Quick BioPIC User's Guide

USER'S GUIDE
ER-201730

October 2021

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The tool is intended to help users follow OSWER directive 9200.4-17P on MNA of chlorinated ethenes. While the USEPA has yet to develop a similar directive for chlorinated ethanes and 1,4-dioxane, the tool follows a very similar technical approach in evaluating MNA for these compounds.

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Quick BioPIC User Guide

Updated October 2021

This Quick Guide is intended for users of the application BioPIC (Bio Pathway Identification Criteria), which uses the Microsoft Excel 2020 platform. This is an updated version of the original BioPIC, which was first developed under ESTCP Project ER-201129 for evaluating Monitored Natural Attenuation (MNA) for chlorinated ethenes. Separate modules for 1,4-dioxane (1,4-D) and chlorinated ethanes have recently been added to BioPIC under ESTCP Project ER-201730 (note that no change to the decision framework for chlorinated ethenes were made as part of this update).

OBJECTIVE

The tool is intended to help users follow OSWER directive 9200.4-17P on MNA of chlorinated ethenes. While the USEPA has yet to develop a similar directive for chlorinated ethanes and 1,4-dioxane, the tool follows a very similar technical approach in evaluating MNA for these compounds.

OVERVIEW OF FRAMEWORK

BioPIC is organized around the USEPA lines of evidence for MNA Framework (USEPA, 1998 and 1998) where the first line of evidence is *Historical groundwater … data that demonstrates a clear and meaningful trend of decreasing contaminant* … Therefore, use of BioPIC requires that the user first applies a groundwater fate and transport model to determine whether the rate of attenuation of the contaminants will bring the highest concentrations of the contaminants in groundwater to acceptable concentrations before the groundwater reaches a receptor or a sentry well. If the predicted concentrations are acceptable, MNA is appropriate. As part of the 2021 update, a model for predicting contaminant trends over time and distance, including a method to estimate site-specific biodegradation rate constants for chlorinated ethenes, chlorinated ethanes, and 1,4-dioxane, has been included within BioPIC.

If MNA is appropriate, BioPIC offers guidance on developing information that can meet the USEPA requirement for a second lines of evidence *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 chlorinated ethenes, BioPIC offers guidance on alternative remedies in cases where MNA is not appropriate, specifically the use of in situ bioremediation, and whether it is useful to bioaugment the site with active microorganisms as well as biostimulate with nutrients.

BIOPIC START-UP AND HOME PAGE

Please begin by opening the file titled BioPIC_2021.xlsm. When the screen first opens, click on “enable macros” or enable these within the application settings. These macros are required for using the software; consult with IT or system administrators as needed.

Upon opening the file, you’ll see 3 different red “Start” buttons and 3 different blue “Overview” buttons.

- By clicking on one of the red “Start” buttons for a set of target compounds, you will be led to a stepwise diagnostic process with several YES/NO questions following the framework logic available in the blue “Overview” buttons.

- By clicking on the one of the blue "Overview" buttons for a set of target compounds, you will see a flowchart representation of the entire Decision Framework for that set of compounds. This serves as a reference for users so that they get a sense of the decision logic, and it may be valuable to print out and include in deliverables.

You'll also see 5 tabs (worksheets) at the bottom of the screen. The first tab—the Home tab—is the starting point. A user can always click the Home tab to return to the home page and chose another option (or to start over). The three "Guided Tour" tabs lead to the same screens as the red "Start" tabs for each of the targeted compounds; these are redundant but are included for users who were accustomated to the previous version of BioPIC. The final tab is a "FILES" tab that contains several useful calculators (described in more detail below) that can be launched or downloaded separately as needed.

NAVIGATION TIPS:

After clicking on one of the red "Start" buttons, you are taken to a separate page that provides a guided tour through the relevant decision framework. A few simple rules for navigating these pages:

- Most questions can be answered by clicking on one of 5 (five) potential options: YES, NO, Decision Criteria, Help and Back.
- When a YES or NO button is chosen, the next question will appear.
- If users are uncertain how to answer the question, a click on the Decision Criteria or Help button displays more detailed background information that should help the user to select the appropriate answer.
- By clicking the Back button, the user will be directed back to the previous question.

"FILES" TAB (last tab on the Home Page):

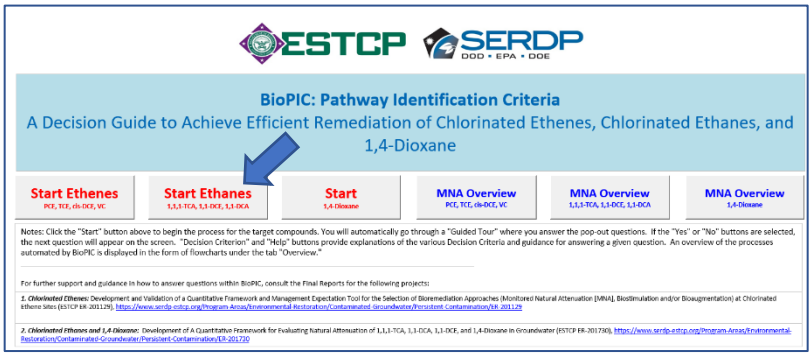
The last (5th) tab (worksheet) on the BioPIC Home Page is titled "Files" and contains several Excel files as separate objects to aid users to enter data for further analysis. Users, for example, can click the "CSIA.XLSX", "Dhc.XLSX", "FeS.XLSX", "Magnetic Susceptibility.XLSX" or "Mole Percent Calculator.XLSX" buttons in the "Decision Criteria" box, and will be automatically directed to these tab "Files." By double-clicking the Excel button, the corresponding Excel file will be displayed. Note that this includes all files that were part of the original release of BioPIC, as well as several new files developed as part of the 2021 update. The latter include a new "MNA Rate Constant Estimator" that serves as a standalone contaminant fate and transport model. It was patterned after BIOCHLOR (though using a slightly different code) but incorporates more compounds and some other features (described in more detail in the User's Guide for this model, which is also Appendix C of the project report for ESTCP ER-201730).

Users are encouraged to provide feedback and report incidents for continuous improvement of the BioPIC tool to Carmen A. Lebron (lebron.carmen.a@gmail.com), John Wilson (john@scissortailenv.com) or David Adamson (dtadamson@gsienv.com).

Detailed BioPIC User Guide:

Chlorinated Ethanes

The following describes the Decision Framework for the compounds that are part of the Ethanes module, including 1,1,1-TCA and 1,1-DCA, as well as 1,1-DCE. The latter compound is part of this module because it is primarily of interest as a by-product of a chlorinated ethane degradation pathway (i.e., the abiotic degradation of 1,1,1-TCA).



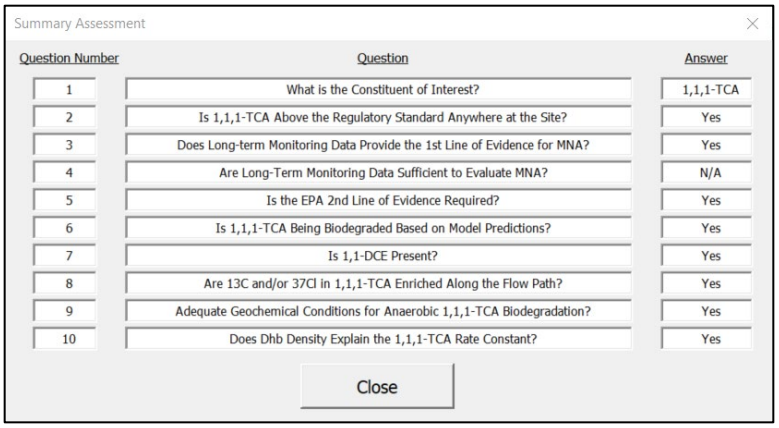
BioPIC Home Page showing start button for entering the chlorinated ethane decision framework

Each of the numbered questions below corresponds to a number in the flowchart/guided tour. After each number, the decision criteria are explained. For most, further information is provided in the Help text. Note that these text descriptions are shown as pop-up boxes within the tool. A graphic showing the entire decision flowchart for these compounds is reproduced at the end of this section.

1. What is the constituent of interest?

Decision Criteria:

Choose the appropriate constituent of interest. Options are 1,1,1-TCA, 1,1-DCA, and 1,1-DCE. This will take the user through the decision logic for that particular compound (i.e., to Question #2 if 1,1,1-TCA is selected). Once finished with the logic for the selected compound, a summary assessment will be displayed that shows the results for that particular compound (see example graphic at right). The process can be then repeated for the remaining compound(s). Note that once the user has selected a constituent of interest and starts answering the subsequent questions, the summary assessment can also be pulled up by clicking the View Summary box that appears to the right of each question. In these cases, it will display answers to only those questions that have been completed.



Example of Summary Assessment pop-up box after completing the stepwise decision framework for 1,1,1-TCA

2. Is 1,1,1-TCA above the regulatory standard anywhere at the site?

Decision Criteria:

Answer YES if: The 1,1,1-TCA concentration at any groundwater monitoring location at the site is above the applicable concentration-based regulatory standard. Note that this standard is site-specific. If no standard has been established for your site, then select a value based on guidance from EPA or other states (see **HELP**) for planning purposes.

Answer NO if: The 1,1,1-TCA concentration at each groundwater monitoring location at the site is already below the concentration-based regulatory standard. The decision tool is intended for sites where the concentration of 1,1,1-TCA must fall below a site-specific regulatory standard before contaminated groundwater reaches the point of compliance (POC). If the 1,1,1-TCA concentration is already below the regulatory standard across the site (including the POC), then it is highly likely that it will remain below this standard in the future. This assumes that the site has been reasonably well characterized (especially the source area) and that no significant changes in conditions at the site are anticipated.

HELP

In the absence of site-specific regulatory standards for 1,1,1-TCA the user may wish to select the federal MCL (200 µg/L) for 1,1,1-TCA to proceed with the BioPIC evaluation.

If 1,1,1-TCA is already below the established or assumed standard, then a future exceedance would likely be associated with one or more of the following: 1) the site is poorly characterized; 2) a new release of 1,1,1-TCA occurs; 3) active remediation is on-going or recently completed, such that steady state conditions have not yet been reached; or 4) any other change in site conditions has occurred that enhance 1,1,1-TCA mass transfer to the aquifer and inhibit 1,1,1-TCA attenuation.

3. Does Long-Term Monitoring Data Provide the 1st Line of Evidence for MNA?

Decision Criteria

Answer YES if: The 1,1,1-TCA concentration is currently below the regulatory standard at the point of compliance (POC) and is predicted to be below the concentration-based regulatory standard at the POC at any time in the future. Use the model provided as part of this tool (see **FILES** for "MNA Rate Constant Estimator") to predict if the concentration will be below the standard at any time in the future (see **HELP** for additional explanation).

Answer NO if: At any time in the future, the 1,1,1-TCA concentration will exceed the regulatory standard at the POC. Use the model provided as part of this tool to predict the concentration in the future at the POC. Note there usually is also a temporal component in the regulatory goals, which involves establishing how long it will take the concentration at a particular location to achieve a regulatory goal. The implementation of more aggressive remedies may reduce time to achieve remediation goals, thereby reducing the overall cost. However, this tool primary deals with the spatial aspects of remediation goals (i.e., will the goal be achieved at a POC) rather than the temporal components.

HELP

If sufficient historical contaminant concentration data are not available to determine if the 1,1,1-TCA plume will reach a POC, then a groundwater flow and solute transport model, such as the "MNA Rate Constant Estimator" provided as part of this tool (see **FILES**) should be used to predict solute plume behavior. In this case, the simulation should account for the effects of advective groundwater flow, dispersion of the relevant solutes, sorption, and degradation of 1,1,1-TCA in groundwater at the site.

For more information on using this type of model for 1,1,1-TCA, consult the project report (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 (ESTCP ER-201730)), which can be found on the project page (copy link into web browser): https://serdp-estcp.org/Program-Areas/Environmental-Restoration/Contaminated-Groundwater/Persistent-Contamination/ER-201730/ER-201730.

A similar approach for chlorinated ethenes can be found in another project report (Development of a Quantitative Framework and Management Expectation Tool for the Selection of Bioremediation Approaches at Chlorinated Ethene Sites (ESTCP Project ER-201129)), which can be downloaded from the project page (copy link into web browser): https://www.serdp-estcp.org/Program-Areas/Environmental-Restoration/Contaminated-Groundwater/Persistent-Contamination/ER-201129/ER-201129. In the ER-201129 report, Section 5.2.3 illustrates the process of calibrating a groundwater flow and transport model (in this case, the "BIOCHLOR" model), and Section 5.2.4 Step 1 illustrates the use of a model to apply the decision criteria.

If available, a robust historical database of contaminant concentrations can be used as an alternative to a computer model. Spatial and temporal trends in solute concentrations can be utilized to determine if the plume is stable or receding and therefore will not reach the POC. When sufficient data are available, using empirical data to ascertain trends is much better than using a model. In many cases, sufficient 1,1,1-TCA concentration data are available to evaluate plume behavior and to determine if solute concentrations will exceed cleanup goals at a regulatory POC.

4. Is Long-Term Monitoring Data Sufficient to Evaluate MNA?

Decision Criteria

Answer YES if: The current long-term monitoring data confirm that one or more of the following conditions are met:

1. The plume is currently beyond the point of compliance at a concentration that is above the applicable standard.
2. The plume is still expanding and is predicted to extend beyond the point of compliance in the future based on modeling.
3. Concentration-based goals have been established within the plume (i.e., upgradient of the point of compliance), and model predictions suggest that these will not be achieved with a reasonable timeframe.

Answer NO if: The current long-term monitoring data confirm that ALL of the following conditions are met:

1. The plume is currently not beyond the point of compliance.
2. The current dataset is too limited to evaluate if plume is expanding vs. receding.
3. The current dataset is too limited to predict using a model whether the plume will expand or whether concentration-based goals will be achieved.
4. There is no current regulatory requirement for active remediation.

HELP

Long-term monitoring data are an important component of site management, and they are particularly important for demonstrating the site-specific viability of MNA.

It is possible that a site's existing dataset for this compound may be limited if it is a recent addition to the monitoring program. It may not be possible to establish a clear trend in the attenuation in concentration of 1,1,1-TCA or its degradation products along a flow path in groundwater at appropriate monitoring points. This means that the data are inadequate to permit a thorough evaluation for the primary line of evidence for MNA. This is typically because one or more of the following apply: 1) data are highly variable across the site; 2) data are highly variable from event-to-event; or 3) data are available from a relatively limited number of monitoring points or events. In each case, these data limitations make it difficult to establish trends with any degree of statistical certainty.

In that case, collecting additional data may be beneficial to provide more certainty that current trends are statistically significant and sustainable. These additional monitoring events may also include data that would be used to support the second line of evidence for MNA (e.g., geochemical data, biomarkers, stable isotopes) or even hydrogeologic parameters to improve fate and transport model predictions.

At sites where the number of monitoring locations is relatively small (e.g., 4 or less wells), this type of analysis will likely benefit by including additional monitoring locations along the plume transect. In part, this is because it can be more challenging to establish that a trend is significant using standard approaches (e.g., that the slope of a best-fit regression line is different than zero). As a result, adding more monitoring locations along the plume transect may provide value.

It is necessary to use modeling software to evaluate if current trends in natural attenuation will meet the standard at the point of compliance (POC). An example is the "MNA Rate Constant Estimator" that has been developed as part of this decision tool (see **FILES**). This type of model allows the user to predict concentrations along a plume transect. However, it relies on the user to calibrate to the model predictions based on field data, and it also assumes that those field data are representative. At sites where data vary considerably from event to event, this type of calibration can be challenging. In any case, the goal is to demonstrate that the plume footprint is stable and/or shrinking and will not result in concentrations at a downgradient POC that exceed an acceptable level. This may require additional monitoring locations (particularly if the plume extent is not yet delineated) or additional monitoring events to demonstrate longer-term stability. Data from additional events are also important to establish that trends are sustainable even if minor (or major) changes in site conditions (e.g., groundwater flow directions, redox conditions) occur that might impact attenuation and/or plume stability.

In some cases where MNA is proposed as a remedy, an estimate of the remediation timeframe is also required by the regulatory agency. The remediation timeframe is typically the date when some or all wells are projected to achieve a concentration goal. Estimating the remediation timeframe is often done using

linear regression of concentration vs. time data at a specific well (or wells), but this requires data from enough events to ensure that the result is statistically significant. Additional monitoring events may be required to achieve this objective at sites with limited concentration vs. time data.

For the case where the dataset is already robust and confirms that MNA is unlikely to be effective, active remediation is likely to be the next course of action. Active remediation should be selected and implemented based on appropriate, well-defined objectives, but one primary goal should be to reduce concentration in the source area enough so that concentrations are below the site-specific standard at the point of compliance. The required reduction in the source concentration can be estimated using models, including the one provided as part of this tool (see **FILES**). Another good option is REMChlor-MD, which allows the user to input the extent of source reduction and then compare the plume behavior for cases with and without remediation. REMChlor-MD also incorporates the effects of matrix diffusion (i.e., contaminants diffusing into and out of lower-permeability intervals within the saturated zone), which has implications for contaminant transport and persistence at many sites.

Additional resources for understanding and developing monitoring objectives for MNA include the following (copy link into web browser):

https://clu-in.org/download/contaminantfocus/dnapl/Treatment_Technologies/performance_monitoring_mns600R04027.pdf

https://www.serdp-estcp.org/content/download/25789/262545/file/FAQ%20ER-201211.V2%20February%202014.pdf

https://clu-in.org/download/techfocus/na/NA-approach-for-eval-2011.pdf

https://www.epa.gov/sites/production/files/2014-02/documents/d9200.4-17.pdf

https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=10004674.TXT

https://pubs.usgs.gov/wri/wri034057/

5. Is the EPA Second Line of Evidence Required?

Decision Criteria

Answer YES if: The appropriate regulatory authority has requested that multiple lines of evidence for 1,1,1-TCA attenuation should be collected before approval of MNA as a site remedy will be granted. The first direct line of evidence requires data that demonstrate a clear and meaningful trend of decreasing contaminant mass and/or concentration over time at appropriate monitoring or sampling points. The second line of evidence originally included “hydrogeologic and geochemical data that can be used to indirectly demonstrate the type(s) of natural attenuation processes active at the site, and the rates at which such processes will reduce contaminant concentrations to required levels”.

Answer NO if: The appropriate regulatory authority has specifically requested that only the first/primary line of evidence for natural attenuation is required for approval of MNA as a site remedy. The first direct line of evidence requires data that demonstrate a clear and meaningful trend of decreasing contaminant

mass and/or concentration over time at appropriate monitoring or sampling points. Note that the final decision to require, or to not require, the second line of evidence is made by the appropriate regulatory authority. If the regulatory authority has not signaled what lines of evidence will be required, then a more conservative approach would be to answer "YES" to this question and proceed with collecting additional lines of evidence.

HELP

As part of EPA's MNA guidance, the agency has listed three lines of evidence that may be part of an MNA evaluation. Collecting data to support the first line of evidence is always required, and data to support the second line of evidence is typically required. The rest of this decision tool is focused on the second and third lines of evidence. It is recommended that the user go through these decision points even if the applicable data are not available.

For sites where the second line of evidence is required, it is expected that one focus will be on establishing that geochemical conditions are favorable for targeted reactions and on estimating degradation rates. However, it should be noted that 1,1,1-TCA will naturally attenuate in aquifers via hydrolysis/dehydrohalogenation, and this reaction occurs at a predictable rate based on the groundwater temperature. This information should be used to support other secondary lines of evidence in supporting natural attenuation.

The following resources provide more information on the lines of evidence approach, including definitions and how they are used (copy link into web browser):

https://www.epa.gov/sites/production/files/2014-02/documents/d9200.4-17.pdf

https://www.serdp-estcp.org/content/download/25789/262545/file/FAQ%20ER-201211.V2%20February%202014.pdf

6. Is 1,1,1-TCA biodegrading based on model predictions?

Decision Criteria

Answer YES if: Using 1,1,1-TCA degradation rate constants greater than zero in the model provides a better fit than the fit when the rate constant is set to zero. This can be evaluated using the same simulation in the "MNA Rate Constant Estimator" model that was used in "Does Long-Term Monitoring Data Provide the 1st Line of Evidence for MNA?" Prepare a new simulation where the rate constant for degradation of 1,1,1-TCA is set to zero (note that the model automatically incorporates degradation due to hydrolysis). Compare the actual in situ concentrations of 1,1,1-TCA against the new simulation. Then enter trial values for the rate constant for 1,1,1-TCA degradation into the simulation to determine if the model projections provide a better fit to the actual field-measured concentrations. A better fit is defined as having a lower value of RMSE (root mean square error) between the field data and the concentrations predicted by the model). If rate constants greater than zero provide a better fit, then 1,1,1-TCA is degrading. Note that this may have already been established as part of the earlier evaluation of the criterion "Does Long-Term Monitoring Data Provide the 1st Line of Evidence for MNA?"

Answer NO if: Setting the 1,1,1-TCA degradation rate constant to zero in the model provides a better fit than the fit when the rate constant is greater than zero. This is evaluated using the same model and simulations described above for the "YES" answer.

7. Is 1,1-DCE present?

Decision Criteria

Answer YES if: 1,1-DCE has been present above the reporting limit at any groundwater monitoring location at the site. This compound is a by-product of 1,1,1-TCA hydrolysis and therefore serves as a confirmatory line of evidence that this reaction is actively contributing to 1,1,1-TCA attenuation.

Answer NO if: 1,1-DCE has not been present at any groundwater monitoring location at the site, either currently or historically.

8. Are ^{13}C and/or ^{37}Cl in 1,1,1-TCA enriched along the flow path?

Decision Criteria

Answer YES if: A clear pattern of carbon and/or chlorine isotope fractionation can be observed in samples collected along a groundwater flow path. For 1,1,1-TCA, values of $\delta^{13}C$ and $\delta^{37}Cl$ can be obtained for individual samples via commercial lab analysis (see **HELP** for further explanation of CSIA principles and analytical considerations). If samples are taken from the source area and then at several locations downgradient, a 2-dimensional plot of these values can then be generated (see CSIA_111TCA in **FILES**). If the values of both $\delta^{13}C$ and $\delta^{37}Cl$ generally increase along the groundwater flow path (i.e., become "less negative" due to depletion of the lighter isotope), then this is taken as evidence for degradation of 1,1,1-TCA.

Answer NO if: No isotope data are available or if there is no clear trend in samples collected along a groundwater flow path. Again, this is best visualized by creating a 2-D plot of the $\delta^{13}C$ and $\delta^{37}Cl$ values (see HELP and FILES for more guidance).

HELP

Additional lines of evidence for 1,1,1-TCA attenuation can be provided by site-specific analysis of samples for stable isotopes of carbon and chloride. Data from a single sample may not provide sufficient evidence for 1,1,1-TCA degradation. This is because little is known about the natural variation in in the isotopic composition of the 1,1,1-TCA that was originally released to groundwater. Collecting multiple samples along the groundwater flow path is a more appropriate approach for establishing degradation because it relies on site-specific isotopic data to document 1,1,1-TCA degradation.

If values for $\delta^{13}C$ and $\delta^{37}Cl$ are available for 1,1,1-TCA, open the tab Files and select the spreadsheet CSIA_111TCA.xlsx. Enter your data in the tab Input. The user can enter data for up to 8 wells. Data from a well located within the source area or in an upgradient area should be entered in the first row (Well 0); this provides a site-specific estimate of the isotopic composition of undegraded 1,1,1-TCA and serves as a baseline for further comparisons. This well should have the lowest (most negative) values for $\delta^{13}C$ and

$\delta^{37}Cl$ (prioritize the well with the lowest $\delta^{37}Cl$). Data from other wells are entered in the remaining rows, following the order that they fall along the groundwater flow path.

Once the available data have been entered, the user can first consult the tab 2-D Chart_simple. Your data should plot on the chart. If not, you may need to extend the scales of the x and/or y axes. Degradation of 1,1,1-TCA is indicated if the data points generally proceed up and/or to the right within the plot in the direction of groundwater flow. This occurs due to the preferential degradation of bonds that contain lighter isotopes, such that the lighter isotopes become depleted and the heavier isotopes become enriched within the remaining portion of the compound as it is transported downgradient. The degree of enrichment can vary depending on the compound, the isotope, and the transformation pathway.

The error bars represent uncertainty in the determination of $\delta^{13}C$ and $\delta^{37}Cl$. The values can be said to increase between two samples if either one or both of the vertical or horizontal error bars do not overlap.

For 1,1,1-TCA, the user can also roughly estimate the amount of 1,1,1-TCA that has been degraded based on different possible degradation pathways (see Step 2 in the Input tab). This relies on published isotopic enrichment factors (Ɛ) for carbon and/or chlorine for three different abiotic transformation pathways: (1) hydrolysis/dehydrohalogenation; (2) reductive dechlorination by zero-valent iron; (3) oxidation via persulfate; and 4) biological reductive dechlorination of 1,1,1-TCA is also known to cause fractionation of carbon isotopes, but the effect is relatively small. Note that for Pathway 4, the chlorine isotope enrichment factor for biological reduction has yet to be established, so it is not included in the 2-D plots described below.

For each of the four possible pathways listed above, the percent of 1,1,1-TCA degraded is presented as a range based on the uncertainty in the isotopic enrichment factors, as well as a user-input uncertainty factor. The latter can be used to perform a limited sensitivity analysis on the degradation estimates.

To better understand how the data compare to the expected isotopic fractionation patterns for each pathway, the user can consult the tab 2-D delta from upgradient. In this chart, the origin is the isotopic composition of the upgradient/source well (Well 0), and the rest of the site-specific data are plotted as symbols. The three solid lines represent the fractionation pattern associated with each of the first three pathways described above as degradation proceeds. The slopes of these lines reflect changes to both elements (carbon and chloride) and are minimally influenced by retardation and other non-destructive processes that may occur during groundwater transport. If the data adhere to a specific pathway line, then this is plausible evidence that this specific pathway may be contributing to the observed fractionation. It should be understood that alternate or multiple transformation pathways may be occurring and cause data to not adhere to any of the plotted lines.

9. Are geochemical conditions adequate for anaerobic 1,1,1-TCA biodegradation?

Decision Criteria

Answer YES if: Dissolved oxygen is routinely absent in groundwater samples from one or more wells in the 1,1,1-TCA plume or in the area downgradient of the plume. This is a qualitative line of evidence that conditions are favorable to support anaerobic reductive dechlorination but does not imply that 1,1,1-TCA is actually being biodegraded. A threshold (maximum) DO value that would preclude anaerobic

biodegradation has not been established, and because of field sampling limitations, dissolved oxygen concentration data on well water are often unreliable. For the purposes of this decision tool, conditions are considered generally favorable for anaerobic biodegradation of 1,1,1-TCA when one of the following criteria are met: Dissolved oxygen concentrations measured in the field are less than 0.1 mg/L, ferrous iron (Fe^{2+}) concentrations are greater than 0.5 mg/L, and methane concentrations are greater than 0.005 mg/L.

Answer NO if: Dissolved oxygen is typically present at elevated levels (> 1 mg/L) across the entire site. This might include sites where the impacted intervals are shallow, unconfined, and/or organic-rich. This type of determination would also rely on other corroborating geochemical data, such as highly positive ORP readings (≥ +100 mV against the AgCl reference electrode), ferrous iron (Fe^{2+}) < 0.5 mg/L or methane < 0.005 mg/L.

HELP

1,1,1-TCA can be naturally attenuated by reactions that occur in primarily anaerobic conditions (e.g., biological reductive dechlorination to 1,1-DCA and abiotic degradation by reactive minerals via several different pathways). 1,1,1-TCA can also be naturally attenuated by a hydrolysis/dehydrohalogenation reaction that will proceed regardless of the redox conditions.

In assessing whether geochemical conditions are favorable for anaerobic vs. aerobic processes, it should be noted that field methods for measuring dissolved oxygen may generate inconsistent and/or erroneous results. One contributing factor is the common use of long-screened (≥ 10 ft) monitoring wells that may be collecting water from multiple zones with different redox conditions. This mixing of groundwater can make it difficult to quantify zones with higher dissolved oxygen that may promote 1,1,1-TCA biodegradation. Consequently, field dissolved oxygen measurements should be used with caution and supported by other lines of evidence. Other data that would corroborate that geochemical conditions are favorable for reductive dechlorination of 1,1,1-TCA include negative ORP readings and elevated dissolved iron and methane concentrations. Total organic carbon (> 20 mg/L) is also a positive indicator because it provides a carbon source and electron donor to promote microbial reductive dechlorination. In addition, portions of the site where groundwater transitions between anaerobic and aerobic should be delineated to identify areas that might be best managed by different natural attenuation pathways.

10. Does *Dhb* Density Explain the 1,1,1-TCA Rate Constant?

Decision Criteria

Answer YES if: The 1,1,1-TCA biodegradation rate constant used in the model is consistent with correlations based on the abundance of the Dhb biomarker (also referred to as DHBt) for strains of *Dehalobacter* bacteria that degrade 1,1,1-TCA. The correlations were derived from other studies and kinetic data. To do this, first refer to the simulation in the "MNA Rate Constant Estimator" model that was used to evaluate the criterion "Does Long-Term Monitoring Data Provide the 1st Line of Evidence for MNA?". Note that this model has the option to use biomarker data to estimate the biodegradation rate constant (i.e., it uses a correlation to predict the representative rate constant based on the biomarker levels measured at the site). If this option was employed and it resulted in a reasonable fit to the actual

data, then this confirms that "YES" is the appropriate answer. See **HELP** for additional guidance on determining if the fit was reasonable.

Answer NO if: No data on the abundance of the Dhb biomarker are available, or if the 1,1,1-TCA biodegradation rate constant used to optimize the model is inconsistent with rate constants predicted using the biomarker correlations. To evaluate the latter condition, first refer to the model simulation that was used to evaluate the criterion "Does Long-Term Monitoring Data Provide the 1st Line of Evidence for MNA?". If the option to use the correlation was not employed OR did not result in an optimal fit, then "NO" is the appropriate answer.

HELP

For 1,1,1-TCA, the "MNA Rate Constant Estimator" model (see **FILES**) has an option to estimate rate constants based on the abundance of Dhb (also referred to as DHBt), which is a qPCR-based biomarker for degradation of this compound. The correlations are designed to help calibrate the model, and they are intended as a starting point for improving the fit between the actual field data and the model simulations. Consequently, they should not be considered a true prediction of the actual degradation rate that is occurring at the site. This is because they are based on empirical data from other studies where conditions may be quite different than those observed at the site being evaluated.

To use the biomarker correlations as part of this decision tool, the following process is recommended:

1. In Box 6b, select the specific biomarker Dhb from the dropdown menu. On the chlorinated ethanes module of this model, this is one of only two biomarker options; only Dhb is applicable for 1,1,1-TCA. Selecting a biomarker from the menu will launch a pop-up box where biomarker abundance data can be entered.
2. In the pop-up box, enter the abundance of the selected biomarker for those wells where data are available. The model will perform a spatial interpolation to estimate a representative biomarker abundance for the site (i.e., a single value that is weighted based on the distance between wells with biomarker data).
3. The rate constant associated with this biomarker abundance will then be automatically entered in the appropriate location in Box 6b.
4. Enter the rate constant from Box 6b into Box 6. This is the rate constant that is used for the model simulation (i.e., to generate a simulated concentration vs. distance curve).
5. The user should then evaluate the fit between the actual field data and the model simulation that is based on this estimated rate constant. Manually adjust the rate constant in Box 6 until an optimal fit between the actual field data and the model simulation is obtained. Use the Root Mean Square Error (RMSE) that is overlaid on the plot as a guide; lower RMSE values generally indicate a better fit. Record the rate constant that provided the optimal fit.
6. Compare the recorded rate constant from the biomarker correlation with the "optimal" rate constant from Box 6. If the optimal rate constant from Box 6 is within a factor of 3 to 5 of the rate constant that was generated from the biomarker correlation, then this is considered reasonable evidence that these biodegradation processes are contributing to the actual field trend in 1,1,1-TCA concentrations.

The derivation of these correlations is described in Appendix D of the project report. They are based on an assumption that anaerobic reductive dechlorination of 1,1,1-TCA follows Michaelis-Menten (Haldane)

kinetics. The rate equation for Michaelis-Menten kinetics can be rearranged to solve for a first-order rate constant that is a function of other kinetic parameters (specifically Km and Vmax expressed in terms of gene copies), the biomarker abundance (expressed in gene copies per mL) and the concentration of the organic chemical being degraded (in this case, 1,1,1-TCA). Derived values for the kinetic parameters for each biomarker are also detailed in Appendix D of the ESTCP ER-201730 project report.

11. Is 1,1-DCE above the regulatory standard anywhere at the site?

Decision Criteria:

Answer YES if: The 1,1-DCE concentration at any groundwater monitoring location at the site is above the applicable concentration-based regulatory standard. Note that this standard is site-specific. If no standard has been established for your site, then select a value based on guidance from EPA or other states (see **HELP**) for planning purposes.

Answer NO if: The 1,1-DCE concentration at each groundwater monitoring location at the site is already below the concentration-based regulatory standard and 1,1,1-TCA is not detected at the site. The decision tool is intended for sites where the concentration of 1,1-DCE must fall below a site-specific regulatory standard before contaminated groundwater reaches the point of compliance (POC). If the 1,1-DCE concentration is already below the regulatory standard across the site (including the POC), then it is likely that it will remain below this standard in the future (see **HELP** for possible exceptions). This assumes that the site has been reasonably well characterized (especially the source area) and that no significant changes in conditions at the site are anticipated.

HELP

In the absence of site-specific regulatory standards for 1,1-DCE, the user may wish to select the federal MCL (7 µg/L) for 1,1-DCE to proceed with the BioPIC evaluation.

If 1,1-DCE is already below the established or assumed standard, then a future exceedance would likely be associated with one or more of the following: 1) the site is poorly characterized; 2) a new release of a highly chlorinated ethene or ethane occurs and results in the formation of 1,1-DCE; 3) active remediation is on-going or recently completed, such that steady state conditions have not yet been reached; or 4) any other change in site conditions has occurred that would contribute to 1,1-DCE formation or inhibit 1,1-DCE attenuation.

12. Does Long-Term Monitoring Data Provide the 1st Line of Evidence for MNA?

Decision Criteria

Answer YES if: The 1,1-DCE concentration is currently below the regulatory standard at the point of compliance (POC) and is predicted to be below the concentration-based regulatory standard at the POC at any time in the future. Use the model provided as part of this tool (see **FILES** for "MNA Rate Constant Estimator") to predict if the concentration will be below the standard at any time in the future (see **HELP** for additional explanation).

Answer NO if: At any time in the future, the 1,1-DCE concentration will exceed the regulatory standard at the POC. Use the model provided as part of this tool to predict the concentration in the future at the POC. Note there usually is also a temporal component in the regulatory goals, which involves establishing how long it will take the concentration at a particular location to achieve a regulatory goal. The implementation of more aggressive remedies may reduce time to achieve remediation goals, thereby reducing the overall cost. However, this tool primary deals with the spatial aspects of remediation goals (i.e., will the goal be achieved at a POC) rather than the temporal components.

HELP

If sufficient historical contaminant concentration data are not available to determine if the 1,1-DCE plume will reach a POC, then a groundwater flow and solute transport model, such as the "MNA Rate Constant Estimator" provided as part of this tool (see **FILES**), should be used to predict solute plume behavior. In this case, the simulation should account for the effects of advective groundwater flow, dispersion of the relevant solutes, sorption, and degradation of 1,1-DCE in groundwater at the site.

For more information on using this type of model for 1,1-DCE, consult the project report (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 (ESTCP ER-201730)), which can be found on the project page (copy link into web browser): https://serdp-estcp.org/Program-Areas/Environmental-Restoration/Contaminated-Groundwater/Persistent-Contamination/ER-201730/ER-201730.

A similar approach for chlorinated ethenes can be found in another project report (Development of a Quantitative Framework and Management Expectation Tool for the Selection of Bioremediation Approaches at Chlorinated Ethene Sites (ESTCP Project ER-201129)), which can be downloaded from the project page (copy link into web browser): https://www.serdp-estcp.org/Program-Areas/Environmental-Restoration/Contaminated-Groundwater/Persistent-Contamination/ER-201129/ER-201129. In the ER-201129 report, Section 5.2.3 illustrates the process of calibrating a groundwater flow and transport model (in this case, the "BIOCHLOR" model), and Section 5.2.4 Step 1 illustrates the use of a model to apply the decision criteria.

If available, a robust historical database of contaminant concentrations can be used as an alternative to a computer model. Spatial and temporal trends in solute concentrations can be utilized to determine if the plume is stable or receding and therefore will not reach the POC. When sufficient data are available, using empirical data to ascertain trends is much better than using a model. In many cases, sufficient 1,1-DCE concentration data are available to evaluate plume behavior and to determine if solute concentrations will exceed cleanup goals at a regulatory POC.

13. Is Long-Term Monitoring Data Sufficient to Evaluate MNA?

Decision Criteria

Answer YES if: The current long-term monitoring data confirm that one or more of the following conditions are met:

1. The plume is currently beyond the point of compliance at a concentration that is above the applicable standard.

2. The plume is still expanding and is predicted to extend beyond the point of compliance in the future based on modeling.
3. Concentration-based goals have been established within the plume (i.e., upgradient of the point of compliance), and model predictions suggest that these will not be achieved with a reasonable timeframe.

Answer NO if: The current long-term monitoring data confirm that ALL of the following conditions are met:

1. The plume is currently not beyond the point of compliance.
2. The current dataset is too limited to evaluate if plume is expanding vs. receding.
3. The current dataset is too limited to predict using a model whether the plume will expand or whether concentration-based goals will be achieved.
4. There is no current regulatory requirement for active remediation.

HELP

Long-term monitoring data are an important component of site management, and they are particularly important for demonstrating the site-specific viability of MNA.

It is possible that a site's existing dataset for this compound may be limited if it is a recent additional to the monitoring program. It may not be possible to establish a clear trend in the attenuation in concentration of 1,1-DCE or its degradation products along a flow path in groundwater at appropriate monitoring points. This means that the data are inadequate to permit a thorough evaluation for the primary line of evidence for MNA. This is typically because one or more of the following apply: 1) data are highly variable across the site; 2) data are highly variable from event-to-event; or 3) data are available from a relatively limited number of monitoring points or events. In each case, these data limitations make it difficult to establish trends with any degree of statistical certainty.

In that case, collecting additional data may be beneficial to provide more certainty that current trends are statistically significant and sustainable. These additional monitoring events may also include data that would be used to support the second line of evidence for MNA (e.g., geochemical data, biomarkers, stable isotopes) or even hydrogeologic parameters to improve fate and transport model predictions.

At sites where the number of monitoring locations is relatively small (e.g., 4 or less wells), this type of analysis will likely benefit by including additional monitoring locations along the plume transect. In part, this is because it can be more challenging to establish that a trend is significant using standard approaches (e.g., that the slope of a best-fit regression line is different than zero). As a result, adding more monitoring locations along the plume transect may provide value.

It is necessary to use modeling software to evaluate if current trends in natural attenuation will meet the standard at the point-of-compliance. An example is the "MNA Rate Constant Estimator" that has been developed as part of this decision tool (see **FILES**). This type of model allows the user to predict concentrations along a plume transect. However, it relies on the user to calibrate to the model predictions based on field data, and it also assumes that those field data are representative. At sites, where data vary considerably from event to event, this type of calibration can be challenging. In any case, the goal is to demonstrate that the plume footprint is stable and/or shrinking and will not result in concentrations at a downgradient POC that exceed an acceptable level. This may require additional monitoring locations

(particularly if the plume extent is not yet delineated) or additional monitoring events to demonstrate longer-term stability. Data from additional events are also important to establish that trends are sustainable even if minor (or major) changes in site conditions (e.g., groundwater flow directions, redox conditions) occur that might impact attenuation and/or plume stability.

In some cases where MNA is proposed as a remedy, an estimate of the remediation timeframe is also required by the regulatory agency. The remediation timeframe is typically the date when some or all wells are projected to achieve a concentration goal. Estimating the remediation timeframe is often done using linear regression of concentration vs. time data at a specific well (or wells), but this requires data from enough events to ensure that the result is statistically significant. Additional monitoring events may be required to achieve this objective at sites with limited concentration vs. time data.

For the case where the dataset is already robust and confirms that MNA is unlikely to be effective, active remediation is likely to be the next course of action. Active remediation should be selected and implemented based on appropriate, well-defined objectives, but one primary goal should be to reduce concentration in the source area enough so that concentrations are below the site-specific standard at the point of compliance. The required reduction in the source concentration can be estimated using models, including the one provided as part of this tool (see "MNA Rate Constant Estimator" in **FILES**). Another good option is REMChlor-MD, which allows the user to input the extent of source reduction and then compare the plume behavior for cases with and without remediation. REMChlor-MD also incorporates the effects of matrix diffusion (i.e., contaminants diffusing into and out of lower-permeability intervals within the saturated zone) which has implications for contaminant transport and persistence at many sites.

Additional resources for understanding and developing monitoring objectives for MNA include the following (copy link into web browser):

https://clu-in.org/download/contaminantfocus/dnapl/Treatment_Technologies/performance_monitoring_mns600R04027.pdf

https://www.serdp-estcp.org/content/download/25789/262545/file/FAQ%20ER-201211.V2%20February%202014.pdf

https://clu-in.org/download/techfocus/na/NA-approach-for-eval-2011.pdf

https://www.epa.gov/sites/production/files/2014-02/documents/d9200.4-17.pdf

https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=10004674.TXT

https://pubs.usgs.gov/wri/wri034057/

14. Is the EPA Second Line of Evidence Required?

Decision Criteria

Answer YES if: The appropriate regulatory authority has requested that multiple lines of evidence for 1,1-DCE attenuation should be collected before approval of MNA as a site remedy will be granted. The first

direct line of evidence requires data that demonstrate a clear and meaningful trend of decreasing contaminant mass and/or concentration over time at appropriate monitoring or sampling points. The second line of evidence originally included "hydrogeologic and geochemical data that can be used to indirectly demonstrate the type(s) of natural attenuation processes active at the site, and the rates at which such processes will reduce contaminant concentrations to required levels".

Answer NO if: The appropriate regulatory authority has specifically requested that only the first/primary line of evidence for natural attenuation is required for approval of MNA as a site remedy. The first direct line of evidence requires data that demonstrate a clear and meaningful trend of decreasing contaminant mass and/or concentration over time at appropriate monitoring or sampling points. Note that the final decision to require, or to not require, the second line of evidence is made by the appropriate regulatory authority. If the regulatory authority has not signaled what lines of evidence will be required, then a more conservative approach would be to answer "YES" to this question and proceed with collecting additional lines of evidence.

HELP

As part of EPA's MNA guidance, the agency has listed three lines of evidence that may be part of an MNA evaluation. Collecting data to support the first line of evidence is always required, and data to support the second line of evidence is typically required. The rest of this decision tool is focused on the second and third lines of evidence. It is recommended that the user go through these decision points even if the applicable data are not available.

The following resources provide more information on the lines of evidence approach, including definitions and how they are used (copy link into web browser):

https://www.epa.gov/sites/production/files/2014-02/documents/d9200.4-17.pdf

https://www.serdp-estcp.org/content/download/25789/262545/file/FAQ%20ER-201211.V2%20February%202014.pdf

15. Is 1,1-DCE biodegrading based on model predictions?

Decision Criteria

Answer YES if: Using 1,1-DCE degradation rate constants greater than zero in the model provide a better fit than the fit when the rate constant is set to zero. This can be evaluated using the same simulation in the "MNA Rate Constant Estimator" model that was used to "Does Long-Term Monitoring Data Provide the 1st Line of Evidence for MNA?" Prepare a new simulation where the rate constant for degradation of 1,1-DCE is set to zero. Compare the actual field-measured concentrations of 1,1-DCE against the new simulation. Then enter trial values for the rate constant for 1,1-DCE degradation into the simulation to determine if the model projections provide a better fit to the actual field-measured concentrations. A better fit is defined as having a lower value of RMSE (root mean square error) between the field data and the concentrations predicted by the model). If rate constants greater than zero provide a better fit, then 1,1-DCE is degrading. Note that this may have already been established as part of the earlier evaluation of the criterion "Does Long-Term Monitoring Data Provide the 1st Line of Evidence for MNA?" In addition,

the model will also have to be calibrated to fit the field-measured concentrations of 1,1,1-TCA and 1,1-DCA.

Answer NO if: Setting the 1,1-DCE degradation rate constant to zero in the model provides a better fit than the fit when the rate constant is greater than zero. This is evaluated using the same model and simulations described above for the "YES" answer.

16. Are ^{13}C and/or ^{37}Cl in 1,1-DCE enriched along the flow path?

Decision Criteria

Answer YES if: A clear pattern of carbon and/or chlorine isotope fractionation can be observed in samples collected along a groundwater flow path. For 1,1-DCE, values of $\delta^{13}C$ and $\delta^{37}Cl$ can be obtained for individual samples via commercial lab analysis (see HELP for further explanation of CSIA principles and analytical considerations). If samples are taken from the source area and then at several locations downgradient, a 2-dimensional plot of these values can then be generated (see CSIA_11DCA_11DCE in **FILES**). If the values of both $\delta^{13}C$ and $\delta^{37}Cl$ generally increase along the groundwater flow path (i.e., become "less negative" due to depletion of the lighter isotope), then this is taken as evidence for degradation of 1,1-DCE.

Answer NO if: No isotope data are available or if there is no clear trend in samples collected along a groundwater flow path. Again, this is best visualized by creating a 2-D plot of the $\delta^{13}C$ and $\delta^{37}Cl$ values (see **HELP** and **FILES** for more guidance).

HELP

Additional lines of evidence for 1,1-DCE attenuation can be provided by site-specific analysis of samples for stable isotopes of carbon and chloride. Data from a single sample may not provide sufficient evidence for 1,1-DCE degradation. This is because it is often a by-product of releases of other contaminants, and isotopic patterns may be difficult to distinguish if data are limited. Collecting multiple samples along the groundwater flow path is a more appropriate approach for establishing degradation because it relies on site-specific isotopic data to document 1,1-DCE degradation. Collecting isotopic data for parent compounds (e.g., 1,1,1-TCA) is also recommended to better establish trends across the site.

If values for $\delta^{13}C$ and $\delta^{37}Cl$ are available for 1,1-DCE, open the tab Files and select the spreadsheet CSIA_11DCA_11DCE.xlsx. Enter your data in the tab Input. The user can enter data for up to 8 wells. Data from a well located within the source area or in an upgradient area should be entered in the first row (Well 0); this provides a site-specific estimate of the isotopic composition of 1,1-DCE at the source and serves as a baseline for further comparisons. This well should have the lowest (most negative) values for $\delta^{13}C$ and $\delta^{37}Cl$ (prioritize the well with the lowest $\delta^{37}Cl$). Data from other wells are entered in the remaining rows, following the order that they fall along the groundwater flow path.

Once the available data have been entered, the user can consult the tab 2-D Chart_simple. Your data should plot on the chart. If not, you may need to extend the scales of the x and/or y axes. Degradation of 1,1-DCE is indicated if the data points generally proceed up and/or to the right within the plot in the direction of groundwater flow. This occurs due to the preferential degradation of bonds that contain lighter isotopes, such that the lighter isotopes become depleted and the heavier isotopes become

enriched within the remaining portion of the compound as it is transported downgradient. The degree of enrichment can vary depending on the compound, the isotope, and the transformation pathway.

The error bars represent uncertainty in the determination of $\delta^{13}C$ and $\delta^{37}Cl$. The values can be said to increase between two samples if either one or both of the vertical or horizontal error bars do not overlap.

Note that the user may also enter isotopic data for 1,1,1-TCA in the Input tab and plot it on the same chart as the 1,1-DCE data. If the $\delta^{13}C$ and $\delta^{37}Cl$ values for 1,1-DCE are less than the corresponding $\delta^{13}C$ and $\delta^{37}Cl$ values for 1,1,1-TCA at the source and near-source wells, then this is confirmatory evidence that the 1,1-DCE originated from 1,1,1-TCA degradation. This pattern occurs because the 1,1-DCE is formed from the preferential degradation of the lighter isotopes in 1,1,1-TCA, which is then reflected in the isotopic signature of the 1,1-DCE in these wells. If the $\delta^{13}C$ and $\delta^{37}Cl$ values for 1,1-DCE in the far downgradient wells exceed those of 1,1,1-TCA in the source wells, then this suggests that 1,1-DCE is degrading during groundwater transport.

17. Are geochemical conditions adequate for aerobic 1,1-DCE cometabolic biodegradation?

Decision Criteria

Answer YES if: Dissolved oxygen is routinely present in groundwater samples from one or more wells in the plume or in the area downgradient of the plume. This is a qualitative line of evidence that conditions are favorable to support aerobic cometabolic oxidation but does not imply that 1,1-DCE is actually being biodegraded. A threshold (minimum) DO value that would preclude aerobic biodegradation has not been established, and because of field sampling limitations, dissolved oxygen concentration data on well water are often unreliable. For the purposes of this decision tool, conditions are considered generally favorable for aerobic biodegradation of 1,1-DCE when one of the following criteria are met: Dissolved oxygen concentrations measured in the field are greater than 1 mg/L, ORP readings are > + 100 mV (against the AgCl reference electrode), ferrous iron (Fe^{2+}) concentrations are less than 0.5 mg/L, and methane concentrations are less than 0.005 mg/L. However, field parameter data should be evaluated with appropriate caution (see HELP).

Answer NO if: Dissolved oxygen is typically present at low levels (< 0.1 mg/L) across the entire site. This might include sites where the impacted intervals are deep, confined, and/or organic-rich. This type of determination would also rely on other corroborating geochemical data, such as highly negative ORP readings (< -100 mV against the AgCl reference electrode), elevated ferrous iron (Fe^{2+}) > 1 mg/L, or methane > 0.5 mg/L.

HELP

1,1-DCE can be naturally attenuated by reactions that occur in both anaerobic conditions (e.g., biological reductive dechlorination) and aerobic conditions. The former reaction can be evaluated as part of the evaluation of other chlorinated ethenes or 1,1,1-TCA and yields transformation products (vinyl chloride, ethene) that are similar to other chlorinated ethenes. Aerobic oxidation of 1,1-DCE results in products that are not easily measurable, so documenting favorable geochemical conditions is an important secondary line of evidence.

In assessing whether geochemical conditions are favorable for anaerobic vs. aerobic processes, it should be noted that field methods for measuring dissolved oxygen may generate inconsistent and/or erroneous results. One contributing factor is the common use of long-screened (≥ 10 ft) monitoring wells that may be collecting water from multiple zones with different redox conditions. This mixing of groundwater can make it difficult to quantify zones with higher dissolved oxygen that may promote 1,1-DCE biodegradation. Consequently, field dissolved oxygen measurements should be used with caution and supported by other lines of evidence. Other data that would corroborate that geochemical conditions are favorable for aerobic biodegradation of 1,1-DCE include positive ORP readings and little or no dissolved iron and methane. Elevated levels of total organic carbon (TOC) (e.g., > 20 mg/L) is also a positive secondary indicator because it provides a carbon source and electron donor to promote biological cometabolic oxidation of 1,1-DCE. To date, there is little evidence that 1,1-DCE can be used as a sole carbon and energy source for microbial activity, so the presence of organic co-substrates is important.

18. Are *Dhc*, *vcrA*, and *bvc* present?

Decision Criteria

Answer YES if: Any of these qPCR-based biomarkers for chlorinated ethene degradation are present in one or more wells at the site. These are gene targets that are associated with organisms and/or enzymes that can reductively dechlorinate several chlorinated ethenes, including 1,1-DCE, and their abundance in site samples can be quantified by several analytical laboratories. If these biomarkers are present, a supplemental evaluation can rely on correlations between biomarker abundance and rate constants developed for chlorinated ethenes (see **HELP**).

Answer NO if: No data on the abundance of qPCR biomarkers are available, or analytical results confirm that none are present above detection limits.

HELP

The presence or absence of biomarkers for chlorinated ethene biodegradation is a starting point for evaluating MNA. When analytical labs quantify the abundance of specific biomarkers, they can typically provide information on how the measured levels compare to those from other sites. At sites where this abundance is comparably high, this helps support the second line of evidence for MNA. It should be understood that the degradation rate needed to achieve a goal concentration at one site may be much different than that at another site. As a result, a relatively high biomarker abundance does not guarantee that MNA will be successful; these data need to be combined with the primary line of evidence for MNA (meaningful concentration/mass trends).

Another approach is to use the biomarker data to help refine model predictions of the biodegradation rate constant. The "MNA Rate Constant Estimator" model (see **FILES**) has an option to estimate rate constants based on the abundance of several different qPCR-based biomarkers for degradation. These correlations are designed to help calibrate the model, and they are intended as a starting point for improving the fit between the actual field data and the model simulations. Consequently, they should not be considered a true prediction of the actual degradation rate that is occurring at the site. This is because they are based on empirical data from other studies where conditions may be quite different than those observed at the site being evaluated.

To use the biomarker correlations as part of this decision tool, the following process is recommended:

1. In Box 6b, select the specific biomarker vcrA from the dropdown menu. On the chlorinated ethanes module of this model, this is one of only two biomarker options; only vcrA is applicable for 1,1-DCE. Selecting a biomarker from the menu will launch a pop-up box where biomarker abundance data can be entered.
2. In the pop-up box, enter the abundance of the selected biomarker for those wells where data are available. The model will perform a spatial interpolation to estimate a representative biomarker abundance for the site (i.e., a single value that is weighted based on the distance between wells with biomarker data).
3. The rate constant associated with this biomarker abundance will then be automatically entered in the appropriate location in Box 6b.
4. Enter the rate constant from Box 6b into Box 6. This is the rate constant that is used for the model simulation (i.e., to generate a simulated concentration vs. distance curve).
5. The user should then evaluate the fit between the actual field data and the model simulation that is based on this estimated rate constant. Manually adjust the rate constant in Box 6 until an optimal fit between the actual field data and the model simulation is obtained. Use the Root Mean Square Error (RMSE) that is overlaid on the plot as a guide; lower RMSE values generally indicate a better fit. Record the rate constant that provided the optimal fit.
6. Compare the recorded rate constant from the biomarker correlation with the "optimal" rate constant from Box 6. If the optimal rate constant from Box 6 is within a factor of 3 to 5 of the rate constant that was generated from the biomarker correlations, then this is considered reasonable evidence that these biodegradation processes are contributing to the actual field trend in 1,1-DCE concentrations.

The derivation of these correlations is described in Appendix XX of the project report. They are based on an assumption that anaerobic biodegradation of 1,1-DCE follows Michaelis-Menten (Haldane) kinetics. The rate equation for Michaelis-Menten kinetics can be rearranged to solve for a first-order rate constant that is a function of other kinetic parameters (specifically Km and Vmax expressed in terms of gene copies), the biomarker abundance (expressed in gene copies per mL) and the concentration of the organic chemical being degraded (in this case, 1,1-DCE). Derived values for the kinetic parameters for each biomarker are also detailed in Appendix D of the project report.

19. Does Magnetic Susceptibility Explain the 1,1-DCE Rate Constant?

Decision Criteria

Answer YES if: The 1,1-DCE degradation rate constant and value of magnetic susceptibility from the field site of concern are in the same range as known values from microcosm studies or from other field sites. This evaluation can be performed using the worksheet provided as part of this tool (see Magnetic Susceptibility_11DCE in the **FILES** tab), and the process is described in the **HELP** screen. If this correlation is observed, then abiotic degradation by magnetite is a plausible mechanism to explain the bulk attenuation rate at the field site of concern.

Answer NO if: No site-specific magnetic susceptibility data are available OR if the 1,1-DCE degradation rate constant and value of magnetic susceptibility from the field site of concern are not in the same range as known values from microcosm studies or from other field sites. The latter can be evaluated using the same worksheet described above for the "YES" answer.

HELP

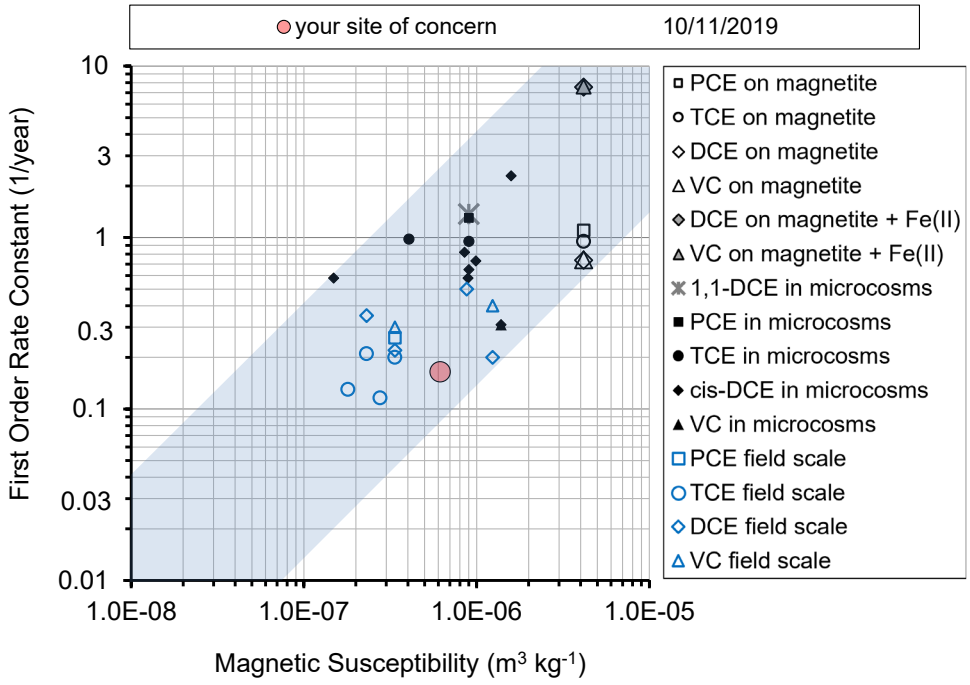
Chlorinated alkenes can be degraded by abiotic reactions with magnetite (He et al., 2009; Lee and Batchelor, 2002; Ferrey et al., 2004). The quantity of magnetite in aquifer sediments can be determined from a measurement of the mass magnetic susceptibility of the sediment. He et al. (2009) summarized rate constants for abiotic degradation of PCE, TCE, *cis*-DCE, and Vinyl Chloride in laboratory microcosm studies that were constructed with sediment with known values of magnetic susceptibility.

Lebrón et al. (2015) developed a worksheet to determine if bulk rate constants for attenuation of PCE, TCE, cis-DCE, and Vinyl Chloride in plumes of contaminated ground water could plausibly be attributed to abiotic degradation by magnetite.

The worksheet compared the field scale rate constant for attenuation of PCE, TCE, cis-DCE or Vinyl Chloride and the magnetic susceptibility of the aquifer sediment to the rate constants and magnetic susceptibilities in the sediments described in He et al. (2009), and to rate constants that had been fitted several field-scale plumes where data were available on magnetic susceptibility. If the rate constant and value of magnetic susceptibility from the field site of concern was in the same range as the values from the microcosm studies or from the other field sites, then abiotic degradation by magnetite was a plausible mechanism to explain the bulk attenuation rate at the field site of concern.

The rate constants for degradation of PCE, TCE, cis-DCE, and Vinyl Chloride by magnetite were very similar (Lee and Batchelor, 2002). There is only one report in the literature that provides a rate constant for abiotic degradation of 1,1-DCE in aquifer material with known magnetic susceptibility (Ferrey et al., 2004). The rate constants for degradation of 1,1-DCE and *cis*-DCE were very similar. The decision logic will assume that the rate constants for abiotic degradation of 1,1-DCE by magnetite are the same as the rate constants for the other chlorinated ethenes.

The **Magnetic Susceptibility_11DCE** worksheet compares the field scale rate constant for degradation of 1,1-DCE at a site of concern and the magnetic susceptibility of the aquifer sediment to the available literature. Data from the field site of concern are entered in the tab ***Data Input***. The evaluation is provided in the tab ***Mag Susceptibility Explain Rate (s***ee figure below for an example)



*Example of the chart in the Tab **Mag Susceptibility Explains Rate** from the **Magnetic Susceptibility Worksheet.xlsx.***

The blue shape encompasses a linear extrapolation of data available in the peer-reviewed literature on the relationship between rate constants and magnetic susceptibility. If the data from the site of concern falls within the blue shape, then abiotic degradation of 1,1-DCE by magnetite is a plausible explanation for the bulk rate constant for attenuation at field scale. Note that the one data point for degradation of 1,1-DCE microcosms constructed with aquifer sediment is consistent with rate constants for degradation of the other chlorinated ethenes in aquifer sediment.

The data in Figure 1 on field scale rate constants includes additional data published in Wiedemeier et al. (2015). The laboratory studies of Lee and Batchelor (2002) on synthetic magnetite are also included in Figure 1**.** Surface area specific first order rate constants reported in Lee and Batchelor (2002) were converted to first order rate constants by multiplying the surface area specific rate constant by the mass of magnetite per unit volume of water in their experimental reactor, and then by the specific surface area of the magnetite suspended in the water.

The following assumptions were used to estimate the magnetic susceptibility of aquifer sediment that would be equivalent to the experimental reactor. The milligram of magnetite per liter of water in the experimental reactor was assumed to be the milligram of magnetite exposed to each liter of pore water in the sediment. Porewater was assumed to occupy 25% of the total volume of the sediment, the dry bulk density of the sediment was assumed to be 2.0 kg/Liter, and magnetite was assumed to represent all the magnetic material in the aquifer sediment. Based on these assumptions, the milligrams of magnetite per kilogram of sediment was calculated, and the equations on page 77 of He et al. (2009) were used to

estimate the magnetic susceptibility of the equivalent aquifer sediment. The calculations are performed in Tab ***Synthetic Magnetite Calculation***.

20. Is 1,1-DCA above the regulatory standard anywhere at the site?

Decision Criteria:

Answer YES if: The 1,1-DCA concentration at any groundwater monitoring location at the site is above the applicable concentration-based regulatory standard. Note that this standard is site-specific. If no standard has been established for your site, then select a value based on guidance from EPA or other states (see **HELP**) for planning purposes.

Answer NO if: The 1,1-DCA concentration at each groundwater monitoring location at the site is already below the concentration-based regulatory standard and 1,1,1-TCA is not detected at the site. The decision tool is intended for sites where the concentration of 1,1-DCA must fall below a site-specific regulatory standard before contaminated groundwater reaches the point of compliance (POC). If the 1,1-DCA concentration is already below the regulatory standard across the site (including the POC), then it is likely that it will remain below this standard in the future (see **HELP** for possible exceptions). This assumes that the site has been reasonably well characterized (especially the source area) and that no significant changes in conditions at the site are anticipated.

HELP

In the absence of site-specific regulatory standards for 1,1-DCA, the user may wish to select one of the following values to proceed with the BioPIC evaluation. Note that there is considerable variability in state-level groundwater and drinking water standards for 1,1-DCA. The information below was compiled on 1 January 2021, so it should also be understood that states are likely to promulgate and/or revised standards over time.

- USEPA Reference Concentration for Drinking Water (10^{-6} risk) = 6.14 µg/L
- California MCL in Drinking Water = 5 µg/L
- North Carolina Groundwater Standard = 6 µg/L
- New Jersey Groundwater Standard = 50 µg/L
- Wisconsin Groundwater Preventive Action Limit = 85 µg/L

If 1,1-DCA is already below the established or assumed standard, then a future exceedance would likely be associated with one or more of the following: 1) the site is poorly characterized; 2) a new release of a highly chlorinated ethane occurs and results in the formation of 1,1-DCA; 3) active remediation is on-going or recently completed, such that steady state conditions have not yet been reached; or 4) any other change in site conditions has occurred that would contribute to 1,1-DCA formation or inhibit 1,1-DCA attenuation.

21. Does Long-Term Monitoring Data Provide the 1st Line of Evidence for MNA?

Decision Criteria

Answer YES if: The 1,1-DCA concentration is currently below the regulatory standard at the point of compliance (POC) and is predicted to be below the concentration-based regulatory standard at the POC at any time in the future. Use the model provided as part of this tool (see **FILES** for "MNA Rate Constant Estimator") to predict if the concentration will be below the standard at any time in the future (see **HELP** for additional explanation).

Answer NO if: At any time, the 1,1-DCA concentration will exceed the regulatory standard at the POC. Use the model provided as part of this tool to predict the concentration in the future at the POC. Note there usually is also a temporal component in the regulatory goals, which involves establishing how long it will take the concentration at a particular location to achieve a regulatory goal. The implementation of more aggressive remedies may reduce time to achieve remediation goals, thereby reducing the overall cost. However, this tool primary deals with the spatial aspects of remediation goals (i.e., will the goal be achieved at a POC) rather than the temporal components.

HELP

If sufficient historical contaminant concentration data are not available to determine if the 1,1-DCA plume will reach a POC, then a groundwater flow and solute transport model, such as the "MNA Rate Constant Estimator" provided as part of this tool (see **FILES**) should be used to predict solute plume behavior. In this case, the simulation should account for the effects of advective groundwater flow, dispersion of the relevant solutes, sorption, and degradation of 1,1-DCA in groundwater at the site.

For more information on using this type of model for 1,1-DCA, consult the project report (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 (ESTCP ER-201730)), which can be found on the project page (copy link into web browser): https://serdp-estcp.org/Program-Areas/Environmental-Restoration/Contaminated-Groundwater/Persistent-Contamination/ER-201730/ER-201730.

A similar approach for chlorinated ethenes can be found in another project report (Development of a Quantitative Framework and Management Expectation Tool for the Selection of Bioremediation Approaches at Chlorinated Ethene Sites (ESTCP Project ER-201129)), which can be downloaded from the project page (copy link into web browser): https://www.serdp-estcp.org/Program-Areas/Environmental-Restoration/Contaminated-Groundwater/Persistent-Contamination/ER-201129/ER-201129. In the ER-201129 report, Section 5.2.3 illustrates the process of calibrating a groundwater flow and transport model (in this case, the "BIOCHLOR" model), and Section 5.2.4 Step 1 illustrates the use of a model to apply the decision criteria.

If available, a robust historical database of contaminant concentrations can be used as an alternative to a computer model. Spatial and temporal trends in solute concentrations can be utilized to determine if the plume is stable or receding and therefore will not reach the POC. When sufficient data are available, using empirical data to ascertain trends is much better than using a model. In many cases, sufficient 1,1-DCA concentration data are available to evaluate plume behavior and to determine if solute concentrations will exceed cleanup goals at a regulatory POC.

22. Is Long-Term Monitoring Data Sufficient to Evaluate MNA?

Decision Criteria

Answer YES if: The current long-term monitoring data confirm that one or more of the following conditions are met:

1. The plume is currently beyond the point of compliance at a concentration that is above the applicable standard.
2. The plume is still expanding and is predicted to extend beyond the point of compliance in the future based on modeling.
3. Concentration-based goals have been established within the plume (i.e., upgradient of the point of compliance), and model predictions suggest that these will not be achieved with a reasonable timeframe.

Answer NO if: The current long-term monitoring data confirm that ALL of the following conditions are met:

1. The plume is currently not beyond the point of compliance.
2. The current dataset is too limited to evaluate if plume is expanding vs. receding.
3. The current dataset is too limited to predict using a model whether the plume will expand or whether concentration-based goals will be achieved.
4. There is no current regulatory requirement for active remediation.

HELP

Long-term monitoring data are an important component of site management, and they are particularly important for demonstrating the site-specific viability of MNA.

It is possible that a site's existing dataset for this compound may be limited if it is a recent additional to the monitoring program. It may not be possible to establish a clear trend in the attenuation in concentration of 1,1-DCA or its degradation products along a flow path in groundwater. This means that the data are inadequate to permit a thorough evaluation for the primary line of evidence for MNA. This is typically because one or more of the following apply: 1) data are highly variable across the site; 2) data are highly variable from event-to-event; or 3) data are available from a relatively limits number of monitoring points or events. In each case, these data limitations make it difficult to establish trends with any degree of statistical certainty.

In that case, collecting additional data may be beneficial to provide more certainty that current trends are statistically significant and sustainable. These additional monitoring events may also include data that would be used to support the second line of evidence for MNA (e.g., geochemical data, biomarkers, stable isotopes) or even hydrogeologic parameters to improve fate and transport model predictions.

At sites where the number of monitoring locations is relatively small (e.g., 4 or less wells), this type of analysis will likely benefit by including additional monitoring locations along the plume transect. In part, this is because it can be more challenging to establish that a trend is significant using standard approaches (e.g., that the slope of a best-fit regression line is different than zero). As a result, adding more monitoring locations along the plume transect may provide value.

It is necessary to use modeling software to evaluate if current trends in natural attenuation will meet the standard at the point of compliance. An example is the "MNA Rate Constant Estimator" that has been developed as part of this decision tool (see **FILES**). This type of model allows the user to predict concentrations along a plume transect. However, it relies on the user to calibrate to the model predictions based on field data, and it also assumes that those field data are representative. At sites where data vary considerably from event to event, this type of calibration can be challenging. In any case, the goal is to demonstrate that the plume footprint is stable and/or shrinking and will not result in concentrations at a downgradient POC that an unacceptable level. This may require additional monitoring locations (particularly if the plume extent is not yet delineated) or additional monitoring events to demonstrate longer-term stability. Data from additional events are also important to establish that trends are sustainable even if minor (or major) changes in site conditions (e.g., groundwater flow directions, redox conditions) occur that might impact attenuation and/or plume stability.

In some cases where MNA is proposed as a remedy, an estimate of the remediation timeframe is also required by the regulatory agency. The remediation timeframe is typically the date when some or all wells are projected to achieve a concentration goal. Estimating the remediation timeframe is often done using linear regression of concentration vs. time data at a specific well (or wells), but this requires data from enough events to ensure that the result is statistically significant. Additional monitoring events may be required to achieve this objective at sites with limited concentration vs. time data.

For the case where the dataset is already robust and confirms that MNA is unlikely to be effective, active remediation is likely to be the next course of action. Active remediation should be selected and implemented based on appropriate, well-defined objectives, but one primary goal should be to reduce concentration in the source area enough so that concentrations are below the site-specific standard at the point of compliance. The required reduction in the source concentration can be estimated using models, including the one provided as part of this tool (see **FILES**). Another good option is REMChlor-MD, which allows the user to input the extent of source reduction and then compare the plume behavior for cases with and without remediation. REMChlor-MD also incorporates the effects of matrix diffusion (i.e., contaminants diffusing into and out of lower-permeability intervals within the saturated zone), which has implications for contaminant transport and persistence at many sites.

Additional resources for understanding and developing monitoring objectives for MNA include the following (copy link into web browser):

https://clu-in.org/download/contaminantfocus/dnapl/Treatment_Technologies/performance_monitoring_mns600R04027.pdf

https://www.serdp-estcp.org/content/download/25789/262545/file/FAQ%20ER-201211.V2%20February%202014.pdf

https://clu-in.org/download/techfocus/na/NA-approach-for-eval-2011.pdf

https://www.epa.gov/sites/production/files/2014-02/documents/d9200.4-17.pdf

https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=10004674.TXT

https://pubs.usgs.gov/wri/wri034057/

23. Is the EPA Second Line of Evidence Required?

Decision Criteria

Answer YES if: The appropriate regulatory authority has requested that multiple lines of evidence for 1,1-DCA attenuation should be collected before approval of MNA as a site remedy will be granted. The first direct line of evidence requires data that demonstrate a clear and meaningful trend of decreasing contaminant mass and/or concentration over time at appropriate monitoring or sampling points. The second line of evidence originally included "hydrogeologic and geochemical data that can be used to indirectly demonstrate the type(s) of natural attenuation processes active at the site, and the rates at which such processes will reduce contaminant concentrations to required levels".

Answer NO if: The appropriate regulatory authority has specifically requested that only the first/primary line of evidence for natural attenuation is required for approval of MNA as a site remedy. The first direct line of evidence requires data that demonstrate a clear and meaningful trend of decreasing contaminant mass and/or concentration over time at appropriate monitoring or sampling points. Note that the final decision to require, or to not require, the second line of evidence is made by the appropriate regulatory authority. If the regulatory authority has not signaled what lines of evidence will be required, then a more conservative approach would be to answer "YES" to this question and proceed with collecting additional lines of evidence.

HELP

As part of EPA's MNA guidance, the agency has listed three lines of evidence that may be part of an MNA evaluation. Collecting data to support the first line of evidence is always required, and data to support the second line of evidence is typically required. The rest of this decision tool is focused on the second and third lines of evidence. It is recommended that the user go through these decision points even if the applicable data are not available.

The following resources provide more information on the lines of evidence approach, including definitions and how they are used (copy link into web browser):

https://www.epa.gov/sites/production/files/2014-02/documents/d9200.4-17.pdf

https://www.serdp-estcp.org/content/download/25789/262545/file/FAQ%20ER-201211.V2%20February%202014.pdf

24. Is 1,1-DCA biodegrading based on model predictions?

Decision Criteria

Answer YES if: Using 1,1-DCA degradation rate constants greater than zero in the model provide a better fit than the fit when the rate constant is set to zero. This can be evaluated using the same simulation in the "MNA Rate Constant Estimator" model that was used to "Does Long-Term Monitoring Data Provide the 1st Line of Evidence for MNA?" Prepare a new simulation where the rate constant for degradation of 1,1-DCA is set to zero. Compare the actual in situ concentrations of 1,1-DCA against the new simulation.

Then enter trial values for the rate constant for 1,1-DCA degradation into the simulation to determine if the model projections provide a better fit to the actual field-measured concentrations. A better fit is defined as having a lower value of RMSE (root mean square error) between the field data and the concentrations predicted by the model). If rate constants greater than zero provide a better fit, then 1,1-DCE is degrading. Note that this may have already been established as part of the earlier evaluation of the criterion "Does Long-Term Monitoring Data Provide the 1st Line of Evidence for MNA?" In addition, the model will also have to be calibrated to fit the field-measured concentrations of 1,1,1-TCA and 1,1-DCE.

Answer NO if: Setting the 1,1-DCA degradation rate constant to zero in the model provides a better fit than the fit when the rate constant is greater than zero. This is evaluated using the same model and simulations described above for the "YES" answer.

25. Are ^{13}C and/or ^{37}Cl in 1,1-DCA enriched along the flow path?

Decision Criteria

Answer YES if: A clear pattern of carbon and/or chlorine isotope fractionation can be observed in samples collected along a groundwater flow path. For 1,1-DCA, values of $\delta^{13}C$ and $\delta^{37}Cl$ can be obtained for individual samples via commercial lab analysis (see HELP for further explanation of CSIA principles and analytical considerations). If samples are taken from the source area and then at several locations downgradient, a 2-dimensional plot of these values can then be generated (see CSIA_11DCA_11DCE in **FILES**). If the values of both $\delta^{13}C$ and $\delta^{37}Cl$ generally increase along the groundwater flow path (i.e., become "less negative" due to depletion of the lighter isotope), then this is taken as evidence for degradation of 1,1-DCA.

Answer NO if: No isotope data are available or if there is no clear trend in samples collected along a groundwater flow path. Again, this is best visualized by creating a 2-D plot of the $\delta^{13}C$ and $\delta^{37}Cl$ values (see **HELP** and **FILES** for more guidance).

HELP

Additional lines of evidence for 1,1-DCA attenuation can be provided by site-specific analysis of samples for stable isotopes of carbon and chloride. Data from a single sample may not provide sufficient evidence for 1,1-DCA degradation. This is because 1,1-DCA is often a by-product of releases of other contaminants, and isotopic patterns may be difficult to distinguish if data are limited. Collecting multiple samples along the groundwater flow path is a more appropriate approach for establishing degradation because it relies on site-specific isotopic data to document 1,1-DCA degradation. Collecting isotopic data for parent compounds (e.g., 1,1,1-TCA) is also recommended to better establish trends across the site.

If values for $\delta^{13}C$ and $\delta^{37}Cl$ are available for 1,1-DCA, open the tab Files and select the spreadsheet CSIA_11DCA_11DCE.xlsx. Enter your data in the tab Input. The user can enter data for up to 8 wells. Data from a well located within the source area or in an upgradient area should be entered in the first row (Well 0); this provides a site-specific estimate of the isotopic composition of 1,1-DCA at the source and serves as a baseline for further comparisons. This well should have the lowest (most negative) values for

$\delta^{13}C$ and $\delta^{37}Cl$ (prioritize the well with the lowest $\delta^{37}Cl$). Data from other wells are entered in the remaining rows, following the order that they fall along the groundwater flow path.

Once the available data have been entered, the user can consult the tab 2-D Chart_simple. Your data should plot on the chart. If not, you may need to extend the scales of the x and/or y axes. Degradation of 1,1-DCA is indicated if the data points generally proceed up and/or to the right within the plot in the direction of groundwater flow. This occurs due to the preferential degradation of bonds that contain lighter isotopes, such that the lighter isotopes become depleted and the heavier isotopes become enriched within the remaining portion of the compound as it is transported downgradient. The degree of enrichment can vary depending on the compound, the isotope, and the transformation pathway.

The error bars represent uncertainty in the determination of $\delta^{13}C$ and $\delta^{37}Cl$. The values can be said to increase between two samples if one or both of the error bars do not overlap. Note that the user may also enter isotopic data for 1,1,1-TCA in the Input tab and plot it on the same chart as the 1,1-DCA data. If the $\delta^{13}C$ and $\delta^{37}Cl$ values for 1,1-DCA are less than the corresponding $\delta^{13}C$ and $\delta^{37}Cl$ values for 1,1,1-TCA at the source and near-source wells, then this is confirmatory evidence that the 1,1-DCA originated from 1,1,1-TCA degradation. This pattern occurs because the 1,1-DCA is formed from the preferential degradation of the lighter isotopes in 1,1,1-TCA, which is then reflected in the isotopic signature of the 1,1-DCA in these wells. If the $\delta^{13}C$ and $\delta^{37}Cl$ values for 1,1-DCA in the far downgradient wells exceed those of 1,1,1-TCA in the source wells, then this suggests that 1,1-DCA is degrading during groundwater transport.

26. Are geochemical conditions adequate for aerobic 1,1-DCA biodegradation?

Decision Criteria

Answer YES if: Dissolved oxygen is routinely present in groundwater samples from one or more wells in the plume or in the area downgradient of the plume. This is a qualitative line of evidence that conditions are favorable to support aerobic oxidation but does not imply that 1,1-DCA is actually being degraded. A threshold (minimum) DO value that would preclude aerobic biodegradation has not been established, and because of field sampling limitations, dissolved oxygen concentration data on well water are often unreliable. For the purposes of this decision tool, conditions are considered generally favorable for aerobic biodegradation of 1,1-DCE when one of the following criteria are met: Dissolved oxygen concentrations measured in the field are greater than 1 mg/L, ORP readings are > + 100 mV (against the AgCl reference electrode), ferrous iron (Fe^{2+}) concentrations are less than 0.5 mg/L, and methane concentrations are less than 0.005 mg/L. However, field parameter data should be evaluated with appropriate caution (see **HELP**).

Answer NO if: Dissolved oxygen is typically present at low levels (< 0.1 mg/L) across the entire site. This might include sites where the impacted intervals are deep, confined, and/or organic-rich. This type of determination would also rely on other corroborating geochemical data, such as highly negative ORP readings (< -100 mV against the AgCl reference electrode), elevated ferrous iron (Fe^{2+}) > 1 mg/L, or methane > 0.5 mg/L.

HELP

1,1-DCA can be naturally attenuated by reactions that occur in both anaerobic conditions (e.g., biological reductive dechlorination) and aerobic conditions. The former reaction can be evaluated as part of the evaluation of 1,1,1-TCA. Aerobic oxidation of 1,1-DCA can occur via direct metabolism (i.e., 1,1-DCA is used as a carbon and energy source by the microbes that perform the reaction) or via co-metabolism (i.e., 1,1-DCA is transformed fortuitously and does not support growth). In either case, the products of these reactions are not easily measurable, so documenting favorable geochemical conditions is an important secondary line of evidence.

In assessing whether geochemical conditions are favorable for anaerobic vs. aerobic processes, it should be noted that field methods for measuring dissolved oxygen may generate inconsistent and/or erroneous results. One contributing factor is the common use of long-screened (≥ 10 ft) monitoring wells that may be collecting water from multiple zones with different redox conditions. This mixing of groundwater can make it difficult to quantify zones with higher dissolved oxygen that may promote 1,1-DCA biodegradation. Consequently, field dissolved oxygen measurements should be used with caution and supported by other lines of evidence. Other data that would corroborate that geochemical conditions are favorable for aerobic biodegradation of 1,1-DCA include positive ORP readings and little or no dissolved iron and methane. Total organic carbon (TOC) may also be a positive indicator because it provides a carbon source and electron donor to promote biological cometabolic oxidation of 1,1-DCA; TOC > 20 mg/L may also serve as a positive line of evidence for the anerobic natural attenuation pathway. In addition, portions of the site where groundwater transitions between anaerobic and aerobic should be delineated to identify areas that might be best managed by different natural attenuation pathways.

27. Is chloroethane present?

Decision Criteria

Answer YES if: Chloroethane has been present above the reporting limit at any groundwater monitoring location at the site. This compound is a by-product of 1,1-DCA reductive dechlorination and therefore serves as a confirmatory line of evidence that this reaction is actively contributing to 1,1-DCA attenuation.

Answer NO if: Chloroethane has not been present at any groundwater monitoring location at the site, either currently or historically.

28. Does *Dhb* Density Explain the 1,1-DCA Rate Constant?

Decision Criteria

Answer YES if: The 1,1-DCA biodegradation rate constant used in the model is consistent with correlations based on the abundance of the Dhb biomarker (also referred to as DHBt) for strains of *Dehalobacter* bacteria that degrade 1,1-TCA. The correlations were derived from other studies and kinetic data. To do this, first refer to the simulation in the "MNA Rate Constant Estimator" model that was used to evaluate the criterion "Does Long-Term Monitoring Data Provide the 1st Line of Evidence for MNA?". Note that this model has the option to use biomarker data to estimate the biodegradation rate constant (i.e., it uses a

correlation to predict the representative rate constant based on the biomarker levels measured at the site). If this option was employed and it resulted in a reasonable fit to the actual data, then this confirms that "YES" is the appropriate answer. See **HELP** for additional guidance on determining if the fit was reasonable.

Answer NO if: No data on the abundance of the Dhb biomarker are available, or if 1,1-DCA biodegradation rate constant used to optimize the model is inconsistent with rate constants predicted using the biomarker correlations. To evaluate the latter condition, first refer to the model simulation that was used to evaluate the criterion "Does Long-Term Monitoring Data Provide the 1st Line of Evidence for MNA?". If the option to use the correlation was not employed OR did not result in an optimal fit, then "NO' is the appropriate answer.

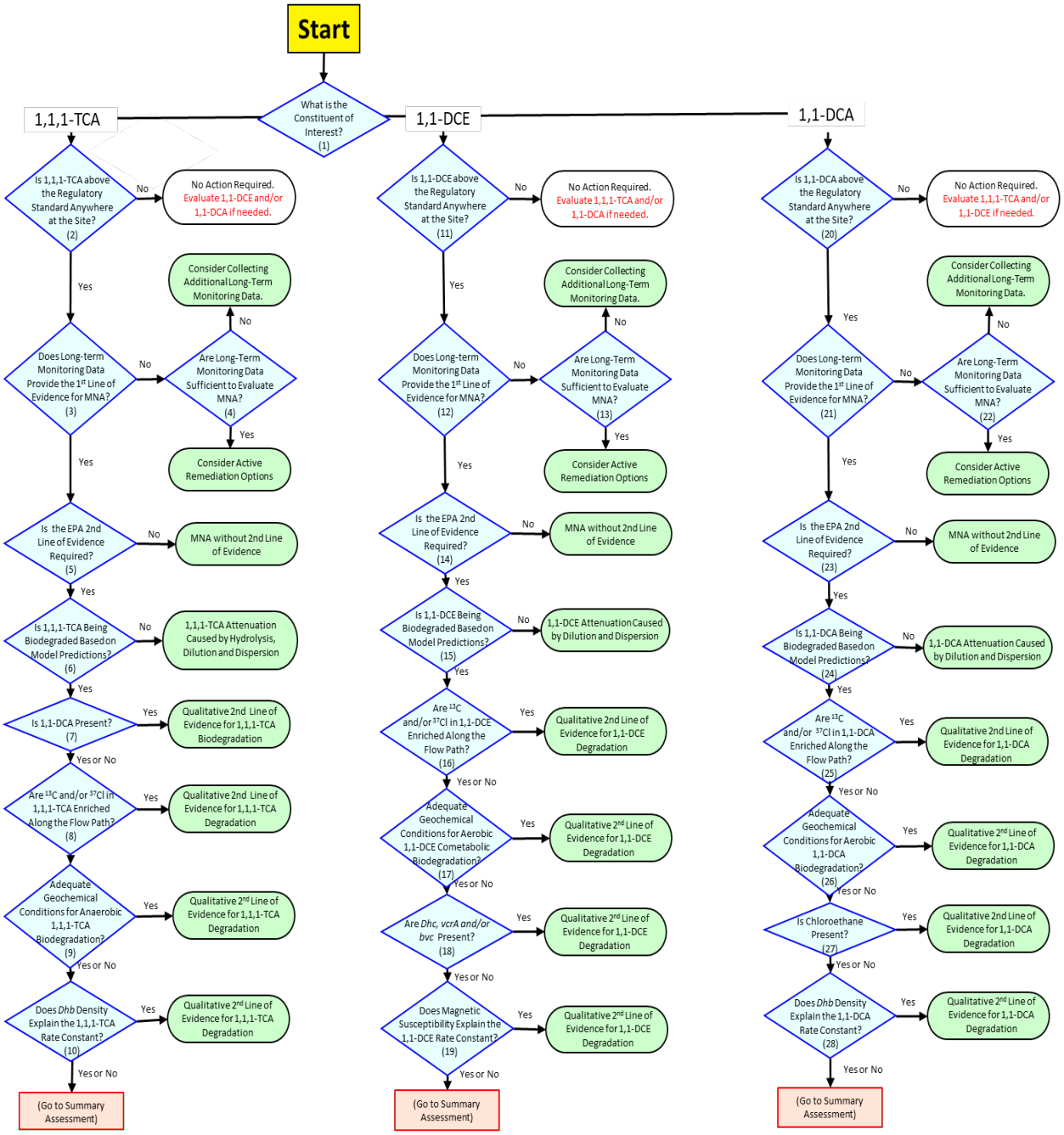
HELP

For 1,1-DCA, the "MNA Rate Constant Estimator" model (see **FILES**) has an option to estimate rate constants based on the abundance of Dhb (also referred to as DHBt), which is a qPCR-based biomarker for degradation of this compound (as well as 1,1,1-TCA). The correlations are designed to help calibrate the model, and they are intended as a starting point for improving the fit between the actual field data and the model simulations. Consequently, they should not be considered a true prediction of the actual degradation rate that is occurring at the site. This is because they are based on empirical data from other studies where conditions may be quite different than those observed at the site being evaluated.

To use the biomarker correlations as part of this decision tool, the following process is recommended:

1. In Box 6b, select the specific biomarker Dhb from the dropdown menu. On the chlorinated ethanes module of this model, this is one of only two biomarker options; only Dhb is applicable for 1,1-DCA. Selecting a biomarker from the menu will launch a pop-up box where biomarker abundance data can be entered.
2. In the pop-up box, enter the abundance of the selected biomarker for those wells where data are available. The model will perform a spatial interpolation to estimate a representative biomarker abundance for the site (i.e., a single value that is weighted based on the distance between wells with biomarker data).
3. The rate constant associated with this biomarker abundance will then be automatically entered in the appropriate location in Box 6b.
4. Enter the rate constant from Box 6b into Box 6. This is the rate constant that is used for the model simulation (i.e., to generate a simulated concentration vs. distance curve).
5. The user should then evaluate the fit between the actual field data and the model simulation that is based on this estimated rate constant. Manually adjust the rate constant in Box 6 until an optimal fit between the actual field data and the model simulation is obtained. Use the Root Mean Square Error (RMSE) that is overlaid on the plot as a guide; lower RMSE values generally indicate a better fit. Record the rate constant that provided the optimal fit.
6. Compare the recorded rate constant from the biomarker correlation with the "optimal" rate constant from Box 6. If the optimal rate constant from Box 6 is within a factor of 3 to 5 of the rate constant that was generated from the biomarker correlation, then this is considered reasonable evidence that these biodegradation processes are contributing to the actual field trend in 1,1-DCA concentrations.

The derivation of these correlations is described in Appendix D of the project report. They are based on an assumption that anaerobic reductive dechlorination of 1,1-DCA follows Michaelis-Menten (Haldane) kinetics. The rate equation for Michaelis-Menten kinetics can be rearranged to solve for a first-order rate constant that is a function of other kinetic parameters (specifically Km and Vmax expressed in terms of gene copies), the biomarker abundance (expressed in gene copies per mL) and the concentration of the organic chemical being degraded (in this case, 1,1-DCA). Derived values for the kinetic parameters for each biomarker are also detailed in Appendix D of the project report.



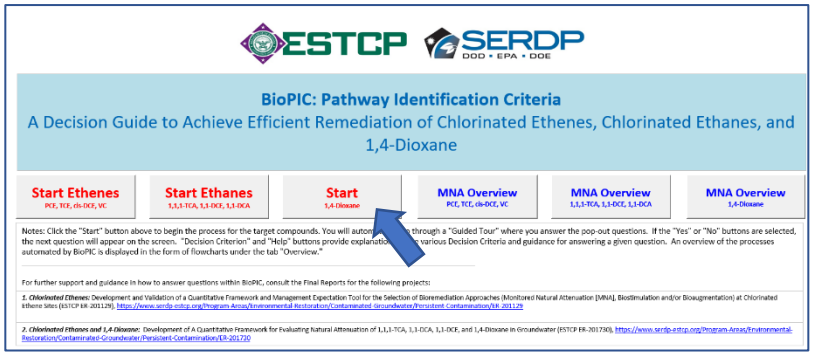
Decision Framework for Chlorinated Ethanes

1,1-DCE is a chlorinated ethene but is included in the decision framework for chlorinated ethanes because it is a key degradation product of the parent compound 1,1,1-TCA. This flowchart was coded into the updated BIOPIC tool.

Detailed BioPIC User Guide:

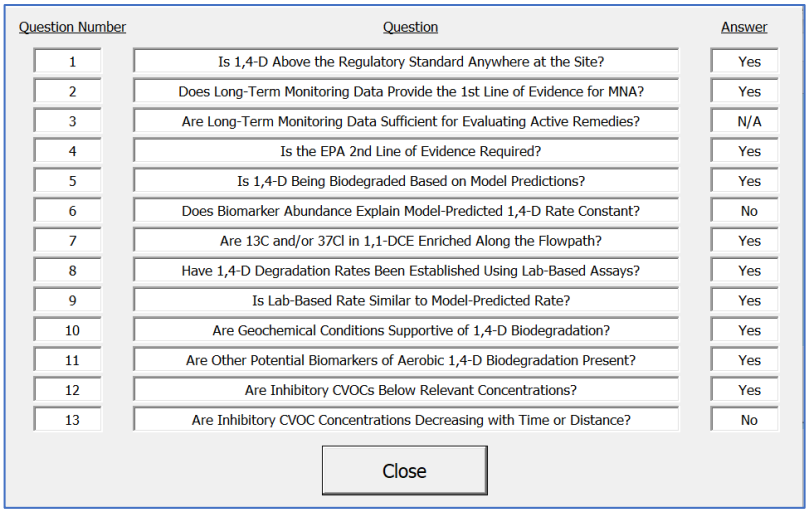
1,4-Dioxane

The following describes the Decision Framework for the 1,4-Dioxane (1,4-D) module.



BioPIC Home Page showing start button for entering the chlorinated ethane decision framework

Each of the numbered questions below corresponds to a number in the flowchart/guided tour. After each number, the decision criteria are explained. For most, further information is provided in the Help text. Note that these text descriptions are shown as pop-up boxes within the tool.



Question Number	Question	Answer
1	Is 1,4-D Above the Regulatory Standard Anywhere at the Site?	Yes
2	Does Long-Term Monitoring Data Provide the 1st Line of Evidence for MNA?	Yes
3	Are Long-Term Monitoring Data Sufficient for Evaluating Active Remedies?	N/A
4	Is the EPA 2nd Line of Evidence Required?	Yes
5	Is 1,4-D Being Biodegraded Based on Model Predictions?	Yes
6	Does Biomarker Abundance Explain Model-Predicted 1,4-D Rate Constant?	No
7	Are 13C and/or 37Cl in 1,1-DCE Enriched Along the Flowpath?	Yes
8	Have 1,4-D Degradation Rates Been Established Using Lab-Based Assays?	Yes
9	Is Lab-Based Rate Similar to Model-Predicted Rate?	Yes
10	Are Geochemical Conditions Supportive of 1,4-D Biodegradation?	Yes
11	Are Other Potential Biomarkers of Aerobic 1,4-D Biodegradation Present?	Yes
12	Are Inhibitory CVOCs Below Relevant Concentrations?	Yes
13	Are Inhibitory CVOC Concentrations Decreasing with Time or Distance?	No

Close

Example of Summary Assessment pop-up box after completing the stepwise decision framework for 1,4-dioxane

As with the other compounds, a summary assessment that shows the all of the results for 1,4-dioxane will be displayed once the user has gone through the entire decision logic (i.e., evaluated all of the possible lines of evidence). A graphic showing the entire decision flowchart for 1,4-dioxane is reproduced at the end of this section. Note that once the user starts answering the questions, the summary assessment can also be pulled up by clicking the View Summary box that appears to the right of each question. In these cases, it will display answers to only those questions that have been completed.

1. Is 1,4-D above the regulatory standard anywhere at the site?

Decision Criteria:

Answer YES if: The 1,4-D concentration at any groundwater monitoring location at the site is above the applicable concentration-based regulatory standard. Note that this standard is site-specific. If no standard has been established for your site, then select a value based on guidance from EPA or other states (see **HELP**) for planning purposes.

Answer NO if: The 1,4-D concentration at each groundwater monitoring location at the site is already below the concentration-based regulatory standard. The decision tool is intended for sites where the concentration of 1,4-D must fall below a site-specific regulatory standard before contaminated groundwater reaches the point of compliance (POC). If the 1,4-D concentration is already below the regulatory standard across the site (including the POC), then it is highly likely that it will remain below this standard in the future. This assumes that the site has been reasonably well characterized (especially the source area) and that no significant changes in conditions at the site are anticipated.

HELP

In the absence of site-specific regulatory standards for 1,4-dioxane, the user may wish to select one of the following values to proceed with the BioPIC evaluation. Note that there is considerable variability in state-level groundwater and drinking water standards for 1,4-dioxane. The information below was compiled on 1 January 2021, so it should also be understood that states are likely to promulgate and/or revised standards over time.

- USEPA Reference Concentration for Drinking Water (10-6 risk) = 0.35 µg/L
- USEPA Reference Concentration for Drinking Water (10-4 risk) = 35 µg/L
- California Notification Level in Drinking Water = 1 µg/L
- Massachusetts Groundwater Standard = 0.3 µg/L
- Colorado Groundwater Standard = 0.35 µg/L
- Florida Groundwater Standard = 3.2 µg/L
- Illinois Groundwater Standard = 7.7 µg/L
- Missouri Groundwater Standard = 61 µg/L
- New Hampshire Groundwater Standard = 0.32 µg/L
- New Jersey Groundwater Standard = 0.4 µg/L
- North Carolina Groundwater Standard = 3 µg/L
- Texas Groundwater Standard = 9.1 µg/L
- Wisconsin Groundwater Preventive Action Limit = 3 µg/L

If 1,4-dioxane is already below the established or assumed standard, then a future exceedance would likely be associated with one or more of the following: 1) the site is poorly characterized; 2) a new release of 1,4-dioxane occurs; 3) active remediation is on-going or recently completed, such that steady state conditions have not yet been reached; or 4) any other change in site conditions has occurred that enhance 1,4-dioxane mass transfer to the aquifer and inhibit 1,4-dioxane attenuation.

2. Does Long-Term Monitoring Data Provide the 1st Line of Evidence for MNA?

Decision Criteria

Answer YES if: The 1,4-D concentration is currently below the regulatory standard at the point of compliance (POC) and is predicted to be below the concentration-based regulatory standard at the POC at any time in the future. Use the model provided as part of this tool (see MNA Rate Constant Estimator in **FILES**) to predict if the concentration will be below the standard at any time in the future (see **HELP** for additional explanation).

Answer NO if: At any time in the future, the 1,4-D concentration will exceed the regulatory standard at the POC. Use the model provided as part of this tool to predict the concentration in the future at the POC. Note there usually is also a temporal component in the regulatory goals, which involves establishing how long it will take the concentration at a particular location to achieve a regulatory goal. The implementation of more aggressive remedies may reduce time to achieve remediation goals, thereby reducing the overall cost. However, this tool primary deals with the spatial aspects of remediation goals (i.e., will the goal be achieved at a POC) rather than the temporal components.

HELP

If sufficient historical contaminant concentration data are not available to determine if the 1,4-D plume will reach a POC, then a groundwater flow and solute transport model, such as the "MNA Rate Constant Estimator" provided as part of this tool (see MNA Rate Constant Estimator in **FILES**), should be used to predict solute plume behavior. In this case, the simulation should account for the effects of advective groundwater flow, dispersion of the relevant solutes, sorption, and degradation of 1,4-D in groundwater at the site.

For more information on using this type of model for 1,4-D, consult the project report (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 (ESTCP ER-201730)), which can be found on the project page (copy link into web browser): https://serdp-estcp.org/Program-Areas/Environmental-Restoration/Contaminated-Groundwater/Persistent-Contamination/ER-201730/ER-201730. The model is also explained in the User's Guide for BioPIC.

A similar approach for chlorinated ethenes can be found in another project report (Development of a Quantitative Framework and Management Expectation Tool for the Selection of Bioremediation Approaches at Chlorinated Ethene Sites (ESTCP Project ER-201129)), which can be downloaded from the project page (copy link into web browser): https://www.serdp-estcp.org/Program-Areas/Environmental-Restoration/Contaminated-Groundwater/Persistent-Contamination/ER-201129/ER-201129. In the ER-201129 report, Section 5.2.3 illustrates the process of calibrating a groundwater flow and transport model (in this case, the "BIOCHLOR" model), and Section 5.2.4 Step 1 illustrates the use of a model to apply the decision criteria.

If available, a robust historical database of contaminant concentrations can be used as an alternative to a computer model. Spatial and temporal trends in solute concentrations can be utilized to determine if the plume is stable or receding and therefore will not reach the POC. When sufficient data are available, using empirical data to ascertain trends is much better than using a model. In many cases, sufficient 1,4-D concentration data are available to evaluate plume behavior and to determine if solute concentrations will exceed cleanup goals at a regulatory POC.

3. Is Long-Term Monitoring Data Sufficient to Evaluate MNA?

Decision Criteria

Answer YES if: The current long-term monitoring data confirm that one or more of the following conditions are met:

1. The plume is currently beyond the point of compliance at a concentration that is above the applicable standard.
2. The plume is still expanding and is predicted to extend beyond the point of compliance in the future based on modeling.
3. Concentration-based goals have been established within the plume (i.e., upgradient of the point of compliance), and model predictions suggest that these will not be achieved with a reasonable timeframe.

Answer NO if: The current long-term monitoring data confirm that ALL of the following conditions are met:

1. The plume is currently not beyond the point of compliance.
2. The current dataset is too limited to evaluate if plume is expanding vs. receding.
3. The current dataset is too limited to predict using a model whether the plume will expand or whether concentration-based goals will be achieved.
4. There is no current regulatory requirement for active remediation.

HELP

Long-term monitoring data are an important component of site management, and they are particularly important for demonstrating the site-specific viability of MNA.

In the case of 1,4-D, it is possible that a site's existing dataset for this compound may be limited if it is a recent additional to the monitoring program. It may not be possible to establish a clear trend in the attenuation in concentration of 1,4-D along the flow path in groundwater. This means that the data are inadequate to permit a thorough evaluation for the primary line of evidence for MNA. This is typically because one or more of the following apply: 1) data are highly variable across the site; 2) data are highly variable from event-to-event; or 3) data are available from a relatively limited number of monitoring points or events. In each case, these data limitations make it difficult to establish trends with any degree of statistical certainty.

In that case, collecting additional data may be beneficial to provide more certainty that current trends are statistically significant and sustainable. These additional monitoring events may also include data that would be used to support the second line of evidence for MNA (e.g., geochemical data, biomarkers, stable isotopes) or even hydrogeologic parameters to improve fate and transport model predictions.

At sites where the number of monitoring locations is relatively small (e.g., 4 or less wells), this type of analysis will likely benefit by including additional monitoring locations along the plume transect. In part, this is because it can be more challenging to establish that a trend is significant using standard approaches (e.g., that the slope of a best-fit regression line is different than zero). As a result, adding more monitoring locations along the plume transect may provide value.

It is necessary to use modeling software to evaluate if current trends in natural attenuation will meet the standard at the point of compliance (POC). An example is the "MNA Rate Constant Estimator" that has been developed as part of this decision tool (see MNA Rate Constant Estimator in **FILES**). This type of model allows the user to predict concentrations along a plume transect. However, it relies on the user to calibrate to the model predictions based on field data, and it also assumes that those field data are representative. At sites, where data vary considerably from event to event, this type of calibration can be

challenging. In any case, the goal is to demonstrate that the plume footprint is stable and/or shrinking and will not result in concentrations at a downgradient POC that exceed an acceptable level. This may require additional monitoring locations (particularly if the plume extent is not yet delineated) or additional monitoring events to demonstrate longer-term stability. Data from additional events are also important to establish that trends are sustainable even if minor (or major) changes in site conditions (e.g., groundwater flow directions, redox conditions) occur that might impact attenuation and/or plume stability.

In some cases where MNA is proposed as a remedy, an estimate of the remediation timeframe is also required by the regulatory agency. The remediation timeframe is typically the date when some or all wells are projected to achieve a concentration goal. Estimating the remediation timeframe is often done using linear regression of concentration vs. time data at a specific well (or wells), but this requires data from enough events to ensure that the result is statistically significant. Additional monitoring events may be required to achieve this objective at sites with limited concentration vs. time data.

For the case where the dataset is already robust and confirms that MNA is unlikely to be effective, active remediation is likely to be the next course of action. Active remediation should be selected and implemented based on appropriate, well-defined objectives, but one primary goal should be to reduce concentration in the source area enough so that concentrations are below the site-specific standard at the point of compliance. The required reduction in the source concentration can be estimated using models, including the one provided as part of this tool (see MNA Rate Constant Estimator in **FILES**). Another good option is REMChlor-MD, which allows the user to input the extent of source reduction and then compare the plume behavior for cases with and without remediation. REMChlor-MD also incorporates the effects of matrix diffusion (i.e., contaminants diffusing into and out of lower-permeability intervals within the saturated zone) which has implications for contaminant transport and persistence at many sites.

Additional resources for understanding and developing monitoring objectives for MNA include the following (copy link into web browser):

https://clu-in.org/download/contaminantfocus/dnapl/Treatment_Technologies/performance_monitoring_mns600R04027.pdf

https://www.serdp-estcp.org/content/download/25789/262545/file/FAQ%20ER-201211.V2%20February%202014.pdf

https://clu-in.org/download/techfocus/na/NA-approach-for-eval-2011.pdf

https://www.epa.gov/sites/production/files/2014-02/documents/d9200.4-17.pdf

https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=10004674.TXT

https://pubs.usgs.gov/wri/wri034057/

4. Is the EPA Second Line of Evidence Required?

Decision Criteria

Answer YES if: The appropriate regulatory authority has requested that multiple lines of evidence for 1,4-D attenuation should be collected before approval of MNA as a site remedy will be granted. The first direct line of evidence requires data that demonstrate a clear and meaningful trend of decreasing contaminant mass and/or concentration over time at appropriate monitoring or sampling points. The second line of evidence originally included "hydrogeologic and geochemical data that can be used to indirectly demonstrate the type(s) of natural attenuation processes active at the site, and the rates at which such processes will reduce contaminant concentrations to required levels".

Answer NO if: The appropriate regulatory authority has specifically requested that only the first/primary line of evidence for natural attenuation is required for approval of MNA as a site remedy. The first direct line of evidence requires data that demonstrate a clear and meaningful trend of decreasing contaminant mass and/or concentration over time at appropriate monitoring or sampling points. Note that the final decision to require, or to not require, the second line of evidence is made by the appropriate regulatory authority. If the regulatory authority has not signaled what lines of evidence will be required, then a more conservative approach would be to answer "YES" to this question and proceed with collecting additional lines of evidence.

HELP

As part of EPA's MNA guidance, the agency has listed three lines of evidence that may part of an MNA evaluation. Collecting data to support the first line of evidence is always required, and data to support the second line of evidence is typically required. The rest of this decision tool is focused on the second and third lines of evidence. It is recommended that the user go through these decision points even if the applicable data are not available.

The following resources provide more information on the lines of evidence approach, including definitions and how they are used (copy link into web browser):

https://www.epa.gov/sites/production/files/2014-02/documents/d9200.4-17.pdf

https://www.serdp-estcp.org/content/download/25789/262545/file/FAQ%20ER-201211.V2%20February%202014.pdf

5. Is 1,4-D biodegrading based on model predictions?

Decision Criteria

Answer YES if: Using 1,4-D biodegradation rate constants greater than zero in the model provide a better fit than the fit when the rate constant is set to zero. This can be evaluated using the same simulation in the "MNA Rate Constant Estimator" model that was used to "Does Long-Term Monitoring Data Provide the 1st Line of Evidence for MNA?" Prepare a new simulation where the rate constant for degradation of 1,4-D is set to zero. Compare the actual field-measured concentrations of 1,4-D against the new simulation. Then enter trial values for the rate constant for 1,4-D degradation into the simulation to determine if the model projections provide a better fit to the actual field-measured concentrations. A better fit is defined as having a lower value of RMSE (root mean square error between the field data and the concentrations predicted by the model). If rate constants greater than zero provide a better fit, then

1,4-D is degrading. Note that this may have already been established as part of the earlier evaluation of the criterion "Does Long-Term Monitoring Data Provide the 1st Line of Evidence for MNA?"

Answer NO if: Setting the 1,4-D biodegradation rate constant to zero in the model provides a better fit than the fit when the rate constant is greater than zero. This is evaluated using the same model and simulations described above for the "YES" answer.

6. Does Biomarker Abundance explain model-predicted 1,4-D rate constant?

Decision Criteria

Answer YES if: The 1,4-D biodegradation rate constant used in the model is consistent with biomarker correlations that were derived from other studies and kinetic data. To do this, first refer to the simulation in the "MNA Rate Constant Estimator" model that was used to evaluate the criterion "Does Long-Term Monitoring Data Provide the 1st Line of Evidence for MNA?". Note that this model has the option to use biomarker data to estimate the biodegradation rate constant (i.e., it uses a correlation to predict the representative rate constant based on the biomarker levels measured at the site). If this option was employed and it resulted in a reasonable fit to the actual data, then this confirms that "YES" is the appropriate answer. See **HELP** for additional guidance on determining if the fit was reasonable.

Answer NO if: No data on the abundance of DXMO (also known as THFMO) or other qPCR biomarkers are available, or if 1,4-D biodegradation rate constant used to optimize the model is inconsistent with rate constants predicted using the biomarker correlations. To evaluate the latter condition, first refer to the model simulation that was used to evaluate the criterion "Does Long-Term Monitoring Data Provide the 1st Line of Evidence for MNA?". If the option to use the correlation was not employed OR did not result in an optimal fit, then "NO' is the appropriate answer.

HELP

The "MNA Rate Constant Estimator" model (see MNA Rate Constant Estimator in **FILES**) has an option to estimate rate constants based on the abundance of several different qPCR-based biomarkers for degradation. These correlations are designed to help calibrate the model, and they are intended as a starting point for improving the fit between the actual field data and the model simulations. Consequently, they should not be considered a true prediction of the actual degradation rate that is occurring at the site. This is because they are based on empirical data from other studies where conditions may be quite different than those observed at the site being evaluated.

To use the biomarker correlations as part of this decision tool, the following process is recommended:

1. In Box 6b, select a specific biomarker from the dropdown menu. For 1,4-dioxane, there is an option to enter the following biomarkers: DXMO, prmA, RDEG, and RMO. The DXMO biomarker (also known as THFMO) is associated with organisms that can grow by degrading 1,4-dioxane. The prmA biomarker (also known by the enzyme name PrMO or PPO) is associated with organisms that cometabolize 1,4-dioxane while growing on propane. The RDEG and RMO biomarkers are associated with organism that cometabolize 1,4-dioxane while growing on toluene, or native organic matter. Only 1 biomarker can be entered at a time; start with DXMO if available. Selecting

a biomarker from the menu will launch a pop-up box where biomarker abundance data can be entered.

2. In the pop-up box, enter the abundance of the selected biomarker for those wells where data are available. The model will perform a spatial interpolation to estimate a representative biomarker abundance for the site (i.e., a single value that is weighted based on the distance between wells with biomarker data).
3. The rate constant associated with this biomarker abundance will then be automatically entered in the appropriate location in Box 6b.
4. Enter the rate constant from Box 6b into Box 6. This is the rate constant that is used for the model simulation (i.e., to generate a simulated concentration vs. distance curve).
5. The user should then evaluate the fit between the actual field data and the model simulation that is based on this estimated rate constant.
6. Manually adjust the rate constant in Box 6 until an optimal fit between the actual field data and the model simulation is obtained. Use the Root Mean Square Error (RMSE) that is overlaid on the plot as a guide; lower RMSE values generally indicate a better fit. Record the rate constant that provided the optimal fit.
7. Return to Box 6b and repeat Steps 1 - 3 for all remaining biomarkers. In each case, record the rate constant that is generated in Box 6b (i.e., after the biomarker data are entered in the pop-up box).
8. Compare the recorded rate constants from the biomarker correlations with the "optimal" rate constant from Box 6. If the optimal rate constant from Box 6 is within a factor of 3 to 5 of one or more of the rate constants that were generated from the biomarker correlations, then this is considered reasonable evidence that this particular biodegradation process is contributing to the actual field trend in 1,4-dioxane concentrations.
9. The biomarkers target different genes in different organisms. Ideally, all the organisms could be present in the groundwater at the same time, and act on 1,4-dioxane concomitantly. Add all the rate constants associated with DXMO, prmA, RDEG, and RMO together, and if the optimal rate constant from Box 6 is within a factor of 3 to 5 of the sum of the rate constants that were generated from the biomarker correlations, then this is considered reasonable evidence that biodegradation processes are contributing to the actual field trend in 1,4-dioxane concentrations.

The derivation of these correlations is described in Appendix D of the project report. They are based on an assumption that aerobic biodegradation of 1,4-dioxane follows Michaelis-Menten (Haldane) kinetics (Mahendra and Alvarez-Cohen, 2006; Mahendra et al., 2013; Ye et al. 2017; Grostern et al., 2009; Parthasarathy et al., 2015). The rate equation for Michaelis-Menten kinetics can be rearranged to solve for a first-order rate constant that is a function of other kinetic parameters (specifically Km and Vmax expressed in terms of gene copies), the biomarker abundance (expressed in gene copies per mL) and the concentration of the organic chemical being degraded (in this case, 1,4-dioxane). Derived values for the kinetic parameters for each biomarker are also detailed in Appendix D of the project report.

Additional information on 1,4-dioxane biomarkers is also provided in Question #11.

7. Are ^{13}C and/or ^{2}H in 1,4-dioxane enriched along the flow path?

Decision Criteria

Answer YES if: A clear pattern of carbon and hydrogen isotope fractionation can be observed in samples collected along a groundwater flow path. For 1,4-D, values of $\delta^{13}C$ and $\delta^{2}H$ can be obtained for individual samples via commercial lab analysis (see **HELP** for further explanation of CSIA principles and analytical considerations). If samples are taken from the source area and then at several locations downgradient, a 2-dimensional plot of these values can then be generated (see CSIA_14D in **FILES**). If the values of both $\delta^{13}C$ and $\delta^{2}H$ (particularly the latter) generally increase along the groundwater flow path (i.e., become "less negative" due to depletion of the lighter isotope), then this is taken as evidence for degradation of 1,4-D.

Answer NO if: No isotope data are available or if there is no clear trend in samples collected along a groundwater flow path. Again, this is best visualized by creating a 2-D plot of the $\delta^{13}C$ and $\delta^{2}H$ values (see **HELP** and CSIA_14D in **FILES** for more guidance).

HELP

Additional lines of evidence for 1,4-dioxane attenuation can be provided by site-specific analysis of samples for stable isotopes of carbon and hydrogen. Data from a single sample is unlikely to provide evidence for 1,4-D biodegradation. This is because there is significantly variability in the known isotopic composition of undegraded 1,4-dioxane sources, as well as data that suggests that these known source compositions do not represent the full range that might be encountered at contaminated sites. As a result, any attempt to establish biodegradation by comparing the isotopic composition of a groundwater sample to known source compositions (similar to the CSIA approach described for chlorinated ethenes) is subject to considerable uncertainty for 1,4-D and unlikely to serve as a convincing line of evidence at this time. Collecting multiple samples along the groundwater flow path is a more appropriate approach because it relies on site-specific isotopic data to document 1,4-D degradation.

If values for $\delta^{13}C$ and $\delta^{2}H$ are available for 1,4-D, open the tab **FILES** and select the spreadsheet CSIA_14D.xlsx. Enter your data in the tab Input. The user can enter data for up to 8 wells. Data from a well located within the source area or in an upgradient area should be entered in the first row (Well 0); this provides a site-specific estimate of the isotopic composition of 1,4-dioxane at the source and serves as a baseline for further comparisons. This well should have the lowest (most negative) values for $\delta^{13}C$ and δH (prioritize the well with the lowest $\delta^{2}H$). Data from other wells are entered in the remaining rows, following the order that they fall along the groundwater flow path.

Once the available data have been entered, the user can first consult the tab 2-D Chart_simple. Your data should plot on the chart. If not, you may need to extend the scales of the x and/or y axes. Degradation of 1,4-dioxane is indicated if the data points generally proceed up and/or to the right within the plot in the direction of groundwater flow. This occurs due to the preferential degradation of bonds that contain lighter isotopes, such that the lighter isotopes become depleted and the heavier isotopes become enriched within the remaining portion of the compound as it is transported downgradient. The degree of enrichment can vary depending on the compound, the isotope, and the transformation pathway.

The error bars represent uncertainty in the determination of $\delta^{13}C$ and $\delta^{2}H$. The values can be said to increase between two samples if either one or both of the vertical or horizontal error bars do not overlap.

For 1,4-dioxane, the user can also roughly estimate the amount of 1,4-dioxane that has been degraded based on different possible degradation pathways (see Step 2 in the Input tab). This relies on published isotopic enrichment factors (Ɛ) for carbon and hydrogen for three different biological transformation pathways: (1) co-metabolic oxidation by Rhodococcus rhodochrous strain 21198 grown on propane; (2) co-metabolic oxidation by Rhodococcus rhodochrous strain 21198 grown on isobutane; and (3) co-metabolic oxidation by Pseudonocardia tetrahydrofurans strain K1 grown on tetrahydrofuran (THF).

For each of the three possible pathways listed above, the percent of 1,4-dioxane degraded is presented as a range based on the uncertainty in the isotopic enrichment factors, as well as a user-input uncertainty factor. The latter can be used to perform a limited sensitivity analysis on the degradation estimates.

To better understand how the data compare to the expected isotopic fractionation patterns for each pathway, the user can consult the tab 2-D delta from upgradient. In this chart, the origin is the isotopic composition of the upgradient/source well (Well 0), and the rest of the site-specific data are plotted as symbols. The three solid lines represent the fractionation pattern associated each of the three pathways described above as degradation proceeds. The slopes of these lines reflect changes to both elements (carbon and hydrogen) and are minimally influenced by retardation and other non-destructive processes that may occur during groundwater transport. If the data adhere to a specific pathway line, then this is plausible evidence that this specific pathway may be contributing to the observed fractionation. It should be understood that alternate or multiple transformation pathways may be occurring and cause data to not adhere to any of the plotted lines.

8. Have 1,4-D degradation rates been established using lab-based assays?

Decision Criteria

Answer YES if: A statistically significant 1,4-D degradation rate constant has been established using concentration vs. time data generated from a lab-based test of site material. This can include standard microcosms constructed with site groundwater (and possibly soil) or more advanced techniques such as an assay based on adding radiolabeled ^{14}C-1,4-D (see **HELP**) to site groundwater. In each case, samples are collected from bottles at periodic intervals to monitor 1,4-D disappearance (and in the case of the ^{14}C assay, product accumulation) over time. An abiotic control must also be included to accurately quantify the rate associated with biological activity. The 1,4-D rate constant is then calculated from the concentration vs. time dataset under the assumption that degradation follows a first-order relationship. For MNA studies, this type of testing is traditionally considered a third (or tertiary) line of evidence. However, it also provides rate information that is consistent with the second line of evidence.

Answer NO if: No lab-based tests have been performed, or if the results of lab-based tests are negative. The latter is true if rate constants are not statistically significant (i.e., not greater than zero or if they are not different than controls). If lab-based tests are used to obtain lines of evidence for 1,4-D biodegradation, it is recommended that samples be collected from multiple locations at the site and tested individual. This reduces the possibility of “false negative” results.

HELP

The predominant product formed from biodegradation of 1,4-D is carbon dioxide (CO_2). Because many other processes result in formation of CO_2, it is not possible to document in situ biodegradation of 1,4-D based on product accumulation. It is possible, however, to document this process in the laboratory using ^{14}C-1,4-D in microcosms. Using ^{14}C material makes it possible to identify $^{14}CO_2$ as a product from ^{14}C-1,4-D. It is also possible to identify other biodegradation products that may be released. Furthermore, by measuring the rate at which ^{14}C-labeled products accumulate, it is possible to determine a pseudo-first order biodegradation rate constant. The rate at which 1,4-D biodegrades can also be determined in microcosms without using ^{14}C-1,4-D. However, this often requires at least several months of incubation, in order to detect an adequate level of decrease in 1,4-D. The ^{14}C assay is typically complete within six weeks, and it is sensitive enough to detect rate constants as low as 0.0069 yr^{-1}, equivalent to a half-life of 100 years.

The assay beings by collecting groundwater samples and shipping them overnight on ice to a laboratory that is equipped to use ^{14}C-labeled compounds. In the lab, a purified stock solution of ^{14}C-1,4-D is added to the microcosms and measurements are made at time zero for the amount of ^{14}C initially present, along with GC analysis of total 1,4-D and headspace analysis of VOCs. At weekly intervals, samples of groundwater are removed from the microcosms to determine the amount of ^{14}C-labeled products formed. When the incubation period is complete, the product data is evaluated using a mass balance model to estimate the pseudo first order rate constant. Data from a filter-sterilized control is also evaluated and if the rate of accumulation from the control is statistically significant, a net rate constant is calculated and evaluated for statistical significance. The procedures are very similar to those outlined in Mills IV et al. (Quantification of TCE co-oxidation in groundwater using a ^{14}C–Assay. Groundwater Monitoring & Rem. 2018, 38, 57-67).

In a study performed for ESTCP, groundwater samples were collected from 10 sites and a total of 49 wells. Of these, statistically significant first order rate constants were measured for 1,4-D in groundwater from 15 of the wells based on the ^{14}C assay. It should be noted that most of the half-lives determined were in excess of 50 years. That may be a consequence of the assay being performed with groundwater alone (i.e., no soil present), which may present limitations in terms of the amount of biomass and nutrients available. For this reason, a statistically significant result in the ^{14}C assay may be viewed as justification for performing additional laboratory studies with soil present, to further refine the estimate of a biodegradation rate constant.

9. Is lab-based degradation rate for 1,4-D similar to the model-predicted rate?

Decision Criteria

Answer YES if: If the 1,4-D biodegradation rate constant from one or more locations in the lab-based tests is greater than the biodegradation rate predicted from modeling. To do this, first refer to the model simulation that was used to evaluate the criterion “Does Natural Attenuation Current Meet the Goal?”. The model estimates the biodegradation rate that would result in the actual 1,4-D concentration vs. distance pattern observed at the site being evaluated. The lab-based tests establish a biodegradation rate under controlled conditions and are unequivocal evidence that 1,4-biodegredation can occur.

Consequently, lab-based rates greater than the model-predicted rates are seen as strong quantitative evidence of biodegradation potential, and in some cases, may be used to refine the rate constants estimated by the model. Additional lines of evidence beyond these lab-based assays should be evaluated as needed.

Answer NO if: If the 1,4-D biodegradation rate constant from all locations in the lab-based tests are less than the biodegradation rate predicted from modeling. This is evaluated using the same model and simulation described above for the "YES" answer. For cases where the lab-based tests yield a very slow 1,4-D degradation rate (e.g., half-lives greater than 100 years, degradation rates that are more than an order of magnitude smaller than the model-predicted rate), the user should consider performing supplemental lab-based tests to confirm if nutrient limitations and other factors may have suppressed the 1,4-D biodegradation rate. Additional lines of evidence beyond these lab-based assays should be evaluated as needed.

10. Are geochemical conditions supportive of 1,4-D biodegradation?

Decision Criteria

Answer YES if: Dissolved oxygen is routinely present in groundwater samples from one or more wells in the 1,4-D plume or in the area downgradient of the 1,4-D plume. This is a qualitative line of evidence and does not imply that 1,4-D is actually degrading. Dissolved oxygen is needed to support aerobic 1,4-D biodegradation, although a threshold (minimum) value to support in situ biodegradation has not been established. Lab studies have shown that degradation rates decrease below 2 mg/L, but 1,4-D biodegradation has been observed in wells with lower field measurements of dissolved oxygen. For the purposes of this decision tool, conditions are considered generally favorable for aerobic biodegradation of 1,4-dioxane when one of the following criteria are met: Dissolved oxygen concentrations measured in the field are greater than 0.1 mg/L, ferrous iron (Fe^{2+}) concentrations are less than 0.5 mg/L, and methane concentrations are less than 0.005 mg/L. However, field parameter data should be evaluated with caution (see **HELP**).

Answer NO if: Dissolved oxygen is typically present at low levels (<< 1 mg/L) across the entire site. This might include sites where the impacted intervals are deep, confined, and/or organic-rich. This type of determination would also rely on other corroborating geochemical data, such as highly negative ORP readings, elevated dissolved iron, and methane.

HELP

1,4-dioxane can be naturally attenuated by reactions that occur in primarily aerobic conditions. No naturally occurring abiotic or anaerobic degradation reactions have been established. In assessing whether geochemical conditions are favorable for anaerobic vs. aerobic processes, it should be noted that field methods for measuring dissolved oxygen may generate inconsistent and/or erroneous results. One contributing factor is the common use of long-screened (≥ 10 ft) monitoring wells that may be collecting water from multiple zones with different redox conditions. This mixing of groundwater can make it difficult to quantify zones with higher dissolved oxygen that may promote 1,4-dioxane biodegradation. Consequently, field dissolved oxygen measurements should be used with caution and supported by other lines of evidence. Other data that would corroborate that geochemical conditions are favorable include

positive ORP readings and low dissolved iron and methane concentrations. Total organic carbon is also a positive indicator, although carbon may create reducing conditions if oxygen availability is limited. This highlights the importance of delineating those portions of the site where groundwater transitions between anaerobic and aerobic to identify areas that might be best managed by natural attenuation.

11. Are potential biomarkers of aerobic 1,4-D biodegradation present?

Decision Criteria

Answer YES if: The presence of genes encoding DXMO/THFMO and/or ALDH has been established using quantitative polymerase chain reaction (qPCR) testing OR the presence of several other less-specific biomarkers has been established. DXMO/THFMO and ALDH have been identified as enzymes that are involved in the initial steps of 1,4-dioxane metabolism and/or co-metabolism. Other monooxygenases such as SCAM (short chain alkane monooxygenase), RMO and RDEG (both of which are ring-hydroxylating toluene monooxygenases) have also been identified as enzymes that can be involved in 1,4-dioxane co-metabolism. In addition, various propane monooxygenases have been evaluated for 1,4-dioxane capacity, and at least one (encoded by prmA) may be capable of both metabolic and co-metabolic degradation of 1,4-dioxane. However, it is not well-established if other propane monooxygenases (e.g., PPO) or various methane monooxygenases are capable of degrading 1,4-dioxane. In some cases, these enzymes may be expressed at the same time as other monooxygenases that are more directly involved in 1,4-dioxane degradation, such that they would serve as a secondary indicator that conditions are favorable for biodegradation. See **HELP** for additional information.

Note that genes that encode oxygenases are frequently found in a variety of environmental samples, including groundwater that would be considered anaerobic based on field measurements. These oxygenase enzymes also have broad metabolic capabilities, and the presence of an oxygenase-encoding gene does not ensure that 1,4-dioxane is actually degrading (see **HELP** for additional information). Consequently, qPCR results showing the presence of non-specific oxygenase genes should be used with caution and supported by other lines of evidence.

Answer NO if: No qPCR data are available OR if these biomarkers were not detected in any of the samples analyzed

HELP

The metabolic pathway for aerobic biodegradation of 1,4-D includes several enzymes that appear to be relevant to this process. For that reason, detection of the DNA responsible for coding the formation of these enzymes can provide a useful line of evidence for 1,4-D biodegradation. For example, an aldehyde dehydrogenase (ALDH) has been identified as a secondary biomarker 14D biodegradation by THFMO-expressing strains such as *P. dioxanivorans* CB1190.

Biodegradation of 1,4-D may also occur by a cometabolic process, whereby microbes grow on a substrate other than 1,4-D, but they express non-specific oxygenase enzymes that are capable of initiating oxidation of 1,4-D. There are numerous primary substrates that result in expression of enzymes capable of oxidizing 1,4-D, including tetrahydrofuran, propane, and butane. Monooxygenases investigated as possible or likely to be able to cometabolize 1,4-dioxane include soluble methane monooxygenase (sMMO), ring

hydroxylating toluene monooxygenase (RMO and RDEG), phenol hydroxlyase (PHE), and short-chain alkane monooxygenases (SCAM). Recently, a toluene-oxidizing monooxygenase has been described that can oxidize low concentrations of 1,4-dioxane and can also oxidize propane at sufficiently high rates that its activity can support the growth of the host bacterium using propane as a sole source of carbon and energy (Deng et al. 2020). The majority of these have been classified as soluble di-iron monooxygenases (SDIMO) (He *et al*., 2017; Deng et al., 2018). For example, the SCAM enzyme is frequently found in bacteria that can grow on a broad range of gaseous and short chain alkanes (C_2-C_6). SCAM is thought to catalyze the terminal oxidation of alkanes to primary alcohol products. Bacteria that express SCAM can cometabolically degrade 1,4-dioxane at low, environmentally relevant concentrations (≤100 ppb) and have also been shown to oxidize a wide variety of chlorinated 1,4-dioxane-associated co-contaminants. It has been shown that model strains expressing other monooxygenases such as sMMO or one of several toluene-oxidizing monooxygenases can degrade high (≥50 ppm) concentrations of 1,4-dioxane. However, the activity of sMMO towards 1,4-dioxane has not been reproduced, even at the level of the purified enzyme (Hatzinger et al., 2017). The activity of the model toluene-oxidizing monooxygenases towards lower concentrations of 1,4-dioxane (≤100 ppb) also has not been confirmed.

A *q*PCR assay has been developed for many of the genes that encode the enzymes described above, and in most cases these assays are now commercially available (e.g., Microbial Insights) or can be completed by academic labs (e.g., SCAM at North Carolina State University in Dr. Michael Hyman's research lab).

In the case of 1,4-dioxane, collecting data on multiple gene targets may be useful. For example, the term propane monooxygenase has been widely used in the literature and was historically used to generically describe any undefined propane-oxidizing monooxygenase. More recently, two distinctly different enzymes have been referred to as propane monooxygenase. One of these enzymes is SCAM. The second enzyme is found in a wide diversity of hydrocarbon-oxidizing bacteria including organisms that can grow on substrates including methane, non-methane alkanes, alkenes (e.g., propene or isoprene), MTBE, and even 1,4-dioxane. Unlike SCAM, this enzyme (PrMO) has a restricted substrate range and is thought to sub-terminally oxidize propane to 2-propanol. Although expression of PrMO can enable some bacteria to grow on propane (and potentially ethane and *n*-butane), the only contaminants unequivocally known to be degraded by this enzyme are NDMA and phenol. This enzyme is encoded by the *prm*ABCD gene cluster and can be quantified qPCR using the PPO assay. The dramatically different catalytic capabilities of PrMO and SCAM justifies a nomenclature that distinguishes these two enzymes, especially as genome analyses now indicate that many gaseous alkane-oxidizing bacteria possess genes that encode both PrMO and SCAM. Consequently, qPCR-based analyses demonstrating changes in the abundance of one of these genes can potentially also exhibit quantitatively equivalent changes in the other. This issue of multiple monooxygenases within a single organism also extends to bacteria such as *P. dioxanivorans* CB1190 that also possess genes encoding PrMO in addition to genes encoding THFMO/DXMO.

Detection of these oxygenase genes provides an indirect line of evidence for the capacity for oxidation of 1,4-D. However, just because a gene is detected does not mean that it is being expressed, i.e., the active enzyme needed for oxidation of 1,4-D may not be undergoing synthesis. It is possible to test for mRNA (messenger ribonucleic acid), which is present only if the gene is being expressed (i.e., DNA makes RNA makes proteins). However, it is considerably more challenging to obtain good quantification of mRNA. 1,4-D is often present at levels below 1 mg/L, at which point there is not much substrate available to support growth that will allow for detection of DNA, let alone mRNA.

The appeal of using qPCR to quantify specific genes is the relatively low cost of this measurement. The results may be viewed as supportive but, taken alone, not sufficient to document the occurrence of 1,4-D biodegradation. In other words, if biodegradation is occurring, it is likely that the necessary DNA will be present in a groundwater sample. However, the presence of the DNA does not ensure that biodegradation is occurring, and the absence of the DNA does not exclude the possibility that biodegradation is occurring.

12. Are inhibitory CVOCs present at relevant concentrations?

Decision Criteria

Answer YES if: The concentration of 1,1-DCE currently exceeds 10 µg/L in one or more wells. 1,4-D biodegradation may proceed at this level, but the rate is likely to slow given the various mechanisms by which 1,1-DCE can inhibit 1,4-D biodegradation.

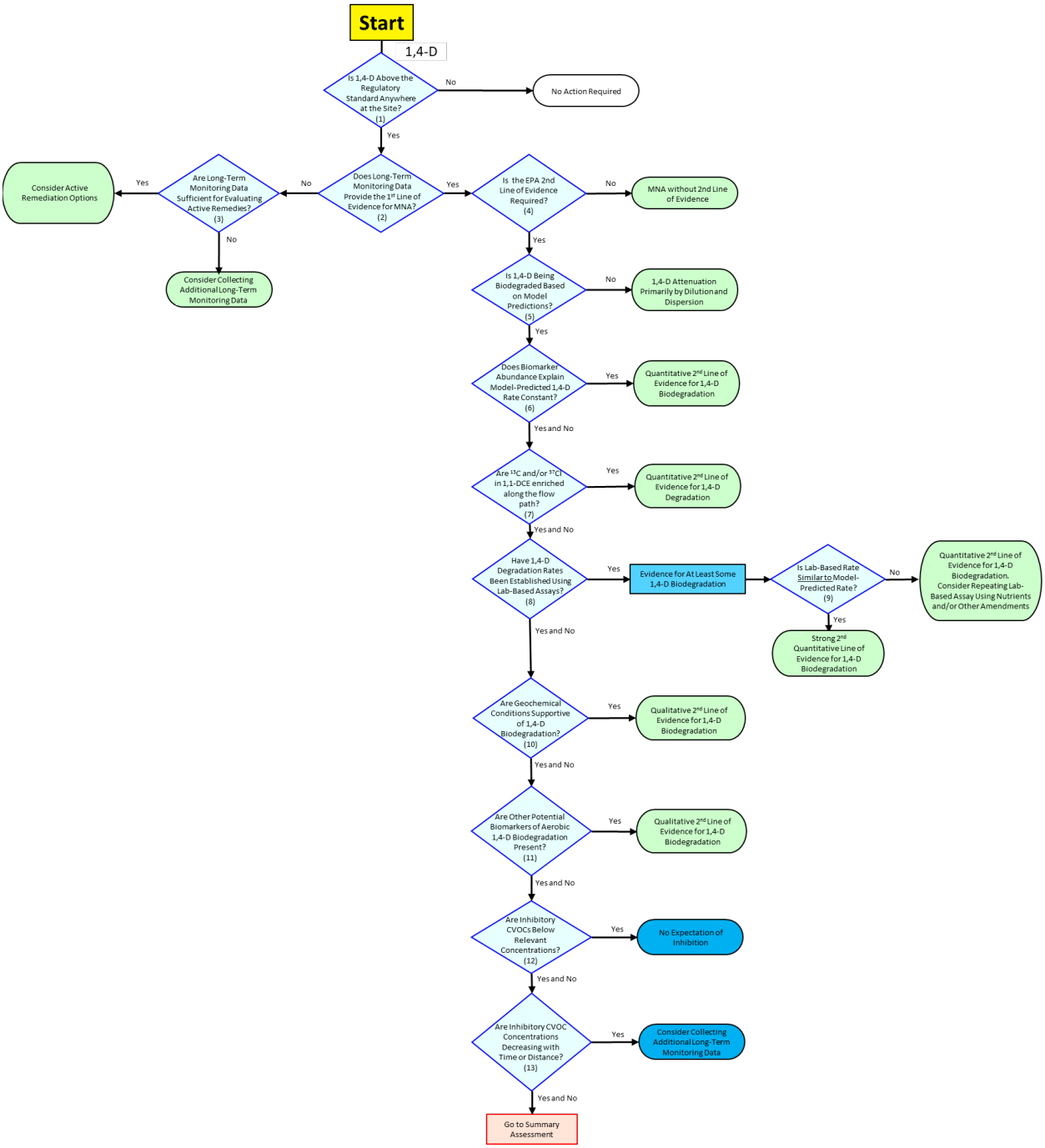
Answer NO if: No CVOCs are currently present in any wells at the site OR if the concentration of individual CVOCs is generally lower than 10 µg/L. Given the uncertainty, low CVOC concentrations should be combined with other lines of evidence that conditions are favorable for 1,4-D biodegradation.

13. Are inhibitory CVOC concentrations declining with time or distance?

Decision Criteria

Answer YES if: Declining trends in CVOC concentrations (total and/or individually) over time or along the groundwater flow path can be established. This can be accomplished using the model provided as part of this tool (see MNA Rate Constant Estimator in **FILES**, or go to the **GUIDED TOUR** for Chlorinated Ethenes and/or Chlorinated Ethanes). It provides evidence that these compounds are degrading, which can lessen their inhibitory effects based on lab and field studies. It also would help delineate portions of the 1,4-D plume where CVOCs are not present (e.g., in the toe of the 1,4-D plume) that might be better candidates for 1,4-D natural attenuation activity. Regardless, this is a qualitative indicator that conditions may be favorable for 1,4-D biodegradation and is more valuable if supported by other lines of evidence. It also suggests that collecting additional long-term monitoring data may be the most appropriate next step to determine if these favorable trends continue and eventually contribute to more rapid 1,4-D attenuation.

Answer NO if: CVOC concentrations exhibit stable trends over time OR along the groundwater flow path.

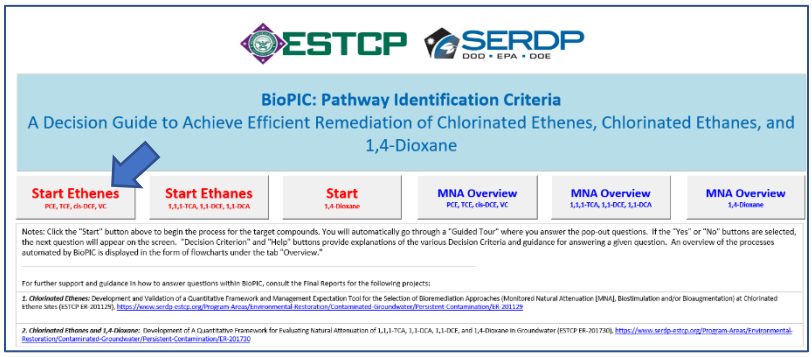


Decision Framework for 1,4-Dioxane.
This flowchart was coded into the updated BIOPIC tool.

Detailed BioPIC User Guide:

Chlorinated Ethenes

The following describes the Decision Framework for the compounds that are part of the Ethenes module, including PCE, TCE, cis-1,2-DCE, and VC.



BioPIC Home Page showing start button for entering the chlorinated ethene decision framework

Each of the numbered questions below corresponds to a number in the flowchart/guided tour. After each number, the decision criteria are explained. For most, further information is provided in the Help text. Note that these text descriptions are shown as pop-up boxes within the tool. A graphic showing the entire decision flowchart for these compounds is reproduced at the end of this section. If the user has selected a constituent of interest for evaluating the 2nd line of evidence for MNA (Question #3), a summary assessment can be displayed that shows the results for that particular compound. The summary assessment can be pulled up by clicking the View Summary box that appears to the right of each question, and it will display answers to only those questions that have been completed.

Note that these descriptions are retained from the 2015 version of BioPIC; no changes to the Chlorinated Ethene decision framework were made as part of the 2021 update to BioPIC. This means that the questions and associated decision criteria/help formats in this module differ somewhat from those found in the Chlorinated Ethane module and in the 1,4-Dioxane module.

1. Does natural attenuation currently meet the goal?

Decision Criteria

If at any time, the concentrations of PCE, TCE, DCE and VC will exceed the regulatory standard at the POC, then natural attenuation will not meet the cleanup goal.

There usually is also a temporal component in the regulatory goals, and the implementation of more aggressive remedies may reduce time to achieve remediation goals, thereby reducing the overall cost. This tool only deals with the spatial, not temporal, aspects of remediation goals.

HELP

If sufficient historical contaminant concentration data are not available to determine if a solute plume will reach a POC, then a groundwater flow and solute transport model such as BIOCHLOR should be used to predict solute plume behavior. In this case, the simulation should account for the effects of advective groundwater flow, dispersion of the relevant solutes, sorption, and degradation of the PCE, TCE, DCE and VC in groundwater at the site.

For more information, consult Development and Validation of a Quantitative Framework and Management Expectation Tool for the Selection of Bioremediation Approaches at Chlorinated Ethene Sites ESTCP Project ER-201129 https://www.serdp-estcp.org/Program-Areas/Environmental-Restoration/Contaminated-Groundwater/Persistent-Contamination/ER-201129/ER-201129. Section 5.2.3 illustrates the process of calibrating a groundwater flow and transport model. Section 5.2.4 Step 1 illustrates the use of a model to apply the decision criteria.

If available, a robust historical database of contaminant concentrations can be used as an alternative to a computer model. Spatial and temporal trends in solute concentrations can be utilized to determine if the plume is stable or receding and therefore will not reach the POC. When sufficient data are available, using empirical data to ascertain trends is much better than using a model. In many cases, sufficient solute concentration data are available to evaluate plume behavior and to determine if solute concentrations will exceed cleanup goals at a regulatory POC.

If historical data are used to determine whether NA currently meets the goal, it is still necessary to build a transport and fate model of the plume. The model is necessary to extract degradation rate constants that will be used in BioPIC to evaluate whether biological reductive dechlorination or abiotic degradation are a second line of evidence for MNA. Any computer application that simulates the fate and migration of PCE, TCE, DCE and VC in groundwater can be used to assess solute plume behavior. The simulation time for the model should be sufficient for concentrations of PCE, TCE, DCE and VC to reach their maximum concentrations at the POC. Most computer applications (i.e., software) cannot distinguish between cDCE, tDCE and 1,1- DCE. If this is true for the software you're using, then the simulations should be run to determine if natural attenuation will meet the remediation goal using the sum of the cDCE, tDCE, and 1,1-DCE isomers. When analyzing the degradation of PCE, TCE, DCE, and VC, different combinations of DCE isomers should be used in the analysis, depending upon the compound for which degradation pathways are being analyzed. This is discussed in the relevant sections that follow. For example, when evaluating degradation of TCE, only the cDCE and tDCE isomers should be included in the analysis because these are the relevant compounds produced from the degradation of TCE. When evaluating DCE degradation and therefore the possible production of VC, the sum of all DCE isomers should be used in the simulations, regardless of DCE origin, because all three DCE isomers can be reduced to VC by specialized bacteria. Again, when DCE is discussed in this document, if one of the isomers is specified, for example, cDCE, then it is that isomer that is relevant and that isomer only that should be considered. If the general term DCE is used, then the reader should assume that all three isomers of DCE should be considered (i.e., cDCE, tDCE, and 1,1-DCE).

2. Are reductive dechlorination genes present?

Decision Criteria

This decision box is reached if natural attenuation does not meet remediation goals. For the purpose of this decision support system, relevant RDase genes (e.g., tceA, bvcA, vcrA) are determined by the quantitative polymerase chain reaction (qPCR). Based on the current qPCR technology, a specific RDase gene is considered to be present if its abundance exceeds 10E+03 gene copies per liter of groundwater.

HELP

Some Dhc strains possess the bvcA or vcrA genes, which encode VC reductive dehalogenases (RDases). Assays to specifically assess bvcA and vcrA gene abundances are commercially available. If bvcA and vcrA can be quantified, Dhc strains with the potential to dechlorinate VC to ethene are present. Dhc can only grow at the expense of reductive dechlorination reactions. Therefore, if Dhc biomarker genes (i.e., specific RDase genes and the Dhc 16S rRNA gene) are detected in samples collected from a chlorinated ethene plume, it is highly probable that these Dhc strains grew with chlorinated ethenes as electron acceptors. Without growth, Dhc biomarkers are unlikely to exceed 10E+03 gene copies per liter of groundwater, and therefore would not be quantified with qPCR.

Note that not all Dhc strains carry VC RDase genes and therefore not all Dhc strains contribute to VC reductive dechlorination to ethene. The vcrA and/or bvcA genes are typically found at sites where ethene is formed; however, not all VC RDases have been identified and it is possible that at some sites ethene formation occurs even in the absence of vcrA and bvcA. Quantitative real-time polymerase reactions (qPCR) targeting Dhc and bacterial 16S rRNA genes should accompany the VC RDase gene analysis. This information is useful to calculate the ratio of Dhc to total bacterial 16S rRNA gene copies and the ratio of VC RDase genes to Dhc cells, which inform about the potential for ethene formation. In general, qPCR assays can detect and enumerate Dhc biomarker genes when at least 100 to 1,000 Dhc cells, respectively, are present per liter of groundwater.

3. Is the EPA 2nd line of evidence required?

Decision Criteria

The final decision to require, or to not require, the second line of evidence is made by the appropriate regulatory authority.

The USEPA may require two lines of evidence before approval of Monitored Natural Attenuation (MNA) as a site remedy will be granted. The first direct line of evidence requires data that demonstrate a clear and meaningful trend of decreasing contaminant mass and/or concentration over time at appropriate monitoring or sampling points. The second line of evidence originally included “hydrogeologic and geochemical data that can be used to indirectly demonstrate the type(s) of natural attenuation processes active at the site, and the rates at which such processes will reduce contaminant concentrations to required levels”.

HELP

Lines of evidence for MNA are described in Use of Monitored Natural Attenuation at Superfund, RCRA Corrective Action, and Underground Storage Tank Sites (EPA. 1999)http://www2.epa.gov/sites/production/files/2014-02/documents/d9200.4-17.pdf. The intent of the second line of evidence was to corroborate that degradation is occurring. Since the 1999 release of the EPA document, several additional methodologies have been developed. These include compound-specific isotope analysis (CSIA) and various molecular biological tools such as qPCR targeting biomarker genes of dechlorinating bacteria. In addition, our understanding of degradation mechanisms affecting chlorinated ethenes has increased, and previously unknown degradation mechanisms, particularly abiotic degradation mechanisms such as degradation using magnetite or FeS, have been identified.

The first line of evidence is always required. A regulator will require the second line of evidence based on the regulator's level of understanding of the processes that control the distribution and fate of the contaminants. If the critical processes for natural attenuation are already well understood and the processes are ubiquitous at sites, and there is extensive experience from other sites that documents that the processes are reliable, then a regulator may not require the second line of evidence.

If the processes are not ubiquitous, or the critical process(es) operate effectively at some sites but not at others, a regulator will often require the second line of evidence. The focus on this decision support system is to evaluate natural attenuation processes and provide a creditable second line of evidence.

There is a third line of evidence, which can be provided by field or microcosm studies, that directly demonstrate the occurrence of a particular natural attenuation process at the site and its ability to degrade the contaminant(s) of concern. Regulators rarely require the third line of evidence, which is usually reserved for compounds that have not been studied and little is known about their fate and transport. This framework or decision support system does not address the third line of evidence.

4. Is VC present?

Decision Criteria

For the purposes of this decision support system, VC is considered present when the concentration of VC exceeds the site-specific VC cleanup goal. If no cleanup goal for VC has been established, VC is considered present when the concentration is equal to or exceeds 2 µg/L. Other criteria may apply depending on the specific site conditions and the regulatory authority.

HELP

The cleanup goal is not always the U.S. EPA MCL established for drinking water. In many cases, the use of risk-based cleanup goals is appropriate. Consult the regulator for the cleanup goals that apply to the site of interest.

5. Is VC degrading?

Decision Criteria

Access the computer simulation that you prepared to evaluate the criterion "Does Natural Attenuation Currently Meet the Goal? Prepare a new simulation where the rate constant for degradation of VC is set to zero. Compare the actual in situ concentrations of VC against the new simulation. Then enter trial values for the rate constant for VC degradation into the simulation to see if the model projections provide a better fit to the in situ concentrations.

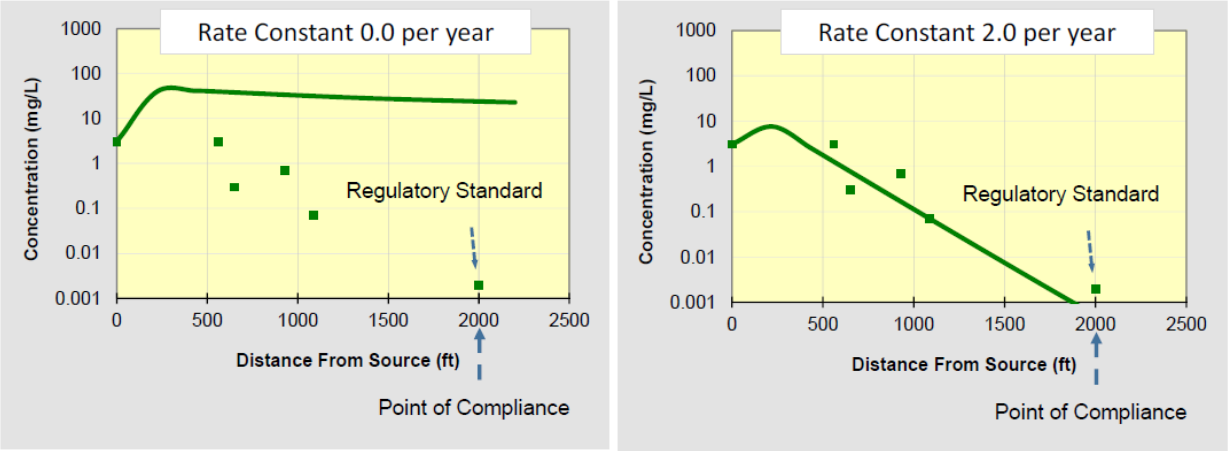
If rate constants greater than zero provide a better fit, then VC degradation is occurring.

Additional information can be provided from an analysis of stable isotopes of carbon in VC. If values of δ13C are available for VC, open the tab Files and select the spreadsheet CSIA.xlsx. Enter your data in the tab Input Data CSIA + Concentration. Then open the tab Kuder Plot VC.

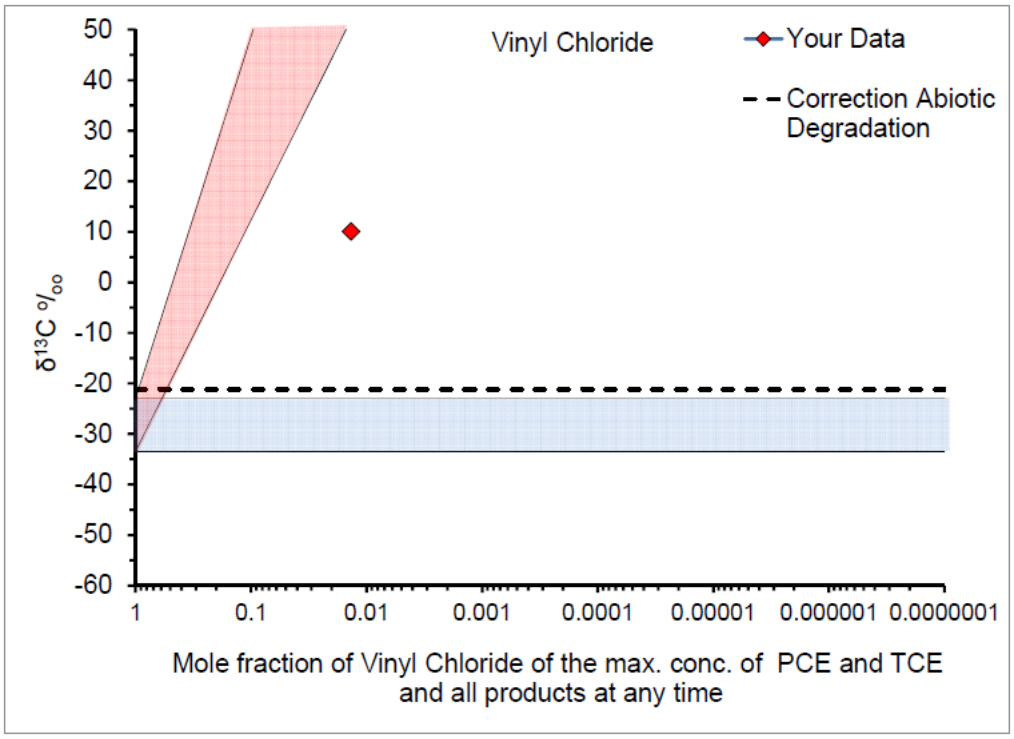
If your data fall above the blue rectangular shape in the chart Kuder Plot for VC, the stable isotopes of carbon in VC have been fractionated, which is evidence that VC degradation has occurred. If your data fall above the blue shape and within the red shape, then microbial reductive dechlorination to ethene can explain the fractionation. If the data falls to the right of red shape, some other process that does not degrade the VC, such as dispersion or dilution, has contributed to the reduction in VC concentrations.

HELP

A computer simulation of the transport and fate of VC can reveal when VC is degrading. The figure below is a hypothetical example where the Point of Compliance (POC) is 2,000 feet from the source of contamination, and the concentrations of VC at the POC are below the MCL for VC. The distance from the source and the acceptable concentration were entered in the input screen of BIOCHLOR so that it would plot in the RUN CENTERLINE output. The value for the rate constant for VC degradation was set at zero to simulate the concentrations that would be expected without VC degradation. The in situ VC concentrations were lower than the simulation with no degradation of VC, indicating that degradation was occurring. Trial values of the rate constant for degradation of VC were selected. The rate constant for degradation of VC that provided the best fit was 2.0 per year.



Data from Compound Specific Isotope Analysis (CSIA) can reveal when DCE is degrading. The figure below is the chart in the spreadsheet CSIA.xlsx under the tab Kuder Plot VC for a hypothetical data set. In this example, the point plotted above the blue box and the dotted line, indicating that degradation had changed the ratio of stable isotopes. The point plotted to the right of the red shape, indicating that processes other than biological reductive dechlorination had contributed to attenuation of the concentrations of VC. These processes can include dispersion in the aquifer or dilution in the monitoring well.



Your data should plot in the chart. If not, you may need to extend the scales of the x and/or y axes.

6. Does Dhc density explain the VC rate constant?

Decision Criteria

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.

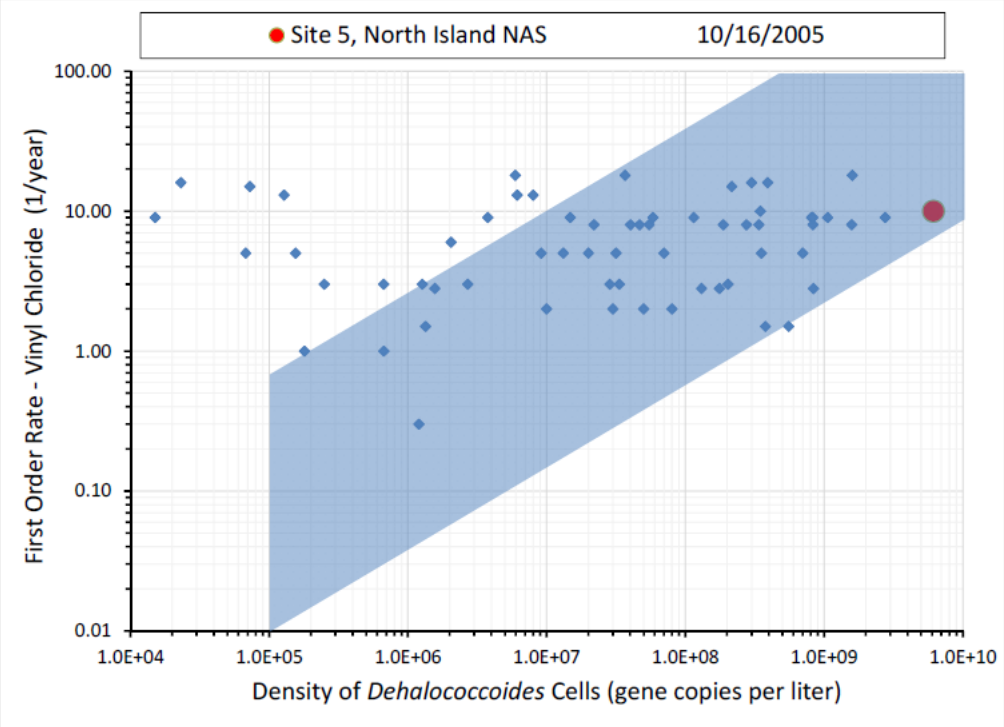
Open the tab Files and select the spreadsheet Reductase Genes.xlsx. Enter your data in the tab Input Data. Then open the tab VC Rase and Dhc. If your data plot in the blue shape, transformation of VC is plausible based on the abundance of the VC reductase genes in the groundwater.

HELP

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.



7. Does Dhc density explain the VC rate constant?

Decision Criteria

Consult the simulation that you prepared to evaluate the criterion "Does Natural Attenuation Currently Meet the Goal?" Prepare a new simulation. Do not include any portion of the plume where biological reductive dechlorination might contribute to the bulk rate constant that is extracted by the model. Exclude any portion of the flow path where the concentrations of any daughter products are increasing with distance from the source. By trial and error, identify the rate constant for degradation of VC that provides the best match between the new simulation and the field data.

Open the tab Files and select the spreadsheet Magnetic Susceptibilty.xlsx. Enter your values for the rate constant for degradation of VC and for mass magnetic susceptibility in the tab Input Data. Then open the tab Mag. Sus. Explains VC.

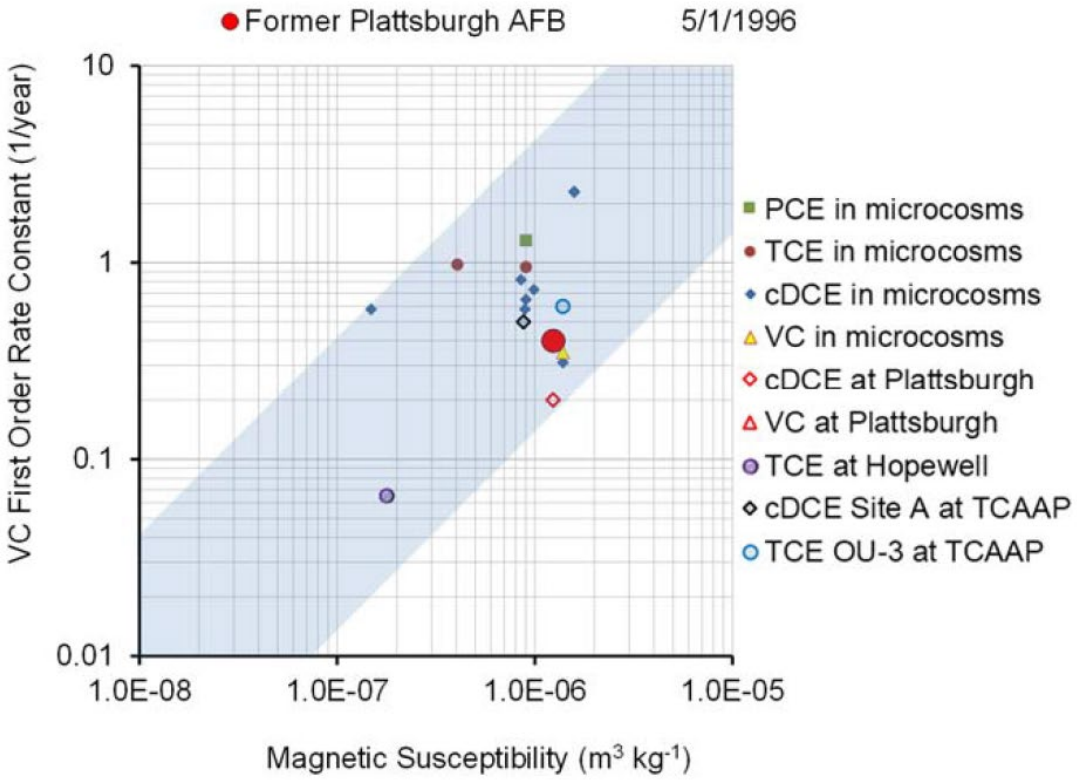
If the site-specific values fall within the blue shape, then mass magnetic susceptibility can explain the apparent in situ rate of VC degradation.

HELP

Magnetite can mediate abiotic degradation of VC. The amount of magnetite in aquifer material can be estimated from the mass magnetic susceptibility of core samples. Empirical data are available that associate degradation rate constants for VC with mass magnetic susceptibility. The available data were used to define the blue shape in the figure. The figure is the chart in tab Mag. Sus. Explains VC for an example data set. If the rate constant plots within the blue shape, then abiotic degradation mediated by magnetite can explain the observed rate constant.

If the rate constant plots above the blue shape, other processes are likely contributing to the rate of VC degradation.

If the rate constant plots below the shape, inappropriate sampling locations may have been selected for mass magnetic susceptibility measurements. Mass magnetic susceptibility should be determined with aquifer material that is most transmissive to water since this is where most solute transport will occur. In addition, the input values used for the rate constant calculation with BIOCHLOR should be verified.



8. Adequate oxygen for aerobic VC biodegradation?

Decision Criteria

Bacteria that degrade VC if oxygen is available are generally present in aquifers. These bacteria require very low dissolved oxygen concentrations to metabolize VC. Because of field sampling limitations, dissolved oxygen concentration data on well water are generally unreliable to determine if sufficient oxygen is available to support oxygen-dependent VC oxidation.

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 (Fe2+) concentrations are below 0.5 mg/L, and methane concentrations are below 0.005 mg/L.

HELP

Bacteria that degrade VC if oxygen is available are generally present in aquifers. These bacteria require very low dissolved oxygen concentrations to metabolize VC. Because of field sampling limitations, dissolved oxygen concentration data on well water are generally unreliable to determine if sufficient oxygen is available to support oxygen-dependent VC oxidation.

It is easy to contaminate a groundwater sample with oxygen because, among other things, the sampling of monitoring wells frequently causes mixing of water from different depth intervals. It is possible the VC in a sample of well water came from one depth interval and the oxygen from another. If this is the case, oxygen may not be available to the VC-degrading bacteria in the aquifer, leading to the erroneous conclusion that VC can be degraded aerobically.

The absence of ferrous iron (Fe2+) and methane are good indicators for the presence of oxygen that supports aerobic biodegradation of organic compounds. The absence of ferrous iron and methane in water collected from a well generally indicates that all of the flowpaths to the well had adequate concentrations of oxygen to support aerobic VC degradation.

Note that aerobic VC oxidizers are able to degrade VC at very low oxygen concentrations. Therefore, aerobic VC oxidation may contribute to VC attenuation in aquifers characterized as "anoxic" (i.e., the answer to the decision criterion is "No"). While aerobic VC degraders will likely contribute to VC degradation in the presence of oxygen, establishing quantitative relationships is difficult. As a result, the presence of oxygen is only a qualitative line of evidence for aerobic biodegradation of VC.

9. Is DCE present?

Decision Criteria

For the purposes of this decision support system, DCE is present when the concentrations of cDCE, tDCE, and/or 1,1-DCE exceed the cleanup goal that has been established for the site. If no cleanup goal for DCE has been established, DCE is considered present when the concentration equals or exceeds 7 µg/L. Other criteria may apply depending on the regulatory authority.

HELP

The cleanup goal is not always the U.S. EPA MCL established for drinking water. Consult the regulator and verify the cleanup goals that apply to the site.

10. Is DCE degrading?

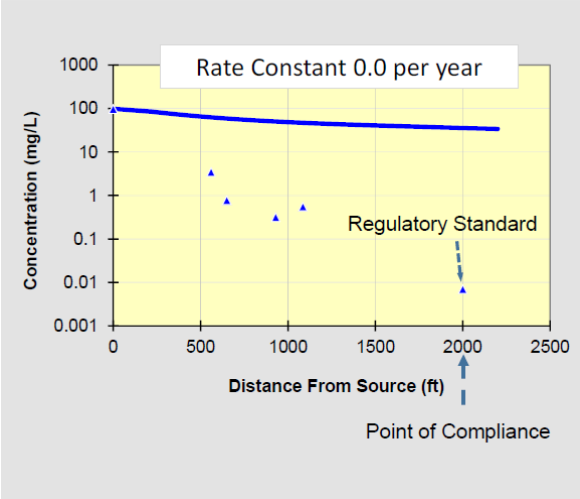
Decision Criteria

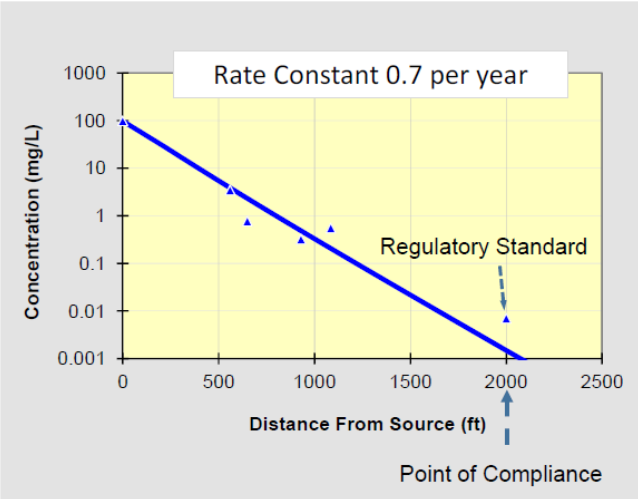
Prepare a new simulation where the rate constant for degradation of DCE is set to zero. Compare the actual in situ concentrations of the sum of cDCE + tDCE + 1,1-DCE against the new simulation. Then enter trial values for the rate constant for DCE degradation into the simulation to see if the model projections provide a better fit to the in situ concentrations.

If rate constants greater than zero provide a better fit, then DCE is degrading.

Additional information can be provided from an analysis of stable isotopes of carbon in DCE. If values for δ13C are available for DCE, open the tab Files and select the spreadsheet CSIA.xlsx. Enter your data in the tab Input Data CSIA + Concentration. Then open the tab Kuder Plot cDCE and examine the chart.

If your data fall above the blue shape, the stable isotopes of carbon in DCE have been fractionated and that is evidence that DCE is degrading. If your data fall above the blue shape and within the red shape, then microbial reductive dechlorination to DCE can explain the fractionation. If the data fall to the right of red shape, some other process that does not degrade the DCE, such as dispersion or dilution, has contributed to the reduction in contaminant concentrations.

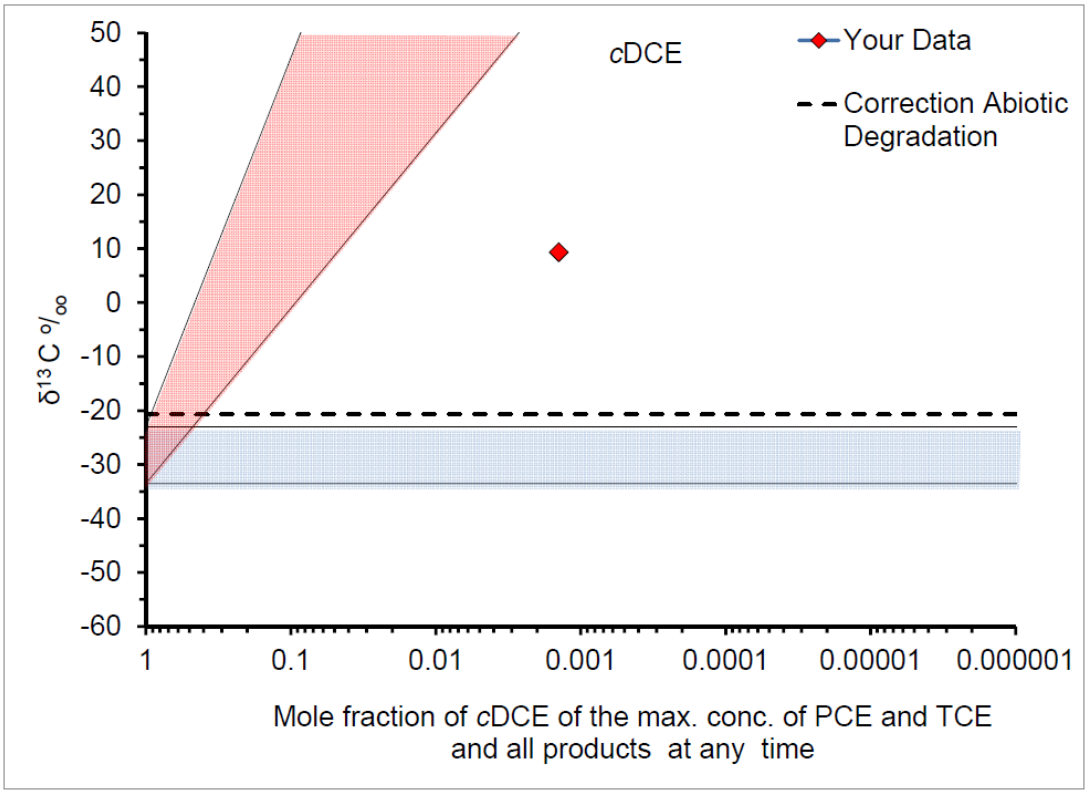




A computer simulation of the transport and fate of DCE can reveal when DCE is degrading. The figure below is a hypothetical example, where the POC is 2,000 feet from the source of contamination, and the acceptable concentration of DCE at the POC was the MCL for 1,1-DCE. The BIOCHLOR model does not discriminate between DCE isomers. The value entered in the model is the sum of the cDCE, tDCE and 1,1-DCE isomers for the total DCE concentration. Regardless of this, in this case the acceptable concentration for DCE was set at the MCL for 1,1-DCE because this isomer has the lowest MCL. The distance from the source and the acceptable concentration was entered in the input screen of BIOCHLOR so that it would plot in the RUN CENTERLINE output. The value for the rate constant for DCE degradation was set at zero to simulate the concentrations that would be expected if there were no degradation of DCE. The concentrations of DCE in the field were lower than the simulation with no degradation of DCE. Trial values of the rate constant for degradation of DCE were selected. The rate constant for degradation of DCE that provided the best fit was 0.7 per year.

Data from Compound Specific Isotope Analysis (CSIA) can reveal when DCE is degrading. The figure below is the chart in the spreadsheet CSIA.xlsx under the tab Kuder Plot DCE for a hypothetical data set. In this example, the point plotted above the blue box and the dotted line, indicating that degradation had changed the ratio of stable isotopes. The point plotted to the right of the red shape, indicating that processes other than biological reductive dechlorination had contributed to attenuation of the concentrations of VC. These processes can include dispersion in the aquifer or dilution in the monitoring well.

Your data should plot in the chart. If not, you may need to extend the scales of the x and/or y axes.



11. Does Dhc density explain the DCE rate constant?

Decision Criteria

Consult the simulation that you prepared to evaluate the criterion "Does Natural Attenuation Currently Meet the Goal?" Identify the rate constant for degradation of DCE. Access information about the abundance of Dhc cells in site groundwater. Open the tab Files and select the spreadsheet Dhc.xlsx. Input values for the first order rate constant for degradation of DCE 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 cDCE. If your data plot in the blue shape, then the abundance of Dhc in groundwater can explain the in situ rate of DCE degradation.

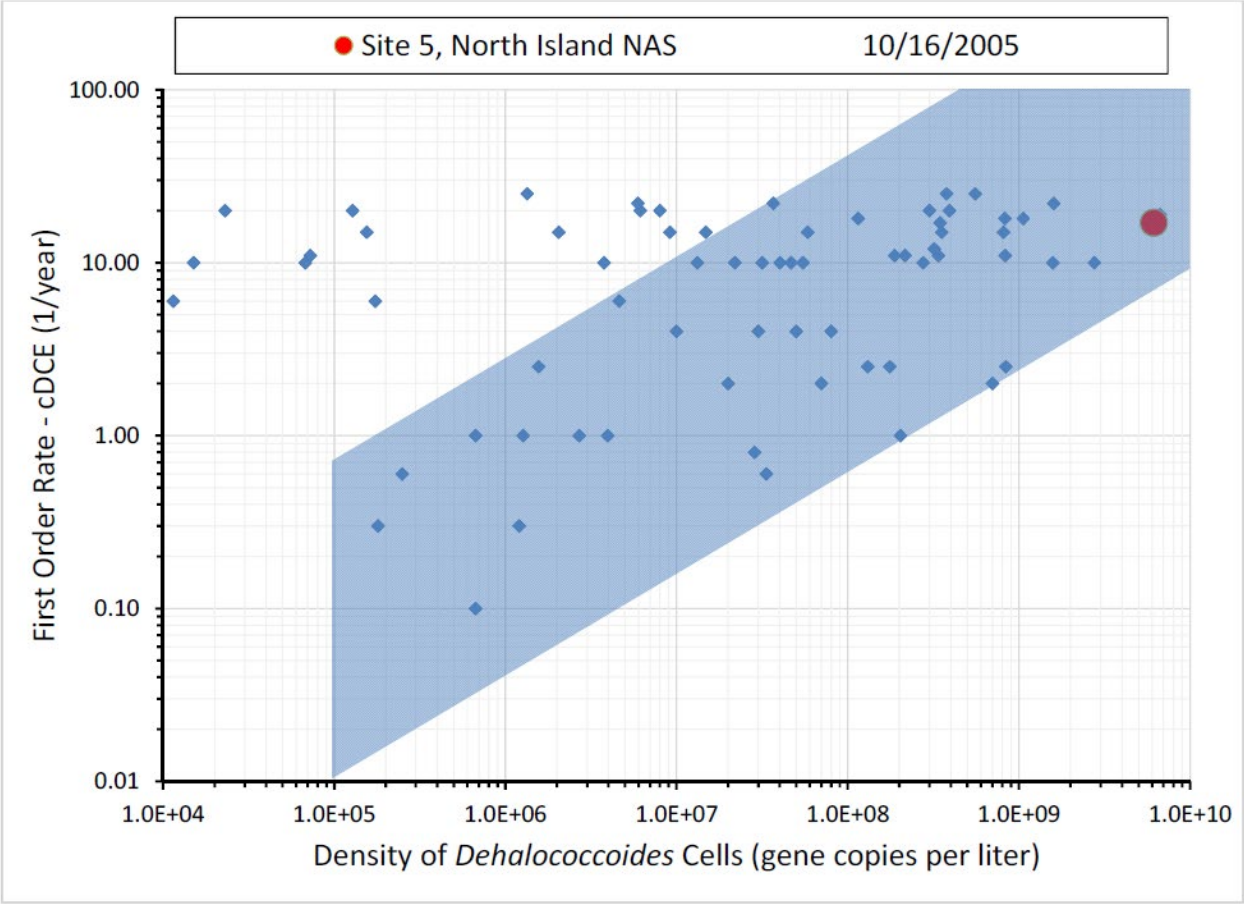
Not every bacterium with the Dhc 16S rRNA gene can degrade DCE. A qPCR assay is commercially available for two of the known genes that code for enzymes that reductively dechlorinate DCE. The reductase genes have been designated vcrA and bvcA. If there is a concern that the Dehalococcoides strains at your site cannot degrade cDCE, 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. Input values for the abundance of vcrA, bvcA and Dhc gene copies into the tab Input Data. Then open the tab VC Rase and Dhc. If your data plot in the blue shape, transformation of cDCE to ethene is plausible based on the abundance of the reductase genes in the groundwater.

HELP

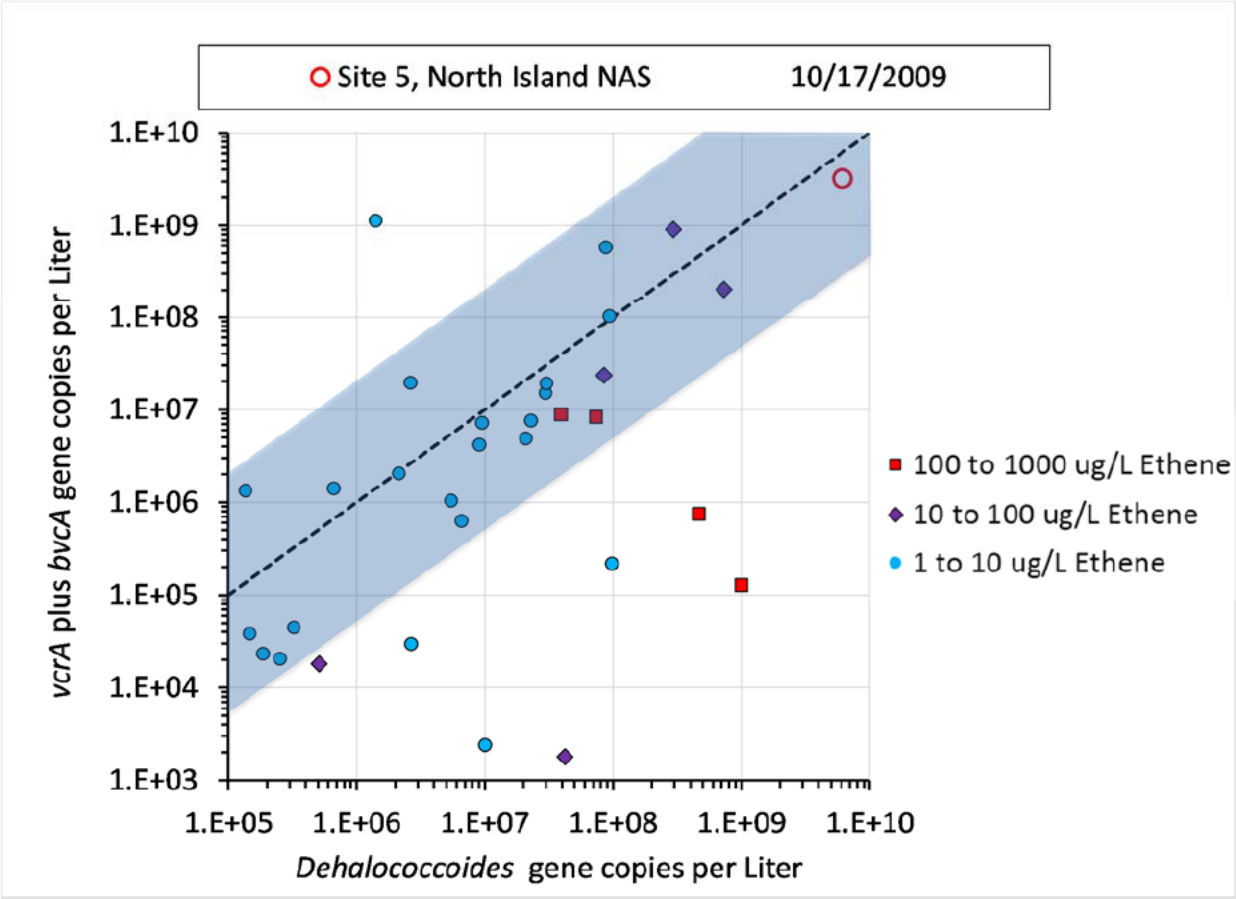
Note that the chart has data points that are outside of the blue shape and have first order rate constants that are larger than can be plausibly explained by the Dhc cell abundance in the groundwater. Possible explanations for the observed rates of cDCE degradation include:

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 DCE-to-VC-to-ethene reductive dechlorination step has been exclusively associated with Dhc strains carrying the Reductase genes vcrA or bvcA; however, it is conceivable that not-yet-recognized bacteria may contribute to DCE- to-VC-to-ethene reductive dechlorination.
3. Microbial DCE oxidation can occur at very low dissolved oxygen concentrations, and areas of the aquifer may have sufficient oxygen to sustain aerobic DCE degradation.
4. Abiotic DCE degradation mediated by reactive iron-bearing mineral phases (e.g., iron sulfides, magnetite) contributes to DCE degradation.



Dhc strains have been described that contribute to reductive dechlorination of polychlorinated ethenes but cannot efficiently dechlorinate DCE. If such strains dominate the Dhc population, a high Dhc cell abundance may not correlate with DCE-to-VC-to-ethene reductive dechlorination activity. Two Dhc RDase

genes involved in DCE-to-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 DCE to ethane is plausible based on the abundance of vcrA and bvcA in the groundwater.



12. Does magnetic susceptibility explain the DCE rate constant?

Decision Criteria

Consult the simulation that you prepared to evaluate the criterion "Does Natural Attenuation Currently Meet the Goal?" Prepare a new simulation. Do not include any portion of the plume where biological reductive dechlorination might contribute to the bulk rate constant that is extracted by the model. Exclude any portion of the flow path where the concentrations of any daughter products are increasing with distance from the source. By trial and error, identify the rate constant for degradation of DCE that provides the best match between the new simulation and the field data.

Open the tab Files and select the spreadsheet Magnetic Susceptibilty.xlsx. Enter your values for the rate constant for degradation of DCE and for mass magnetic susceptibility in the tab Input Data. Then open the tab Mag. Sus. Explains cDCE.

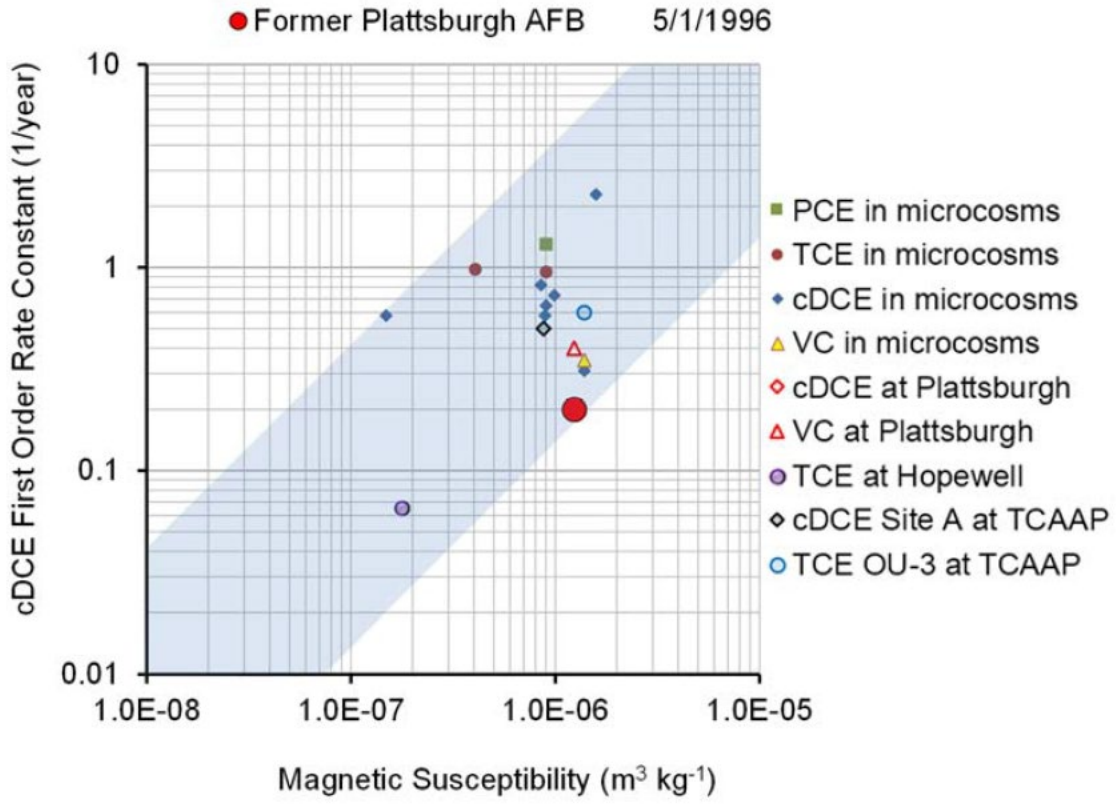
If the site-specific values fall within the blue shape, then mass magnetic susceptibility can explain the apparent in situ rate of DCE degradation.

HELP

Magnetite can mediate abiotic degradation of cDCE. The amount of magnetite in aquifer material can be estimated from the mass magnetic susceptibility of core samples. Empirical data are available that associate degradation rate constants for cDCE with mass magnetic susceptibility. The available data were used to define the blue shape in the figure. The figure is the chart in tab Mag. Sus. Explains DCE for an example data set. If the rate constant plots within the blue shape, then abiotic degradation mediated by magnetite can explain the observed rate constant.

If the rate constant plots above the blue shape, other processes are likely contributing to the rate of VC degradation.

If the rate constant plots below the shape, inappropriate sampling locations may have been selected for mass magnetic susceptibility measurements. Mass magnetic susceptibility should be determined with aquifer material that is most transmissive to water since this is where most solute transport will occur. In addition, the input values used for the rate constant calculation with BIOCHLOR should be verified.



13. Adequate oxygen for aerobic DCE degradation?

Decision Criteria

Bacteria that degrade DCE with oxygen are generally present in aquifers, even when the groundwater has been characterized as anoxic. Because of field sampling limitations, dissolved oxygen concentration data on well water are generally unreliable to determine if sufficient oxygen is available to support oxygen-dependent DCE degradation.

For the purposes of this decision support system, oxygen is considered to be available for aerobic biodegradation of DCE when all of the following criteria are met: Dissolved oxygen concentrations measured in the field exceed 0.1 mg/L, ferrous iron (Fe2+) concentrations are less than 0.5 mg/L, and methane concentrations are less than 0.005 mg/L.

HELP

It is easy to contaminate a groundwater sample with oxygen. Sampling monitoring wells often causes mixing of water from different depth intervals. It is possible the DCE in a sample of well water came from one depth interval and the oxygen from another. If this is the case, oxygen may not be available to the DCE-degrading bacteria in the aquifer, leading to the erroneous conclusion that DCE is degraded aerobically. The absence of ferrous iron (Fe2+) and methane are good indicators for the presence of concentrations of oxygen that support aerobic biodegradation of organic compounds. The absence of ferrous iron or methane in water collected from a well indicates that all of the flow paths to the well had adequate concentrations of oxygen to support aerobic DCE degradation.

14. Is TCE present?

Decision Criteria

For the purposes of this decision support system, TCE is present in groundwater when the concentration of TCE exceeds a cleanup goal for TCE that has been established for the site. If no cleanup goal for TCE has been established, TCE is considered present when the concentration is ≥ 5 µg/L. Other criteria may apply depending on the regulatory authority.

HELP

The cleanup goal is not always the U.S. EPA MCL established for drinking water. Consult the regulator for the cleanup goals that apply to the site of interest.

15. Is TCE degrading?

Decision Criteria

Access the computer simulation that you prepared to evaluate the criterion “Does Natural Attenuation Currently Meet the Goal?” Prepare a new simulation where the rate constant for degradation of TCE is set to zero. Compare the actual in situ concentrations of TCE against the new simulation. Then enter trial

values for the rate constant for TCE degradation into the simulation to see if the model projections provide a better fit to the in situ concentrations.

If rate constants greater than zero provide a better fit, then TCE is degrading.

As an alternative, CSIA can be used to determine if TCE is degrading.

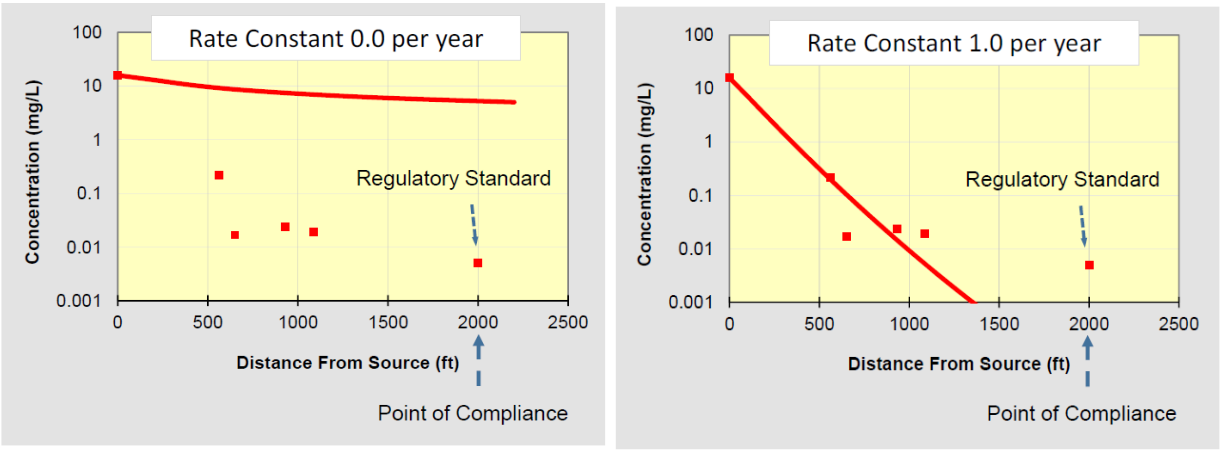
If the value of δ13C of TCE in a down gradient well is larger (less negative) than the value in an up gradient well by more than 0.5‰, that can be taken as evidence for degradation of TCE.

The highest value that has been reported for the δ13C of TCE used in commerce is -23.2‰.

As a general rule, a value of δ13C for TCE that is greater than -22.7‰ can be taken as evidence of degradation of TCE.

HELP

A computer simulation of the transport and fate of TCE can reveal when TCE is degrading. The figure below is a hypothetical example, where the POC is 2,000 feet from the source of contamination, and the acceptable concentration of TCE at the POC was the MCL for TCE. The distance from the source and the acceptable concentration was entered in the input screen of BIOCHLOR so that it would plot in the RUN CENTERLINE output. The value for the rate constant for TCE degradation was set at zero to simulate the concentrations that would be expected if there were no degradation of TCE. The concentrations of TCE in the field were lower than the simulation with no degradation of TCE. Trial values of the rate constant for degradation of DCE were selected. The rate constant for degradation of TCE that provided the best fit was 1.0 per year.



As an alternative, CSIA can be used to determine if TCE is degrading. Microbial degradation of TCE would make the value of δ13C a larger (less negative) number. The precision of the analysis is near 0.5‰. If the value of δ13C of TCE in a down gradient well is larger (less negative) than the value in an up gradient well by more than 0.5‰, that can be taken as evidence for degradation of TCE.

The increase in δ13C in TCE in areas close to a NAPL source area containing TCE may be difficult to discern and may not become apparent until the NAPL source becomes significantly depleted. In addition, the continued formation of TCE from PCE will reduce the value of δ13C for TCE in the pool of TCE until the PCE is consumed, either over time or along the flow path.

16. Are DCE or VC present?

Decision Criteria

Evaluate data on concentrations of TCE, cDCE, tDCE and VC in groundwater. If the sum of cDCE, tDCE and VC is more than 5 mole % of the concentration of TCE, then cDCE, tDCE and VC are present. The presence of cDCE or tDCE or VC indicates that reductive dechlorination of TCE has occurred.

The calculation of mole % can be easily performed using the Excel file Mole Percent Calculator.xlsx, which is included in the BioPIC program, and also found in Appendix D.

HELP

The detection of cDCE or tDCE or VC at TCE-impacted sites suggests that TCE reductive dechlorination has occurred and this process may still be ongoing. Enumeration of pceA genes (present in PCE- and/or TCE-dechlorinating bacteria and implicated in PCE-to-TCE and PCE-to-cDCE reductive dechlorination) and the tceA gene (present in some Dhc strains and implicated in TCE-to-VC reductive dechlorination) with qPCR provides support that bacteria capable of TCE reductive dechlorination to cDCE or VC are present.

17. Are DCE or VC present in relevant concentrations?

Decision Criteria

Evaluate data on concentrations of TCE, cDCE, tDCE and VC in wells downgradient of the source of contamination. If the sum of cDCE, tDCE and VC is more than 25 mole % of the concentration of TCE, then cDCE, tDCE and VC are present in relevant concentrations. The presence of daughter products at these concentrations indicates that microbial reductive dechlorination is an important pathway for TCE fate, and explains in a qualitative manner why TCE is degrading.

The calculation of mole % can easily be performed using the Excel file included with the BioPIC program titled Mole Percent Calculator.xlsx, and also found in Appendix D.

HELP

The detection of cDCE, tDCE or VC at TCE-impacted sites suggests that TCE reductive dechlorination has occurred and this process may still be ongoing. Enumeration of pceA genes with qPCR provides support that bacteria capable of TCE reductive dechlorination to cDCE are present. The pceA gene is present in TCE-dechlorinating bacteria and implicated in TCE-to-cDCE reductive dechlorination. The presence of the Dhc RDase genes tceA, bvcA or vcrA implicated in reductive dechlorination of DCE can explain the formation of VC and ethene.

18. Are DCE or VC present in relevant concentrations?

Decision Criteria

Consult the simulation that you prepared to evaluate the criterion "Does Natural Attenuation Currently Meet the Goal?" Prepare a new simulation. Do not include any portion of the plume where biological reductive dechlorination might contribute to the bulk rate constant that is extracted by the model. Exclude any portion of the flow path where the concentrations of any daughter products are increasing with distance from the source. By trial and error, identify the rate constant for degradation of TCE that provides the best match between the new simulation and the field data.

Open the tab Files and select the spreadsheet Magnetic Susceptibilty.xlsx. Enter your values for the rate constant for degradation of TCE and for mass magnetic susceptibility in the tab Input Data. Then open the tab Mag. Sus. Explains TCE.

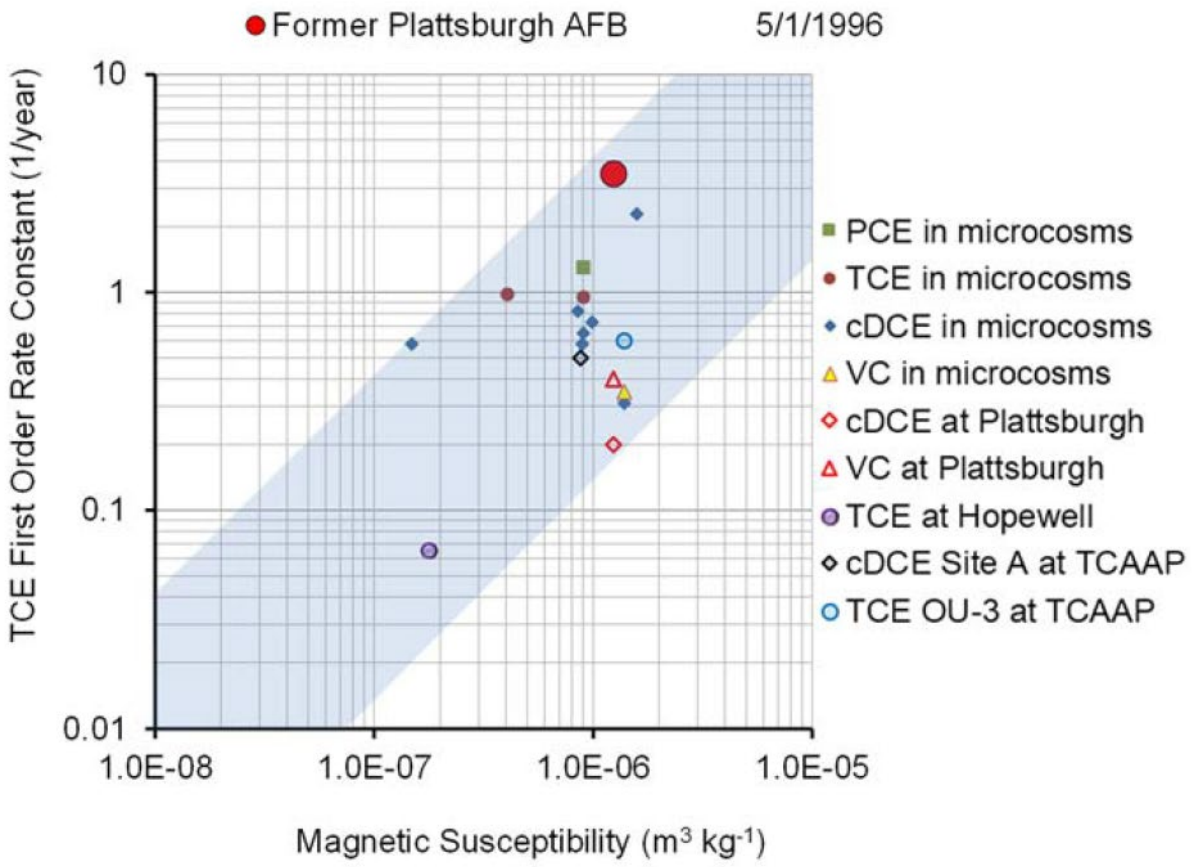
If the site-specific values fall within the blue shape, then mass magnetic susceptibility can explain the apparent in situ rate of TCE degradation.

HELP

Magnetite can mediate abiotic degradation of TCE. The amount of magnetite in aquifer material can be estimated from the mass magnetic susceptibility of core samples. Empirical data are available that associate degradation rate constants for TCE with mass magnetic susceptibility. The available data were used to define the blue shape in the figure. The figure is the chart in tab Mag. Sus. Explains TCE for an example data set. If the rate constant plots within the blue shape, then abiotic degradation mediated by magnetite can explain the observed rate constant.

If the rate constant plots above the blue shape, other processes are likely contributing to the rate of TCE degradation.

If the rate constant plots below the shape, inappropriate sampling locations may have been selected for mass magnetic susceptibility measurements. Mass magnetic susceptibility should be determined with aquifer material that is most transmissive to water since this is where most solute transport will occur. In addition, the input values used for the rate constant calculation with BIOCHLOR should be verified.



19. Does iron sulfide explain the TCE rate constant?

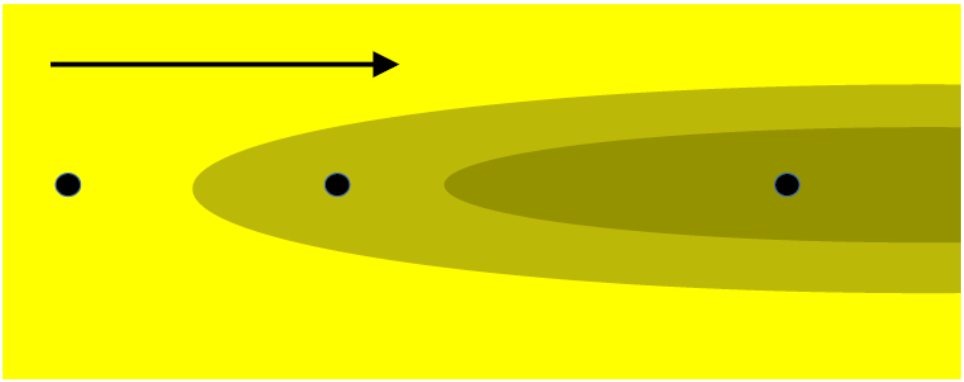
Decision Criteria

Open the tab Files and select the spreadsheet FeS.xlsx. If the distribution of sulfate shows a decrease in sulfate concentration along the flowpath, open the tab Sulfate Sag Along Flow Path and enter values for aquifer properties and for concentrations of sulfate.

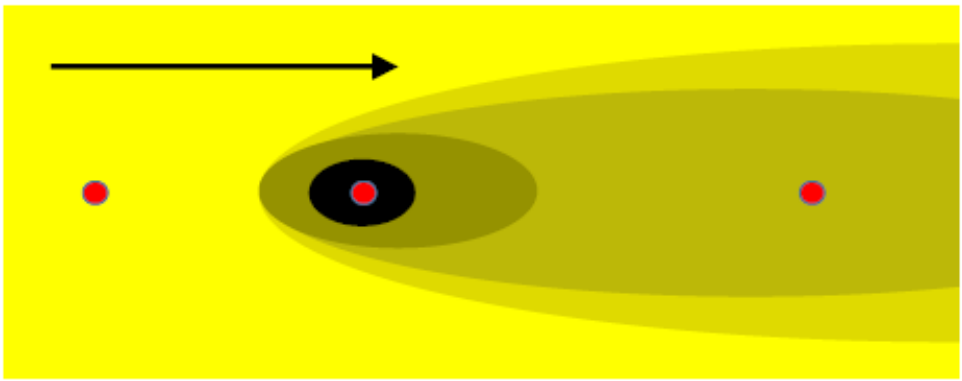
If the value of the rate constant in cell D28 or D29 (whichever is applicable) of the spreadsheet in the tab Sulfate Sag Along Flow Path is equal to or greater than the rate constant estimated using BIOCHLOR, then abiotic degradation by reactive iron sulfide minerals can explain the degradation rate constant for TCE.

If the lowest concentrations of sulfate are at the source of contamination, open the tab Lowest Sulfate at Source and enter values for aquifer properties and for concentrations of sulfate.

If the value of the rate constant in cell D31 or D32 of the tab Lowest Sulfate at Source (whichever is applicable) is equal to or greater than the rate constant from the BIOCHLOR simulation, then abiotic degradation by reactive iron sulfide minerals can explain the degradation rate constant for TCE.



Distribution of sulfate at a site where there is a decrease in sulfate concentration along the flowpath.



Distribution of sulfate at a site where the extent of sulfate depletion is greatest at the source.

HELP

Reactive iron sulfide minerals can mediate TCE degradation. 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.

The spreadsheets use data on the effective porosity, hydraulic gradient and hydraulic conductivity to estimate a seepage velocity of groundwater along a flow path. Then the spreadsheet uses the volumetric sulfate loading to estimate the consumption of sulfate and production of sulfide between an up-gradient well and a down-gradient well along the flow path. The spreadsheet assumes that excess Fe (III) is available in minerals in the aquifer matrix, and that the sulfide produced from the reduction of sulfate reacts to form FeS. The spreadsheet calculates the rate of production of FeS over time.

The spreadsheet models the inactivation of FeS as a first order process on the concentration of FeS present at any time. The user provides the elapsed time since sulfate reduction began at the site, and the

spreadsheet uses the volumetric sulfate loading and the rate of FeS inactivation to calculate the pool of accumulated reactive FeS. Then the spreadsheet uses the rate of degradation of TCE on reactive FeS to estimate a rate constant for TCE degradation along the flowpath between the two wells.

20. Is PCE present?

Decision Criteria

For the purposes of this decision support system, PCE is considered present when the concentration of PCE exceeds a cleanup goal for PCE that has been established for the site. If no cleanup goal for PCE has been established, PCE is considered present when the concentration is ≥ 5 µg/L. Other criteria may apply depending on the regulatory authority.

HELP

The cleanup goal is not always the U.S. EPA MCL established for drinking water. Consult the regulator for the cleanup goals that apply to the site of interest.

21. Is PCE degrading?

Decision Criteria

Access the computer simulation that you prepared to evaluate the criterion "Does Natural Attenuation Currently Meet the Goal?" Prepare a new simulation where the rate constant for degradation of PCE is set to zero. Compare the actual in situ concentrations of PCE against the new simulation. Then enter trial values for the rate constant for PCE degradation into the simulation to see if the model projections provide a better fit to the in situ concentrations.

If rate constants greater than zero provide a better fit, then TCE is degrading.

As an alternative, CSIA can be used to determine if PCE is degrading.

If the value of δ13C of TCE in a down gradient well is larger (less negative) than the value in an up gradient well by more than 0.5‰, that can be taken as evidence for degradation of PCE.

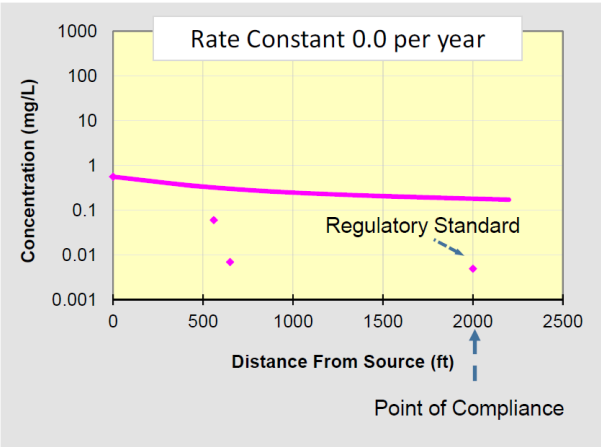
The highest value that has been reported for the δ13C of PCE used in commerce is -23.2‰.

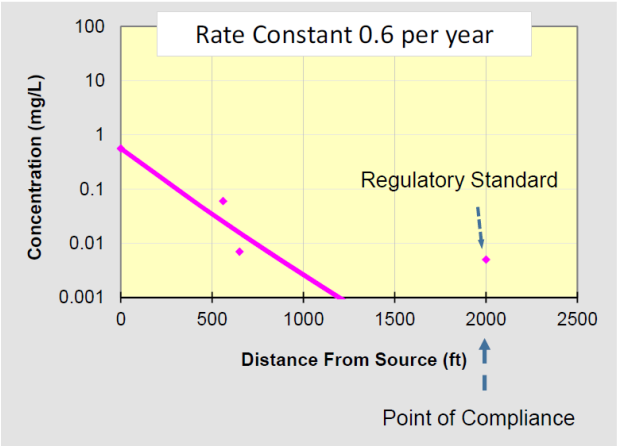
As a general rule, a value of δ13C for PCE that is greater than -22.7‰ can be taken as evidence of degradation of TCE.

HELP

A computer simulation of the transport and fate of PCE can reveal when PCE is degrading. The figure below is a hypothetical example, where the POC is 2,000 feet from the source of contamination, and the acceptable concentration of PCE at the POC was the MCL for PCE. The distance from the source and the acceptable concentration was entered in the input screen of BIOCHLOR so that it would plot in the RUN CENTERLINE output. The value for the rate constant for PCE degradation was set at zero to simulate the concentrations that would be expected if there were no degradation of PCE. The concentrations of PCE

in the field were lower than the simulation with no degradation of PCE. Trial values of the rate constant for degradation of DCE were selected. The rate constant for degradation of PCE that provided the best fit was 0.6 per year.





22. Are TCE, DCE, or VC present?

Decision Criteria

Evaluate data on concentrations of PCE, TCE, cDCE, tDCE and VC in wells down-gradient of the source of contamination. If the sum of TCE, cDCE, tDCE and VC is more than 5 mole % of the concentration of PCE, then TCE, cDCE, tDCE and VC are considered present. The presence of TCE, cDCE, tDCE or VC indicates that reductive dechlorination of PCE has occurred.

The calculation of mole % can easily be performed using the Excel file Mole Percent Calculator.xlsx. This tool is included in the BioPIC program as well as Appendix D.

HELP

The detection of TCE, cDCE, or VC at PCE-impacted sites suggests that PCE reductive dechlorination has occurred and this process may still be ongoing. Enumeration of pceA genes (present in PCE- and/or TCE-dechlorinating bacteria and implicated in PCE-to-TCE and PCE-to-cDCE reductive dechlorination) with qPCR provides support that bacteria capable of PCE reductive dechlorination to TCE or cDCE are present. The presence of the Dhc RDase genes tceA, bvcA or vcrA implicated in reductive dechlorination of DCE can explain the formation of VC and ethene.

23. Are TCE, DCE, or VC present in relevant concentrations?

Decision Criteria

Evaluate data on concentrations of PCE, TCE, cDCE, tDCE and VC in wells downgradient of the source of contamination. If the sum of TCE, cDCE, tDCE and VC is more than 25 mole % of the concentration of PCE,

then TCE, cDCE, tDCE and VC are present at relevant concentrations. The presence of daughter products at these concentrations indicates that microbial reductive dechlorination is an important pathway for TCE fate, and explains in a qualitative manner why TCE is degrading.

The calculation of mole % can easily be performed using the Excel file Mole Percent Calculator.xlsx. This tool is included in the BioPIC program and included in Appendix D.

24. Does magnetic susceptibility explain the PCE rate constant?

Decision Criteria

Consult the simulation that you prepared to evaluate the criterion "Does Natural Attenuation Currently Meet the Goal?" Prepare a new simulation. Do not include any portion of the plume where biological reductive dechlorination might contribute to the bulk rate constant that is extracted by the model. Exclude any portion of the flow path where the concentrations of any daughter products are increasing with distance from the source. By trial and error, identify the rate constant for degradation of TCE that provides the best match between the new simulation and the field data.

Open the tab Files and select the spreadsheet Magnetic Susceptibilty.xlsx. Enter your values for the rate constant for degradation of PCE and for mass magnetic susceptibility in the tab Input Data. Then open the tab Mag. Sus. Explains PCE.

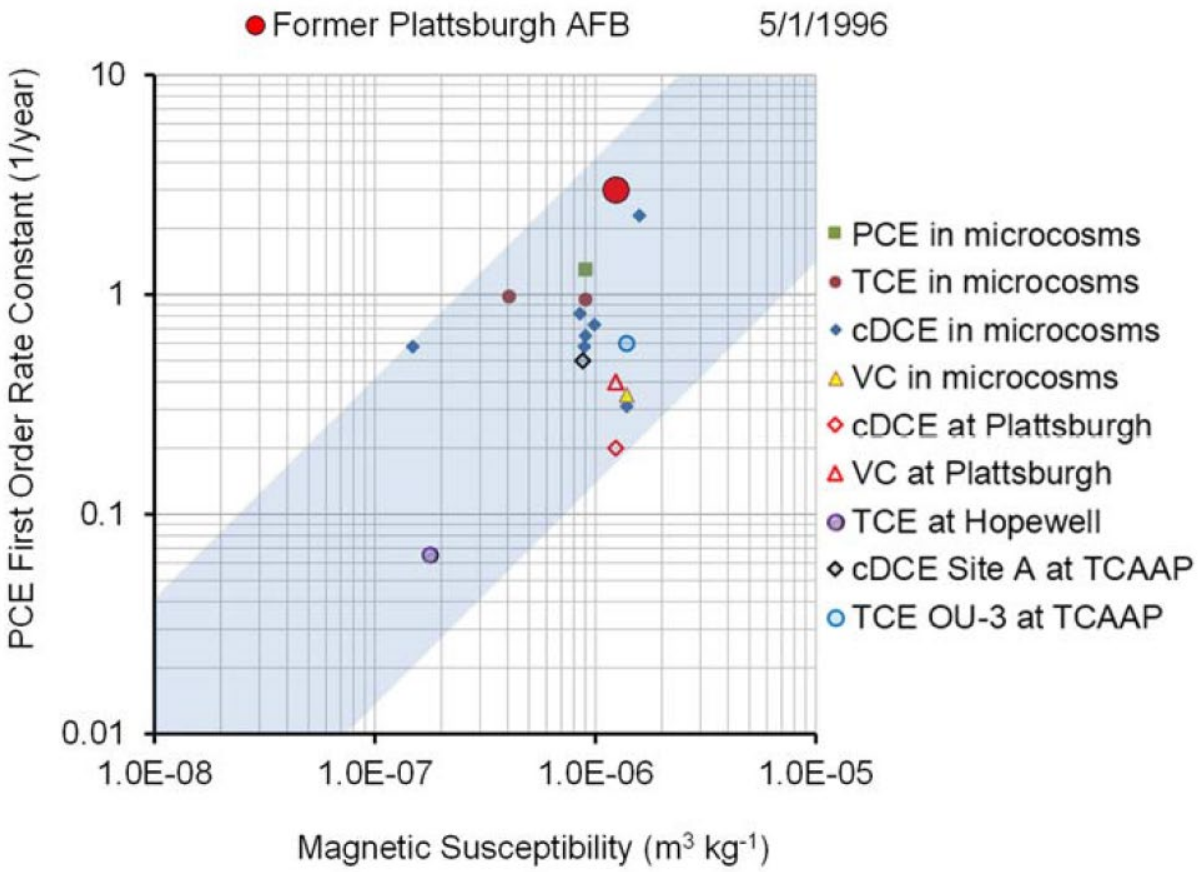
If the site-specific values fall within the blue shape, then mass magnetic susceptibility can explain the apparent in situ rate of PCE degradation.

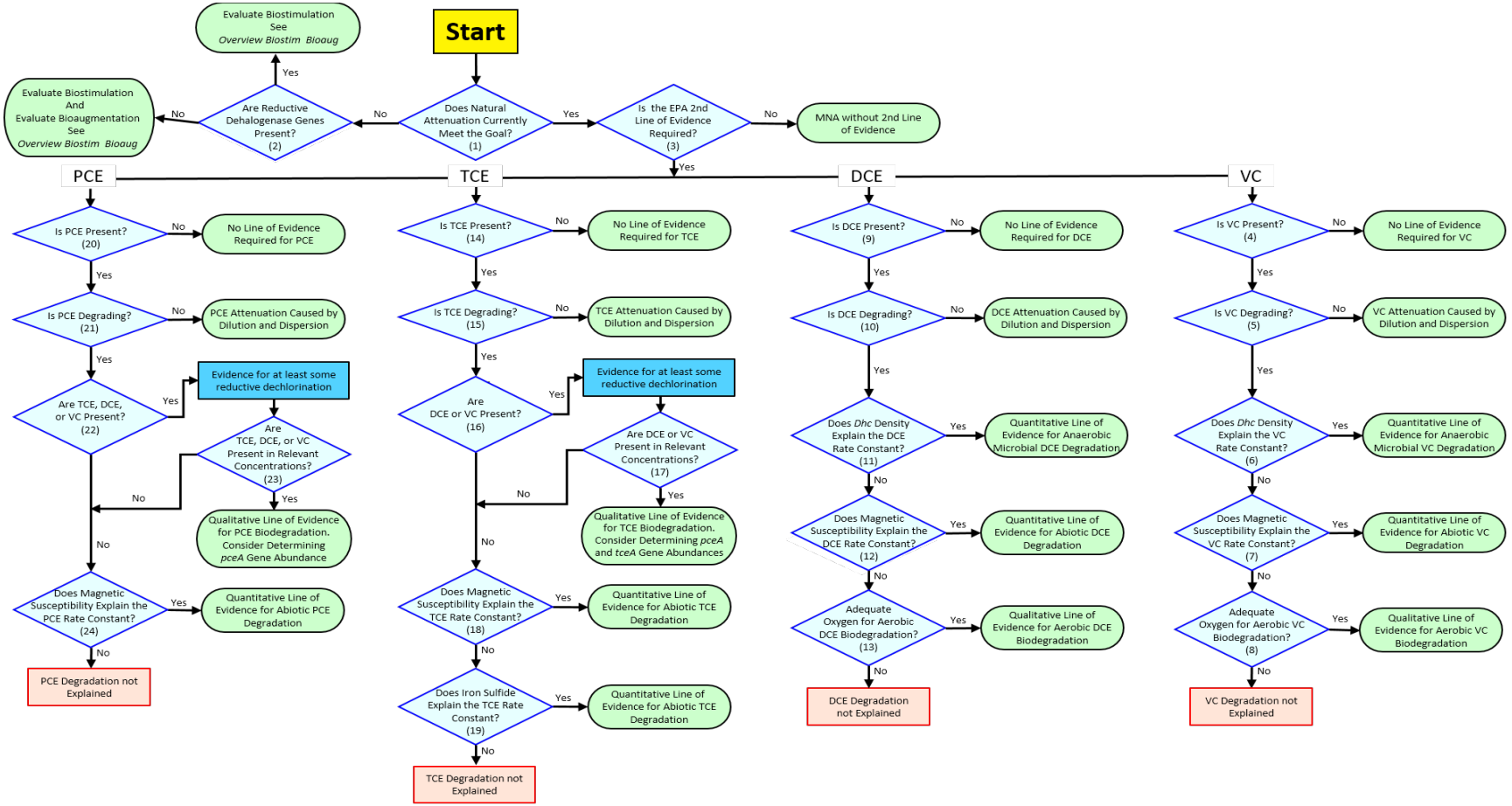
HELP

Magnetite can mediate abiotic degradation of PCE. The amount of magnetite in aquifer material can be estimated from the mass magnetic susceptibility of core samples. Empirical data are available that associate degradation rate constants for PCE with mass magnetic susceptibility. The available data were used to define the blue shape in the figure. The figure is the chart in tab Mag. Sus. Explains PCE for an example data set. If the rate constant plots within the blue shape, then abiotic degradation mediated by magnetite can explain the observed rate constant.

If the rate constant plots above the blue shape, other processes are likely contributing to the rate of PCE degradation.

If the rate constant plots below the shape, inappropriate sampling locations may have been selected for mass magnetic susceptibility measurements. Mass magnetic susceptibility should be determined with aquifer material that is most transmissive to water since this is where most solute transport will occur. In addition, the input values used for the rate constant calculation with BIOCHLOR should be verified.


Former Plattsburgh AFB
5/1/1996
PCE 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 (m3 kg-1)
PCE in microcosms
TCE in microcosms
cDCE in microcosms
VC in microcosms
cDCE at Plattsburgh
VC at Plattsburgh
TCE at Hopewell
cDCE Site A at TCAAP
TCE OU-3 at TCAAP



Decision Framework for Chlorinated Ethenes.

This flowchart was created as part of a previous ESTCP project (ER-201129) and was transferred into the updated BIOPIC tool.