USER GUIDANCE

FOCUS_DEGKIN V2

VERSION 1 CREATED 12 OCTOBER 2006

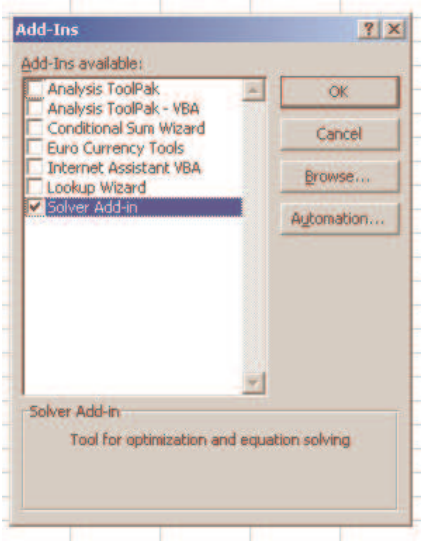
An Excel file was provided by the FOCUS workgroup on degradation kinetics to facilitate kinetic analysis for parent compounds. This is not the tool that is preferred by FOCUS, it is simply one of several options.

The file consists of 10 worksheets. . **Note that only worksheets for datasets without replicates and datasets with 2 replicates have been created. Chi2 statistics for datasets with more than 2 replicates can be calculated by entering the average value for each sampling time in the sheet "chi2 all models no reps".**

FOCUS_DEGKIN v2		
May 2007		
Name of worksheet	**Use this worksheet to**	**User must enter**
SFO no reps	Calculate chi2 statistics and create graphs (conc. vs. time plot and residual plot) for datasets without replicates and SFO kinetics	Observed data and SFO parameters (M0, k) derived elsewhere
SFO 2 reps	Calculate chi2 statistics and create graphs (conc. vs. time plot and residual plot) for datasets with 2 replicates and SFO kinetics	Observed data and SFO parameters (M0, k) derived elsewhere
SFO no reps with fit	Fit SFO kinetics to datasets without replicates, calculate chi2 statistics and create graphs (conc. vs. time plot and residual plot)	Observed data and starting values for SFO parameters (M0, k)
SFO 2 reps with fit	Fit SFO kinetics to datasets with 2 replicates, calculate chi2 statistics and create graphs (conc. vs. time plot and residual plot)	Observed data and starting values for SFO parameters (M0, k)
FOMC no reps	Calculate chi2 statistics and create graphs (conc. vs. time plot and residual plot) for datasets without replicates and FOMC kinetics	Observed data and FOMC parameters (M0, alpha, beta) derived elsewhere
FOMC 2 reps	Calculate chi2 statistics and create graphs (conc. vs. time plot and residual plot) for datasets with 2 replicates and FOMC kinetics	Observed data and FOMC parameters (M0, alpha, beta) derived elsewhere
Chi2 all models no reps	Calculate chi2 statistics for datasets without replicates (observed data and concentrations calculated with any model must be entered)	Observed and calculated data and number of parameters
Chi2 all models 2 reps	Calculate chi2 statistics for datasets with 2 replicates (observed data and concentrations calculated with any model must be entered)	Observed and calculated data and number of parameters (replicate values)
t-test	Calculate t-test statistics to assess whether a parameter estimate is significantly different from zero	Parameter estimate derived elsewhere, standard error, number of observed values used in fitting and number of fitted model parameters
DFOP_DT50,DT90	Calculate DT50 and DT90 values for DFOP kinetics	Parameters of the DFOP model derived elsewhere

GETTING STARTED

You will have to use the Excel Solver Add-In. Prior to using the file for the first time, the Solver must be activated. Go to Tools Add-Ins and click the Solver Add-In box.



USING THE SPREADSHEETS

Load the provided Excel file. In each sheet, the user must enter text or values in the blue cells. All other cells will be automatically updated.

FOCUS_DEGKIN v2

Visual assessment and chi2-test for SFO kinetics

For datasets without replicates

Change number of parameters if M0 was fixed in optimisation!

User input, all other cells calculated

Name of dataset: Example dataset 1

No	Time	Observed	Calculated
1	0	97.80	74.87
2	3	60.00	69.41
3	7	51.00	62.73
4	14	43.00	52.56
5	30	35.00	35.08
6	60	22.00	16.44
7	91	15.00	7.51
8	120	12.00	3.61
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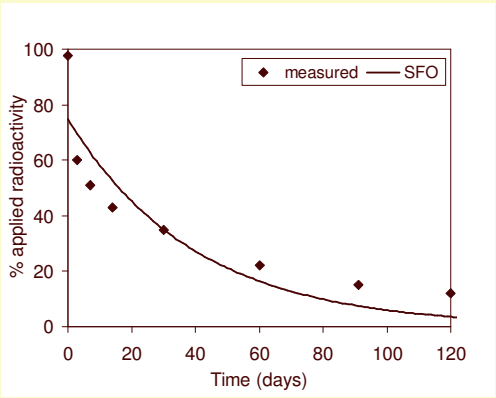
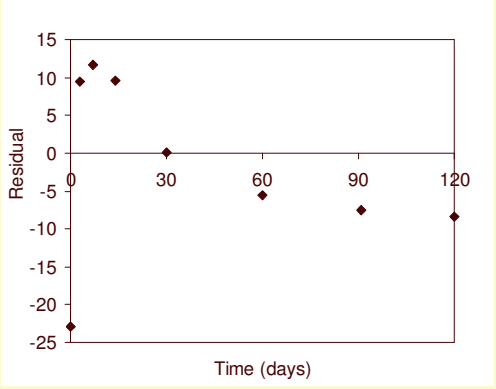
SFO parameters and endpoints

M0	74.87	DT50	27.4
k	0.025	DT90	91.1

Error level Chi2 test 21.2

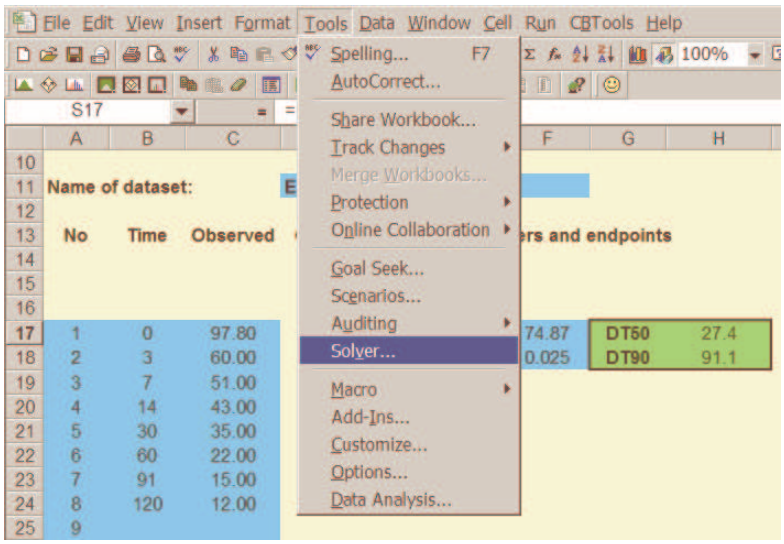
1000.727	Residual Sum of Squares
8	Number of observations
2	Number of parameters
42.0	Average of observed
8.91	Scaled Error
12.592	Chi2 calculated
12.592	Chi2 Table



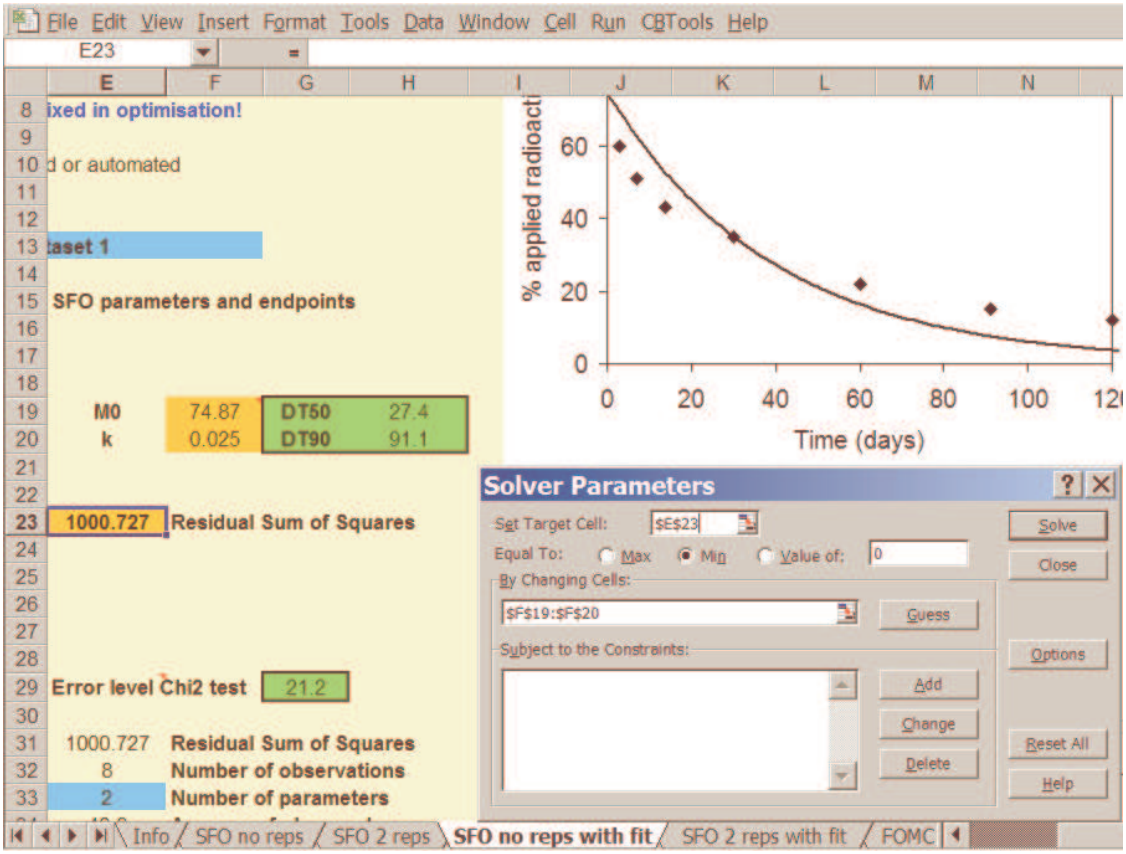


Excel sheets have been created to allow the automatic fitting SFO kinetics to the data. Once the measured data have been entered, appropriate starting values have to be specified for M0 and k. Excel may not be able to obtain a good fit unless adequate starting values are provided. First, the plot of concentrations versus time should be investigated. If the calculated curve is very different from the measured concentrations, the starting values must be changed. Modify the values for M0 and k manually until the curve is close to the measured concentrations.

Once appropriate starting values have been found, the Excel Solver will be run to find the best possible combination of parameter values. The aim is to minimise the residual sum of squares Σ (calculated – observed)^2. Go to Tools, Solver



A new window opens. The target cell is the cell with the residual sum of squares. In this example, the target cell is E29. Check the mimimise box. The smallest possible value will then be derived automatically by the Excel Solver. This will be achieved by testing different combinations of the values for the input parameters, here M0 (initial concentration) and k (degradation rate constant). Enter the reference to the cells with these parameters (here F19 and F20) under ‘by changing cells’,. Click on Solve. Click OK to keep the optimised values.



Check the visual fit. If the curve is not close to the measured data, try again with different starting values. Different optimised parameters may be returned by the Excel Solver routine for different starting values. A number of starting values should, thus, be tested. The combination that gives the smallest residual sum of squares should be used, provided the visual fit is acceptable.

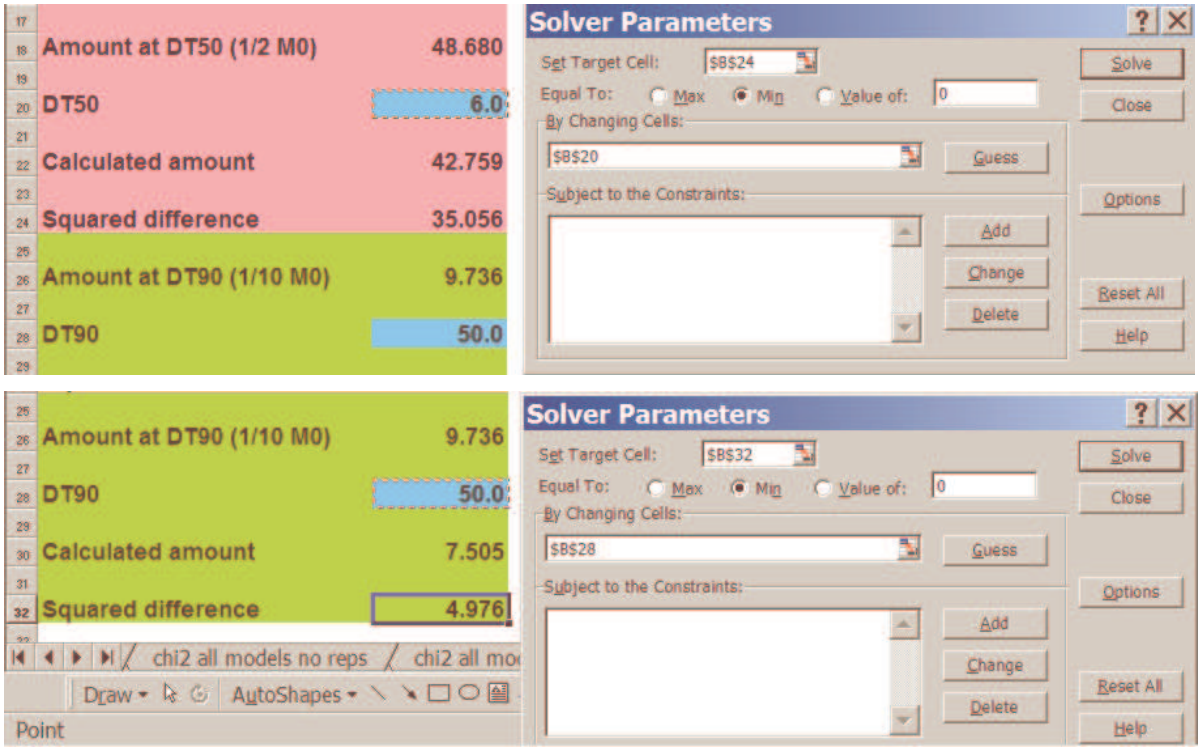
Visual plots are automatically created. Chi2 statistics are also calculated. The error level is set to the smallest error value for which the Chi2 test is passed by solving the equation:

$$err = 100 \sqrt{\frac{1}{\chi^2_{tabulated}} \sum \frac{(C-O)^2}{\overline{O}^2}}$$

Two Excel spreadsheets have been created that allow the calculation of chi2 statistics for any model. One spreadsheet is for datasets with a single measurement per time point (chi2 all models no reps), the second spreadsheet is for two replicates (chi2 all models 2 reps). The observed and calculated concentrations of the pesticide and the number of fitted model parameters must be entered. The model fit must be carried out elsewhere.

An additional Excel spreadsheet (t-test) facilitates the calculation of t-statistics. These indicate whether a parameter estimate derived elsewhere is significantly different from zero. The user must enter the parameter estimate, the standard error and the number of observations and parameters included in the fitting.

The Excel spreadsheet DFOP_DT50,DT90 facilitates the calculation of DT50 and DT90 values of the DFOP model. There is no analytical equation to calculate these endpoints. Instead, the times at which 50% or 90% of the initial amount are lost must be found by iteration. In this spreadsheet, the user must enter an initial guess for the DT50 and DT90. The concentrations remaining at the estimated DT50 is compared with 50% of the initial concentration. The squared difference between the two values is minimised using the Excel Solver. The procedure is repeated for the DT90.



TEST DATASETS

SFO no reps with fit

Time (days)	% applied radioactivity
0	97.80
3	60.00
7	51.00
14	43.00
30	35.00
60	22.00
91	15.00
120	12.00

Starting values: M0 = 100 %AR, k = 0.030 $days^{-1}$

SFO 2 reps with fit

Time (days)	% applied radioactivity
0	88.30
0	91.40
1	85.60
1	84.50
2	78.90
2	77.60
3	72.00
3	71.90
5	50.30
5	59.40
7	47.00
7	45.10
14	27.70
14	27.30
21	10.00
21	10.40
30	2.90
30	4.00

Starting values: M0 = 100 %AR, k = 0.080 $days^{-1}$

Chi2 all models no reps

Time (days)	% applied radioactivity measured	% applied radioactivity calculated
0	99.00	92.83
7	85.00	86.37
14	80.00	80.36
21	70.00	74.77
28	65.00	69.57
56	55.00	52.13
84	40.00	39.07
100	35.00	33.13

Number of parameters: 4

Chi2 all models 2 reps

Time (days)	% applied radioactivity measured	% applied radioactivity calculated
0	95.00	95.37
0	94.00	95.37
3	81.00	77.56
3	83.00	77.56
5	65.00	68.99
5	64.00	68.99
10	58.00	54.08
10	52.00	54.08
20	40.00	37.81
20	35.00	37.81
35	28.00	26.09
35	25.00	26.09
48	22.00	20.58
48	20.00	20.58
60	15.00	17.23
60	18.00	17.23
90	15.00	12.26
90	10.00	12.26

Number of parameters: 3

t-test

parameter estimate =	0.001490
standard error of parameter =	0.001950
number of observed values used in fitting =	18
number of fitted model parameters =	4

DFOP DT50,DT90

M0 =	97.36
g =	0.5149
k1 =	0.38814
k2 =	0.03679

starting value DT50 = 6 days, DT90 = 50 days