

# Annex B – BIOCHLOR-ISO User's Guide

## 1. Introduction

This step by step user's guide is aimed to show the functioning of the Freeware Software for Compound-Specific Isotope Analysis (CSIA) BIOCHLOR-ISO version 2015 for data analysis for remediation assessment (Höhener et al., 2015). The Software is an integrative approach to better constrain the extent of total chloroethene degradation in groundwater samples. It is based on fitting of measured concentration and compound-specific stable carbon isotope data to an analytical reactive transport equation simulating steady-state plumes in two dimensions using an EXCEL spreadsheet. The fitting also yields estimates of degradation rates, of source width and of dispersivities.

This software allows to interpret and evaluate the concentrations and isotope data in order to assess remediation processes at a contaminated site. The User's Guide shows the necessary steps to achieve results applying the approach to a synthetic case. In the benchmark case, the true extent of degradation is well known and data come from real field cases known from literature.

Real data from a contaminated site was used at last to verify and demonstrate the program's functionality, advantages and limits applying to a real case with lower measurement data.

## 2. Using BIOCHLOR-ISO for remediation assesement

The Freeware Software for CSIA data analysis is attached to this manuscript or available online is the first of the three spreadsheets from the "Appendix A. Supplementary data" attached to the manuscript of Höhener (Höhener et al., 2015), available at the link:

<https://www.sciencedirect.com/science/article/pii/S0169772215300280?via%3Dihub>

This initial version contains default data values that need to be replaced with the correct values .

The Freeware Software is divided into 7 sheets, but the first sheet to examine is the "New User Tour" (Figure 1), where a concise guide of the Freeware Software is present.

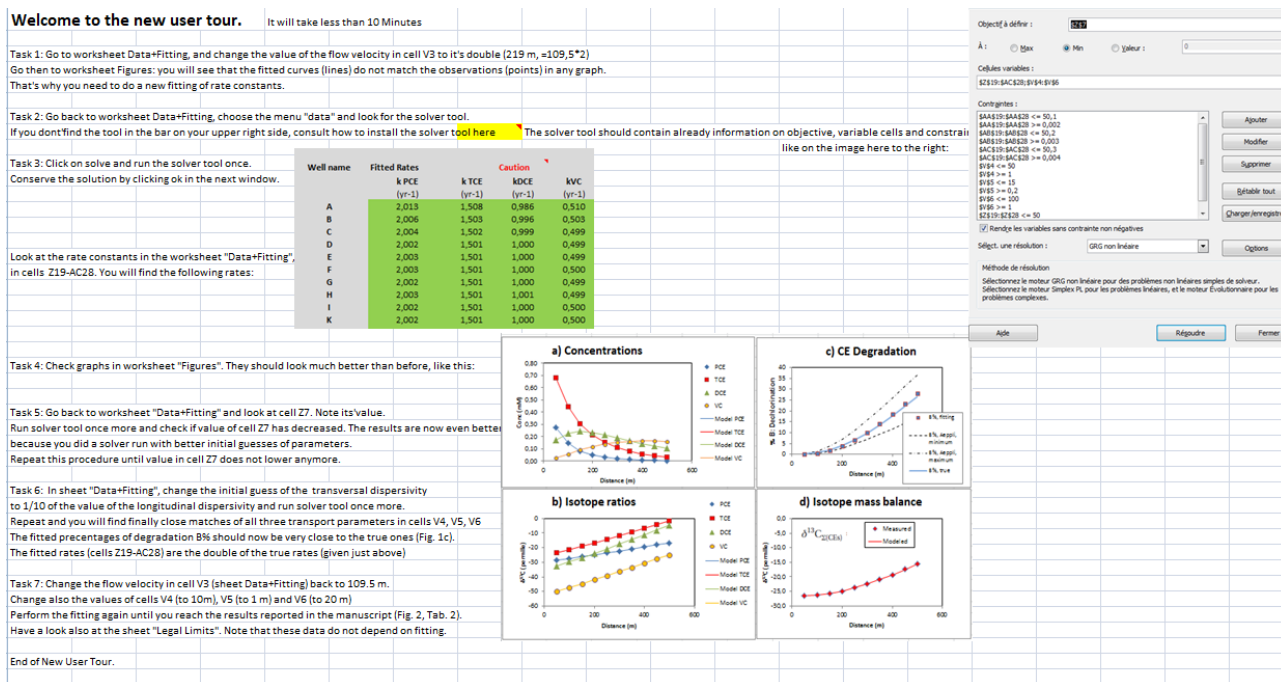


Figure 1 – Front page of the sheet “New user tour”

The remaining worksheets are “Data+Fitting”, where the users can enter the input and read the outputs; “Figures”, where output graphs are shown; “Legal limits”, where tables of contaminants limits (PCE, TCE, DCE and VC) in different countries are present; “References”, where list of references of the documents; “Data+FittingCase2”, where the users can insert the input and read the outputs of the Benchmark case 2; “FiguresCase2”, where output graphs of the Benchmark case 2 are shown.

The Benchmark case 1 is implemented in the software attached, therefore worksheets “Data+FittingCase2” and “FiguresCase2” (attached to (Höhener et al., 2015)) are not present, because they discuss two real cases.

### *Mandatory properties for the freeware software for CSIA*

Before implementing correct values in the freeware software for CSIA, it is mandatory to verify:

- ✓ The version of Microsoft Office: software not supported by previous versions of Microsoft Office 2010; previous versions are therefore not recommended.
- ✓ The presence of the solver tool;

The solver tool (if installed) is found in the upper right angle of the tool bar "Data" (to the right hand side of the menu list on the top row, third last menu). If solver tool does not show up, it must be activated:

- I. Go to Menu File, and chose "Options", that is the second last line;
- II. Click on "Add-Ins (Complements)", that is the second last choice;
- III. Go to bottom of windows, after "manage": choose "Excel Add-ins" from the drop down menu and click "Go...";
- IV. Now a new window opens and here tick only "Solver Add-in Component" (no tick "Euro Currency Tools", "Analysis tools" and "Analysis tools – VBA") and click "OK";
- V. Return to "Data" toolbar and find the solver add-in in the right hand side of the menu list.

For a full description of this tool, the users need to refer to "Help" (F1). Whereas, for screenshots of installation, users can look at the link:

<http://www.addictivetips.com/windows-tips/microsoft-office-excel-2010-solver-add-in/>

The solver tool should contain already information on objective, variable cells and constraints (as shown in Figure 2); users can see them clicking on "Solver" in the right hand side of the menu bar of "Data".

- ✓ The correct operation of the solver: the window containing variable cells and constraints must be equal to Figure 2; if different, verify that in "Control Panel → Change date time or number format → Additional settings" the decimal separator is the "." and not the ",".

**Solver Parameters**

Set Objective:

To: ☐ Max ☒ Min ☐ Value Of:

By Changing Variable Cells:

**Subject to the Constraints:**

\$A\$19:\$A\$28 <= 50.1  
 \$A\$19:\$A\$28 >= 0.002  
 \$B\$19:\$B\$28 <= 50.2  
 \$B\$19:\$B\$28 >= 0.003  
 \$C\$19:\$C\$28 <= 50.3  
 \$C\$19:\$C\$28 >= 0.004  
 \$V\$4 <= 50  
 \$V\$4 >= 1  
 \$V\$5 <= 15  
 \$V\$5 >= 0.2  
 \$V\$6 <= 100  
 \$V\$6 >= 1  
 \$Z\$19:\$Z\$28 <= 50

☒ Make Unconstrained Variables Non-Negative

Select a Solving Method:

**Solving Method**  
 Select the GRG Nonlinear engine for Solver Problems that are smooth nonlinear. Select the LP Simplex engine for linear Solver Problems, and select the Evolutionary engine for Solver problems that are non-smooth.

Figure 2 – Example of the solver tool implemented in the “Data+Fitting” worksheet

#### Parameters implementation for BIOCHLOR-ISO

The main worksheet of the freeware software for CSIA is “Data+Fitting”, where all the inputs have to be entered for the correct execution of the solver. A helpful coloured legend (Figure 3) is implemented in this worksheet to better understand if the cell type is a mandatory input, an optional input or a calculations cell.

| Colour codes of fields |  |                                                                     |
|------------------------|--|---------------------------------------------------------------------|
|                        |  | Mandatory data entry, maximum 15 observation wells                  |
|                        |  | Optional data. No problem if data are not entered.                  |
|                        |  | Fields with fitted results. Initial guesses needed.                 |
|                        |  | Calculated data or comments, no changes allowed                     |
|                        |  | Fields with objective functions for use in fitting with solver tool |
|                        |  | Isotope ratios or Concentrations, no entries.                       |

Figure 3 – Fields colour code

Be aware that colour intensities of calculated and measured cells are indicative of highest concentrations and enrichment.

In this example, the worksheet “Data+Fitting” was filled of random data and the users will find all the analytical passages and the correct input data (for benchmark case 1) to apply and run the software within this paragraph. The “Data+Fitting” worksheet is divided into several “zones” and the users need to enter parameters values only in Zone 1, 2, 3 and 4 (marked with yellow cells).

- ✓ In Zone 1 (named “measured isotope data”), enter the data concerning the distance where the water sample was taken (in meters) and the relative isotopic ratio measured for each contaminant. Replace the default data values entered in the excel file attached to this manuscript with the data entered in Figure 4. In specific, the values corresponding to the “X coord”, “PCE”, “TCE”, “cis-DCE” and “VC” need to be replaced. When available, you have the possibility to enter the isotopic trans-DCE, 1,1-DCE and ETH ratios. The “Y coord” reproduces the distance of the sampling point from the centerline of the plume: if Y=0, sampling points are along the centerline of the plume.

| Zone 1. MEASURED ISOTOPE DATA: Entry in ‰ |                 |                 |                                   |                                   |                                       |                                         |                                       |                                  |                                   |                                                 |                                                     |
|-------------------------------------------|-----------------|-----------------|-----------------------------------|-----------------------------------|---------------------------------------|-----------------------------------------|---------------------------------------|----------------------------------|-----------------------------------|-------------------------------------------------|-----------------------------------------------------|
| Well name                                 | X coord.<br>(m) | Y coord.<br>(m) | PCE<br>$\delta^{13}\text{C}$<br>‰ | TCE<br>$\delta^{13}\text{C}$<br>‰ | cis-DCE<br>$\delta^{13}\text{C}$<br>‰ | trans-DCE<br>$\delta^{13}\text{C}$<br>‰ | 1,1-DCE<br>$\delta^{13}\text{C}$<br>‰ | VC<br>$\delta^{13}\text{C}$<br>‰ | ETH<br>$\delta^{13}\text{C}$<br>‰ | $\delta^{13}\text{C}$ $\Sigma(\text{CEs})$<br>‰ | $\Sigma\text{CE+Eth}$<br>$\delta^{13}\text{C}$<br>‰ |
| Source                                    | 0               | 0               | ‰                                 | ‰                                 | ‰                                     | ‰                                       | ‰                                     | ‰                                | ‰                                 | ‰                                               | ‰                                                   |
| A                                         | 50              | 0               | -28.8                             | -23.3                             | -32.6                                 |                                         |                                       | -49.8                            | -72.8                             | -26.6                                           | -26.7                                               |
| B                                         | 100             | 0               | -27.6                             | -21.3                             | -29.8                                 |                                         |                                       | -47.3                            | -71.3                             | -26.3                                           | -26.7                                               |
| C                                         | 150             | 0               | -26.2                             | -19.1                             | -26.8                                 |                                         |                                       | -44.6                            | -69.6                             | -25.7                                           | -26.7                                               |
| D                                         | 200             | 0               | -24.9                             | -16.8                             | -23.8                                 |                                         |                                       | -41.9                            | -67.8                             | -24.9                                           | -26.7                                               |
| E                                         | 250             | 0               | -23.6                             | -14.4                             | -20.8                                 |                                         |                                       | -39.1                            | -66.0                             | -23.8                                           | -26.7                                               |
| F                                         | 300             | 0               | -22.2                             | -12.0                             | -17.6                                 |                                         |                                       | -36.3                            | -64.3                             | -22.5                                           | -26.7                                               |
| G                                         | 350             | 0               | -20.9                             | -9.4                              | -14.5                                 |                                         |                                       | -33.5                            | -62.5                             | -21.0                                           | -26.7                                               |
| H                                         | 400             | 0               | -19.5                             | -6.9                              | -11.3                                 |                                         |                                       | -30.7                            | -60.8                             | -19.3                                           | -26.7                                               |
| I                                         | 450             | 0               | -18.2                             | -4.3                              | -8.0                                  |                                         |                                       | -27.8                            | -59.2                             | -17.5                                           | -26.7                                               |
| K                                         | 500             | 0               | -16.8                             | -1.6                              | -4.7                                  |                                         |                                       | -24.9                            | -57.5                             | -15.5                                           | -26.7                                               |

Figure 4 – Measured isotope data of the benchmark case 1 in downgradient sampling points

- ✓ In Zone 2 (named “chemical data”), entering the distance of each sampling points is not necessary, because the worksheet automatically refers to those in zone 1, but it is necessary to enter the detected concentration values (in mg/L) as in Figure 5, related to the “PCE”, “TCE”, “cis-DCE” and “VC” columns. When available entering concentration values in “trans-DCE”, “1,1-DCE” and “ETH” columns is possible but not mandatory.

| Zone 2. CHEMICAL DATA: Entry in mg/L |                 |                 |                          |             |                 |                   |                 |            |             |
|--------------------------------------|-----------------|-----------------|--------------------------|-------------|-----------------|-------------------|-----------------|------------|-------------|
| Well name                            | X coord.<br>(m) | Y coord.<br>(m) | Measured data<br>in mg/L |             | cis-DCE<br>mg/L | trans-DCE<br>mg/L | 1,1-DCE<br>mg/L | VC<br>mg/L | ETH<br>mg/L |
|                                      |                 |                 | PCE<br>mg/L              | TCE<br>mg/L |                 |                   |                 |            |             |
| A                                    | 50              | 0               | 45.3                     | 89.2        | 16.4            |                   |                 | 1.6        | 0.1         |
| B                                    | 100             | 0               | 24.3                     | 58.6        | 22.1            |                   |                 | 3.7        | 0.2         |
| C                                    | 150             | 0               | 13.8                     | 40.2        | 23.3            |                   |                 | 5.7        | 0.4         |
| D                                    | 200             | 0               | 8.1                      | 28.3        | 22.5            |                   |                 | 7.3        | 0.7         |
| E                                    | 250             | 0               | 4.9                      | 20.2        | 20.7            |                   |                 | 8.6        | 1.1         |
| F                                    | 300             | 0               | 3.1                      | 14.5        | 18.5            |                   |                 | 9.5        | 1.5         |
| G                                    | 350             | 0               | 1.9                      | 10.5        | 16.1            |                   |                 | 10.0       | 1.9         |
| H                                    | 400             | 0               | 1.2                      | 7.6         | 13.9            |                   |                 | 10.3       | 2.2         |
| I                                    | 450             | 0               | 0.8                      | 5.5         | 11.8            |                   |                 | 10.3       | 2.6         |
| K                                    | 500             | 0               | 0.5                      | 4.0         | 9.9             |                   |                 | 10.1       | 3.0         |

Figure 5 – Measured concentration values of the benchmark case 1 in downgradient sampling points

- ✓ In Zones 2b (“chemical data”) and 2c (“molar percentages”), entering data is not necessary, because implemented calculations allow to achieve both the molar concentrations at each sampling point for contaminants and the percentages of each contaminant related to the total.
- ✓ In Zone 3 (“isotope parameters”) entering the hypothesized enrichment factors ( $\epsilon_i$ ), the isotopic signatures ( $\delta^{13}C_0$ ) and the concentrations (expressed in mM) detected in the source is mandatory
- ✓ In the case the initial concentrations of the source is known and expressed in mg/lit has to be converted in mM for the BIOCHLOR-ISO calculations. The concentration in mM/l are obtained dividing the concentration in mg/l by the gram formula weight of the compound as follows:

$$C_{PCE} = \frac{C_{PCE}}{165.8} \quad C_{TCE} = \frac{C_{TCE}}{(165.8 - 34.4)} \quad C_{\Sigma TCE} = \frac{C_{\Sigma TCE}}{96.9} \quad C_{VC} = \frac{C_{VC}}{62.5}$$

Where the denominator represents the gram formula weight of the compound.

Enter in the Excel sheet the correct data shown in Figure 6.

| Zone 3. ISOTOPE PARAMETERS    |                   |                      | Isotope ratios in source |                 |          |
|-------------------------------|-------------------|----------------------|--------------------------|-----------------|----------|
|                               | Enrichment factor | Fractionation factor |                          |                 |          |
|                               | $\epsilon$        | $\alpha$             | $\delta^{13}C_0$         | $R_0 + R_{ref}$ | Conc. mM |
| PCE                           | -4                | 0.996                | -30.0                    | 0.01090         | 0.5000   |
| TCE                           | -12               | 0.988                | -25.0                    | 0.01096         | 1.0000   |
| DCE                           | -20               | 0.98                 | 9999                     | 0.12360         | 0.0000   |
| VC                            | -25               | 0.975                | 9999                     | 0.12360         | 0.0000   |
| $\epsilon_{\Sigma(CB)}^{min}$ | -60               | Average              | -26.7                    | 0.011237        | 1.5000   |

Figure 6 – Isotope parameters detected in the contaminant source

The entered values are highlighted in yellow color, whereas the grey columns ( $\alpha$  and  $R_0 + R_{ref}$ ) reproduce the calculations of the program to achieve the fractionation factor ( $\alpha$ ) and isotope ratios ( $R_0 + R_{ref}$ ), by means of formulas:

$$\alpha = \frac{(1000 + \epsilon_i)}{1000}$$

$$R_0 + R_{ref} = \frac{\delta^{13}C_0}{1000 \cdot (R_0 + R_{ref})} + (R_0 + R_{ref})$$

- ✓ In Zone 4 (“transport parameters”), the implementation of the real groundwater flow velocity value (yellow cell) is mandatory and is calculated as:

$$v = \frac{k \cdot i}{\theta}$$

where  $k$  is the hydraulic conductivity,  $i$  the hydraulic gradient (calculated as ratio between two hydraulic heads and related distance) and  $\theta$  the effective porosity. Here, the users have to replace the value of 365 m/yr with the value of Figure 7.

| Zone 4. TRANSPORT PARAMETERS |       |      | True Values |
|------------------------------|-------|------|-------------|
| Flow velocity                | 109.5 | m/yr |             |
| long. dispersivity           | 19.97 | m    | 20          |
| trans. dispersivity          | 1.94  | m    | 2           |
| Source width                 | 29.58 | m    | 30          |

Figure 7 – Transport parameters

The longitudinal and transverse dispersivities, as source width (highlighted with green cells), are the results of a fitting procedure executed by the excel solver. The implementation of the initial values is mandatory and, following the run of the solver, values will be automatically updated. The transverse dispersivity value needs to be always inserted as 1/10 of the longitudinal dispersivity value (Gelhar et al., 1992). In grey cells the correct values of dispersivities and source width are reported (20, 2 and 30 m).

- ✓ Zone 5 (“fitting”) includes the parameters needed to the fitting procedure. Here, the measured and modelled isotope ratios values (5a) are compared: measured values are the same entered in Zone 1, whereas the first value entered (grey cell in Figure 8) is the isotope ratio of the source; where the values are missing, the “na” value appears; modelled values are calculated as function of  $k_i$ ,  $\Omega(K_i, x, y)$ ,  $\Omega_h(K_i, x, y)$ ,  $\Omega_i$  and  $(R_0 + R_{ref})_i$  for PCE, TCE,  $\Sigma$ DCE and VC (Figure 8). For the formulas see the supporting information attached (Hohener et al., 2015).

| Zone 5. FITTING                          |                       |                       |                       |                       |                       |                       |                       |                                            |                                            |
|------------------------------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|--------------------------------------------|--------------------------------------------|
| all values in ‰                          |                       |                       |                       |                       |                       |                       |                       |                                            |                                            |
| 5a: Measured and modelled isotope ratios |                       |                       |                       |                       |                       |                       |                       |                                            |                                            |
| Measured                                 | Modelled              | Measured              | Modelled              | Measured              | Modelled              | Measured              | Modelled              | Measured                                   | Modelled                                   |
| PCE                                      | PCE                   | TCE                   | TCE                   | $\Sigma$ DCE          | $\Sigma$ DCE          | VC                    | VC                    | $\delta^{13}\text{C}$ $\Sigma(\text{CEs})$ | $\delta^{13}\text{C}$ $\Sigma(\text{CEs})$ |
| $\delta^{13}\text{C}$                    | $\delta^{13}\text{C}$ | $\delta^{13}\text{C}$ | $\delta^{13}\text{C}$ | $\delta^{13}\text{C}$ | $\delta^{13}\text{C}$ | $\delta^{13}\text{C}$ | $\delta^{13}\text{C}$ | $\delta^{13}\text{C}$                      | $\delta^{13}\text{C}$                      |
| ‰                                        | ‰                     | ‰                     | ‰                     | ‰                     | ‰                     | ‰                     | ‰                     | ‰                                          | ‰                                          |
| -30.0                                    |                       | -25.0                 |                       |                       |                       |                       |                       |                                            |                                            |
| -28.8                                    | -28.8                 | -23.3                 | -23.3                 | -32.6                 | -32.6                 | -49.8                 | -49.8                 | -26.6                                      | -26.6                                      |
| -27.6                                    | -27.6                 | -21.3                 | -21.3                 | -29.8                 | -29.8                 | -47.3                 | -47.3                 | -26.3                                      | -26.3                                      |
| -26.2                                    | -26.2                 | -19.1                 | -19.1                 | -26.8                 | -26.8                 | -44.6                 | -44.6                 | -25.7                                      | -25.7                                      |
| -24.9                                    | -24.9                 | -16.8                 | -16.8                 | -23.8                 | -23.8                 | -41.9                 | -41.9                 | -24.9                                      | -24.9                                      |
| -23.6                                    | -23.6                 | -14.4                 | -14.4                 | -20.8                 | -20.8                 | -39.1                 | -39.1                 | -23.8                                      | -23.8                                      |
| -22.2                                    | -22.2                 | -12.0                 | -12.0                 | -17.6                 | -17.6                 | -36.3                 | -36.3                 | -22.5                                      | -22.5                                      |
| -20.9                                    | -20.9                 | -9.4                  | -9.4                  | -14.5                 | -14.5                 | -33.5                 | -33.5                 | -21.0                                      | -21.0                                      |
| -19.5                                    | -19.5                 | -6.9                  | -6.9                  | -11.3                 | -11.3                 | -30.7                 | -30.7                 | -19.3                                      | -19.3                                      |
| -18.2                                    | -18.2                 | -4.3                  | -4.3                  | -8.0                  | -8.0                  | -27.8                 | -27.8                 | -17.5                                      | -17.5                                      |
| -16.8                                    | -16.8                 | -1.6                  | -1.6                  | -4.7                  | -4.7                  | -24.9                 | -24.9                 | -15.5                                      | -15.5                                      |

Figure 8 – Comparison between measured and modelled isotope ratios

Always in Zone 5, the users need to implement positive values for constant rates (green cells in Figure 9): for initial guesses, values of 4, 3, 2, and 1  $\text{y}^{-1}$  for PCE, TCE, DCE, and VC were implemented and copied down vertically in all fields in columns “k PCE”, “k TCE”, “k DCE” and “k VC”. These values will be automatically changed by the worksheet after running the solver (fitting procedure). Be careful that identical numbers in one horizontal line create failure of calculations. After the solver run, the fitted values of the constant rates are shown in green cells,

as reported in Figure 9. In the blue cells no values need to be entered by users, because these cells are related to the calculations needed by the solver algorithm.

| Average Rates |                 |                 |                 |                |                                               |                   |           |           |           |           |
|---------------|-----------------|-----------------|-----------------|----------------|-----------------------------------------------|-------------------|-----------|-----------|-----------|-----------|
|               | 1.000           | 0.750           | 0.500           | 0.250          |                                               |                   |           |           |           |           |
| True          | 1               | 0.75            | 0.5             | 0.25           |                                               |                   |           |           |           |           |
| Well name     | Fitted Rates    |                 |                 |                | Calculation of Objectives for Isotope fitting |                   |           |           |           |           |
|               | k PCE<br>(yr-1) | k TCE<br>(yr-1) | k DCE<br>(yr-1) | k VC<br>(yr-1) | $\delta^{13}\text{C}$ $\Sigma(\text{CEs})$    | $\Sigma$ Isotopes | PCE       | TCE       | DCE       | VC        |
|               |                 |                 |                 |                | Objective                                     | Objective         | Objective | Objective | Objective | Objective |
|               |                 |                 |                 |                | 17.186                                        | 0.000             | 0.000     | 0.000     | 0.000     | 0.000     |
| A             | 1.000           | 0.750           | 0.500           | 0.250          | 9.11E-07                                      | 3.82E-08          | 3.31E-08  | 6.87E-10  | 7.05E-10  | 3.80E-09  |
| B             | 1.000           | 0.750           | 0.500           | 0.250          | 4.58E-07                                      | 1.92E-08          | 1.72E-08  | 5.29E-11  | 3.99E-10  | 1.54E-09  |
| C             | 1.000           | 0.750           | 0.500           | 0.250          | 1.41E-07                                      | 8.64E-09          | 6.61E-09  | 1.42E-10  | 8.30E-10  | 1.06E-09  |
| D             | 1.000           | 0.750           | 0.500           | 0.250          | 9.96E-09                                      | 5.17E-09          | 3.00E-09  | 5.31E-11  | 1.08E-09  | 1.04E-09  |
| E             | 1.000           | 0.750           | 0.500           | 0.250          | 1.72E+01                                      | 1.68E-09          | 3.83E-10  | 0.00E+00  | 6.80E-10  | 6.18E-10  |
| F             | 1.000           | 0.750           | 0.500           | 0.250          | 1.08E-07                                      | 1.60E-09          | 2.41E-11  | 4.78E-10  | 4.01E-10  | 6.92E-10  |
| G             | 1.000           | 0.750           | 0.500           | 0.250          | 2.32E-07                                      | 1.97E-09          | 6.05E-10  | 5.76E-10  | 1.90E-10  | 6.01E-10  |
| H             | 1.000           | 0.750           | 0.500           | 0.250          | 3.55E-07                                      | 3.01E-09          | 2.08E-09  | 3.92E-10  | 2.06E-10  | 3.34E-10  |
| I             | 1.000           | 0.750           | 0.500           | 0.250          | 4.65E-07                                      | 4.09E-09          | 3.38E-09  | 4.18E-10  | 1.21E-10  | 1.79E-10  |
| K             | 1.000           | 0.750           | 0.500           | 0.250          | 5.57E-07                                      | 5.91E-09          | 5.23E-09  | 4.67E-10  | 1.08E-10  | 1.10E-10  |

Figure 9 – Degradation constant rates, average constant rates and calculation of objectives for isotope fitting

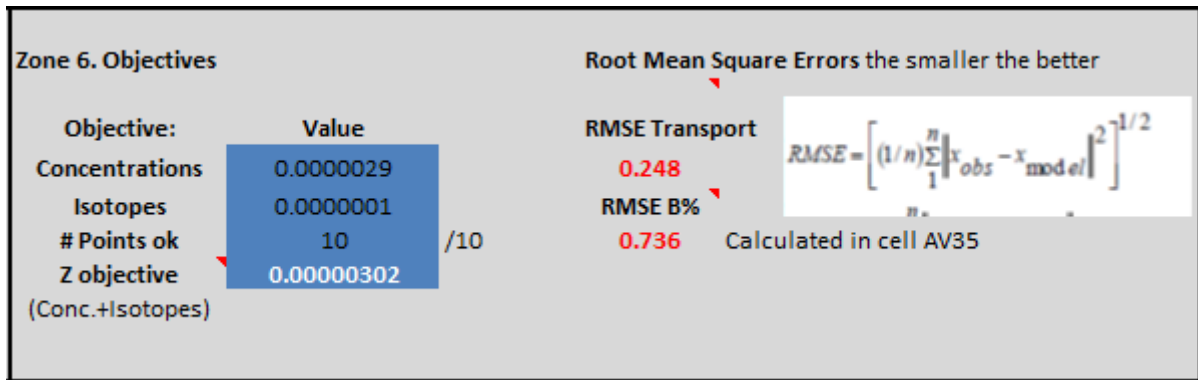
- ✓ In Zone 5b, measured and modelled concentrations values are compared: measured values are the same entered in Zone 2; where the values are missing, the “na” value appears; modelled values are calculated as function of  $k_i$ ,  $\Omega_{(K_i,x,y)}$ ,  $\Omega_{h(K_i,x,y)}$  and measured concentrations for PCE, TCE,  $\Sigma$ DCE and VC (Figure 10). For the formulas see the supporting information attached to (Höhener et al., 2015). A least squares approach is used to minimize the difference between modelled and measured parameters.

| 5b: Measured and modelled concentrations |          |          |          |                   |              |          |          |
|------------------------------------------|----------|----------|----------|-------------------|--------------|----------|----------|
| Measured                                 | Modelled | Measured | Modelled | Measured          | Modelled     | Measured | Modelled |
| PCE (mM)                                 | PCE (mM) | TCE (mM) | TCE (mM) | $\Sigma$ DCE (mM) | $\Sigma$ DCE | VC (mM)  | VC (mM)  |
| 0.2731                                   | 0.2728   | 0.6790   | 0.6784   | 0.1694            | 0.1693       | 0.0254   | 0.0254   |
| 0.1463                                   | 0.1462   | 0.4461   | 0.4459   | 0.2281            | 0.2281       | 0.0589   | 0.0589   |
| 0.0831                                   | 0.0831   | 0.3060   | 0.3060   | 0.2410            | 0.2409       | 0.0906   | 0.0906   |
| 0.0491                                   | 0.0491   | 0.2154   | 0.2154   | 0.2324            | 0.2324       | 0.1173   | 0.1173   |
| 0.0298                                   | 0.0298   | 0.1537   | 0.1537   | 0.2137            | 0.2137       | 0.1377   | 0.1377   |
| 0.0184                                   | 0.0184   | 0.1105   | 0.1105   | 0.1906            | 0.1906       | 0.1519   | 0.1519   |
| 0.0115                                   | 0.0115   | 0.0798   | 0.0798   | 0.1665            | 0.1665       | 0.1606   | 0.1606   |
| 0.0073                                   | 0.0073   | 0.0578   | 0.0578   | 0.1432            | 0.1432       | 0.1644   | 0.1644   |
| 0.0046                                   | 0.0046   | 0.0419   | 0.0419   | 0.1217            | 0.1218       | 0.1644   | 0.1644   |
| 0.0030                                   | 0.0030   | 0.0304   | 0.0304   | 0.1026            | 0.1026       | 0.1612   | 0.1612   |

Figure 10 – Measured and simulated concentrations

- ✓ In Zone 6 (“objectives”): measured concentrations are compared to concentrations modelled by the exact analytical Biochlor model and the sum of squared errors are reported in blue cell named “Concentrations” (cell Z4 and Figure 11). Measured isotope ratios are likewise compared to a “Isotope-Biochlor” model and squared errors sums are reported in cell “Isotopes” (cell Z5). The two objectives are then added to the best objective (cell Z7 = “Z objective”), as in the equation 5 of manuscript (Höhener et al., 2015). “RMSE Transport” is the Sum of error for the three parameters longitudinal and transverse dispersivity and source width, whereas RMSE B% is the error for the prediction of biodegradation beyond Vinyl chloride, B%/fitted. Fitting using the solver function aims at finding the parameters which give the lowest value in the Z objective cell. Alternatively, if concentrations are hard to fit, only isotope ratios may be fitted. They hardly depend on source

width and transversal dispersivity. The results of the fitting are controlled on the worksheet figures and in Zone 7.



**Figure 11** – Measured and modelled concentrations of contaminants

- ✓ In Zone 7 (“calculation of overall dechlorination”), no need to implement any parameters but the percentage of the overall dechlorination of chloroethene is calculated. Different approaches are used to calculate the parameter  $B_{(CE)}$ :
  - Aeppli et al. (Aeppli et al., 2010) quantify in situ transformation rates based on groundwater dating and isotope mass balances; they introduce the target  $B_{\Sigma(CE)}$ , to designate the percentage of dechlorination of parent chloroethene beyond vinyl chloride to non-chlorinated breakdown products (see the parameter “B%CE” in Figure 12).
  - Höyng et al. (Höyng et al., 2015) developed an alternative approach without the use of isotope data when concentrations of a non-reactive tracer are known (see the parameter “B True %” in Figure 12)
  - Höhener et al. (Höhener et al., 2015) take example from both previous approaches and calculate the target using both isotope and concentrations data, starting from the set of 4 degradation rates constants for each monitoring well (see the parameter “B fitted %” in Figure 12); they also replace  $\Omega_{(ki,t)}$  with  $\Omega_{(ki,x,y)}$  which is a solution for a 2D reactive transport at steady state.

The formulas of each approach are discussed in (Höhener et al., 2015): see Equations 1a, 1b, 1c, 1d, 2, 3a, 3b and 4.

Be careful because if negative B% values are calculated, something is wrong in the fitted parameters.

| Calculation of Objectives for Concentration fitting |                   |                     |                     |         |                       |                |           |           |           |           |           |
|-----------------------------------------------------|-------------------|---------------------|---------------------|---------|-----------------------|----------------|-----------|-----------|-----------|-----------|-----------|
| Zone 7. Calculation of overall dechlorination       |                   |                     |                     |         |                       | Concentrations | PCE       | TCE       | DCE       | VC        | Points ok |
| Well                                                | B fitted %        | B % CE              | B % CE              | Epsilon | Overall rate          | Objective      | Objective | Objective | Objective | Objective |           |
|                                                     | from rates, eq. 3 | Aeppli, min, eq. 1d | Aeppli, max, eq. 1c | %       | Dechlorination (yr-1) |                |           |           |           |           |           |
| A                                                   | 0.1               | 0.2                 | 0.4                 | -106.4  | 0.250                 | 1.61E-06       | 9.42E-07  | 6.34E-07  | 2.47E-08  | 5.59E-09  | 1.0       |
| B                                                   | 0.6               | 0.7                 | 1.6                 | -62.8   | 0.250                 | 3.77E-07       | 2.42E-07  | 1.16E-07  | 1.86E-08  | 5.66E-10  | 1.0       |
| C                                                   | 1.9               | 1.6                 | 3.8                 | -51.2   | 0.250                 | 9.31E-08       | 6.91E-08  | 1.88E-08  | 4.87E-09  | 3.07E-10  | 1.0       |
| D                                                   | 3.9               | 3.0                 | 7.0                 | -45.6   | 0.250                 | 1.38E-08       | 1.27E-08  | 2.51E-10  | 4.39E-10  | 3.80E-10  | 1.0       |
| E                                                   | 6.7               | 4.8                 | 11.0                | -42.2   | 0.250                 | 5.93E-09       | 4.18E-12  | 4.80E-09  | 5.02E-10  | 6.29E-10  | 1.0       |
| F                                                   | 10.1              | 6.9                 | 15.7                | -39.8   | 0.250                 | 3.27E-08       | 8.78E-09  | 1.92E-08  | 3.54E-09  | 1.18E-09  | 1.0       |
| G                                                   | 14.2              | 9.3                 | 20.7                | -38.0   | 0.250                 | 8.25E-08       | 3.26E-08  | 3.90E-08  | 8.82E-09  | 2.06E-09  | 1.0       |
| H                                                   | 18.6              | 11.8                | 26.0                | -36.6   | 0.250                 | 1.50E-07       | 6.75E-08  | 6.33E-08  | 1.58E-08  | 3.44E-09  | 1.0       |
| I                                                   | 23.3              | 14.6                | 31.4                | -35.4   | 0.250                 | 2.34E-07       | 1.14E-07  | 9.02E-08  | 2.45E-08  | 5.23E-09  | 1.0       |
| K                                                   | 28.2              | 17.4                | 36.7                | -34.5   | 0.250                 | 3.32E-07       | 1.70E-07  | 1.20E-07  | 3.47E-08  | 7.52E-09  | 1.0       |

| Auxiliary Calculations: Evolution of concentrations as a function of fitted rates |      |      |      |      |            |        |                | Check | For Benchmark Problems only: Zone with tracer data and true B% |            |                                                                                |          |
|-----------------------------------------------------------------------------------|------|------|------|------|------------|--------|----------------|-------|----------------------------------------------------------------|------------|--------------------------------------------------------------------------------|----------|
| PCE                                                                               | TCE  | DCE  | VC   | Sum  | Correction | ε SCE  | B fitted recal |       | RMSE                                                           | 0.73601001 | $RMSE = \left[ \frac{1}{n} \sum_{i=1}^n (x_{obs} - x_{model})^2 \right]^{1/2}$ |          |
|                                                                                   |      |      |      |      |            | %      | %              |       |                                                                |            |                                                                                |          |
| 0.32                                                                              | 0.86 | 0.29 | 0.03 | 1.50 | 1.0        | -106.4 | 0.1            |       |                                                                |            | True Tracer                                                                    | B True % |
| 0.20                                                                              | 0.71 | 0.47 | 0.11 | 1.49 | 1.0        | -62.8  | 0.6            |       |                                                                |            | A                                                                              | 0.766    |
| 0.13                                                                              | 0.57 | 0.57 | 0.21 | 1.47 | 0.0        | -51.2  | 1.9            |       |                                                                |            | B                                                                              | 0.591    |
| 0.08                                                                              | 0.44 | 0.60 | 0.32 | 1.44 | 0.0        | -45.6  | 3.9            |       |                                                                |            | C                                                                              | 0.491    |
| 0.05                                                                              | 0.34 | 0.60 | 0.41 | 1.40 | 0.0        | -42.2  | 6.7            |       |                                                                |            | D                                                                              | 0.427    |
| 0.03                                                                              | 0.26 | 0.57 | 0.49 | 1.35 | 0.0        | -39.8  | 10.1           |       |                                                                |            | E                                                                              | 0.382    |
| 0.02                                                                              | 0.19 | 0.52 | 0.56 | 1.29 | 0.0        | -38.0  | 14.2           |       |                                                                |            | F                                                                              | 0.349    |
| 0.01                                                                              | 0.14 | 0.46 | 0.60 | 1.22 | 0.0        | -36.6  | 18.6           |       |                                                                |            | G                                                                              | 0.323    |
| 0.01                                                                              | 0.10 | 0.41 | 0.63 | 1.15 | 0.0        | -35.4  | 23.3           |       |                                                                |            | H                                                                              | 0.302    |
| 0.01                                                                              | 0.08 | 0.35 | 0.65 | 1.08 | 0.0        | -34.5  | 28.2           |       |                                                                |            | I                                                                              | 0.285    |
|                                                                                   |      |      |      |      |            |        |                |       |                                                                |            | K                                                                              | 0.270    |

Figure 12 – Parameters of the overall dichlorination of chloroethene

- ✓ Zone 8 is optional and is only a space where users can enter detailed site specification
- ✓ In Zone 9 (“numerical integrations of eq S4”) no need to enter values, because the section introduce all the calculation for different  $\Omega$  parameters (Figure 13), that are reactive transport equations for the heavy isotope species.

| Zone 9. Numerical Integrations of eq. S4 |                     |                    |           |           |           |           |           |           |            |            |           |           |             |
|------------------------------------------|---------------------|--------------------|-----------|-----------|-----------|-----------|-----------|-----------|------------|------------|-----------|-----------|-------------|
| Omega 1                                  |                     | No changes, please |           |           |           |           |           |           |            |            |           |           |             |
| Well                                     | $\Omega(k_1, x, y)$ | dt                 | 0.001     | 0.00115   | 0.0013225 | 0.0015209 | 0.001749  | 0.0020114 | 0.002313   | 0.00266002 | 0.0031    | 0.0035179 | 0.0040456   |
|                                          |                     | Sum dt             | 0.001     | 0.00215   | 0.0034725 | 0.0049934 | 0.0067424 | 0.0087537 | 0.011067   | 0.01372682 | 0.0168    | 0.0203037 | 0.0243493   |
|                                          |                     | Time (yrs)         | 0.001     | 0.002     | 0.003     | 0.004     | 0.006     | 0.008     | 0.01       | 0.01       | 0.02      | 0.02      | 0.03        |
| A                                        | 0.5456              | 3.5207E-246        | 1.989E-77 | 4.336E-43 | 1.815E-28 | 1.886E-20 | 1.933E-15 | 4.78E-12  | 1.2712E-09 | 8E-08      | 1.889E-06 | 2.218E-05 | 0.000155811 |
| B                                        | 0.2925              | 0                  | 0         | 5.28E-175 | 6.79E-116 | 2.288E-83 | 5.885E-63 | 4.46E-49  | 4.0741E-39 | 1E-31      | 5.499E-26 | 1.626E-21 | 5.98224E-18 |
| C                                        | 0.1661              | 0                  | 0         | 0         | 5.73E-262 | 1.37E-188 | 1.63E-142 | 3.7E-111  | 1.232E-88  | 8E-72      | 6.579E-59 | 9.06E-49  | 1.13464E-40 |
| D                                        | 0.0982              | 0                  | 0         | 0         | 0         | 0         | 4.13E-254 | 2.7E-198  | 3.515E-158 | 3E-128     | 3.23E-105 | 3.839E-87 | 1.06313E-72 |
| E                                        | 0.0596              | 0                  | 0         | 0         | 0         | 0         | 0         | 0         | 9.462E-248 | 7E-201     | 6.51E-165 | 1.24E-136 | 4.9209E-114 |
| F                                        | 0.0368              | 0                  | 0         | 0         | 0         | 0         | 0         | 0         | 0          | 7E-290     | 5.39E-238 | 3.03E-197 | 1.1252E-164 |
| G                                        | 0.0230              | 0                  | 0         | 0         | 0         | 0         | 0         | 0         | 0          | 0          | 0         | 5.64E-269 | 1.271E-224  |
| H                                        | 0.0145              | 0                  | 0         | 0         | 0         | 0         | 0         | 0         | 0          | 0          | 0         | 0         | 7.0926E-294 |
| I                                        | 0.0093              | 0                  | 0         | 0         | 0         | 0         | 0         | 0         | 0          | 0          | 0         | 0         | 0           |
| K                                        | 0.0059              | 0                  | 0         | 0         | 0         | 0         | 0         | 0         | 0          | 0          | 0         | 0         | 0           |
| O                                        | 0.0000              | 178.7628757        | 36.717185 | 17.676769 | 10.980973 | 7.719808  | 5.836026  | 4.625825  | 3.78939619 | 3.1795     | 2.7160515 | 2.3522614 | 2.058984283 |
| O                                        | 0.0000              | 178.7628757        | 36.717185 | 17.676769 | 10.980973 | 7.719808  | 5.836026  | 4.625825  | 3.78939619 | 3.1795     | 2.7160515 | 2.3522614 | 2.058984283 |
| O                                        | 0.0000              | 178.673536         | 36.659414 | 17.627156 | 10.934599 | 7.6746516 | 5.7909926 | 4.580218  | 3.74271983 | 3.1313     | 2.6661579 | 2.3003367 | 2.00479762  |
| O                                        | 0.0000              | 178.6735166        | 36.659401 | 17.627145 | 10.934589 | 7.6746418 | 5.7909829 | 4.580208  | 3.74270975 | 3.1313     | 2.6661471 | 2.3003255 | 2.004786002 |
| O                                        | 0.0000              | 178.7628757        | 36.717185 | 17.676769 | 10.980973 | 7.719808  | 5.836026  | 4.625825  | 3.78939619 | 3.1795     | 2.7160515 | 2.3522614 | 2.058984283 |

| Omega 2 |                     | No changes, please |           |           |           |           |           |           |            |            |           |           |             |
|---------|---------------------|--------------------|-----------|-----------|-----------|-----------|-----------|-----------|------------|------------|-----------|-----------|-------------|
| Well    | $\Omega(k_2, x, y)$ | dt                 | 0.001     | 0.00115   | 0.0013225 | 0.0015209 | 0.001749  | 0.0020114 | 0.002313   | 0.00266002 | 0.0031    | 0.0035179 | 0.0040456   |
| A       | 0.5899              | 3.5211E-246        | 1.99E-77  | 4.339E-43 | 1.817E-28 | 1.889E-20 | 1.937E-15 | 4.79E-12  | 1.2752E-09 | 8E-08      | 1.898E-06 | 2.23E-05  | 0.000156854 |
| B       | 0.3436              | 0                  | 0         | 5.28E-175 | 6.8E-116  | 2.292E-83 | 5.897E-63 | 4.47E-49  | 4.0868E-39 | 1E-31      | 5.525E-26 | 1.635E-21 | 6.02226E-18 |
| C       | 0.2127              | 0                  | 0         | 0         | 5.74E-262 | 1.37E-188 | 1.64E-142 | 3.7E-111  | 1.2358E-88 | 8E-72      | 6.604E-59 | 9.111E-49 | 1.14223E-40 |
| D       | 0.1373              | 0                  | 0         | 0         | 0         | 0         | 4.14E-254 | 2.7E-198  | 3.526E-158 | 3E-128     | 3.24E-105 | 3.86E-87  | 1.07024E-72 |
| E       | 0.0910              | 0                  | 0         | 0         | 0         | 0         | 0         | 0         | 9.492E-248 | 7E-201     | 6.54E-165 | 1.24E-136 | 4.9538E-114 |
| F       | 0.0614              | 0                  | 0         | 0         | 0         | 0         | 0         | 0         | 0          | 7E-290     | 5.41E-238 | 3.04E-197 | 1.1327E-164 |
| G       | 0.0420              | 0                  | 0         | 0         | 0         | 0         | 0         | 0         | 0          | 0          | 0         | 5.67E-269 | 1.2795E-224 |
| H       | 0.0290              | 0                  | 0         | 0         | 0         | 0         | 0         | 0         | 0          | 0          | 0         | 0         | 7.14E-294   |
| I       | 0.0201              | 0                  | 0         | 0         | 0         | 0         | 0         | 0         | 0          | 0          | 0         | 0         | 0           |
| K       | 0.0141              | 0                  | 0         | 0         | 0         | 0         | 0         | 0         | 0          | 0          | 0         | 0         | 0           |
| O       | 0.0000              | 178.7628757        | 36.717185 | 17.676769 | 10.980973 | 7.719808  | 5.836026  | 4.625825  | 3.78939619 | 3.1795     | 2.7160515 | 2.3522614 | 2.058984283 |
| O       | 0.0000              | 178.7628757        | 36.717185 | 17.676769 | 10.980973 | 7.719808  | 5.836026  | 4.625825  | 3.78939619 | 3.1795     | 2.7160515 | 2.3522614 | 2.058984283 |
| O       | 0.0000              | 178.6958647        | 36.673847 | 17.639545 | 10.946173 | 7.6859149 | 5.8022173 | 4.591576  | 3.75433372 | 3.1433     | 2.6785436 | 2.3132083 | 2.018207919 |
| O       | 0.0000              | 178.6958521        | 36.673839 | 17.639538 | 10.946167 | 7.6859085 | 5.802211  | 4.59157   | 3.75432716 | 3.1433     | 2.6785366 | 2.313201  | 2.018200335 |
| O       | 0.0000              | 178.7628757        | 36.717185 | 17.676769 | 10.980973 | 7.719808  | 5.836026  | 4.625825  | 3.78939619 | 3.1795     | 2.7160515 | 2.3522614 | 2.058984283 |

Figure 13 – Table of the  $\Omega$  parameters

### *Run of the solver*

After all zones are filled, the users can run the solver tool (upper right angle of the tool bar "Data", as in Figure 2), targeting Z objective (cell Z7) in Zone 6. The variables are: degradation rate constants (cells Z19:AC28) in Zone 5b, dispersivities and source width in Zone 4 (cells V4, V5 and V6). Now, the users can check the graphs on worksheet figures. If the graphs are the same as those discussed in "Results and graphs" paragraph, the correct operations were executed.

If the results are not similar the users can try these procedures:

- ✓ Verify that all degradation rate constants are correct and do not show unusual values (for instance values greater than 50 or lower than 0.004); if unusual values present, a manual correction setting typical rate constants in the same column is needed and then the users can re-run solver tool.
- ✓ Repeat all the needed manual corrections and run the solver again, until the longitudinal dispersivity does not change any more.
- ✓ Estimate transversal dispersivity as 1/10 of longitudinal and re-run solver tool until the Z objective does not decrease anymore.

Users can check graphs again and look at the fitted results in Zone 4 (transport parameters), Zone 5 (constant rates) and Zone 7. If the values don't fit again, running the solver for many times until the Z objective will no longer change is suggested.

### *Results and graphs*

After implementing the correct data and running the solver (almost 3 times until Z objective is not varying anymore), the users must analyse the worksheet "Figures", which reproduces the outputs of the software. Figure 14 case (a) shows the real concentrations values of each contaminant compared with the modelled concentrations downgradient of the source along the distance. The modelled data fit very well the experimental data. Moving away from the source, PCE and TCE decrease along the distance, whereas DCE and VC slightly increase.

Figure 14 case (b) shows the real isotope ratios of each contaminant compared with the modelled isotope ratios downgradient of the source along the distance. Even in this case, the modelled data fit very well the experimental data. Therefore, the chosen enrichment factors correctly reproduce the curve slopes and are within an acceptable value range according to the literature (from -4 to -25).

Figure 14 case (c) shows the total chloroethene degradation for different approach. The target "B% fitting" and "B% true" curves are correctly overlapped and are within the interval of "B% Aeppli minimum" and "B% Aeppli maximum". For this benchmark case, the degradation of chloroethene increases moving away from the source. "B% true" can be calculated only when the tracer values are present, otherwise the section related to "B% true" (AS34:AY52) can be deleted (as shown in field case examples).

Figure 14 case (d) shows the isotope mass balance. The modelled results fit very well the measured values. The isotope signatures (measured and modelled) increase along the distance, proving that biodegradation processes take place in the specific time considered. Otherwise, if the biodegradation did not occur, the graph would have showed a horizontal line, i.e. no change in time and space of the isotopic signature.

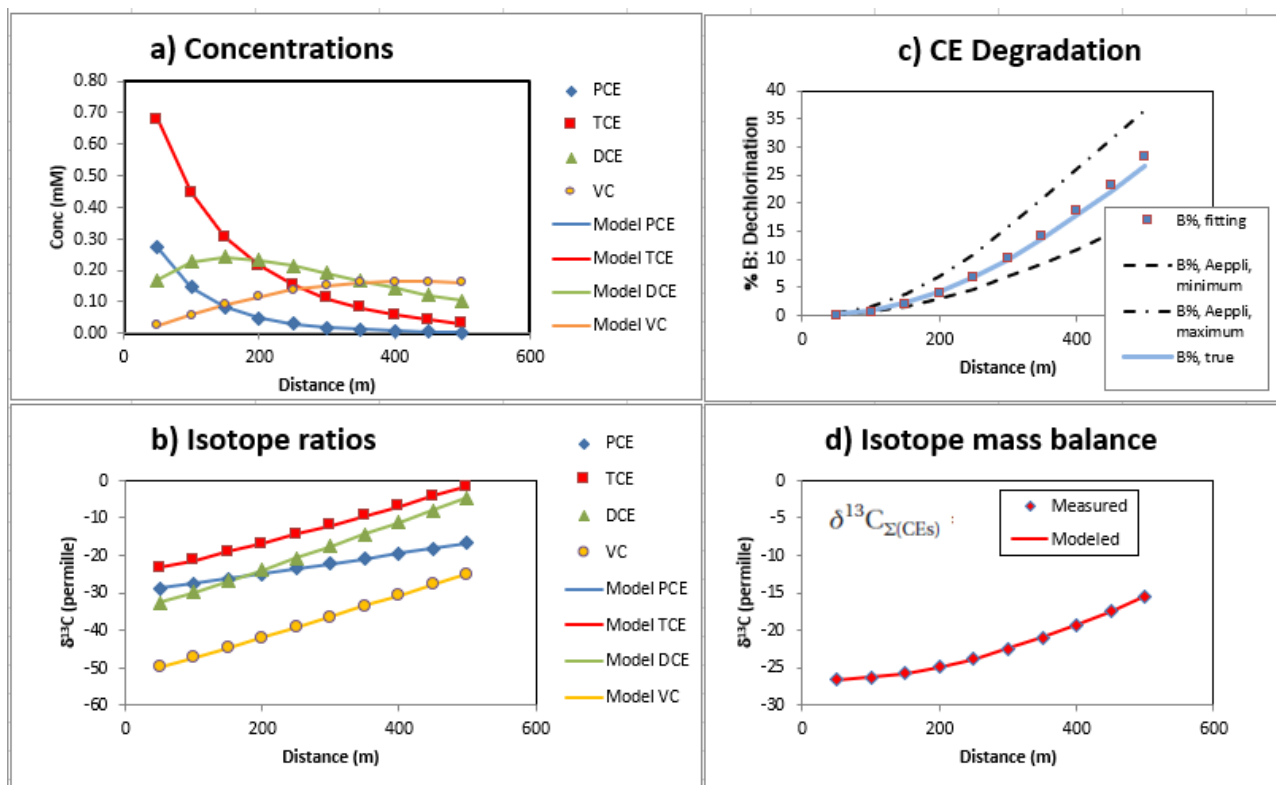


Figure 14 – Output graphs of the Freeware Software for CSIA analysis

All the modelled results fit very well the experimental data because of the completeness of the entered data related to the benchmark case 1: 8 isotope ratios and 8 concentration values downgradient the source were available. Generally, sampling many measurement points along the center line of a contamination plume is difficult.

The other outputs returned after 3 run of the solver are the transport parameters and degradation constant rates. To analyse them, users need to return to the worksheet “Data+Fitting” and read the green cells in Zone 4 and 5 (as shown in Figure 15). They fit with the true values entered as comments by the authors in the Excel file.

Looking at the Z objective, that is very low (0.00000305) and does not vary again after new run of the solver.

| Zone 4. TRANSPORT PARAMETERS |       |      | True Values |  | Zone 6. Objectives |            |     | Root Mean Square Errors the smaller the better |                                                                                |
|------------------------------|-------|------|-------------|--|--------------------|------------|-----|------------------------------------------------|--------------------------------------------------------------------------------|
| Flow velocity                | 109.5 | m/yr |             |  | Objective:         | Value      |     | RMSE Transport                                 | $RMSE = \left[ \frac{1}{n} \sum_{i=1}^n (x_{obs} - x_{model})^2 \right]^{1/2}$ |
| long. dispersivity           | 19.97 | m    | 20          |  | Concentrations     | 0.0000029  |     | 3.267                                          |                                                                                |
| trans. dispersivity          | 2.81  | m    | 2           |  | Isotopes           | 0.0000001  |     | RMSE B%                                        |                                                                                |
| Source width                 | 35.60 | m    | 30          |  | # Points ok        | 10         | /10 | 0.736                                          | Calculated in cell AV35                                                        |
|                              |       |      |             |  | Z objective        | 0.00000305 |     |                                                |                                                                                |
|                              |       |      |             |  | (Conc.+Isotopes)   |            |     |                                                |                                                                                |

| Average Rates |       |       |       |       |
|---------------|-------|-------|-------|-------|
|               | 1.000 | 0.750 | 0.500 | 0.250 |
| True          | 1     | 0.75  | 0.5   | 0.25  |

| Well name | Fitted Rates    |                 |                 |                |
|-----------|-----------------|-----------------|-----------------|----------------|
|           | k PCE<br>(yr-1) | k TCE<br>(yr-1) | k DCE<br>(yr-1) | k VC<br>(yr-1) |
| A         | 1.000           | 0.750           | 0.500           | 0.250          |
| B         | 1.000           | 0.750           | 0.500           | 0.250          |
| C         | 1.000           | 0.750           | 0.500           | 0.250          |
| D         | 1.000           | 0.750           | 0.500           | 0.250          |
| E         | 1.000           | 0.750           | 0.500           | 0.250          |
| F         | 1.000           | 0.750           | 0.500           | 0.250          |
| G         | 1.000           | 0.750           | 0.500           | 0.250          |
| H         | 1.000           | 0.750           | 0.500           | 0.250          |
| I         | 1.000           | 0.750           | 0.500           | 0.250          |
| K         | 1.000           | 0.750           | 0.500           | 0.250          |

Figure 15 – Output values of the Freeware Software for CSIA analysis

### *Synthetic operating guide*

Here, a summary of each operation that a user needs to carry out:

- I. Open the Biochlor Excel file;
- II. Verify the Microsoft Office version;
- III. Verify the presence of the solver;
- IV. Verify the correct operation of the solver by means of variables and constraints analysis;
- V. Replace each fake wrong data values with the correct ones (yellow cells in Zone 1,2,3 and 4);
- VI. Run the solver tool once (it is found in the upper right angle of the tool bar "Data") and save the solution by clicking ok in the next window;
- VII. Verify the values of Z objective and run the solver tool again, look at the value if varies or not (maybe necessary 3 run of the solver);
- VIII. Verify graphs and fitted results (green cells in Zone 4 and 5).
- IX. Save the file as "Biochlor2015\_filled.xls".

### 3. Examples of application of BIOCHLOR-ISO to Benchmark case 1

Using the file “Biochlor2015\_Filled” related to the Benchmark case 1, where all correct values and graphs are implemented, users can modify the groundwater flow velocity in order to analyse how the results will change. Here the suggestions:

- I. Into the worksheet “Data+Fitting”, the users have to replace the groundwater flow velocity in cell V3 (now 109.5 m/yr) to its double (219 m/yr).
- II. Into the worksheet “Figures”: the fitted curves (modelled lines) do not match the experimental data (points) in any graph (Figure 18). Now a new fitting of degradation rate constants and transport parameters is necessary.

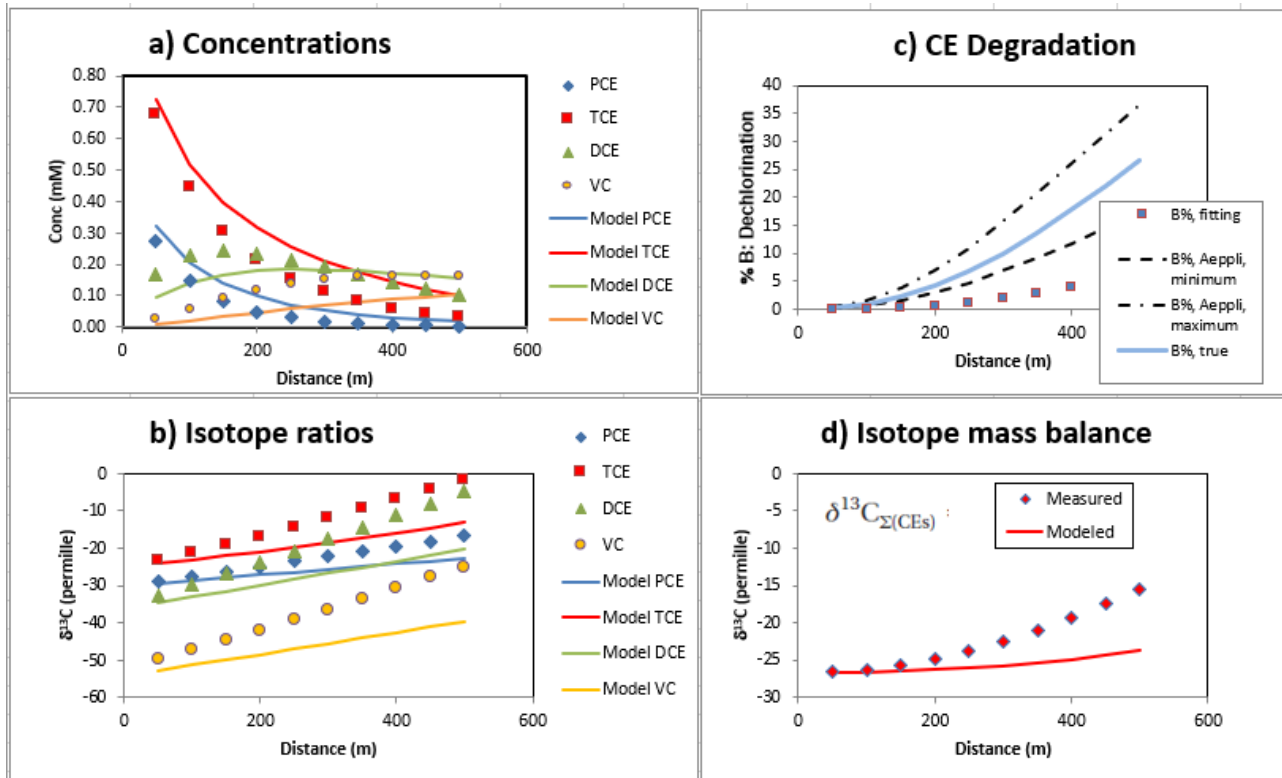


Figure 16 – Output graphs of benchmark case 1, when the groundwater flow velocity varies

- III. Again, into the worksheet “Data+Fitting”, users need to run the solver tool once and save the solution by clicking ok in the next window.
- IV. Looking at the degradation rate constants in the worksheet "Data+Fitting" (in cells Z19-AC28), users will find the values reported in Figure 19:

| Average Rates |                 |                 |                |               |
|---------------|-----------------|-----------------|----------------|---------------|
|               | 2.004           | 1.502           | 0.998          | 0.501         |
| True          |                 |                 |                |               |
|               | 1               | 0.75            | 0.5            | 0.25          |
| Well name     | Fitted Rates    |                 |                |               |
|               | k PCE<br>(yr-1) | k TCE<br>(yr-1) | kDCE<br>(yr-1) | kVC<br>(yr-1) |
| A             | 2.013           | 1.508           | 0.987          | 0.510         |
| B             | 2.006           | 1.503           | 0.996          | 0.503         |
| C             | 2.004           | 1.502           | 0.999          | 0.499         |
| D             | 2.002           | 1.501           | 1.000          | 0.499         |
| E             | 2.002           | 1.501           | 1.000          | 0.499         |
| F             | 2.002           | 1.501           | 1.000          | 0.500         |
| G             | 2.002           | 1.501           | 1.000          | 0.499         |
| H             | 2.002           | 1.501           | 1.001          | 0.499         |
| I             | 2.002           | 1.501           | 1.000          | 0.500         |
| K             | 2.002           | 1.501           | 1.000          | 0.500         |

Figure 17 – Values of degradation rate constant after the first run of the solver

- V. Looking at graphs in worksheet "Figures", the users can see that modelled curves fit much better than before the experimental data (Figure 20).

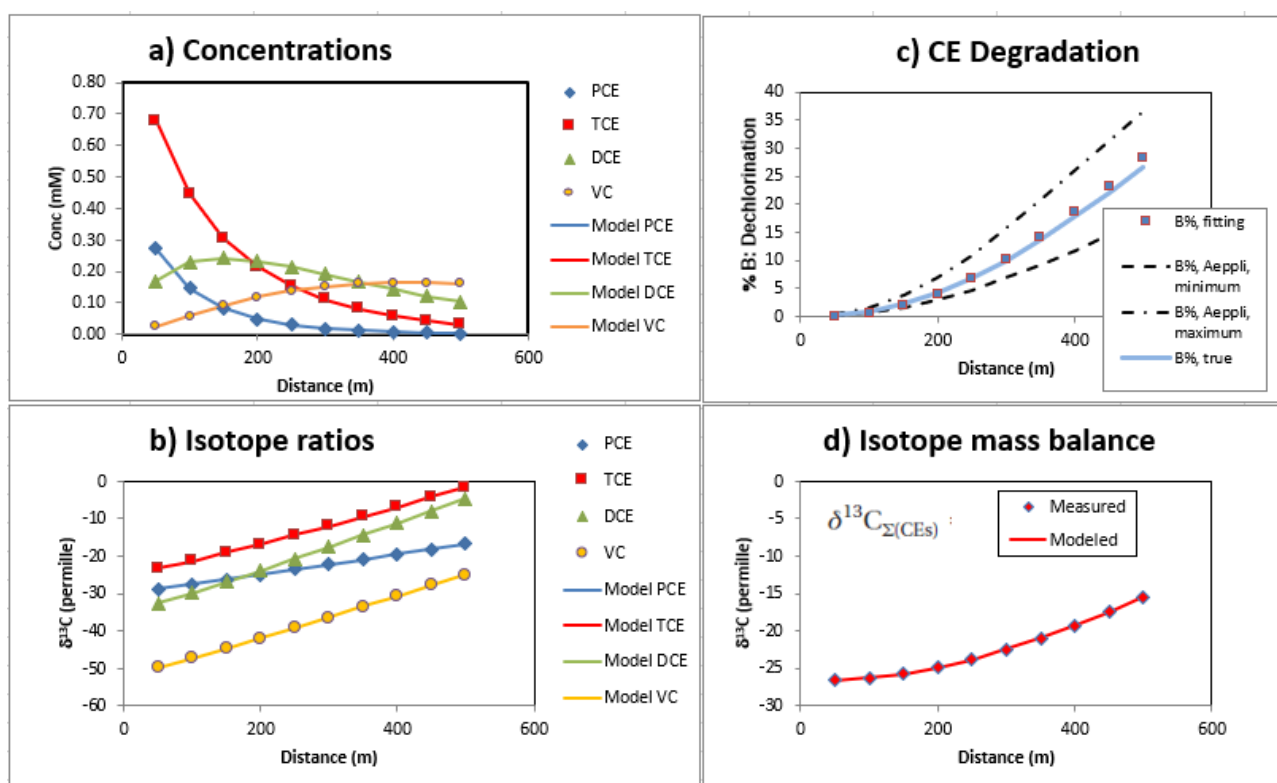


Figure 18 – Output graphs of benchmark case 1, after the first run of the solver

- VI. Returning to the worksheet "Data+Fitting", users have to read the value of Z objective (cell Z7): 0.001476. Run solver tool once more and check again the value (now Z objective is equal to 0.00000251). The results are once better than before because running the solver with better guess of initial parameter values. Users need to repeat this procedure until the Z objective value keeps constant as well as the graphs. The output of unknown parameters (after 3 run of the solver tool) is shown in Figure 21.

| Zone 4. TRANSPORT PARAMETERS |       |      | True Values |  | Zone 6. Objectives |            |     | Root Mean Square Errors the smaller the better |                                                                                                                                              |  |
|------------------------------|-------|------|-------------|--|--------------------|------------|-----|------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------|--|
| Flow velocity                | 219   | m/yr |             |  | Objective:         | Value      |     | RMSE Transport                                 | <div><math display="block">RMSE = \left[ \frac{1}{n} \sum_{i=1}^n (x_{obs} - x_{model})^2 \right]^{1/2}</math></div> Calculated in cell AV35 |  |
| long. dispersivity           | 19.99 | m    | 20          |  | Concentrations     | 0.0000024  |     | 4.560                                          |                                                                                                                                              |  |
| trans. dispersivity          | 3.17  | m    | 2           |  | Isotopes           | 0.0000001  |     | RMSE B%                                        |                                                                                                                                              |  |
| Source width                 | 37.81 | m    | 30          |  | # Points ok        | 10         | /10 | 0.736                                          |                                                                                                                                              |  |
|                              |       |      |             |  | Z objective        | 0.00000251 |     |                                                |                                                                                                                                              |  |
|                              |       |      |             |  | (Conc.+Isotopes)   |            |     |                                                |                                                                                                                                              |  |

|               |       |       |       |       |
|---------------|-------|-------|-------|-------|
| Average Rates | 2.000 | 1.500 | 1.000 | 0.500 |
| True          | 1     | 0.75  | 0.5   | 0.25  |

| Well name | Fitted Rates    |                 |                |               | Caution |
|-----------|-----------------|-----------------|----------------|---------------|---------|
|           | k PCE<br>(yr-1) | k TCE<br>(yr-1) | kDCE<br>(yr-1) | kVC<br>(yr-1) |         |
| A         | 2.001           | 1.500           | 1.000          | 0.500         |         |
| B         | 2.000           | 1.500           | 1.000          | 0.500         |         |
| C         | 2.000           | 1.500           | 1.000          | 0.500         |         |
| D         | 2.000           | 1.500           | 1.000          | 0.500         |         |
| E         | 2.000           | 1.500           | 1.000          | 0.500         |         |
| F         | 2.000           | 1.500           | 1.000          | 0.500         |         |
| G         | 2.000           | 1.500           | 1.000          | 0.500         |         |
| H         | 2.000           | 1.500           | 1.000          | 0.500         |         |
| I         | 2.000           | 1.500           | 1.000          | 0.500         |         |
| K         | 2.000           | 1.500           | 1.000          | 0.500         |         |

**Figure 19** – Output values of benchmark case 1, after three runs of the solver

VII. Close the excel file and save as “Biochlor2015\_Filled\_v”

The graphs show good accordance between modelled and experimental data and new value of degradation constant rates and transport parameters are achieved. To obtain a good fit between the modelled and experimental data in the case of doubled groundwater flow velocity, is also necessary to double the degradation constants rates, as calculated by the solver.

#### 4. BIBLIOGRAPHY

- Aeppli, C., Hofstetter, T.B., Amaral, H.I.F., Kipfer, R., Schwarzenbach, R.P., Berg, M., 2010. Quantifying in situ transformation rates of chlorinated ethenes by combining compound-specific stable isotope analysis, groundwater dating, and carbon isotope mass balances. *Environ. Sci. Technol.* <https://doi.org/10.1021/es903895b>
- Gelhar, L.W., Welty, C., Rehfeldt, K.R., 1992. A Critical Review of Data on Field-Scale Dispersin in Aquifers. *Water Resour. Res.* 28, 1955–1974. <https://doi.org/10.1029/92WR00607>
- Höhener, P., Elsner, M., Eisenmann, H., Atteia, O., 2015. Improved constraints on in situ rates and on quantification of complete chloroethene degradation from stable carbon isotope mass balances in groundwater plumes. *J. Contam. Hydrol.* <https://doi.org/10.1016/j.jconhyd.2015.09.006>
- Höyng, D., Prommer, H., Blum, P., Grathwohl, P., Mazo D' Affonseca, F., 2015. Evolution of carbon isotope signatures during reactive transport of hydrocarbons in heterogeneous aquifers. *J. Contam. Hydrol.* <https://doi.org/10.1016/j.jconhyd.2014.12.005>