Using Molecular Tools to Predict Rate Constants for Anaerobic Biodegradation of *cis*-DCE and Vinyl Chloride in Ground Water

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Validation of Advanced Molecular Biological Tools to Monitor Chlorinated Solvent Bioremediation and Estimate CVOC Degradation Rates

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ESTCP Project ER-201726

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Development of a Quantitative Framework for Evaluating Natural Attenuation of 1,1,1-TCA, 1,1-DCA, 1,1-DCE, and 1,4-Dioxane in Groundwater

Anthony Danko | NAVFAC EXWC

ESTCP Project ER-201730

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Carmen Lebrón- Private Consultant

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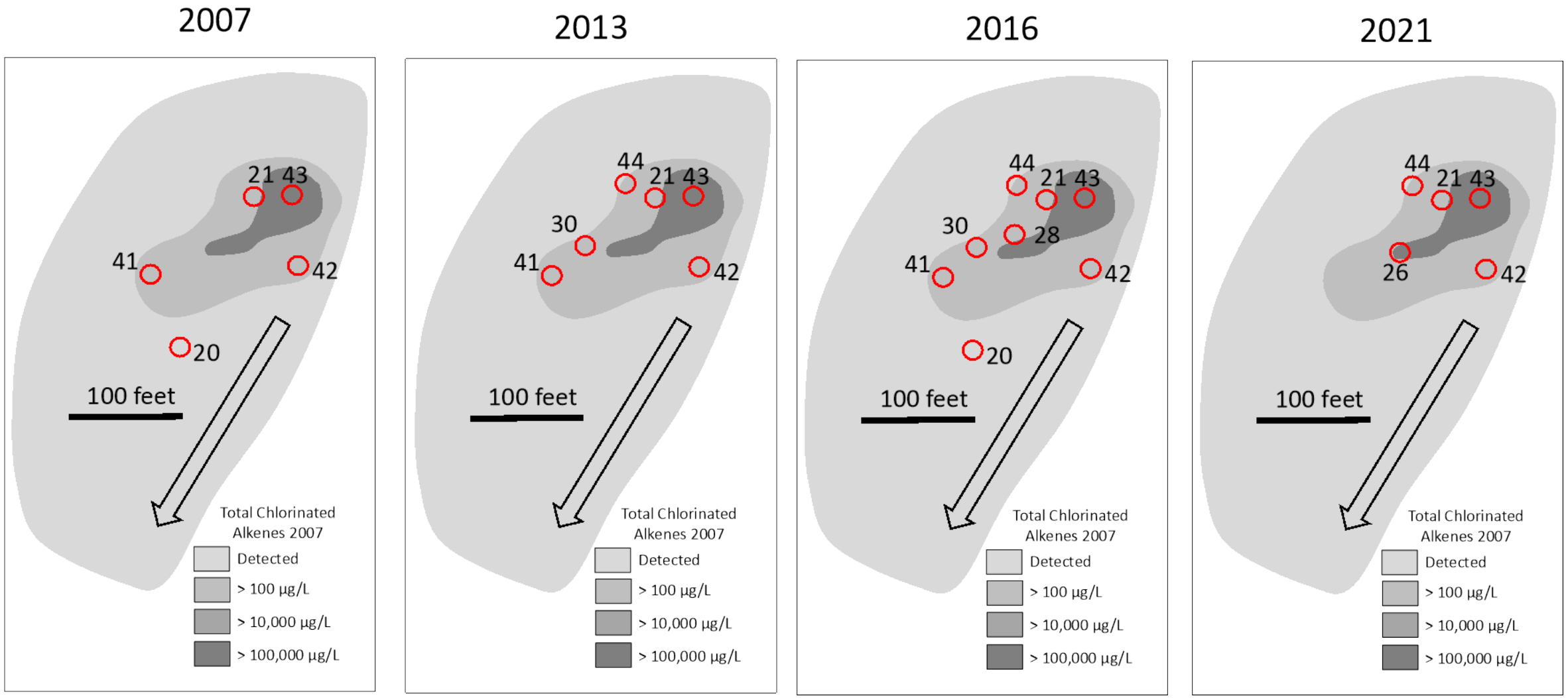
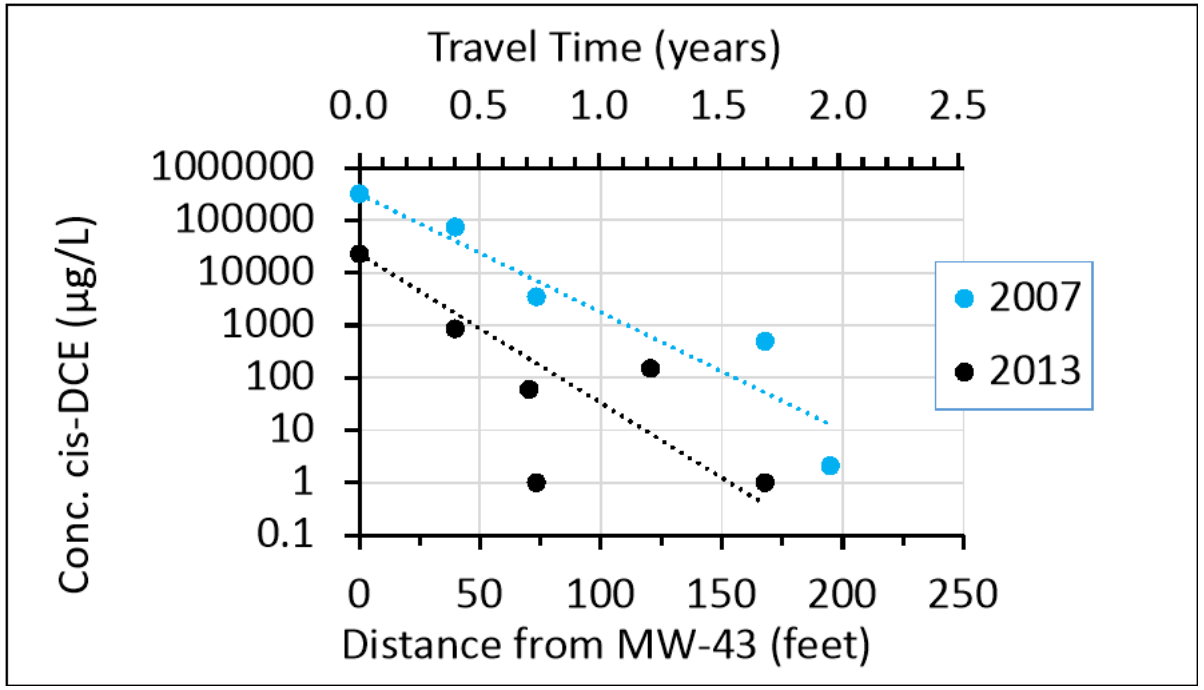
The Study Site is near San Diego, CA.

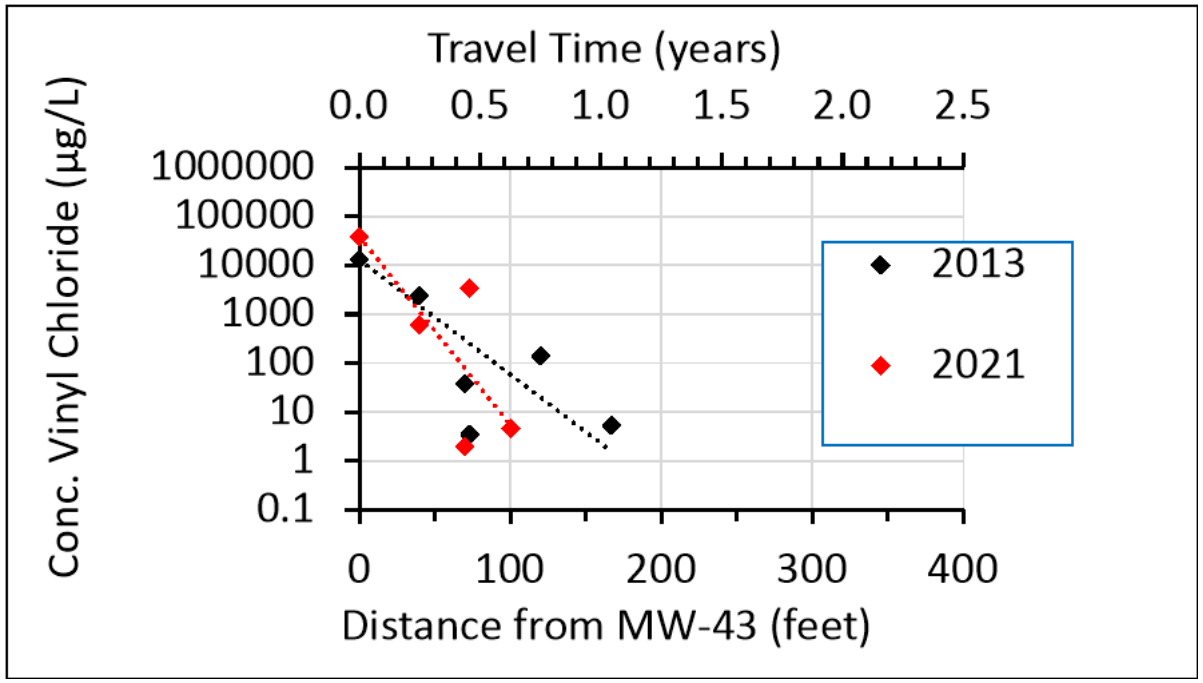


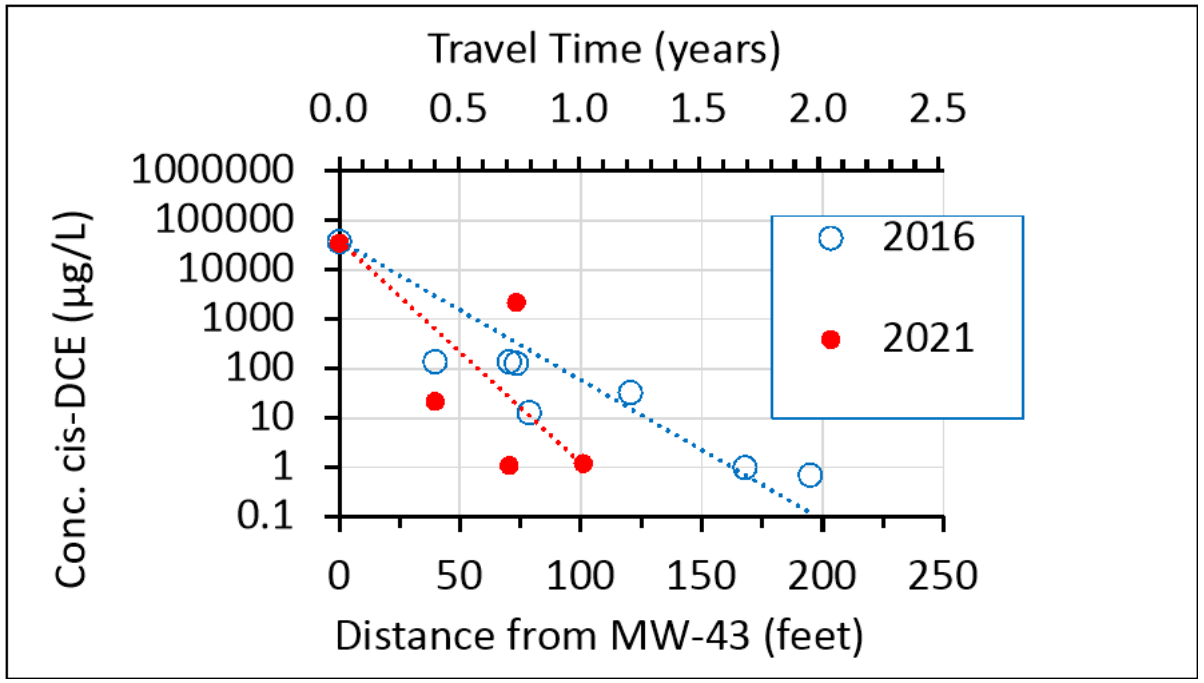
Wells that were used in particular years to estimate the field-scale rate of biodegradation.

Date	Field Rate *c*-DCE per year	Field Rate Vinyl Chloride per year
2007	**5.3** ± 1.7*	
2013	**5.0** ± 3.3*	**6.7** ± 4.6*
2016	**4.8** ± 1.2*	
2021	**8.6** ± 7.8*	**13.6** ± 10.8*

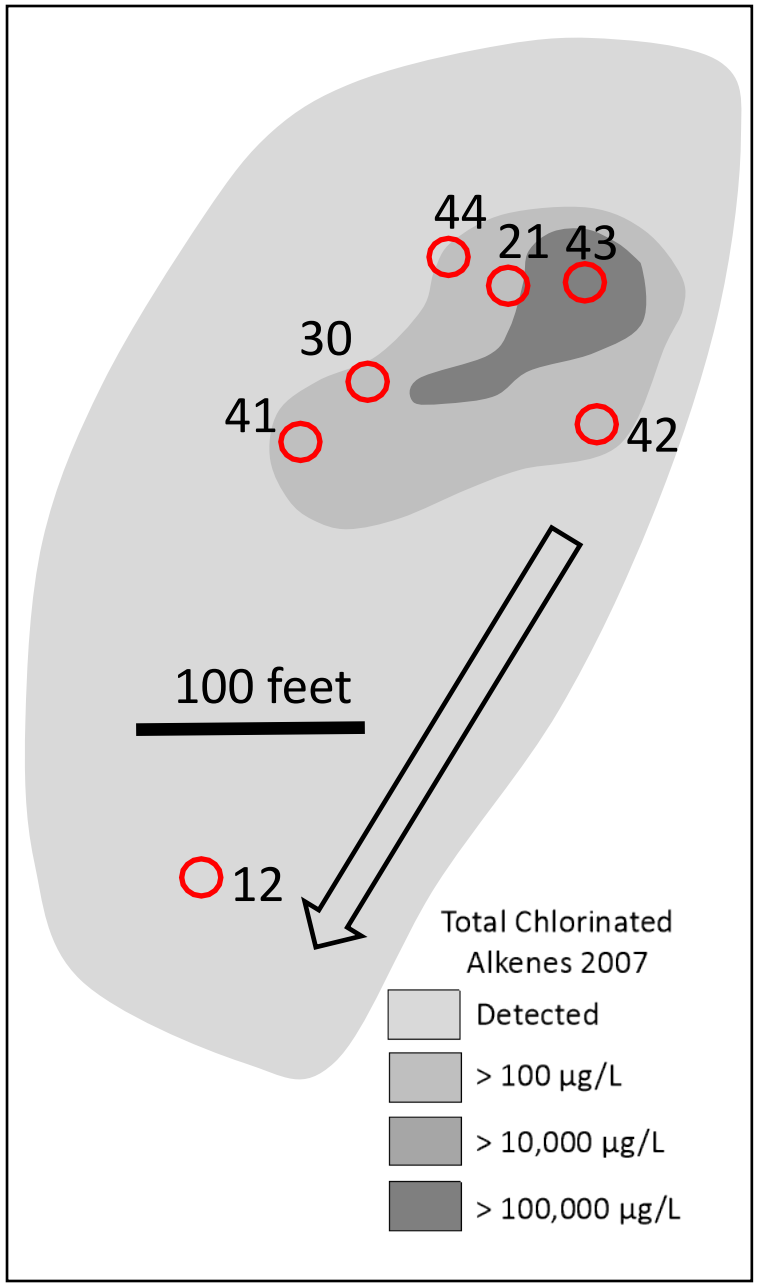
*80% Confidence Interval







2013



The abundance of qPCR biomarkers when the site was sampled in the second quarter of 2013

Well	*Dhc*	*tceA*	*bvcA*	*vcrA*
	Gene Copies per mL			
43	1.3E+05	7.7E+06	1.4E+05	2.3E+01
21	8.4E+05	4.8E+07	1.9E+06	5.1E+05
44	3.1E+04	1.9E+06	6.2E+03	3.3E+04
42	3.6E+03	1.2E+06	5.0E+02	5.9E+03
30	1.8E+05	8.4E+06	3.0E+05	1.5E+05
41	1.6E+03	6.6E+04	5.4E+01	2.7E+03
12	3.7E+01	7.2E+02	4.4E+01	5.3E+02

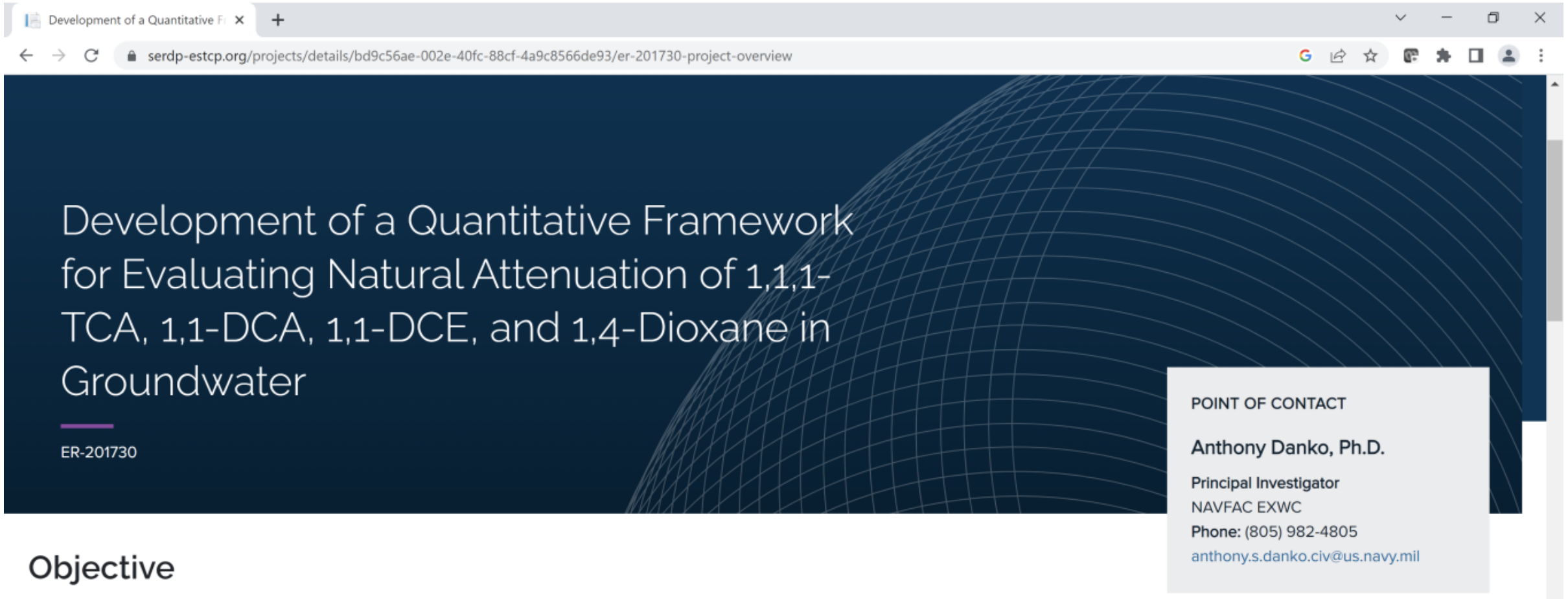
For the *Dhc* qPCR biomarker the following kinetic parameters are available:

Culture	V_{max} *cis*-DCE	V_{max} VC	K_m *cis*-DCE	K_m *VC*	Reference
	mg/gene copy* year		mg/L		
Dhc VC Stanford U. Victoria, TX	6.6E-07	4.3E-07	0.32	0.16	Cupples et al. 2004 ES&T 38, 1101-1107

The

(1) kinetic parameters,
(2) the concentrations of *cis*-DCE or Vinyl Chloride,
(3) the abundance of the biomarker in groundwater, and
(4) the retardation coefficient of *cis*-DCE
or Vinyl Chloride

were used in **MNA Rate Constant Estimator** to estimate a rate constant for biological reductive dechlorination in the groundwater sampled at each monitoring well.



Search ER-201730 under Groundwater Remediation and Management in the SERDP/ESTCP Webpage

Audio Summary of ER-201730

0:00 / 4:52

Monitored natural attenuation (MNA) has emerged as a preferred remedial option at many sites impacted by chlorinated solvents because it offers a cost-effective and practical approach for cleanup of solutes in groundwater. However, existing MNA protocols do not include 1,4-dioxane and commonly co-occurring chlorinated solvents like 1,1,1-trichoroethan (1,1,1-TCA), 1,1-dichloroethan (1,1-DCA), and 1,1-dichloroethene (1,1-DCE). The objectives of this project were to:

1. Develop a modified model and framework for evaluating natural attenuation of these compounds.
2. Develop and validate a protocol to directly measure rate constants for natural biodegradation of 1,4-dioxane using ^{14}C- labeled 1,4-dioxane and groundwater from 10 different field sites.
3. Use the field and laboratory data to establish if there is consistency between various lines of evidence for 1,4-dioxane attenuation.

Technology Description

An evaluation of MNA relies on establishing various lines of evidence, including secondary and tertiary lines of evidence that help demonstrate degradation processes and associated rates that are responsible for the primary line of evidence (decreasing concentrations of the target compound(s)). This project developed a new fate and transport model to easily evaluate historical monitoring data to predict biodegradation rate constants as well as new decision matrices (flowcharts) that serve as a guided tour on how to interpret potential lines of evidence for MNA. These were then integrated into an existing software platform (BioPIC) that allows users to access both the model and the decision matrices. Several approaches also were used to generate input data to support and validate the model and framework. First, rate coefficients and lines of evidence for attenuation were calculated and/or measured at multiple sites using a focused sampling program at 10 field sites. Second, degradation and the associated rate constants for 1,4-dioxane at these same sites were determined using a ^{14}C-labeled 1,4-dioxane assay developed for this project.

PRODUCTS

Final Report

ER-201730 Final Report.pdf

12/8/2022

Executive Summary

ER-201730 Executive Summary.pdf

5/4/2022

User's Guide

BioPIC User's Guide and Tool

ER-201730 BioPIC User's Guide and Tool.zip

1/16/2023

User's Guide

MNA Rate Constant Estimator User's Guide and Tool

ER-201730 MNA Rate Constant Estimator User's Guide and Tool.zip

1/16/2023

MNA Rate Constant Estimator

Site Name: Generic Site | Run Name: | Date/Other:

Chlorinated Ethenes (PCE, TCE)

1. ADVECTION

Seepage Velocity (Restore Formula)	Vs	90.0	(ft/yr)
or			
Hydraulic Conductivity	K	1.5E+04	(ft/yr)
Hydraulic Gradient	i	0.0012	(ft/ft)
Effective Porosity	ne	0.2	(-)

2. ADSORPTION

Total Porosity	n	0.23	(-)
Fraction Organic Carbon	foc	0.004	(-)
Retardation Factor (Restore Formula)	Rf	5.6	(-)

3. GENERAL

Calibrate Model to Data From this Year	2013	(xxxx)
See Output in this Year	2020	(xxxx)
Modeled Area Length	200	(ft)
Distance from Source to Receptor	180	(ft)

4. SOURCE DATA

Source Width: 150 (feet)

			Enter: 2007 Source Concentration (ug/L)	2013 Actual Source Conc.* (ug/L)	2013 Modeled Source Conc. (ug/L)
Year Source Released	2007	(xxxx)	PCE			0
Year for Initial Source Concentration	2007	(xxxx)	TCE			0
Source Attenuation Rate (Select a typical rate below, or adjust the rate sc; Column O best matches Column P)	0.400	(per year)	c-DCE	320,000	23,000	29,030
			VC	18,000	13,000	1,633

Typical Source Attenuation Rates: (for simplest way to run model enter zero)

Constant Source: enter 0 per year	Some source atten.: 0.07 per year	Faster source atten.: 0.14 per year

KEY:

Enter directly	115
Calculated, can overide	0.02
Calculated, locked	0.02

* Leave blank if source rate is zero or if calibration year is same as year your source data starts.

5. FIELD DATA FROM WELLS

Year Data was Collected: 2013

	(ug/L)	(ug/L)	(ug/L)	(ug/L)	(ug/L)	(ug/L)	(ug/L)	(ug/L)	Criteria (ug/L)
PCE	0								5
TCE	0								5
c-DCE	23,000	860	60	1	150	1			70
VC	13,000	2400	38	3.5	140	5			2
Distance from Source (ft)	*0*	*38*	*71*	74	*121*	*168*			
Well Name (optional)	*43*	*21*	*44*	42	*30*	*41*			

6. BIODEGRADATION: ADJUST TO MATCH FIELD DATA; USE 6B OR 6C FOR HELP

First Order Rate Constant

PCE	0.070	(per year)
TCE	0.040	(per year)
c-DCE	4.500	(per year)
VC	3.800	(per year)

Preliminary plume rate estimates can be pulled from 6b or 6c. Change to better match field conditions or site knowledge.

Biodegradation Rate Constant Estimation Tools (Optional)

6b: Estimate from Biomarker Data

Biomarker Type: DHC

Enter Biomarker Data | Reset

First Order Rate Constant

PCE	--	(per year)
TCE - RDEG	--	(per year)
TCE - RMO	--	(per year)
TCE - TOD	--	(per year)
TCE - SMMO	--	(per year)
TCE - PHE	--	(per year)
TCE - Total	--	(per year)
c-DCE	88.239	(per year)
VC	79.834	(per year)

6c: Initial Estimate from Field Data (Above)

First Order Rate Constant

PCE	--	(per year)
TCE	--	(per year)
c-DCE	4.497	(per year)
VC	3.750	(per year)

RUN

Toggle Automatic Recalculation: **Currently OFF**

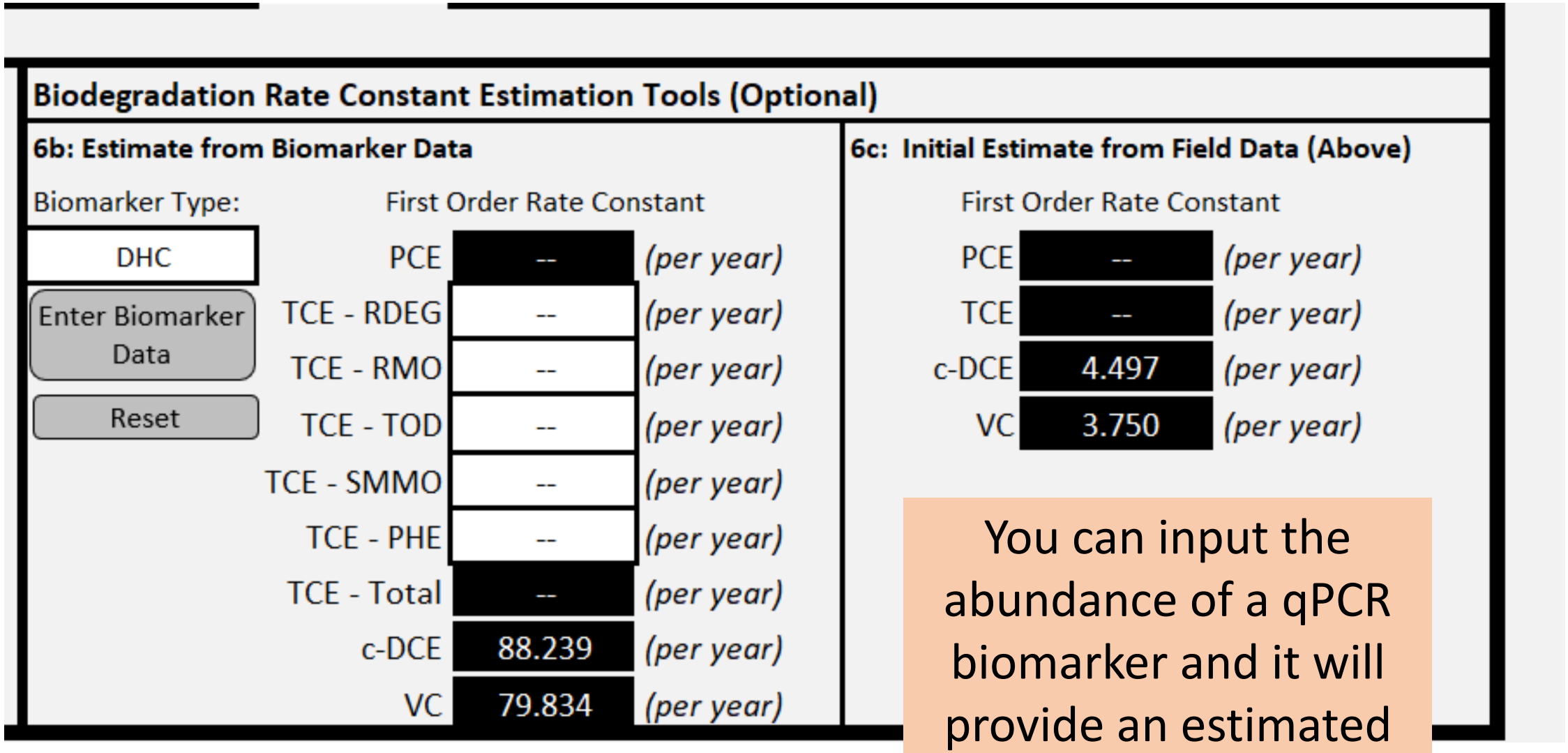
Plots Below

Chlorinated Ethenes Complex Model

Back to Select Model Page

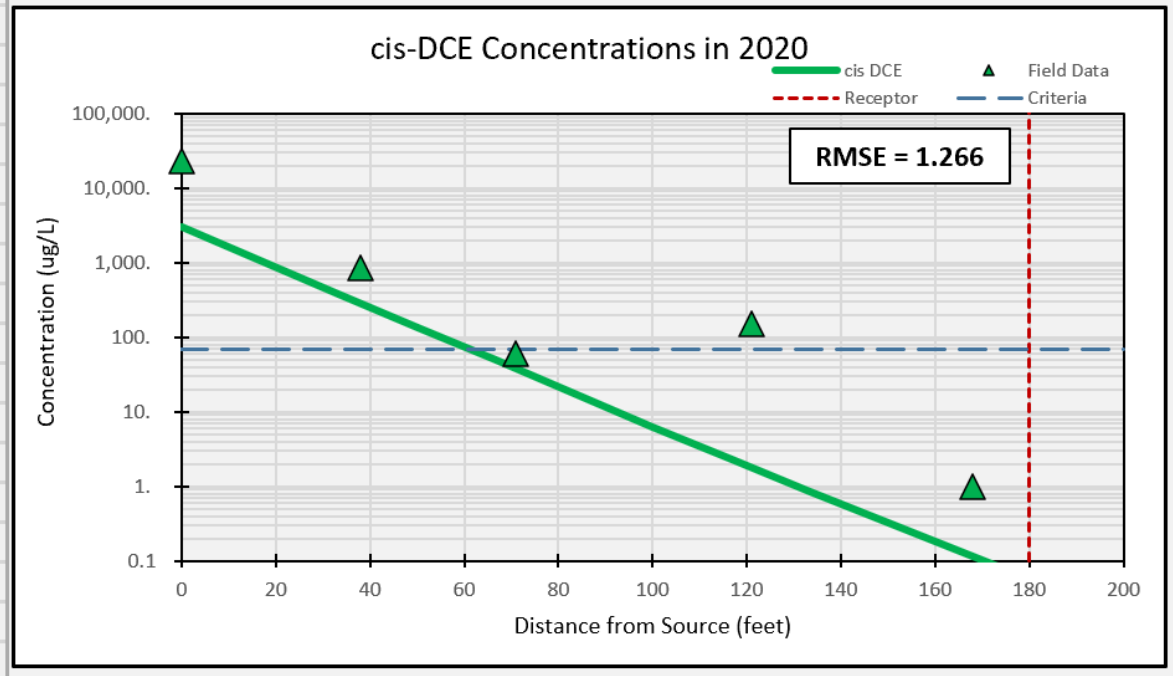
MNA Rate Constant Calculator is an upgraded version of BIOSCREEN or BIOCHLOR

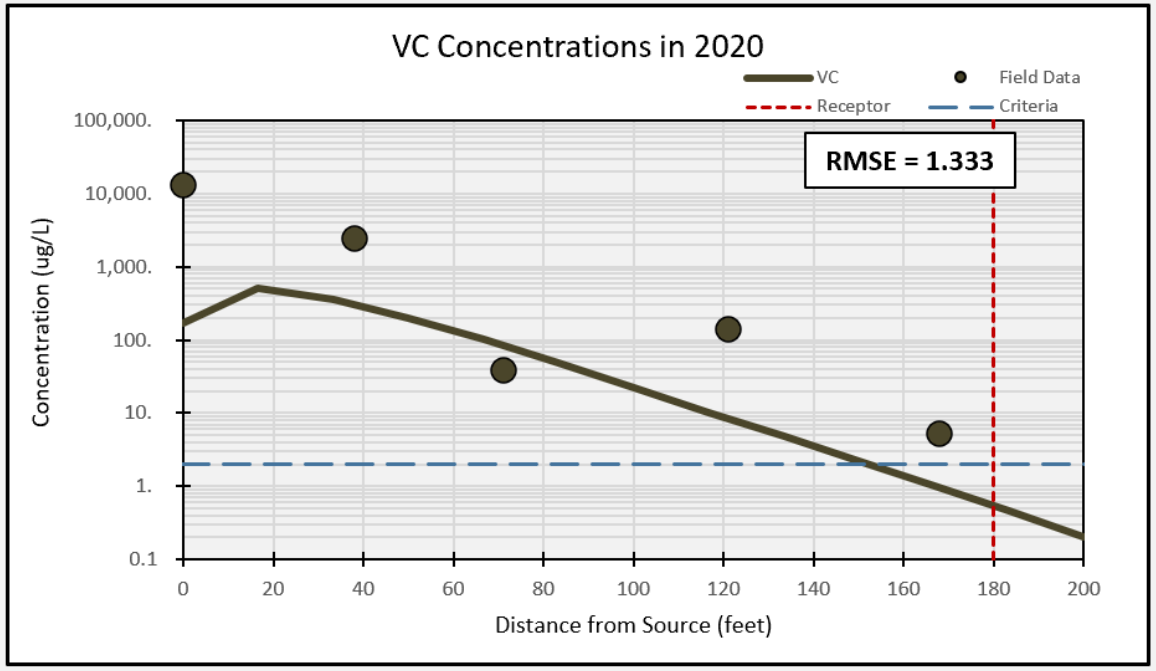
You can input the abundance of a qPCR biomarker and it will provide an estimated rate constant for biodegradation.



You can input the abundance of a qPCR biomarker and it will provide an estimated rate constant for biodegradation.

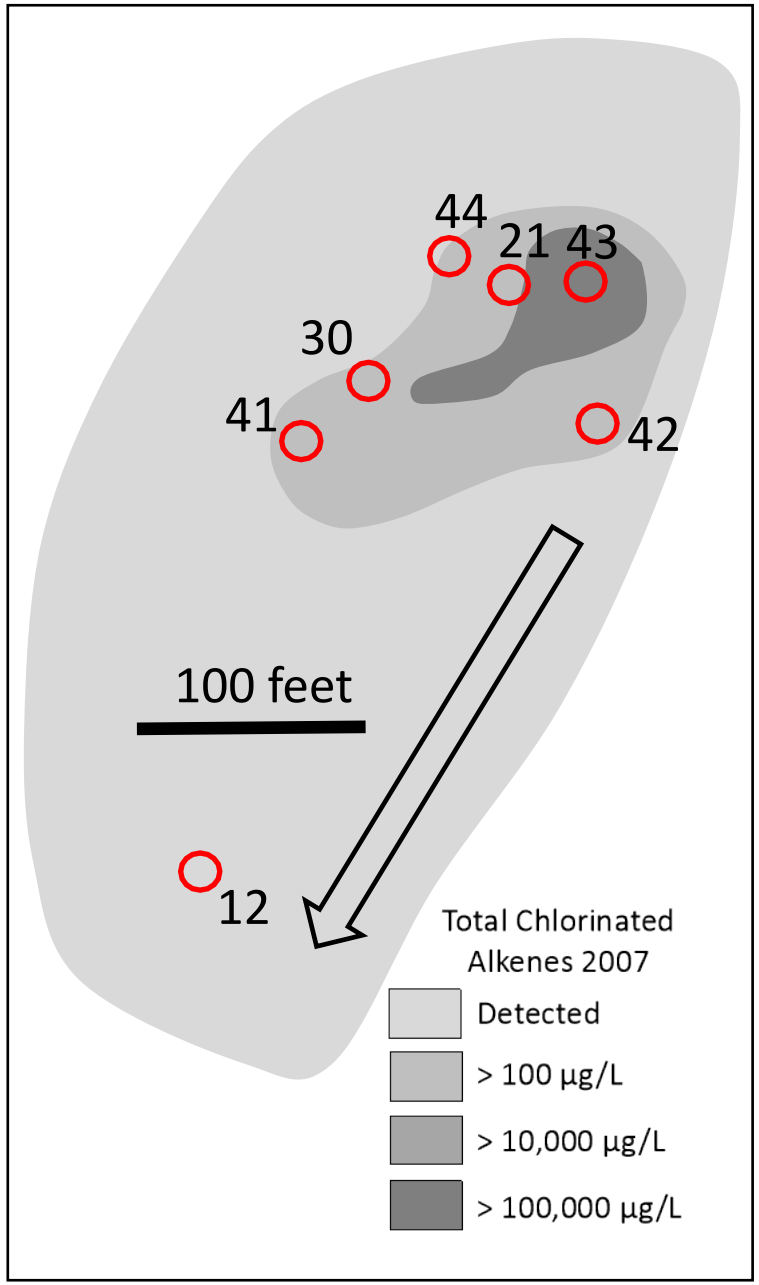
Output of MNA Rate Constant Estimator





RMSE: "Root Mean Square Error". The lower the number, the better fit between the model and the field data. The number is the typical error between a measured point and the model results.

2013

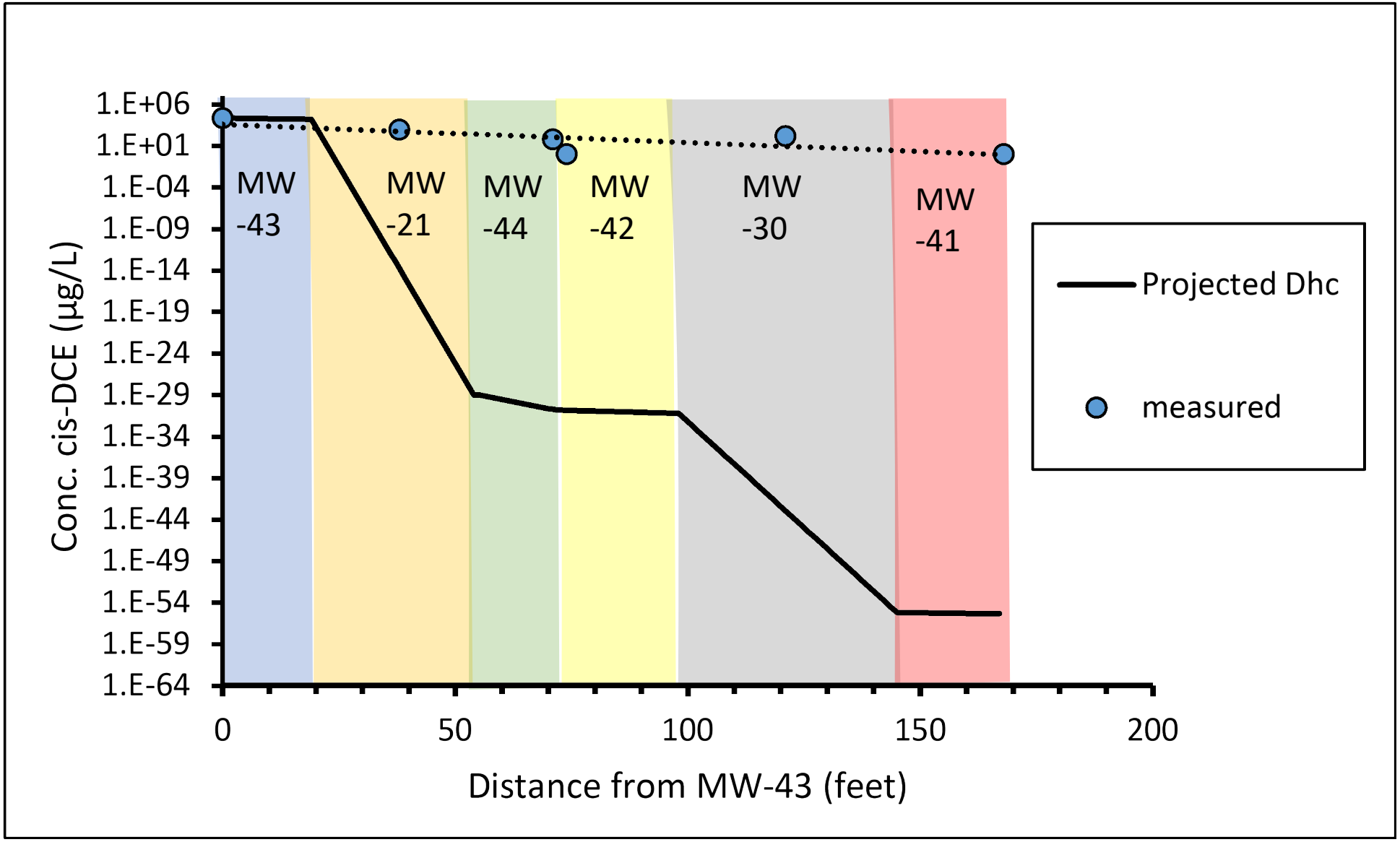


Well	Distance	*cis*-DCE	Vinyl Chloride	*Dhc*
	feet	µg/L	µg/L	cells/mL
S5-MW-43	0	23000	13000	1.3E+05
S5-MW-21	38	860	2400	8.4E+05
S5-MW-44	71	60	38	3.1E+04
S5-MW-42	74	1	3.5	3.6E+03
S5-MW-30	121	150	140	1.8E+05
S5-MW-41	168	1	5.3	1.6E+03

Well	*cis*-DCE	VC
	per year	per year
S5-MW-43	1.7	4.1
S5-MW-21	217	121
S5-MW-44	25	92
S5-MW-42	3.4	2.6
S5-MW-30	117	256
S5-MW-41	1.5	0.8

MNA Rate Constant Estimator uses a time-weighted average of the rate constants in the individual wells to estimate an overall rate constant associated with the biomarkers.

c-DCE degradation in 2013



Date	Field Rate	Field Rate	qPCR *Dhc*	qPCR *Dhc*
	cis-DCE	Vinyl Chloride	*cis*-DCE	Vinyl Chloride
	per year	per year	per year	per year
2007	**5.3** ± 1.7*			
2013	**5.0** ± 3.3*	**6.7** ± 4.6*	84	98
2016	**4.8** ± 1.2*			
2021	**8.6** ± 7.8*	**13.6** ± 10.8*		

The rate constants estimated from the qPCR biomarkers over-estimated the field rate constant for biodegradation of *cis*-DCE by more than an order of magnitude.

Rate Constants Predicted from abundance of *vcrA* gene copy or *Dhc* gene copy

Date	Field Rate	Field Rate	qPCR *vcrA*	qPCR *Dhc*	qPCR *vcrA*	qPCR *Dhc*
	cis-DCE	Vinyl Chloride	*cis*-DCE	*cis*-DCE	Vinyl Chloride	Vinyl Chloride
	per year	per year	per year	per year	per year	per year
2007	**5.3** ± 1.7*					
2013	**5.0** ± 3.3*	**6.7** ± 4.6*	59	84	67	98
2016	**4.8** ± 1.2*					
2021	**8.6** ± 7.8*	**13.6** ± 10.8*				

At the study site, it did not make much difference if the abundance of *Dhc* or *vcrA* gene copies were used to estimate the rate constants.

ACS ES&T Engineering

2022, 2, 43-53

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Article

Quantitative Proteomics and Quantitative PCR as Predictors of *cis*-1,2-Dichlorethene and Vinyl Chloride Reductive Dechlorination Rates in Bioaugmented Aquifer Microcosms

Mandy M. Michalsen, Fadime Kara Murdoch, Frank E. Löffler, John Wilson, Paul B. Hatzinger, Jack D. Istok, Larry Mullins, Amy Hill, Robert W. Murdoch, Charles Condee, and Katarzyna H. Kucharzyk*

Cite This: *ACS EST Engg.* 2022, 2, 43–53

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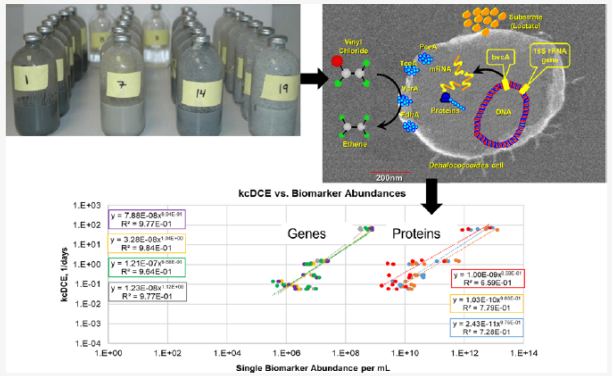
ABSTRACT: Quantitative measurement of process-specific biomarker genes of *Dehalococcoides mccartyi* (*Dhc*) supports monitoring at chlorinated ethene contaminated sites. In this study, we varied *Dhc* cell abundances from ~10^3 to 10^8 cells/mL in aquifer microcosms and correlated the corresponding reductive dehalogenase (RDase) gene and RDase protein abundances with measured reductive dechlorination (RD) rates of *cis*-1,2-dichloroethene (cDCE) and vinyl chloride (VC). An additional set of microcosms tested the RD rate-predictive power of the regression analyses. These efforts revealed (1) that targeted proteomics quantifies *Dhc* biomarker proteins (e.g., TceA and VcrA, OmeA) over a relevant range of *Dhc* cell densities, and (2) that protein and gene abundances can predict RD rates. Protein detection limits translated to a rate coefficient of 10^{-4} day^{-1} (0.04 year^{-1}) for both k_{cDCE} and k_{vc}, which is within the range observed at sites undergoing monitored natural attenuation (MNA) (i.e., without the implementation of enhanced bioremediation treatment). Rates predicted using a combination of quantitative biomarker gene and protein measurements generally resulted in the best match with experimentally determined rate constants. These new findings provide evidence that quantitative biomarker measurements may be useful predictors of *in situ* RD rates, which would constitute a major advance for the cost-effective management of contaminated sites.



Can we do better if we measure the abundance of the Reductase Enzymes?

Thursday Platform Sessions—1:00–2:40 p.m.

	A SESSIONS Waterloo 1-2 (Level 5)	B SESSIONS Waterloo 3 (Level 5)	C SESSIONS Waterloo 4 (Level 5)	D SESSIONS Waterloo 5-6 (Level 5)	E SESSIONS Waller A-B (Level 3)
1:00	**Achieving Project Success through Remediation Failure.** *R. Oesterreich.* Ryan Oesterreich (Arcadis/USA)	**Results from a 1,4-Dioxane Biogeochemical Reactor Field Pilot Test.** *C. Walecka-Hutchison, J. Sprague, J. Gamlin, R. Caird, Y. Miao, I. Kwok, and S. Mahendra.* Claudia Walecka Hutchison (Dow/USA)	**Combining Biotic and Abiotic Treatment Processes Post In Situ Thermal Treatment (ISTT).** *J.G. Booth, R.D. Collins, R. Hogdahl, and R. Simon.* J. Greg Booth (Woodard & Curran/USA)	**Role of Stratigraphic Models to Refine Site Assessments.** *B. Campanaro, J. Sadeque, R. Samuels, and D. Parse.* Ben Campanaro (AECOM/USA)	**Natural Occurrence of Feammox Conditions and Anammox Microbiota within a PFAS Plume at the Groundwater-to-Surface Water Interface.** *B. Harding, R. Gwinn, and J. Buzzell.* Barry Harding (AECOM/USA)
1:25	**Comparison of In Situ Bioremediation of Perchlor Chlorinated Solvents at Th in Close Proximity: Challer Lessons Learned.** *W.A. Fos P. Srivastav, and R.E. Mayer* William Foss (APTIM/USA)			**Where is the Vinyl Chloride? Alternative Natural and Enhanced Degradation Pathways for Chlorinated Solvents.** *J.R. Hesemann.* John Hesemann (Burns & McDonnell/USA)	**Groundwater/Surface Water Interactions at the Transition Zone: Utilizing an In Situ Passive Sampling Program to Evaluate Groundwater Upwelling.** *B.G. Pautler, M. Healey, J. Roberts, J. Conder, D. Toler, L. Fontenot, and S. Aufdenkampe.* Sandra Dworatzek (SiREM/Canada)
1:50	**EVO Use in Hard Water Aq Implications and Strategie Successful Substrate Distr** *J.F. Ortiz-Medina, L. Ross, a R.C. Borden.* Fausto Ortiz (EOS Remediat			**Groundwater Plume Analytics® Tools for Improved Conceptual Site Models at Bioremediation Sites.** *J.A. Ricker and D.C. Winchell.* Joseph Ricker (WSP/USA)	**A Seep Origin Story: Using Electrical Hydrogeology to Find Mysterious Deep LNAPL Source.** *T. Halihan, K.W. Spears, and S.W. McDonald.* Todd Halihan (Oklahoma State University/USA)
2:15	**Successful Enhanced Reductive Dechlorination in Bedrock with Long-Term Monitoring: Two Case Studies.** *P.M. Dombrowski, P. Kakarla, M. Temple, M. Lee, D. Raymond, and C. Weeden.* Paul Dombrowski (In-Situ Oxidative Technologies, Inc. [ISOTEC]/USA)	**In Situ Bioremediation of 1,4-Dioxane in Mixed Contaminant Plume with Metabolic Bioaugmentation and Cometabolism.** *F.J. Krembs, K. McDonald, M. Olson, and S. Dworatzek.* Fritz Krembs (Trihydro Corporation/USA)	**In Situ Enhanced Bioremediation to Reduce Large TCE/PCE Plumes and Government's Life Cycle Costs.** *P. Srivastav, W.A. Foss, and R.E. Mayer.* Praveen Srivastav (APTIM/USA)	**Quantitative Proteomics Approach to Monitor cVOC Bioremediation and Degradation Rates.** *K.H. Kucharzyk, F. Kara Murdoch, F.E. Loffler, J. Wilson, P.B. Hatzinger, J.D. Istok, R.W. Murdoch, L. Mullins, A. Hill, and M. Michalsen.* Kate Kucharzyk (Battelle/USA)	**ssessing the Origin of roundwater Springs and mplications for PFAS Fate and ransport at Mountain Home Air orce Base, Idaho.** *M.R. Shultz and . Anding.* ike Shultz (Burns & McDonnell/SA)

A11. Challenges in Application of Bioremediation Tools

C7. Bioremed ation App

Tools for Site Assessment and Bioremediation Monitoring

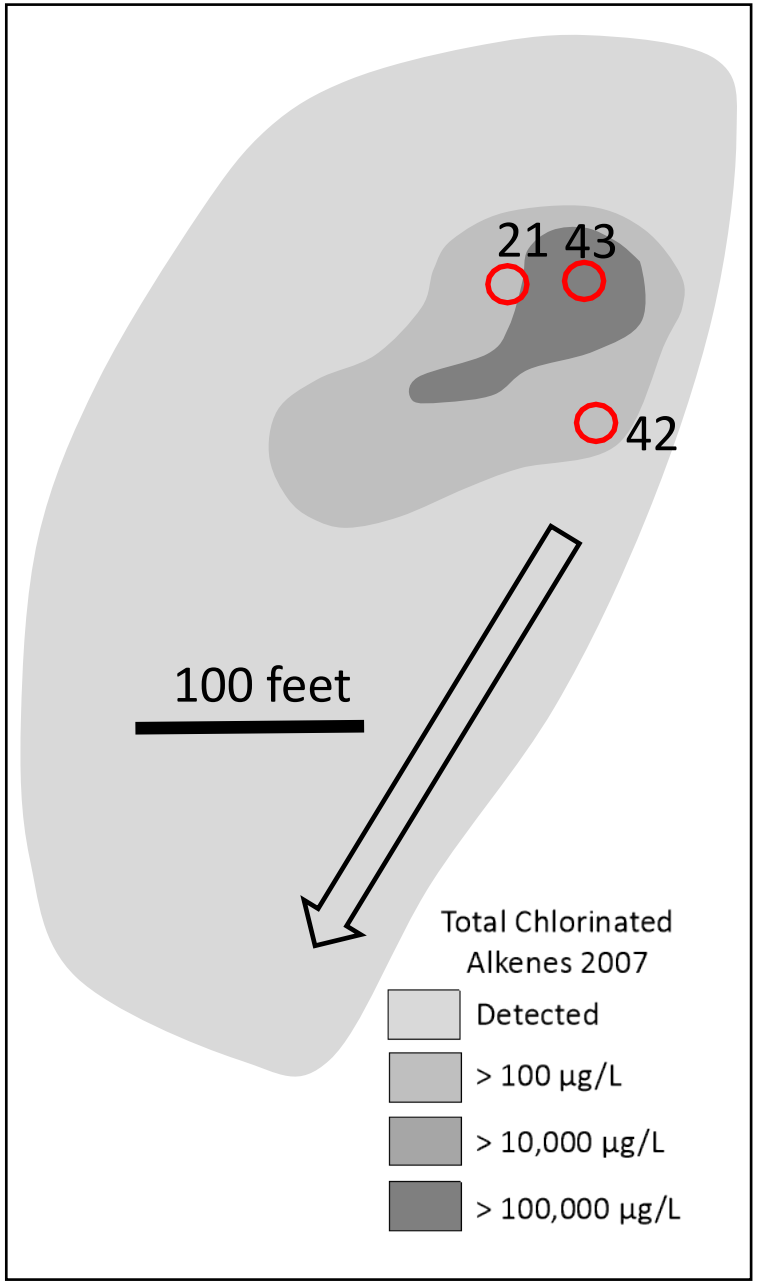
E9. Groundwater/Surface Water Interactions

Kate Kucharzyk provides more details on the proteomics approach this afternoon.

For the *TceA* Reductase Enzyme the following is available:

Culture	V_{max} *cis*-DCE	K_m *cis*-DCE	Reference
	mg/peptide* year	mg/L	
Dhc DMC195 Cornell U. Ithaca, NY	1.2E-10	0.28	Rowe et al. 2013 *ES&T* 47,3724-3733

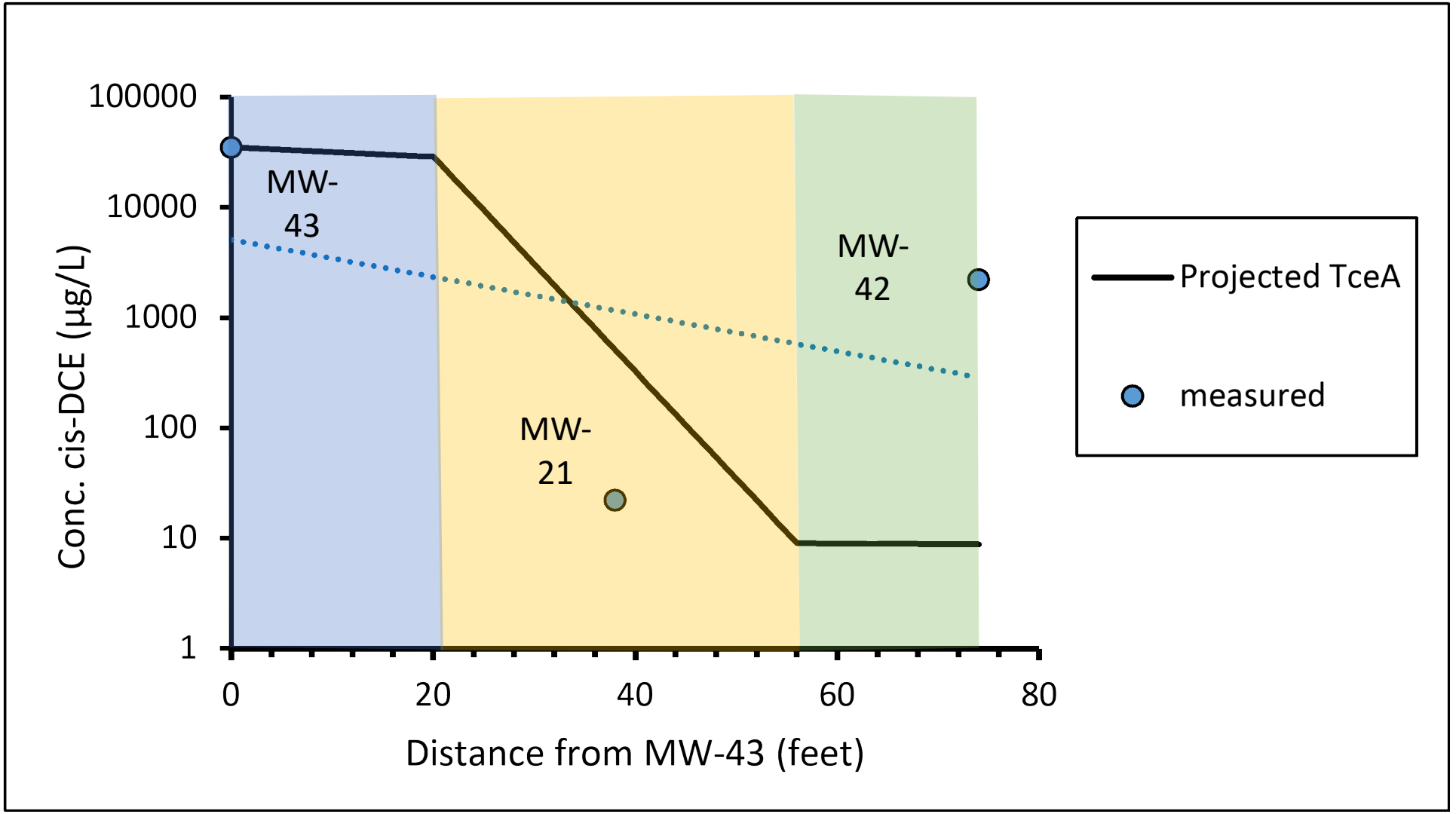
2021



Well	Distance	*cis*-DCE	*TceA*
	feet	µg/L	peptides/mL
S5-MW-43	0	35000	6.3E+08
S5-MW-21	38	22	1.2E+08
S5-MW-42	74	2200	7.7E+06

Well	*cis*-DCE by TceA
	per year
S5-MW-43	1.01
S5-MW-21	22.3
S5-MW-42	0.17

cis-DCE degradation in 2021



Date	Field Rate	qPCR *Dhc*	qProt TceA
	cis-DCE	*cis*-DCE	*cis*-DCE
	per year	per year	per year
2007	**5.3** ± 1.7*		
2013	**5.0** ± 3.3*	84	
2016	**4.8** ± 1.2*		
2021	**8.6** ± 7.8*		11.5

The rate constant for biodegradation of *cis*-DCE estimated from the abundance of the *TceA* peptides fell within the 80% confidence interval of the field scale rate constant.

Date	Field Rate	qPCR *Dhc*	qProt TceA
	cis-DCE	*cis*-DCE	*cis*-DCE
	per year	per year	per year
2007	**5.3** ± 1.7*		
2013	**5.0** ± 3.3*	84	
2016	**4.8** ± 1.2*		
2021	**8.6** ± 7.8*		11.5

The rate constant for biodegradation of *cis*-DCE estimated from the abundance of the *TceA* peptides was a closer match to the field data than the rate constant estimated from the abundance of the *Dhc* qPCR marker.

Summary Evaluation:

- Use of the published kinetic parameters allow a quantitate evaluation of the contribution of biological reductive dechlorination.

- A comparison of field scale rate constants to rate constants predicted using the biomarkers can determine if the biological reductive dechlorination is a plausible explanation of the field scale rate constant, and thus provides the USEPA second line of evidence.

Summary Evaluation:

- The predicted rate constants vary widely from well to well.

- The uncertainty in the time-weighted average suggests that it will be **prudent to repeat the sampling and analysis of the biomarkers over several quarters and perhaps several years** to confirm the central tendency of the time weighted averages.

Summary Evaluation:

- Targeted Proteomics shows promise of being able to provide more precise predictions of rate constants compared to biomarkers that measure the abundance of DNA.

- Unfortunately, there are no kinetic parameters for the *VcrA* reductase or for the *BvcA* reductase available in the literature.

- Having these parameters would expand the application of targeted proteomics to understand biological reductive dechlorination in groundwater.