Data Needs for Effective Application of MNA and In-Situ Bioremediation Featuring *Framework to Apply Novel Molecular and Other Screening Tools for MNA Evaluations*

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Development and Validation of a Quantitative Framework and Management Expectation Tool for the Selection of Bioremediation Approaches at Chlorinated Solvent Sites

ER-201129

Objective | Technology | Results | Implementation

Project of the Year 2015

Objectives of the Demonstration

The overarching objective of this project was to develop and validate a framework that can be used to make bioremediation decisions based on site-specific physical and biogeochemical characteristics and constraints. This framework represents an update to the U.S. Environmental Protection Agency's (EPA) *Technical Protocol for Evaluating the Natural Attenuation of Chlorinated Solvents in Ground Water.*

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Technology Description

The quantitative framework developed is a systematic approach that uses the relationships between specific biogeochemical parameters and degradation rates to

Points of Contact

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Products

Cost and Performance Report (Posted 01/16) Final Report (Posted 12/15)

Model/Software - BioPic Tool Guidance Document - BioPic Tool

Blog Post (01/19/2016) Blog Post (12/07/2015)
Webinar Series (03/19/2015)

You want the BioPIC Tool. Section 5 of the Final Report provides guidance of using a model to extract rate constants for biodegradation, and gives more detail than is provided in the decision criteria and help buttons of the BioPIC tool.

ESTCP Project ER-201129

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EPA/600/R-98/128
September 1998

EPA

Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Ground Water

EPA/600/R-98/128
September 1998

Use of Monitored Natural Attenuation at Superfund, RCRA Corrective Action, and Underground Storage Tank Sites

U.S. Environmental Protection Agency
Office of Solid Waste and Emergency Response
Directive 9200.4-17P

(1) Historical groundwater and/or soil chemistry data that demonstrate a clear and meaningful trend of decreasing **contaminant mass and/or concentration** over time at appropriate monitoring or sampling points.

(In the case of a groundwater plume, decreasing concentrations should not be solely the result of plume migration. In the case of inorganic contaminants, the primary attenuating mechanism should also be understood.)

(2) Hydrogeologic and geochemical data that can be used to demonstrate indirectly the type(s) of natural attenuation processes active at the site, and the rate at which such processes will reduce contaminant concentrations to required levels. For example, characterization data may be used to quantify the rates of contaminant sorption, dilution, or volatilization, or to demonstrate and quantify the **rates of biological degradation processes** occurring at the site.

Unless EPA or the overseeing regulatory authority determines that historical data (Number 1 above) are of sufficient quality and duration to support a decision to use MNA, data characterizing the nature and rates of natural attenuation processes at the site (Number 2 above) should be provided.

Is the entire plume required to meet the goal?

If so, at what date must concentrations in the plume meet the cleanup level?

The performance depends on the success of source treatment, and the kinetics of natural attenuation of the source.

These processes can **not** be evaluated or understood using Compound Specific Isotope Analysis (CSIA) or Molecular Biological Tools (MBT).

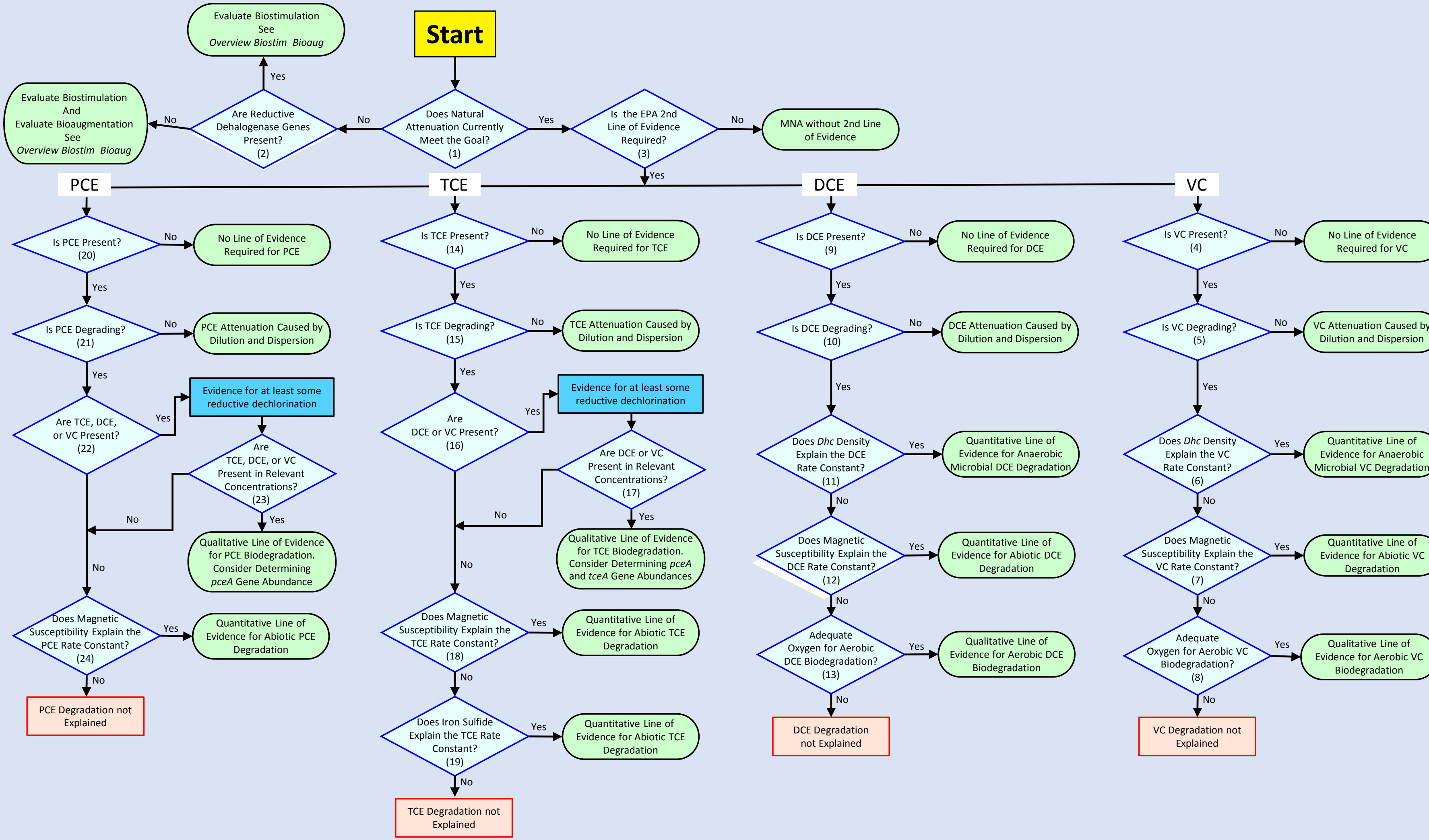
How far can the plume be allowed to extend?

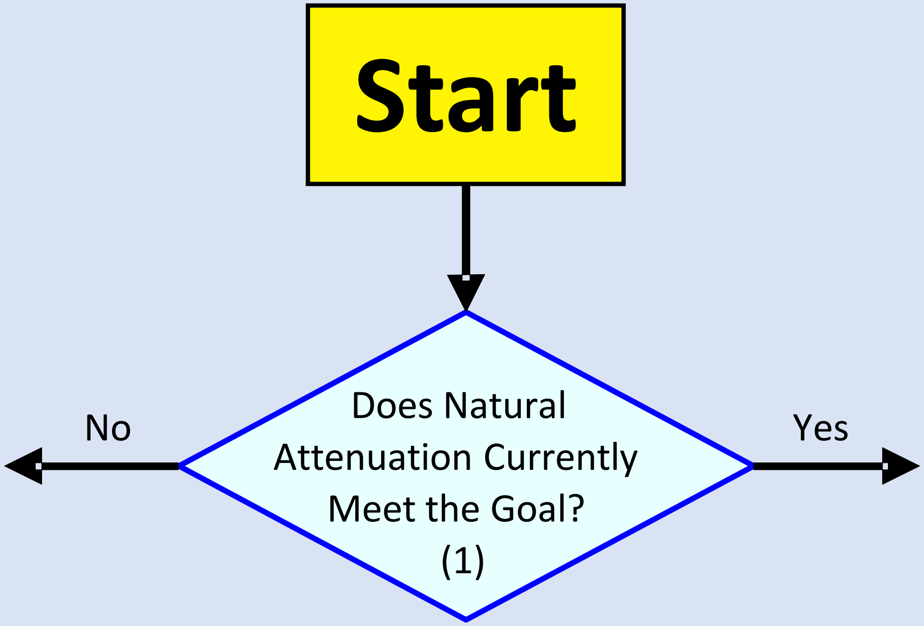
Will the rate of attenuation bring the highest concentrations in groundwater to acceptable concentrations before the groundwater reaches the receptor of the sentry well?

Evaluated by extracting a rate constant from field data for the rate of degradation necessary to meet the goal.

Compound Specific Isotope Analysis (CSIA) or Molecular Biological Tools (MBT) can provide a second line evidence to support a site conceptual model.

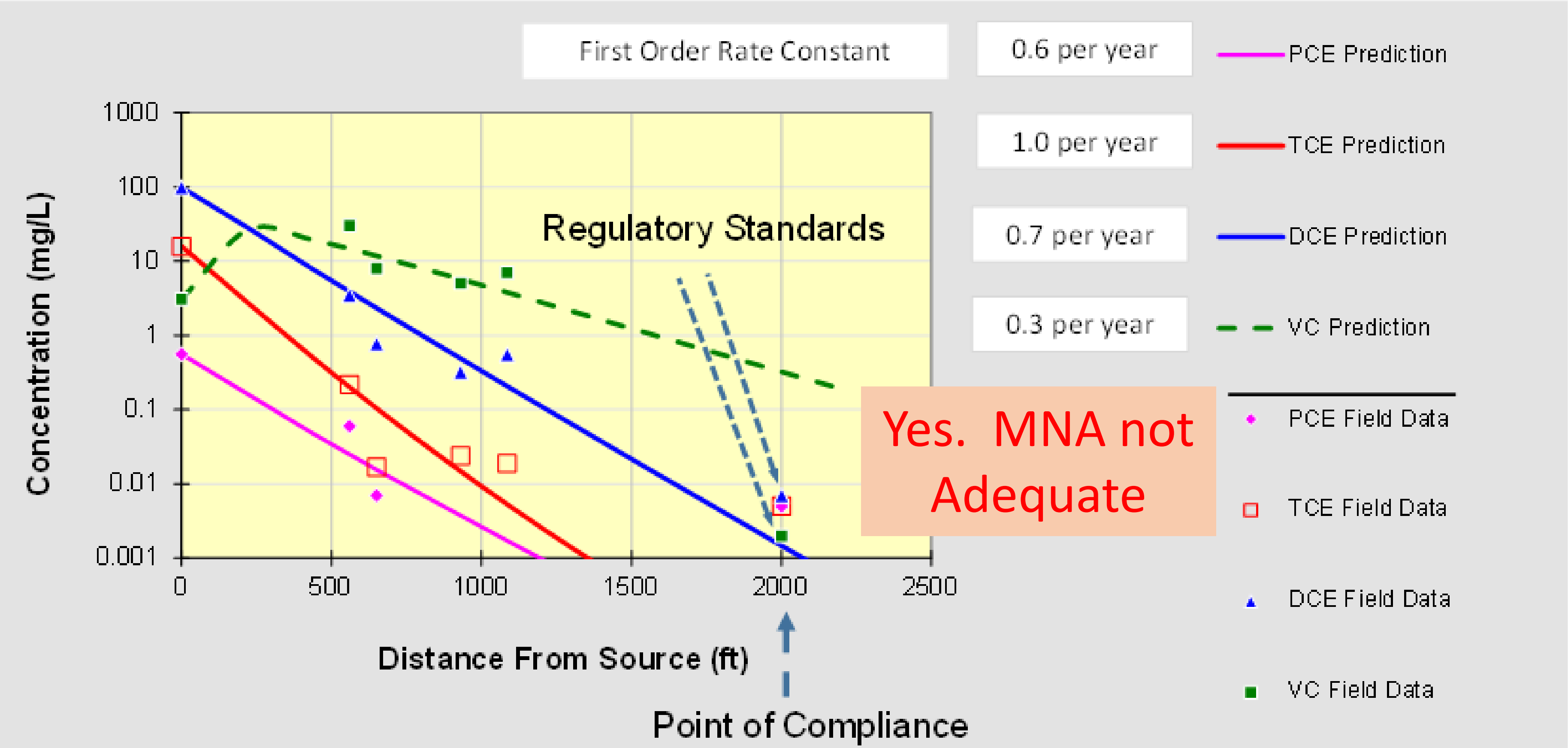
The decision logic in BioPIC




Start
Does Natural Attenuation Currently Meet the Goal? (1)
No
Yes

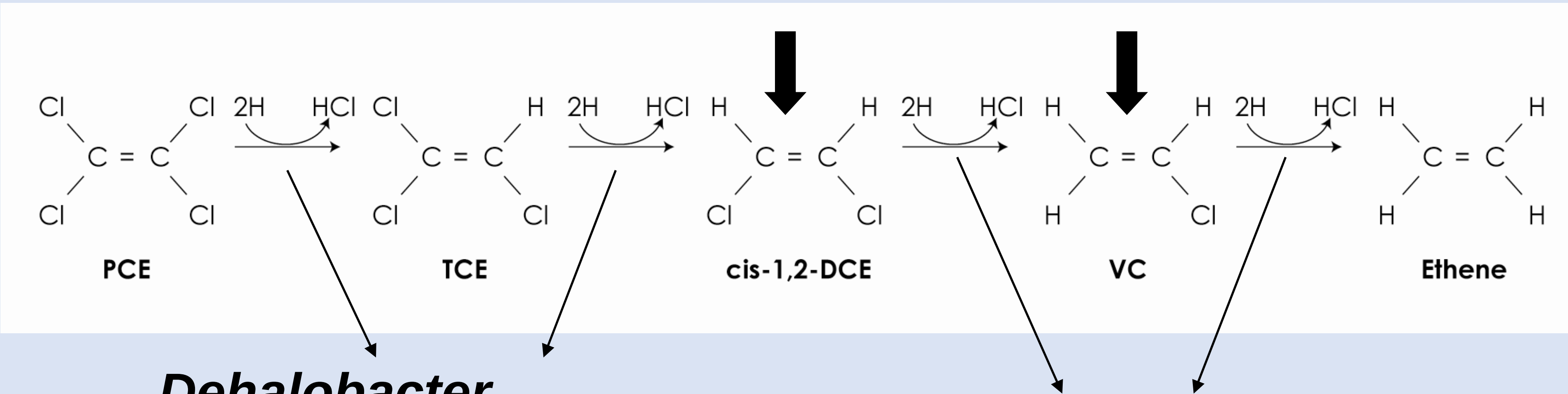
Use a computer model to project the previous behavior of the contamination forward in space and time.

Will contaminants in the plume extend past the point of compliance at unacceptable concentrations?



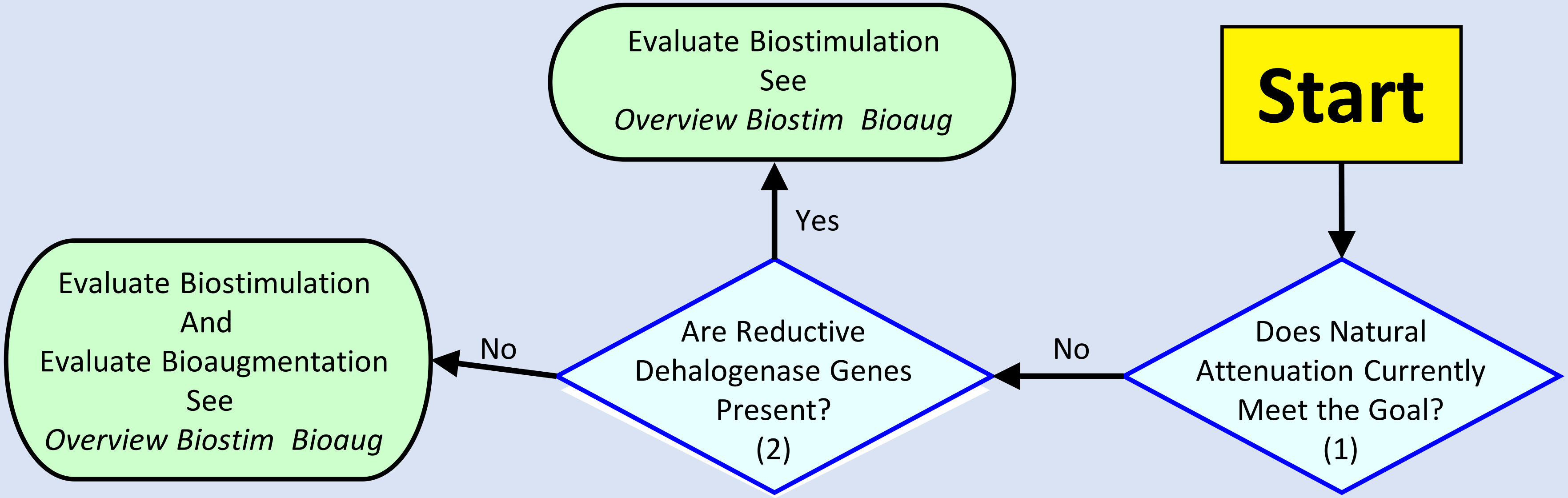
Microbiology of Reductive Dechlorination of Chloroethenes

Can accumulate if requisite bacteria are not present



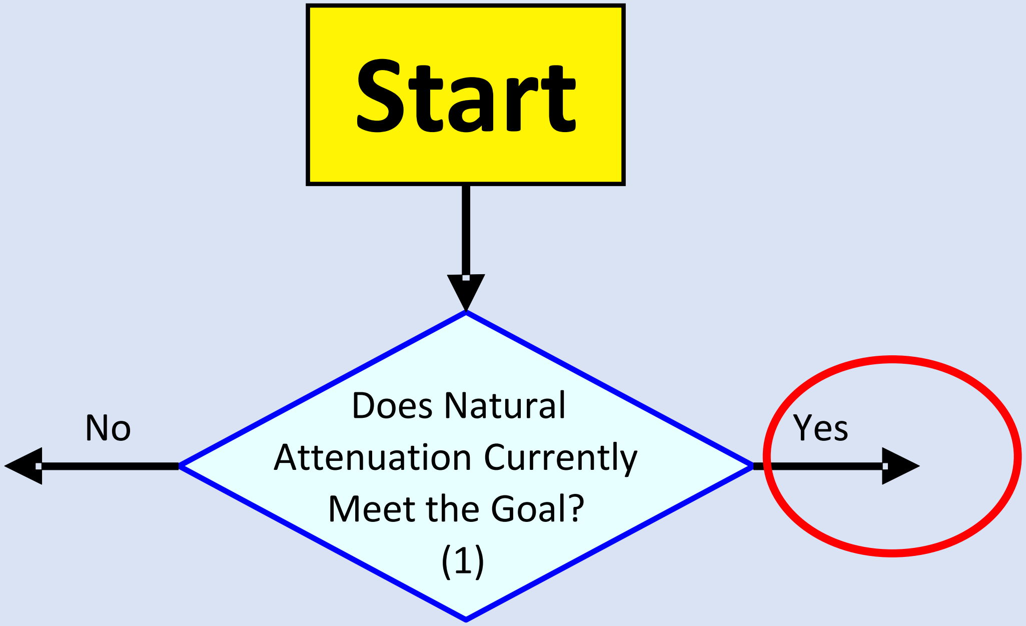
Dehalobacter
Dehalospirillum
Desulfitobacterium
Desulfuromonas
Dehalococcoides

some strains of
Dehalococcoides



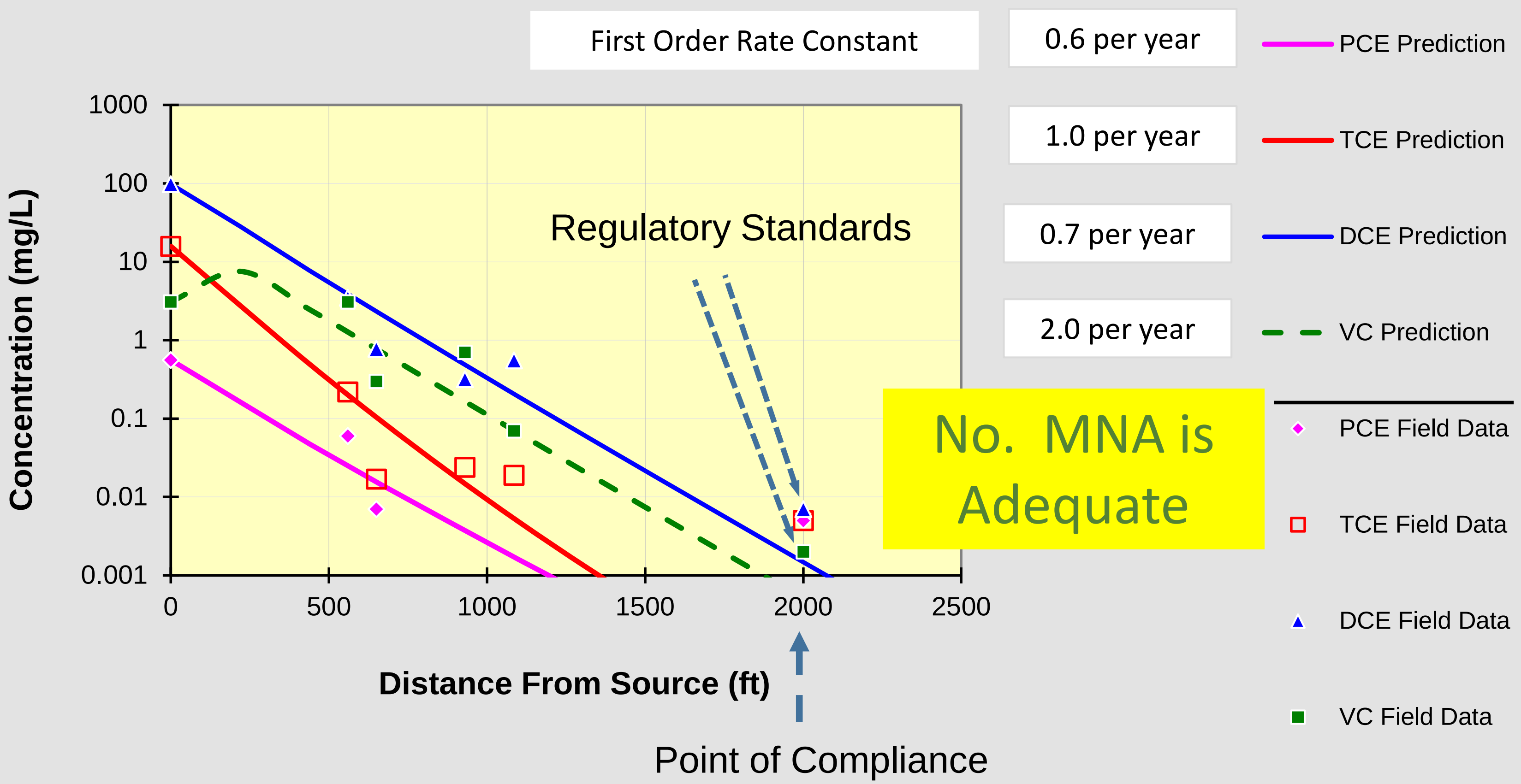
Assays based on the Quantitative Polymerase Chain Reaction (qPCR) are used to identify the presence of organisms with genes that can degrade chlorinatred alkenes to harmless end products.

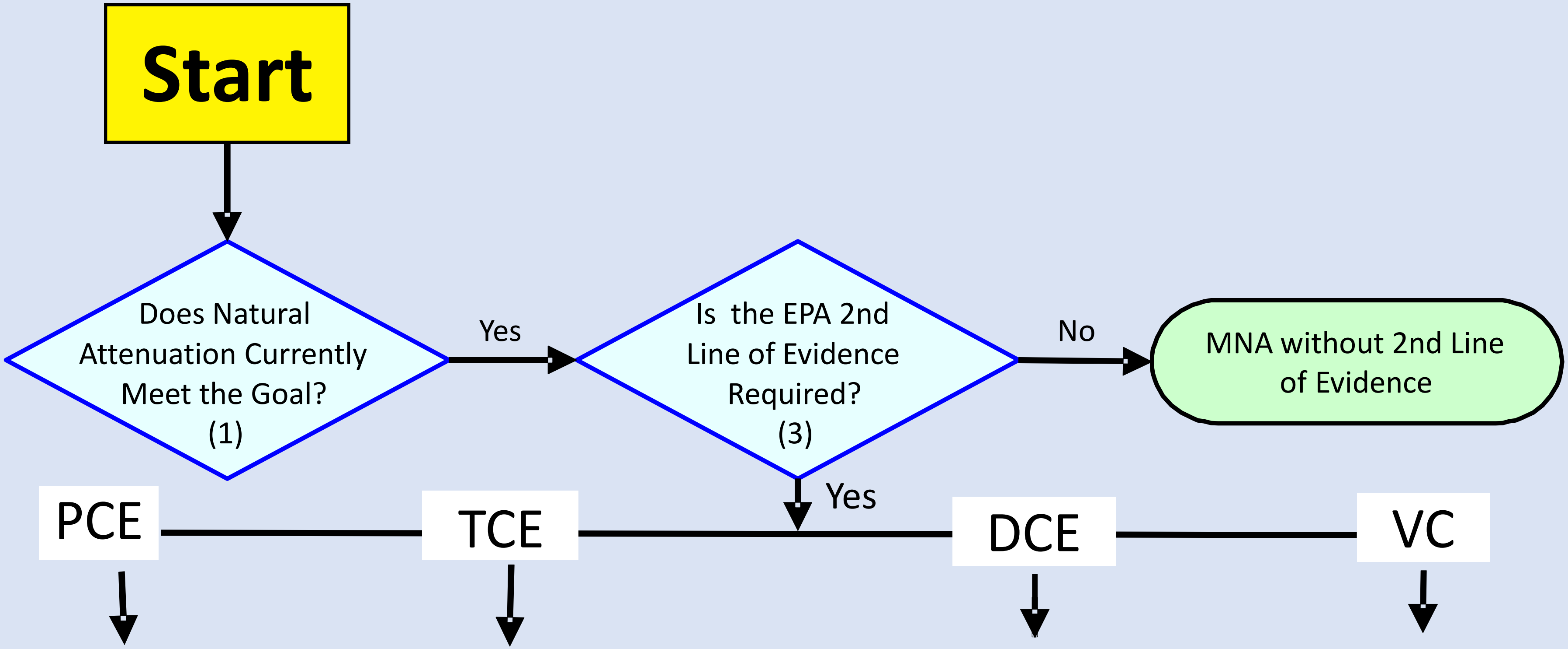
If the density of the pceA, tceA, bvcA, or vcrA genes are greater than 1000 gene copies per liter of groundwater, that gene is considered to be present.


Start
Does Natural Attenuation Currently Meet the Goal?
(1)
No
Yes

Use a computer model to project the previous behavior of the contamination forward in space and time.

Will contaminants in the plume extend past the point of compliance at unacceptable concentrations?

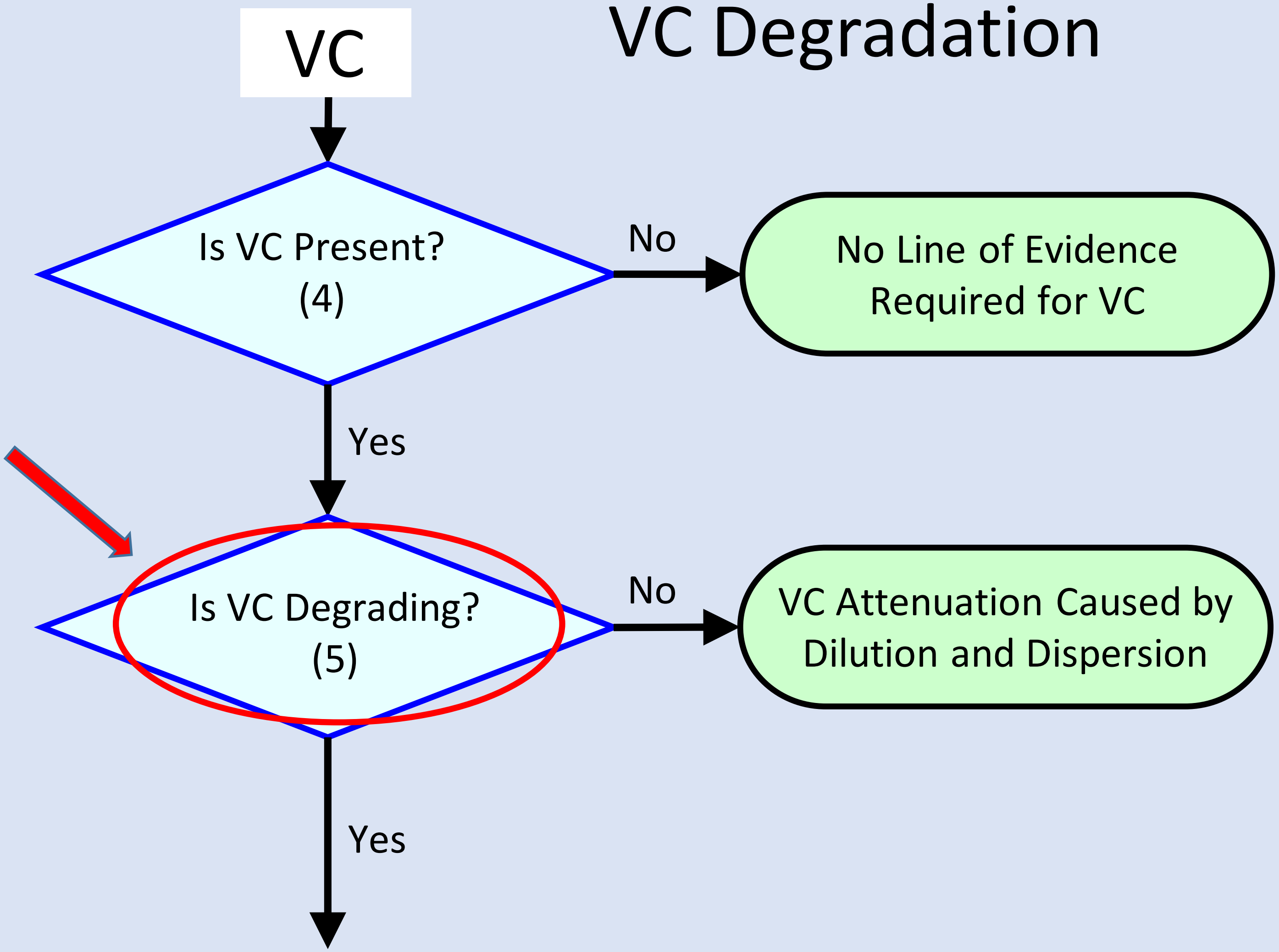



Start
Does Natural Attenuation Currently Meet the Goal? (1)
Yes
Is the EPA 2nd Line of Evidence Required? (3)
No
MNA without 2nd Line of Evidence
Yes
PCE
TCE
DCE
VC

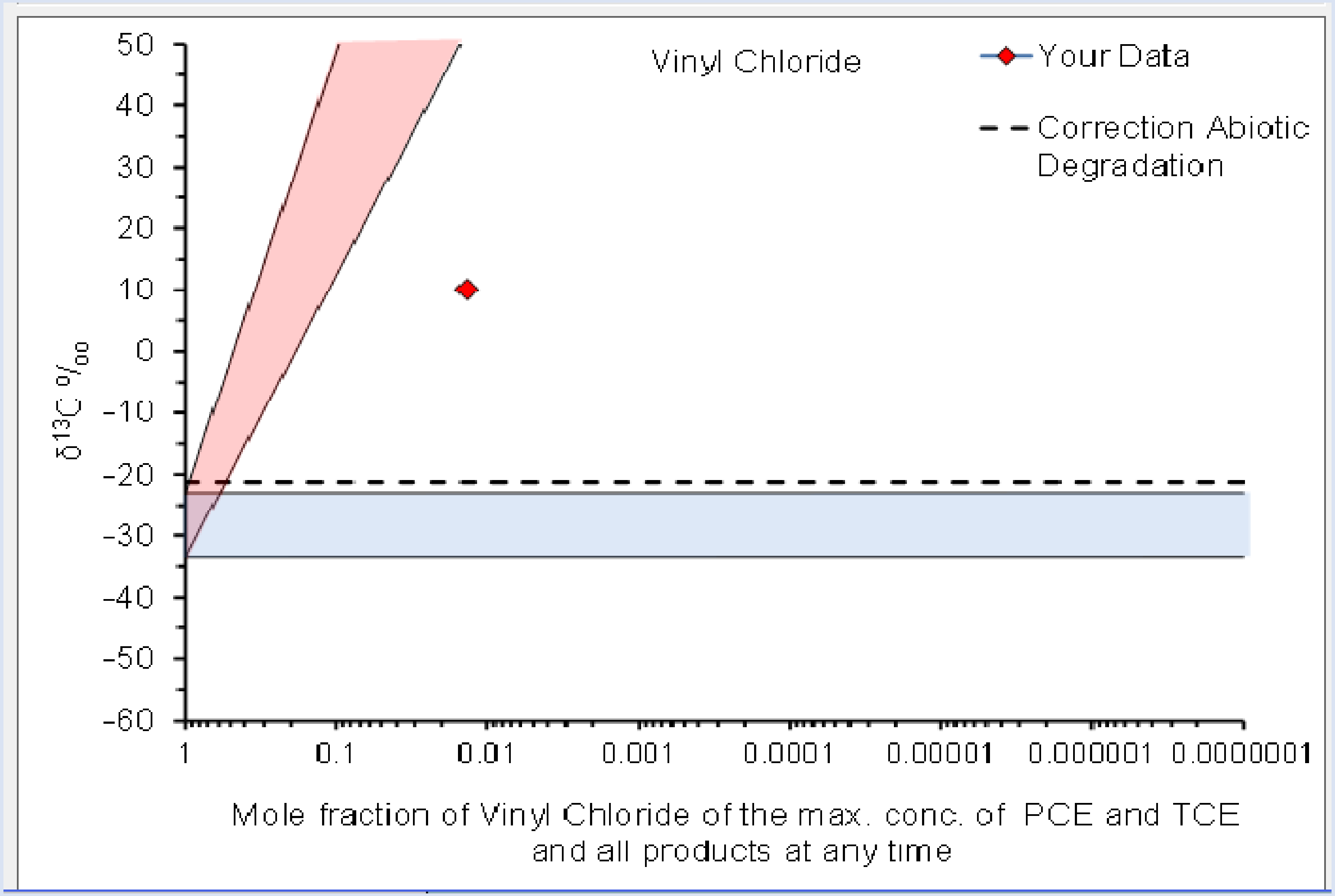
VC Degradation

The primary line of evidence for degradation is the projection of the computer model.

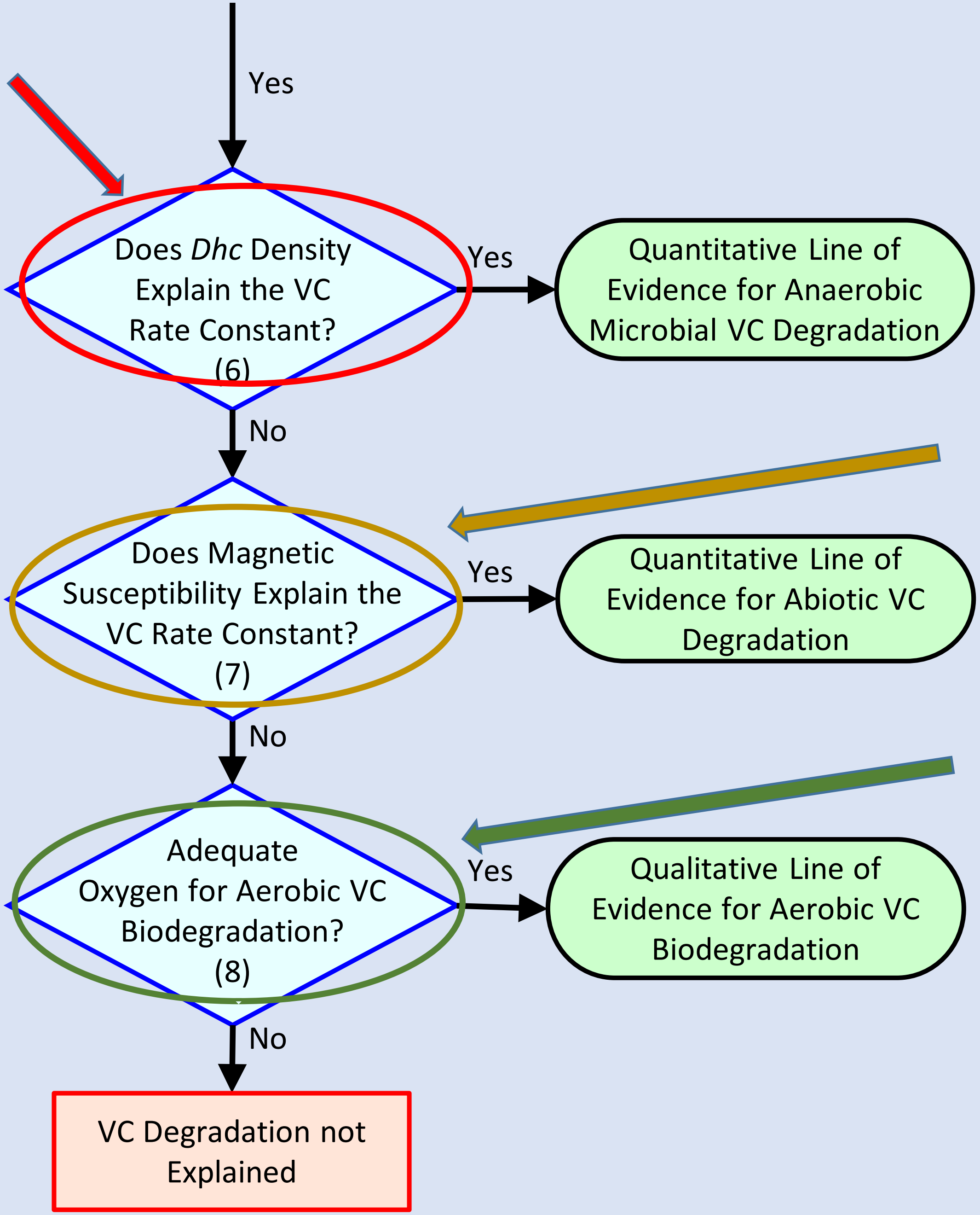
The secondary line of evidence is stable isotopic fractionation as revealed by CSIA.



The secondary line of evidence is stable isotopic fractionation as revealed by CSIA.



Compare the rate of degradation and the density of *Dehalococcoides* bacteria at the site to the rate of degradation and the density of *Dehalococcoides* at benchmark sites.



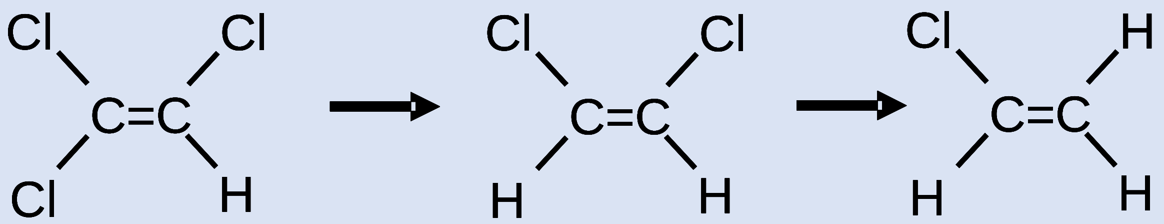
Compare the rate of degradation and the magnetic susceptibility of sediment at the site to the rate of degradation and magnetic susceptibility at benchmark sites.

Adequate oxygen is defined by the absence of Fe^{+2} and methane in addition to the presence of oxygen.

Carried out by Biological Reductive Dechlorination

Sequential Reductive Dechlorination

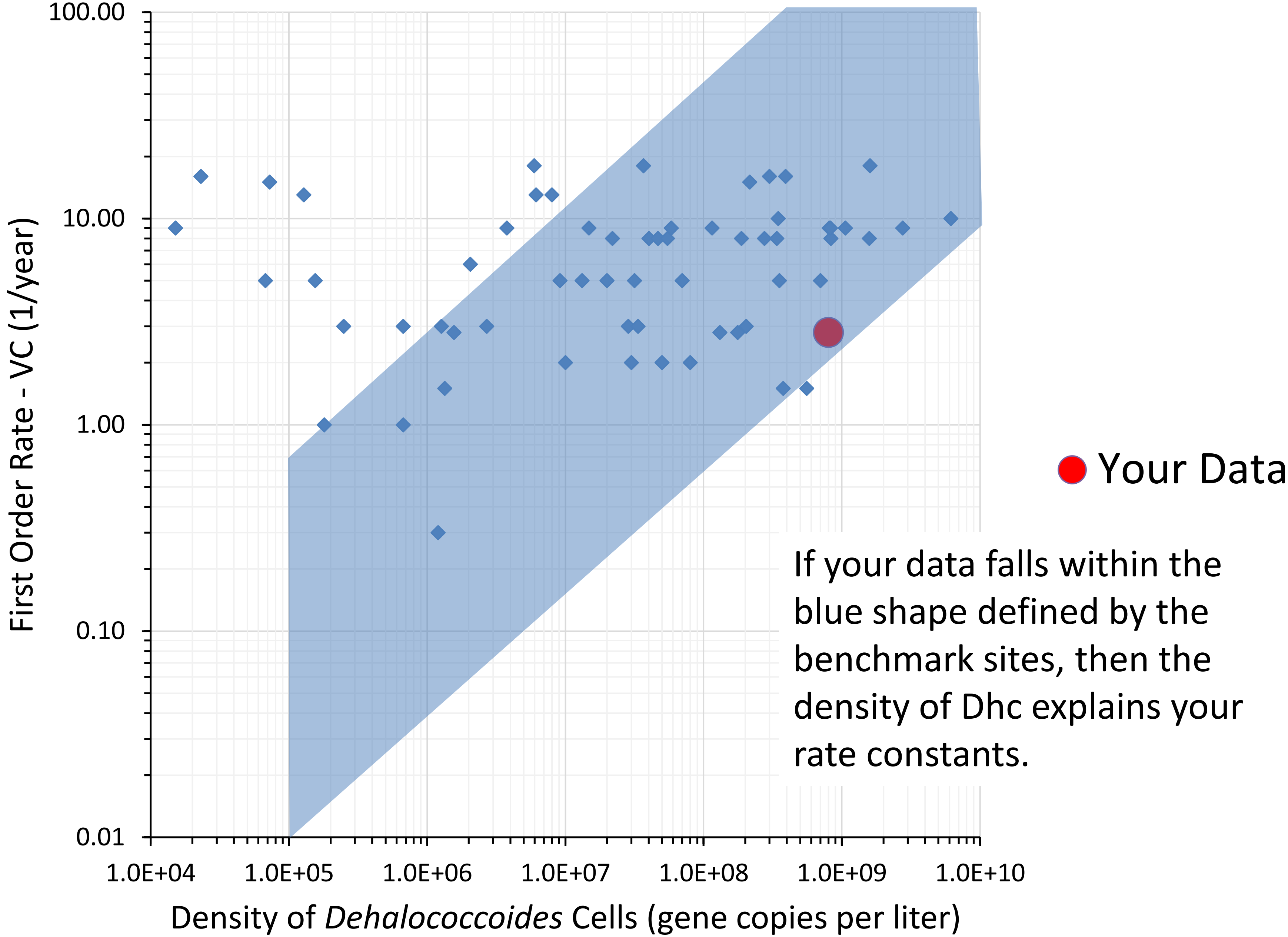
also called Hydrogenolysis



Can biotic degradation by *Dehalococcoides* bacteria explain the field-scale rate constant for degradation?

		Overwrite Input Cells with Data Specific to Your Site				
		Input				
		First order rate constant for degradation per year			pCR Assay Gene Copies per Liter	
	TCE			*Dehaloccoides* 16sRNA	8.00E+08	
	cis-DCE	2.5				
	Vinyl Chloride	2.8				

Excel Spreadsheet ***Dhc explain rates.xlsx***.



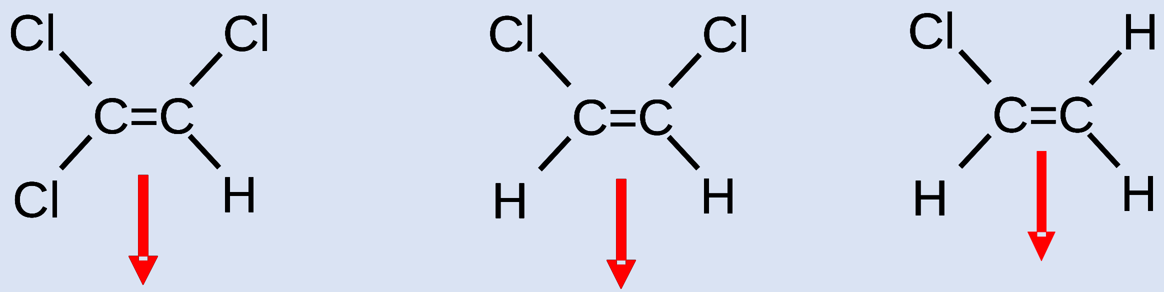
If your data falls within the blue shape defined by the benchmark sites, then the density of Dhc explains your rate constants.

Magnetite ($FeO.Fe_2O_3$) often occurs naturally in sediments formed by weathering of igneous or metamorphic rock.

Magnetite can also be produced in situ by iron-reducing bacteria.

Magnetite can degrade TCE or *cis*-DCE or Vinyl Chloride to oxidized products under either aerobic or anaerobic conditions.

If the TCE or *cis*-DCE is degraded by magnetite, there is no production of Vinyl Chloride.



CO_2 and other oxidized products

Overwrite input cells with data specific to your site

Input

	First order rate constant for degradation per year	Fraction of benchmark rate constants that are comparatively faster than the rate constant for this site*
PCE		rate slower than expected
TCE		rate slower than expected
cis-DCE	0.2	<20%
Vinyl Chloride	0.4	<20%

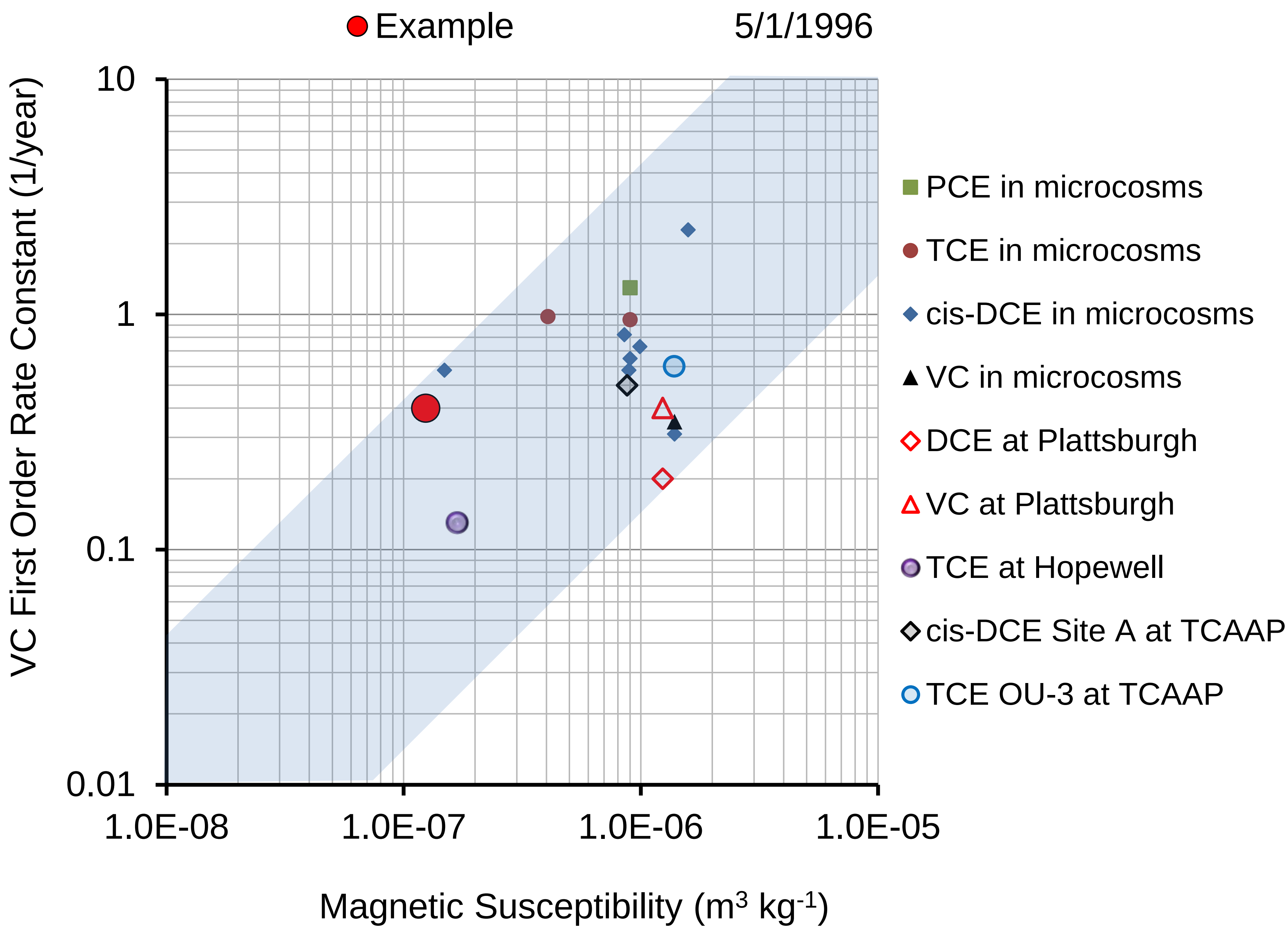
	Magnetic Suceptibility SI Units (m^3kg^{-1})
	1.25E-07
Location and Site	Example
Date	5/1/1996

The BASELINE is the lower boundary of the blue shape that encompases plausibe rate constants associated with abiotic degradation on magnetite.

*The fraction of the benchmark rate constants that exceed the BASELINE to a greater extent than the rate constant for this site exceeds the BASELINE

Data Input | Mag. Sus. Explains PCE | Mag. Sus. Explains TCE | Mag. Sus. Explains cDCE | Mag. Su

READY


Example
5/1/1996
VC First Order Rate Constant (1/year)
10
1
0.1
0.01
1.0E-08
1.0E-07
1.0E-06
1.0E-05
Magnetic Susceptibility ($m^3\ kg^{-1}$)
PCE in microcosms
TCE in microcosms
cis-DCE in microcosms
VC in microcosms
DCE at Plattsburgh
VC at Plattsburgh
TCE at Hopewell
cis-DCE Site A at TCAAP
TCE OU-3 at TCAAP

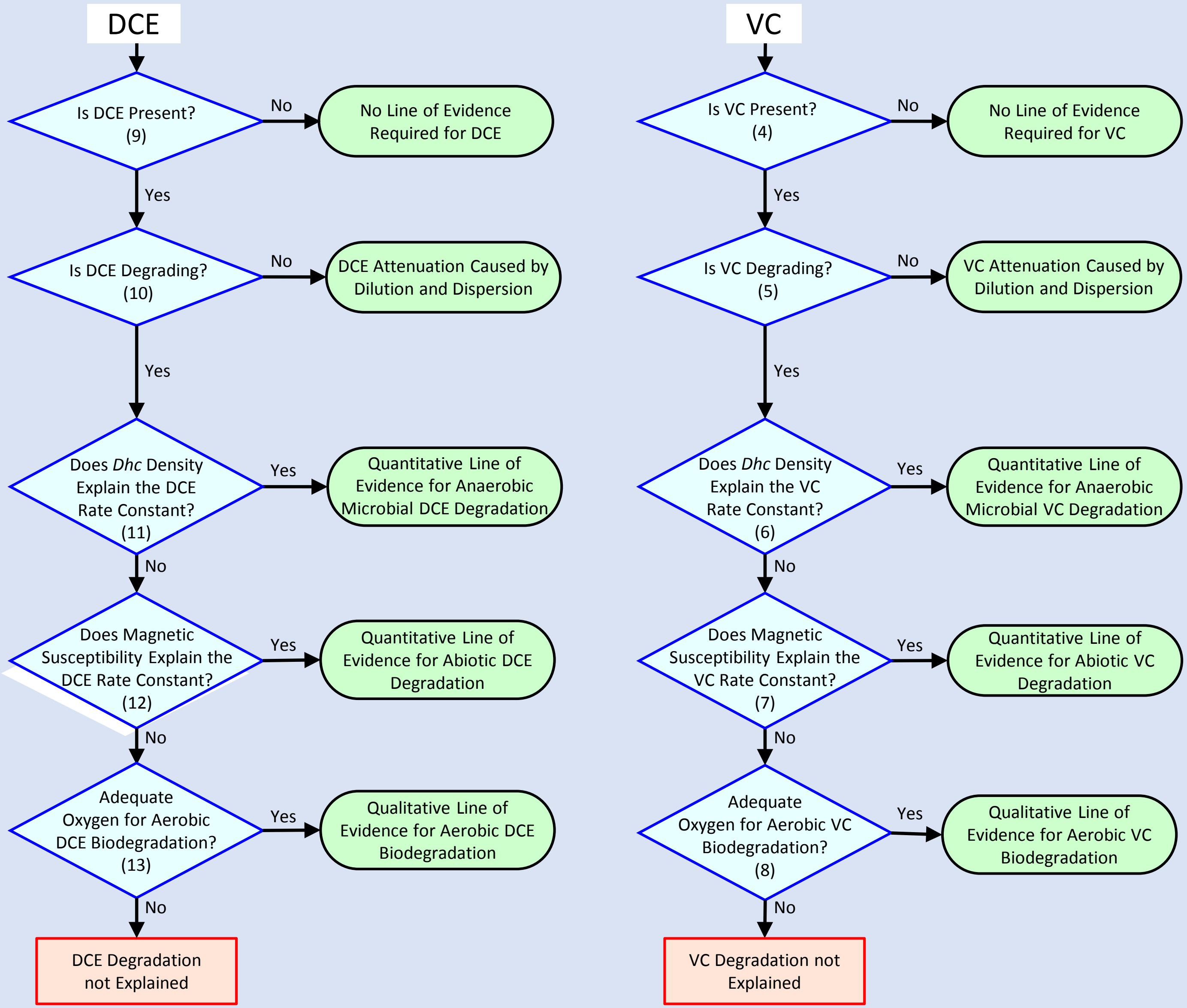
For the purposes of this decision support system, oxygen is considered to be available for aerobic biodegradation of VC when all of the following criteria are met:

Dissolved oxygen concentrations measured in the field exceed 0.1 mg/L.

Ferrous iron (Fe^{2+}) concentrations are below 0.5 mg/L.

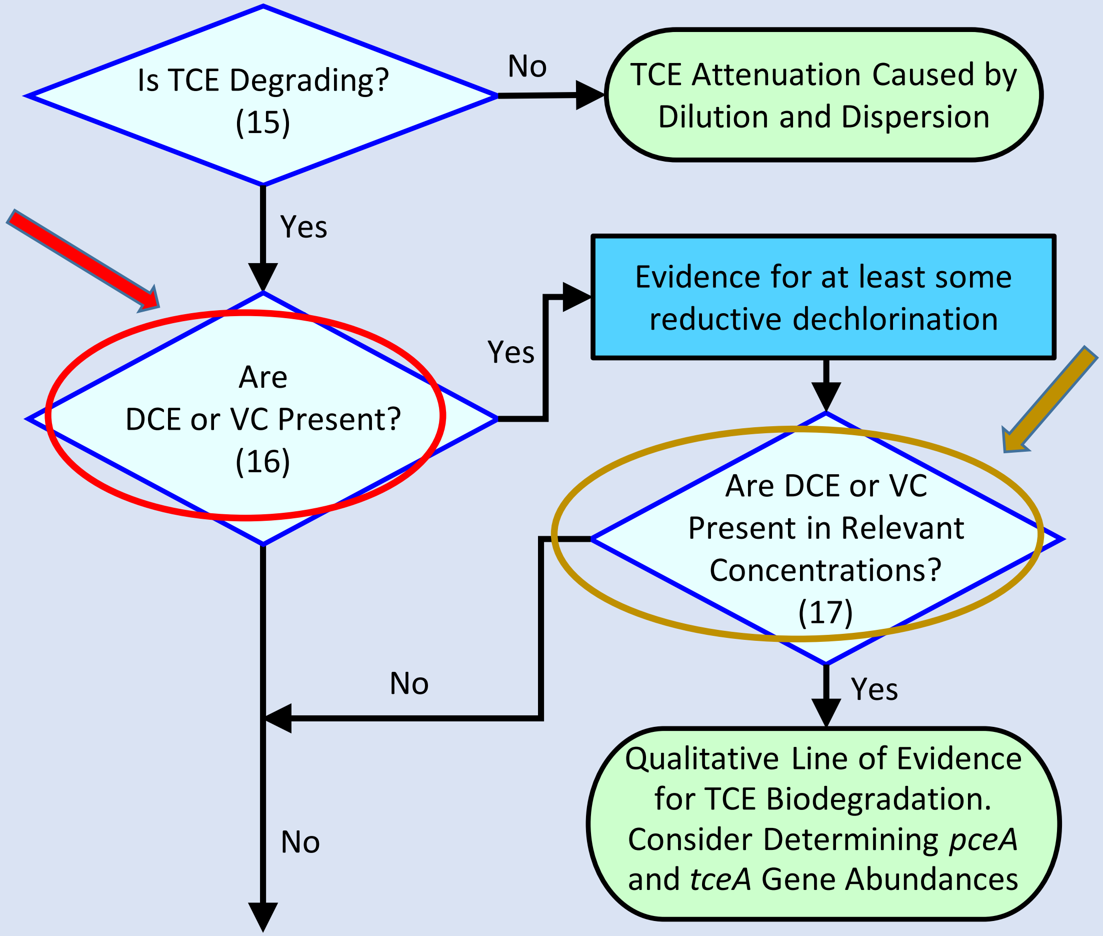
Methane concentrations are below 0.005 mg/L.

The decision logic for DCE parallels the decision logic for Vinyl Chloride.

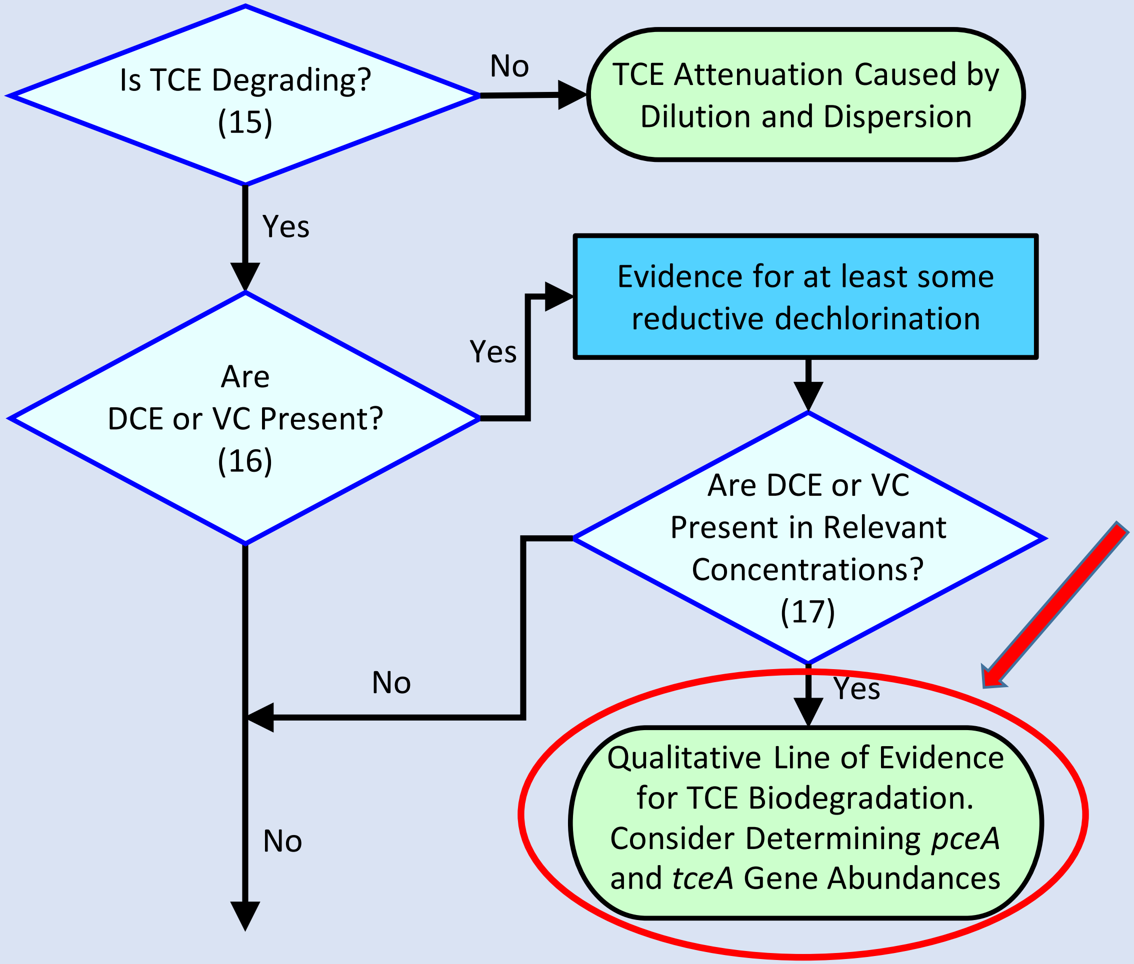


TCE Degradation

If the sum of cDCE, tDCE and VC is more than 5% of the concentration of TCE on a mole basis, then cDCE, tDCE and VC are present.



If the sum of cDCE, tDCE and VC is more than 25% of the concentration of TCE on a mole basis, then cDCE, tDCE and VC are present at relevant concentrations.



Many organisms can degrade TCE.

There is no simple association of the rate of degradation and the density of *Dehalococcoides* bacteria at the site.

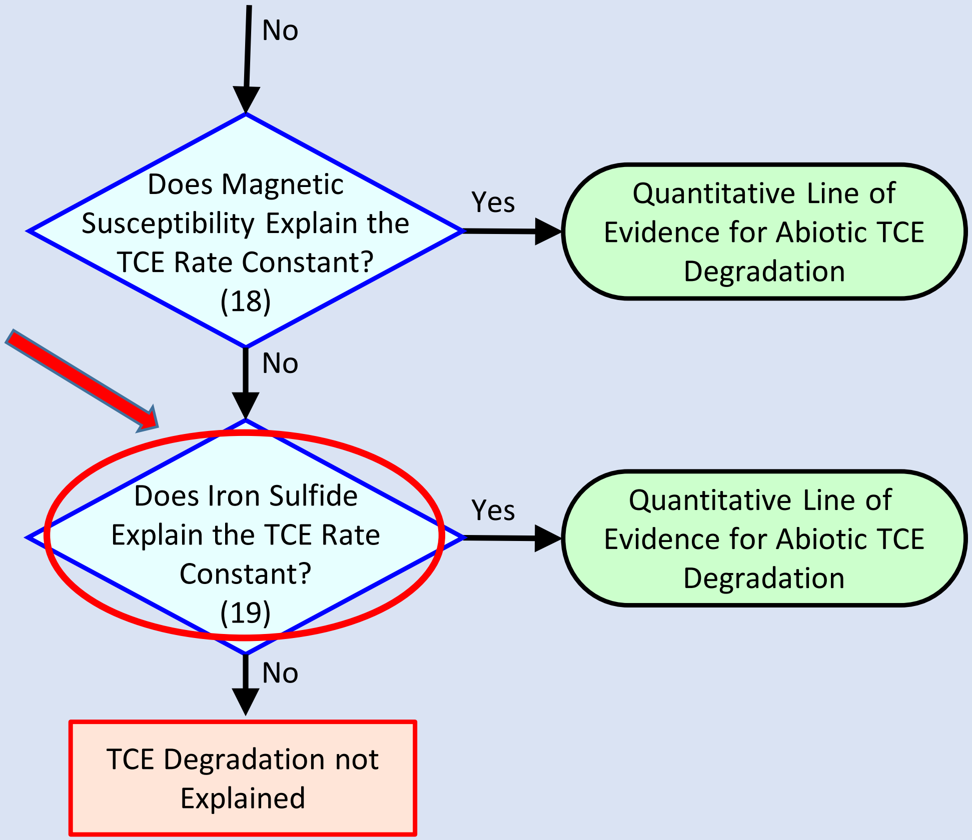
The density of the reductase genes provides a qualitative line of evidence.

Reactive iron sulfide minerals are formed during sulfate reduction and will form over time as sulfate reduction progresses and ferrous iron is dissolved in the groundwater.

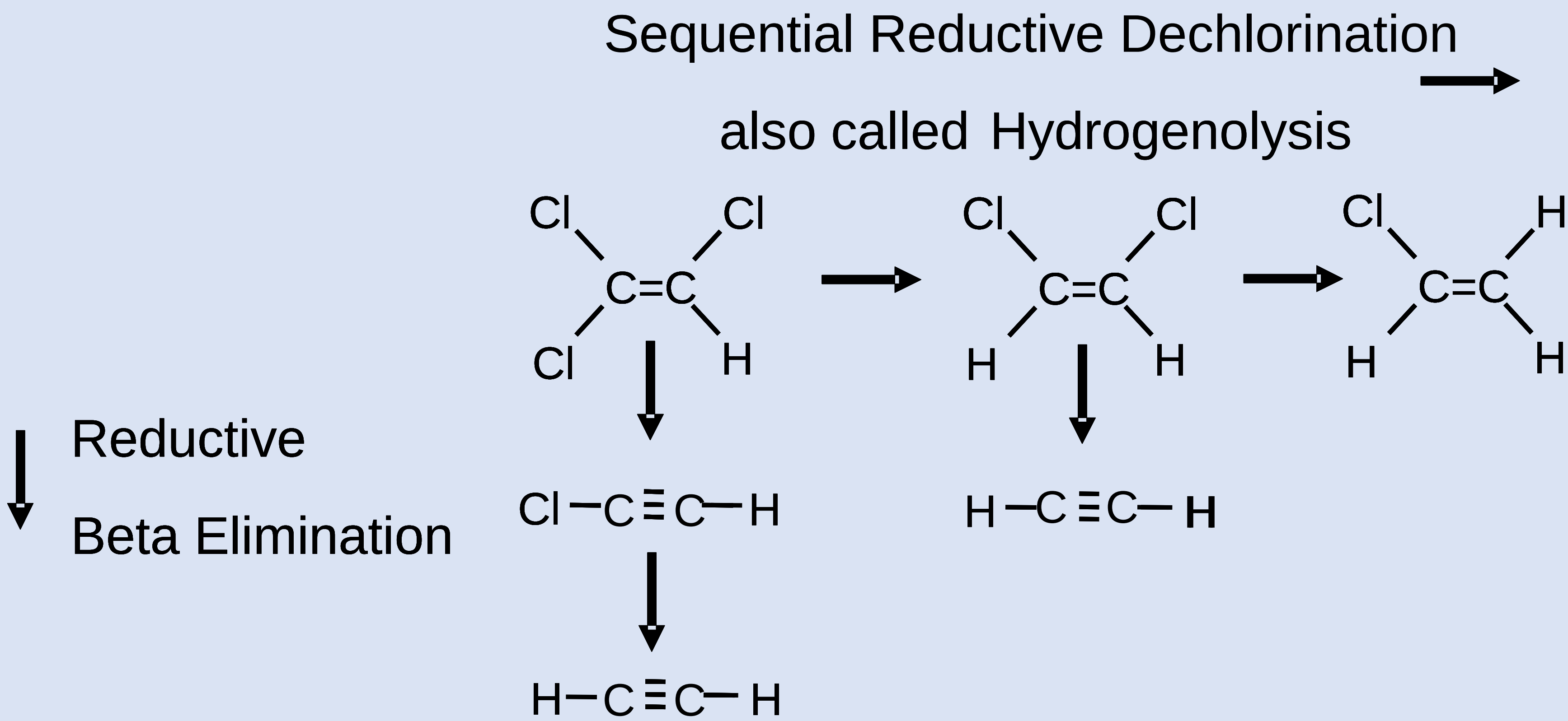
However, the reactive iron sulfide minerals are inactivated over time at a rate that is proportional to the amount of reactive minerals that have already accumulated.

The pool of reactive iron sulfide will increase until the rate of production from sulfate reduction is balanced by the rate of inactivation.

The rate of TCE degradation mediated by reactive iron sulfide minerals is related to the steady-state pool of reactive iron sulfide.



Carried out by FeS and Pyrite (FeS_2) and Green Rusts



FeS does not react at a significant rate with DCE or Vinyl Chloride.

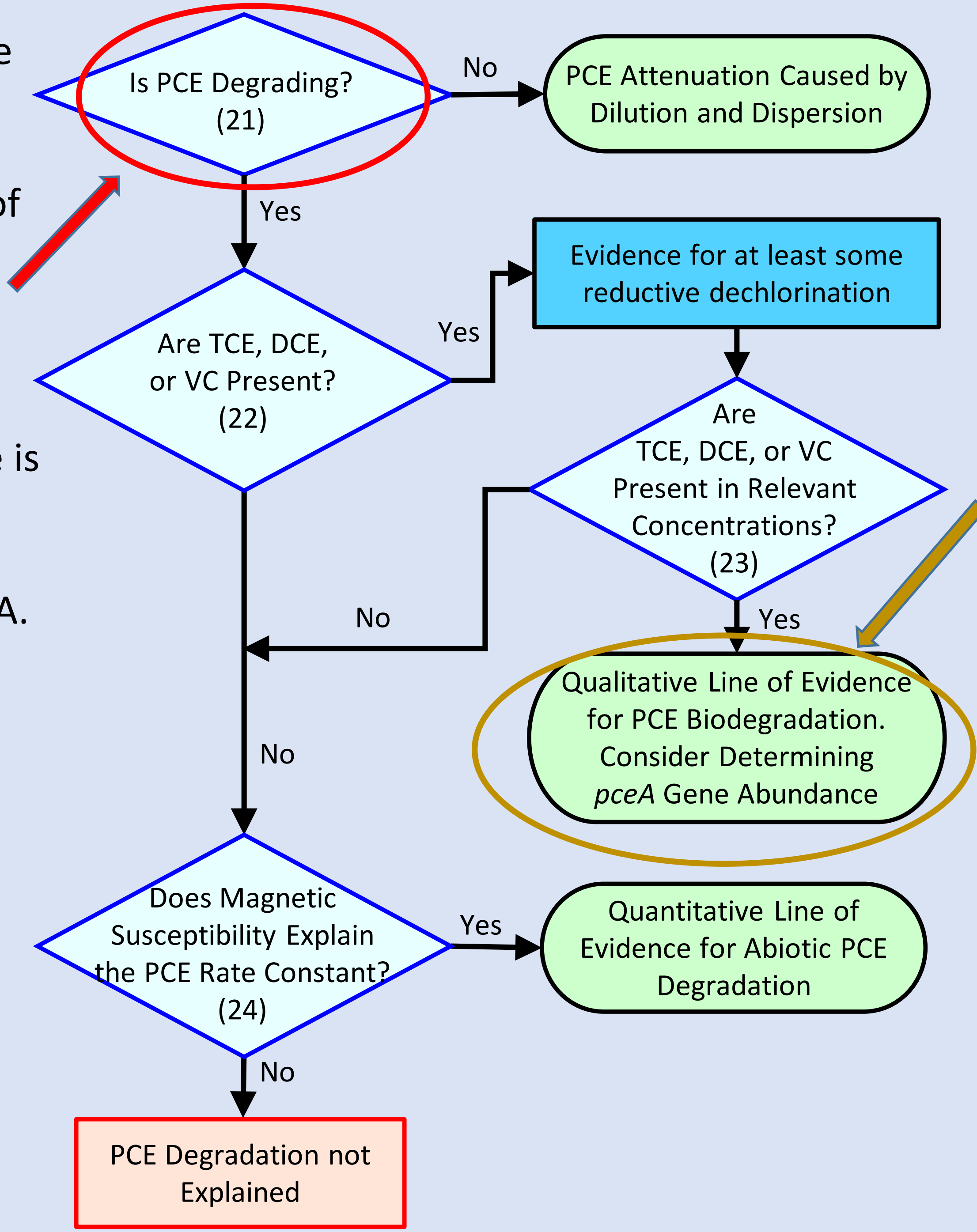
The spreadsheets use data on the effective porosity, hydraulic gradient and hydraulic conductivity, distance between wells, concentrations of sulfate and sulfide in groundwater, and pH.

	A	B	C	D
1				
2		Identify wells along the flow path of the contaminant plume.		Overwrite Input Cells with
3		Find a down-gradient well with lower concentrations of TCE.		Data Specific to Your Site
4		Find an up-gradient well with higher concentrations of TCE		
5		The concentration of sulfate should be higher in the up-gradient well than in the down-gradient well.		
6			Unit	Input
7			Unit	Interim Calculation
8		Parameter	Unit	Final Output
9				
10		Hydraulic Gradient	foot per foot	0.0012
11		Hydraulic Conductivity	feet per day	51
12		Effective Porosity	cm^3/cm^3	0.2
13		Seepage Velocity of Ground Water	feet per year	111.69
14		Distance from down gradient well with lowest sulfate to up-gradient well with	feet	194
15		Concentration sulfate in up-gradient well	mg/L	613
16		Concentration sulfate in down-gradient well	mg/L	94.6
17		Concentration of soluble sulfide in up-gradient well	mg/L	0
18		Concentration of soluble sulfide in down-gradient well	mg/L	0
19		Time since plume first reached the down gradient well	years	20
20		Yearly production of FeS along flow path	moles FeS per liter groundwater	0.00207
21		Average pH		6.50
22		Average Total Soluble Sulfide	mg/L	12.00
23		k (the first order rate constant for inactivation of FeS) from Rickard's Equation	per year	1.169
24		k (the first order rate constant for inactivation of FeS) Input from Literature	per year	0.162
25		Reactive Iron Sulfide that is accumulated calculated from Rickard's equation based on pH and soluble sulfide	moles FeS per liter groundwater	1.77E-03
26		Reactive Iron Sulfide that is accumulated based on the first order rate constant for inactivation of FeS.	moles FeS per liter groundwater	1.23E-02
27		First Order Rate Constant for Attenuation of TCE over Time explained by Reactive FeS		
28		k (the first order rate constant for inactivation of FeS) from Rickard's Equation	per year	4.81E-01
29		k (the first order rate constant for inactivation of FeS) Input from Literature	per year	3.34E+00
30				

PCE Degradation

The primary line of evidence for degradation is the projection of the computer model.

The secondary line of evidence is stable isotopic fractionation as revealed by CSIA.

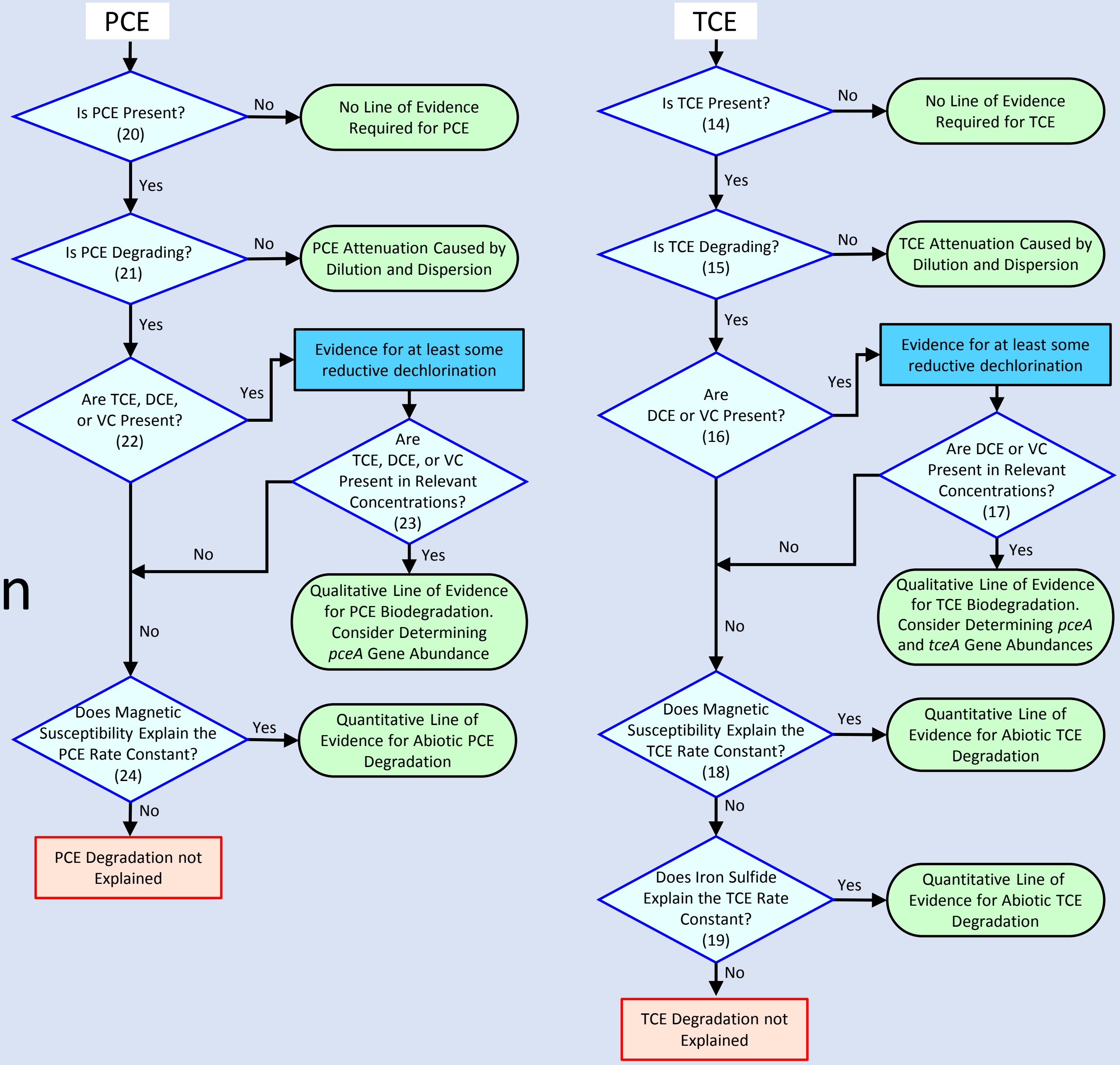


Many organisms can degrade TCE.

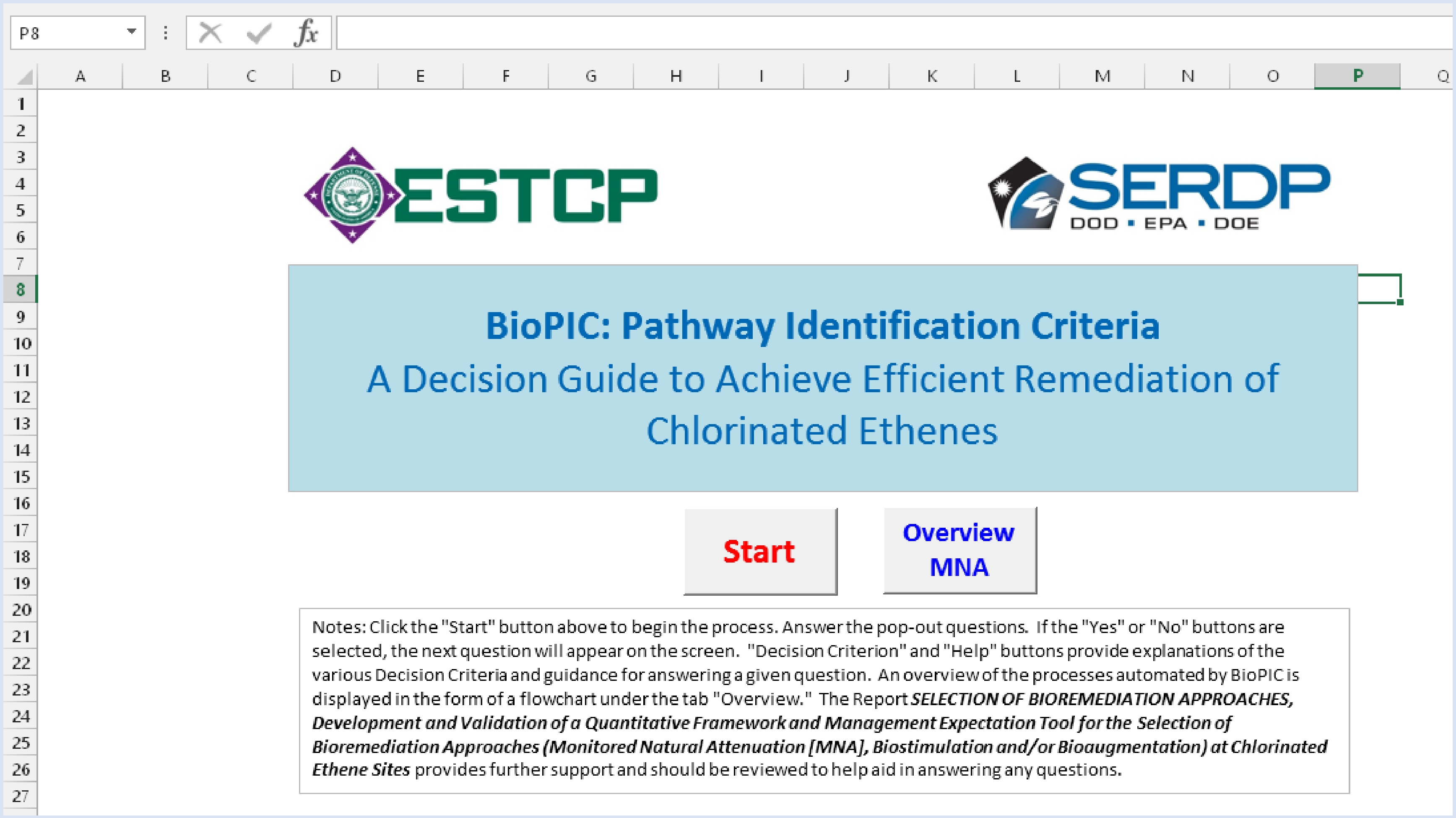
There is no simple association of the rate of degradation and the density of *Dehalococcoides* bacteria at the site.

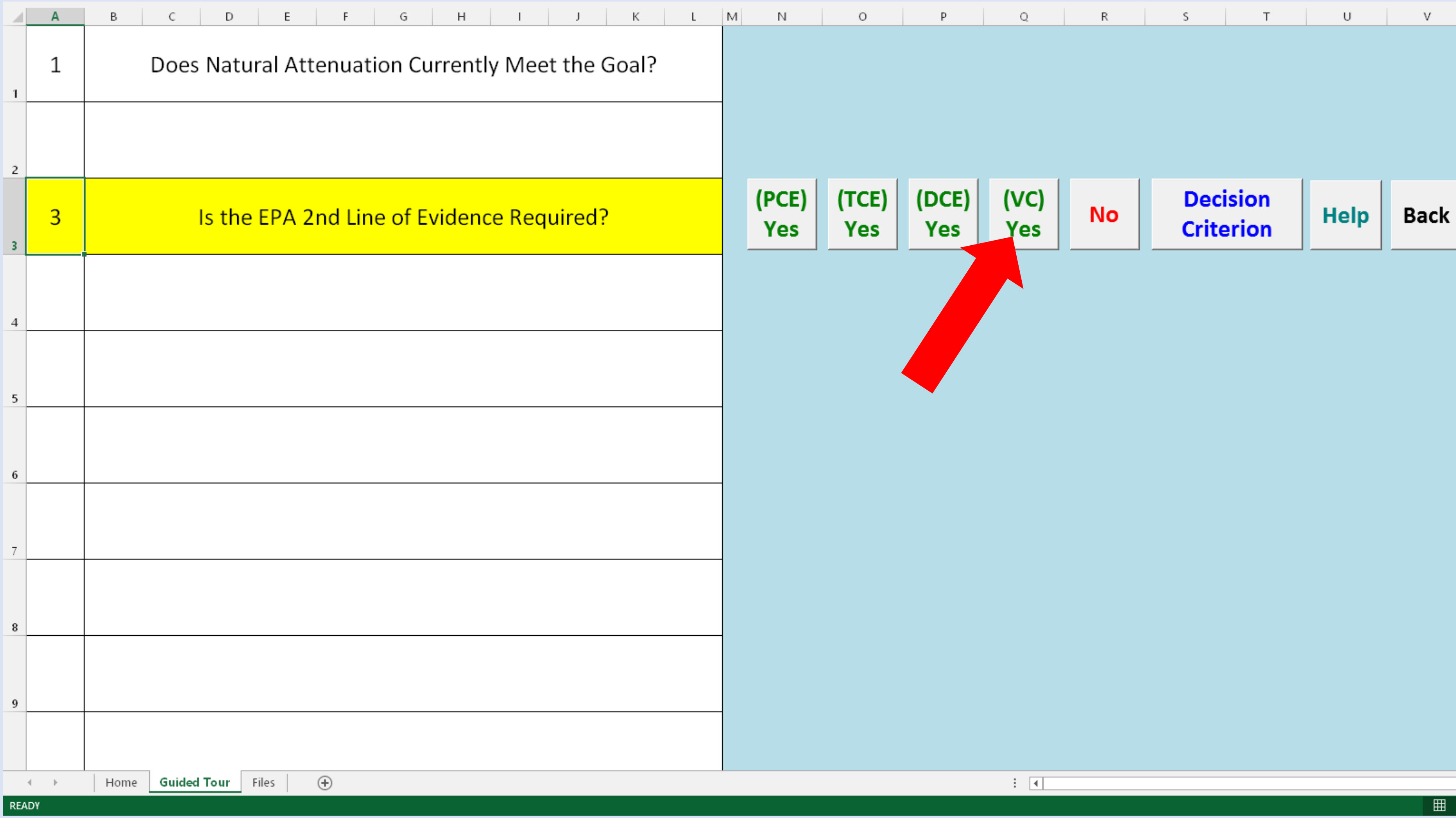
The density of the reductase genes provides a qualitative line of evidence.

The decision logic for PCE parallels the decision logic for TCE, except there is no evaluation of abiotic degradation on FeS.



It is all organized in a decision guide.




1
Does Natural Attenuation Currently Meet the Goal?
3
Is the EPA 2nd Line of Evidence Required?
(PCE) Yes
(TCE) Yes
(DCE) Yes
(VC) Yes
No
Decision Criterion
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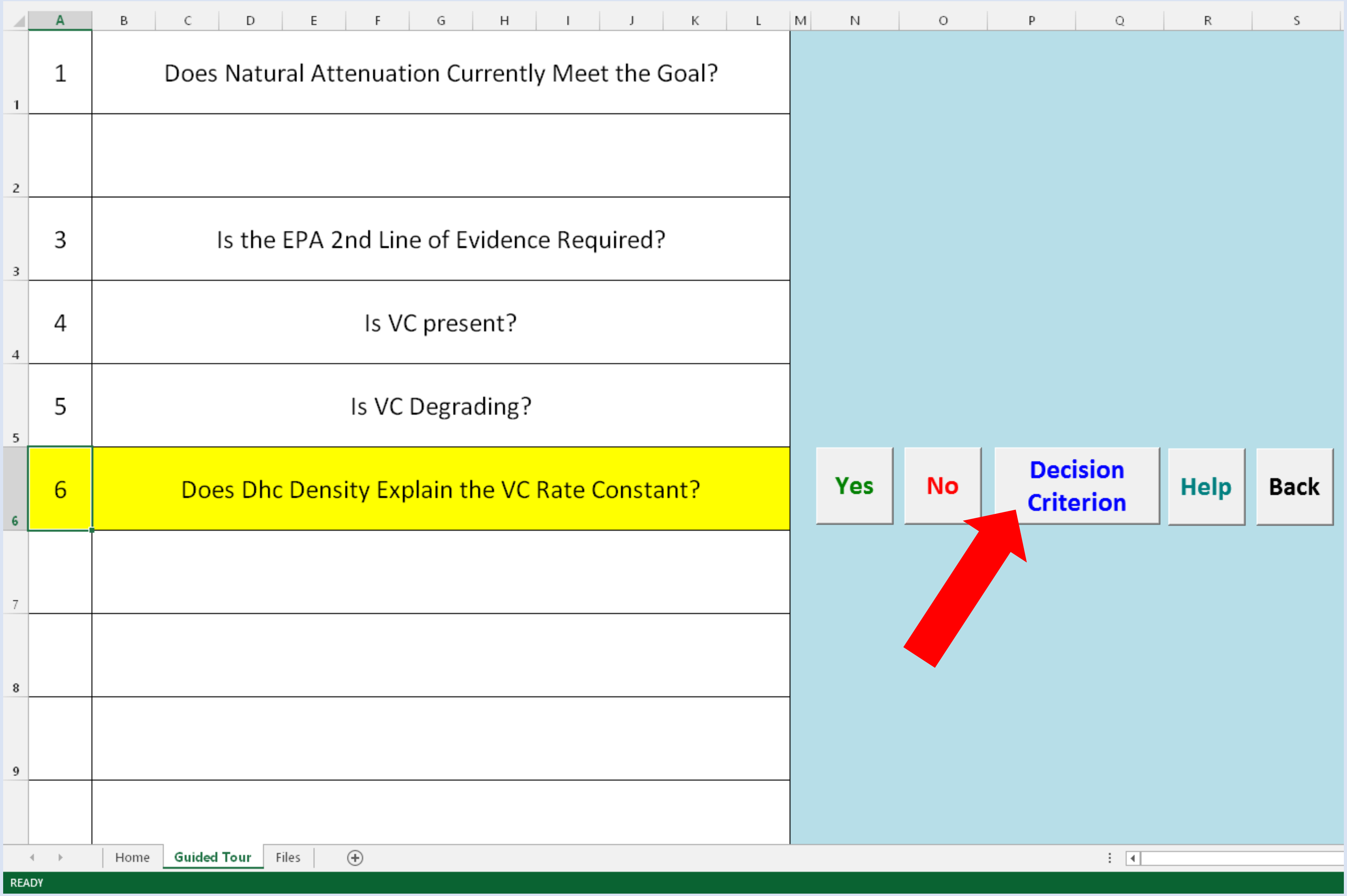

1
Does Natural Attenuation Currently Meet the Goal?
3
Is the EPA 2nd Line of Evidence Required?
4
Is VC present?
5
Is VC Degrading?
6
Does Dhc Density Explain the VC Rate Constant?
Yes
No
Decision Criterion
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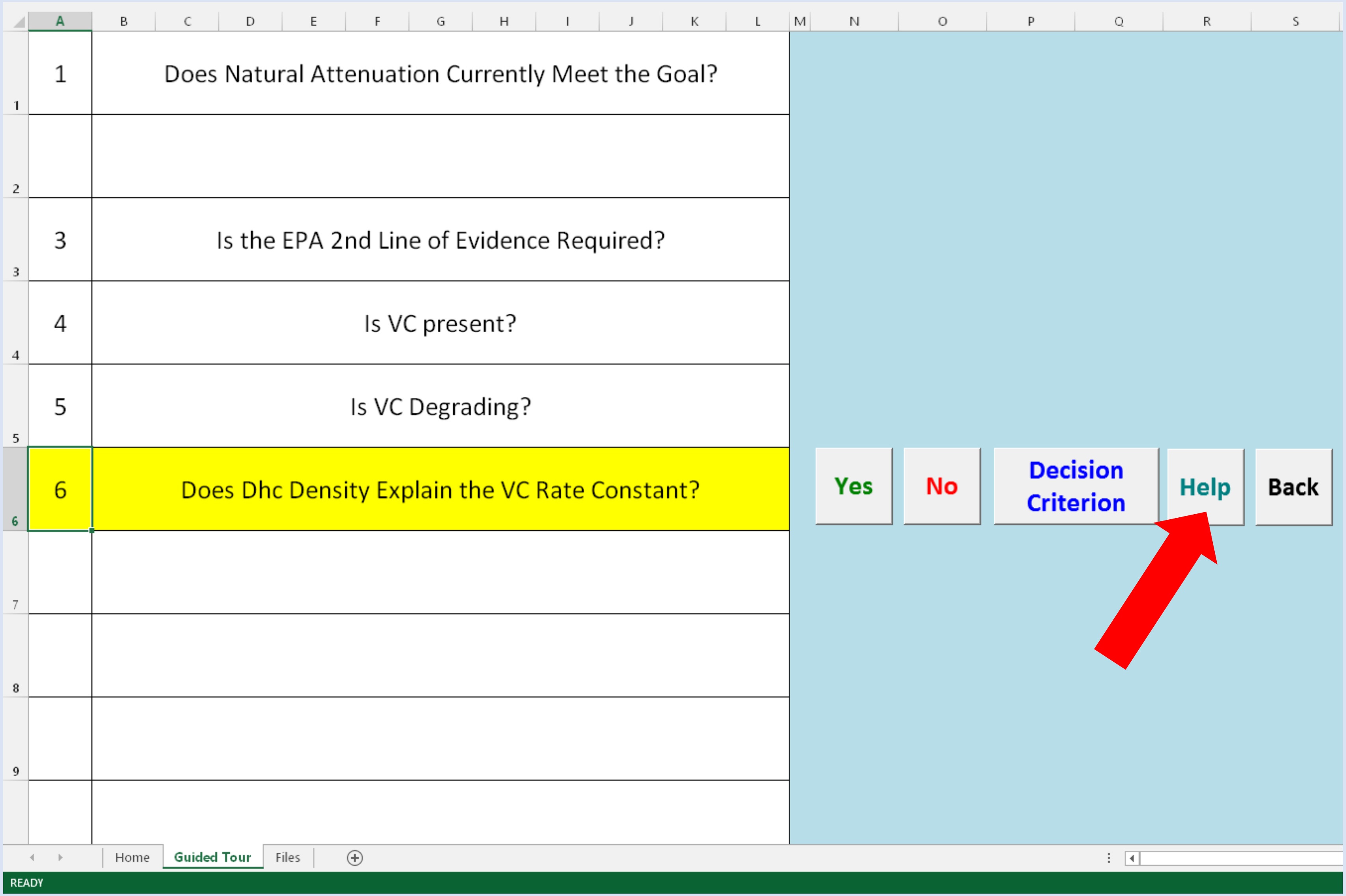
Decision Criterion Box 6

Consult the simulation that you prepared to evaluate the criterion "Does Natural Attenuation Currently Meet the Goal?" Identify the rate constant for degradation of VC. Access information about the abundance of Dhc cells in groundwater at the site. Open the tab Files and select the spreadsheet Dhc.xlsx. Input values for the first order rate constant for degradation of VC and the abundance of Dhc biomarker gene copies on the tab Input Dhc data. If you have more than one value for the abundance of Dhc gene copies, input the highest value, not the average. Then open the tab Dhc Explains VC. If your data plot in the blue shape, then the abundance of Dhc in groundwater can explain the in situ rate of VC degradation.

Not every bacterium with the Dhc 16S rRNA gene can degrade VC. A qPCR assay is commercially available for two of the known genes that code for enzymes that reductively dechlorinate VC. The reductase genes have been designated vcrA and bvcA. If there is a concern that the Dehalococcoides strains at your site cannot degrade VC, access information on the abundance of vcrA and bvcA genes in groundwater at the site.
Open the tab Files and select the spreadsheet Reductase Genes.xlsx. Enter your data in the tab Input Data. Then open the tab VC Rase and Dhc. If your data plot in the blue shape, transformation of VC is plausible based on the abundance of the VC reductase genes in the groundwater.

Close

Dhc.xlsx


1
Does Natural Attenuation Currently Meet the Goal?
3
Is the EPA 2nd Line of Evidence Required?
4
Is VC present?
5
Is VC Degrading?
6
Does Dhc Density Explain the VC Rate Constant?
Yes
No
Decision Criterion
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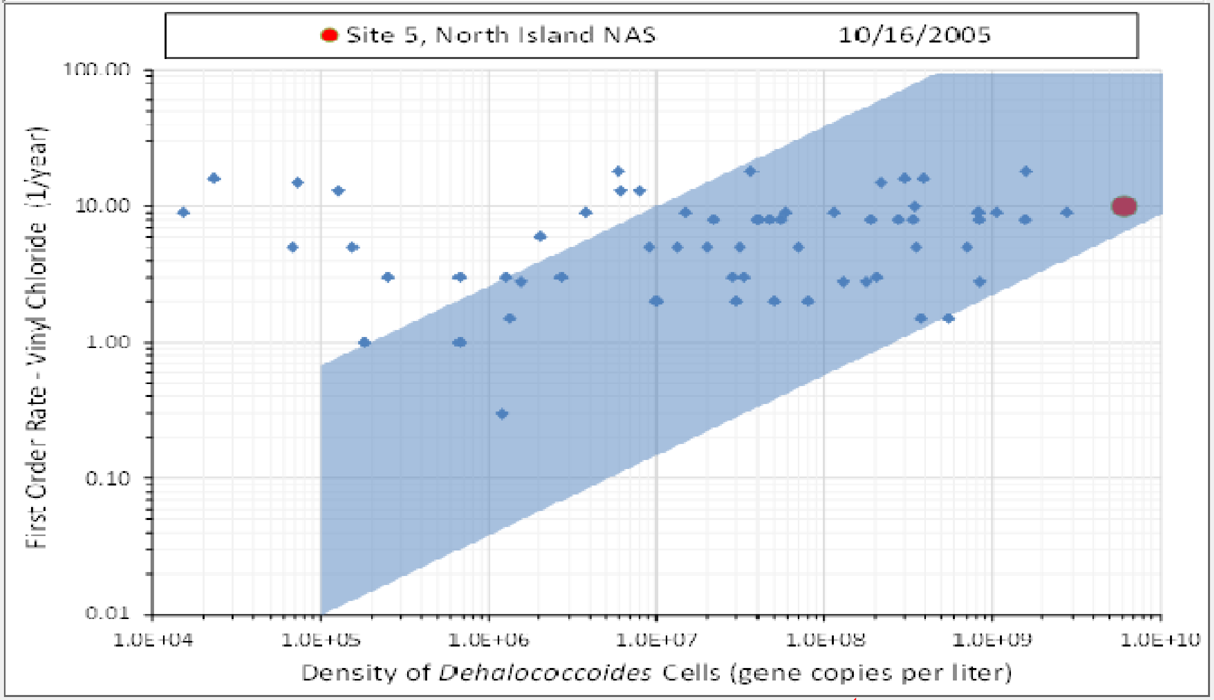
Help Box 6

The figure below is the chart in tab Dhc Explains VC for an example data set. In this example the density of Dehalococcoides gene copies does explain the rate.

Note that the chart has data points that are outside of the blue shape and have first order rate constants that are larger than can be plausibly explained by the Dhc cell abundance in the groundwater. Possible explanations for the observed rates of VC degradation include:

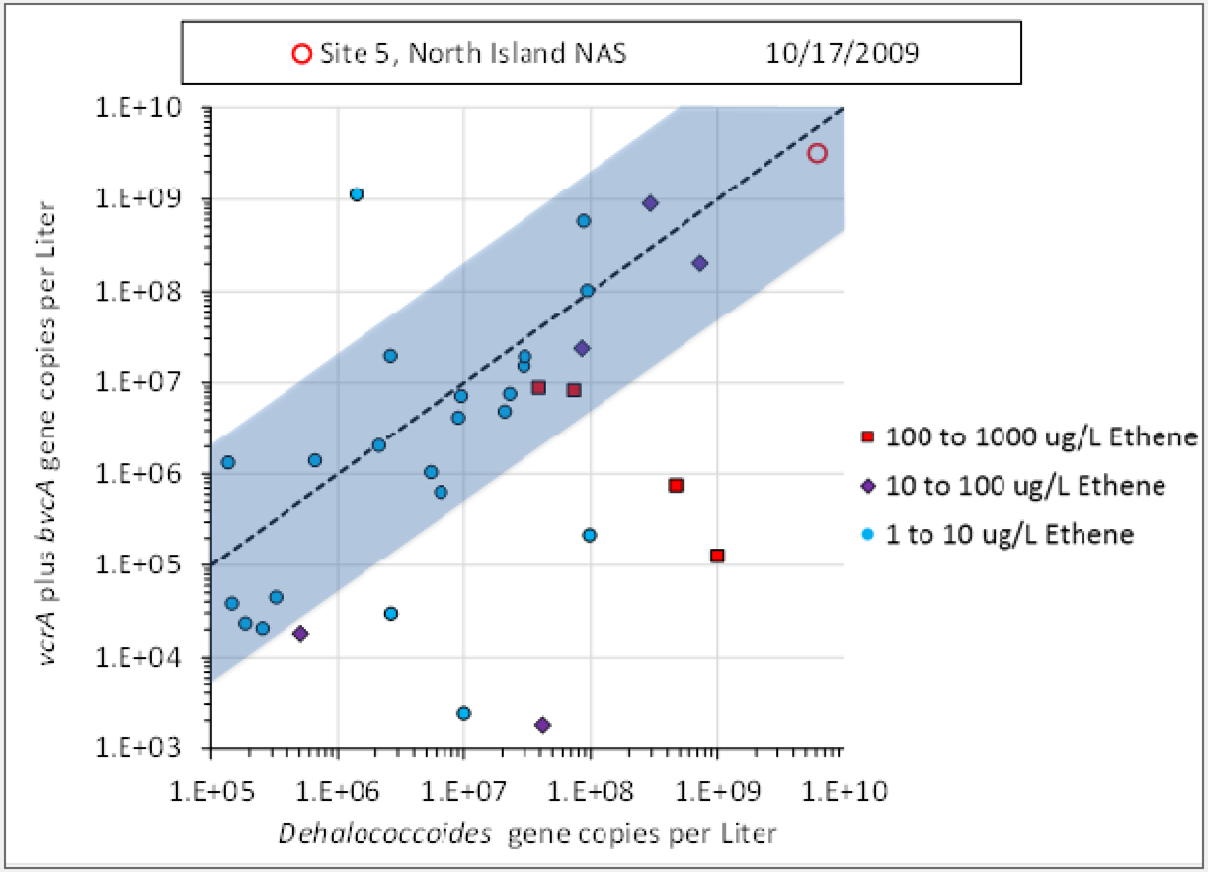
1. The groundwater Dhc analysis underestimates the actual Dhc abundance in the aquifer due to Dhc cell attachment to the aquifer solids.
2. To date, the VC-to-ethene reductive dechlorination step has been exclusively associated with Dhc strains carrying the VC RDase genes vcrA or bvcA; however, it is conceivable that not-yet-recognized bacteria may contribute to VC-to-ethene reductive dechlorination.
3. Microbial VC oxidation can occur at very low dissolved oxygen concentrations, and areas of the aquifer may have sufficient oxygen to sustain aerobic VC (and ethene) degradation.
4. Abiotic VC degradation mediated by reactive iron-bearing mineral phases (e.g., iron sulfides, magnetite) contributes to VC degradation.



Close

More Information

Dhc strains have been described that contribute to reductive dechlorination of polychlorinated ethenes but cannot efficiently dechlorinate VC. If such strains dominate the Dhc population, a high Dhc cell abundance may not correlate with VC-to-ethene reductive dechlorination activity. Two Dhc RDase genes involved in VC-to-ethene reductive dechlorination have been identified, vcrA and bvcA, and commercial qPCR assays targeting these genes are available. The combined application of Dhc 16S rRNA gene- and RDase gene-targeted qPCR can provide additional valuable information about VC degradation at the site. The figure below is the chart in tab RDase and Dhc for an example data set. If the data plot near the dotted line, the abundance of genes for the reductase enzymes is near the abundance of Dhc cells. In this example, the data plot in the blue shape, and transformation of VC is plausible based on the abundance of vcrA and bvcA in the groundwater.



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Monitored Natural Attenuation (MNA)

- MNA of Petroleum Hydrocarbons and Fuel Components
- MNA of Chlorinated Solvents