concentrations attributed to transformation reactions may also result from unrelated physical or chemical processes due to site heterogeneity and sparse monitoring. Moreover, transformation rate estimates obtained from microcosm experiments, which do not always accurately mimic *in situ* conditions, should be interpreted with caution.. Thus, there is a demand for the development of field methods that verify the biotransformation of contaminants and quantify *in situ* transformation rates (Madsen, 1991; 1998), especially under MNA conditions, where reaction rates may be slow.

Several previous studies have demonstrated that single-well, push-pull tests are capable of detecting and quantifying a wide range of biotransformation reactions including reductive precipitation of radionuclides (Istok et al., 2004), anaerobic

degradation of aromatic hydrocarbons (Reusseur et al., 2002), and aerobic cometabolism of chlorinated ethenes (Kim et al., 2004). Recently, push-pull tests have been used to measure *in situ* rates of reductive dechlorination of chlorinated ethenes using injected reactive tracers with similar chemical structures and reactivities to the targeted contaminants. These tracer analogs have included trichlorofluoroethene (TCFE) as a reactive tracer for trichloroethene (TCE), *cis*-dichlorofluoroethene (DCFE) for *cis*-DCE, chlorofluoroethene (CFE) for VC, and fluoroethene (FE) for ethene (Figure 1) (Hageman et al., 2001; Ennis et al., 2005; Field et al., 2005). Using gas chromatography/mass spectroscopy, it is possible to selectively and sensitively detect and quantify these reactive tracers and their transformation products in the presence of complex contaminant mixtures. Moreover, laboratory and field studies have demonstrated the correlation between the observed transformation pathways and measured rates of these reactive tracers and their targeted contaminants (Vancheeswaran et al., 1999; Hageman et al., 2001 and 2003; Pon and Semprini, 2003; Field et al., 2005; and Ennis et al., 2005). Thus, the observed transformation of an injected fluorinated reactive tracer during a push-pull test provides unambiguous evidence that subsurface conditions are favorable for the reductive dechlorination of the corresponding chlorinated ethene. Push-pull tests have also been shown to be highly reproducible. Over 100 push-pull tests have been conducted at the Oak Ridge Field Research Center in Oak Ridge, Tennessee investigating the microbial reduction of Tc and U in various aqueous geochemistries.

Previous studies have used the push-pull test methodology to investigate reductive dechlorination of chlorinated ethenes subsequent to biostimulation with exogenous electron donors (e.g. lactate or $H_2$) (see Field et al., 2005). This study represents the first

attempt to apply these techniques to a contaminated environment under MNA conditions. Push-pull tests were conducted in existing monitoring wells using injected TCFE as a reactive tracer for TCE. Because site groundwater also contained trace concentrations of carbon tetrachloride (CTET, tetrachloromethane), we attempted for the first time to measure *in situ* reductive dechlorination rates for a chlorinated ethane by injecting trichlorofluoromethane (TCFM) as an analog for CTET. Given its similar chemical structure to CTET, we hypothesized that TCFM should be reductively dechlorinated following an analogous pathway (Figure 2). As part of this study, we also collected a comprehensive suite of geochemical and microbial indicator data from the wells prior to the push-pull tests. Although geochemical and microbial data are routinely collected during MNA assessments, this study represents the first attempt to investigate relationships between these data and measured *in situ* contaminant transformation rates. While fully investigating the relationships among these disparate data types in a systematic and comprehensive way was beyond the scope of this study, the preliminary data reported here will serve as a platform for future research in MNA assessments.

## METHODS

**Study Area:** Field tests were conducted at The Department of Energy's Savannah River Site (SRS). The C Area Burning/Rubble Pit (CBRP), located near C Reactor, was a shallow, unlined excavation used to dispose of solvents between 1951 and 1973. Characterization and monitoring activities near CBRP have shown that the groundwater is contaminated with volatile organic compounds, primarily TCE, CTET, and their transformation products (Flach et al., 1999).

**Test Wells:** Within the study area, the principal water-bearing formation is an unconfined aquifer comprised of massive beds of sand and clayey sand containing minor interbeds of clay, which are bounded below by low permeable clay. Groundwater in the unconfined aquifer is recharged by surface infiltration and discharges to wetlands and streams (Flach et al., 1999). A suite of test wells was selected to examine the transformation behavior of injected reactive tracers along groundwater flowpaths from the source zone, through the main plume, to a discharge zone at the Twin Lakes wetlands (Figure 3).

**Baseline (Pre-Test) Groundwater Characterization:** Groundwater samples were collected from all wells prior to testing using conventional groundwater sampling protocols (i.e. low flow rate purging until the pH, dissolved oxygen, electrical conductivity, and oxidation-reduction potential stabilized, followed by sample collection). Geochemical analyses performed and methods used for baseline characterization are described in Table 1. Microbiological sampling was accomplished by filtering groundwater through a 0.2 μm filter and freezing the filter onsite until shipment and processing in the laboratory. Phospholipid fatty acid (PLFA) analysis was performed on the samples according to the methods described by Pinkart et al. (2002). 16S rRNA gene diversity was assessed via denatured gradient gel electrophoresis (DGGE) with band excision and sequencing as described by Peacock et al. (2004). For real-time PCR analyses, samples were extracted using MoBio Laboratories (Solana Beach, CA) Power Soil DNA kits according to the manufacturer's recommendations. Real-time PCR was performed on each sample with oligonucleotides designed to target the region of interest (Suzuki, 2000). For Taqman-based assays, one of the

oligonucleotides was a probe containing 6-carboxy-fluorescein (FAM) as a reporter fluorochrome on the 5' end, and N,N,N,N-tetramethyl-6-carboxyrhodamine (TAMRA) as a quencher on the 3' end. Each 30 µl reaction contained 1X TaqMan Universal PCR Master Mix (Applied Biosystems), forward primer, reverse primer, Taqman probe and DNA template from the extracted samples. The PCR conditions were as follows: 2 min at 50°C and 10 min at 95°C, followed by 50 cycles of 15 s at 95°C and 1 min at 58°C. The PCR reaction was carried out in an ABI Prism 7300 Sequence Detection System (Applied Biosystems, Foster City, CA) (Harms, 2003 and Stultz, 2001). For SYBR green-based assays, real-time PCR was performed on an ABI 7000 Sequence Detection System (Applied Biosystems, Foster City, CA). PCR mixtures contained 1 X Cloned Pfu Buffer (Stratagene, LaJolla, CA), 0.2 mM of each of the four deoxynucleoside triphosphates, SYBR green (diluted 1:30,000; Molecular Probes, Eugene, OR), and 1 U of Pfu Turbo HotStart DNA polymerase (Stratagene, LaJolla, CA). Annealing temperatures, primer concentrations, and $MgCl_2$ concentrations varied and were dependent on the primer sets (Greene, 2003 and Hales, 1996). A calibration curve was obtained via serial dilution of a known concentration of positive control DNA. The CT values obtained from each sample were compared with the standard curve to determine the original sample DNA concentration. $\delta\ ^{13}C$ values for TCE and DCE were assayed according to methods described in Sherwood-Lollar et al. (2001).

**Test Solution Preparation and Injection:** Trichlorofluoroethene (TCFE) was obtained from ABCR Chemicals (Karlsruhe, Germany), and trichlorofluoromethane (TCFM) was obtained from SynQuest Laboratories, Inc. (Alachua, FL). Test solutions were prepared by collecting groundwater from each well in a plastic tank, adding sodium bromide as a

conservative tracer, and degassing with nitrogen gas to mix and deoxygenate the test solution prior to injection. Concentrated aqueous solutions of the reactive tracers were stored in collapsible metalized-film (for TCFE) or teflon (for TCFM) gas-sampling bags (Chromatography Research Supplies, Addison, IL) to prevent volatilization losses. For each push-pull test, ~ 200 L of test solution were injected into the well using a peristaltic pump. TCFE and TCFM were added by metering the solution from the collapsible bag into the main injection line using a second pump head installed on the peristaltic pump. The chemical composition of the test solution was determined by collecting and analyzing samples during injection.

**Transport Tests:** Transport tests were conducted in three wells to obtain retardation factors for site-scale modeling and interpreting transformation test data. Test solutions were prepared and injected as described above. Immediately after completion of the injection phase, a peristaltic pump was used to continuously extract groundwater from the test well until ~ 3 times the injected volume had been extracted. Sufficient samples were collected during the extraction phase and analyzed to prepare breakthrough curves for all injected tracers and their transformation products. Tests were completed within 4 to 6 hours. Retardation factors were estimated from transport test data using the method of Schroth et al. (2001), which assumes that TCFE and TCFM sorption behavior can be described by linear equilibrium isotherms.

**Transformation Tests:** Transformation tests were conducted in ten wells. Test solutions were prepared and injected as described above. Samples of the test solution/groundwater mixture were collected approximately once per week for up to 10 weeks following injection. Dilution-adjusted concentrations for the injected reactive

tracers and their transformation products formed *in situ* were prepared using the "forced mass balance" (FMB) technique developed by Hageman et al. (2003). The FMB technique requires estimates of retardation factors for injected reactive tracers and their transformation products. Retardation factors for TCFE and TCFM were obtained from the transport test data as described above. Retardation factors for TCFE and TCFM transformation products were estimated using the estimated retardation factor for TCFE or TCFM and the octanol:water partition coefficient (Kow) for each compound as follows (using DCFE as an example):

$$R_{TCFE} = 1 + \frac{\rho_b K_{TCFE}}{n} \quad (1a)$$

$$R_{DCFE} = 1 + \frac{\rho_b}{n} \frac{Kow_{DCFE}}{Kow_{TCFE}} K_{TCFE} \quad (1b)$$

where $R_{TCFE}$ is the estimated TCFE retardation factor obtained from a transport test, $K_{TCFE}$ is the computed sediment:water distribution coefficient for TCFE, $Kow_{DCFE}$ and $Kow_{TCFE}$ are octanol:water partition coefficients and $R_{DCFE}$ is the estimated DCFE retardation factor. An analogous procedure was used for all TCFE and TCFM transformation products. Dilution-adjusted concentration profiles were fitted by minimized least squares to obtain *in situ* transformation rates.

**Analytical Methods:** Samples for bromide analysis were collected in 15-mL plastic vials. Samples for volatiles analysis including TCFE, TCFM, and their transformation products were collected in 40-mL glass acid-preserved VOA vials without headspace. Samples were stored and shipped on ice until analyzed. Bromide concentrations were determined using a Dionex model DX-120 ion chromatograph equipped with an electrical

conductivity detector and AS14 column. Concentrations of TCFE, DCFE, CFE, TCFM, DCFM, and FM were determined by headspace analysis with gas chromatography/mass spectrometry (GC/MS) consisting of a Hewlett-Packard model 5890 GC equipped with a 5972 series MS detector. Chromatographic separations were performed on a Supelco (30 m × 0.32 mm × 4 μm) SPD-1 column. The fluorinated transformation products were positively identified by comparing their spectra, which were obtained by operating the MS in scan mode, to published spectra. The MS was operated in selected ion monitoring mode for quantitation. 1-Chloropropane and 1-chlorobutane were used as internal standards for gas chromatography.

## RESULTS and DISCUSSION

### Groundwater Characterization:

TCE was detected in every monitoring well except well CRP-41A. TCE concentrations ranged from 3100 μg/L in CRP-20CL (near the source and in the plume) to 30 μg/L in CRP-44B (the wetland groundwater discharge area) (Table 2). Although *cis*-1,2-DCE was detected in every monitoring well except CRP-41A, *trans*-1,2-DCE was only detected in five wetland wells. Vinyl chloride (VC) was only detected in wetland well CRP-48B. Surprisingly, ethene was detected in all of the wells sampled except CRP-42B. The highest ethene concentrations were detected in source well CRP-3D, plume well CRP-18D, and wetland well CRP-48B. Collectively, the chlorinated ethene data provide evidence that reductive dechlorination is occurring at the site, as demonstrated by the detection of *cis*- and *trans*-1,2-DCE in the wetland wells. The detection of VC in one wetland well, which also had the highest measured ethene

concentration, suggests that indigenous microorganisms may have the ability to completely transform TCE to ethene. However, this interpretation is confounded by the widespread distribution of trace ethene in wells without VC .

Stable isotope data for wells CRP-3D, 18D, 29CL, 41A, and 41B had $\delta^{13}C$ values of ~ -26 to -28 ‰ for TCE and -21 to -23 ‰ for *cis*-DCE. More positive (i.e. less negative) $\delta^{13}C$ values for TCE and more negative $\delta^{13}C$ values for *cis*-DCE were obtained for wetland wells CRP-42A, 42B, 43B, 44B. The only positive $\delta^{13}C$ value for TCE (1 ‰) occurred in wetland well CRP-44B. In laboratory studies, reductive dechlorination of TCE has resulted in $\delta^{13}C$ values for TCE as large as 14 ‰ (Bloom et al. 2000; Sherwood Lollar et al. 1999), although field values are usually much lower (Sherwood Lollar et al., 2001). The wetland well data demonstrated more positive $\delta^{13}C$ values for TCE, suggesting microbial preference for the lighter isotope and thus evidence for TCE reductive dechlorination.. These findings are also consistent with the higher measured concentrations of *cis*- and *trans*-DCE, VC, and ethene in those wells.

Carbon tetrachloride was detected above trace levels in wells CRP-20CL and CRP-41A. Chloroform was only detected in these wells and well CRP-3D. Although chloroform is a known reductive dechlorination product of anaerobic CTET transformation (Figure 1), it is also a common co-contaminant with carbon tetrachloride. Therefore, its presence does not provide unequivocal evidence for ongoing carbon tetrachloride reductive dechlorination.

The conclusion that conditions in the wetland wells are favorable for the reductive dechlorination of chlorinated solvents was also supported by geochemical data. However, not all of the measured parameters yielded consistent results (Table 2). For example,

although reductive dechlorination often proceeds under sulfate- or iron-reducing conditions, dissolved oxygen was detected in all wells, with concentrations ranging from 1.5 to 8.2 mg/L. Nitrate was detected in every well except CRP-43D, and nitrite was detected in wells CRP-3D, 41A, 43B and 44B, suggesting that denitrification is an important microbial process. Sulfate but not sulfide was detected at low levels throughout the site, suggesting that sulfate reduction may be a relatively minor process. $Fe^{2+}$ was detected in three wetland wells (CRP-41A, 41B and 48B), indicative of iron reduction. Wells CRP-41A, 41B and 48B also contained the highest methane concentrations. Fermentation products were also detected in several wells. Acetate was detected at trace concentrations (< 1 mg/L) in every well except CRP-18D and 42B, and the highest concentration occurred in wetland well CRP-44B (2.25 mg/L). Lactate was detected in wells CRP-20CL and 44B, and propionate was detected in wells CRP-41A and 44B. Curiously, soluble organic carbon was not detected in any well. Oxygen reduction potential (ORP) values were highly variable and ranged from -125.3 to 135 mV, and the most negative ORP values occurred in two wetland wells.

Microbial data provided additional evidence of increased microbial activity in wetland wells (Table 3). Biomass estimates derived from Eubacterial Q-PCR results were highest in wetland well CRP-43B (~$10^6$ copies/mL) and lowest in plume well CRP-18D (~$10^3$ copies/mL). Q-PCR results were generally consistent with trends in microbial activity inferred from geochemical data. The highest numbers of iron- and sulfate-reducing bacteria were detected in the wetland wells, and methanogens were detected in all wells except well CRP-18D. *Dehalococcoides* spp. (DHC), which are capable of complete dechlorination of TCE, were detected in six wells. The highest abundance of

DHC was detected in well CRP-48B, which also had the highest VC and ethene concentrations.

Biomass estimates derived from phospholipid fatty acids (PLFA) analysis ranged from below detection to ~$10^7$ per mL, with the largest value in well CRP-44B. PLFA profiles showed that the microbial community compositions varied considerably among the wells. In three of the four wells with the lowest biomass (CRP-3D, 20 CL, 41B, and 41A), only monoenoic PLFA and normal saturated PLFA were detected, indicating the presence of relatively simple microbial communities. Monoenoic PLFA are indicative of Gram negative Proteobacteria. Normal saturated PLFA are general biomarkers found in all living organisms, and therefore, their presence does not provide significant insight into the microbial community composition. Well CRP-48B had a notable proportion of eukaryotic biomarkers (polyenoic PLFA) but primarily contained normal saturated PLFA. Eukaryotic biomarkers were detected in five of the wells, in proportions ranging from ~1 to 12 %, with the highest proportion in well CRP-48B. The specific biomarker detected in these wells was 18:2ω6, which is often attributed to the presence of fungi (Pinkart et al., 2002). Three wells contained biomarkers indicative of the presence of anaerobes, and these wells also had the highest biomass estimates. Well CRP-42A had a large proportion of mid-chain-branched saturated PLFA (~30%), which are attributed to the presence of sulfate-reducing bacteria and/or Actinomycetes. Wells CRP-44B and CRP-43B had notable proportions of Firmicutes (shown by terminally-branched saturated PLFA) (~6 % and ~2 %, respectively). Firmicutes include *Clostridia*/*Bacteroides*-like fermenting bacteria. The presence of fermenting bacteria is important at locations

contaminated with chlorinated solvents because fermenters can provide the $H_2$ necessary for reductive dechlorination.

The DGGE profiles contained numerous bands, several of which were faint (Figure 4). Several prominent bands were excised and subjected to PCR and sequence analysis to identify the closest known bacterial genus based on sequence similarity. Phylogenetic affiliations were determined by comparing the sample 16S rRNA gene sequences to known bacterial sequences in the Ribosomal Database Project (RDP) and/or the National Center for Biotechnology Information database (GenBank). For each sequenced PCR product, the closest described match is reported (Table 4).

A principal component analysis (PCA) of the geochemical and microbial data was used to investigate overall patterns of similarity among the wells (Figure 5). The results show that the wells can be separated into three distinct groups. The first group contained wells CRP-3D, 20CL, and 18D, which had relatively higher concentrations of dissolved oxygen, nitrate and TCE, and relatively lower concentrations of less-chlorinated ethenes. Group 2 contained wells CRP-42A, 43B, 44B and possibly CRP-42B, and had higher concentrations of $CO_2$, methane, *trans*-1,2-DCE, acetate, mid-chain-branched saturated PLFA, terminally-branched saturated PLFA, and methanogens. Group 3 contained wells CRP-41A, 41B, and 48A, and exhibited relatively higher concentrations of $Fe^{2+}$, VC, and ethene, as well as higher abundances of *Dehalococcoides* spp.

**Transport Tests:** Extraction phase breakthrough curves for TCFE and TCFM exhibited slightly larger apparent dispersion compared to the coinjected $Br^-$ tracer, which is attributed to sorption (Figure 6). Model fits obtained using the method of Schroth et al. (2001) were generally good, and the estimated retardation factors for TCFE were

between 1.1 and 2.5, with an average of 1.9 (Table 5). Estimated retardation factors for TCFM were somewhat larger, ranging from 1.9 to 5.1, with an average of 3.3 (Table 5). These results were expected because TCFM is more hydrophobic, having a lower aqueous solubility and higher octanol:water partition coefficient than TCFE. The method of Schroth et al. (2001) also provides estimates for aquifer dispersivity, and these values ranged from 0.5 to 2.0 cm, with an average of 1.1 cm (Table 5).

**Transformation Tests:** *In situ* transformation of injected TCFE was observed in wells CRP-3D, 18D, 41A, 41B, 22CL, and 48A. *In situ* transformation of injected TCFM was also observed in well CRP-3D. Unequivocal evidence that aquifer conditions support the reductive dechlorination of TCFE and TCFM (and, by analogy, TCE and CTET) was demonstrated by the detection of diagnostic reductive dechlorination products from these tracers including *cis*- and *trans*-DCFE, CFE, FE, DCFM, and CFM. However, in most tests FE was not detected (see below), and our analytical method was unable to quantify FM. In this test we were only able to monitor the reductive daughter products of TCFE and TCFM. Doubtless there may be other mechanisms at work in regards to the attenuation of the reactive tracers (e.g. oxidation or mineralization). We were not able to measure these processes, but using the mass balance approach that the push-pull affords we could quantify the reductive processes.

In well CRP-41B, injected TCFE was transformed to DCFE and CFE during the ~1,800-hour duration test (Figure 7). As in all tests, transformation product concentrations were very low, and only a small fraction of the injected TCFE was transformed to less-chlorinated products. For this test, the maximum DCFE concentration after dilution-adjustment was only ~ 270 nM, which was ~1 % of the

injected TCFE concentration (Figure 7). The estimated TCFE transformation rate was 0.001/day. FE was not detected, which may be due to an inability of the indigenous microorganisms to dechlorinate CFE or limitations of the methodology in measuring extremely low transformation rates. Variable groundwater flow velocities in the test wells resulted in variable dilution rates for injected reactive tracers. If transformation proceeds slowly, then the test solution may be transported downgradient from the well before the terminal transformation products are detected. For this reason Ennis et al. (2005) proposed the use of injected CFE to improve the sensitivity of detecting the transformation of CFE to FE.

Transformation of TCFE to DCFE, CFE, and FE was observed in well CRP-48A (Figure 8). The detection of FE is significant and provides definitive evidence that indigenous microorganisms at this location have the metabolic capability to completely dechlorinate TCFE, and by analogy, TCE. The estimated TCFE transformation rate was 0.002/day (Table 6).

Measured reductive dechlorination rates for TCFE across all tests were highly variable, ranging from < 0.0005 to 0.002/day (Table 6). These rates are orders of magnitude slower than TCFE transformation rates (0.05 to 1.6/day) reported in previous field tests by Hageman et al. (2001, 2003) and Field et al. (2005). The slower rates in this study may be attributed to differences in aquifer and experimental conditions compared to the other field sites. The Hageman et al. (2001) study was conducted under aquifer conditions with significantly higher concentrations of TCE (approaching 4000 μM) and other chlorinated solvents, as well as a wide range of organic contaminants (esp. petroleum hydrocarbons) which could serve as electron donors during reductive

dechlorination. In contrast, the TCE concentrations in the aquifer at the SRS site were very low, and aqueous concentrations of potential electron donors were very low (Table 2). More importantly, in the Hageman et al. (2003) and Field et al. (2005) studies, the microbial activity was stimulated via fumarate, hydrogen, and lactate amendments to increase reductive dechlorination rates.

These results represent the first *in situ* measurements of TCFE reductive dechlorination rates under low chlorinated solvent concentrations and low microbial activity conditions. These aquifer conditions are typical of many dilute contaminant plumes for which MNA may be an appropriate remedy. In practice, the rate measurements (and retardation factors obtained from transport tests) can be used as inputs to site-scale numerical flow and transport models, which would then be used to evaluate the ability of MNA to meet site cleanup goals. The sensitivity of the push-pull tests are dependent on the analytical methods used to assess the daughter products of the reactive tracers.

Interestingly, measured TCFE reductive dechlorination rates were in qualitative agreement with the results of the principal component analysis of geochemical and microbial indicator data (Figure 8). Wells in Groups 1 and 2 had the lowest estimated TCFE transformation rates (<= 0.001/day), while wells in Group 3 had the highest transformation rates (~ 0.002/day). Although a larger data set would be needed to evaluate the statistical significance of this correlation, the results suggest that it may be possible to evaluate relationships among geochemical and microbial indicator and reductive dechlorination rates.

TCFM was injected in a single test in source well CRP-3D, and unequivocal evidence for *in situ* TCFM transformation was demonstrated by the detection of the diagnostic transformation products DCFM and CFM. FM could not be resolved by our analytical method (Figure 9). The maximum dilution-adjusted concentration for DCFM was ~ 0.9 μM, which is ~ 5 % of the injected TCFM concentration. The estimated TCFM transformation rate was 0.001 /day.

## CONCLUSIONS

This study presented the first successful detection and quantification of *in situ* reductive dechlorination activity for injected TCFE in the absence of exogenous donors to stimulate microbial activity in the subsurface. Because the test solutions were prepared with site groundwater and interrogated a large volume of the subsurface (~ 0.1 to 0.3 $m^3$), they are reasonably representative of the metabolic capabilities of indigenous microorganisms under the specific biogeochemical conditions existing in this aquifer. This study also showed that reductive dechlorination of TCFM can be detected and suggests that the suitability of this compound as a reactive tracer to monitor the reductive dechlorination of carbon tetrachloride (CTET) should be explored in additional laboratory and field studies. An important first step would be to investigate the ability of known dechlorinating organisms to transform TCFM and to compare transformation rates for TCFM and CTET under similar conditions.

*In situ* monitored natural attenuation or enhanced attenuation are often the most cost-effective methods of remediating groundwater contaminated with low concentrations of chlorinated solvents. The key to demonstrating the potential efficacy of monitored

natural attenuation (MNA) at a site is establishing cause-and-effect relationships, which provide direct evidence that biodegradative processes are either occurring or that these processes can be enhanced. The use of reactive tracers like TCFE and TCFM provides direct and unequivocal evidence that biotransformation is occurring *in situ* and should be a useful approach for performing monitored natural attenuation assessments at chlorinated solvent sites.

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**Table 1. Geochemical analyses and methodologies.**

| Analyte Class | Analyte | Method |
|---|---|---|
| Alkalinity | Alkalinity | EPA 310.1[i] |
| IC-Anions | Chloride<br>Nitrate<br>Nitrite<br>Sulfate | SW846-9056[ii] |
| IC-Cations | Divalent Manganese<br>Ferric Iron<br>Ferrous Iron | SW846-7199M[ii, iii] |
| Soluble Organic Carbon | Soluble Organic Carbon | SW846-9060[ii] |
| Sulfide | Sulfide | EPA 376.1[i] |
| Volatiles | 1,1-Dichloroethene<br>*cis*-1,2-Dichloroethene<br>Tetrachloroethene<br>*trans*-1,2-Dichloroethene<br>Trichloroethene<br>Vinyl Chloride | SW846-5030/8260[ii,iv] |
| Volatile Fatty Acids | Acetic Acid<br>Butyric Acid<br>Hexanoic Acid<br>*iso*-Hexanoic Acid<br>*iso*-Pentanoic Acid<br>Lactic Acid<br>Pentanoic Acid<br>Propionic Acid<br>Pyruvic Acid | Microseeps AM23G (ion chromatography) |
| Dissolved Gases | Acetylene<br>Carbon Dioxide<br>Ethane<br>Ethene<br>*iso*-Butane<br>Methane<br>*n*-Butane<br>Propane<br>Propene | Microseeps PM01/AM20GAx (sample preparation through static headspace followed by gas chromatographic analysis of the headspace with either thermocouple detection or flame-ionization detection) |
| Total Inorganic Carbon | Total Inorganic Carbon | Microseeps PM01/AM20GAx[v] |

[i] Refers to USEPA "Methods for Chemical Analysis of Water and Wastes"
[ii] Refers to USEPA "Test Methods for Evaluating Solid Waste"
[iii] SW846—7199M refers to a modification of the method made simply to apply that method to the indicated analytes.
[iv] SW846-5030/8260 refers to the sample introduction method SW846-5030 and the analysis method SW-846 8260.
[v] This is listed separately because the analysis of Total Inorganic Carbon requires a separate analysis from that used for the other dissolved gasses. This implies another set of QC data, etc.

**Table 2. Results of geochemical characterization**

| Sample | Source | Plume | | Buffer | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CRP-3D | CRP-18D | CRP-20CL | CRP-41A | CRP-41B | CRP-42A | CRP-42B | CRP-43B | CRP-44B | CRP-48B |
| Temperature $^{o}$C | 17.6 | 17.5 | 19.8 | 15.8 | 16.9 | 15.2 | 16.6 | 14.9 | 14.9 | 15.5 |
| pH | 5.0 | 6.2 | 5.0 | 6.2 | 6.6 | 4.8 | 4.4 | 5.1 | 4.9 | 6.2 |
| Oxygen reducing potential (r | 100 | 81 | 135 | -80 | -125 | 74 | 101 | 75 | 30 | -77 |
| Electrical conductivity | 38 | 48 | 26 | 53 | 52 | 40 | 21 | 13 | 20 | 56 |
| Oxygen mg/L | 8.2 | 8.0 | 2.8 | 2.1 | 1.5 | 5.8 | 4.6 | 4.5 | 1.7 | 1.8 |
| $NO_3^-$ mg/L | 4.0 | 3.4 | 4.2 | 1.0 | 1.0 | 1.0 | 1.3 | *ND* | 1.0 | 1.0 |
| $NO_2^-$ mg/L | 0.53 | *ND* | ND | 0.53 | *ND* | *ND* | *ND* | 0.53 | 0.53 | *ND* |
| $SO_4^{2-}$ mg/L | 3.3 | 3.8 | 3.2 | 3.5 | 3.2 | 9.7 | 5.1 | 3.6 | 3.3 | 3.5 |
| $HS^-$ mg/L | *ND* | *ND* | *ND* | *ND* | *ND* | *ND* | *ND* | *ND* | *ND* | *ND* |
| $Fe^{2+}$ mg/L | *ND* | *ND* | *ND* | 13 | 13 | *ND* | *ND* | *ND* | *ND* | 13 |
| Methane ug/L | 0.24 | 0.14 | 170 | 0.92 | 0.38 | 4.6 | 1.8 | 130 | 250 | 130 |
| Hydrogen | ND | *ND* | 99 | 7.6 | 0.45 | 0.97 | 0.39 | *ND* | 2.7 | 1.1 |
| TIC mg/L | 51 | 90 | 110 | 97 | 86 | 230 | 57 | 100 | 200 | 96 |
| TCE ug/L | 220 | 220 | 3100 | *ND* | 570 | 440 | 530 | 87 | 30 | 280 |
| 1,1-DCE ug/L | *ND* | *ND* | *ND* | *ND* | *ND* | *ND* | *ND* | *ND* | *ND* | *ND* |
| cis-1,2-DCE ug/L | 58 | 17 | 78 | *ND* | 21 | 200 | 230 | 210 | 400 | 600 |
| trans-1,2-DCE ug/L | *ND* | *ND* | *ND* | *ND* | *ND* | 9.9 | 12 | 8.9 | 21 | 18 |
| VC ug/L | *ND* | *ND* | *ND* | *ND* | *ND* | *ND* | *ND* | *ND* | *ND* | 14 |
| Ethene ng/L | 430 | 160 | 9.8 | 10 | 6.2 | 5.4 | *ND* | 30 | 7.5 | 4800 |
| Acetic Acid mg/L | 0.079 | *ND* | 0.098 | 0.38 | 0.089 | 0.477 | *ND* | 0.095 | 2.25 | 0.092 |
| Carbon Dioxide mg/L | 18 | 16 | 26 | 6.2 | 5.9 | 73 | 46 | 33 | 44 | 10 |
| Ethane ng/L | 190 | 50 | *ND* | *ND* | *ND* | *ND* | *ND* | *ND* | *ND* | 210 |
| Lactic Acid and HIBA mg/L | *ND* | *ND* | 0.334 | *ND* | *ND* | *ND* | *ND* | *ND* | 0.027 | *ND* |
| Propionic acid mg/L | *ND* | *ND* | *ND* | 0.122 | *ND* | *ND* | *ND* | *ND* | 0.146 | *ND* |

**Table 3. Results of microbiological characterization**

| | Source | Plume | | Buffer | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **qPCR (copies/mL)** | **CRP-3D** | **CRP-18D** | **CRP-20CL** | **CRP-41A** | **CRP-41B** | **CRP-42A** | **CRP-42B** | **CRP-43B** | **CRP-44B** | **CRP-48B** |
| Eubacteria16S | 1.84E+06 | 2.03E+03 | 4.96E+04 | 4.81E+06 | 1.57E+06 | 2.02E+06 | 2.10E+06 | 6.98E+06 | 5.86E+03 | 4.05E+05 |
| Methanogens | 3.94E+03 | *ND* | 9.05E+00 | 4.48E+04 | 2.47E+04 | 2.24E+05 | 5.67E+04 | 1.00E+05 | 1.95E+03 | 3.85E+04 |
| Iron reducing bacteria/sulfate reducing bacteria | 2.26E+01 | *ND* | 1.00E+00 | 6.80E+05 | 6.26E+03 | 2.60E+03 | 7.72E+02 | 2.50E+04 | *ND* | 6.61E+02 |
| *Dehalococcoides ethenogenes* | 5.01E+00 | *ND* | *ND* | *ND* | 1.61E+01 | 1.24E+00 | 2.43E+01 | 2.61E+01 | *ND* | 3.38E+03 |
| **DGGE (X = Detected; NS = Not sequenced; % match)** | | | | | | | | | | |
| Unique | | | | | | | | X | | |
| NS | | | | | | X | | X | | |
| Deinococcus (87 %) | | | | | | | | X | | |
| Unc. AY510168 (85 %) | | | | | | | | X | | |
| Pseudomonas (87 %) | | | | X | | | | X | | |
| Unc. AY093473 (89 %) | | | X | X | X | X | | | | X |
| Erythrobacter (97 %) | X | | | | | | | | | |
| Sulfuricurvum (97 %) | | | | X | X | | X | X | | X |
| Rhodoferax (89 %) | | | | X | | | | | | X |
| NS | | | | | X | | | | | |
| Rhodoferax (100 %) | | | | X | | | | X | | |
| Propionovibrio (81 %) | | | | X | | | | | | |
| Acidobacterium (69 %) | | | | | | | | | | X |
| Ralstonia (96 %) | X | | | | | | | | | |
| Propionovibrio (94 %) | | | | | X | | | | | |
| Beijerinckia (93 %) | | | | X | | X | X | | | |
| Pelistega (82 %) | X | | | | | | X | | | |
| NS | | | | | | X | | | | |
| NS | | | | | | X | | | | |
| Unique | | | | X | X | X | | | | |
| Sphingomonas (68 %) | X | | | | | | X | | | |
| Arthrobacter (95 %) | | | X | | | | | | | X |
| NS | | | | | | | | | | X |
| NS | | | | | | | | X | | |
| Sphingomonas (72 %) | X | | | | | X | | | | |

| **PLFA** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Viable biomass, cells/ml | 8.60E+03 | 0.00E+00 | 9.27E+02 | 3.49E+03 | 2.95E+03 | 3.29E+04 | 1.11E+04 | 1.19E+05 | 9.11E+06 | 3.96E+03 |
| picomoles prokaryote PLFA/ml | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 6 | 444 | 0 |
| picomoles eukaryote PLFA/ml | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 0 |
| ratio prokaryote:eukaryote | NC | NC | Nc | NC | NC | 9 | 17 | 98 | 39 | 7 |
| Metabolic Status: (Ratio) | | | | | | | | | | |
| group A (cy17:0/16:1w7c) | 0 | *NC* | *NC* | 0 | 0 | 0 | 0.00 | 0 | 0.09 | *NC* |
| group B (cy19:0/18:1w7c) | 0 | *NC* | *NC* | 0 | *NC* | 0 | 0.40 | 0 | 0.00 | *NC* |
| Total | 0 | 0 | 0 | 0 | 0 | 0 | 0.40 | 0 | 0.09 | 0 |
| group A (16:1w7t/16:1w7c) | 0 | *NC* | *NC* | 0 | 0 | 0.00 | 0 | 0.02 | 0.06 | *NC* |
| group B (18:1w7t/18:1w7c) | 0 | *NC* | *NC* | 0 | *NC* | 0.17 | 0 | 0.00 | 0.09 | *NC* |
| Total | 0 | 0 | 0 | 0 | 0 | 0.17 | 0 | 0.02 | 0.14 | 0 |
| Community Structure: (% of Total PLFA) | | | | | | | | | | |
| Firmicutes (TerBrSats) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.49 | 6.25 | 0 |
| Proteobacteria (Monos) | 82.3 | 0 | 100.0 | 60.6 | 38.8 | 41.9 | 64.5 | 67.0 | 68.6 | 21.5 |
| Anaerobic metal reducers (BrMonos) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Actinomycetes (MidBrSats) | 0 | 0 | 0 | 0 | 0 | 30.1 | 0 | 0.6 | 0.8 | 0 |
| General (Nsats) | 17.7 | 0 | 0 | 39.4 | 61.2 | 18.3 | 29.8 | 29.9 | 21.9 | 66.2 |
| Eukaryotes (polyenoics) | 0 | 0 | 0 | 0 | 0 | 9.8 | 5.7 | 1.0 | 2.5 | 12.4 |

[1] method Modified Bligh & Dyer: Detection limit 7 pmoles total PLFA
NA: Not Analyzed NC: Not Calculated ND: Not Detected
[ratios > 0.1 adapting to environmentally induced stress
Starvation Membrane Stress[

**Table 4. Sequence analysis results**

| Band | Similar genus | Similarity Index | Donors | Acceptors | Habitat |
|---|---|---|---|---|---|
| A, C, | *Failed* | | | | |
| B<br>I | *Beijerinckia* | 0.935<br>0.935 | | | |
| C | *Erythrobacter* | 0.972 | organics | $O_2$ | Marine aerobic phototrophic bacteria that are not capable of anaerobic photosynthesis |
| D<br>P | *Ralstonia* | 0.964<br>0.791 | Chlorinated phenols, organics | $O_2$, | Freshwater and marine habitats |
| E | *Pelistega* | 0.828 | | | |
| F,<br>G<br>J | *Sphingomonas* | 0.680<br>0.724<br>0.680 | many organics, recalcitrant compounds | $O_2$ | freshwater & soil aerobe |
| H | *Roseateles* | 1.000 | Organic compounds | $O_2$, ($NO_3^-$) | Aerobic phototrophic bacteria that can survive varying concentrations of oxygen |
| K | *Hydrogenophaga* | 0.836 | $H_2$, organics | $O_2$ | soil |
| L | *Aquaspirillum* | 0.949 | organics | $O_2$ | freshwater, soil |
| M<br>N | *Rhodoferax* | 0.893<br>1.000 | Low [ Sulfer] | $CO_2$ | Contaminated soils Phototrophic |
| O<br>R | *Propionovibrio* | 0.812<br>0.941 | Ferment aromatic hydrocarbons | | Wastewater, Anaerobic, |
| Q | *Unique?* | | | | Closest *Muricauda* 0.527 |
| S | *Unique?* | | | | Closest *Muricauda* 0.491 |
| T | *Uncultured bacterium* | 0.894 | | | AY093473 |
| U | *Uncultured bacterium* | 0.757 | | | AY093464 |
| V | *Acidobacterium* | 0.693 | | | |
| W | *Arthrobacter* | 0.949 | wide range of.organics | $O_2$ | a common soil organism,, canfix nitrogen |
| X | *Unique?* | | | | Closest *Lactobacillus* 0.424 |
| Y | *Deinococcus* | 0.873 | | | |
| Z | *Uncultured* | 0.849 | | | AY510168 |
| aa | *Pseudomonas* | 0.866 | small organics | $O_2$, ($NO_3^-$) | soil, opportunist |
| bb | *Sulfuricurvum* | 0.974 | $S^=$, $S^0$, $S_2O_3^=$, $H_2$, organo-sulfurs | $NO_3^-$, low $O_2$ | anaerobe to microaerophile capable of growing on crude oil under anaerobic conditions. |

**Table 5. Transport test summary**

| Monitoring Well | Estimated dispersivity αL (cm) | Estimated TCFE retardation factor, R | Estimated TCFM retardation factor, R |
|---|---|---|---|
| 20 CL | 2.0 | 1.1 | 1.9 |
| 43B | 0.7 | 2.5 | 5.1 |
| 44A | 0.5 | 2.1 | 3.0 |

**Table 6. Estimated *in situ* reductive dechlorination rates for TCFE**

| Monitoring Well | Rate (1/day) |
|---|---|
| 3D | 0.001 |
| 18D | <0.0005 |
| 41A | 0.002 |
| 41B | 0.001 |
| 22CL | 0.004 |
| 48A | 0.002 |
| 48B | <0.0005 |
| 42A | <0.0005 |
| 42B | <0.0005 |
| 44B | <0.0005 |

**Table 7. Model predictions and uncertainty analysis results**

| Year | Best Estimate of Source Concentration (μg/L) | Mass Flux to River (g/d) | |
|---|---|---|---|
| | | Best Estimate | 95% UCL |
| 1999 | 1143 | 3.2 | 210 |
| 2010 | 628 | 3.8 | 37 |
| 2025 | 277 | 1.7 | 11 |
| 2050 | 69 | 0.4 | 2.4 |

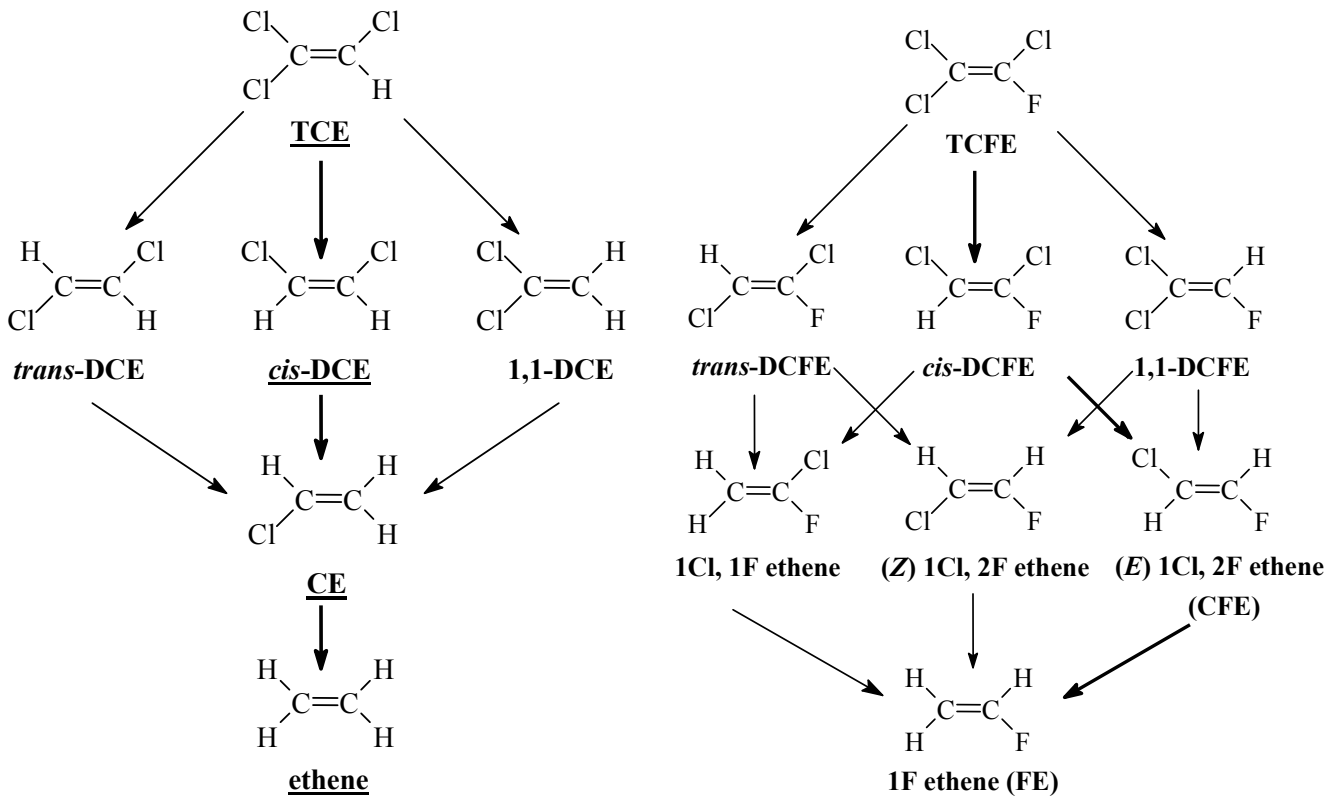


**Figure 1: Reductive dechlorination pathways for TCE and TCFE showing transformation products that may be detected in field push-pull tests. Underlined compounds are the most commonly observed TCE transformation products. Heavy arrows show major transformation pathways observed in laboratory and field studies.**

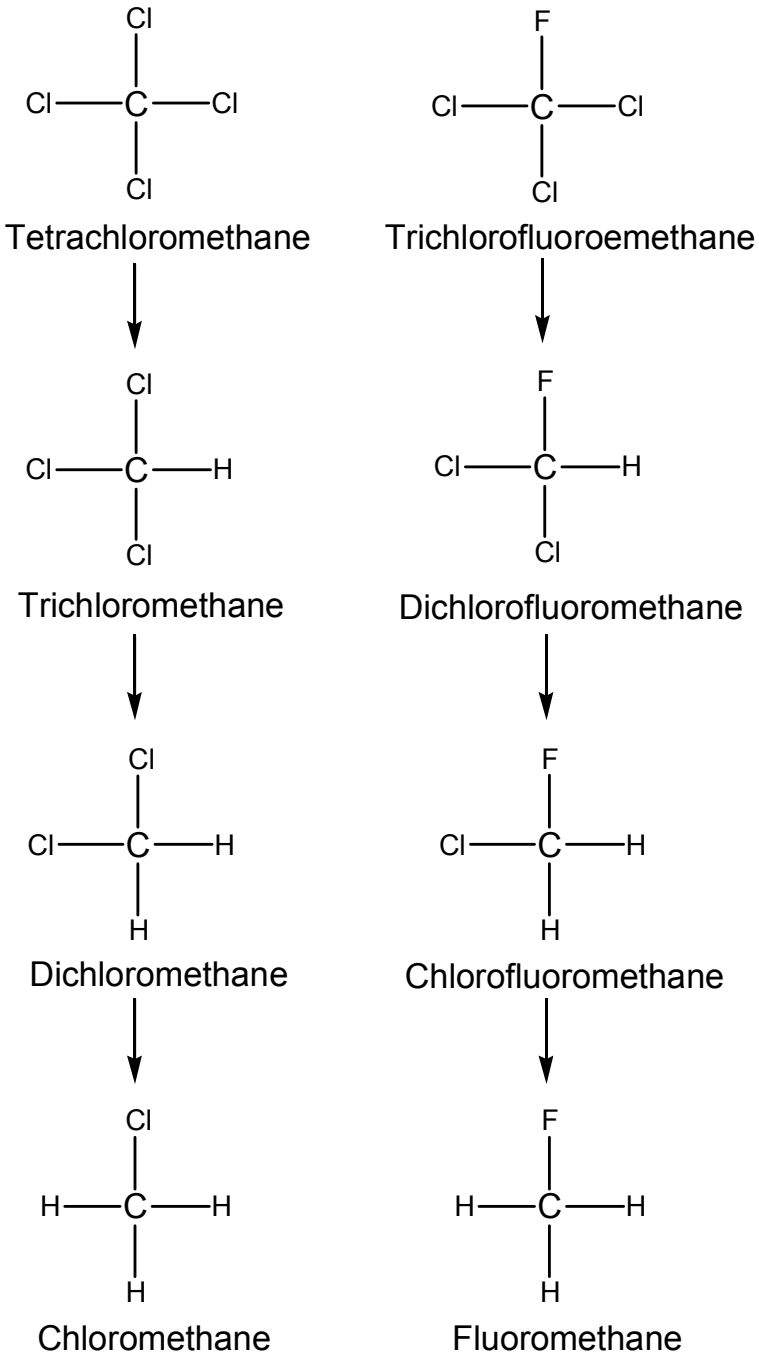


**Figure 2: Reductive dechlorination pathways for tetrachloromethane and TCFM showing transformation products that may be detected in field push-pull tests.**


**Figure 3: Location of test wells near CBRP.**

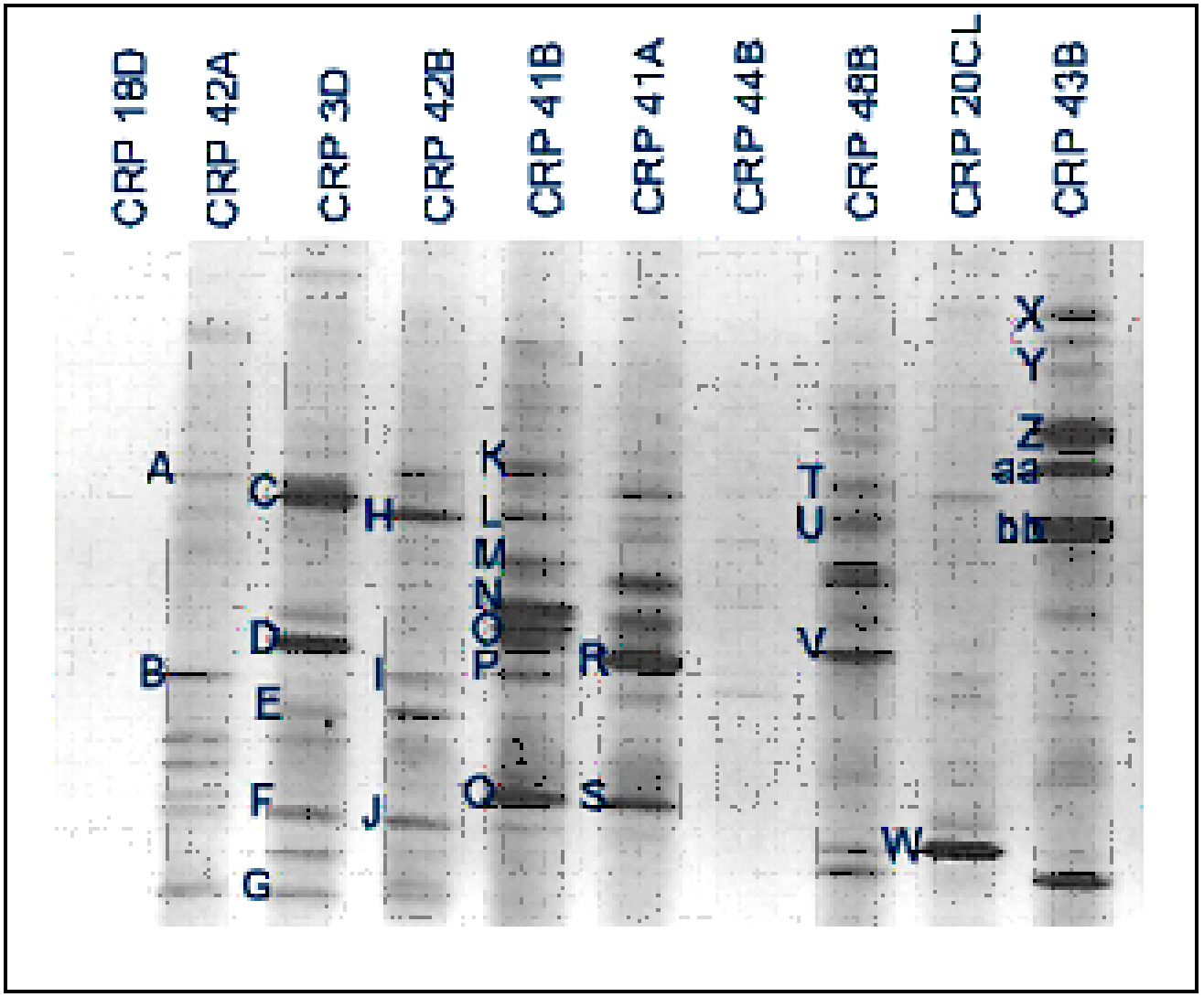


**Figure 4.** DGGE profiles of 16S rRNA genes amplified from DNA extracted from ground water samples

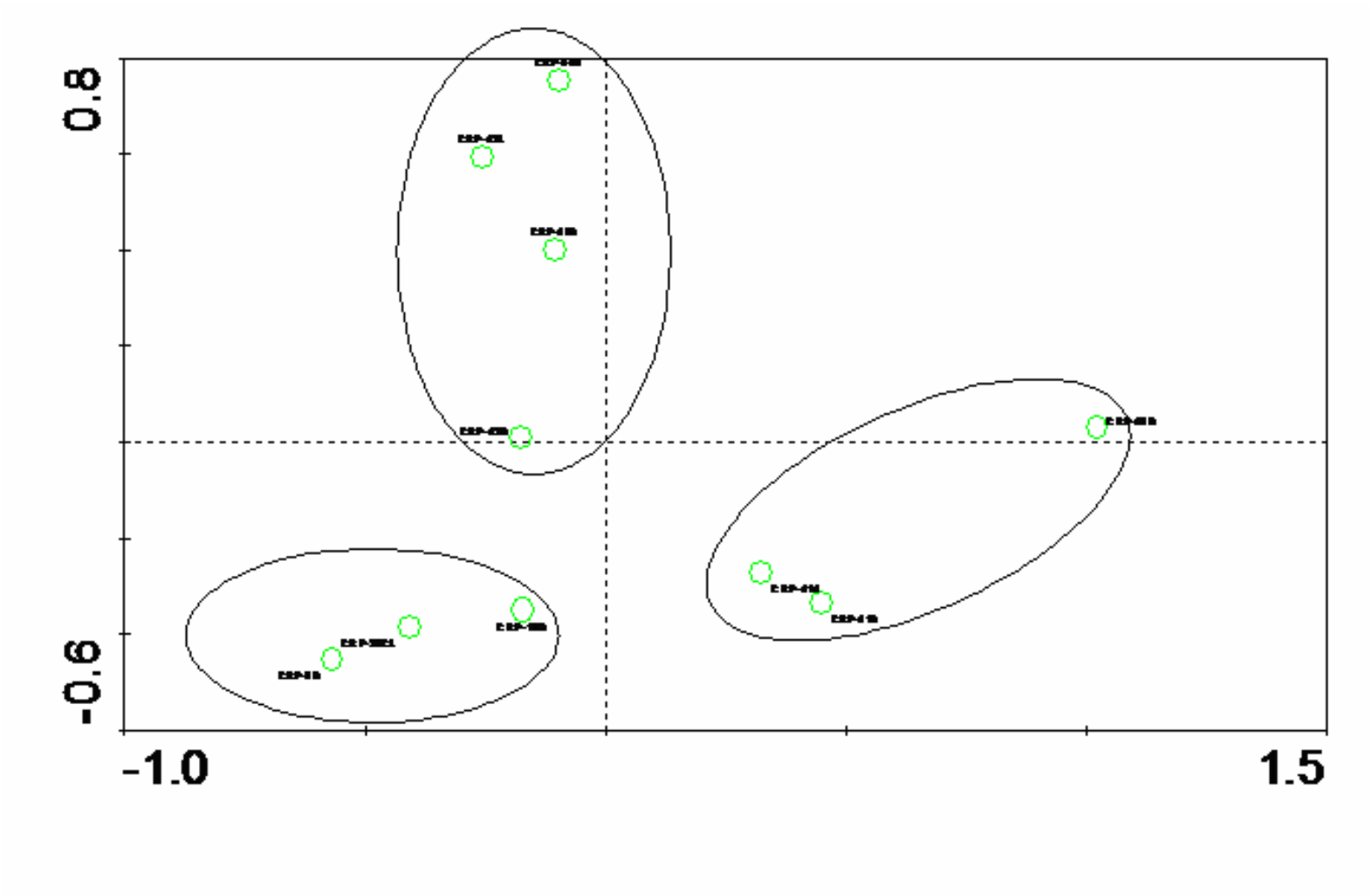


**Figure 5. Principal component analysis (PCA) group separations.**

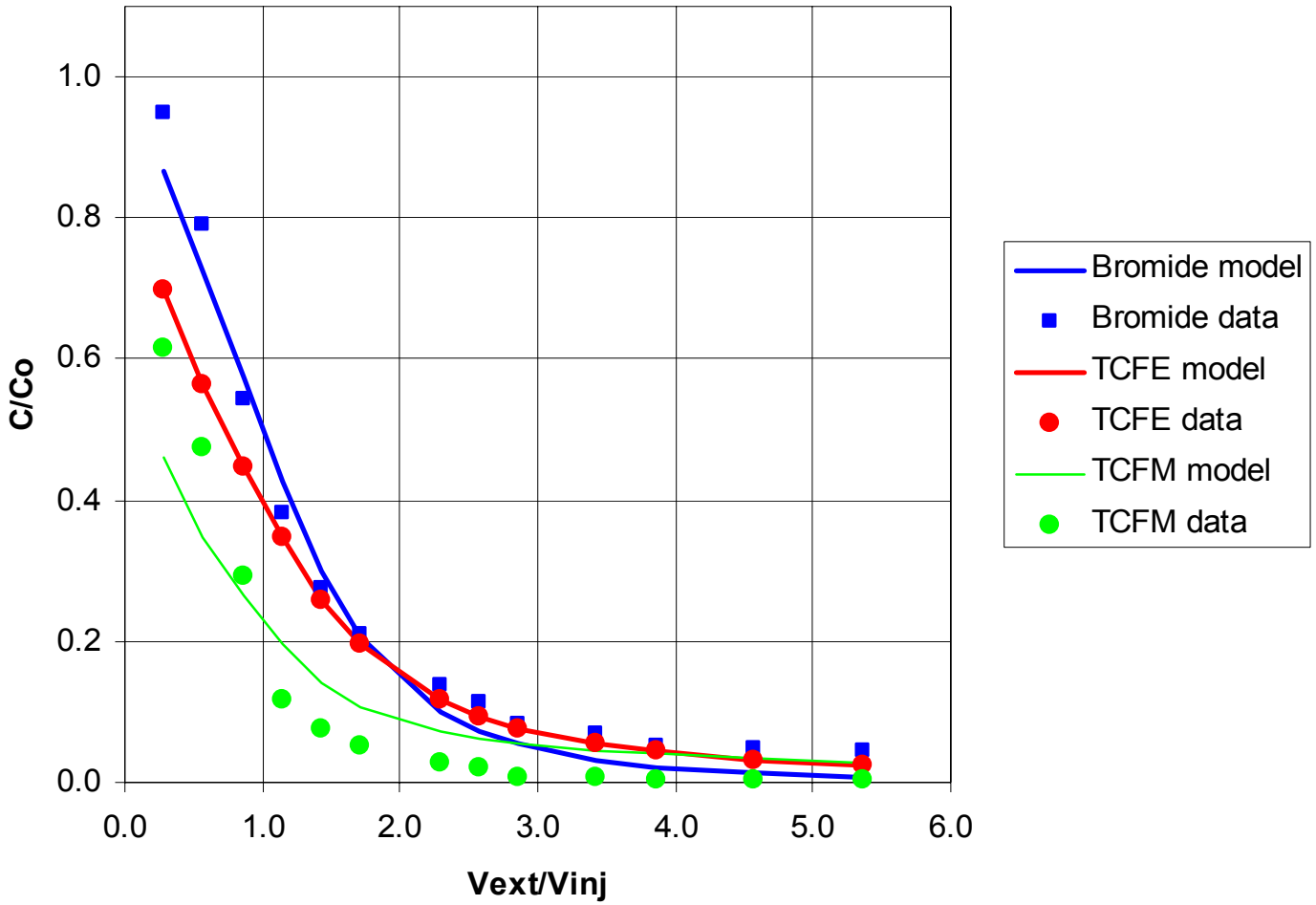


**Figure 6. Transport test results for Test 10 (CRP-43B) showing increased dispersion for TCFE and TCFM compared to co-injected $Br^-$. For TCFE, fitted R = 2.5; for TCFM, fitted R = 5.1.**

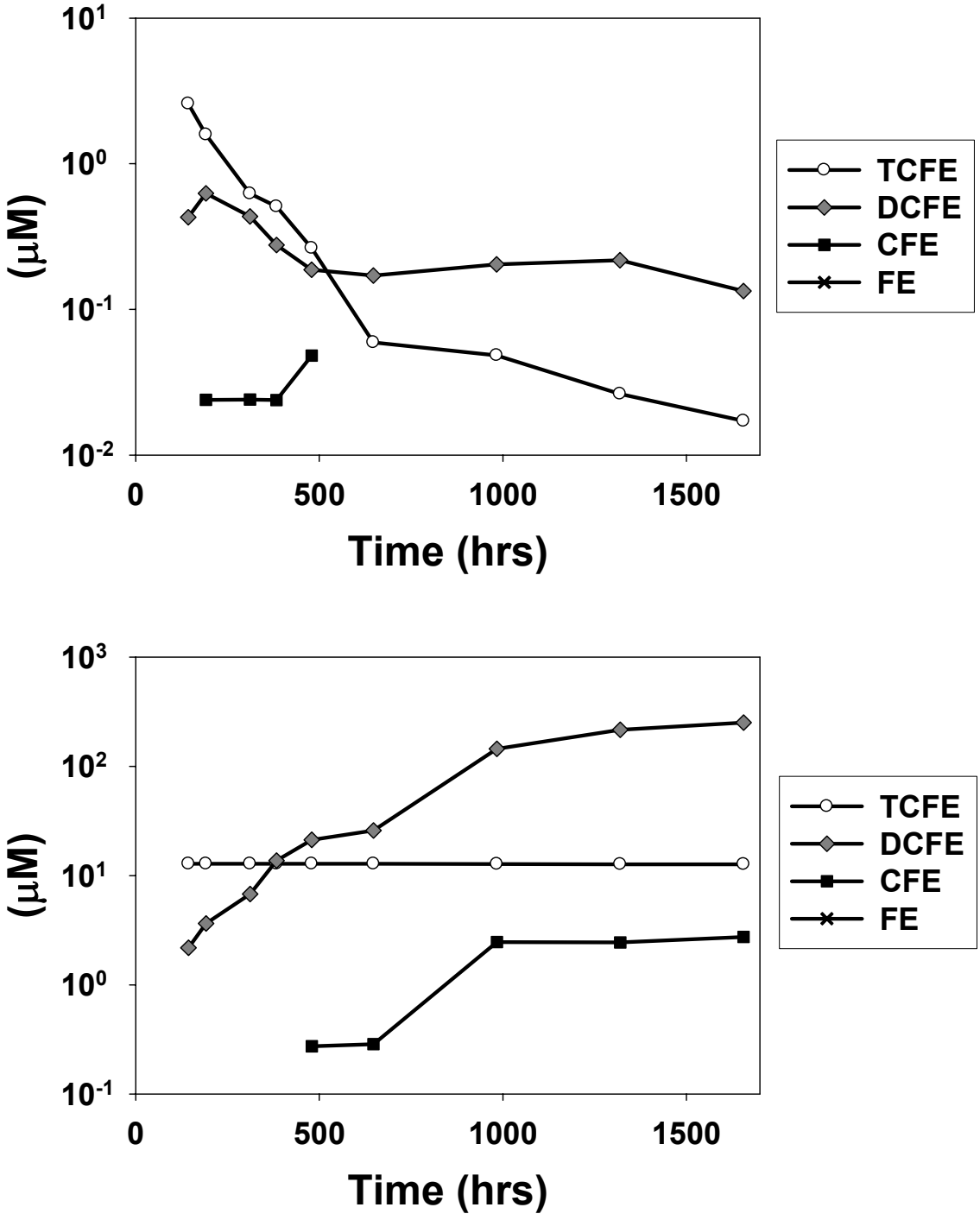


Figure 7. TCFE transformation test results for test 5, well 41B. Raw data (upper) and dilution adjusted data (lower).

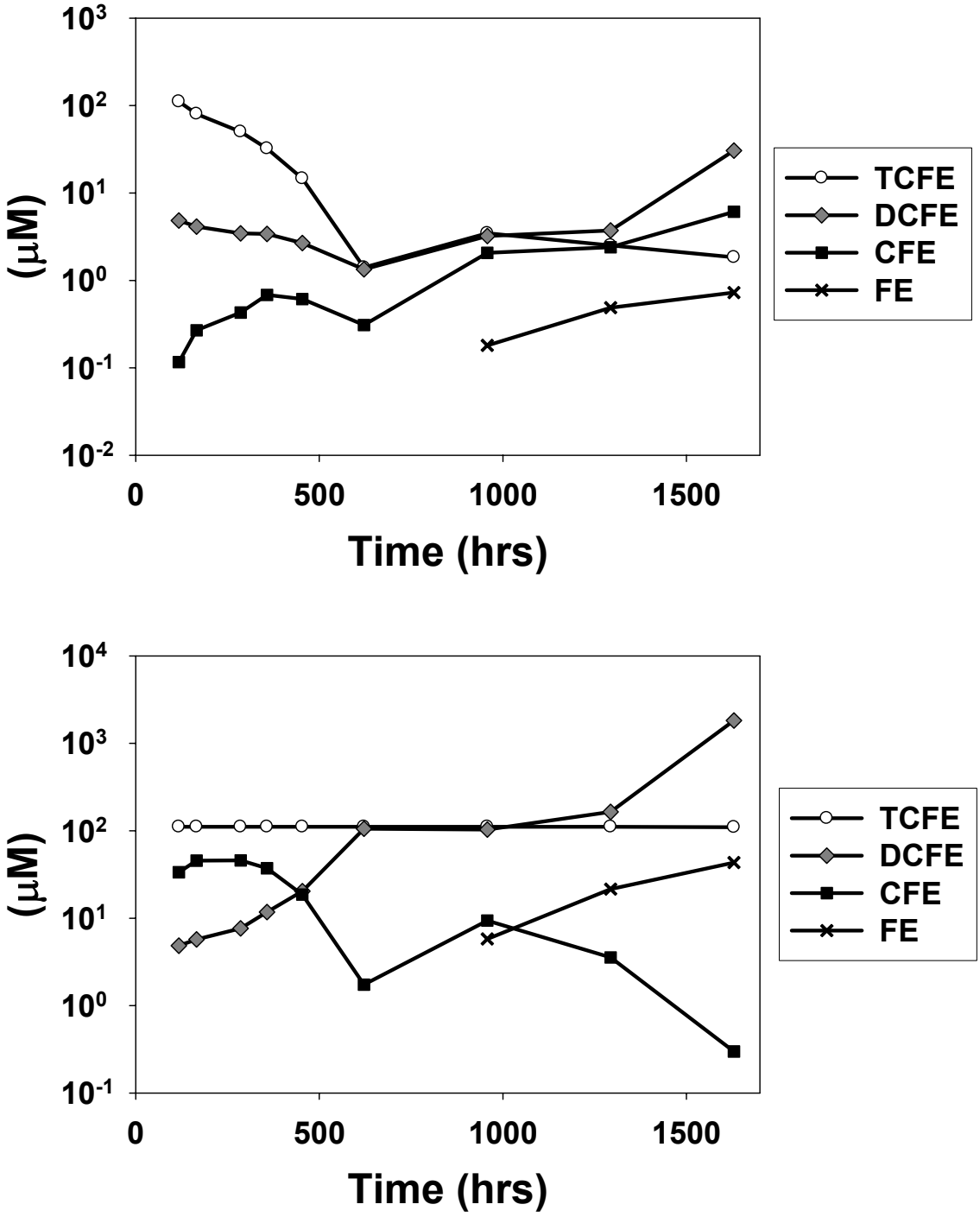


Figure 8. TCFE transformation test results for test 12, well 48A. Raw data (upper) and dilution adjusted data (lower).

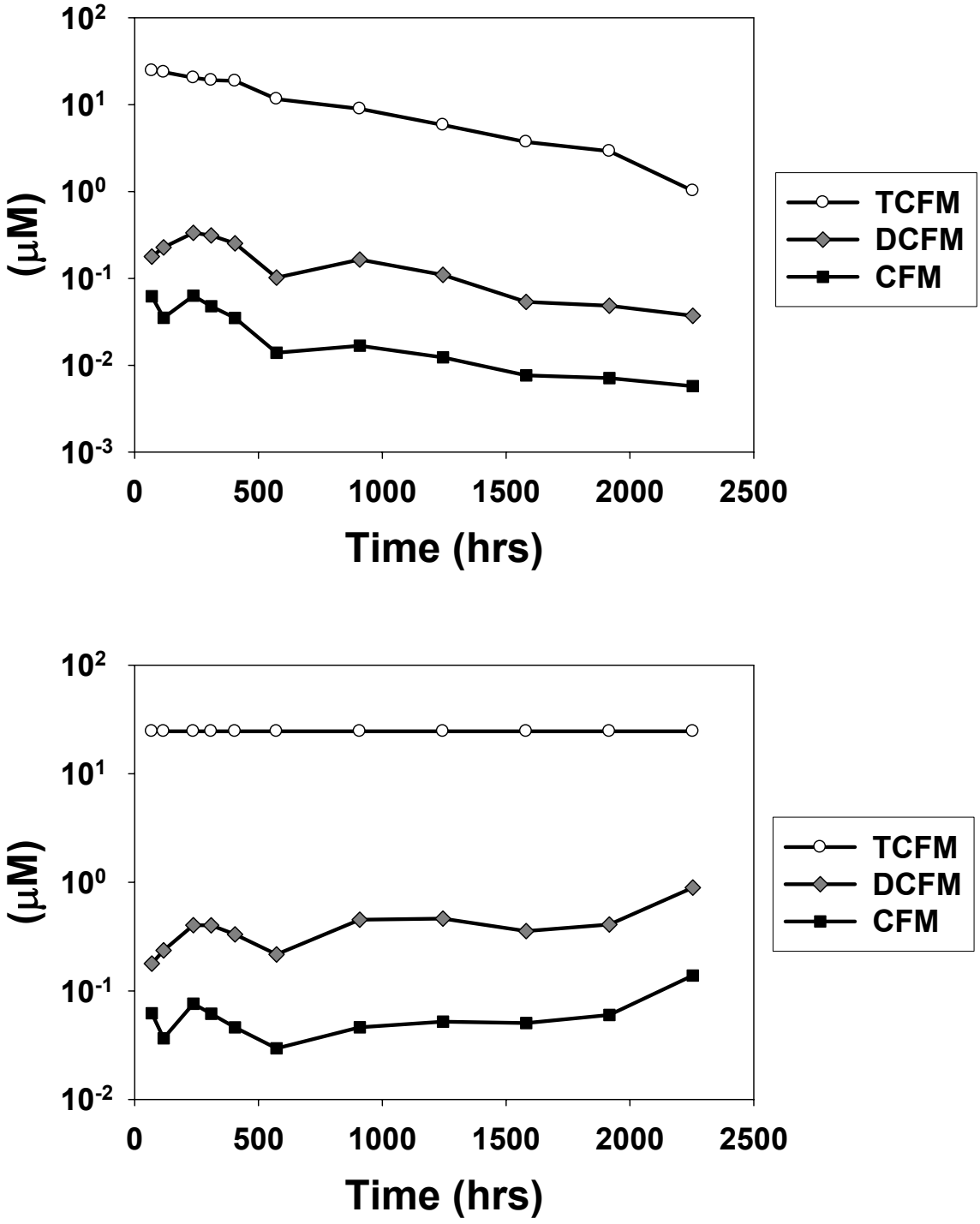


Figure 9. TCFM transformation test results for test 1, well 3D. Raw data (upper) and dilution adjusted data (lower).