EXECUTIVE SUMMARY

Identification of Abiotic Degradation Pathways of Chlorinated Ethenes by Compound-specific Stable Isotope Analysis: A Proof-of-Concept Study

SERDP Project ER-2623

JULY 2021

Tomasz Kuder
University of Oklahoma

SERDP
DOD ▪ EPA ▪ DOE

EXECUTIVE SUMMARY

Project: ER-2623

TABLE OF CONTENTS

LIST OF FIGURES

ACRONYMS AND ABBREVIATIONS

CE	chlorinated ethenes
CSIA	compound-specific stable isotope analysis
CSM	conceptual site model
DoD	U.S. Department of Defense
MBT	molecular biological tools
MNA	monitored natural attenuation
PCE	perchloroethylene (tetrachloroethene)
TCE	trichloroethene
ZVI	zero-valent iron

ACKNOWLEDGMENTS

I would like to thank Dr. Frank Loeffler (The University of Tennessee, Knoxville) for providing anaerobic cultures for this study, and Dr. Lee Krumholz (University of Oklahoma, Norman) for helpful advice on anaerobic microcosm setup is acknowledged. I thank Dr. Anat Bernstein and Dr. Almog Gafni (Ben Gurion University Negev, Israel), for sharing samples from their trichloroethene (TCE) degradation experiments for hydrogen isotope analysis of TCE.

1.0 INTRODUCTION

Chlorinated ethenes (CE) have been among the most widely used industrial solvents, with numerous large volume users, within the structure of the U.S. Department of Defense (DoD). CE solvents such as TCE and tetrachloroethene (PCE) are among the most commonly detected groundwater contaminants. Legacy CE spills remain one of the key environmental challenges, at DoD facilities and elsewhere, in the U.S. and worldwide.

Monitored natural attenuation (MNA) and miscellaneous active remediation solutions aim to eliminate the environmental impact of contaminants, either though *in situ* destruction of the contaminant mass or by physical removal from the impacted aquifer or soil profile. Historically, MNA of CE relied on documenting the efficacy of biological reductive dechlorination. The challenge of degradation pathway-specific assessment is a limiting factor for incorporating abiotic degradation of CE into conceptual site models (CSM) and realizing the benefits of such abiotic processes in contaminated sites remediation.

This project investigated several lines of evidence based on compound-specific stable isotope analysis (CSIA) as a prerequisite to applications in the assessment of abiotic degradation at contaminated sites. While CSIA was proven to be very useful in the assessment of biodegradation of CE (Hunkeler et al., 2008), fewer studies centered on abiotic degradation. There are data gaps on isotope effects determined in controlled studies of key degradation mechanisms, in respect to hydrogen and for certain aspects of chlorine isotope fractionation. The incomplete reference data make CSIA less informative, in particular for scenarios with mixed biological/abiotic degradation, due to the inability to attribute changes of isotope ratios of contaminants specifically to abiotic degradation as compared to biological degradation.

Prior results from laboratory studies of abiotic degradation systems demonstrated that abiotic degradation of CE can be expected to produce measurable carbon isotope fractionation, and for certain degradation pathways, chlorine and hydrogen fractionation. However, biodegradation is likely to contribute to the overall CE mass destruction in nearly every plausible field scenario involving abiotic degradation. To be useful in the assessment of such mixed mechanism systems, CSIA results must be pathway-specific, so that the abiotic and biological degradation effects can be deconvoluted. Ideally, CSIA would be able to discriminate between alternative biological versus abiotic mechanisms and permit quantitative allocation of contaminant degradation if more than one pathway were involved. For certain compounds, such as methyl tert-butyl ether and benzene, CSIA has been demonstrated to provide such pathway-specific information.

The general objective of this project was to reduce the uncertainty of future contaminated site assessment projects, through exploration of C-Cl-H isotope data from several key CE degradation pathways (biological and abiotic, to allow for comparing/contrasting between the two categories). The criterion of success for this exploration is to identify isotope characteristics that can be adopted as specific pathway indicators, suitable for differentiation of abiotic and biotic pathways that can plausibly overlap under field conditions. Ultimately, CSIA data would serve to improve the quality of conceptual models and permit more efficient and cost-effective contaminated site management.

2.0 OBJECTIVES

The present proof-of-concept is a demonstration of multi-element CSIA (including CSIA of chlorinated degradation products if such compounds are present) for discrimination between abiotic degradation and common biodegradation pathways. Isotope data from laboratory degradation experiments were obtained to address two specific field scenarios. The scenarios have been chosen to constrain the selection of degradation pathways to be discriminated from each other.

> Scenario 1: Identification/discrimination of reaction mechanisms in oxic/suboxic environments conducive to aerobic biodegradation and to abiotic degradation on iron mineral surfaces. A typical field example of such site is an TCE plume with MNA Tier-1 evidence of TCE attenuation, but without confirmatory evidence of reductive dechlorination by geochemical footprints and degradation product.
>
> Scenario 2: Identification/discrimination of reaction mechanisms in anoxic environments conducive to reductive dechlorination, biological or abiotic. Typical field examples of such sites are locations with zero-valent iron (ZVI) permeable reactive barriers (with biological reductive dechlorination augmenting the yield from ZVI degradation) or sites conducive to precipitation of reactive iron minerals, such as ferrous sulfide.

3.0 TECHNICAL APPROACH

Interpretation of CSIA data from field sites requires sound understanding of isotope effects (isotope fractionations) associated with (bio)chemical transformations of the CE. This is most important in applications of CSIA in assessment of contaminant transformations by competing degradation pathways, each associated with its own specific pattern of isotope fractionation. In the present case, the assumption is that abiotic degradation is not the sole or perhaps not even the dominant mechanism of CE mass destruction; so, it is necessary to also consider isotope fractionation in biodegradation pathways that can be plausibly expected to impact the contaminant plumes under assessment.

The following categories of degradation systems were compared/contrasted with each other, using data from laboratory degradation experiment:

- Abiotic degradation – ZVI degradation of TCE as a model for abiotic degradation. The pros and cons of using ZVI as the model abiotic reaction are discussed in a following paragraph.
- Biodegradation – dechlorination of PCE and TCE by reductive dechlorination cultures.
- Biodegradation – dechlorination of TCE by co-metabolic aerobic cultures (that data set combines carbon and chlorine isotope ratios obtained previously by a partner research group and hydrogen data obtained using archived samples from that former project).

The results from those controlled degradation experiments were evaluated, to specifically answer the question if CSIA can be informative in reaction pathway discrimination for two specific field scenarios.

4.0 RESULTS AND DISCUSSION

Two promising lines of CSIA evidence for discrimination between competing biotic and abiotic degradation pathways were defined using present experimental data and published reference data. The first line of evidence is provided by hydrogen isotope ratios of reductive dechlorination precursors (TCE) and products (TCE in PCE degradation, cDCE in TCE degradation). **Figure ES-1** illustrates the contrast between the abiotic ZVI reaction and biodegradation. Clearly, the mechanisms of product hydrogenation favor incorporation of ^{1}H over ^{2}H in the abiotic pathway. The difference is in the order of hundreds per mil units and can be readily determined by current analytical methodology, for differentiation of abiotic and biological reductive dechlorination (cf. Scenario 2 defined in the preceding section).

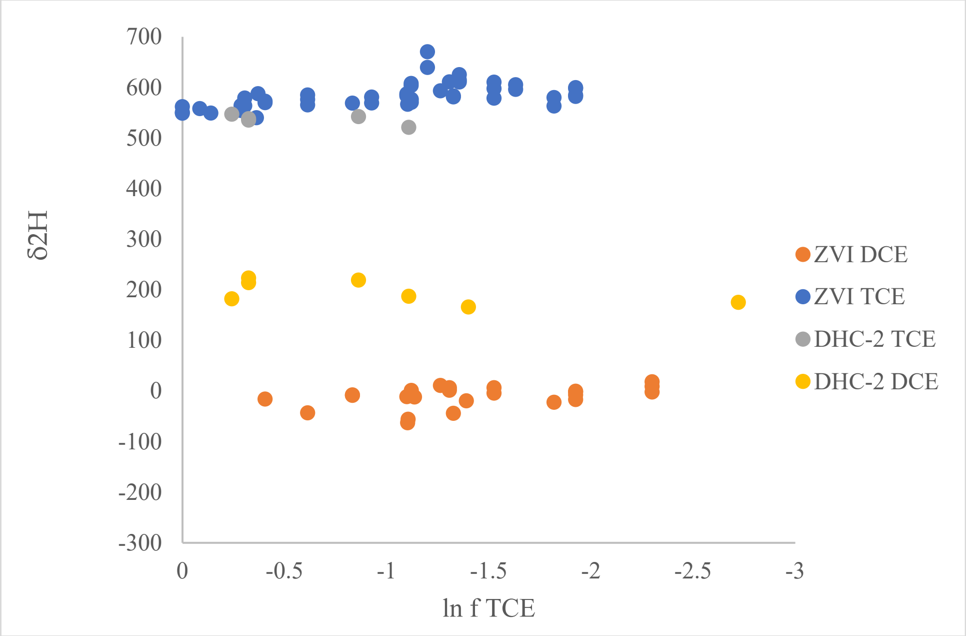

Figure ES-1. Hydrogen Isotope Ratios of TCE and cDCE Versus Remaining Mass of TCE, in Degradation of TCE on ZVI and in Biodegradation of Identical TCE by *Dehalococcoides Mccartyi* Strain 195.

ZVI data represent four rounds of degradation experiments. The same lot of TCE was used in both experiments.

Hydrogen isotope ratios of reductive dechlorination products depend in part on the isotope signature of the precursor compound (along the dechlorination chain, new hydrogen atoms are inserted into the degradation products and hydrogen atoms already present in the precursor compounds are transferred to the products as well). Moreover, the isotope signature of product hydrogenation is most likely anchored to ambient water, which serves as hydrogen donor or equilibrates rapidly with hydrogen produced by fermentation of organic substrates. Common isotope composition (δ^2H) of TCE falls between +600 and -200, depending on the origin of TCE (manufactured TCE solvent is commonly but not always at the positive end of the range) and whether TCE was impacted by isotope exchange with ambient water. Groundwater δ^2H is near zero in coastal areas, trending towards more negative values in the continental interior.

Accordingly, numerical values shown in Figure ES-1 are representative of specific experimental conditions but would not necessarily apply at any specific field site. **Figure ES-2** presents a comprehensive picture of the isotope ratios to be expected for different combinations of TCE precursor and water isotope signatures.

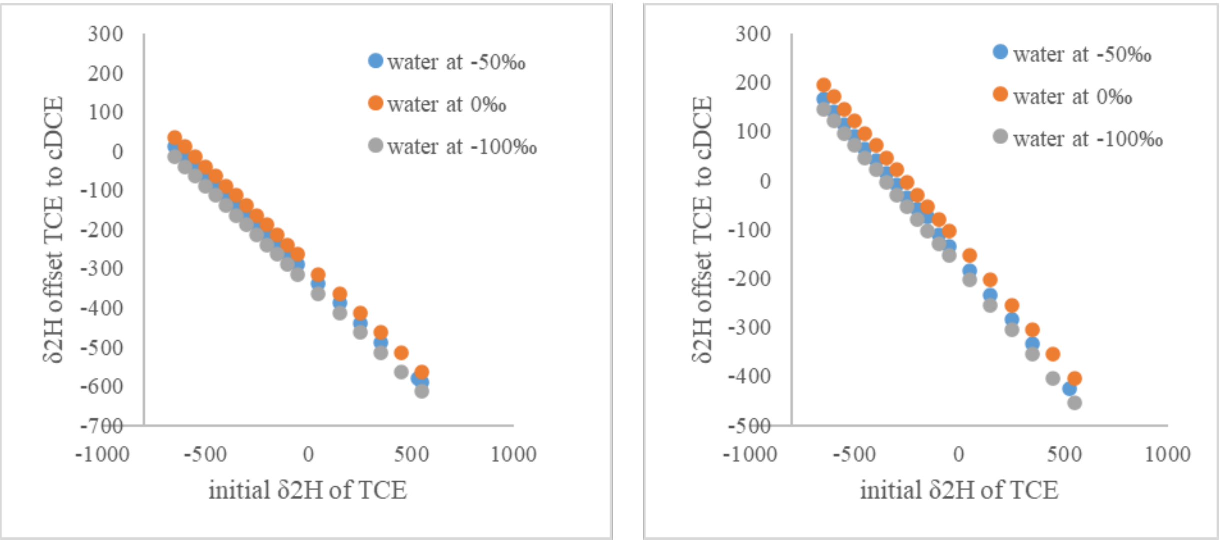

Figure ES-2. Offsets of δ^2H Between TCE and cDCE in Abiotic (A) and Biological (B) Reductive Dechlorination.

Panel B is based on isotope data in degradation of PCE to cDCE by Desulfuromonas michiganensis experiment (see the following section). Net value of δ^2H of cDCE is obtained by subtracting the value of offset (Y axis) from the corresponding δ^2H of TCE (X-axis).

The second line of CSIA evidence is dual element C-Cl CSIA applied to discrimination of aerobic biodegradation from reductive processes (biotic and abiotic alike). C-Cl characterization of aerobic organisms was included in the original proposal, but identical data were made available elsewhere in early stages of the project (Gafni et al., 2018; Gafni et al., 2020). This line of evidence applies to Scenario 1 defined in the preceding section. C-Cl trends are clearly divergent from reductive dechlorination trends (**Figure ES-3**). The role of hydrogen CSIA (dual element C-H) remains unknown pending future studies, since ZVI may not be an ideal model reaction for abiotic reactivity under oxic/suboxic conditions.

Other lines of CSIA evidence were assessed. While it is possible to define discernible differences for pair-wise comparisons of abiotic and biological pathways, interpretation of those is more ambiguous and at this point they do not offer significant site assessment benefit specifically in biotic-abiotic reaction pathway differentiation. However, comprehensive, multi-element and multi-compound CSIA offers other benefits in site assessment. For example, by decreasing the uncertainty of dealing with commingled contaminant sources, where changes of isotope ratios over distance or over time could result from mixing of divergent source signatures rather than from degradation.

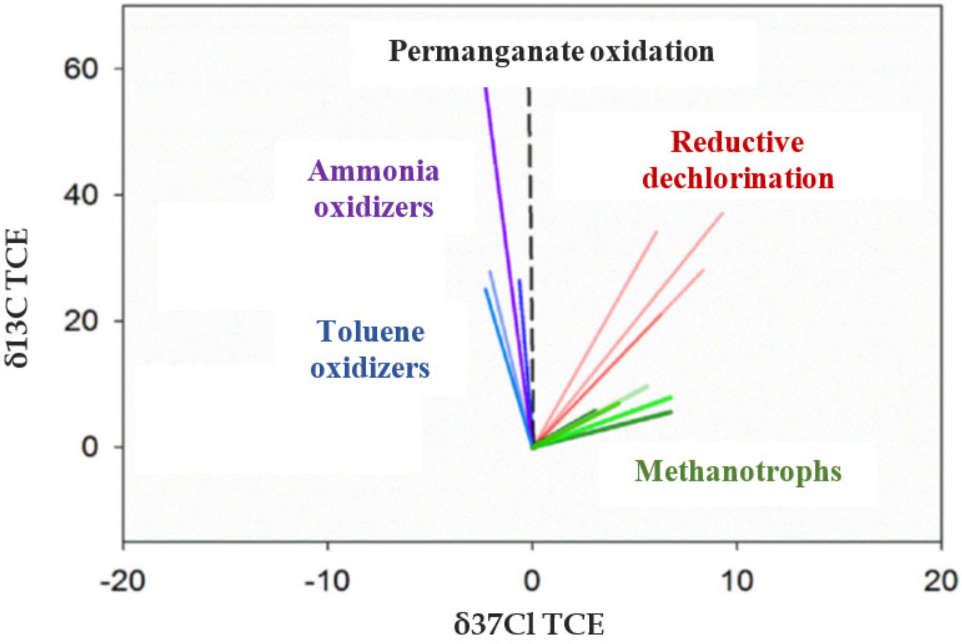

Figure ES-3. Dual Element C-Cl Trends in Aerobic Co-metabolic Biodegradation of TCE Contrasted with Reductive Dechlorination.

Isotope ratios are normalized to zero for the initial TCE isotope composition. Figure modified after Gafni et al. (2020).

The third and final highlight of this project is the assessment of hydrogen isotope effects resulting from hydrogen exchange between CE and water. Common perception of hydrogen exchange of carbon-bound hydrogen prevalent in isotope earth and environmental science community is that the process is only significant over geological time scales or at extreme geological conditions in deep subsurface. This turns out to be incorrect for certain chlorinated hydrocarbons, such as chloroform and CE. The reaction is a simple acid-base proton exchange, with the dominant base being hydroxide ions and chlorinated hydrocarbons acting as weak Brønsted acids. The rate of the exchange process was shown to be dependent on the concentrations of hydroxide in solution (higher rates at higher pH). In this project, exchange experiments were conducted under a range of pH and temperatures similar to those encountered in groundwater or microcosm media to identify the rates and the attendant changes of TCE and cDCE isotope composition. The overall message from that study is that the process is too slow to impact cDCE data at the laboratory or field settings, but the exchange can impact δ^2H of TCE in certain aquifers over timescales as short as months and certainly over years to decades. In general, hydrogen CSIA data from TCE would be increasingly affected for groundwater pH increasing above 7 and for aquifers situated in warm climates (**Figure ES-4**).

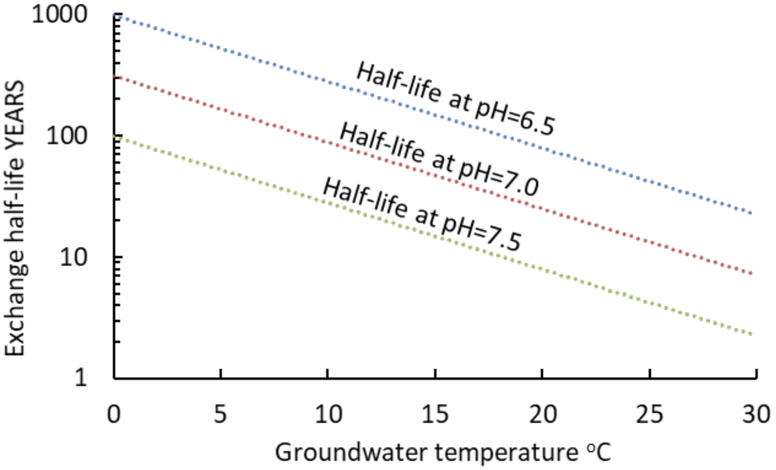

Figure ES-4. Hydrogen Exchange Half-lives as a Function of Aquifer pH and Temperature.

The rates are based on kinetic experiments performed as part of this project.

5.0 IMPLICATIONS FOR FUTURE RESEARCH AND BENEFITS

Higher degree of precision of contaminated site assessment translates to cost savings though more effective site remediation approach.

Two lines of CSIA evidence yielded most conclusive results in the laboratory demonstration. The first one is the discrimination between biotic and abiotic reductive dechlorination based on the differences in the extent of δ^2H depletion in dechlorination products, TCE and cDCE. The approach could be employed to assess the efficacy of ZVI remedies (by deconvolution of mass destruction by ZVI from that of ambient biodegradation) and possibly also to identify overlooked abiotic processes in anoxic aquifers conducive to such abiotic reductive dechlorination with FeS and other ferrous minerals.

The second line of evidence is the dual-element C-Cl, which appears to be a robust discriminative parameter for separation of aerobic degradation of TCE from reductive pathways (biotic and abiotic alike). The approach C-Cl could be implemented at any of the aerobic TCE plumes where aerobic degradation or abiotic degradation is part of or a feasible addition to the CSM. An additional lab study to confirm the C-Cl trend attendant of the Fenton-like pathway would be advisable to decrease the risk of uncertainty in data interpretation.

The results from this project illustrate the benefits of comprehensive, multi-element characterization of CE. While the issue has not been part of this project, contributions from multiple sources of CE can complicate interpretation of degradation pathways. While certain lines of evidence can have no direct value in the discrimination of biotic versus abiotic pathways (e.g., chlorine in the context of reductive pathways), additional dimensions of isotope composition characterization may help in addressing isotope signature interferences from commingled sources of CE. While the cost of analysis is proportionally higher for multi-element CSIA, such data sets are most informative for source discrimination and for degradation pathway assessment.

None of the presented lines of CSIA evidence are a silver bullet in terms of providing a specificity of a DNA fingerprint. Ideally, field site assessment should combine CSIA with other technologies, such as molecular biological tools (MBT). MBT can constrain the biological processes, to decrease the challenge for pathway discrimination by eliminating certain endmember reactions. For example, C-Cl evidence can be expected to be very robust for identification of dominant reaction process, in pair-wise reaction pathway comparison. For example, degradation of TCE by toluene oxygenase organisms is readily discernible from reductive elimination. On the other hand, C-Cl results can be more ambiguous at sites with significant contributions from multiple pathways. Using the example of co-metabolic degradation of TCE, net isotope fractionation caused by mixed aerobic consortia utilizing a combination of toluene and methane oxygenases could in theory overlap with that resulting for reductive processes. Using MBT to exclude significant contributions from methane monooxygenases would decrease the overall uncertainty.

Another valuable contribution from this project is better understanding of the role of hydrogen isotope exchange between CE and water. While that line of research was initiated as a side project to assure the quality of microcosm data, the results are of general interest for users of hydrogen CSIA. Reaction kinetic of the exchange will help to avoid data misinterpretations in field site assessment, from sites where exchange processes overprinted contaminant source or degradation signatures.

6.0 LITERATURE CITED

Gafni A, Lihl C, Gelman F, Elsner M, Bernstein A. delta C-13 and delta Cl-37 Isotope Fractionation to Characterize Aerobic vs Anaerobic Degradation of Trichloroethylene. Environmental Science & Technology Letters. 2018;5(4):202-208.

Gafni A, Gelman F, Ronen Z, Bernstein A. Variable carbon and chlorine isotope fractionation in TCE co-metabolic oxidation. Chemosphere. 2020;242.

Hunkeler D, Meckenstock RU, Sherwood Lollar B, et al. A guide for assessing biodegradation and source identification of organic ground water contaminants using compound specific isotope analysis (CSIA). Ada, Oklahoma, USA: US EPA;2008. EPA 600/R-08/148.