Examples for kinetic evaluations using mkin

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February 18, 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.

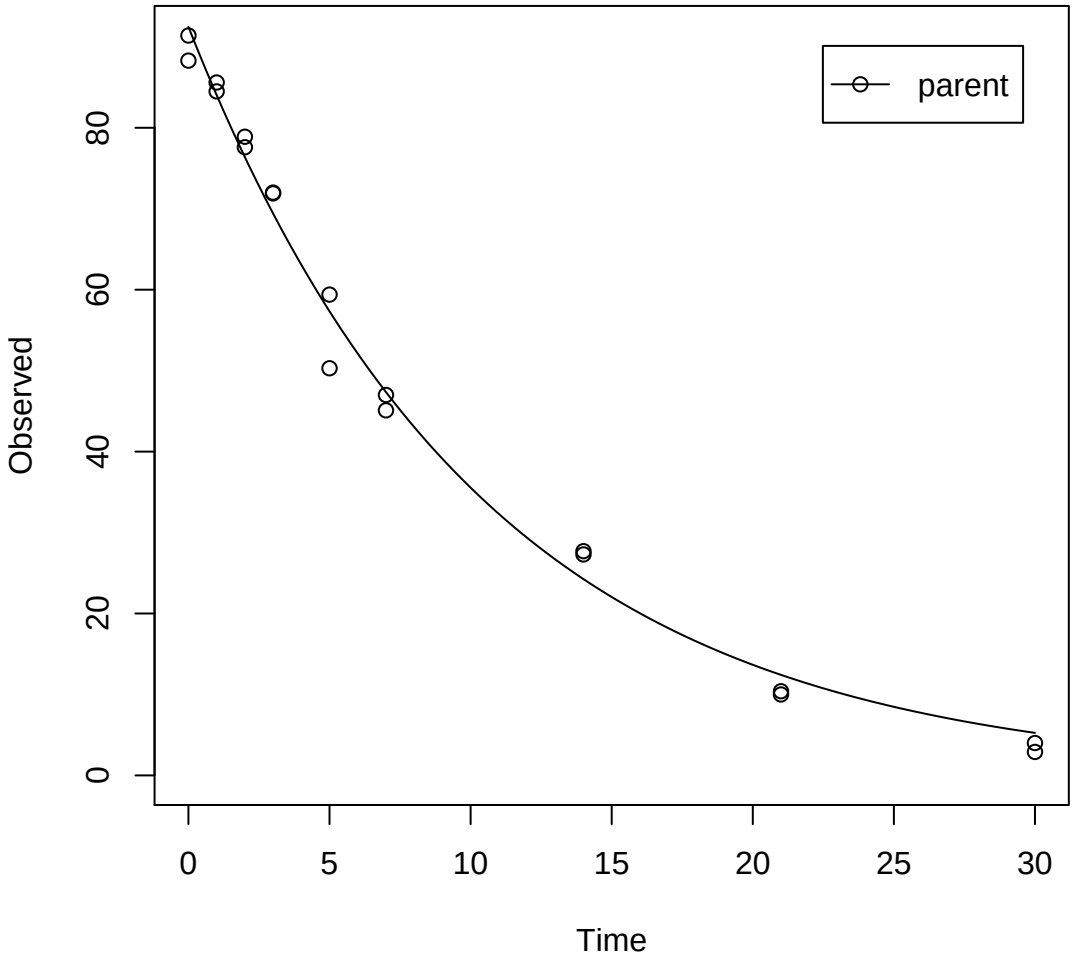
```
R> m.L1.SFO <- mkinfit(SFO, FOCUS_2006_L1_mkin, quiet=TRUE)
R> summary(m.L1.SFO)
```

```
mkin version:    0.9.13
R version:       2.15.2
Date of fit:     Mon Feb 18 22:54:21 2013
Date of summary: Mon Feb 18 22:54:21 2013

Equations:
[1] d_parent = - k_parent_sink * parent

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
parent_0        92.471      1.368
k_parent_sink   -2.347      0.041

Backtransformed parameters:
              Estimate
parent_0        92.471
k_parent_sink    0.096

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
```

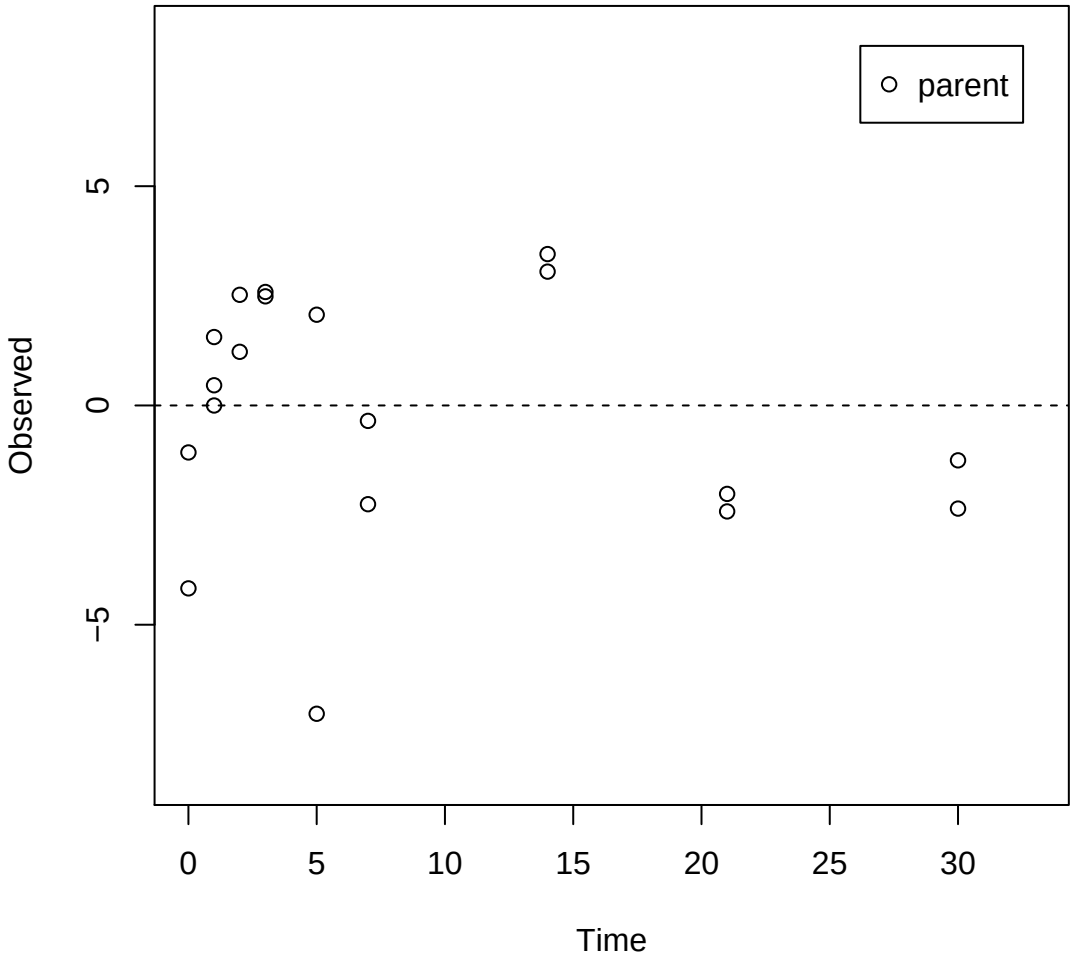
A plot of the fit is obtained with the plot function for mkinfit objects.

```
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:    0.9.13
R version:       2.15.2
Date of fit:     Mon Feb 18 22:54:21 2013
Date of summary: Mon Feb 18 22:54:21 2013

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

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
parent_0    92.47         NA
alpha       25.63         NA
beta        27.98         NA

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

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

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%).

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.

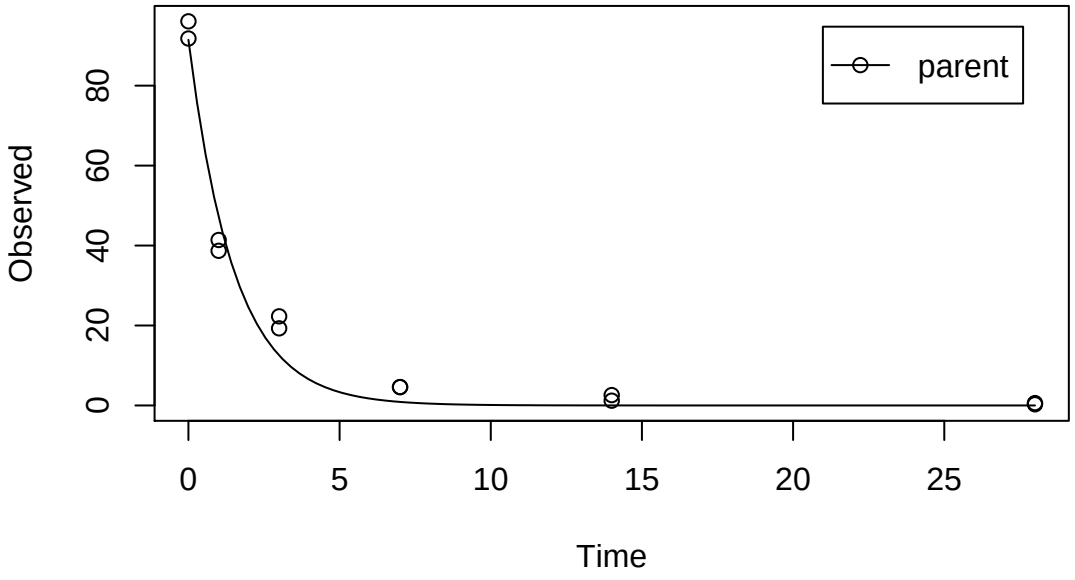
```
R> m.L2.SFO <- mkinfit(SFO, FOCUS_2006_L2_mkin, quiet=TRUE)
R> summary(m.L2.SFO)
```

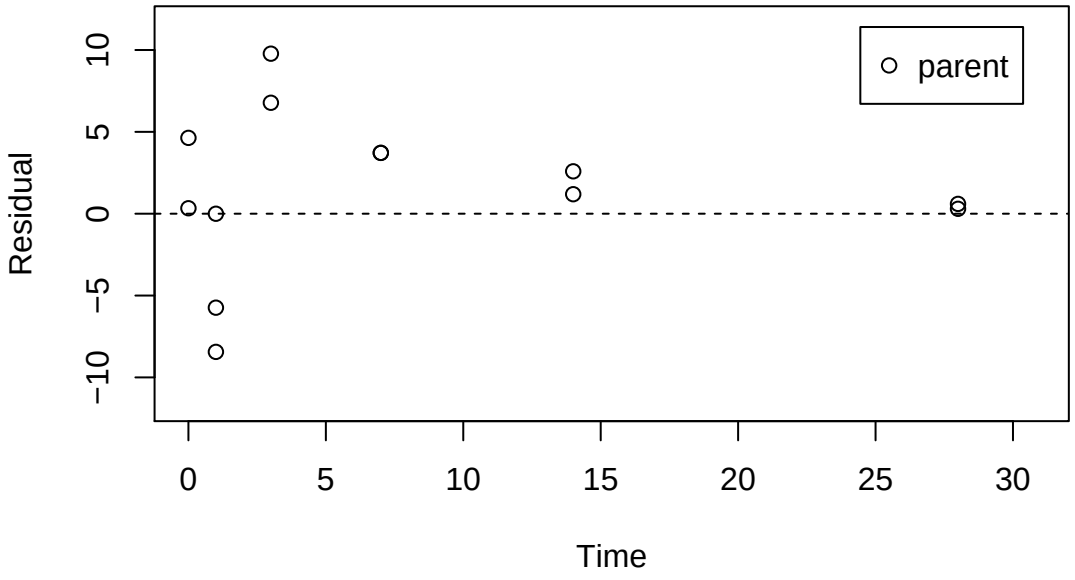
```
mkin version:    0.9.13
R version:       2.15.2
Date of fit:     Mon Feb 18 22:54:21 2013
Date of summary: Mon Feb 18 22:54:21 2013

Equations:
[1] d_parent = - k_parent_sink * parent

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
parent_0       91.4656      3.807
k_parent_sink  -0.4112      0.107

Backtransformed parameters:
              Estimate
parent_0        91.466
k_parent_sink    0.663

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 91.4656079103   4.6344
    0   parent     91.8 91.4656079103   0.3344
    1   parent     41.4 47.1395280371  -5.7395
    1   parent     38.7 47.1395280371  -8.4395
    3   parent     19.3 12.5210295280   6.7790
    3   parent     22.3 12.5210295280   9.7790
    7   parent      4.6  0.8833842647   3.7166
    7   parent      4.6  0.8833842647   3.7166
   14   parent      2.6  0.0085318162   2.5915
   14   parent      1.2  0.0085318162   1.1915
   28   parent      0.3  0.0000007958   0.3000
   28   parent      0.6  0.0000007958   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

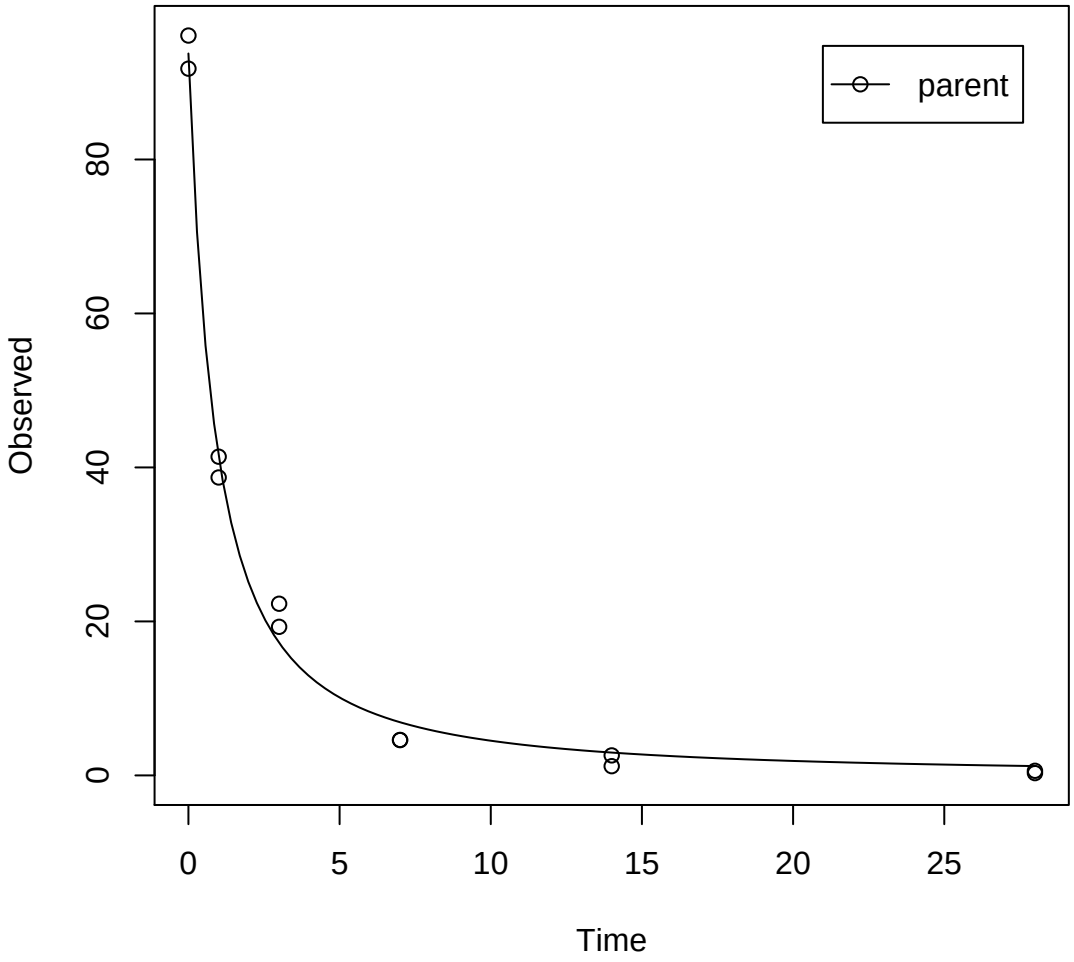
there is an underestimation beyond that point.

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> plot(m.L2.FOMC)
R> s.m.L2.FOMC <- summary(m.L2.FOMC)
R> s.m.L2.FOMC$errmin
```

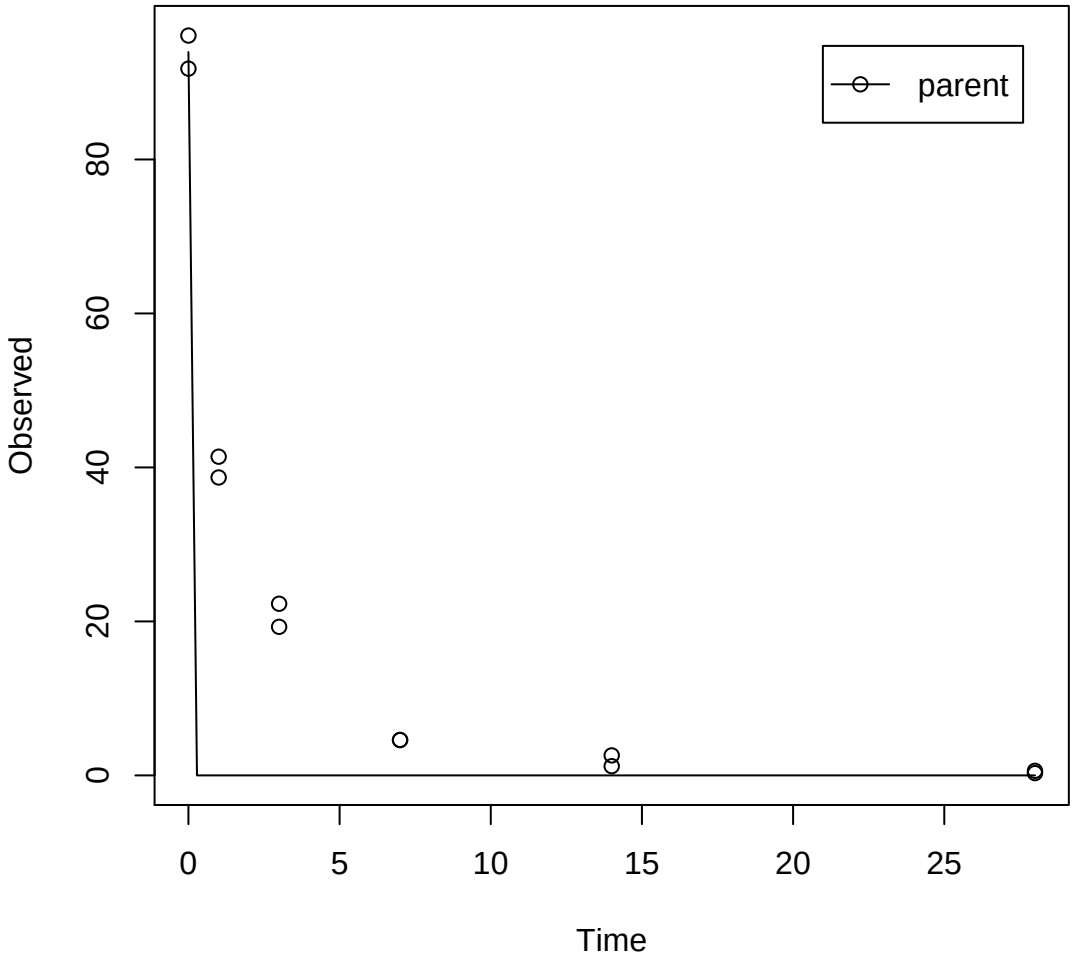
```
            err.min n.optim df
All data 0.06204245       3  3
parent   0.06204245       3  3
```



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 does not further reduce the χ^2 error level.

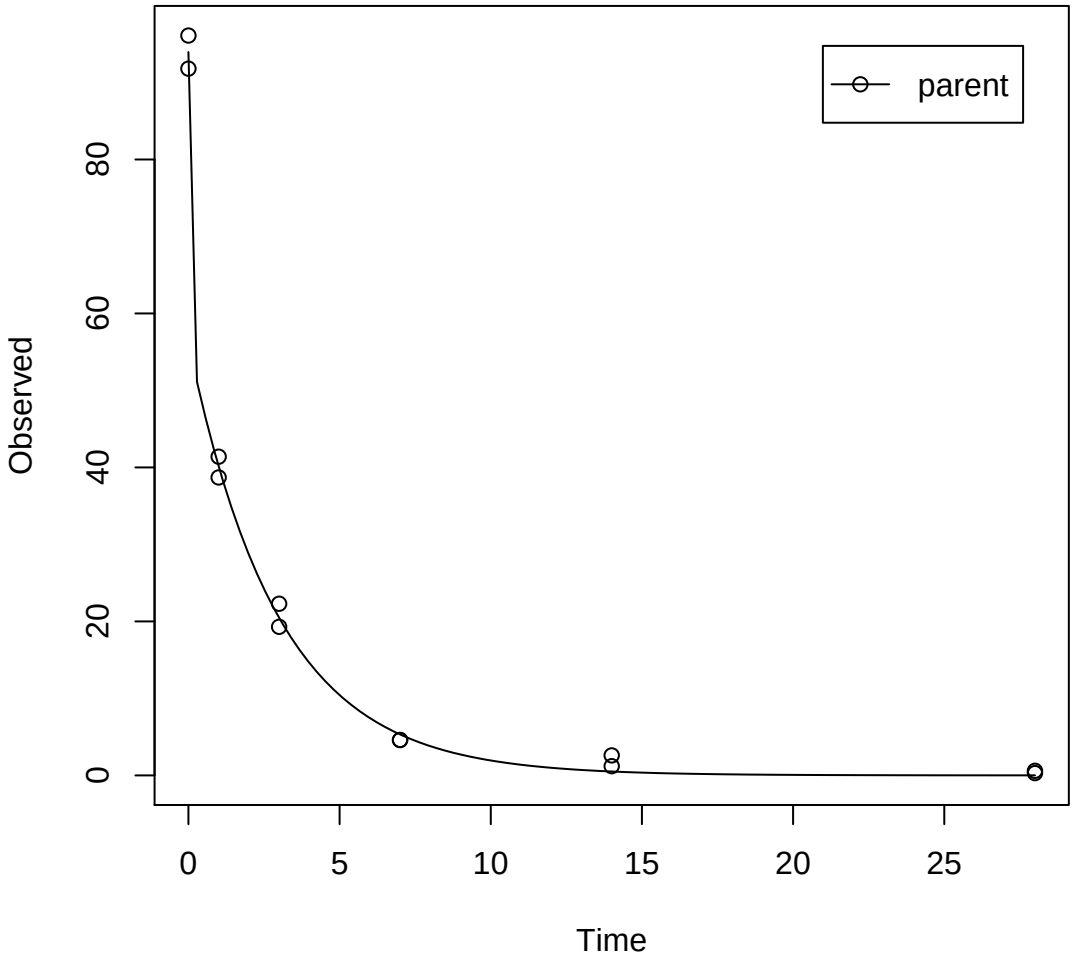
```
R> m.L2.DFOP <- mkinfit(DFOP, FOCUS_2006_L2_mkin, quiet=TRUE)
R> plot(m.L2.DFOP)
```



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> s.m.L2.DFOP <- summary(m.L2.DFOP)
R> s.m.L2.DFOP$errmin
```

```
            err.min n.optim df
All data 0.02528763       4  2
parent   0.02528763       4  2
```



Therefore, the FOMC model is clearly the best-fit model for dataset L1 based on the χ^2 error level criterion.

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)
```

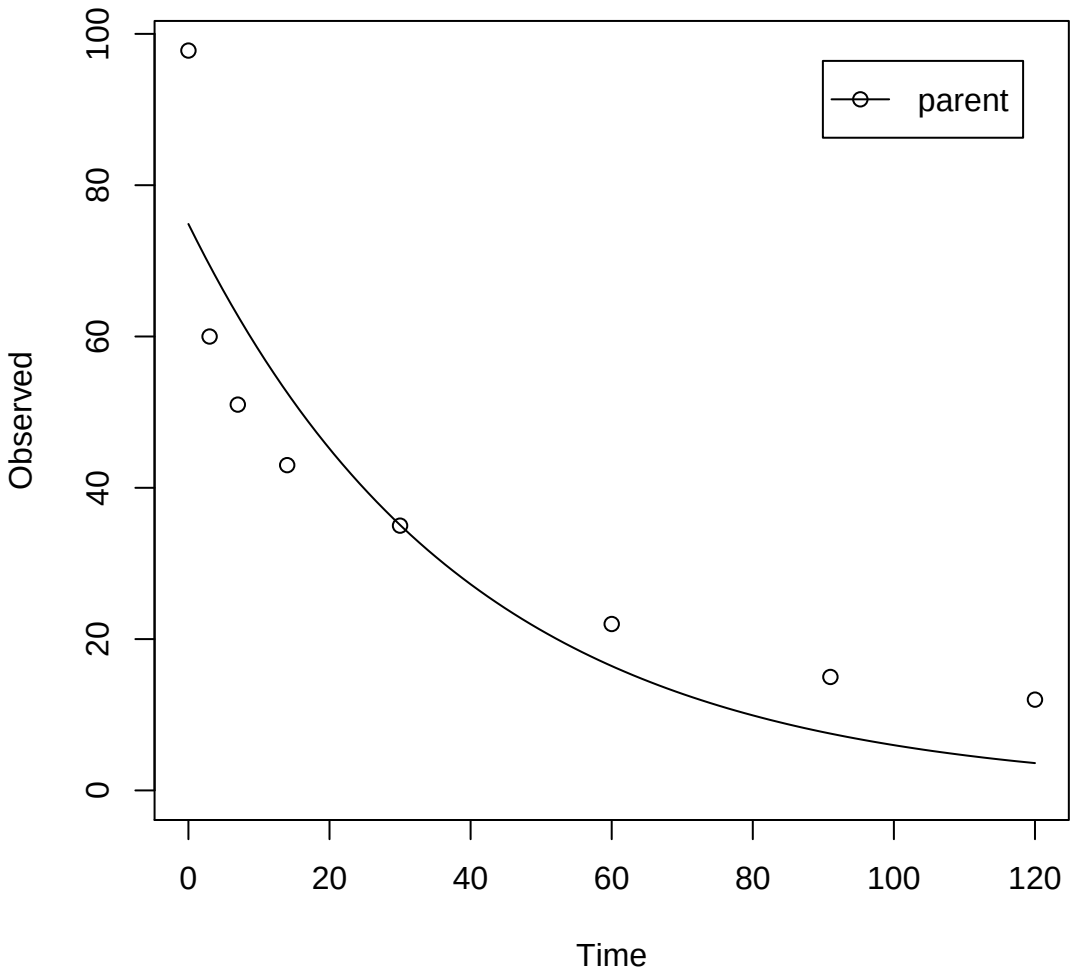
SFO model, summary and plot:

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

```
mkin version:    0.9.13
R version:       2.15.2
Date of fit:     Mon Feb 18 22:54:22 2013
Date of summary: Mon Feb 18 22:54:22 2013

Equations:
[1] d_parent = - k_parent_sink * parent

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
parent_0        74.873      8.458
k_parent_sink   -3.678      0.326

Backtransformed parameters:
              Estimate
parent_0        74.873
k_parent_sink    0.025

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
```

```
R> plot(m.L3.SFO)
```



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

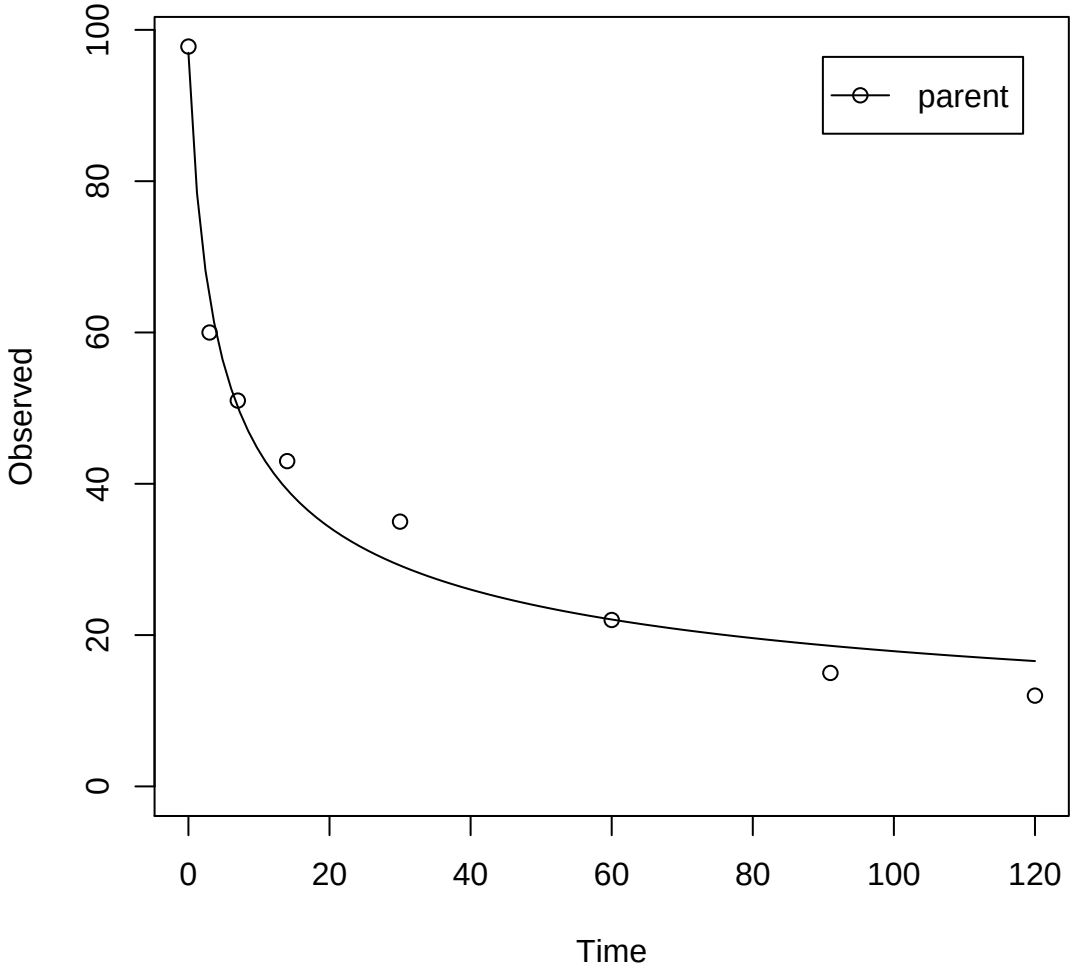
The FOMC model performs better:

```
R> m.L3.FOMC <- mkinfit(FOMC, FOCUS_2006_L3_mkin, quiet = TRUE)
R> plot(m.L3.FOMC)
R> s.m.L3.FOMC <- summary(m.L3.FOMC)
R> s.m.L3.FOMC$errmin
```

```
            err.min n.optim df
All data 0.07321867       3  5
parent   0.07321867       3  5
```

```
R> endpoints(m.L3.FOMC)
```

```
$distimes
           DT50     DT90
parent 7.729478 431.2428

$ff
logical(0)

$SFORB
logical(0)
```

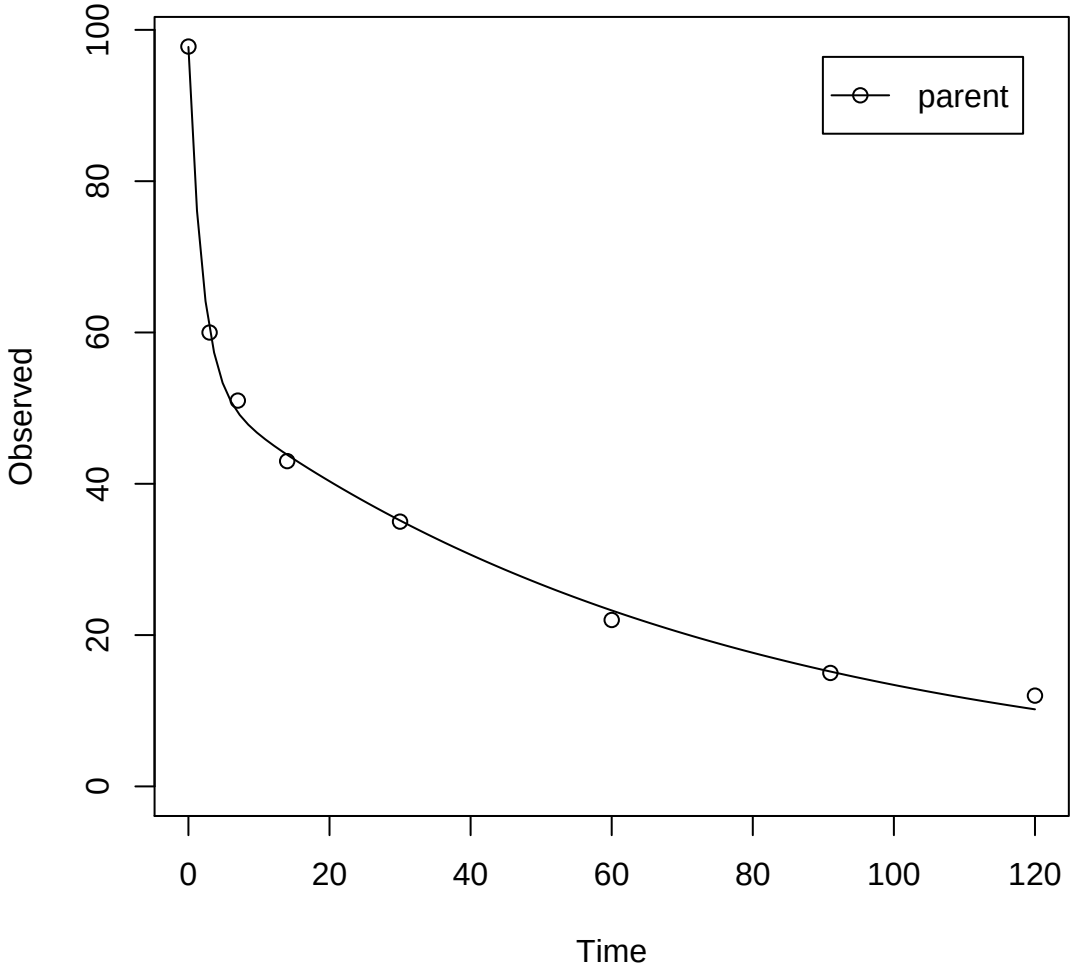


The error level at which the χ^2 test passes is 7% in this case.

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> s.m.L3.DFOP <- summary(m.L3.DFOP)
R> s.m.L3.DFOP$errmin
```

```
            err.min n.optim df
All data 0.02223992       4  4
parent   0.02223992       4  4
```



Therefore, the DFOP model is 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)
```

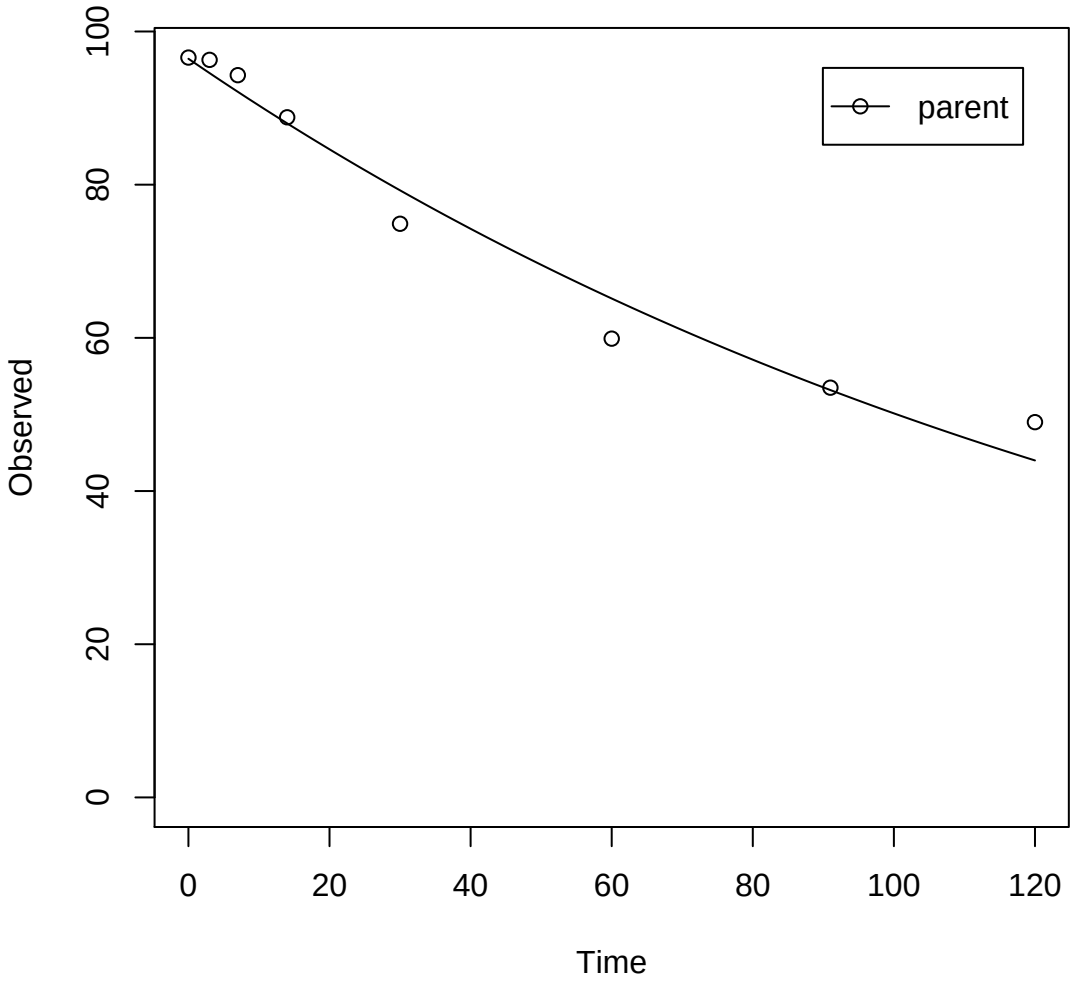
SFO model, summary and plot:

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

```
mkin version:    0.9.13
R version:       2.15.2
Date of fit:     Mon Feb 18 22:54:23 2013
Date of summary: Mon Feb 18 22:54:23 2013

Equations:
[1] d_parent = - k_parent_sink * parent

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
parent_0         96.44      1.949
k_parent_sink    -5.03      0.080

Backtransformed parameters:
              Estimate
parent_0        96.442
k_parent_sink    0.007

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

Data:
 time variable observed predicted residual
    0   parent     96.6     96.44   0.1585
    3   parent     96.3     94.57   1.7324
    7   parent     94.3     92.13   2.1744
   14   parent     88.8     88.00   0.7972
   30   parent     74.9     79.26  -4.3589
   60   parent     59.9     65.14  -5.2376
   91   parent     53.5     53.18   0.3167
  120   parent     49.0     43.99   5.0054
```

```
R> plot(m.L4.SFO)
```

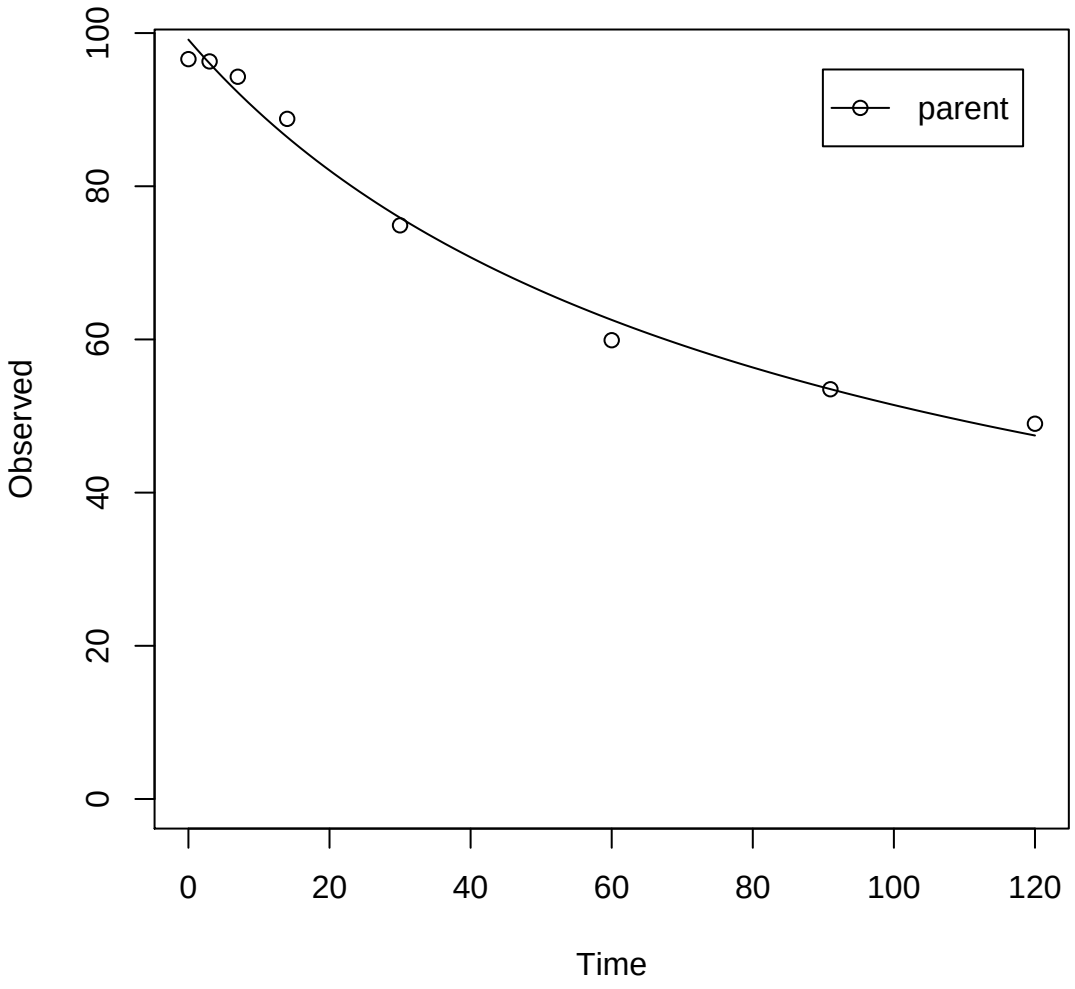


The χ^2 error level of 3.3% as well as the plot suggest that the model fits very well.

The FOMC model for comparison

```
R> m.L4.FOMC <- mkinfit(FOMC, FOCUS_2006_L4_mkin, quiet = TRUE)
R> plot(m.L4.FOMC)
R> s.m.L4.FOMC <- summary(m.L4.FOMC)
R> s.m.L4.FOMC$errmin
```

```
            err.min n.optim df
All data 0.02027643       3  5
parent   0.02027643       3  5
```



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)
```

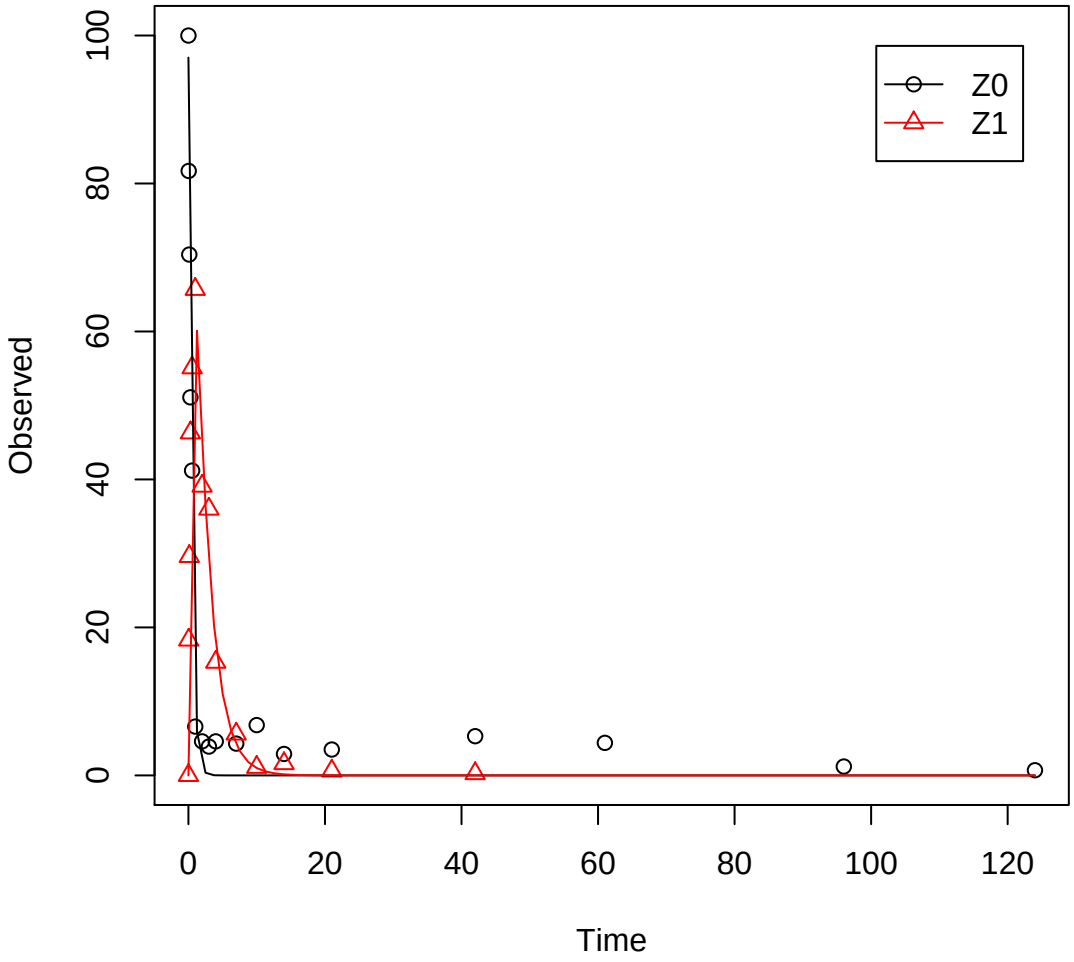
The next step is to set up the models used for the kinetic analysis. As the simultaneous fit of parent and the first metabolite is usually straightforward, Step 1 (SFO for parent only) is skipped here. We start with the model 2a, with formation and decline of metabolite Z1 and the pathway from parent 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> summary(m.Z.2a, data = FALSE)
```

```
mkin version:    0.9.13
R version:       2.15.2
Date of fit:     Mon Feb 18 22:54:24 2013
Date of summary: Mon Feb 18 22:54:24 2013

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

```
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
Z0_0       97.0149         NA
k_Z0_sink -36.2915         NA
k_Z0_Z1     0.8047         NA
k_Z1_sink  -0.7296         NA

Backtransformed parameters:
          Estimate
Z0_0        97.015
k_Z0_sink    0.000
k_Z0_Z1      2.236
k_Z1_sink    0.482

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
```

```
R> plot(m.Z.2a)
```



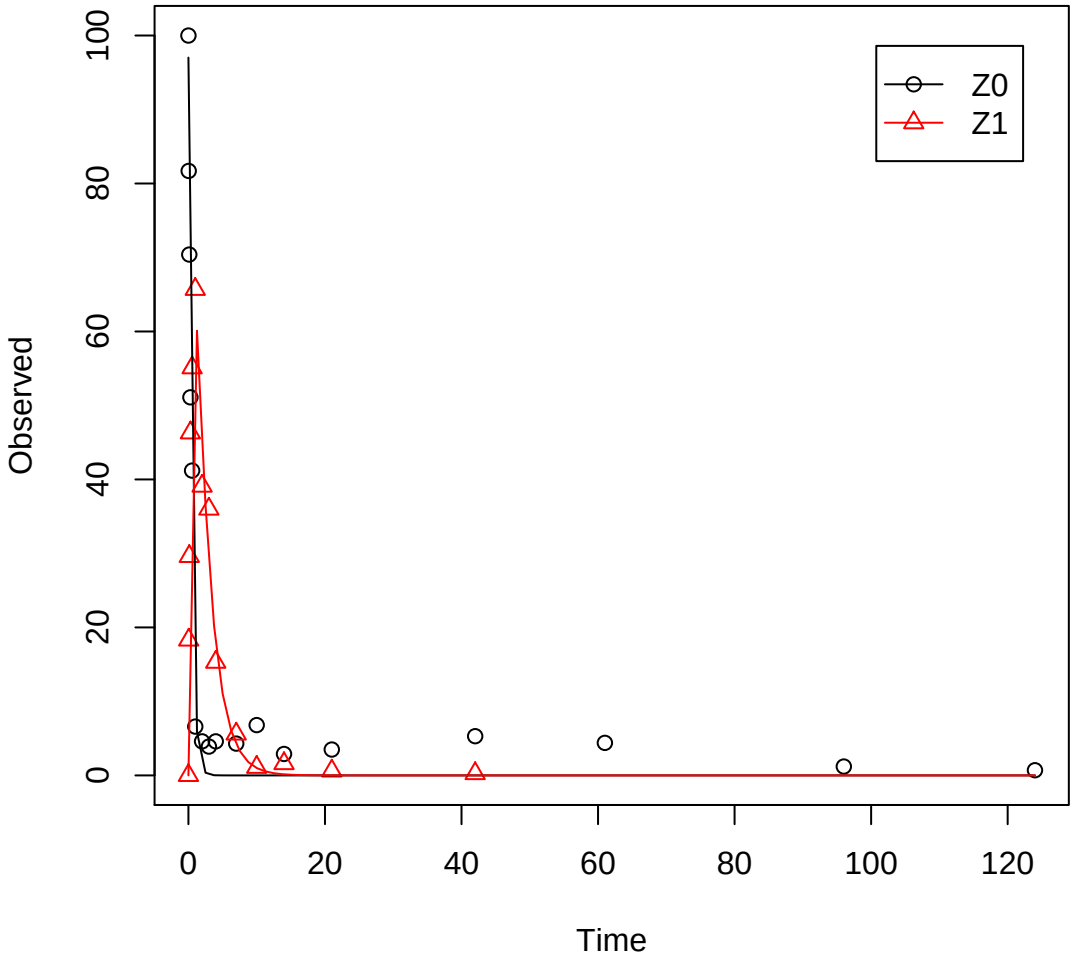
As obvious from the summary, the kinetic rate constant from parent compound Z to sink is negligible. Accordingly, the exact magnitude of the fitted parameter `log k_Z_sink` is ill-defined and the covariance matrix is not returned. This suggests, in agreement to the analysis in the FOCUS kinetics report, 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> summary(m.Z.2a.ff, data = FALSE)
```

```
mkin version:    0.9.13
R version:       2.15.2
Date of fit:     Mon Feb 18 22:54:25 2013
Date of summary: Mon Feb 18 22:54:25 2013

Equations:
[1] d_Z0 = - k_Z0 * Z0
[2] d_Z1 = + f_Z0_to_Z1 * k_Z0 * Z0 - k_Z1 * Z1

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
Z0_0        9.701e+01         NA
k_Z0        8.047e-01         NA
f_Z0_to_Z1  8.862e+06         NA
k_Z1       -7.296e-01         NA

Backtransformed parameters:
           Estimate
Z0_0         97.015
k_Z0          2.236
f_Z0_to_Z1    1.000
k_Z1          0.482

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
```

```
R> plot(m.Z.2a.ff)
```



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.

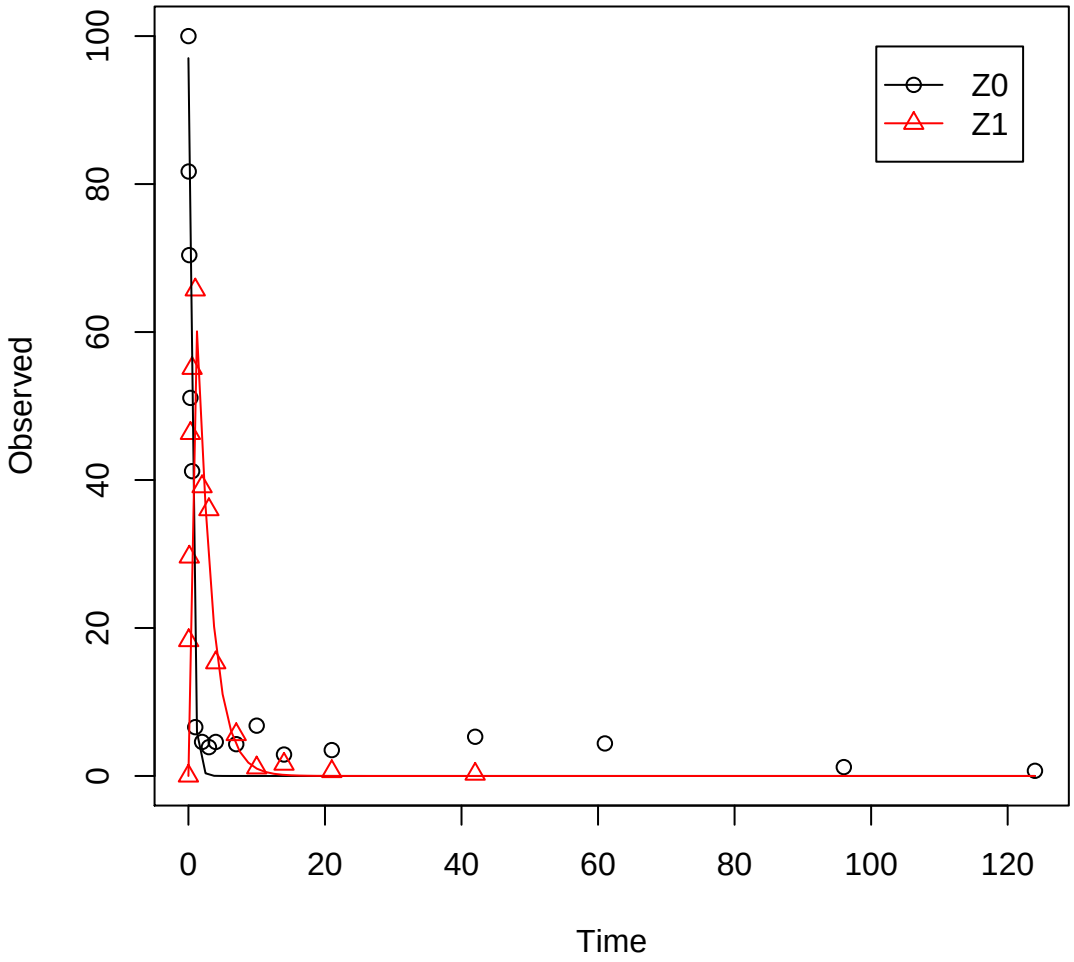
```
R> Z.3 <- mkinmod(Z0 = list(type = "SFO", to = "Z1", sink = FALSE),
+                 Z1 = list(type = "SFO"))
R> m.Z.3 <- mkinfit(Z.3, FOCUS_2006_Z_mkin, parms.ini = c(k_Z0_Z1 = 0.5),
+                   quiet = TRUE)
R> m.Z.3 <- mkinfit(Z.3, FOCUS_2006_Z_mkin, solution_type = "deSolve",
+     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

```
R> summary(m.Z.3, data = FALSE)
```

```
mkin version:    0.9.13
R version:       2.15.2
Date of fit:     Mon Feb 18 22:54:31 2013
Date of summary: Mon Feb 18 22:54:31 2013

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

Starting values for optimised parameters:
          initial   type transformed
Z0_0        100.0  state  100.000000
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
Z0_0       97.0149      2.682
k_Z0_Z1     0.8047      0.066
k_Z1_sink  -0.7296      0.089

Backtransformed parameters:
          Estimate
Z0_0        97.015
k_Z0_Z1      2.236
k_Z1_sink    0.482

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   0.04346
k_Z1_sink 0.4104 0.04346   1.00000
```

```
R> plot(m.Z.3)
```



The first attempt to fit the model fails, 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

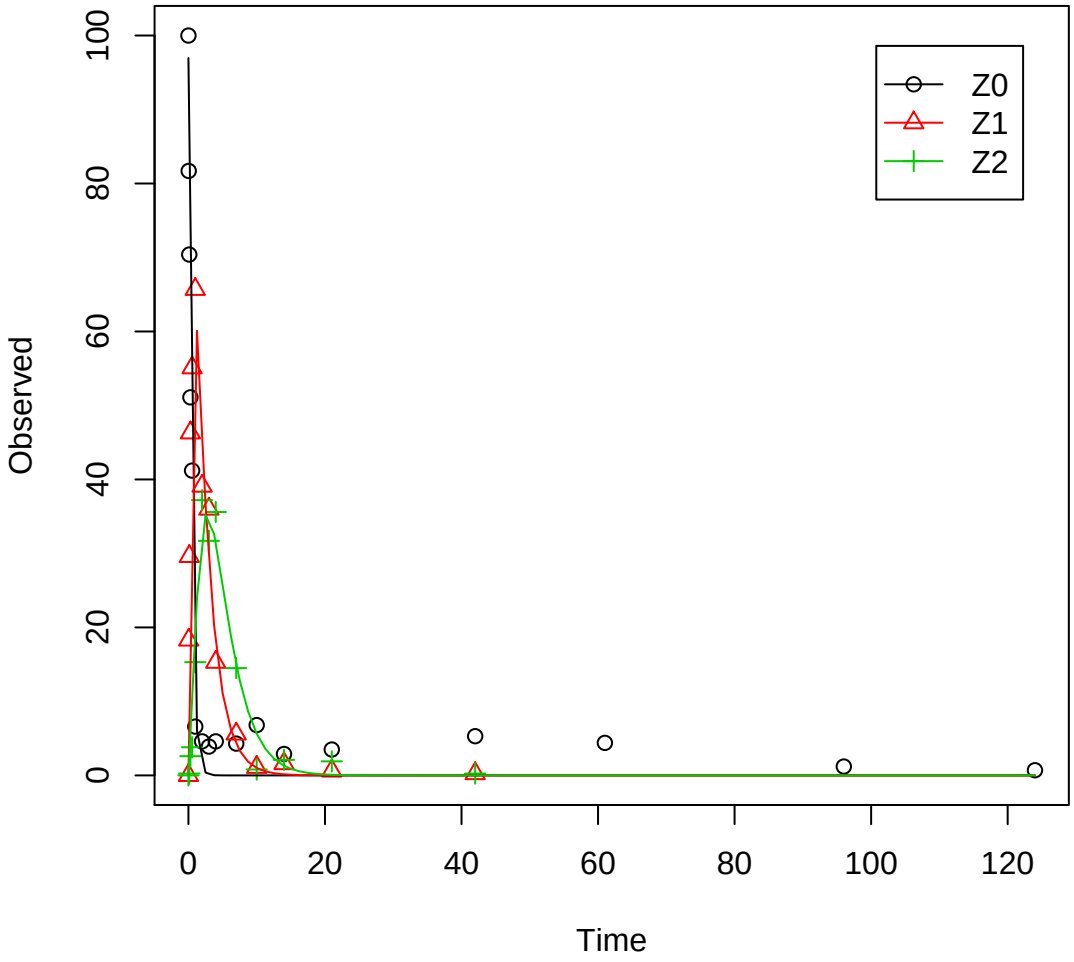
value for one of the parameters overcomes this. Alternatively, the **deSolve** based model solution can be chosen, at the cost of a bit more computing time.

```
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> summary(m.Z.4a, data = FALSE)
```

```
mkin version:    0.9.13
R version:       2.15.2
Date of fit:     Mon Feb 18 22:54:33 2013
Date of summary: Mon Feb 18 22:54:33 2013

Equations:
[1] d_Z0 = - 0 - k_Z0_Z1 * Z0
[2] d_Z1 = + k_Z0_Z1 * Z0 - k_Z1_sink * Z1 - k_Z1_Z2 * Z1
[3] d_Z2 = + k_Z1_Z2 * Z1 - k_Z2_sink * Z2

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
Z0_0       96.9587      2.447
k_Z0_Z1     0.7970      0.060
k_Z1_sink  -4.1044      4.847
k_Z1_Z2    -0.7667      0.146
k_Z2_sink  -0.8410      0.229

Backtransformed parameters:
          Estimate
Z0_0        96.959
k_Z0_Z1      2.219
k_Z1_sink    0.017
k_Z1_Z2      0.465
k_Z2_sink    0.431
```

```
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
```

```
R> plot(m.Z.4a)
```



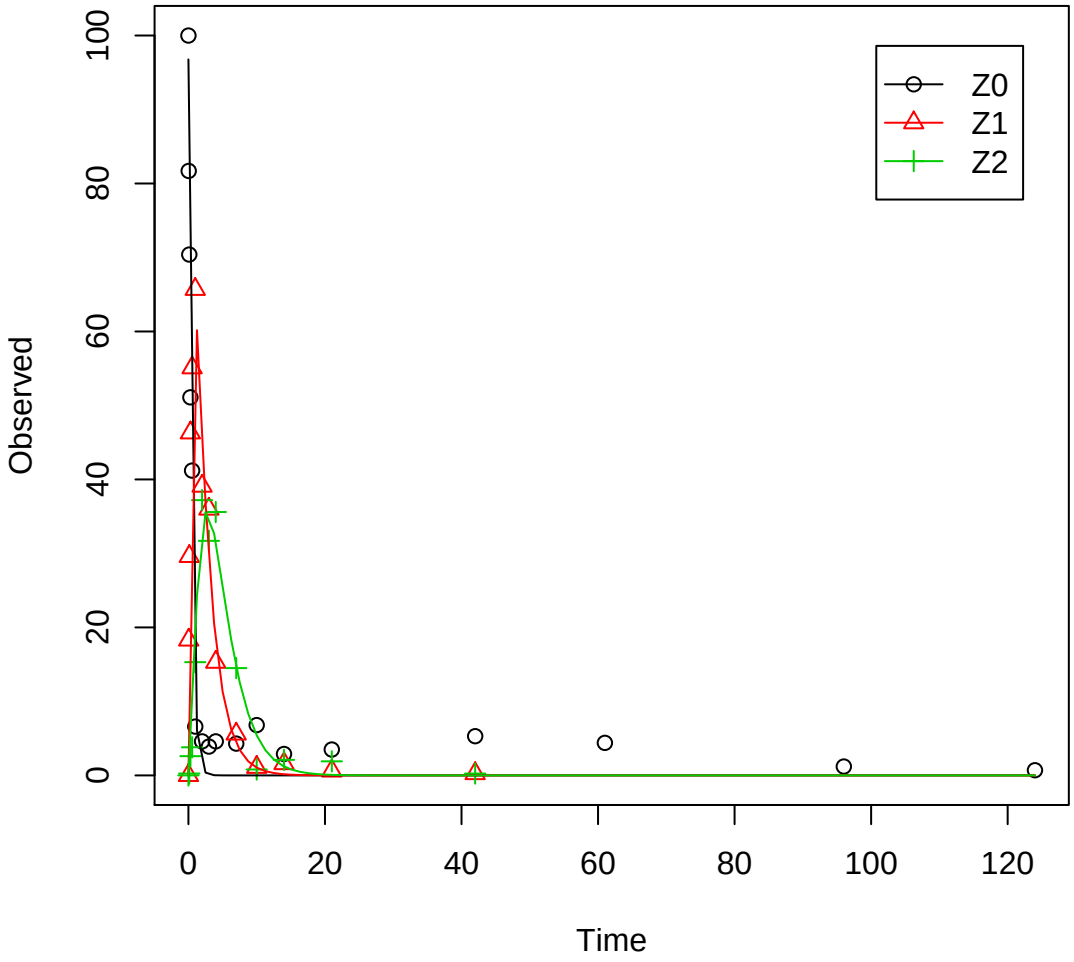
As suggested in the FOCUS report, the pathway to sink was removed for metabolite Z1 as well. While this step appears questionable on the basis of the above results, it is followed here for the purpose of comparison. Also, in the FOCUS report, it is assumed that there is additional empirical evidence 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> summary(m.Z.5, data = FALSE)
mkin version:    0.9.13
R version:       2.15.2
Date of fit:     Mon Feb 18 22:54:34 2013
Date of summary: Mon Feb 18 22:54:34 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

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
Z0_0       96.7690      2.266
k_Z0_Z1     0.7948      0.058
k_Z1_Z2    -0.7410      0.068
k_Z2_sink  -0.8027      0.111

Backtransformed parameters:
          Estimate
Z0_0        96.769
k_Z0_Z1      2.214
k_Z1_Z2      0.477
k_Z2_sink    0.448

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
```

```
R> plot(m.Z.5)
```



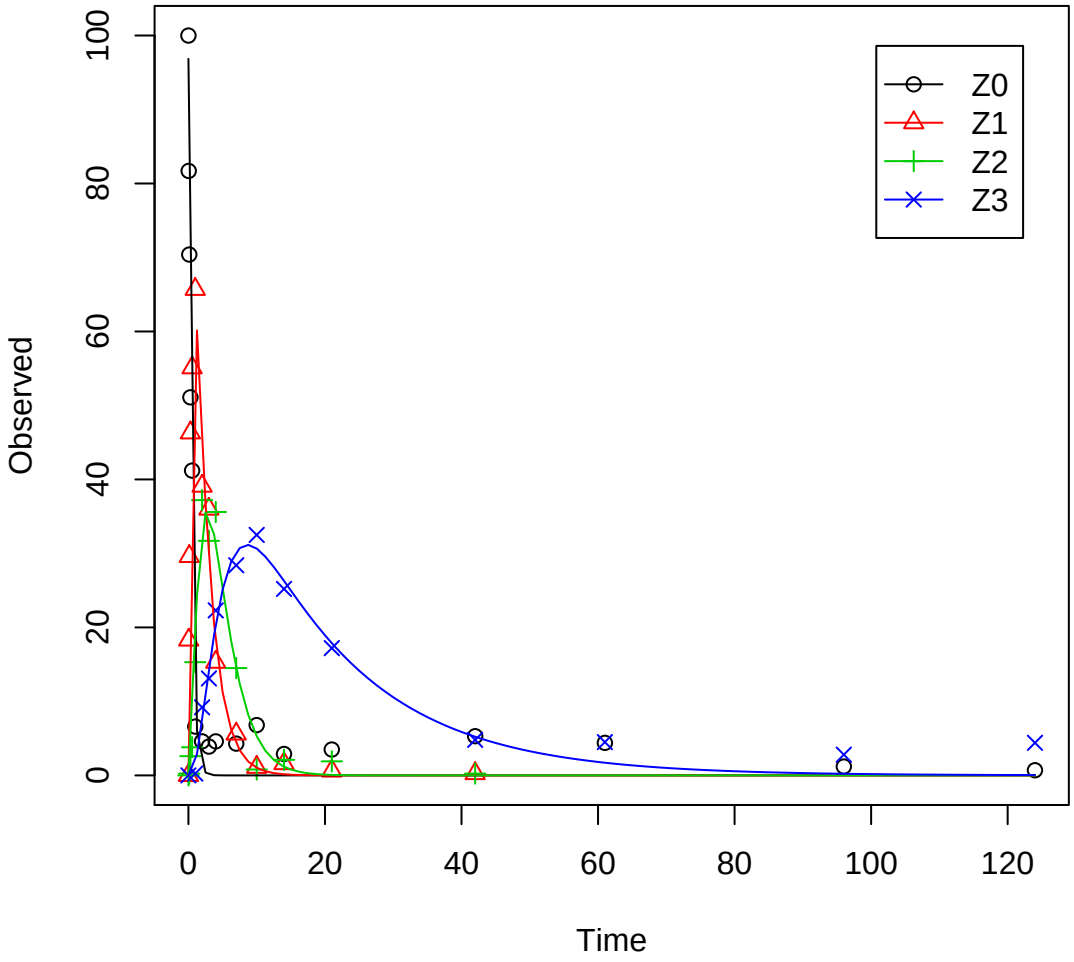
Finally, metabolite Z3 is added to the model.

```
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> summary(m.Z.FOCUS, data = FALSE)

mkin version:    0.9.13
R version:       2.15.2
```

```
Date of fit:     Mon Feb 18 22:54:35 2013
Date of summary: Mon Feb 18 22:54:35 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 * Z2
[4] d_Z3 = + k_Z2_Z3 * Z2 - k_Z3_sink * Z3

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
Z0_0       96.8386      2.059
k_Z0_Z1     0.7954      0.053
k_Z1_Z2    -0.7375      0.061
k_Z2_Z3    -1.5467      0.123
k_Z2_sink  -1.4326      0.172
k_Z3_sink  -2.8355      0.244

Backtransformed parameters:
          Estimate
Z0_0        96.839
k_Z0_Z1      2.215
k_Z1_Z2      0.478
k_Z2_Z3      0.213
k_Z2_sink    0.239
k_Z3_sink    0.059

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
```

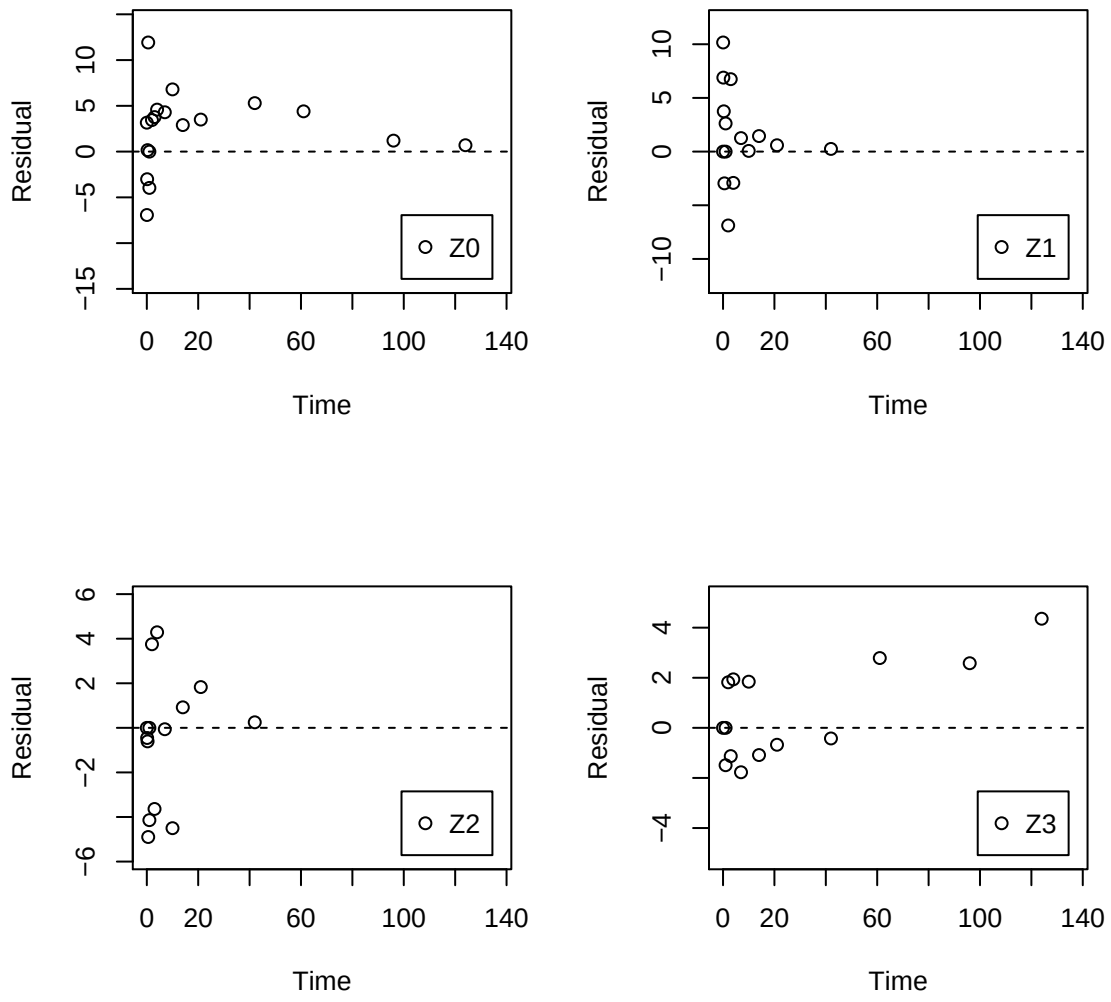
```
Z2         21.24       2 11
Z3         12.46       1 12

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
```

```
R> plot(m.Z.FOCUS)
```



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.

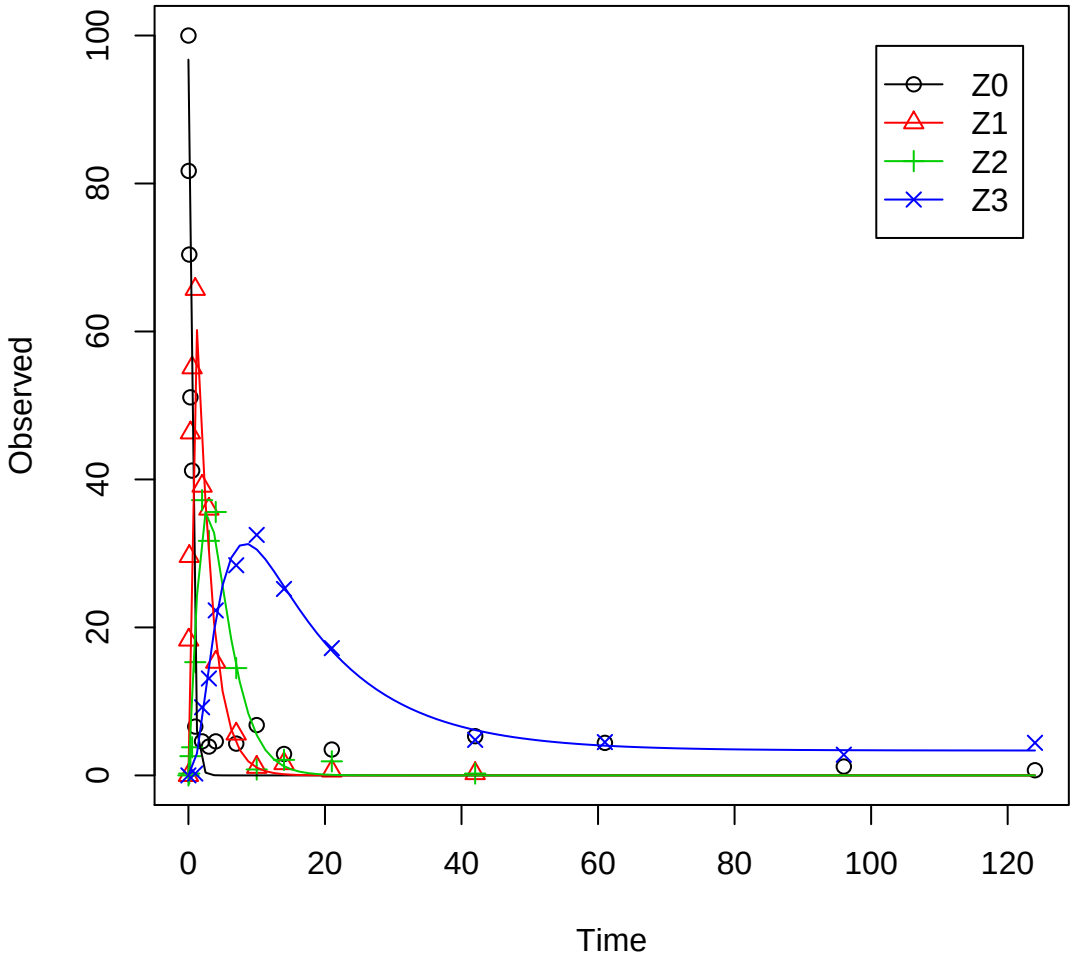
```
R> Z.mkin.1 <- mkinmod(Z0 = list(type = "SFO", to = "Z1", sink = FALSE),
+                  Z1 = list(type = "SFO", to = "Z2", sink = FALSE),
+                  Z2 = list(type = "SFO", to = "Z3"),
+                  Z3 = list(type = "SFORB"))
```

```
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, k_Z2_Z3 = 0.2),
+                   quiet = TRUE)
R> summary(m.Z.mkin.1, data = FALSE)
```

```
mkin version:    0.9.13
R version:       2.15.2
Date of fit:     Mon Feb 18 22:54:40 2013
Date of summary: Mon Feb 18 22:54:40 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

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_Z3           2e-01 deparm  -1.6094379
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
Z0_0             96.7375         NA
k_Z0_Z1           0.7947         NA
k_Z1_Z2          -0.7426         NA
k_Z2_Z3          -1.6094         NA
k_Z2_sink        -1.4946         NA
k_Z2_Z3_free     -1.5042         NA
k_Z3_free_sink   -2.6544         NA
k_Z3_free_bound  -5.2440         NA
k_Z3_bound_free -19.8427         NA

Backtransformed parameters:
```

```
                Estimate
Z0_0              96.738
k_Z0_Z1            2.214
k_Z1_Z2            0.476
k_Z2_Z3            0.200
k_Z2_sink          0.224
k_Z2_Z3_free       0.222
k_Z3_free_sink     0.070
k_Z3_free_bound    0.005
k_Z3_bound_free    0.000

Residual standard error: 4.149 on 48 degrees of freedom

Chi2 error levels in percent:
         err.min n.optim df
All data  19.931       9 48
Z0        17.429       2 15
Z1        15.949       1 13
Z2        21.967       3 10
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 10.1977 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 2.244e-09
```

```
R> plot(m.Z.mkin.1)
```



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.

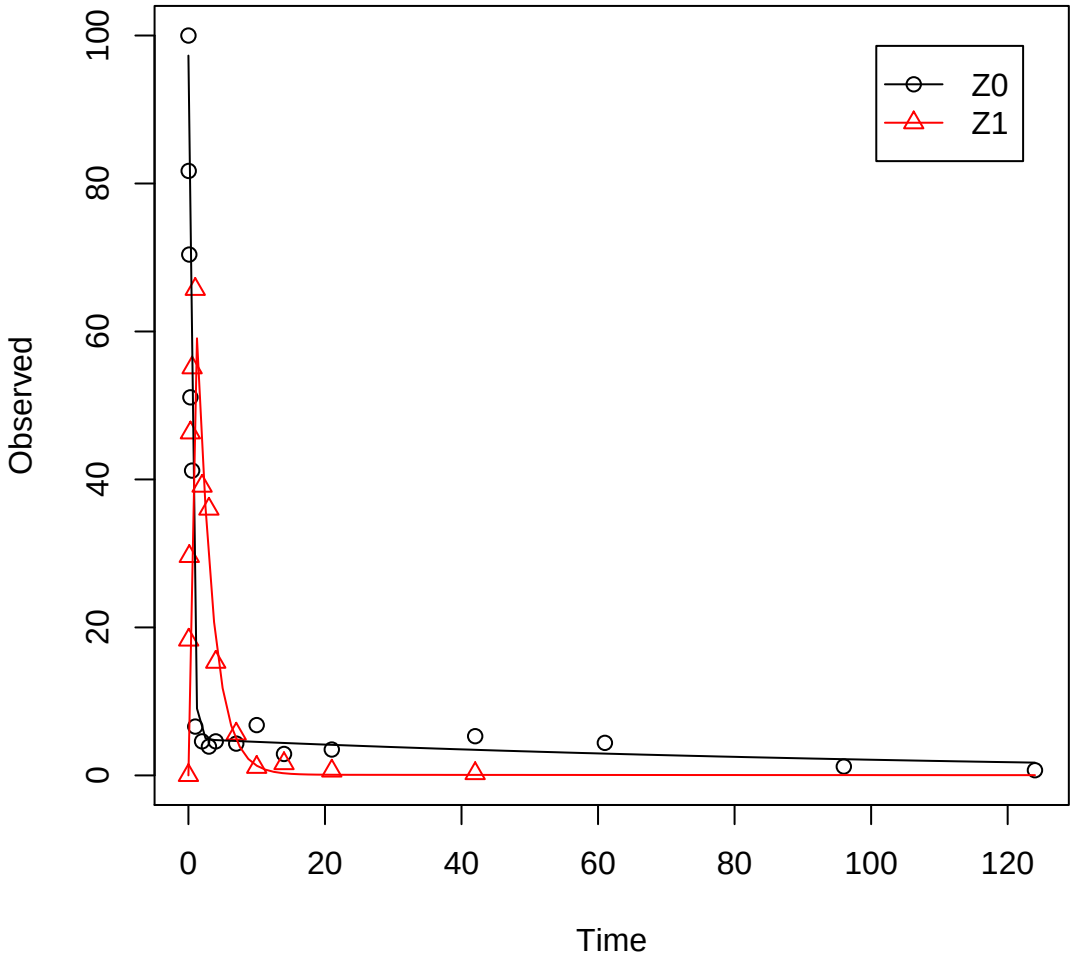
```
R> Z.mkin.2 <- mkinmod(Z0 = list(type = "SFORB", to = "Z1", sink = FALSE),
+                  Z1 = list(type = "SFO"))
R> m.Z.mkin.2 <- mkinfit(Z.mkin.2, FOCUS_2006_Z_mkin, quiet = TRUE)
R> summary(m.Z.mkin.2, data = FALSE)
```

```
mkin version:    0.9.13
R version:       2.15.2
Date of fit:     Mon Feb 18 22:54:41 2013
Date of summary: Mon Feb 18 22:54:41 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

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
Z0_free_0       97.2851      2.395
k_Z0_free_bound -2.0821      0.432
k_Z0_bound_free -4.7204      1.605
k_Z0_free_Z1     0.8549      0.064
k_Z1_sink       -0.7934      0.085

Backtransformed parameters:
               Estimate
Z0_free_0        97.285
k_Z0_free_bound   0.125
k_Z0_bound_free   0.009
k_Z0_free_Z1      2.351
k_Z1_sink         0.452

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

```
R> plot(m.Z.mkin.2)
```



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

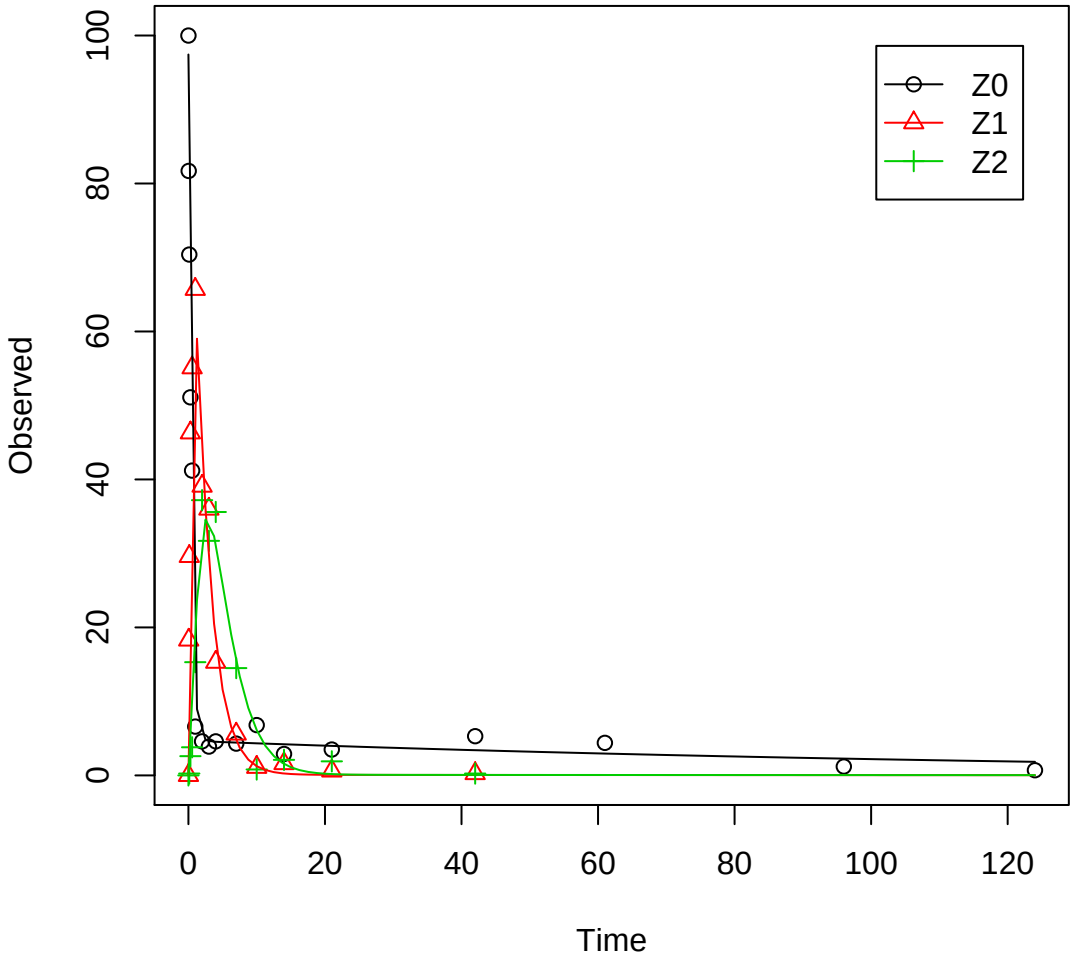
```
R> Z.mkin.3 <- mkinmod(Z0 = list(type = "SFORB", to = "Z1", sink = FALSE),
+                  Z1 = list(type = "SFO", to = "Z2"),
+                  Z2 = list(type = "SFO"))
R> m.Z.mkin.3 <- mkinfit(Z.mkin.3, FOCUS_2006_Z_mkin, quiet = TRUE)
R> summary(m.Z.mkin.3, data = FALSE)
```

```
mkin version:    0.9.13
R version:       2.15.2
Date of fit:     Mon Feb 18 22:54:45 2013
Date of summary: Mon Feb 18 22:54:45 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

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
Z0_free_0        97.4356  2.124e+00
k_Z0_free_bound  -2.1488  4.120e-01
k_Z0_bound_free  -4.8383  1.636e+00
k_Z0_free_Z1      0.8457  5.900e-02
k_Z1_sink       -19.3794  1.751e+06
k_Z1_Z2          -0.7812  8.800e-02
k_Z2_sink        -0.8606  1.440e-01

Backtransformed parameters:
                Estimate
Z0_free_0         97.436
k_Z0_free_bound    0.117
k_Z0_bound_free    0.008
k_Z0_free_Z1       2.330
k_Z1_sink          0.000
k_Z1_Z2            0.458
k_Z2_sink          0.423

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
Z0_free_0         1.00000         0.05615         0.06063      0.09324
k_Z0_free_bound   0.05615         1.00000         0.54490      0.41896
k_Z0_bound_free   0.06063         0.54490         1.00000      0.16141
k_Z0_free_Z1      0.09324         0.41896         0.16141      1.00000
k_Z1_sink         0.15262        -0.11467        -0.06038      0.02314
k_Z1_Z2           0.08997        -0.09395        -0.02475     -0.09097
k_Z2_sink         0.11068        -0.07920        -0.01859     -0.05193
                k_Z1_sink  k_Z1_Z2 k_Z2_sink
Z0_free_0         0.15262  0.08997   0.11068
k_Z0_free_bound  -0.11467 -0.09395  -0.07920
k_Z0_bound_free  -0.06038 -0.02475  -0.01859
k_Z0_free_Z1      0.02314 -0.09097  -0.05193
k_Z1_sink         1.00000 -0.66065  -0.66738
k_Z1_Z2          -0.66065  1.00000   0.59341
k_Z2_sink        -0.66738  0.59341   1.00000
```

```
R> plot(m.Z.mkin.3)
```



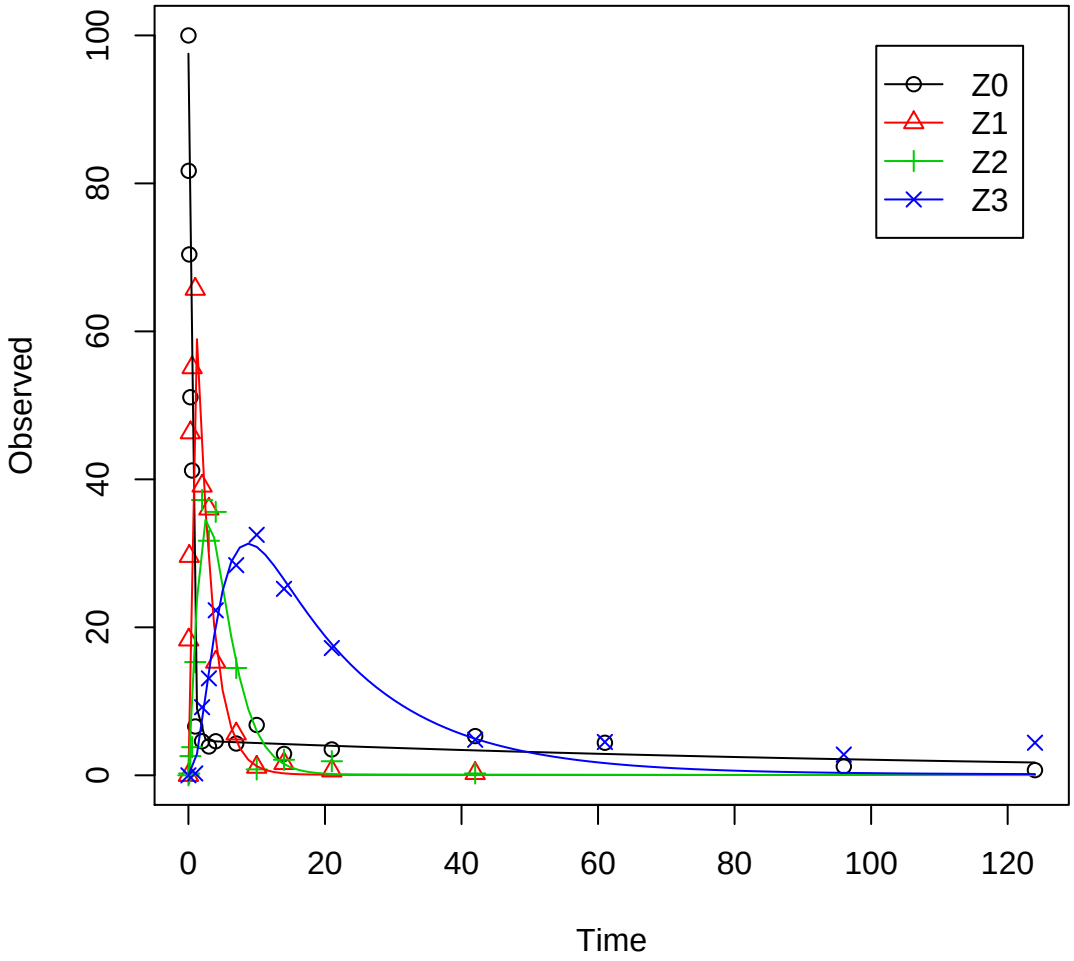
Finally, Z3 is added as well. This model appears overparameterised (no covariance matrix returned) if the sink for Z1 is left in the model.

```
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> summary(m.Z.mkin.4, data = FALSE)

mkin version:    0.9.13
R version:       2.15.2
```

```
Date of fit:     Mon Feb 18 22:54:49 2013
Date of summary: Mon Feb 18 22:54:49 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

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
Z0_free_0        97.5296      1.887
k_Z1_Z2          -0.7769      0.058
k_Z0_free_bound  -2.1362      0.368
k_Z0_bound_free  -4.7653      1.417
k_Z0_free_Z1      0.8470      0.053
k_Z2_sink        -1.5610      0.183
k_Z2_Z3          -1.5276      0.114
k_Z3_sink        -2.7691      0.225

Backtransformed parameters:
                Estimate
Z0_free_0         97.530
k_Z1_Z2            0.460
k_Z0_free_bound    0.118
k_Z0_bound_free    0.009
k_Z0_free_Z1       2.333
k_Z2_sink          0.210
k_Z2_Z3            0.217
k_Z3_sink          0.063
```

```
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
Z0_free_0         1.00000  0.24238         0.07823         0.06921      0.08883
k_Z1_Z2           0.24238  1.00000        -0.22742        -0.08934     -0.10841
k_Z0_free_bound   0.07823 -0.22742         1.00000         0.53976      0.42766
k_Z0_bound_free   0.06921 -0.08934         0.53976         1.00000      0.16281
k_Z0_free_Z1      0.08883 -0.10841         0.42766         0.16281      1.00000
k_Z2_sink         0.32993  0.34052        -0.26331        -0.12743     -0.05310
k_Z2_Z3          -0.07494 -0.14885         0.06698         0.06082     -0.01281
k_Z3_sink        -0.10456 -0.22486         0.13845         0.12526      0.01858
                k_Z2_sink  k_Z2_Z3 k_Z3_sink
Z0_free_0          0.3299 -0.07494  -0.10456
k_Z1_Z2            0.3405 -0.14885  -0.22486
k_Z0_free_bound   -0.2633  0.06698   0.13845
k_Z0_bound_free   -0.1274  0.06082   0.12526
k_Z0_free_Z1      -0.0531 -0.01281   0.01858
k_Z2_sink          1.0000 -0.25473  -0.68320
k_Z2_Z3           -0.2547  1.00000   0.56390
k_Z3_sink         -0.6832  0.56390   1.00000
```

```
R> plot(m.Z.mkin.4)
```



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. Therefore, the model `Z.mkin.5` is proposed as the best-fit model for the dataset from Appendix 7 of the FOCUS report.

```
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"),
```

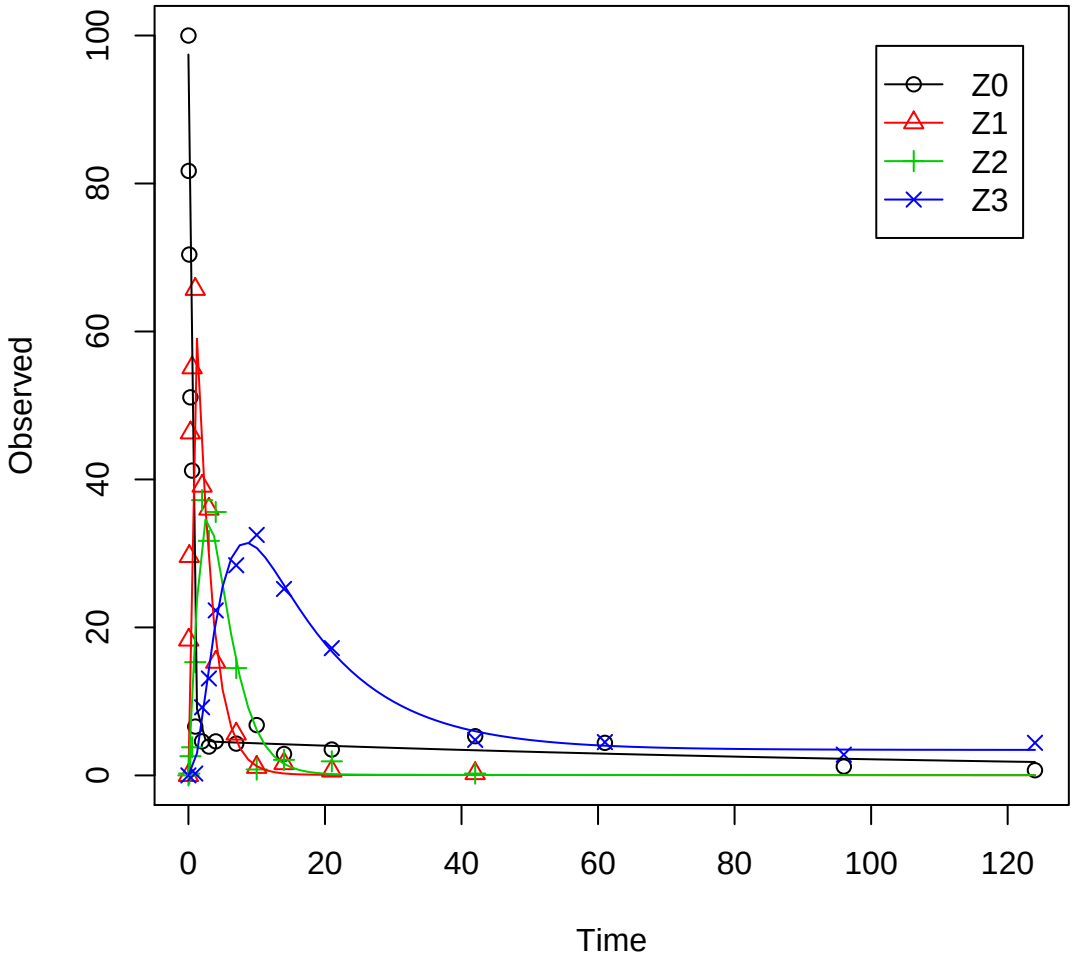
```
+                 Z3 = list(type = "SFORB"))
R> m.Z.mkin.5 <- mkinfit(Z.mkin.5, FOCUS_2006_Z_mkin,
+   parms.ini = c(k_Z1_Z2 = 0.2), quiet = TRUE)
R> summary(m.Z.mkin.5, data = FALSE)
```

```
mkin version:    0.9.13
R version:       2.15.2
Date of fit:     Mon Feb 18 22:55:03 2013
Date of summary: Mon Feb 18 22:55:03 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

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
Z0_free_0       97.4307      1.887
k_Z1_Z2         -0.7813      0.059
k_Z0_free_bound -2.1467      0.369
k_Z0_bound_free -4.8228      1.463
k_Z0_free_Z1     0.8459      0.053
k_Z2_sink       -1.6296      0.221
k_Z2_Z3_free    -1.4854      0.196
k_Z3_free_sink  -2.5954      0.385
```

```
k_Z3_free_bound  -5.2565      1.372
k_Z3_bound_free -13.4386    282.033

Backtransformed parameters:
                Estimate
Z0_free_0         97.431
k_Z1_Z2            0.458
k_Z0_free_bound    0.117
k_Z0_bound_free    0.008
k_Z0_free_Z1       2.330
k_Z2_sink          0.196
k_Z2_Z3_free       0.226
k_Z3_free_sink     0.075
k_Z3_free_bound    0.005
k_Z3_bound_free    0.000

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
Z0_free_0         1.00000  0.24874        0.079292        0.073662      0.09072
k_Z1_Z2           0.24874  1.00000       -0.223885       -0.082769     -0.10368
```

```
k_Z0_free_bound   0.07929 -0.22389        1.000000        0.542876      0.42696
k_Z0_bound_free   0.07366 -0.08277        0.542876        1.000000      0.16540
k_Z0_free_Z1      0.09072 -0.10368        0.426958        0.165399      1.00000
k_Z2_sink         0.31619  0.36741       -0.287122       -0.168222     -0.08266
k_Z2_Z3_free     -0.03185 -0.05441        0.008287        0.013478     -0.01908
k_Z3_free_sink   -0.12037 -0.22367        0.070584        0.024242     -0.01842
k_Z3_free_bound   0.01629  0.01906        0.009670        0.031837      0.03607
k_Z3_bound_free   0.03781  0.08868       -0.055136       -0.008975     -0.06574
                k_Z2_sink k_Z2_Z3_free k_Z3_free_sink k_Z3_free_bound
Z0_free_0         0.31619    -0.031847       -0.12037         0.01629
k_Z1_Z2           0.36741    -0.054405       -0.22367         0.01906
k_Z0_free_bound  -0.28712     0.008287        0.07058         0.00967
k_Z0_bound_free  -0.16822     0.013478        0.02424         0.03184
k_Z0_free_Z1     -0.08266    -0.019083       -0.01842         0.03607
k_Z2_sink         1.00000    -0.069361       -0.66596         0.02603
k_Z2_Z3_free     -0.06936     1.000000       -0.26493         0.73477
k_Z3_free_sink   -0.66596    -0.264933        1.00000        -0.36064
k_Z3_free_bound   0.02603     0.734774       -0.36064         1.00000
k_Z3_bound_free   0.23931     0.774434       -0.71123         0.81143
                k_Z3_bound_free
Z0_free_0              0.037814
k_Z1_Z2                0.088677
k_Z0_free_bound       -0.055136
k_Z0_bound_free       -0.008975
k_Z0_free_Z1          -0.065741
k_Z2_sink              0.239308
k_Z2_Z3_free           0.774434
k_Z3_free_sink        -0.711226
k_Z3_free_bound        0.811433
k_Z3_bound_free        1.000000
```

```
R> plot(m.Z.mkin.5)
```



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