Anaerobic Reductive Dechlorination of 1-Chloro-1-fluoroethene To Track the Transformation of Vinyl Chloride

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1-Chloro-1-fluoroethene (1,1-CFE) was studied as a reactive tracer to quantify the anaerobic transformation of vinyl chloride (VC). Batch kinetic studies of 1,1-CFE and VC transformation were performed with an enrichment culture obtained from the Evanite site in Corvallis, OR. The culture is capable of completely transforming trichloroethene (TCE) through cis-dichloroethene (c-DCE) and VC to ethene. The culture also transforms fluorinated analogues, such as trichlorofluoroethene (TCFE), to fluoroethene (FE) as a final product. The transformation sequence of the fluorinated analogue was correlated with that achieved for the chlorinated ethene with the same degree of chloride substitution. For example, the production of 1,1-CFE, the major CFE isomer formed from TCFE transformation, was correlated with the production of VC from TCE transformation. Since the 1,1-CFE and its product, FE, have a distinct analytical signature, 1,1-CFE may be used as a reactive in situ tracer to evaluate the VC transformation potential. The half-saturated constants ($K_s$) of VC and 1,1-CFE were 63 and 87 $\mu$M, respectively, while similar maximum utilization rates ($k_{\text{max}}X$) of 334 and 350 $\mu$M/d were achieved. Acetylene inhibited both VC and 1,1-CFE transformation. A competitive inhibition model with the independently measured $K_s$ values used as the inhibition constants predicted rates of transformation of both VC and 1,1-CFE when both compounds were present. 1,1-CFE transformation was also tested with three different cultures. With all the cultures, 1,1-CFE transformation was associated with VC transformation to ethene, and the rates of transformation were comparable. The results demonstrated that 1,1-CFE was a good reactive surrogate for evaluating the rates of VC transformation.

Introduction

Chlorinated aliphatic hydrocarbons (CAHs), such as tetrachloroethene (PCE) and trichloroethene (TCE), are major groundwater contaminants (1). Anaerobic biotransformation of PCE and TCE sequentially generates cis-dichloroethene (c-DCE) and VC with ethene as a harmless end product. Incomplete transformation of PCE and TCE to c-DCE or VC is frequently observed in CAH-contaminated groundwater, and this limits the usefulness of anaerobic biotransformation as a natural attenuation process (2). Among these products, VC is the most undesirable as it is a known human carcinogen and has the lowest drinking water standard (2 $\mu$g/L) (3).

VC transformation to ethene is generally the most difficult transformation step. High $K_s$ values of c-DCE and VC cause incomplete transformation of TCE and PCE to c-DCE or VC (4). PCE and TCE can also inhibit the transformation of c-DCE and VC (5). Dehalococcoides ethenogenes strain 195 (6) has the ability to transform PCE to ethene; however, the final step of VC to ethene is cometabolic in nature (7). VC halorespiration by mixed cultures (8, 9) has led to the isolation of a pure culture (10) that is capable of using c-DCE and VC as electron acceptors, but not PCE and TCE (9, 10).

Chemical surrogates of TCE and PCE, such as trichlorofluoroethene (TCFE), have been used to track the transformation of PCE and TCE (11, 12) in laboratory and field studies. The advantage of using a surrogate compound in situ is that a unique identification of biotransformation is possible even when the CAHs of interest are present. CAH transformation is difficult to demonstrate when physical processes, such as sorption and desorption and non-aqueous-phase liquid (NAPL) dissolution, are occurring. Fluorinated analogues are resistant to defluorination, and fluorinated products are easily detected by standard gas chromatographic methods. TCFE, for example, has been used in push–pull field tests to determine the PCE and TCE transformation potential (12). However, the observation of the production of dechlorination products beyond cis-1,2-dichloro-fluoroethene (c-DCFE) proved difficult, partly due to dilution by groundwater flow. The direct addition of 1,1-CFE as a surrogate for VC in field tests would limit dilution effects, and 1,1-CFE can be added at desired concentrations to detect product formation, even when dilution occurs.

In laboratory microcosm studies (11), TCFE was transformed into three dichlorofluoroethene isomers (DCFEs), with c-DCE being the main isomer. The DCFE isomers can be potentially transformed into three chlorofluoroethene isomers (CFEs), which can be further transformed into fluoroethene (FE). Vancheeswaran et al. (11) observed limited DCFE transformation to CFE; thus, the major DCFE transformation product was not clearly determined. In our study, we identified the major transformation intermediate of TCFE through c-DCE as 1-chloro-1-fluoroethene (1,1-CFE). This led us to evaluate 1,1-CFE as a potential analogue for the transformation of VC.

The objectives of this study were to (i) identify 1,1-CFE as the main isomer formed by TCFE transformation by the Evanite culture, (ii) determine the transformation kinetic parameters ($k_{\text{max}}X$ and $K_s$) for VC and 1,1-CFE with the Evanite culture, (iii) study the inhibition of VC and 1,1-CFE on the transformation of each other, and acetylene inhibition on the transformation of both compounds, and (iv) evaluate how 1,1-CFE tracks the transformation of VC with different dehalogenating cultures.

Kinetic and inhibition studies were performed to determine the correlation of 1,1-CFE with VC transformation. Values of $k_{\text{max}}X$ and $K_s$ for VC and 1,1-CFE were determined. Inhibition studies were performed to probe whether the same enzyme was likely responsible for transforming the compounds. In a previous study with the Evanite culture, we found acetylene was a reversible inhibitor of the reductive dechlorination (13). Acetylene inhibition of 1,1-CFE and VC transformations was therefore investigated. VC and 1,1-CFE inhibition of each other was tested, and the results were evaluated using a competitive inhibition model. 1,1-CFE transformation was also tested concurrent with TCE and c-DCE transformation to determine whether its transformation correlated with the VC transformation step. Cultures enriched from three different contaminated sites that had different transforming abilities were tested.
Materials and Methods

Chemicals. PCE (99%) and c-DFE (97%) were purchased from Acros (Fisher Scientific, Pittsburgh, PA), and TCE (99.9%) was purchased from Aldrich (Milwaukee, WI). TCFF (97%), c-DFE (98%) consisting of 1–2% t-DFE, 1,1-DFE (98%), and 1,2-DFE (98%) (E/Z mixture consisting of 50% E and 50% Z isomers) were purchased from SynQuest Labs., Inc. (Alachua, FL). Gas standards, ethene (1000 ppm Z isomers) were purchased from Scott Specialty Gases (Alltech Associates, Inc., Deerfield, IL). Methane (99%), CO2 (99%), acetylene (99.6%), and H2 (99%) were obtained from AIRCO (Vancouver, WA). Yeast extract was purchased from Fisher Scientific (Fair Lawn, NJ).

Analytical Methods. The concentrations of TCE, TCFE, c-DFE, c-DFE, 1,1-DFE, VC, acetylene, ethene, and methane in the headspace of batch reactors were determined by gas chromatographic analysis. A headspace sample (100 µL) was injected into an HP-5890 gas chromatograph equipped with a 30 m megabore GSQ-Plot column (by J&W Scientific, Folsom, CA) and connected to a flame-ionized detector (FID). H2 was analyzed by injecting 100 µL of headspace sample into an HP-5890 gas chromatograph. Chromatographic separation was achieved using argon as a carrier gas, with a Supelco 60/80 Carboxen 100 column (15 ft × 0.125 in.; Bellefonte, PA), followed by quantification using a thermal conductivity detector (TCD).

TCFF Transformation Pathway. To evaluate the transformation pathway of TCFE to FE, different isomers of DCFE and CFE had to be identified. Authentic standards were used, and identification was based on GC retention times. The retention times of TCFE, t-DFE, c-DFE, 1,1-DFE, and FE were 8.3, 7.3, 7.1, 5.0, and 2.3 min, respectively. 1,2-DFE (Z) and 1,2-DFE (E) were present as a 50/50 mixture in the authentic standard, yielding two peaks with retention times of 5.3 and 5.6 min.

Growth of the Cultures. The Evanite anaerobic culture was obtained from TCE-contaminated groundwater and sediment at the Evanite site in Corvallis, OR. The growth conditions for the Evanite culture were described by Pon et al. (13). This culture completely transforms PCE through TCE, c-DFE, and VC to ethene. For the VC and 1,1-DFE kinetic tests, the culture was further enriched in the 1000 mL batch reactors fed 5 mL of H2 (200 µmol), 20 mL of VC (800 µmol), and 20 mL of CO2. The growth medium was maintained at a pH of 7 using a phosphate buffer. Additional H2 (5 mL) was added when gas-phase H2 levels fell below 0.5% v/v. The cells were harvested when the VC removal rate in the reactors reached 80 µmol/d.

Two other cultures were tested. The Point Mugu culture was maintained using the same incubation procedures as the Evanite culture, except TCE was used as the electron acceptor instead of PCE. The Point Mugu culture required a substantially longer time to transform VC to ethene than the Evanite culture. The Lawrence Livermore culture that transforms TCE mainly to VC was grown under conditions presented by Yu (14). Methane was not produced by any of the cultures.

The three cultures were examined by using a PCR assay developed to detect the 16S ribosomal RNA gene (rDNA) sequence with Dehalococcoides-specific primers Dhg 728F and 1155 R (15) and Dhc 1F and 1377R (16) and Desulfuromonas-specific primers Dsm 205F and 1015R (15). Specific PCR results showed all three cultures contained Dehalococcoides-like microorganisms, but Desulfuromonas-like microorganisms were not found.

Kinetic Tests. The kinetic tests were performed in 125 mL batch bottles (Wheaton, Millville, NJ) capped with gray butyl rubber septa (Wheaton) to allow temporal sampling. Studies were conducted in single reactors. A 50 mL sample of medium containing cells from the growth reactor was transferred in an anaerobic glovebox to the VC batch reactors. The reactors were purged for 10 min to remove the glovebox H2 and any residual VC or ethene with nitrogen gas that was furnace-treated at 600 °C to remove residual oxygen. H2 (5 mL), CO2 (20 mL) (furnace-treated), and VC or 1,1-DFE were then added to the reactor’s headspace. The reactors were incubated at 20 °C and shaken in an inverted position at 200 rpm. H2 was supplied in excess during the experiment.

$k_{max}X$ and $K_d$ Determinations. A multiple-equilibration method in a single batch reactor was used to determine VC and CFE $k_{max}X$ and $K_d$ values. The test was performed as follows: The transformation rate was first determined at the lowest substrate concentration. The concentration was then increased through VC or 1,1-DFE addition, and the rate was determined at the next desired concentration. Concentrations were successively increased until a maximum rate was achieved. At each concentration the transformation rate of 1,1-DFE or VC was determined on the basis of product formation, FE and ethene, respectively. Rates were determined using the liquid- and gas-phase mass balances and Henry’s law equilibrium, and by linear regression of five product formation data points versus time. Rates of product formation were measured over a wide range of substrate concentrations (10–575 µM for CFE and 25–975 µM for VC). The kinetic parameters $k_{max}X$ and $K_d$ of the 1,1-DFE and VC transformations were determined by nonlinear regression of the Monod equation using the S-Plus program (Insightful Corp., Seattle, WA). The tests were completed within a 12 h period so that cell growth would be limited. An estimated doubling time of the ethene-producing population with growth on VC was approximately 60 h (13).

Acetylene Inhibition Test. The inhibition experiments consisted of three stages, an initial rate determination stage, an inhibition stage, and a recovery stage, as described by Pon et al. (13). The initial transformation rates of 1,1-DFE and VC were determined in the first stage in the absence of acetylene. Acetylene was added as an inhibitor in the second stage. Prior to addition, the acetylene was scrubbed with 10% (v/v) Cu2(SO4)2·5H2O to remove trace amounts of acetone (17). In the third stage, the reactors were purged for 15 min with furnace-treated nitrogen gas to remove the acetylene, and the transformation rates were remeasured upon VC or 1,1-DFE and H2 addition.

1,1-DFE Transformation Tests with Three Different Cultures. Tests were performed with the three different cultures previously described to verify that 1,1-DFE transformation solely tracks the transformation of VC. The studies were conducted in single reactors using the same procedures as the kinetic tests, except 1,1-DFE was added along with TCE. Henry’s law constants for PCE, TCE, c-DFE, VC, and ethene were those published by Gossett (18). Dimensionless Henry’s constants for 1,1-DFE and FE of 3.55 and 2.66, respectively, were determined using the successive air–water equilibration method at 20 °C (19).

Results

PCE and TCE Transformation. An initial study was performed to determine the transformation pathway of TCFE and to verify that 1,1-DFE was the main isomer produced by the Evanite culture. Figure 1 shows the culture transformed PCE through TCE, c-DFE, and VC to ethene, while TCFE was transformed through c-DFE and 1,1-DFE to FE. PCE was transformed to c-DFE during the first 70 d of the incubation, and TCFE was transformed to c-DFE over the same time interval. VC and 1,1-DFE were produced at similar rates from the transformation of c-DFE and c-DFE, respectively. The results indicated c-DFE was a good surrogate for c-DFE transformation. A similar transformation trend was also observed for the subsequent transformation of VC and 1,1-
CFE to ethene and FE, respectively. This initial study demonstrated the fluorinated analogues c-DCFE and 1,1-CFE followed c-DCE and VC transformation patterns, respectively. The parallel transformation pathways of TCE and TCFE are shown in Figure 2. TCFE is potentially transformed to three DCFE isomers, with c-DCFE being the main isomer formed. c-DCFE is mainly transformed to 1,1-CFE with FE as the end product, while TCE is transformed to c-DCE, VC, and ethene. TCFE was transformed mainly to c-DCFE with some t-DCFE (two GC peaks had relative percent areas of 96% and 4%). 1,1-Dichloro-2-fluoroethene (1,1-DCFE) was not detected. c-DCFE was further transformed mainly to 1,1-CFE. A small GC peak with a retention time of 5.3 min was probably 1,2-CFE (E), since it is more likely produced from c-DCFE transformation due to the location of the fluoride substituent (Figure 2).

**Kinetic Study for 1,1-CFE and VC.** The results of the multiple-equilibration method to determine \( k_{\text{max}}X \) and \( K_s \) values for 1,1-CFE are presented in Figure 3. The increases in 1,1-CFE concentration and the corresponding increases in FE production rates are shown in Figure 3A. 1,1-CFE concentrations were increased until transformation rates close to the maximum were reached. The resulting Monod plot of transformation rate versus concentration is shown in Figure 3B, along with the nonlinear regression fit to the Monod equation and the 95% confidence intervals. \( k_{\text{max}}X \) and \( K_s \) for 1,1-CFE transformation were 350 \((\pm 10) \mu \text{M/day}\) and 87 \(\pm 8 \mu \text{M}\), respectively. The same procedure was used to determine the \( k_{\text{max}}X \) and \( K_s \) values for VC transformation (data not shown), with estimated values of 334 \((\pm 11) \mu \text{M/day}\) and 63 \(\pm 7 \mu \text{M}\), respectively. The results showed 1,1-CFE and VC had very similar \( k_{\text{max}}X \) and \( K_s \) values, and therefore similar transformation rates.

**VC and Acetylene Inhibition Studies on 1,1-CFE Transformation.** Results of experiments on the inhibition between VC and 1,1-CFE and the inhibition of the transformation of both compounds by acetylene are presented in Figure 4 and Table 1. Two reactors were initially given the same amount of 1,1-CFE. Different amounts of VC were then added at 85 h to evaluate VC inhibition of 1,1-CFE transformation (Table 1).

Figure 4A shows a fairly constant rate of FE production until VC was added at 85 h. VC addition inhibited 1,1-CFE transformation, as indicated by the decrease in the FE production rate. Ethene and FE were produced at similar rates over the period from 85 to 270 h. When acetylene (24 \(\mu \text{M}\)) was added at 270 h, both VC and CFE transformations...
TABLE 1. Comparison of Measured and Predicted Rates of 1,1-CFE and VC Transformation for the Results Presented in Figure 5

<table>
<thead>
<tr>
<th>CFE</th>
<th>VC</th>
<th>FE production rate, measd/pred</th>
<th>Ethene production rate, measd/pred</th>
<th>Ethene/FE production rate, ratio, measd/pred</th>
</tr>
</thead>
<tbody>
<tr>
<td>101</td>
<td>139</td>
<td>0.082/0.067</td>
<td>0.091/0.127</td>
<td>1.1/1.9</td>
</tr>
<tr>
<td>96.6</td>
<td>154</td>
<td>0.015/0.061</td>
<td>0.033/0.140</td>
<td>2.2/2.3</td>
</tr>
<tr>
<td>103</td>
<td>1300</td>
<td>0.012/0.012</td>
<td>0.132/0.227</td>
<td>11.0/18.9</td>
</tr>
<tr>
<td>680</td>
<td>1160</td>
<td>0.032/0.073</td>
<td>0.075/0.170</td>
<td>2.3/2.3</td>
</tr>
</tbody>
</table>

a Reactor A from 85 to 269 h. b Reactor A after the acetylene inhibition test (from 405 to 600 h). c Reactor B from 85 to 269 h. d Reactor B from 269 to 600 h.

For rate estimates using eq 1 the product of the maximum transformation rate and microbial concentration for the estimations (k_{max}X) of 5.0 (μmol/h)/L was determined from the initial 85 h of FE production (Figure 4). Both VC and 1,1-CFE production rates are a function of substrate and inhibitor concentrations. VC and 1,1-CFE were complementary substrate and inhibitor pairs, as defined in eq 1. The inhibition was based on the ratio of the measured K_{i} values as proposed by Haston (21). The reactor ethene and FE production rates were estimated using eq 1, by including mass balances for partitioning between the aqueous and gas phases.

The measured and predicted rates of ethene and FE production and ratio of ethene and FE production rates (measured/predicted) are presented in Table 1 for periods when both compounds were present. There is good agreement between the measured and predicted ratios. The changes in production rates are shown to be correlated to the aqueous concentrations, as expected by the competitive inhibition model. The results indicate VC and 1,1-CFE transformation rates could be predicted reasonably well using the measured K_{i} values in the competitive inhibition model. Differences in estimated and measured rates might result from culture growth and decay during the course of the experiment. The ratios, however, of measured to predicted rates, which would normalize growth and decay, are in good agreement. The greatest difference between measured and predicted rates for both ethene (0.033 μmol/h) and FE (0.015 μmol/h) occurred after the acetylene inhibition test. Despite this difference, the ratios in measured and predicted rates were in good agreement, considering the order in magnitude of the concentrations used in several of the tests.

Both VC and 1,1-CFE transformation rates recovered to only 36% and 18% of the rates achieved prior to acetylene inhibition. The reason for the loss of activity is not known. Microbial decay over 125 h (5.2 d), without electron acceptor utilization, is one potential factor causing a loss in activity. Potential acetylene toxicity on the microbial community could be another factor. Consistent with previous observations (13), acetylene was not transformed during the experiments.

1,1-CFE Transformation Studies with Three Different Enrichment Cultures. Results of experiments performed with the three different cultures to further explore 1,1-CFE as a reactive surrogate of VC transformation are shown in Figure 5. 1,1-CFE and VC were both transformed by the Evianite culture in the presence of TCE and d-DCE (Figure 5A). Ethene and FE were produced at similar rates, and essentially complete conversion of 1,1-CFE and VC was achieved within 10 d. The results are consistent with those shown in Figure 1, which was conducted over a longer time scale. The results obtained with the Point Mugu culture are shown in Figure 5B. Both VC and 1,1-CFE transformation occurred after the control reactor (data not shown), further supporting VC inhibition of 1,1-CFE transformation.

The rates of ethene and FE production from VC and 1,1-CFE transformation were estimated for the different stages of the tests when both compounds were present. The analysis assumed competition for the same enzyme and used a competitive inhibition model. Using the previously determined K_{i} values for VC and 1,1-CFE, the ethene and FE production rates were predicted using a competitive inhibition model (20):

$$\frac{dC_{\text{substrate}}}{dt} = \frac{(k_{\text{max}}X)C_{\text{substrate}}}{K_{s(\text{substrate})} + K_{s(\text{inhibitor})}C_{\text{inhibitor}}}$$

(1)
The Evanite culture transformed TCFE to VC and ethene. The enrichment cultures include the Evanite (A), Point Magu (B), and Lawrence Livermore (C) cultures. c-DCE concentration was reduced to a low level. 1,1-CFE transformation tracked both the initial slow and then later accelerated rates of VC transformation. The correlation of very limited 1,1-CFE transformation with very limited VC transformation is demonstrated with the Lawrence Livermore culture (Figure 5C). The culture transformed TCE through c-DCE to VC. 1,1-CFE was very slowly transformed to FE, while VC was very slowly transformed to ethene. The tests with the three cultures showed 1,1-CFE tracked VC transformation well in the presence of higher chlorinated CAHs, despite the different transformation abilities of the cultures.

**Discussion**

The Evanite culture transformed TCFE to c-DCE and t-DCE, with c-DCE being the main isomer formed. This result was consistent with previous observations (11). The Evanite culture transformed c-DCE to 1,1-CFE as the major intermediate. This observation differs from those of Vanchesevaran et al. (11), who reported minor amounts of 1,2-CFE (E) produced in Lawrence Livermore microcosms.

FE was not defluorinated to ethene by the Evanite culture. The transformation pathway indicates the fluoride substituent acts more like a hydrogen substituent than a chloride substituent. This might result from the combined effect of it being a poorer leaving group than chloride, and its size being smaller than that of chloride. Thus, the CFE dechlorination step is associated with the VC step, rather than the DCE dechlorination step, as indicated in Figures 1 and 5.

The multiple-equilibration method yielded $K_X$ and $k_{\text{max}}X$ via nonlinear regression that had standard deviations of less than 20%. Previous studies using the traditional multireactor method resulted in larger standard deviations of approximately 50% of the $K_X$ value and 20% of the $k_{\text{max}}X$ values (4, 21). The multireactor method assumes all the reactors have the same initial conditions, which may be difficult to achieve with anaerobic cultures. The multiple-equilibration method is simple to apply, and these initial results indicate it is an effective means of obtaining kinetic parameters. Studies are needed with direct comparisons of the different methods. VC and 1,1-CFE had very similar $K_X$ and $k_{\text{max}}X$ values (4, 21) indicating VC and 1,1-CFE were transformed at similar rates by the Evanite culture. The kinetic values indicate the same enzyme likely transformed both compounds. This is supported by data shown in Figure 4 that show VC and 1,1-CFE inhibit each other’s transformation. Predicted rates and measured rates matched well when the measured $K_X$ values were used in the competitive inhibition model, for different VC and 1,1-CFE concentrations (Table 1). The ratios of the $K_X$ values simulated the competitive inhibition well, consistent with earlier reports of Haston (21) for c-DCE on VC transformation, providing additional evidence that the two compounds are transformed by the same enzyme. Acetylene inhibited both VC and 1,1-CFE transformation to similar extents, and the transformation rate partially recovered upon removal of acetylene (Figure 4A). The mode of acetylene inhibition is not known, but acetylene appears to inhibit VC and 1,1-CFE transformation, similarly.

1,1-CFE transformation tracked VC transformation well with three different cultures that transformed VC to ethene at different rates. No false-positive results were observed where 1,1-CFE was transformed when VC was not. The results suggest 1,1-CFE is an excellent surrogate for determining rates and extents of VC transformation.

FE was a stable product of 1,1-CFE transformation, within our ability to measure FE concentration or ethene production. Thus, FE production rates could be used to estimate 1,1-CFE transformation rates. Rates of VC transformation were estimated from measured rates of 1,1-CFE transformation and measured kinetic parameters for VC and 1,1-CFE transformation by the Evanite culture, using a competitive inhibition model with concentrations of VC and 1,1-CFE included. VC transformation rates observed upon its addition to batch reactors were estimated from initial 1,1-CFE rates (Table 1). The inhibition that was observed illustrates the importance of including the effects of 1,1-CFE and VC concentration when VC transformation rates are estimated in field studies when 1,1-CFE is added as a reactive surrogate.

Vinyl bromide (VB) has also been evaluated as an indicator of VC transformation potential (22). In these studies, VB reduction to ethene resulted in bromide ion release, which might also track VC transformation. Vinyl bromide transformation was much more rapid than VC transformation, and can be potentially used to rapidly screen for VC transformation potential. However, 1,1-CFE transformation rates more closely track those of VC when they are present at similar aqueous concentrations. Thus, there is a potential for using VB to rapidly screen for VC transformation potential and for 1,1-CFE to help quantify VC dechlorination potential.

1,1-CFE was demonstrated here to be an excellent surrogate to track VC transformation, and has potential use for estimating rates of intrinsic or enhanced in situ VC transformation. Regulatory approval would be required to add 1,1-CFE, since FE would likely persist and should be considered as toxic as VC. However, regulatory approval to add TCFE was obtained for previous push–pull tests in a contaminated aquifer (12) to study PCE and TCE transformation. An advantage of adding 1,1-CFE, compared to TCFE, is that the last step is directly studied so the effects of groundwater dilution can be minimized, and known concentrations can be injected. Results of such push–pull tests will be described in a future paper.

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