Examples for kinetic evaluations using mkin

Johannes Ranke

Eurofins Regulatory AG
Weidenweg 15, CH–4310 Rheinfelden, Switzerland

and

University of Bremen

April 14, 2013

Contents

Key words: Kinetics, FOCUS, nonlinear optimisation

1 Kinetic evaluations for parent compounds

These examples are also evaluated in a parallel vignette of the **kinfit** package (Ranke, 2012). The datasets are from Appendix 3, of the FOCUS kinetics report (FOCUS Work Group on Degradation Kinetics, 2006, 2011).

1.1 Laboratory Data L1

The following code defines example dataset L1 from the FOCUS kinetics report, p. 284

```
R> library("mkin")
R> FOCUS_2006_L1 = data.frame(
+   t = rep(c(0, 1, 2, 3, 5, 7, 14, 21, 30), each = 2),
+   parent = c(88.3, 91.4, 85.6, 84.5, 78.9, 77.6,
+              72.0, 71.9, 50.3, 59.4, 47.0, 45.1,
+              27.7, 27.3, 10.0, 10.4, 2.9, 4.0))
R> FOCUS_2006_L1_mkin <- mkin_wide_to_long(FOCUS_2006_L1)
```

The next step is to set up the models used for the kinetic analysis. Note that the model definitions contain the names of the observed variables in the data. In this case, there is only one variable called `parent`.

```
R> SFO <- mkinmod(parent = list(type = "SFO"))
R> FOMC <- mkinmod(parent = list(type = "FOMC"))
R> DFOP <- mkinmod(parent = list(type = "DFOP"))
```

The three models cover the first assumption of simple first order (SFO), the case of declining rate constant over time (FOMC) and the case of two different phases of the kinetics

(DFOP). For a more detailed discussion of the models, please see the FOCUS kinetics report.

The following two lines fit the model and produce the summary report of the model fit. This covers the numerical analysis given in the FOCUS report.

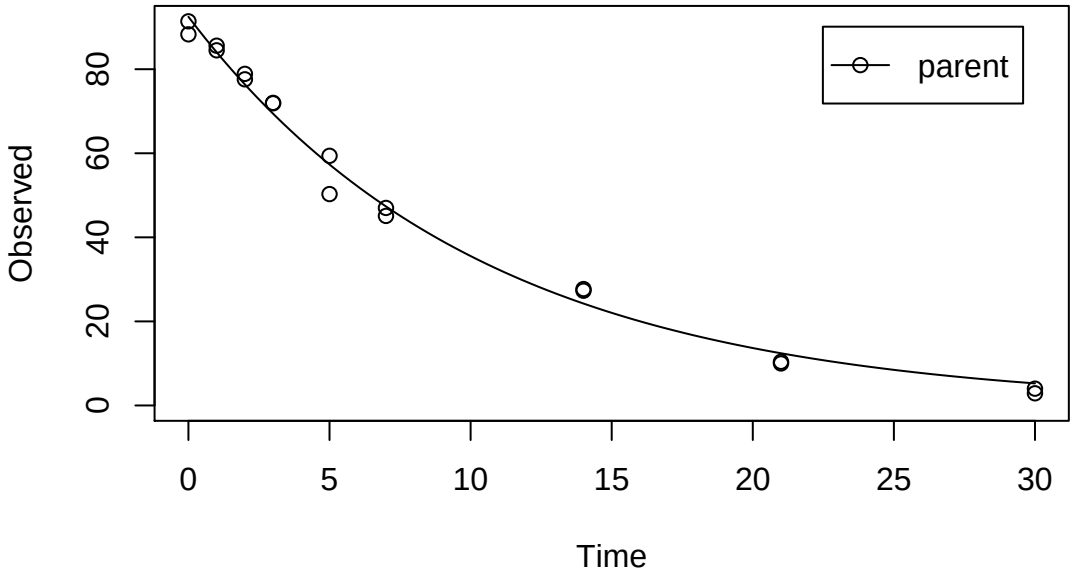
```
R> m.L1.SFO <- mkinfit(SFO, FOCUS_2006_L1_mkin, quiet=TRUE)
R> summary(m.L1.SFO)
```

```
mkin version:    0.9.19
R version:       2.15.3
Date of fit:     Sun Apr 14 14:28:40 2013
Date of summary: Sun Apr 14 14:28:40 2013

Equations:
[1] d_parent = - k_parent_sink * parent

Method used for solution of differential equation system:
analytical

Starting values for optimised parameters:
              initial   type transformed
parent_0        100.0  state  100.000000
k_parent_sink     0.1 deparm   -2.302585

Fixed parameter values:
None

Optimised, transformed parameters:
              Estimate Std. Error  Lower  Upper
parent_0        92.470    1.36800 89.570 95.370
k_parent_sink   -2.347    0.04057 -2.433 -2.261

Backtransformed parameters:
              Estimate    Lower   Upper
parent_0      92.47000 89.57000 95.3700
k_parent_sink  0.09561  0.08773  0.1042

Residual standard error: 2.948 on 16 degrees of freedom

Chi2 error levels in percent:
         err.min n.optim df
All data   3.424       2  7
parent     3.424       2  7

Estimated disappearance times:
        DT50  DT90
parent 7.249 24.08
```

```
Estimated formation fractions:
            ff
parent_sink  1

Parameter correlation:
              parent_0 k_parent_sink
parent_0        1.0000        0.6248
k_parent_sink   0.6248        1.0000

Data:
 time variable observed predicted residual
    0   parent     88.3    92.471  -4.1710
    0   parent     91.4    92.471  -1.0710
    1   parent     85.6    84.039   1.5610
    1   parent     84.5    84.039   0.4610
    2   parent     78.9    76.376   2.5241
    2   parent     77.6    76.376   1.2241
    3   parent     72.0    69.412   2.5884
    3   parent     71.9    69.412   2.4884
    5   parent     50.3    57.330  -7.0301
    5   parent     59.4    57.330   2.0699
    7   parent     47.0    47.352  -0.3515
    7   parent     45.1    47.352  -2.2515
   14   parent     27.7    24.247   3.4527
   14   parent     27.3    24.247   3.0527
   21   parent     10.0    12.416  -2.4163
   21   parent     10.4    12.416  -2.0163
   30   parent      2.9     5.251  -2.3513
   30   parent      4.0     5.251  -1.2513
```

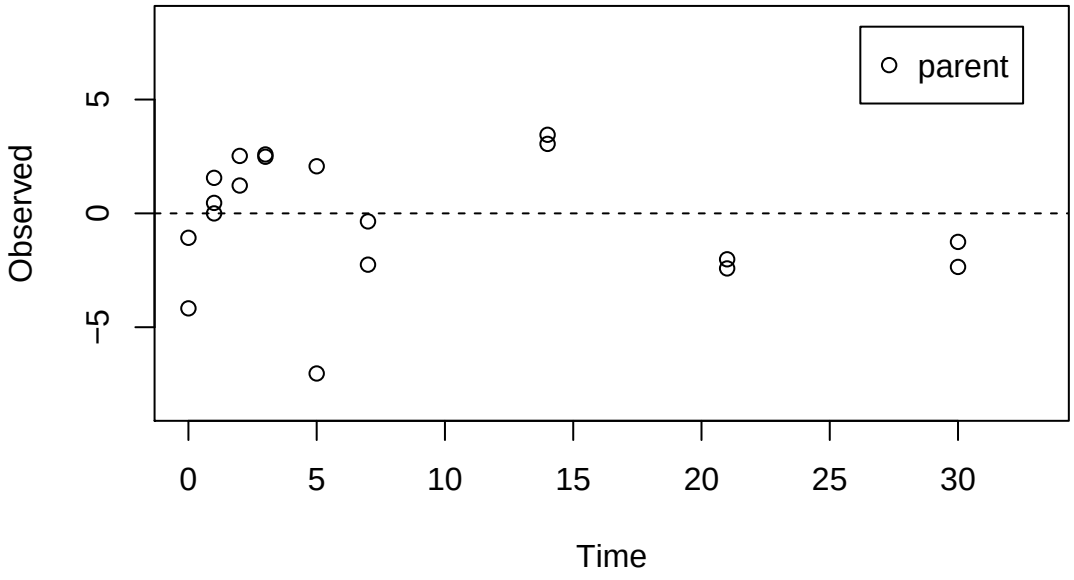
A plot of the fit is obtained with the plot function for mkinfit objects.

```
R> plot(m.L1.SFO)
```



The residual plot can be easily obtained by

```
R> mkinresplot(m.L1.SFO, ylab = "Observed", xlab = "Time")
```



For comparison, the FOMC model is fitted as well, and the χ^2 error level is checked.

```
R> m.L1.FOMC <- mkinfit(FOMC, FOCUS_2006_L1_mkin, quiet=TRUE)
R> summary(m.L1.FOMC)
```

```
mkin version:    0.9.19
R version:       2.15.3
Date of fit:     Sun Apr 14 14:28:40 2013
Date of summary: Sun Apr 14 14:28:40 2013

Equations:
[1] d_parent = - (alpha/beta) * ((time/beta) + 1)^-1 * parent

Method used for solution of differential equation system:
analytical

Starting values for optimised parameters:
         initial   type transformed
parent_0     100  state  100.000000
alpha          1 deparm    0.000000
beta          10 deparm    2.302585

Fixed parameter values:
None

Optimised, transformed parameters:
```

```
         Estimate Std. Error Lower Upper
parent_0    92.47         NA    NA    NA
alpha       25.63         NA    NA    NA
beta        27.98         NA    NA    NA

Backtransformed parameters:
          Estimate Lower Upper
parent_0 9.247e+01    NA    NA
alpha    1.350e+11    NA    NA
beta     1.412e+12    NA    NA

Residual standard error: 3.045 on 15 degrees of freedom

Chi2 error levels in percent:
         err.min n.optim df
All data   3.619       3  6
parent     3.619       3  6

Estimated disappearance times:
        DT50  DT90
parent 7.249 24.08

Parameter correlation:
Could not estimate covariance matrix; singular system:

Data:
 time variable observed predicted residual
    0   parent     88.3    92.471  -4.1711
    0   parent     91.4    92.471  -1.0711
    1   parent     85.6    84.038   1.5618
    1   parent     84.5    84.038   0.4618
    2   parent     78.9    76.377   2.5233
    2   parent     77.6    76.377   1.2233
    3   parent     72.0    69.412   2.5884
    3   parent     71.9    69.412   2.4884
    5   parent     50.3    57.331  -7.0306
    5   parent     59.4    57.331   2.0694
    7   parent     47.0    47.351  -0.3510
    7   parent     45.1    47.351  -2.2510
   14   parent     27.7    24.247   3.4526
   14   parent     27.3    24.247   3.0526
   21   parent     10.0    12.416  -2.4162
   21   parent     10.4    12.416  -2.0162
   30   parent      2.9     5.251  -2.3513
   30   parent      4.0     5.251  -1.2513
```

Due to the higher number of parameters, and the lower number of degrees of freedom of the fit, the χ^2 error level is actually higher for the FOMC model (3.6%) than for the SFO model (3.4%). Additionally, the covariance matrix can not be obtained, indicating

overparameterisation of the model.

The χ^2 error levels reported in Appendix 3 and Appendix 7 to the FOCUS kinetics report are rounded to integer percentages and partly deviate by one percentage point from the results calculated by `mkin`. The reason for this is not known. However, `mkin` gives the same χ^2 error levels as the **kinfit** package. Furthermore, the calculation routines of the kinfit package have been extensively compared to the results obtained by the KinGUI software, as documented in the kinfit package vignette. KinGUI is a widely used standard package in this field. Therefore, the reason for the difference was not investigated further.

1.2 Laboratory Data L2

The following code defines example dataset L2 from the FOCUS kinetics report, p. 287

```
R> FOCUS_2006_L2 = data.frame(
+   t = rep(c(0, 1, 3, 7, 14, 28), each = 2),
+   parent = c(96.1, 91.8, 41.4, 38.7,
+              19.3, 22.3, 4.6, 4.6,
+              2.6, 1.2, 0.3, 0.6))
R> FOCUS_2006_L2_mkin <- mkin_wide_to_long(FOCUS_2006_L2)
```

Again, the SFO model is fitted and a summary is obtained.

```
R> m.L2.SFO <- mkinfit(SFO, FOCUS_2006_L2_mkin, quiet=TRUE)
R> summary(m.L2.SFO)
```

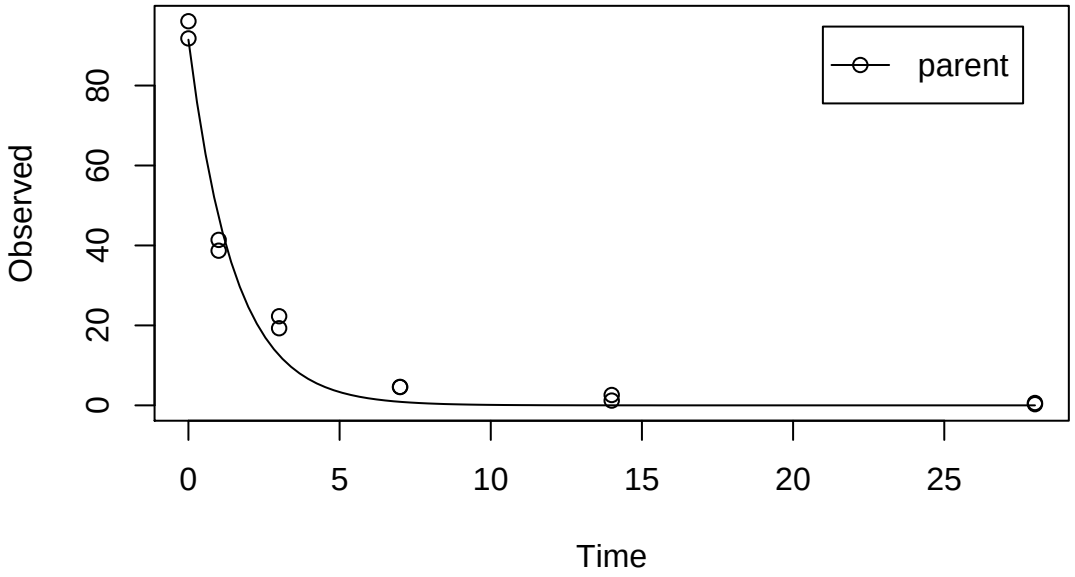
```
mkin version:    0.9.19
R version:       2.15.3
Date of fit:     Sun Apr 14 14:28:41 2013
Date of summary: Sun Apr 14 14:28:41 2013

Equations:
[1] d_parent = - k_parent_sink * parent

Method used for solution of differential equation system:
analytical

Starting values for optimised parameters:
              initial   type transformed
parent_0        100.0  state  100.000000
k_parent_sink     0.1 deparm   -2.302585

Fixed parameter values:
None

Optimised, transformed parameters:
```

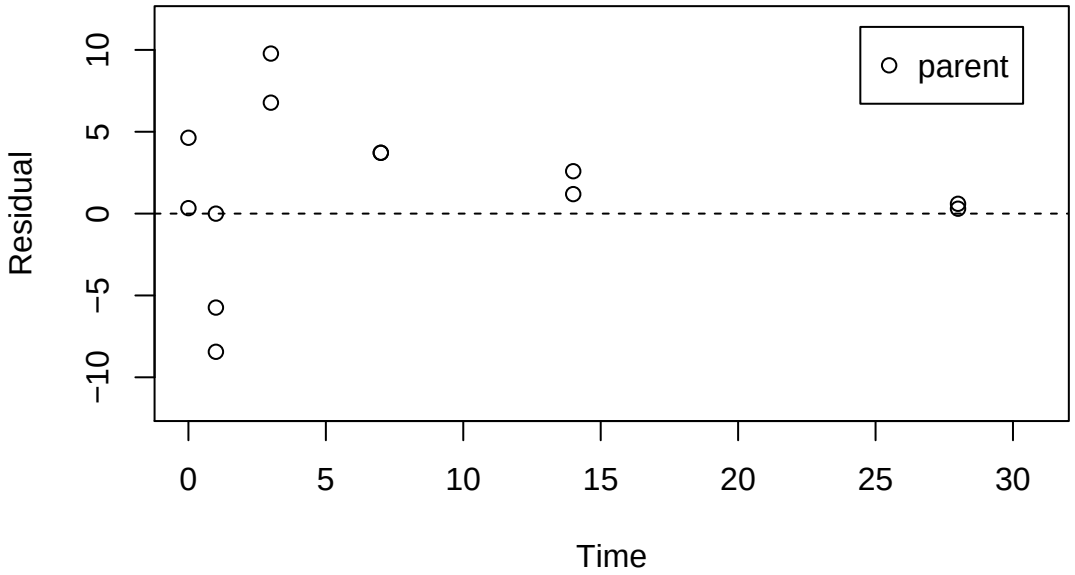
```
              Estimate Std. Error   Lower   Upper
parent_0       91.4700     3.8070 82.9800 99.9500
k_parent_sink  -0.4112     0.1074 -0.6505 -0.1719

Backtransformed parameters:
              Estimate   Lower   Upper
parent_0       91.4700 82.9800 99.9500
k_parent_sink   0.6629  0.5218  0.8421

Residual standard error: 5.51 on 10 degrees of freedom

Chi2 error levels in percent:
         err.min n.optim df
All data   14.38       2  4
parent     14.38       2  4

Estimated disappearance times:
        DT50  DT90
parent 1.046 3.474

Estimated formation fractions:
            ff
parent_sink  1

Parameter correlation:
              parent_0 k_parent_sink
parent_0        1.0000        0.4295
k_parent_sink   0.4295        1.0000

Data:
 time variable observed predicted residual
    0   parent     96.1 9.147e+01   4.6344
    0   parent     91.8 9.147e+01   0.3344
    1   parent     41.4 4.714e+01  -5.7395
    1   parent     38.7 4.714e+01  -8.4395
    3   parent     19.3 1.252e+01   6.7790
    3   parent     22.3 1.252e+01   9.7790
    7   parent      4.6 8.834e-01   3.7166
    7   parent      4.6 8.834e-01   3.7166
   14   parent      2.6 8.532e-03   2.5915
   14   parent      1.2 8.532e-03   1.1915
   28   parent      0.3 7.958e-07   0.3000
   28   parent      0.6 7.958e-07   0.6000
```

The χ^2 error level of 14% suggests that the model does not fit very well. This is also obvious from the plots of the fit and the residuals.

```
R> par(mfrow = c(2, 1))
R> plot(m.L2.SFO)
```

```
R> mkinresplot(m.L2.SFO)
```





In the FOCUS kinetics report, it is stated that there is no apparent systematic error observed from the residual plot up to the measured DT90 (approximately at day 5), and there is an underestimation beyond that point.

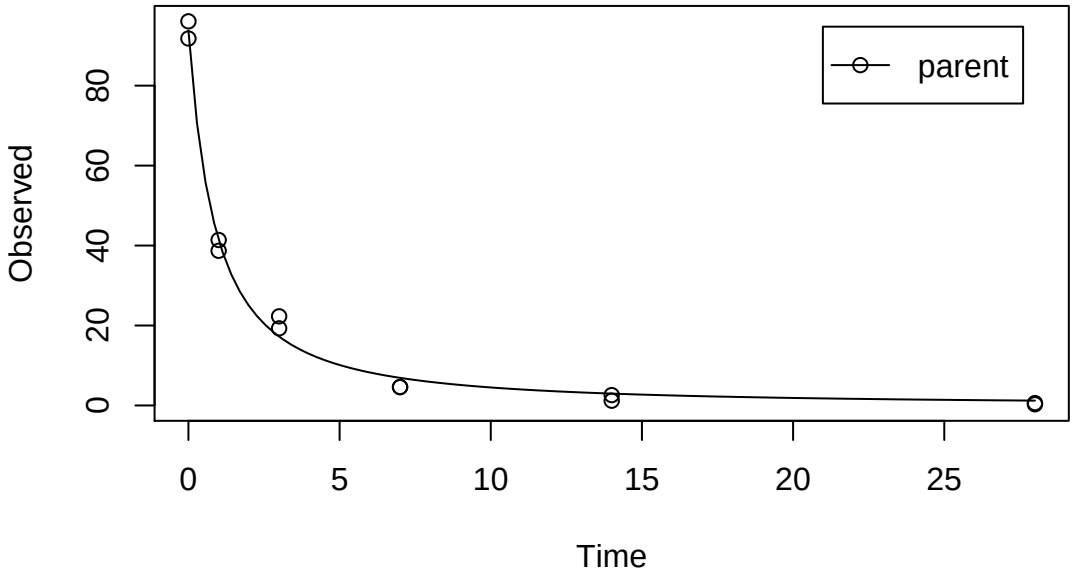
We may add that it is difficult to judge the random nature of the residuals just from the three samplings at days 0, 1 and 3. Also, it is not clear *a priori* why a consistent underestimation after the approximate DT90 should be irrelevant. However, this can be rationalised by the fact that the FOCUS fate models generally only implement SFO kinetics.

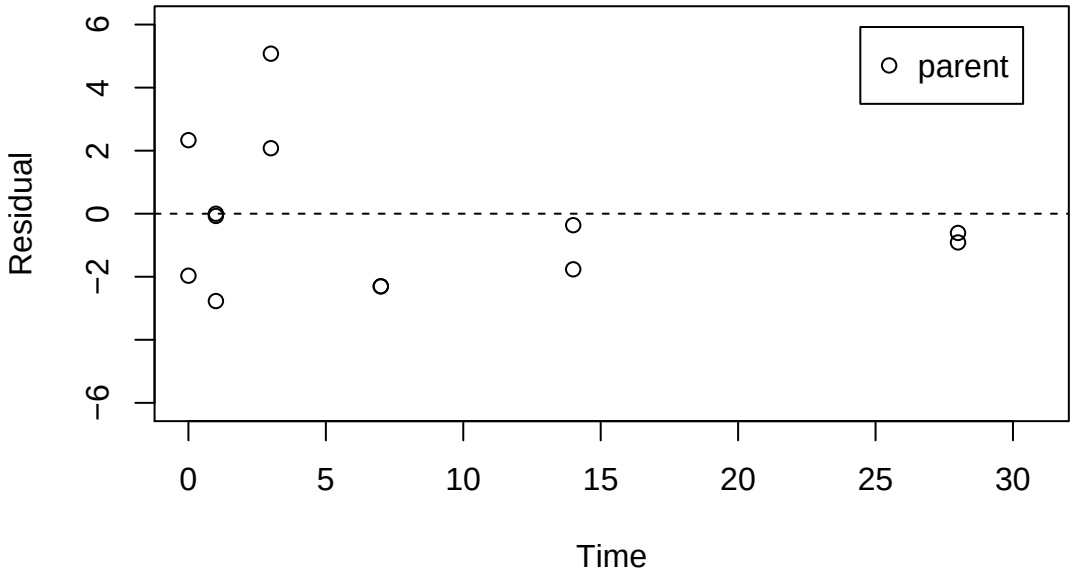
For comparison, the FOMC model is fitted as well, and the χ^2 error level is checked.

```
R> m.L2.FOMC <- mkinfit(FOMC, FOCUS_2006_L2_mkin, quiet = TRUE)
R> par(mfrow = c(2, 1))
R> plot(m.L2.FOMC)
R> mkinresplot(m.L2.FOMC)
R> summary(m.L2.FOMC, data = FALSE)
```

```
mkin version:    0.9.19
R version:       2.15.3
Date of fit:     Sun Apr 14 14:28:41 2013
Date of summary: Sun Apr 14 14:28:41 2013

Equations:
[1] d_parent = - (alpha/beta) * ((time/beta) + 1)^-1 * parent

Method used for solution of differential equation system:
analytical

Starting values for optimised parameters:
         initial   type transformed
parent_0     100  state  100.000000
alpha          1 deparm    0.000000
beta          10 deparm    2.302585

Fixed parameter values:
None

Optimised, transformed parameters:
         Estimate Std. Error   Lower   Upper
parent_0  93.7700     1.8560 89.5700 97.9700
alpha      0.3180     0.1867 -0.1044  0.7405
beta       0.2102     0.2943 -0.4556  0.8759

Backtransformed parameters:
         Estimate   Lower  Upper
parent_0   93.770 89.5700 97.970
alpha       1.374  0.9009  2.097
beta        1.234  0.6341  2.401
```

```
Residual standard error: 2.628 on 9 degrees of freedom

Chi2 error levels in percent:
         err.min n.optim df
All data   6.204       3  3
parent     6.204       3  3

Estimated disappearance times:
         DT50  DT90
parent 0.8092 5.356

Parameter correlation:
         parent_0    alpha    beta
parent_0  1.00000 -0.09553 -0.1863
alpha    -0.09553  1.00000  0.9757
beta     -0.18628  0.97568  1.0000
```



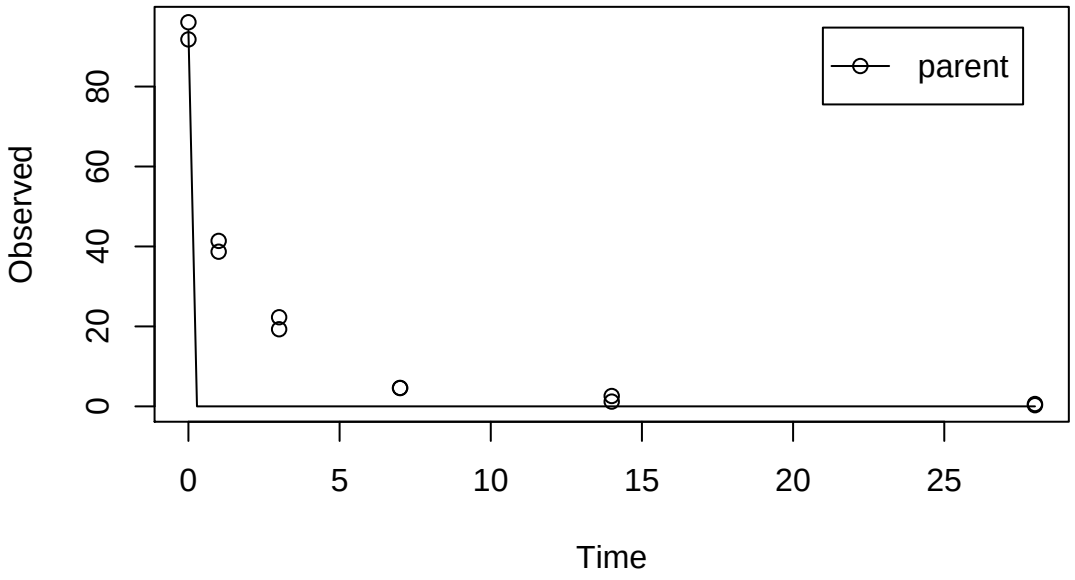


The error level at which the χ^2 test passes is much lower in this case. Therefore, the FOMC model provides a better description of the data, as less experimental error has to

be assumed in order to explain the data.

Fitting the four parameter DFOP model further reduces the χ^2 error level.

```
R> m.L2.DFOP <- mkinfit(DFOP, FOCUS_2006_L2_mkin, quiet = TRUE)
R> plot(m.L2.DFOP)
```

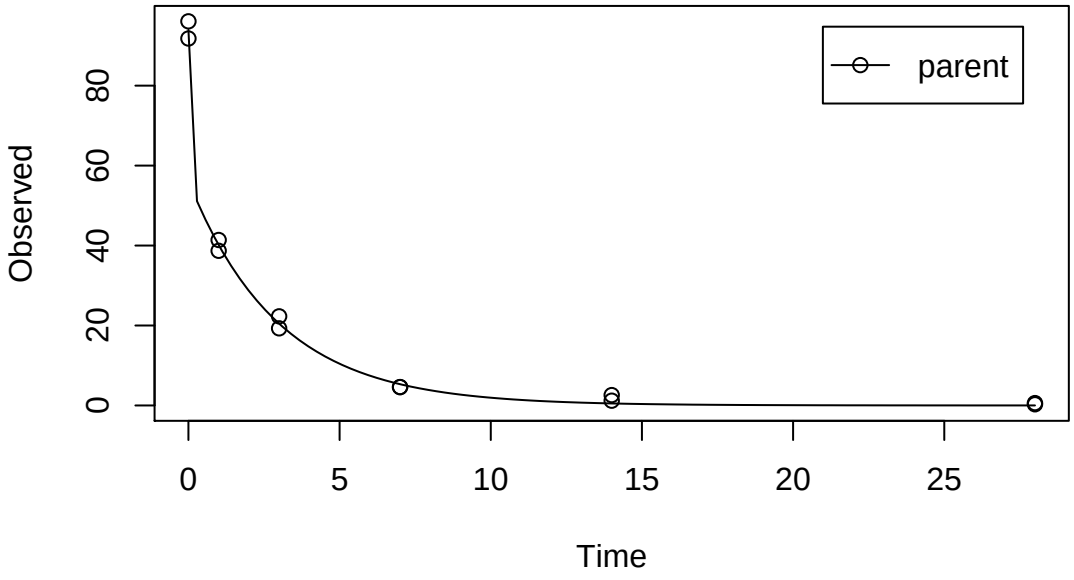


Here, the default starting parameters for the DFOP model obviously do not lead to a reasonable solution. Therefore the fit is repeated with different starting parameters.

```
R> m.L2.DFOP <- mkinfit(DFOP, FOCUS_2006_L2_mkin,
+   parms.ini = c(k1 = 1, k2 = 0.01, g = 0.8),
+   quiet=TRUE)
R> plot(m.L2.DFOP)
R> summary(m.L2.DFOP, data = FALSE)
```

```
mkin version:    0.9.19
R version:       2.15.3
Date of fit:     Sun Apr 14 14:28:42 2013
Date of summary: Sun Apr 14 14:28:42 2013

Equations:
[1] d_parent = - ((k1 * g * exp(-k1 * time) + k2 * (1 - g) * exp(-k2 * time)) / (g * exp

Method used for solution of differential equation system:
```

```
analytical

Starting values for optimised parameters:
         initial   type transformed
parent_0   1e+02  state 100.0000000
k1         1e+00 deparm   0.0000000
k2         1e-02 deparm  -4.6051702
g          8e-01 deparm   0.9802581

Fixed parameter values:
None

Optimised, transformed parameters:
         Estimate Std. Error Lower Upper
parent_0  93.9500         NA    NA    NA
k1         4.9590         NA    NA    NA
k2        -1.0880         NA    NA    NA
g         -0.2821         NA    NA    NA

Backtransformed parameters:
         Estimate Lower Upper
parent_0  93.9500    NA    NA
k1       142.4000    NA    NA
k2         0.3369    NA    NA
g          0.4016    NA    NA

Residual standard error: 1.732 on 8 degrees of freedom

Chi2 error levels in percent:
         err.min n.optim df
All data   2.529       4  2
parent     2.529       4  2

Estimated disappearance times:
       DT50 DT90
parent   NA   NA

Parameter correlation:
Could not estimate covariance matrix; singular system:
```



Here, the DFOP model is clearly the best-fit model for dataset L2 based on the χ^2 error level criterion. However, the failure to calculate the covariance matrix indicates that the parameter estimates correlate excessively. Therefore, the FOMC model may be preferred for this dataset.

1.3 Laboratory Data L3

The following code defines example dataset L3 from the FOCUS kinetics report, p. 290.

```
R> FOCUS_2006_L3 = data.frame(
+   t = c(0, 3, 7, 14, 30, 60, 91, 120),
+   parent = c(97.8, 60, 51, 43, 35, 22, 15, 12))
R> FOCUS_2006_L3_mkin <- mkin_wide_to_long(FOCUS_2006_L3)
```

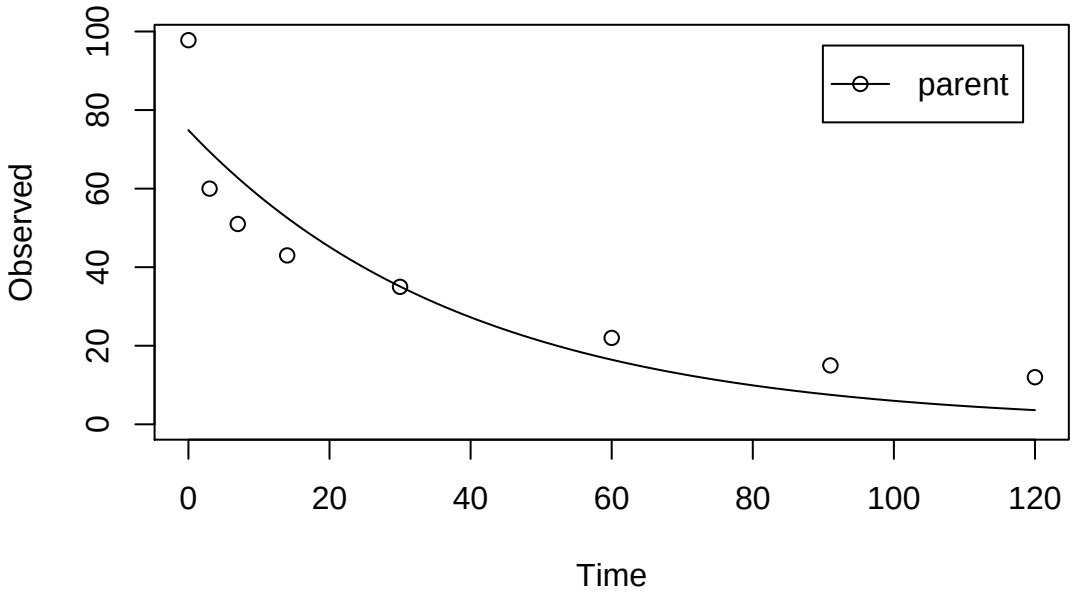
SFO model, summary and plot:

```
R> m.L3.SFO <- mkinfit(SFO, FOCUS_2006_L3_mkin, quiet = TRUE)
R> plot(m.L3.SFO)
R> summary(m.L3.SFO)
```

```
mkin version:    0.9.19
R version:       2.15.3
Date of fit:     Sun Apr 14 14:28:42 2013
```

```
Date of summary: Sun Apr 14 14:28:42 2013

Equations:
[1] d_parent = - k_parent_sink * parent

Method used for solution of differential equation system:
analytical

Starting values for optimised parameters:
              initial   type transformed
parent_0        100.0  state  100.000000
k_parent_sink     0.1 deparm   -2.302585

Fixed parameter values:
None

Optimised, transformed parameters:
              Estimate Std. Error  Lower Upper
parent_0        74.870     8.4580 54.180 95.57
k_parent_sink   -3.678     0.3261 -4.476 -2.88

Backtransformed parameters:
              Estimate    Lower    Upper
parent_0      74.87000 54.18000 95.57000
k_parent_sink  0.02527  0.01138  0.05612

Residual standard error: 12.91 on 6 degrees of freedom

Chi2 error levels in percent:
         err.min n.optim df
All data   21.24       2  6
parent     21.24       2  6

Estimated disappearance times:
        DT50  DT90
parent 27.43 91.12

Estimated formation fractions:
            ff
parent_sink  1

Parameter correlation:
              parent_0 k_parent_sink
parent_0        1.0000        0.5484
k_parent_sink   0.5484        1.0000

Data:
 time variable observed predicted  residual
    0   parent     97.8    74.873  22.92734
```

```
  3   parent     60.0    69.407  -9.40654
  7   parent     51.0    62.734 -11.73403
 14   parent     43.0    52.563  -9.56336
 30   parent     35.0    35.083  -0.08281
 60   parent     22.0    16.439   5.56137
 91   parent     15.0     7.510   7.48961
120   parent     12.0     3.609   8.39083
```



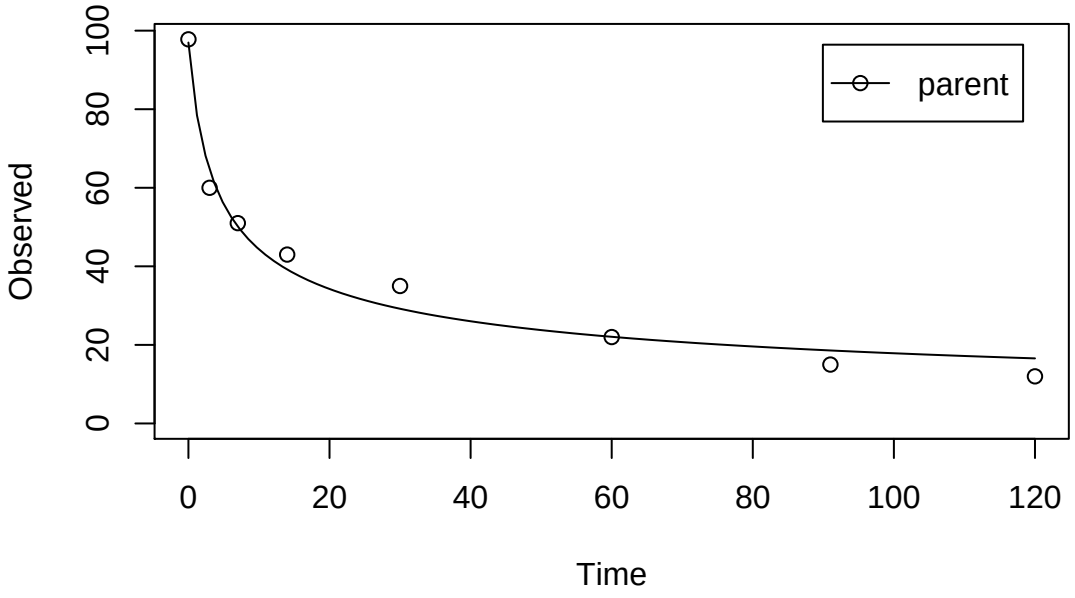
The χ^2 error level of 22% as well as the plot suggest that the model does not fit very well.

The FOMC model performs better:

```
R> m.L3.FOMC <- mkinfit(FOMC, FOCUS_2006_L3_mkin, quiet = TRUE)
R> plot(m.L3.FOMC)
R> summary(m.L3.FOMC, data = FALSE)
```

```
mkin version:    0.9.19
R version:       2.15.3
Date of fit:     Sun Apr 14 14:28:42 2013
Date of summary: Sun Apr 14 14:28:42 2013

Equations:
[1] d_parent = - (alpha/beta) * ((time/beta) + 1)^-1 * parent

Method used for solution of differential equation system:
analytical
```

```
Starting values for optimised parameters:
         initial   type transformed
parent_0     100  state  100.000000
alpha          1 deparm    0.000000
beta          10 deparm    2.302585

Fixed parameter values:
None

Optimised, transformed parameters:
         Estimate Std. Error   Lower    Upper
parent_0  96.9700     4.5500 85.2800 108.7000
alpha     -0.8619     0.1704 -1.3000  -0.4237
beta       0.6193     0.4744 -0.6003   1.8390

Backtransformed parameters:
         Estimate   Lower    Upper
parent_0  96.9700 85.2800 108.7000
alpha      0.4224  0.2725   0.6546
beta       1.8580  0.5487   6.2890

Residual standard error: 4.572 on 5 degrees of freedom

Chi2 error levels in percent:
         err.min n.optim df
All data   7.322       3  5
parent     7.322       3  5

Estimated disappearance times:
        DT50  DT90
parent 7.729 431.2

Parameter correlation:
         parent_0   alpha    beta
parent_0   1.0000 -0.1512 -0.4271
alpha     -0.1512  1.0000  0.9110
beta      -0.4271  0.9110  1.0000
```



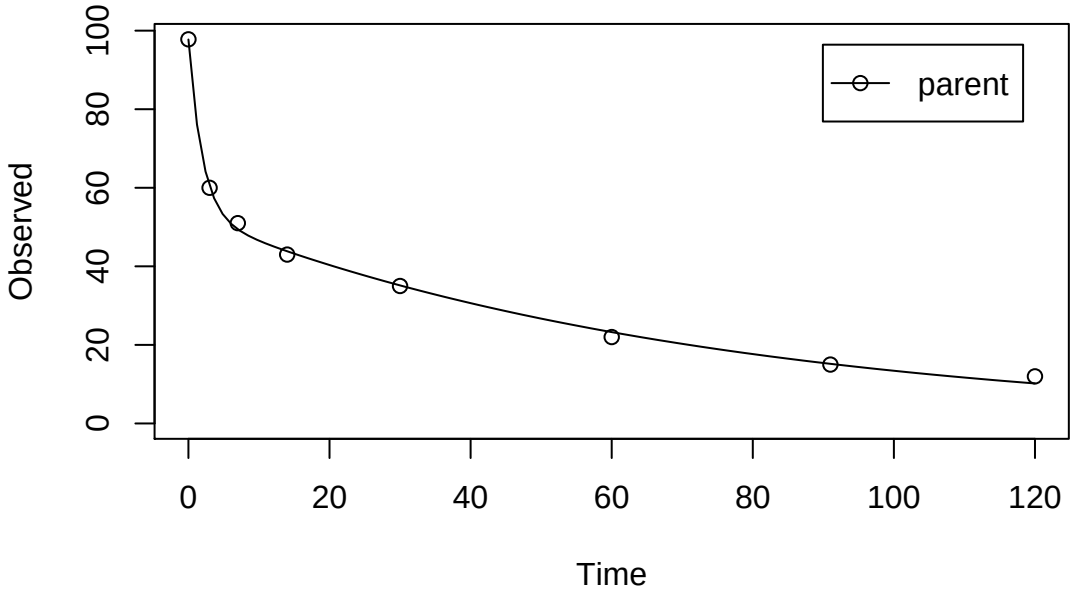
The error level at which the χ^2 test passes is 7% in this case.

Fitting the four parameter DFOP model further reduces the χ^2 error level considerably:

```
R> m.L3.DFOP <- mkinfit(DFOP, FOCUS_2006_L3_mkin, quiet = TRUE)
R> plot(m.L3.DFOP)
R> summary(m.L3.DFOP, data = FALSE)
```

```
mkin version:    0.9.19
R version:       2.15.3
Date of fit:     Sun Apr 14 14:28:42 2013
Date of summary: Sun Apr 14 14:28:42 2013

Equations:
[1] d_parent = - ((k1 * g * exp(-k1 * time) + k2 * (1 - g) * exp(-k2 * time)) / (g * exp

Method used for solution of differential equation system:
analytical

Starting values for optimised parameters:
         initial   type transformed
parent_0   1e+02  state  100.000000
k1         1e-01 deparm   -2.302585
k2         1e-02 deparm   -4.605170
g          5e-01 deparm    0.000000
```

```
Fixed parameter values:
None

Optimised, transformed parameters:
         Estimate Std. Error   Lower     Upper
parent_0  97.7500    1.43800 93.7500 101.70000
k1        -0.6612    0.13340 -1.0310  -0.29100
k2        -4.2860    0.05902 -4.4500  -4.12200
g         -0.1229    0.05121 -0.2651   0.01925

Backtransformed parameters:
         Estimate    Lower     Upper
parent_0 97.75000 93.75000 101.70000
k1        0.51620  0.35650   0.74750
k2        0.01376  0.01168   0.01621
g         0.45660  0.40730   0.50680

Residual standard error: 1.439 on 4 degrees of freedom

Chi2 error levels in percent:
         err.min n.optim df
All data   2.224       4  4
parent     2.224       4  4

Estimated disappearance times:
        DT50 DT90
parent 7.464  123

Parameter correlation:
         parent_0      k1       k2       g
parent_0  1.00000  0.1640  0.01315  0.4253
k1        0.16399  1.0000  0.46477 -0.5526
k2        0.01315  0.4648  1.00000 -0.6631
g         0.42526 -0.5526 -0.66310  1.0000
```



Here, a look to the model plot, the confidence intervals of the parameters and the correlation matrix suggest that the paramter estimates are reliable, and the DFOP model can be used as the best-fit model based on the χ^2 error level criterion for laboratory data L3.

1.4 Laboratory Data L4

The following code defines example dataset L4 from the FOCUS kinetics report, p. 293

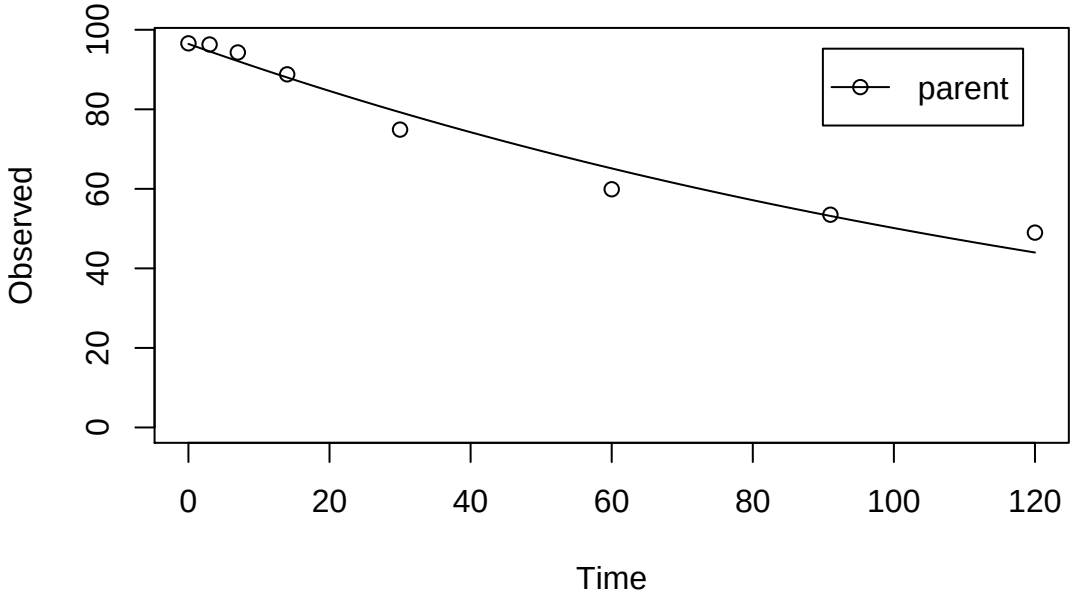
```
R> FOCUS_2006_L4 = data.frame(
+   t = c(0, 3, 7, 14, 30, 60, 91, 120),
+   parent = c(96.6, 96.3, 94.3, 88.8, 74.9, 59.9, 53.5, 49.0))
R> FOCUS_2006_L4_mkin <- mkin_wide_to_long(FOCUS_2006_L4)
```

SFO model, summary and plot:

```
R> m.L4.SFO <- mkinfit(SFO, FOCUS_2006_L4_mkin, quiet = TRUE)
R> plot(m.L4.SFO)
R> summary(m.L4.SFO, data = FALSE)
```

```
mkin version:    0.9.19
R version:       2.15.3
Date of fit:     Sun Apr 14 14:28:43 2013
Date of summary: Sun Apr 14 14:28:43 2013
```

```
Equations:
[1] d_parent = - k_parent_sink * parent

Method used for solution of differential equation system:
analytical

Starting values for optimised parameters:
              initial   type transformed
parent_0        100.0  state  100.000000
k_parent_sink     0.1 deparm   -2.302585

Fixed parameter values:
None

Optimised, transformed parameters:
              Estimate Std. Error  Lower   Upper
parent_0         96.44    1.94900 91.670 101.200
k_parent_sink    -5.03    0.07999 -5.225  -4.834

Backtransformed parameters:
               Estimate     Lower     Upper
parent_0      96.440000 91.670000 1.012e+02
k_parent_sink  0.006541  0.005378 7.955e-03

Residual standard error: 3.651 on 6 degrees of freedom

Chi2 error levels in percent:
         err.min n.optim df
All data   3.288       2  6
parent     3.288       2  6

Estimated disappearance times:
       DT50 DT90
parent  106  352

Estimated formation fractions:
            ff
parent_sink  1

Parameter correlation:
              parent_0 k_parent_sink
parent_0        1.0000        0.5865
k_parent_sink   0.5865        1.0000
```



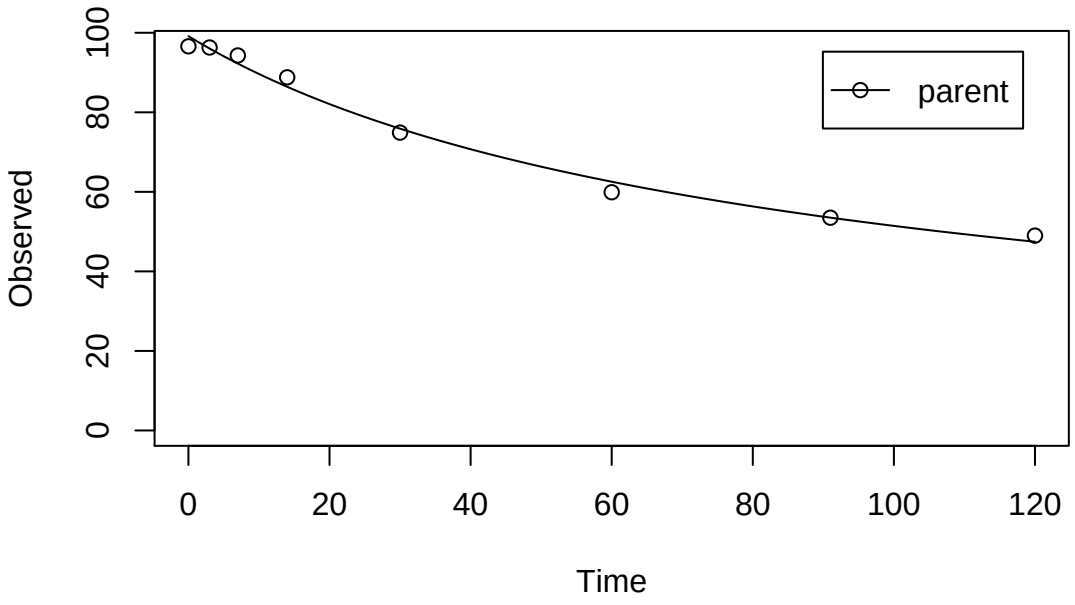
The χ^2 error level of 3.3% as well as the plot suggest that the model fits very well.

The FOMC model for comparison

```
R> m.L4.FOMC <- mkinfit(FOMC, FOCUS_2006_L4_mkin, quiet = TRUE)
R> plot(m.L4.FOMC)
R> summary(m.L4.FOMC, data = FALSE)
```

```
mkin version:    0.9.19
R version:       2.15.3
Date of fit:     Sun Apr 14 14:28:43 2013
Date of summary: Sun Apr 14 14:28:43 2013

Equations:
[1] d_parent = - (alpha/beta) * ((time/beta) + 1)^-1 * parent

Method used for solution of differential equation system:
analytical

Starting values for optimised parameters:
         initial   type transformed
parent_0     100  state  100.000000
alpha          1 deparm    0.000000
beta          10 deparm    2.302585

Fixed parameter values:
```

```
None

Optimised, transformed parameters:
         Estimate Std. Error  Lower    Upper
parent_0  99.1400     1.6800 94.820 103.5000
alpha     -0.3506     0.3725 -1.308   0.6068
beta       4.1740     0.5635  2.725   5.6230

Backtransformed parameters:
         Estimate   Lower   Upper
parent_0  99.1400 94.8200 103.500
alpha      0.7042  0.2703   1.835
beta      64.9800 15.2600 276.600

Residual standard error: 2.315 on 5 degrees of freedom

Chi2 error levels in percent:
         err.min n.optim df
All data   2.028       3  5
parent     2.028       3  5

Estimated disappearance times:
        DT50 DT90
parent 108.9 1644

Parameter correlation:
         parent_0   alpha    beta
parent_0   1.0000 -0.5365 -0.6083
alpha     -0.5365  1.0000  0.9913
beta      -0.6083  0.9913  1.0000
```



The error level at which the χ^2 test passes is slightly lower for the FOMC model. However, the difference appears negligible.

2 Kinetic evaluations for parent and metabolites

2.1 Laboratory Data for example compound Z

The following code defines the example dataset from Appendix 7 to the FOCUS kinetics report, p.350

```
R> LOD = 0.5
R> FOCUS_2006_Z = data.frame(
+   t = c(0, 0.04, 0.125, 0.29, 0.54, 1, 2, 3, 4, 7, 10, 14, 21,
+         42, 61, 96, 124),
+   Z0 = c(100, 81.7, 70.4, 51.1, 41.2, 6.6, 4.6, 3.9, 4.6, 4.3, 6.8,
+          2.9, 3.5, 5.3, 4.4, 1.2, 0.7),
+   Z1 = c(0, 18.3, 29.6, 46.3, 55.1, 65.7, 39.1, 36, 15.3, 5.6, 1.1,
+          1.6, 0.6, 0.5 * LOD, NA, NA, NA),
+   Z2 = c(0, NA, 0.5 * LOD, 2.6, 3.8, 15.3, 37.2, 31.7, 35.6, 14.5,
+          0.8, 2.1, 1.9, 0.5 * LOD, NA, NA, NA),
```

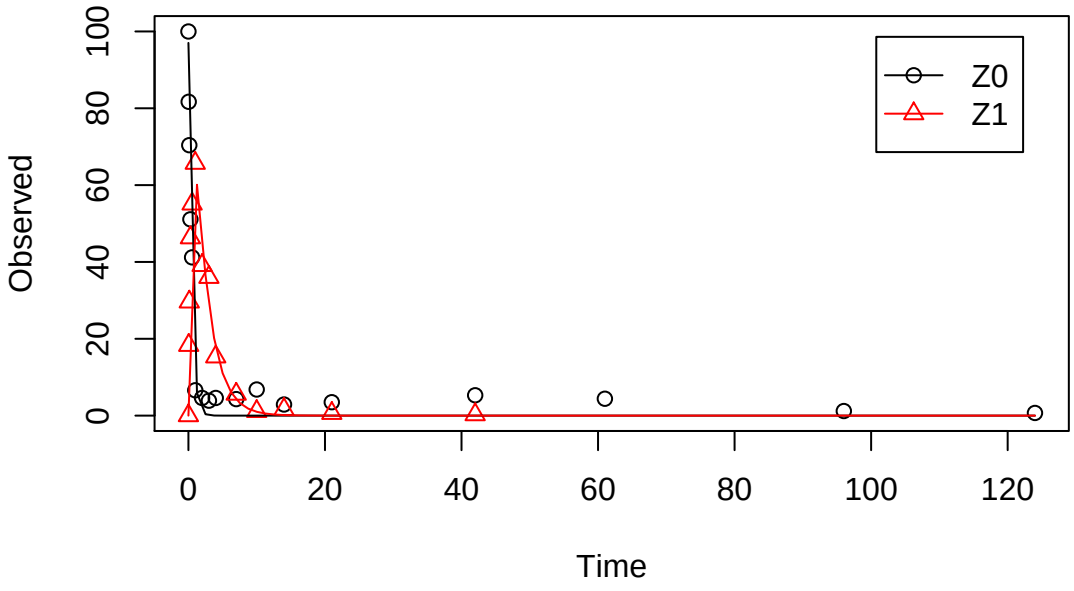
```
+   Z3 = c(0, NA, NA, NA, NA, 0.5 * LOD, 9.2, 13.1, 22.3, 28.4, 32.5,
+          25.2, 17.2, 4.8, 4.5, 2.8, 4.4))
R> FOCUS_2006_Z_mkin <- mkin_wide_to_long(FOCUS_2006_Z)
```

The next step is to set up the models used for the kinetic analysis. As the simultaneous fit of parent and the first metabolite is usually straightforward, Step 1 (SFO for parent only) is skipped here. We start with the model 2a, with formation and decline of metabolite Z1 and the pathway from parent directly to sink included (default in mkin).

```
R> Z.2a <- mkinmod(Z0 = list(type = "SFO", to = "Z1"),
+                  Z1 = list(type = "SFO"))
R> m.Z.2a <- mkinfit(Z.2a, FOCUS_2006_Z_mkin, quiet = TRUE)
R> plot(m.Z.2a)
R> summary(m.Z.2a, data = FALSE)
```

```
mkin version:    0.9.19
R version:       2.15.3
Date of fit:     Sun Apr 14 14:28:44 2013
Date of summary: Sun Apr 14 14:28:44 2013

Equations:
[1] d_Z0 = - k_Z0_sink * Z0 - k_Z0_Z1 * Z0 d_Z1 = + k_Z0_Z1 * Z0 - k_Z1_sink * Z1

Method used for solution of differential equation system:
eigen

Starting values for optimised parameters:
          initial   type transformed
Z0_0        100.0  state  100.000000
k_Z0_sink     0.1 deparm   -2.302585
k_Z0_Z1       0.1 deparm   -2.302585
k_Z1_sink     0.1 deparm   -2.302585

Fixed parameter values:
   value  type
Z1     0 state

Optimised, transformed parameters:
          Estimate Std. Error Lower Upper
Z0_0       97.0100         NA    NA    NA
k_Z0_sink -36.2900         NA    NA    NA
k_Z0_Z1     0.8047         NA    NA    NA
k_Z1_sink  -0.7296         NA    NA    NA

Backtransformed parameters:
           Estimate Lower Upper
Z0_0      9.701e+01    NA    NA
k_Z0_sink 1.733e-16    NA    NA
```

```
k_Z0_Z1   2.236e+00     NA     NA
k_Z1_sink 4.821e-01     NA     NA

Residual standard error: 5.064 on 27 degrees of freedom

Chi2 error levels in percent:
          err.min n.optim df
All data   18.20        4 27
Z0         18.04        3 14
Z1         15.75        1 13

Estimated disappearance times:
    DT50  DT90
Z0 0.310 1.030
Z1 1.438 4.776

Estimated formation fractions:
               ff
Z0_sink 7.75e-17
Z0_Z1   1.00e+00
Z1_sink 1.00e+00

Parameter correlation:
Could not estimate covariance matrix; singular system:
```



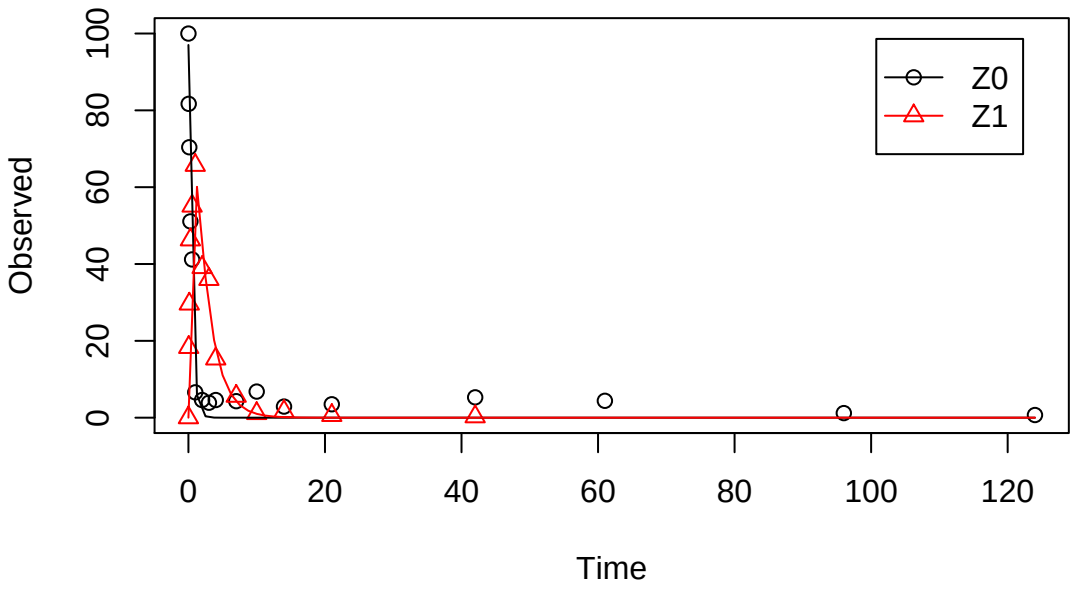
As obvious from the summary, the kinetic rate constant from parent compound Z to sink is negligible. Accordingly, the exact magnitude of the fitted parameter `log k_Z_sink` is ill-defined and the covariance matrix is not returned. This suggests, in agreement with the analysis in the FOCUS kinetics report, to simplify the model by removing the pathway to sink.

A similar result can be obtained when formation fractions are used in the model formulation:

```
R> Z.2a.ff <- mkinmod(Z0 = list(type = "SFO", to = "Z1"),
+                  Z1 = list(type = "SFO"), use_of_ff = "max")
R> m.Z.2a.ff <- mkinfit(Z.2a.ff, FOCUS_2006_Z_mkin, quiet = TRUE)
R> plot(m.Z.2a.ff)
R> summary(m.Z.2a.ff, data = FALSE)
```

```
mkin version:    0.9.19
R version:       2.15.3
Date of fit:     Sun Apr 14 14:28:44 2013
Date of summary: Sun Apr 14 14:28:44 2013

Equations:
[1] d_Z0 = - k_Z0 * Z0                          d_Z1 = + f_Z0_to_Z1 * k_Z0 * Z0 - k_Z1 *

Method used for solution of differential equation system:
eigen

Starting values for optimised parameters:
           initial   type transformed
Z0_0         100.0  state 100.0000000
k_Z0           0.1 deparm  -2.3025851
f_Z0_to_Z1     0.2 deparm  -0.9802581
k_Z1           0.1 deparm  -2.3025851

Fixed parameter values:
   value  type
Z1     0 state

Optimised, transformed parameters:
             Estimate Std. Error Lower Upper
Z0_0        9.701e+01         NA    NA    NA
k_Z0        8.047e-01         NA    NA    NA
f_Z0_to_Z1  8.862e+06         NA    NA    NA
k_Z1       -7.296e-01         NA    NA    NA

Backtransformed parameters:
           Estimate Lower Upper
Z0_0        97.0100    NA    NA
k_Z0         2.2360    NA    NA
```

```
f_Z0_to_Z1    1.0000     NA     NA
k_Z1          0.4821     NA     NA

Residual standard error: 5.064 on 27 degrees of freedom

Chi2 error levels in percent:
          err.min n.optim df
All data   18.20        4 27
Z0         17.56        2 15
Z1         16.25        2 12

Estimated disappearance times:
    DT50  DT90
Z0 0.310 1.030
Z1 1.438 4.776

Parameter correlation:
Could not estimate covariance matrix; singular system:
```



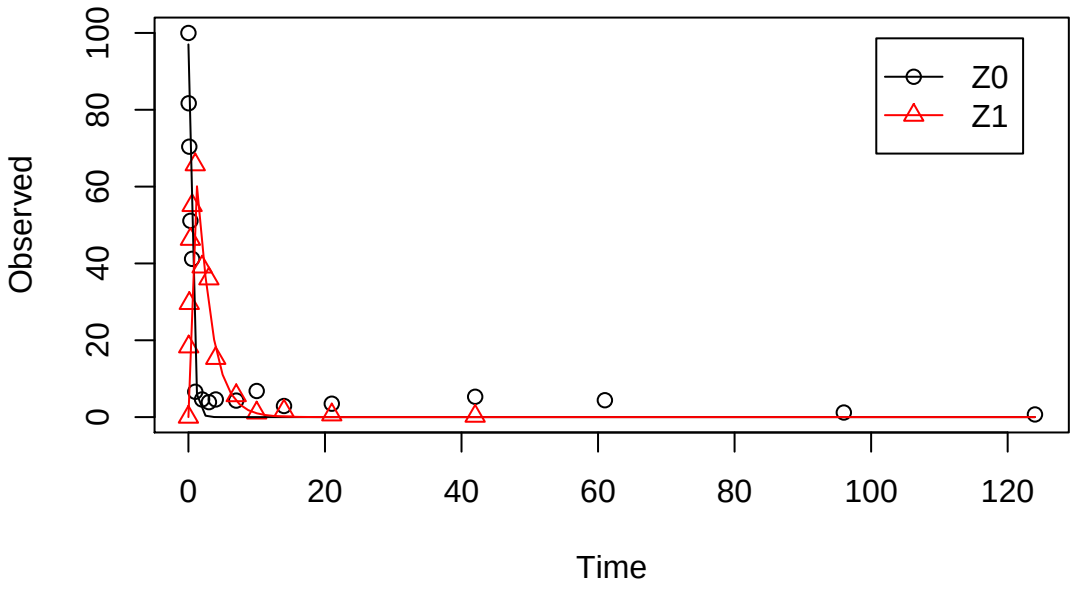
Here, the ilr transformed formation fraction fitted in the model takes a very large value, and the backtransformed formation fraction from parent Z to Z1 is practically unity. Again, the covariance matrix is not returned as the model is overparameterised.

The simplified model is obtained by setting the list component `sink` to `FALSE`. This model definition is not supported when formation fractions are used.

```
R> Z.3 <- mkinmod(Z0 = list(type = "SFO", to = "Z1", sink = FALSE),
+                 Z1 = list(type = "SFO"))
R> m.Z.3 <- mkinfit(Z.3, FOCUS_2006_Z_mkin, parms.ini = c(k_Z0_Z1 = 0.5),
+                  quiet = TRUE)
R> #m.Z.3 <- mkinfit(Z.3, FOCUS_2006_Z_mkin, solution_type = "deSolve")
R> plot(m.Z.3)
R> summary(m.Z.3, data = FALSE)
```

```
mkin version:    0.9.19
R version:       2.15.3
Date of fit:     Sun Apr 14 14:28:45 2013
Date of summary: Sun Apr 14 14:28:45 2013

Equations:
[1] d_Z0 = - 0 - k_Z0_Z1 * Z0              d_Z1 = + k_Z0_Z1 * Z0 - k_Z1_sink * Z1

Method used for solution of differential equation system:
eigen

Starting values for optimised parameters:
          initial   type transformed
Z0_0        100.0  state 100.0000000
k_Z0_Z1       0.5 deparm  -0.6931472
k_Z1_sink     0.1 deparm  -2.3025851

Fixed parameter values:
   value  type
Z1     0 state

Optimised, transformed parameters:
          Estimate Std. Error   Lower    Upper
Z0_0       97.0100    2.68200 91.5200 102.5000
k_Z0_Z1     0.8047    0.06568  0.6702   0.9392
k_Z1_sink  -0.7296    0.08854 -0.9109  -0.5482

Backtransformed parameters:
          Estimate   Lower   Upper
Z0_0       97.0100 91.5200 102.500
k_Z0_Z1     2.2360  1.9550   2.558
k_Z1_sink   0.4821  0.4022   0.578

Residual standard error: 4.973 on 28 degrees of freedom

Chi2 error levels in percent:
         err.min n.optim df
All data   17.93       3 28
Z0         17.56       2 15
Z1         15.75       1 13
```

```
Estimated disappearance times:
    DT50  DT90
Z0 0.310 1.030
Z1 1.438 4.776

Estimated formation fractions:
         ff
Z0_Z1     1
Z1_sink   1

Parameter correlation:
             Z0_0 k_Z0_Z1 k_Z1_sink
Z0_0       1.0000 0.10629   0.41038
k_Z0_Z1    0.1063 1.00000   0.04345
k_Z1_sink 0.4104 0.04345   1.00000
```

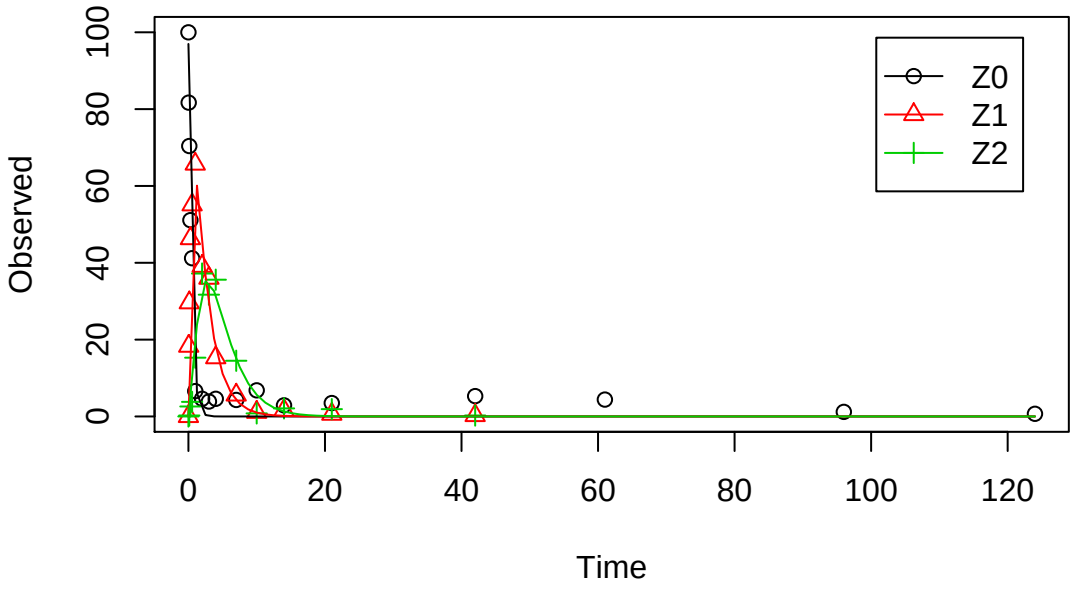


The first attempt to fit the model failed, as the default solution type chosen by mkinfit is based on eigenvalues, and the system defined by the starting parameters is identified as being singular to the solver. This is caused by the fact that the rate constants for both state variables are the same using the default starting paramters. Setting a different starting value for one of the parameters overcomes this. Alternatively, the **deSolve** based model solution can be chosen, at the cost of a bit more computing time.

```
R> Z.4a <- mkinmod(Z0 = list(type = "SFO", to = "Z1", sink = FALSE),
+                  Z1 = list(type = "SFO", to = "Z2"),
+                  Z2 = list(type = "SFO"))
R> m.Z.4a <- mkinfit(Z.4a, FOCUS_2006_Z_mkin, parms.ini = c(k_Z0_Z1 = 0.5),
+                    quiet = TRUE)
R> plot(m.Z.4a)
R> summary(m.Z.4a, data = FALSE)
```

```
mkin version:    0.9.19
R version:       2.15.3
Date of fit:     Sun Apr 14 14:28:46 2013
Date of summary: Sun Apr 14 14:28:46 2013

Equations:
[1] d_Z0 = - 0 - k_Z0_Z1 * Z0                                d_Z1 = + k_Z0_Z1 * Z0 - k_Z1_s
[3] d_Z2 = + k_Z1_Z2 * Z1 - k_Z2_sink * Z2

Method used for solution of differential equation system:
eigen

Starting values for optimised parameters:
          initial   type transformed
Z0_0        100.0  state 100.0000000
k_Z0_Z1       0.5 deparm  -0.6931472
k_Z1_sink     0.1 deparm  -2.3025851
k_Z1_Z2       0.1 deparm  -2.3025851
k_Z2_sink     0.1 deparm  -2.3025851

Fixed parameter values:
   value  type
Z1     0 state
Z2     0 state

Optimised, transformed parameters:
          Estimate Std. Error    Lower    Upper
Z0_0       96.9600    2.44700  92.0100 101.9000
k_Z0_Z1     0.7970    0.05974   0.6762   0.9179
k_Z1_sink  -4.1040    4.84700 -13.9100   5.7000
k_Z1_Z2    -0.7667    0.14630  -1.0630  -0.4707
k_Z2_sink  -0.8410    0.22860  -1.3030  -0.3787

Backtransformed parameters:
          Estimate     Lower    Upper
Z0_0       96.9600 9.201e+01 101.9000
k_Z0_Z1     2.2190 1.966e+00   2.5040
k_Z1_sink   0.0165 9.112e-07 298.8000
k_Z1_Z2     0.4645 3.455e-01   0.6245
k_Z2_sink   0.4313 2.716e-01   0.6848
```

```
Residual standard error: 4.54 on 39 degrees of freedom

Chi2 error levels in percent:
         err.min n.optim df
All data   19.78       5 39
Z0         17.47       2 15
Z1         16.37       2 12
Z2         20.47       1 12

Estimated disappearance times:
     DT50  DT90
Z0 0.3124 1.038
Z1 1.4410 4.787
Z2 1.6072 5.339

Estimated formation fractions:
            ff
Z0_Z1   1.0000
Z1_sink 0.0343
Z1_Z2   0.9657
Z2_sink 1.0000

Parameter correlation:
              Z0_0 k_Z0_Z1 k_Z1_sink k_Z1_Z2 k_Z2_sink
Z0_0       1.0000  0.1064    0.3492 -0.1769   -0.1534
k_Z0_Z1    0.1064  1.0000    0.1479 -0.1501   -0.1226
k_Z1_sink  0.3492  0.1479    1.0000 -0.8745   -0.8656
k_Z1_Z2   -0.1769 -0.1501   -0.8745  1.0000    0.8190
k_Z2_sink -0.1534 -0.1226   -0.8656  0.8190    1.0000
```



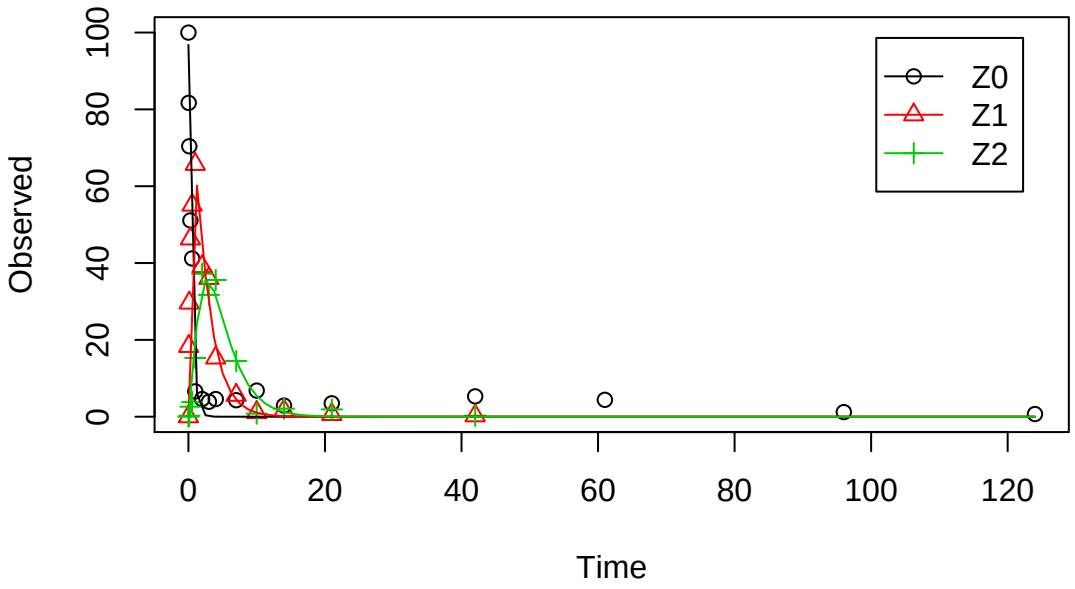
As suggested in the FOCUS report, the pathway to sink was removed for metabolite Z1 as well in the next step. While this step appears questionable on the basis of the above results, it is followed here for the purpose of comparison. Also, in the FOCUS report, it is assumed that there is additional empirical evidence that Z1 quickly and exclusively hydrolyses to Z2. Again, in order to avoid a singular system when using default starting parameters, the starting parameter for the pathway without sink term has to be adapted.

```
R> Z.5 <- mkinmod(Z0 = list(type = "SFO", to = "Z1", sink = FALSE),
+                 Z1 = list(type = "SFO", to = "Z2", sink = FALSE),
+                 Z2 = list(type = "SFO"))
R> m.Z.5 <- mkinfit(Z.5, FOCUS_2006_Z_mkin,
+                   parms.ini = c(k_Z0_Z1 = 0.5, k_Z1_Z2 = 0.2), quiet = TRUE)
R> plot(m.Z.5)
R> summary(m.Z.5, data = FALSE)
```

```
mkin version:    0.9.19
R version:       2.15.3
Date of fit:     Sun Apr 14 14:28:47 2013
Date of summary: Sun Apr 14 14:28:47 2013

Equations:
[1] d_Z0 = - 0 - k_Z0_Z1 * Z0                d_Z1 = + k_Z0_Z1 * Z0 - 0 - k_Z1_Z2 * Z1
[3] d_Z2 = + k_Z1_Z2 * Z1 - k_Z2_sink * Z2
```

```
Method used for solution of differential equation system:
eigen

Starting values for optimised parameters:
          initial   type transformed
Z0_0        100.0  state 100.0000000
k_Z0_Z1       0.5 deparm  -0.6931472
k_Z1_Z2       0.2 deparm  -1.6094379
k_Z2_sink     0.1 deparm  -2.3025851

Fixed parameter values:
   value  type
Z1     0 state
Z2     0 state

Optimised, transformed parameters:
          Estimate Std. Error   Lower    Upper
Z0_0       96.7700    2.26600 92.1900 101.3000
k_Z0_Z1     0.7948    0.05843  0.6767   0.9129
k_Z1_Z2    -0.7410    0.06821 -0.8789  -0.6032
k_Z2_sink  -0.8027    0.11090 -1.0270  -0.5785

Backtransformed parameters:
          Estimate   Lower    Upper
Z0_0       96.7700 92.1900 101.3000
k_Z0_Z1     2.2140  1.9670   2.4920
k_Z1_Z2     0.4766  0.4152   0.5471
k_Z2_sink   0.4481  0.3581   0.5607

Residual standard error: 4.486 on 40 degrees of freedom

Chi2 error levels in percent:
         err.min n.optim df
All data   19.58       4 40
Z0         17.43       2 15
Z1         15.94       1 13
Z2         20.51       1 12

Estimated disappearance times:
     DT50  DT90
Z0 0.3131 1.040
Z1 1.4543 4.831
Z2 1.5468 5.138

Estimated formation fractions:
        ff
Z0_Z1    1
Z1_Z2    1
Z2_sink  1
```

```
Parameter correlation:
             Z0_0  k_Z0_Z1  k_Z1_Z2 k_Z2_sink
Z0_0      1.00000  0.05781  0.28747   0.31786
k_Z0_Z1   0.05781  1.00000 -0.04361   0.01212
k_Z1_Z2   0.28747 -0.04361  1.00000   0.24018
k_Z2_sink 0.31786  0.01212  0.24018   1.00000
```



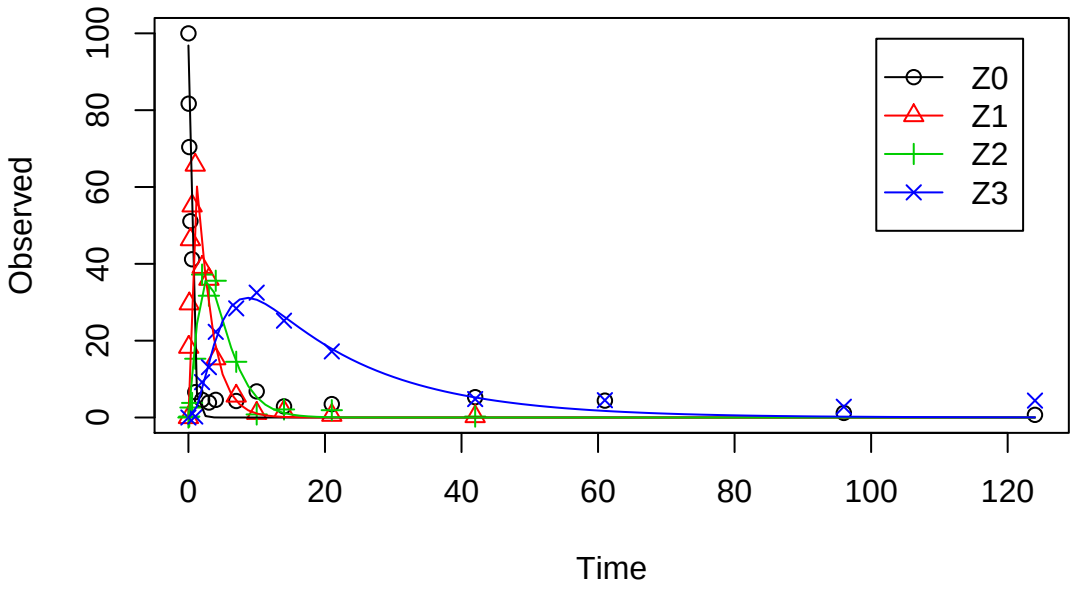
Finally, metabolite Z3 is added to the model.

```
R> Z.FOCUS <- mkinmod(Z0 = list(type = "SFO", to = "Z1", sink = FALSE),
+                     Z1 = list(type = "SFO", to = "Z2", sink = FALSE),
+                     Z2 = list(type = "SFO", to = "Z3"),
+                     Z3 = list(type = "SFO"))
R> m.Z.FOCUS <- mkinfit(Z.FOCUS, FOCUS_2006_Z_mkin,
+                       parms.ini = c(k_Z0_Z1 = 0.5, k_Z1_Z2 = 0.2, k_Z2_Z3 = 0.3),
+                       quiet = TRUE)
R> plot(m.Z.FOCUS)
R> summary(m.Z.FOCUS, data = FALSE)
```

```
mkin version:    0.9.19
R version:       2.15.3
Date of fit:     Sun Apr 14 14:28:48 2013
Date of summary: Sun Apr 14 14:28:48 2013
```

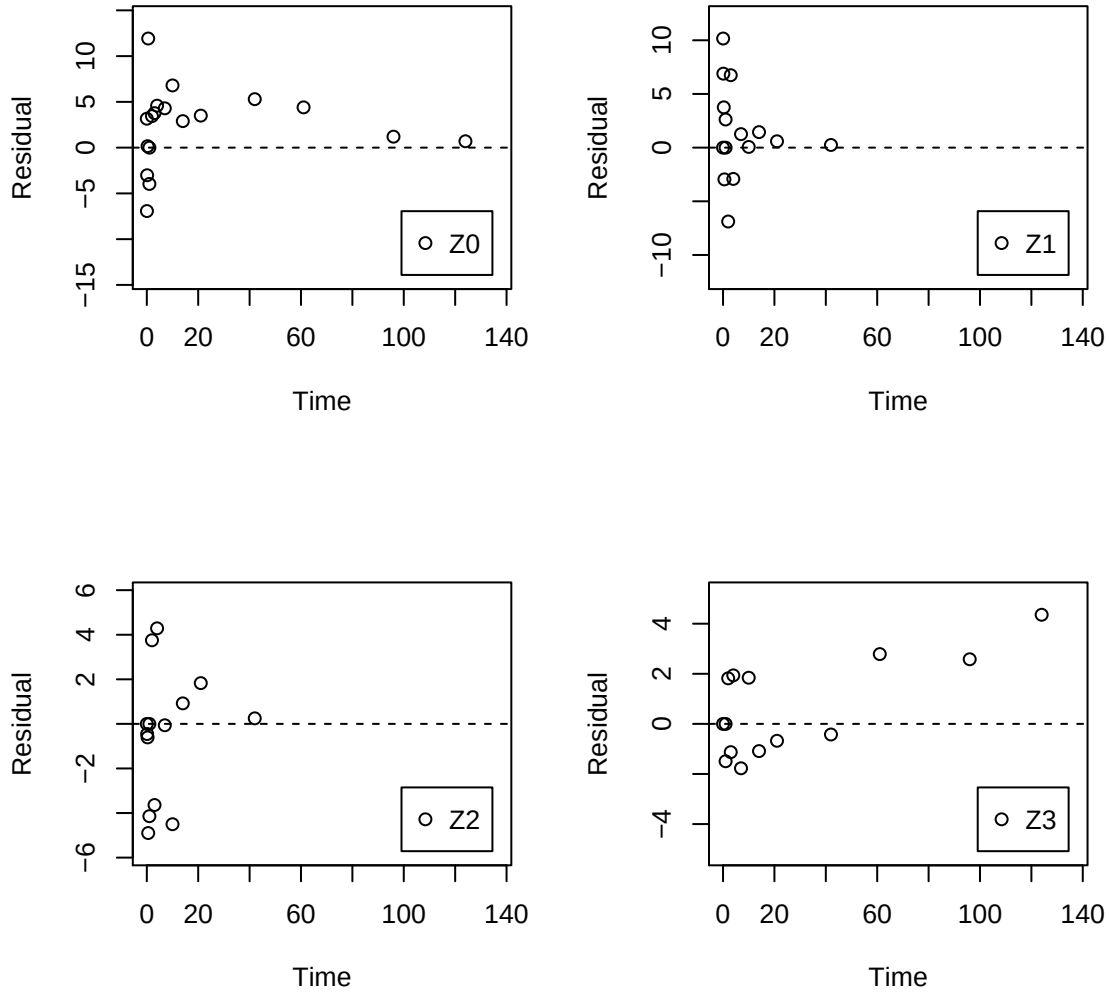
```
Equations:
[1] d_Z0 = - 0 - k_Z0_Z1 * Z0                              d_Z1 = + k_Z0_Z1 * Z0 - 0 - k_
[3] d_Z2 = + k_Z1_Z2 * Z1 - k_Z2_sink * Z2 - k_Z2_Z3 * Z2 d_Z3 = + k_Z2_Z3 * Z2 - k_Z3_s

Method used for solution of differential equation system:
eigen

Starting values for optimised parameters:
          initial   type transformed
Z0_0        100.0  state 100.0000000
k_Z0_Z1       0.5 deparm  -0.6931472
k_Z1_Z2       0.2 deparm  -1.6094379
k_Z2_Z3       0.3 deparm  -1.2039728
k_Z2_sink     0.1 deparm  -2.3025851
k_Z3_sink     0.1 deparm  -2.3025851

Fixed parameter values:
   value  type
Z1     0 state
Z2     0 state
Z3     0 state

Optimised, transformed parameters:
          Estimate Std. Error   Lower    Upper
Z0_0       96.8400    2.05900 92.7100 101.0000
k_Z0_Z1     0.7954    0.05332  0.6884   0.9025
k_Z1_Z2    -0.7375    0.06123 -0.8604  -0.6146
k_Z2_Z3    -1.5470    0.12250 -1.7930  -1.3010
k_Z2_sink  -1.4330    0.17160 -1.7770  -1.0880
k_Z3_sink  -2.8350    0.24360 -3.3240  -2.3470

Backtransformed parameters:
          Estimate    Lower    Upper
Z0_0      96.84000 92.71000 101.0000
k_Z0_Z1    2.21500  1.99100   2.4660
k_Z1_Z2    0.47830  0.42300   0.5409
k_Z2_Z3    0.21290  0.16650   0.2723
k_Z2_sink  0.23870  0.16910   0.3368
k_Z3_sink  0.05869  0.03599   0.0957

Residual standard error: 4.1 on 51 degrees of freedom

Chi2 error levels in percent:
         err.min n.optim df
All data   19.78       6 51
Z0         17.45       2 15
Z1         15.92       1 13
Z2         21.24       2 11
```

```
Z3          12.46       1 12

Estimated disappearance times:
      DT50   DT90
Z0  0.3129  1.039
Z1  1.4492  4.814
Z2  1.5348  5.099
Z3 11.8100 39.232

Estimated formation fractions:
            ff
Z0_Z1   1.0000
Z1_Z2   1.0000
Z2_sink 0.5285
Z2_Z3   0.4715
Z3_sink 1.0000

Parameter correlation:
               Z0_0  k_Z0_Z1 k_Z1_Z2  k_Z2_Z3 k_Z2_sink k_Z3_sink
Z0_0        1.00000  0.05387  0.2727 -0.07295   0.37006  -0.11348
k_Z0_Z1     0.05387  1.00000 -0.0521 -0.03581   0.02442  -0.02521
k_Z1_Z2     0.27275 -0.05210  1.0000 -0.12132   0.29384  -0.19144
k_Z2_Z3    -0.07295 -0.03581 -0.1213  1.00000  -0.18887   0.55154
k_Z2_sink   0.37006  0.02442  0.2938 -0.18887   1.00000  -0.64293
k_Z3_sink -0.11348 -0.02521 -0.1914  0.55154  -0.64293   1.00000
```



This is the fit corresponding to the final result chosen in Appendix 7 of the FOCUS report. The residual plots can be obtained by

```
R> par(mfrow = c(2, 2))
R> mkinresplot(m.Z.FOCUS, "Z0", lpos = "bottomright")
R> mkinresplot(m.Z.FOCUS, "Z1", lpos = "bottomright")
R> mkinresplot(m.Z.FOCUS, "Z2", lpos = "bottomright")
R> mkinresplot(m.Z.FOCUS, "Z3", lpos = "bottomright")
```



As the FOCUS report states, there is a certain tailing of the time course of metabolite Z3. Also, the time course of the parent compound is not fitted very well using the SFO model, as residues at a certain low level remain.

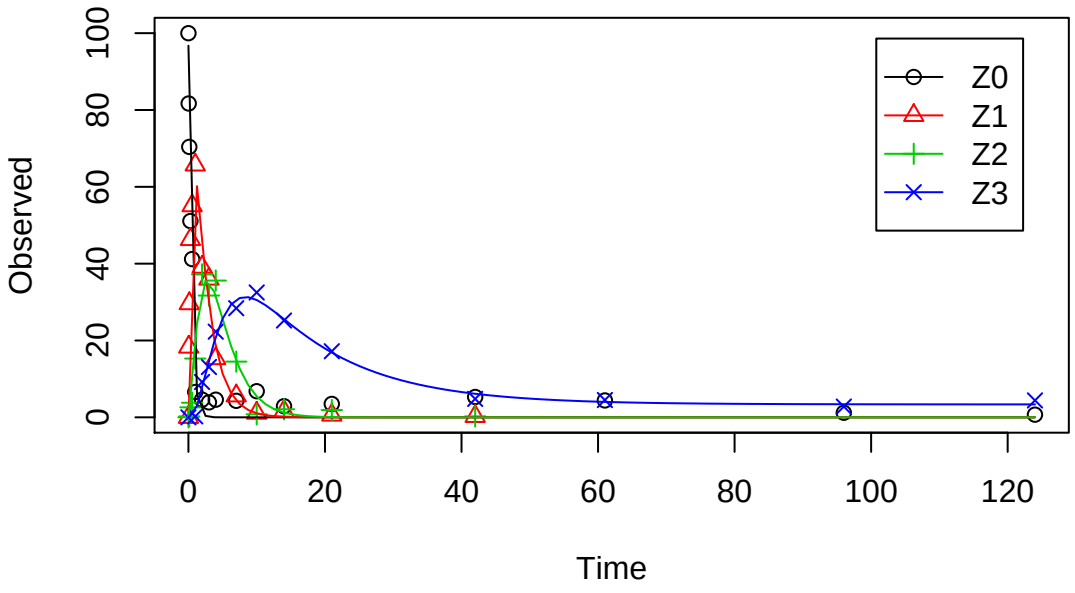
Therefore, an additional model is offered here, using the single first-order reversible binding (SFORB) model for metabolite Z3. As expected, the χ^2 error level is lower for metabolite Z3 using this model and the graphical fit for Z3 is improved. However, the covariance matrix is not returned.

```
R> Z.mkin.1 <- mkinmod(Z0 = list(type = "SFO", to = "Z1", sink = FALSE),
+                  Z1 = list(type = "SFO", to = "Z2", sink = FALSE),
+                  Z2 = list(type = "SFO", to = "Z3"),
+                  Z3 = list(type = "SFORB"))
R> m.Z.mkin.1 <- mkinfit(Z.mkin.1, FOCUS_2006_Z_mkin,
+                    parms.ini = c(k_Z0_Z1 = 0.5, k_Z1_Z2 = 0.3),
+                    quiet = TRUE)
R> plot(m.Z.mkin.1)
R> summary(m.Z.mkin.1, data = FALSE)
```

```
mkin version:    0.9.19
R version:       2.15.3
Date of fit:     Sun Apr 14 14:28:53 2013
Date of summary: Sun Apr 14 14:28:53 2013

Equations:
[1] d_Z0 = - 0 - k_Z0_Z1 * Z0
[2] d_Z1 = + k_Z0_Z1 * Z0 - 0 - k_Z1_Z2 * Z1
[3] d_Z2 = + k_Z1_Z2 * Z1 - k_Z2_sink * Z2 - k_Z2_Z3_free * Z2
[4] d_Z3_free = + k_Z2_Z3_free * Z2 - k_Z3_free_sink * Z3_free - k_Z3_free_bound * Z3_fr
[5] d_Z3_bound = + k_Z3_free_bound * Z3_free - k_Z3_bound_free * Z3_bound

Method used for solution of differential equation system:
eigen

Starting values for optimised parameters:
                initial   type transformed
Z0_0              1e+02  state 100.0000000
k_Z0_Z1           5e-01 deparm  -0.6931472
k_Z1_Z2           3e-01 deparm  -1.2039728
k_Z2_sink         1e-01 deparm  -2.3025851
k_Z2_Z3_free      1e-01 deparm  -2.3025851
k_Z3_free_sink    1e-01 deparm  -2.3025851
k_Z3_free_bound   1e-01 deparm  -2.3025851
k_Z3_bound_free   2e-02 deparm  -3.9120230

Fixed parameter values:
          value  type
Z1            0 state
Z2            0 state
Z3_free       0 state
Z3_bound      0 state
```

```
Optimised, transformed parameters:
                Estimate Std. Error Lower Upper
Z0_0             96.7400         NA    NA    NA
k_Z0_Z1           0.7947         NA    NA    NA
k_Z1_Z2          -0.7426         NA    NA    NA
k_Z2_sink        -1.4950         NA    NA    NA
k_Z2_Z3_free     -1.5040         NA    NA    NA
k_Z3_free_sink   -2.6540         NA    NA    NA
k_Z3_free_bound  -5.2440         NA    NA    NA
k_Z3_bound_free -21.1000         NA    NA    NA

Backtransformed parameters:
                 Estimate Lower Upper
Z0_0            9.674e+01    NA    NA
k_Z0_Z1         2.214e+00    NA    NA
k_Z1_Z2         4.759e-01    NA    NA
k_Z2_sink       2.243e-01    NA    NA
k_Z2_Z3_free    2.222e-01    NA    NA
k_Z3_free_sink  7.034e-02    NA    NA
k_Z3_free_bound 5.279e-03    NA    NA
k_Z3_bound_free 6.894e-10    NA    NA

Residual standard error: 4.107 on 49 degrees of freedom

Chi2 error levels in percent:
         err.min n.optim df
All data  19.755       8 49
Z0        17.429       2 15
Z1        15.949       1 13
Z2        21.190       2 11
Z3         8.561       3 10

Estimated disappearance times:
      DT50   DT90
Z0  0.3131  1.040
Z1  1.4566  4.839
Z2  1.5523  5.157
Z3 10.1978 45.329

Estimated formation fractions:
                ff
Z0_Z1        1.0000
Z1_Z2        1.0000
Z2_sink      0.5024
Z2_Z3_free   0.4976
Z3_free_sink 1.0000

Estimated Eigenvalues of SFORB model(s):
```

```
    Z3_b1      Z3_b2
7.562e-02  6.413e-10

Parameter correlation:
Could not estimate covariance matrix; singular system:
```

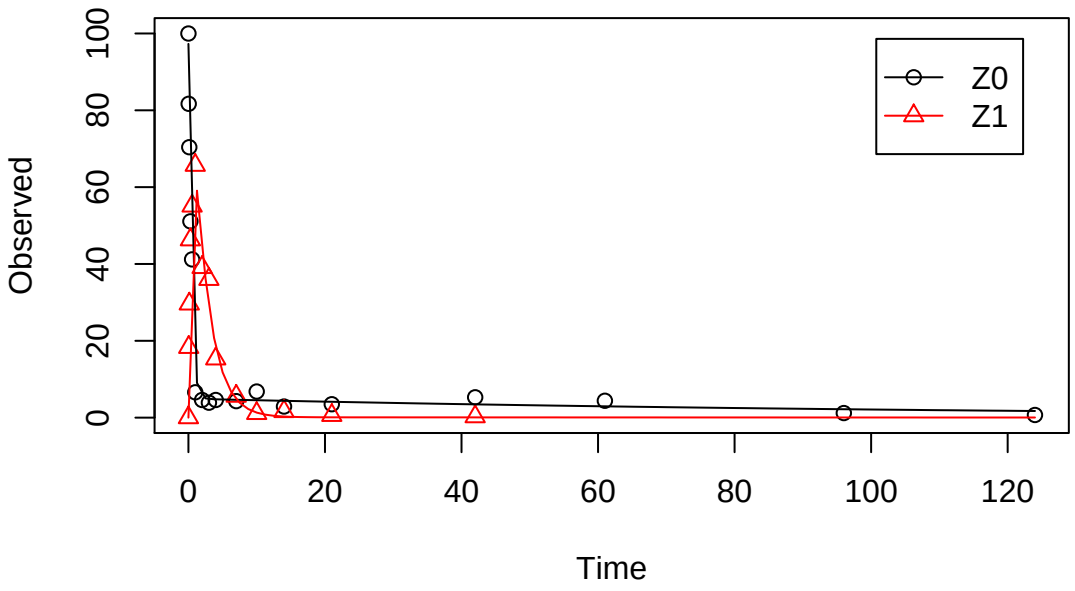


Therefore, a further stepwise model building is performed starting from the stage of parent and one metabolite, starting from the assumption that the model fit for the parent compound can be improved by using the SFORB model.

```
R> Z.mkin.2 <- mkinmod(Z0 = list(type = "SFORB", to = "Z1", sink = FALSE),
+                  Z1 = list(type = "SFO"))
R> m.Z.mkin.2 <- mkinfit(Z.mkin.2, FOCUS_2006_Z_mkin, quiet = TRUE)
R> plot(m.Z.mkin.2)
R> summary(m.Z.mkin.2, data = FALSE)

mkin version:    0.9.19
R version:       2.15.3
Date of fit:     Sun Apr 14 14:28:54 2013
Date of summary: Sun Apr 14 14:28:54 2013

Equations:
[1] d_Z0_free = - 0 - k_Z0_free_bound * Z0_free + k_Z0_bound_free * Z0_bound - k_Z0_free
[2] d_Z0_bound = + k_Z0_free_bound * Z0_free - k_Z0_bound_free * Z0_bound
[3] d_Z1 = + k_Z0_free_Z1 * Z0_free - k_Z1_sink * Z1
```

```
Method used for solution of differential equation system:
eigen

Starting values for optimised parameters:
                initial   type transformed
Z0_free_0         1e+02  state  100.000000
k_Z0_free_bound   1e-01 deparm   -2.302585
k_Z0_bound_free   2e-02 deparm   -3.912023
k_Z0_free_Z1      1e-01 deparm   -2.302585
k_Z1_sink         1e-01 deparm   -2.302585

Fixed parameter values:
          value  type
Z0_bound      0 state
Z1            0 state

Optimised, transformed parameters:
                Estimate Std. Error   Lower    Upper
Z0_free_0        97.2900    2.39500 92.3600 102.2000
k_Z0_free_bound  -2.0820    0.43220 -2.9710  -1.1940
k_Z0_bound_free  -4.7200    1.60500 -8.0190  -1.4220
k_Z0_free_Z1      0.8549    0.06430  0.7227   0.9871
k_Z1_sink        -0.7934    0.08506 -0.9682  -0.6185

Backtransformed parameters:
                 Estimate     Lower    Upper
Z0_free_0       97.290000 9.236e+01 102.2000
k_Z0_free_bound  0.124700 5.128e-02   0.3031
k_Z0_bound_free  0.008911 3.291e-04   0.2413
k_Z0_free_Z1     2.351000 2.060e+00   2.6830
k_Z1_sink        0.452300 3.798e-01   0.5387

Residual standard error: 4.438 on 26 degrees of freedom

Chi2 error levels in percent:
          err.min n.optim df
All data    15.89       5 26
Z0          14.74       4 13
Z1          14.94       1 13

Estimated disappearance times:
    DT50  DT90
Z0 0.302 1.190
Z1 1.532 5.091

Estimated formation fractions:
           ff
Z0_free_Z1  1
```

```
Z1_sink     1

Estimated Eigenvalues of SFORB model(s):
   Z0_b1    Z0_b2
2.476313 0.008461

Parameter correlation:
                 Z0_free_0 k_Z0_free_bound k_Z0_bound_free k_Z0_free_Z1 k_Z1_sink
Z0_free_0         1.000000        0.006494         0.03324      0.11182   0.39155
k_Z0_free_bound   0.006494        1.000000         0.54646      0.41393  -0.29191
k_Z0_bound_free   0.033238        0.546465         1.00000      0.15837  -0.12597
k_Z0_free_Z1      0.111819        0.413926         0.15837      1.00000  -0.04188
k_Z1_sink         0.391553       -0.291912        -0.12597     -0.04188   1.00000
```

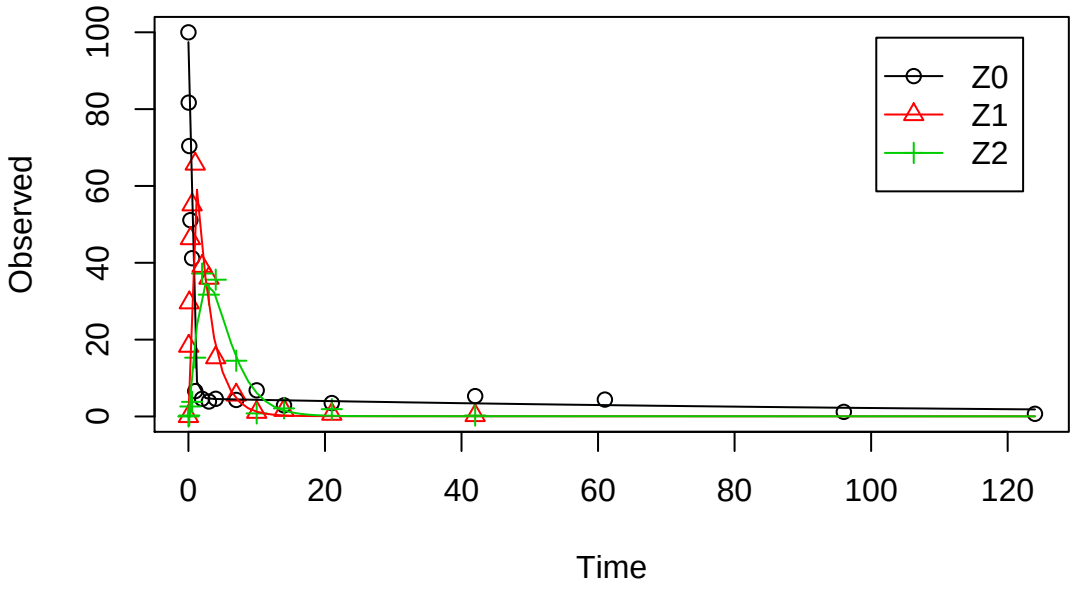


When metabolite Z2 is added, the additional sink for Z1 is turned off again, for the same reasons as in the original analysis.

```
R> Z.mkin.3 <- mkinmod(Z0 = list(type = "SFORB", to = "Z1", sink = FALSE),
+                 Z1 = list(type = "SFO", to = "Z2"),
+                 Z2 = list(type = "SFO"))
R> m.Z.mkin.3 <- mkinfit(Z.mkin.3, FOCUS_2006_Z_mkin, quiet = TRUE)
R> plot(m.Z.mkin.3)
R> summary(m.Z.mkin.3, data = FALSE)
```

```
mkin version:    0.9.19
R version:       2.15.3
Date of fit:     Sun Apr 14 14:28:57 2013
Date of summary: Sun Apr 14 14:28:58 2013

Equations:
[1] d_Z0_free = - 0 - k_Z0_free_bound * Z0_free + k_Z0_bound_free * Z0_bound - k_Z0_free
[2] d_Z0_bound = + k_Z0_free_bound * Z0_free - k_Z0_bound_free * Z0_bound
[3] d_Z1 = + k_Z0_free_Z1 * Z0_free - k_Z1_sink * Z1 - k_Z1_Z2 * Z1
[4] d_Z2 = + k_Z1_Z2 * Z1 - k_Z2_sink * Z2

Method used for solution of differential equation system:
eigen

Starting values for optimised parameters:
               initial   type transformed
Z0_free_0        1e+02  state  100.000000
k_Z0_free_bound  1e-01 deparm   -2.302585
k_Z0_bound_free  2e-02 deparm   -3.912023
k_Z0_free_Z1     1e-01 deparm   -2.302585
k_Z1_sink        1e-01 deparm   -2.302585
k_Z1_Z2          1e-01 deparm   -2.302585
k_Z2_sink        1e-01 deparm   -2.302585

Fixed parameter values:
         value  type
Z0_bound     0 state
Z1           0 state
Z2           0 state

Optimised, transformed parameters:
                Estimate Std. Error      Lower      Upper
Z0_free_0        97.4400  2.124e+00  9.313e+01  1.017e+02
k_Z0_free_bound  -2.1490  4.121e-01 -2.984e+00 -1.314e+00
k_Z0_bound_free  -4.8380  1.636e+00 -8.153e+00 -1.524e+00
k_Z0_free_Z1      0.8457  5.911e-02  7.259e-01  9.655e-01
k_Z1_sink       -19.3800  1.751e+06 -3.547e+06  3.547e+06
k_Z1_Z2          -0.7812  8.755e-02 -9.586e-01 -6.038e-01
k_Z2_sink        -0.8606  1.439e-01 -1.152e+00 -5.691e-01

Backtransformed parameters:
                 Estimate     Lower    Upper
Z0_free_0       9.744e+01 93.130000 101.7000
k_Z0_free_bound 1.166e-01  0.050600   0.2688
k_Z0_bound_free 7.920e-03  0.000288   0.2178
k_Z0_free_Z1    2.330e+00  2.067000   2.6260
k_Z1_sink       3.834e-09  0.000000      Inf
k_Z1_Z2         4.579e-01  0.383400   0.5467
k_Z2_sink       4.229e-01  0.316000   0.5660
```

```
Residual standard error: 4.136 on 37 degrees of freedom

Chi2 error levels in percent:
         err.min n.optim df
All data   17.95       7 37
Z0         14.67       4 13
Z1         15.52       2 12
Z2         21.27       1 12

Estimated disappearance times:
     DT50  DT90
Z0 0.3043 1.185
Z1 1.5138 5.029
Z2 1.6391 5.445

Estimated formation fractions:
                  ff
Z0_free_Z1 1.000e+00
Z1_sink    8.373e-09
Z1_Z2      1.000e+00
Z2_sink    1.000e+00

Estimated Eigenvalues of SFORB model(s):
   Z0_b1    Z0_b2
2.446638 0.007542

Parameter correlation:
                Z0_free_0 k_Z0_free_bound k_Z0_bound_free k_Z0_free_Z1 k_Z1_sink  k_Z1_Z
Z0_free_0         1.00000         0.05615         0.06063      0.09324   0.15262  0.0899
k_Z0_free_bound   0.05615         1.00000         0.54490      0.41896  -0.11467 -0.0939
k_Z0_bound_free   0.06063         0.54490         1.00000      0.16141  -0.06038 -0.0247
k_Z0_free_Z1      0.09324         0.41896         0.16141      1.00000   0.02314 -0.0909
k_Z1_sink         0.15262        -0.11467        -0.06038      0.02314   1.00000 -0.6606
k_Z1_Z2           0.08997        -0.09395        -0.02475     -0.09097  -0.66065  1.0000
k_Z2_sink         0.11068        -0.07920        -0.01859     -0.05193  -0.66738  0.5934
```



This results in a much better representation of the behaviour of the parent compound Z0.

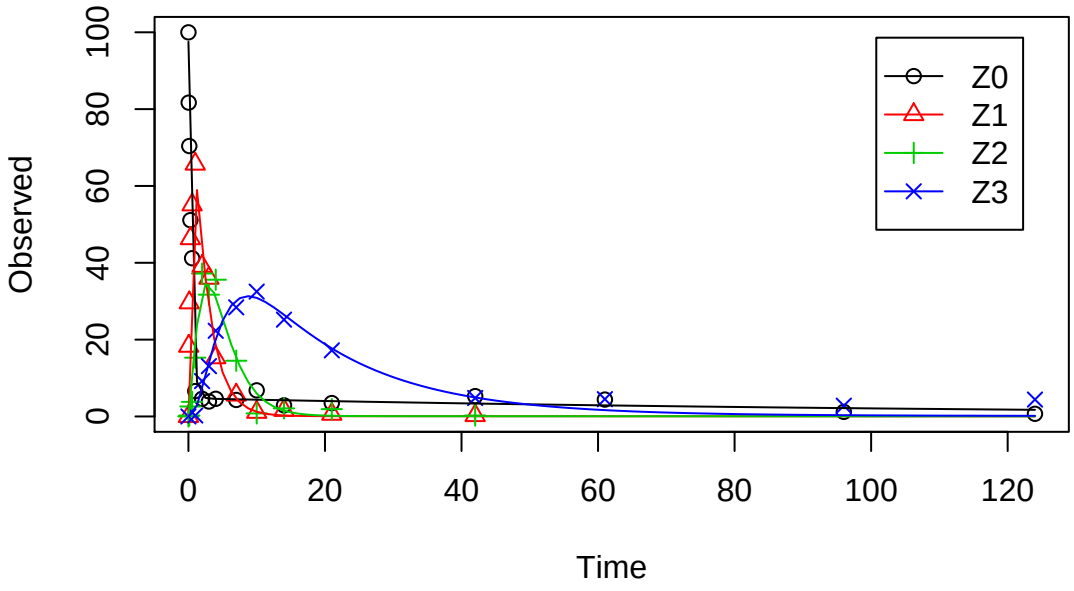
Finally, Z3 is added as well. This model appears overparameterised (no covariance matrix returned) if the sink for Z1 is left in the model.

```
R> Z.mkin.4 <- mkinmod(Z0 = list(type = "SFORB", to = "Z1", sink = FALSE),
+                  Z1 = list(type = "SFO", to = "Z2", sink = FALSE),
+                  Z2 = list(type = "SFO", to = "Z3"),
+                  Z3 = list(type = "SFO"))
R> m.Z.mkin.4 <- mkinfit(Z.mkin.4, FOCUS_2006_Z_mkin,
+   parms.ini = c(k_Z1_Z2 = 0.05), quiet = TRUE)
R> plot(m.Z.mkin.4)
R> summary(m.Z.mkin.4, data = FALSE)
```

```
mkin version:    0.9.19
R version:       2.15.3
Date of fit:     Sun Apr 14 14:29:02 2013
Date of summary: Sun Apr 14 14:29:02 2013

Equations:
[1] d_Z0_free = - 0 - k_Z0_free_bound * Z0_free + k_Z0_bound_free * Z0_bound - k_Z0_free
[2] d_Z0_bound = + k_Z0_free_bound * Z0_free - k_Z0_bound_free * Z0_bound
[3] d_Z1 = + k_Z0_free_Z1 * Z0_free - 0 - k_Z1_Z2 * Z1
[4] d_Z2 = + k_Z1_Z2 * Z1 - k_Z2_sink * Z2 - k_Z2_Z3 * Z2
```

```
[5] d_Z3 = + k_Z2_Z3 * Z2 - k_Z3_sink * Z3

Method used for solution of differential equation system:
eigen

Starting values for optimised parameters:
               initial   type transformed
Z0_free_0        1e+02  state  100.000000
k_Z1_Z2          5e-02 deparm   -2.995732
k_Z0_free_bound  1e-01 deparm   -2.302585
k_Z0_bound_free  2e-02 deparm   -3.912023
k_Z0_free_Z1     1e-01 deparm   -2.302585
k_Z2_sink        1e-01 deparm   -2.302585
k_Z2_Z3          1e-01 deparm   -2.302585
k_Z3_sink        1e-01 deparm   -2.302585

Fixed parameter values:
         value  type
Z0_bound     0 state
Z1           0 state
Z2           0 state
Z3           0 state

Optimised, transformed parameters:
               Estimate Std. Error   Lower    Upper
Z0_free_0       97.5300    1.88700 93.7400 101.3000
k_Z1_Z2         -0.7769    0.05834 -0.8942  -0.6597
k_Z0_free_bound -2.1360    0.36810 -2.8760  -1.3960
k_Z0_bound_free -4.7650    1.41700 -7.6130  -1.9170
k_Z0_free_Z1     0.8470    0.05339  0.7398   0.9543
k_Z2_sink       -1.5610    0.18260 -1.9280  -1.1940
k_Z2_Z3         -1.5280    0.11350 -1.7560  -1.2990
k_Z3_sink       -2.7690    0.22460 -3.2200  -2.3180

Backtransformed parameters:
               Estimate     Lower    Upper
Z0_free_0       97.53000 9.374e+01 101.3000
k_Z1_Z2          0.45980 4.089e-01   0.5170
k_Z0_free_bound  0.11810 5.636e-02   0.2475
k_Z0_bound_free  0.00852 4.938e-04   0.1470
k_Z0_free_Z1     2.33300 2.095e+00   2.5970
k_Z2_sink        0.20990 1.455e-01   0.3030
k_Z2_Z3          0.21700 1.728e-01   0.2727
k_Z3_sink        0.06272 3.994e-02   0.0985

Residual standard error: 3.737 on 49 degrees of freedom

Chi2 error levels in percent:
         err.min n.optim df
```

```
All data   17.98       8 49
Z0         14.69       4 13
Z1         15.03       1 13
Z2         21.99       2 11
Z3         12.32       1 12

Estimated disappearance times:
     DT50   DT90
Z0  0.304  1.186
Z1  1.507  5.008
Z2  1.623  5.393
Z3 11.051 36.712

Estimated formation fractions:
               ff
Z0_free_Z1 1.0000
Z1_Z2      1.0000
Z2_sink    0.4917
Z2_Z3      0.5083
Z3_sink    1.0000

Estimated Eigenvalues of SFORB model(s):
   Z0_b1    Z0_b2
2.451259 0.008108

Parameter correlation:
                Z0_free_0  k_Z1_Z2 k_Z0_free_bound k_Z0_bound_free k_Z0_free_Z1 k_Z2_sin
Z0_free_0         1.00000  0.24238         0.07823         0.06921      0.08883    0.329
k_Z1_Z2           0.24238  1.00000        -0.22742        -0.08934     -0.10841    0.340
k_Z0_free_bound   0.07823 -0.22742         1.00000         0.53976      0.42766   -0.263
k_Z0_bound_free   0.06921 -0.08934         0.53976         1.00000      0.16281   -0.127
k_Z0_free_Z1      0.08883 -0.10841         0.42766         0.16281      1.00000   -0.053
k_Z2_sink         0.32993  0.34052        -0.26331        -0.12743     -0.05310    1.000
k_Z2_Z3          -0.07494 -0.14885         0.06698         0.06082     -0.01281   -0.254
k_Z3_sink        -0.10456 -0.22486         0.13845         0.12526      0.01858   -0.683
```



The error level of the fit, but especially of metabolite Z3, can be improved if the SFORB model is chosen for this metabolite, as this model is capable of representing the tailing of the metabolite decline phase.

Using the SFORB additionally for Z1 or Z2 did not further improve the result.

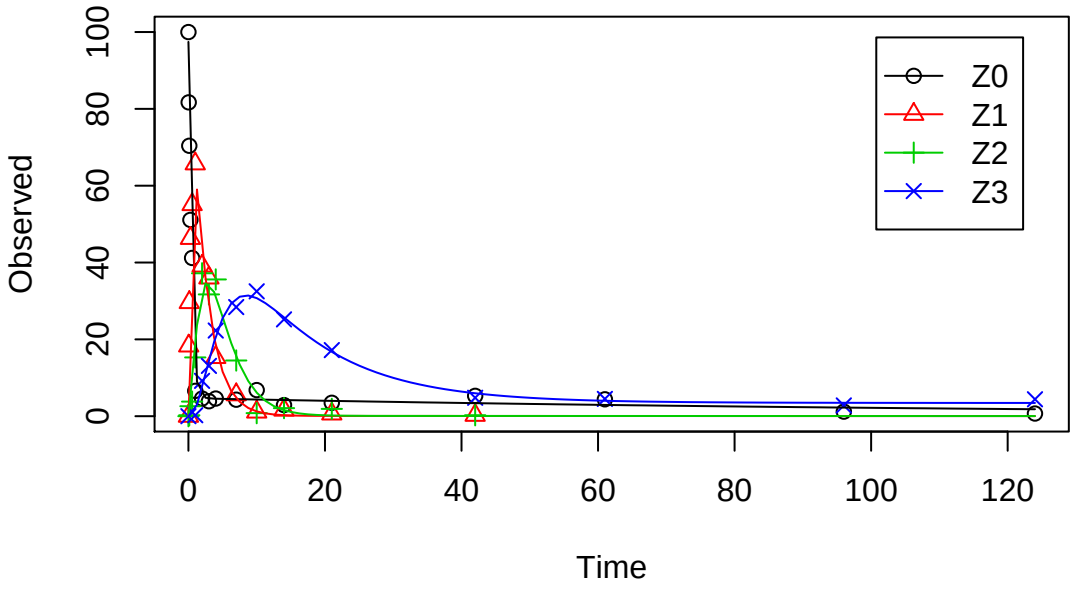
```
R> Z.mkin.5 <- mkinmod(Z0 = list(type = "SFORB", to = "Z1", sink = FALSE),
+                  Z1 = list(type = "SFO", to = "Z2", sink = FALSE),
+                  Z2 = list(type = "SFO", to = "Z3"),
+                  Z3 = list(type = "SFORB"))
R> m.Z.mkin.5 <- mkinfit(Z.mkin.5, FOCUS_2006_Z_mkin,
+   parms.ini = c(k_Z1_Z2 = 0.2), quiet = TRUE)
R> plot(m.Z.mkin.5)
R> summary(m.Z.mkin.5, data = FALSE)
```

```
mkin version:    0.9.19
R version:       2.15.3
Date of fit:     Sun Apr 14 14:29:14 2013
Date of summary: Sun Apr 14 14:29:14 2013

Equations:
[1] d_Z0_free = - 0 - k_Z0_free_bound * Z0_free + k_Z0_bound_free * Z0_bound - k_Z0_free
[2] d_Z0_bound = + k_Z0_free_bound * Z0_free - k_Z0_bound_free * Z0_bound
[3] d_Z1 = + k_Z0_free_Z1 * Z0_free - 0 - k_Z1_Z2 * Z1
```

```
[4] d_Z2 = + k_Z1_Z2 * Z1 - k_Z2_sink * Z2 - k_Z2_Z3_free * Z2
[5] d_Z3_free = + k_Z2_Z3_free * Z2 - k_Z3_free_sink * Z3_free - k_Z3_free_bound * Z3_fr
[6] d_Z3_bound = + k_Z3_free_bound * Z3_free - k_Z3_bound_free * Z3_bound

Method used for solution of differential equation system:
eigen

Starting values for optimised parameters:
                initial   type transformed
Z0_free_0         1e+02  state  100.000000
k_Z1_Z2           2e-01 deparm   -1.609438
k_Z0_free_bound   1e-01 deparm   -2.302585
k_Z0_bound_free   2e-02 deparm   -3.912023
k_Z0_free_Z1      1e-01 deparm   -2.302585
k_Z2_sink         1e-01 deparm   -2.302585
k_Z2_Z3_free      1e-01 deparm   -2.302585
k_Z3_free_sink    1e-01 deparm   -2.302585
k_Z3_free_bound   1e-01 deparm   -2.302585
k_Z3_bound_free   2e-02 deparm   -3.912023

Fixed parameter values:
          value  type
Z0_bound      0 state
Z1            0 state
Z2            0 state
Z3_free       0 state
Z3_bound      0 state

Optimised, transformed parameters:
                Estimate Std. Error     Lower    Upper
Z0_free_0        97.4300    1.88700   93.6400 101.2000
k_Z1_Z2          -0.7813    0.05861   -0.8992  -0.6634
k_Z0_free_bound  -2.1470    0.36930   -2.8900  -1.4040
k_Z0_bound_free  -4.8230    1.46300   -7.7650  -1.8800
k_Z0_free_Z1      0.8459    0.05334    0.7386   0.9532
k_Z2_sink        -1.6300    0.22080   -2.0740  -1.1850
k_Z2_Z3_free     -1.4850    0.19600   -1.8800  -1.0910
k_Z3_free_sink   -2.5950    0.38470   -3.3690  -1.8220
k_Z3_free_bound  -5.2570    1.37200   -8.0160  -2.4970
k_Z3_bound_free -13.4400  282.00000 -580.8000 553.9000

Backtransformed parameters:
                 Estimate      Lower      Upper
Z0_free_0       9.743e+01  9.364e+01  1.012e+02
k_Z1_Z2         4.578e-01  4.069e-01  5.151e-01
k_Z0_free_bound 1.169e-01  5.559e-02  2.457e-01
k_Z0_bound_free 8.044e-03  4.242e-04  1.525e-01
k_Z0_free_Z1    2.330e+00  2.093e+00  2.594e+00
k_Z2_sink       1.960e-01  1.257e-01  3.056e-01
```

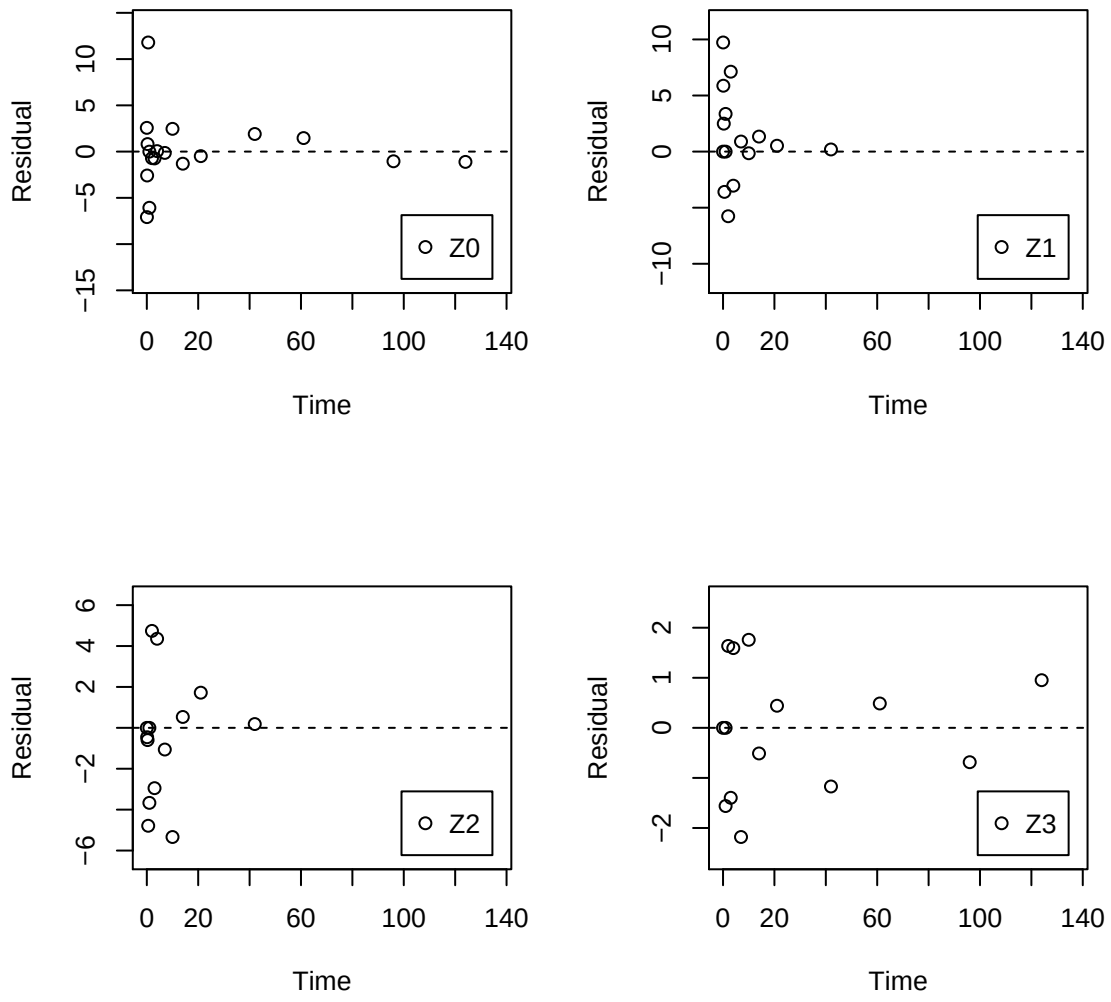
```
k_Z2_Z3_free    2.264e-01  1.526e-01  3.358e-01
k_Z3_free_sink  7.462e-02  3.442e-02  1.618e-01
k_Z3_free_bound 5.214e-03  3.301e-04  8.234e-02
k_Z3_bound_free 1.458e-06 5.692e-253 3.734e+240

Residual standard error: 3.73 on 47 degrees of freedom

Chi2 error levels in percent:
         err.min n.optim df
All data  17.890      10 47
Z0        14.666       4 13
Z1        15.049       1 13
Z2        21.991       2 11
Z3         8.433       3 10

Estimated disappearance times:
     DT50   DT90
Z0 0.3043  1.185
Z1 1.5141  5.030
Z2 1.6409  5.451
Z3 9.5901 41.258

Estimated formation fractions:
                ff
Z0_free_Z1   1.000
Z1_Z2        1.000
Z2_sink      0.464
Z2_Z3_free   0.536
Z3_free_sink 1.000

Estimated Eigenvalues of SFORB model(s):
    Z0_b1     Z0_b2     Z3_b1     Z3_b2
2.447e+00 7.658e-03 7.983e-02 1.363e-06

Parameter correlation:
                Z0_free_0  k_Z1_Z2 k_Z0_free_bound k_Z0_bound_free k_Z0_free_Z1 k_Z2_sin
Z0_free_0         1.00000  0.24874        0.079292        0.073662      0.09072   0.3161
k_Z1_Z2           0.24874  1.00000       -0.223885       -0.082769     -0.10368   0.3674
k_Z0_free_bound   0.07929 -0.22389        1.000000        0.542876      0.42696  -0.2871
k_Z0_bound_free   0.07366 -0.08277        0.542876        1.000000      0.16540  -0.1682
k_Z0_free_Z1      0.09072 -0.10368        0.426958        0.165399      1.00000  -0.0826
k_Z2_sink         0.31619  0.36741       -0.287122       -0.168222     -0.08266   1.0000
k_Z2_Z3_free     -0.03185 -0.05441        0.008287        0.013478     -0.01908  -0.0693
k_Z3_free_sink   -0.12037 -0.22367        0.070584        0.024242     -0.01842  -0.6659
k_Z3_free_bound   0.01629  0.01906        0.009670        0.031837      0.03607   0.0260
k_Z3_bound_free   0.03781  0.08868       -0.055136       -0.008975     -0.06574   0.2393
                k_Z3_free_sink k_Z3_free_bound k_Z3_bound_free
Z0_free_0            -0.12037         0.01629        0.037814
k_Z1_Z2              -0.22367         0.01906        0.088677
```

```
k_Z0_free_bound        0.07058          0.00967       -0.055136
k_Z0_bound_free        0.02424          0.03184       -0.008975
k_Z0_free_Z1          -0.01842          0.03607       -0.065741
k_Z2_sink             -0.66596          0.02603        0.239308
k_Z2_Z3_free          -0.26493          0.73477        0.774434
k_Z3_free_sink         1.00000         -0.36064       -0.711226
k_Z3_free_bound       -0.36064          1.00000        0.811433
k_Z3_bound_free       -0.71123          0.81143        1.000000
```



Looking at the confidence intervals of the SFORB model parameters of Z3, it is clear that nothing can be said about the degradation rate of Z3 towards the end of the experiment. However, this appears to be a feature of the data.

```
R> par(mfrow = c(2, 2))
R> mkinresplot(m.Z.mkin.5, "Z0", lpos = "bottomright")
R> mkinresplot(m.Z.mkin.5, "Z1", lpos = "bottomright")
R> mkinresplot(m.Z.mkin.5, "Z2", lpos = "bottomright")
R> mkinresplot(m.Z.mkin.5, "Z3", lpos = "bottomright")
```



As expected, the residual plots are much more random than in the case of the all SFO model for which they were shown above. In conclusion, the model `Z.mkin.5` is proposed as the best-fit model for the dataset from Appendix 7 of the FOCUS report.

References

FOCUS Work Group on Degradation Kinetics. *Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. Report of the FOCUS Work Group on Degradation Kinetics*, 2006. URL http://focus.jrc.ec.europa.eu/dk. EC Document Reference Sanco/10058/2005 version 2.0.

FOCUS Work Group on Degradation Kinetics. *Generic guidance for estimating persistence and degradation kinetics from environmental fate studies on pesticides in EU registration*, 1.0 edition, November 2011. URL http://focus.jrc.ec.europa.eu/dk.

Johannes Ranke. *kinfit: Routines for fitting simple kinetic models to chemical degradation data*, 2012. URL http://CRAN.R-project.org.