Example evaluation of FOCUS dataset Z

Johannes Ranke

Wissenschaftlicher Berater
Kronacher Str. 8, 79639 Grenzach-Wyhlen, Germany

and

University of Bremen

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Contents

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1 The data

The following code defines the example dataset from Appendix 7 to the FOCUS kinetics report (FOCUS Work Group on Degradation Kinetics, 2011), p.350.

```
require(mkin)
```

```
## Loading required package:  mkin
## Loading required package:  minpack.lm
## Loading required package:  rootSolve
```

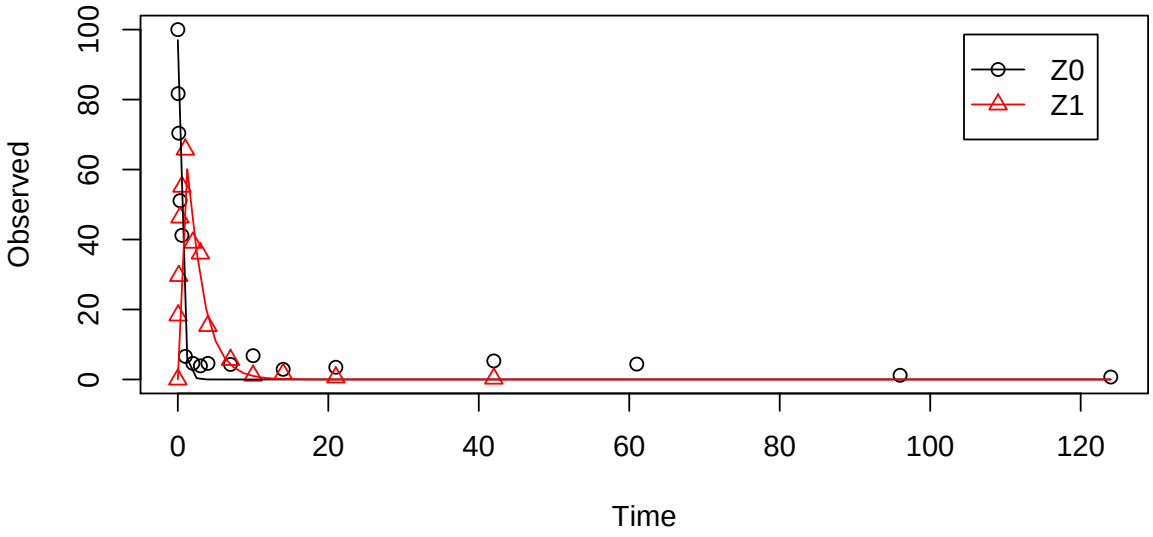
```
LOD = 0.5
FOCUS_2006_Z = data.frame(
  t = c(0, 0.04, 0.125, 0.29, 0.54, 1, 2, 3, 4, 7, 10, 14, 21,
        42, 61, 96, 124),
  Z0 = c(100, 81.7, 70.4, 51.1, 41.2, 6.6, 4.6, 3.9, 4.6, 4.3, 6.8,
         2.9, 3.5, 5.3, 4.4, 1.2, 0.7),
  Z1 = c(0, 18.3, 29.6, 46.3, 55.1, 65.7, 39.1, 36, 15.3, 5.6, 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))

FOCUS_2006_Z_mkin <- mkin_wide_to_long(FOCUS_2006_Z)
```

2 Parent compound and one metabolite

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).

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




```
summary(m.Z.2a, data = FALSE)
```

```
## mkin version:    0.9.34
## R version:       3.1.1
## Date of fit:     Wed Oct 15 00:59:06 2014
## Date of summary: Wed Oct 15 00:59:06 2014
##
## Equations:
## d_Z0 = - k_Z0_sink * Z0 - k_Z0_Z1 * Z0
## d_Z1 = + k_Z0_Z1 * Z0 - k_Z1_sink * Z1
##
## Model predictions using solution type eigen
##
## Fitted with method Port using 349 model solutions performed in 4.08 s
##
## Weighting: none
##
```

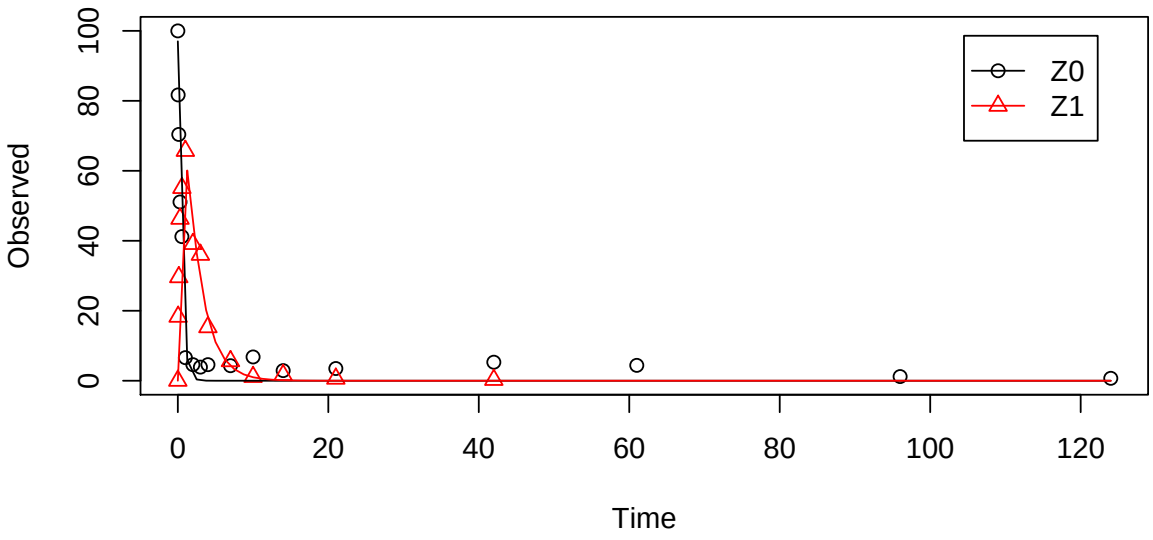
```
## Starting values for parameters to be optimised:
##              value   type
## Z0_0      100.0000  state
## k_Z0_sink   0.1000 deparm
## k_Z0_Z1     0.1001 deparm
## k_Z1_sink   0.1002 deparm
##
## Starting values for the transformed parameters actually optimised:
##                 value lower upper
## Z0_0          100.000  -Inf   Inf
## log_k_Z0_sink  -2.303  -Inf   Inf
## log_k_Z0_Z1    -2.302  -Inf   Inf
## log_k_Z1_sink  -2.301  -Inf   Inf
##
## Fixed parameter values:
##      value  type
## Z1_0     0 state
##
## Optimised, transformed parameters:
##               Estimate Std. Error Lower Upper t value Pr(>|t|) Pr(>t)
## Z0_0            97.000         NA    NA    NA      NA       NA     NA
## log_k_Z0_sink  -21.900         NA    NA    NA      NA       NA     NA
## log_k_Z0_Z1      0.805         NA    NA    NA      NA       NA     NA
## log_k_Z1_sink   -0.730         NA    NA    NA      NA       NA     NA
##
## Parameter correlation:
## Could not estimate covariance matrix; singular system:
##
## Residual standard error: 5.06 on 27 degrees of freedom
##
## Backtransformed parameters:
##           Estimate Lower Upper
## Z0_0      9.70e+01    NA    NA
## k_Z0_sink 3.06e-10    NA    NA
## k_Z0_Z1   2.24e+00    NA    NA
## k_Z1_sink 4.82e-01    NA    NA
##
## Chi2 error levels in percent:
##          err.min n.optim df
## All data    17.9       4 26
## Z0          18.0       3 14
## Z1          15.1       1 12
```

```
##
## Resulting formation fractions:
##                ff
## Z0_sink 1.37e-10
## Z0_Z1   1.00e+00
## Z1_sink 1.00e+00
##
## Estimated disappearance times:
##    DT50 DT90
## Z0 0.31 1.03
## Z1 1.44 4.78
```

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:

```
Z.2a.ff <- mkinmod(Z0 = list(type = "SFO", to = "Z1"),
                   Z1 = list(type = "SFO"),
                   use_of_ff = "max")

m.Z.2a.ff <- mkinfit(Z.2a.ff, FOCUS_2006_Z_mkin, quiet = TRUE)
plot(m.Z.2a.ff)
```




```
summary(m.Z.2a.ff, data = FALSE)
```

```
## mkin version:    0.9.34
## R version:       3.1.1
## Date of fit:     Wed Oct 15 00:59:10 2014
## Date of summary: Wed Oct 15 00:59:10 2014
##
## Equations:
## d_Z0 = - k_Z0 * Z0
## d_Z1 = + f_Z0_to_Z1 * k_Z0 * Z0 - k_Z1 * Z1
##
## Model predictions using solution type eigen
##
## Fitted with method Port using 329 model solutions performed in 3.825 s
##
## Weighting: none
##
## Starting values for parameters to be optimised:
##               value   type
## Z0_0       100.0000  state
## k_Z0         0.1000 deparm
## k_Z1         0.1001 deparm
## f_Z0_to_Z1   0.5000 deparm
##
```

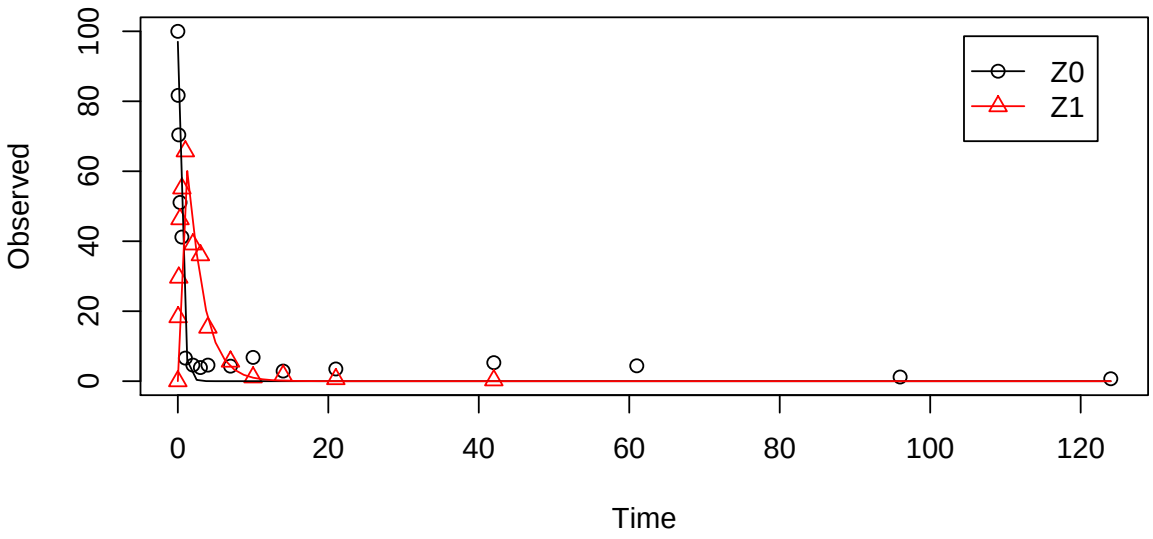
```
## Starting values for the transformed parameters actually optimised:
##              value lower upper
## Z0_0       100.000  -Inf   Inf
## log_k_Z0    -2.303  -Inf   Inf
## log_k_Z1    -2.302  -Inf   Inf
## f_Z0_ilr_1   0.000  -Inf   Inf
##
## Fixed parameter values:
##      value  type
## Z1_0     0 state
##
## Optimised, transformed parameters:
##            Estimate Std. Error Lower Upper t value Pr(>|t|) Pr(>t)
## Z0_0         97.000         NA    NA    NA      NA       NA     NA
## log_k_Z0      0.805         NA    NA    NA      NA       NA     NA
## log_k_Z1     -0.730         NA    NA    NA      NA       NA     NA
## f_Z0_ilr_1   15.900         NA    NA    NA      NA       NA     NA
##
## Parameter correlation:
## Could not estimate covariance matrix; singular system:
##
## Residual standard error: 5.06 on 27 degrees of freedom
##
## Backtransformed parameters:
##            Estimate Lower Upper
## Z0_0         97.000    NA    NA
## k_Z0          2.240    NA    NA
## k_Z1          0.482    NA    NA
## f_Z0_to_Z1    1.000    NA    NA
##
## Chi2 error levels in percent:
##          err.min n.optim df
## All data    17.9       4 26
## Z0          17.6       2 15
## Z1          15.6       2 11
##
## Resulting formation fractions:
##               ff
## Z0_Z1   1.00e+00
## Z0_sink 1.73e-10
##
## Estimated disappearance times:
```

```
##    DT50 DT90
## Z0 0.31 1.03
## Z1 1.44 4.78
```

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`.

```
Z.3 <- mkinmod(Z0 = list(type = "SFO", to = "Z1", sink = FALSE),
               Z1 = list(type = "SFO"), use_of_ff = "max")
m.Z.3 <- mkinfit(Z.3, FOCUS_2006_Z_mkin, quiet = TRUE)
plot(m.Z.3)
```




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

```
## mkin version:    0.9.34
## R version:       3.1.1
## Date of fit:     Wed Oct 15 00:59:12 2014
## Date of summary: Wed Oct 15 00:59:12 2014
##
## Equations:
## d_Z0 = - k_Z0 * Z0
## d_Z1 = + k_Z0 * Z0 - k_Z1 * Z1
```

```
##
## Model predictions using solution type eigen
##
## Fitted with method Port using 100 model solutions performed in 1.141 s
##
## Weighting: none
##
## Starting values for parameters to be optimised:
##         value   type
## Z0_0 100.0000  state
## k_Z0   0.1000 deparm
## k_Z1   0.1001 deparm
##
## Starting values for the transformed parameters actually optimised:
##            value lower upper
## Z0_0     100.000  -Inf   Inf
## log_k_Z0  -2.303  -Inf   Inf
## log_k_Z1  -2.302  -Inf   Inf
##
## Fixed parameter values:
##      value  type
## Z1_0     0 state
##
## Optimised, transformed parameters:
##          Estimate Std. Error  Lower   Upper t value Pr(>|t|)   Pr(>t)
## Z0_0       97.000     2.6800 91.500 103.000   36.20 4.73e-25 2.36e-25
## log_k_Z0    0.805     0.0657  0.670   0.939   12.30 9.12e-13 4.56e-13
## log_k_Z1   -0.730     0.0885 -0.911  -0.548   -8.24 5.74e-09 2.87e-09
##
## Parameter correlation:
##           Z0_0 log_k_Z0 log_k_Z1
## Z0_0     1.000   0.1063   0.4104
## log_k_Z0 0.106   1.0000   0.0435
## log_k_Z1 0.410   0.0435   1.0000
##
## Residual standard error: 4.97 on 28 degrees of freedom
##
## Backtransformed parameters:
##      Estimate  Lower   Upper
## Z0_0   97.000 91.500 103.000
## k_Z0    2.240  1.950   2.560
## k_Z1    0.482  0.402   0.578
```

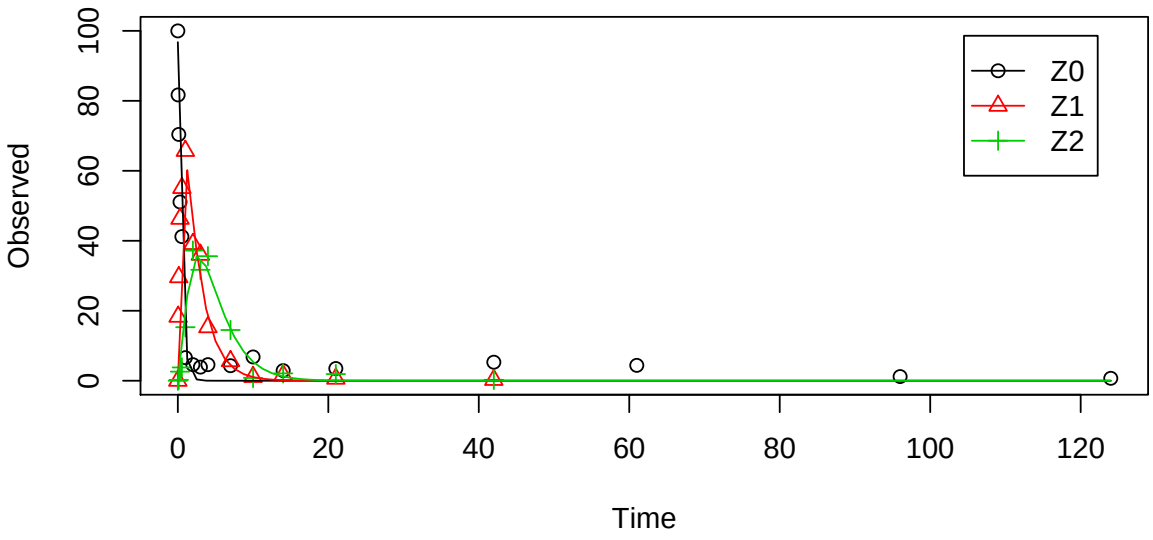
```
##
## Chi2 error levels in percent:
##          err.min n.optim df
## All data    17.6       3 27
## Z0          17.6       2 15
## Z1          15.1       1 12
##
## Estimated disappearance times:
##    DT50 DT90
## Z0 0.31 1.03
## Z1 1.44 4.78
```

As there is only one transformation product for Z0 and no pathway to sink, the formation fraction is internally fixed to unity.

3 Including metabolites Z2 and Z3

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.

```
Z.5 <- mkinmod(Z0 = list(type = "SFO", to = "Z1", sink = FALSE),
               Z1 = list(type = "SFO", to = "Z2", sink = FALSE),
               Z2 = list(type = "SFO"))
m.Z.5 <- mkinfit(Z.5, FOCUS_2006_Z_mkin, quiet = TRUE)
plot(m.Z.5)
```



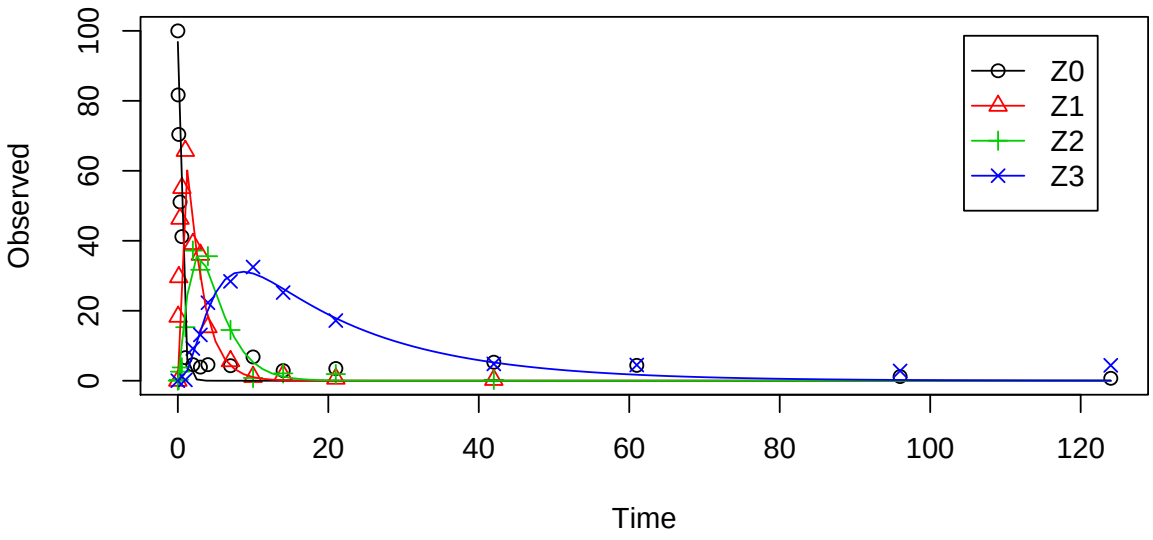

```
summary(m.Z.5, data = FALSE)

## mkin version:    0.9.34
## R version:       3.1.1
## Date of fit:     Wed Oct 15 00:59:15 2014
## Date of summary: Wed Oct 15 00:59:15 2014
##
## Equations:
## d_Z0 = - 0 - k_Z0_Z1 * Z0
## d_Z1 = + k_Z0_Z1 * Z0 - 0 - k_Z1_Z2 * Z1
## d_Z2 = + k_Z1_Z2 * Z1 - k_Z2_sink * Z2
##
## Model predictions using solution type eigen
##
## Fitted with method Port using 201 model solutions performed in 3.356 s
##
## Weighting: none
##
## Starting values for parameters to be optimised:
##               value   type
## Z0_0      100.0000  state
## k_Z0_Z1     0.1000 deparm
## k_Z1_Z2     0.1001 deparm
## k_Z2_sink   0.1002 deparm
```

```
##
## Starting values for the transformed parameters actually optimised:
##                 value lower upper
## Z0_0          100.000  -Inf   Inf
## log_k_Z0_Z1    -2.303  -Inf   Inf
## log_k_Z1_Z2    -2.302  -Inf   Inf
## log_k_Z2_sink  -2.301  -Inf   Inf
##
## Fixed parameter values:
##      value  type
## Z1_0     0 state
## Z2_0     0 state
##
## Optimised, transformed parameters:
##               Estimate Std. Error  Lower   Upper t value Pr(>|t|)
## Z0_0            96.800     2.2700 92.200 101.000   42.70 5.43e-35
## log_k_Z0_Z1      0.795     0.0584  0.677   0.913   13.60 1.36e-16
## log_k_Z1_Z2     -0.741     0.0682 -0.879  -0.603  -10.90 1.68e-13
## log_k_Z2_sink   -0.803     0.1110 -1.030  -0.579   -7.24 8.79e-09
##                 Pr(>t)
## Z0_0          2.72e-35
## log_k_Z0_Z1   6.80e-17
## log_k_Z1_Z2   8.41e-14
## log_k_Z2_sink 4.39e-09
##
## Parameter correlation:
##                 Z0_0 log_k_Z0_Z1 log_k_Z1_Z2 log_k_Z2_sink
## Z0_0          1.0000      0.0578      0.2875        0.3179
## log_k_Z0_Z1   0.0578      1.0000     -0.0436        0.0121
## log_k_Z1_Z2   0.2875     -0.0436      1.0000        0.2402
## log_k_Z2_sink 0.3179      0.0121      0.2402        1.0000
##
## Residual standard error: 4.49 on 40 degrees of freedom
##
## Backtransformed parameters:
##           Estimate  Lower   Upper
## Z0_0        96.800 92.200 101.000
## k_Z0_Z1      2.210  1.970   2.490
## k_Z1_Z2      0.477  0.415   0.547
## k_Z2_sink    0.448  0.358   0.561
##
## Chi2 error levels in percent:
```

```
##          err.min n.optim df
## All data    19.1       4 38
## Z0          17.4       2 15
## Z1          15.3       1 12
## Z2          19.6       1 11
##
## Resulting formation fractions:
##         ff
## Z0_Z1    1
## Z1_Z2    1
## Z2_sink  1
##
## Estimated disappearance times:
##     DT50 DT90
## Z0 0.313 1.04
## Z1 1.454 4.83
## Z2 1.547 5.14
```

Finally, metabolite Z3 is added to the model. The fit is accellerated by using the starting parameters from the previous fit.

```
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"))
m.Z.FOCUS <- mkinfit(Z.FOCUS, FOCUS_2006_Z_mkin,
                     parms.ini = m.Z.5$bparms.ode,
                     quiet = TRUE)
plot(m.Z.FOCUS)
```




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

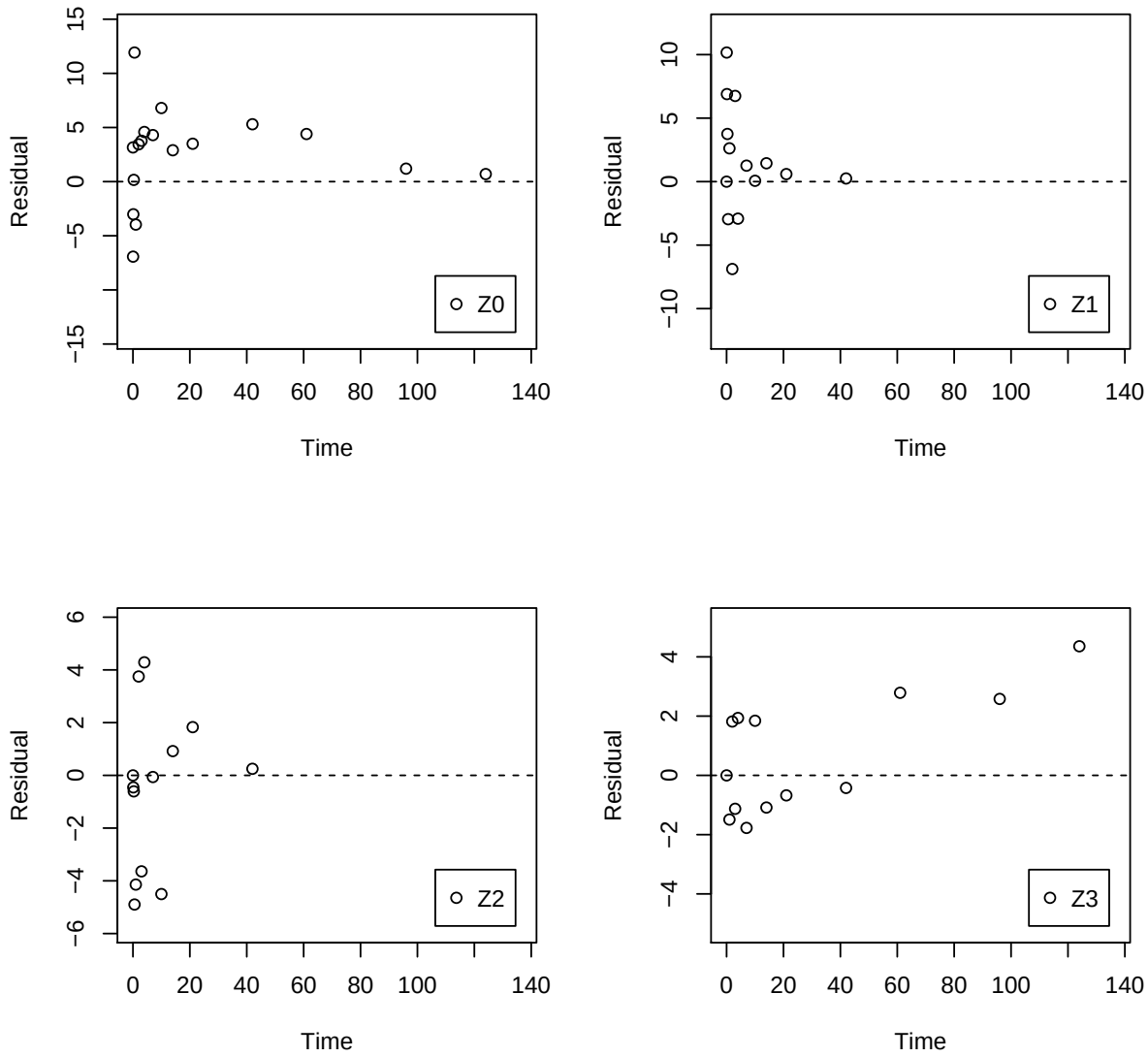
```
## mkin version:    0.9.34
## R version:       3.1.1
## Date of fit:     Wed Oct 15 00:59:23 2014
## Date of summary: Wed Oct 15 00:59:23 2014
##
## Equations:
## d_Z0 = - 0 - k_Z0_Z1 * Z0
## d_Z1 = + k_Z0_Z1 * Z0 - 0 - k_Z1_Z2 * Z1
## d_Z2 = + k_Z1_Z2 * Z1 - k_Z2_sink * Z2 - k_Z2_Z3 * Z2
## d_Z3 = + k_Z2_Z3 * Z2 - k_Z3_sink * Z3
##
## Model predictions using solution type eigen
##
## Fitted with method Port using 353 model solutions performed in 8.237 s
##
## Weighting: none
##
## Starting values for parameters to be optimised:
##               value   type
## Z0_0      100.0000  state
## k_Z0_Z1     2.2140 deparm
## k_Z1_Z2     0.4766 deparm
```

```
## k_Z2_sink   0.4481 deparm
## k_Z2_Z3     0.1000 deparm
## k_Z3_sink   0.1001 deparm
##
## Starting values for the transformed parameters actually optimised:
##                  value lower upper
## Z0_0          100.0000  -Inf   Inf
## log_k_Z0_Z1     0.7948  -Inf   Inf
## log_k_Z1_Z2    -0.7410  -Inf   Inf
## log_k_Z2_sink  -0.8027  -Inf   Inf
## log_k_Z2_Z3    -2.3026  -Inf   Inf
## log_k_Z3_sink  -2.3016  -Inf   Inf
##
## Fixed parameter values:
##      value  type
## Z1_0     0 state
## Z2_0     0 state
## Z3_0     0 state
##
## Optimised, transformed parameters:
##               Estimate Std. Error  Lower   Upper t value Pr(>|t|)
## Z0_0            96.800     2.0600 92.700 101.000   47.00 1.12e-43
## log_k_Z0_Z1      0.795     0.0533  0.688   0.902   14.90 3.08e-20
## log_k_Z1_Z2     -0.738     0.0612 -0.860  -0.615  -12.00 1.57e-16
## log_k_Z2_sink   -1.430     0.1720 -1.780  -1.090   -8.35 4.17e-11
## log_k_Z2_Z3     -1.550     0.1230 -1.790  -1.300  -12.60 2.60e-17
## log_k_Z3_sink   -2.840     0.2440 -3.320  -2.350  -11.60 5.64e-16
##                 Pr(>t)
## Z0_0          5.58e-44
## log_k_Z0_Z1   1.54e-20
## log_k_Z1_Z2   7.84e-17
## log_k_Z2_sink 2.08e-11
## log_k_Z2_Z3   1.30e-17
## log_k_Z3_sink 2.82e-16
##
## Parameter correlation:
##                  Z0_0 log_k_Z0_Z1 log_k_Z1_Z2 log_k_Z2_sink
## Z0_0           1.0000      0.0539      0.2727        0.3701
## log_k_Z0_Z1    0.0539      1.0000     -0.0521        0.0244
## log_k_Z1_Z2    0.2727     -0.0521      1.0000        0.2939
## log_k_Z2_sink  0.3701      0.0244      0.2939        1.0000
## log_k_Z2_Z3   -0.0730     -0.0358     -0.1213       -0.1890
```

```
## log_k_Z3_sink -0.1135     -0.0252     -0.1915       -0.6430
##               log_k_Z2_Z3 log_k_Z3_sink
## Z0_0              -0.0730       -0.1135
## log_k_Z0_Z1       -0.0358       -0.0252
## log_k_Z1_Z2       -0.1213       -0.1915
## log_k_Z2_sink     -0.1890       -0.6430
## log_k_Z2_Z3        1.0000        0.5516
## log_k_Z3_sink      0.5516        1.0000
##
## Residual standard error: 4.1 on 51 degrees of freedom
##
## Backtransformed parameters:
##           Estimate  Lower    Upper
## Z0_0       96.8000 92.700 101.0000
## k_Z0_Z1     2.2200  1.990   2.4700
## k_Z1_Z2     0.4780  0.423   0.5410
## k_Z2_sink   0.2390  0.169   0.3370
## k_Z2_Z3     0.2130  0.166   0.2720
## k_Z3_sink   0.0587  0.036   0.0957
##
## Chi2 error levels in percent:
##          err.min n.optim df
## All data    19.2       6 48
## Z0          17.4       2 15
## Z1          15.2       1 12
## Z2          20.3       2 10
## Z3          11.9       1 11
##
## Resulting formation fractions:
##            ff
## Z0_Z1   1.000
## Z1_Z2   1.000
## Z2_sink 0.528
## Z2_Z3   0.472
## Z3_sink 1.000
##
## Estimated disappearance times:
##      DT50  DT90
## Z0  0.313  1.04
## Z1  1.449  4.81
## Z2  1.535  5.10
## Z3 11.810 39.23
```

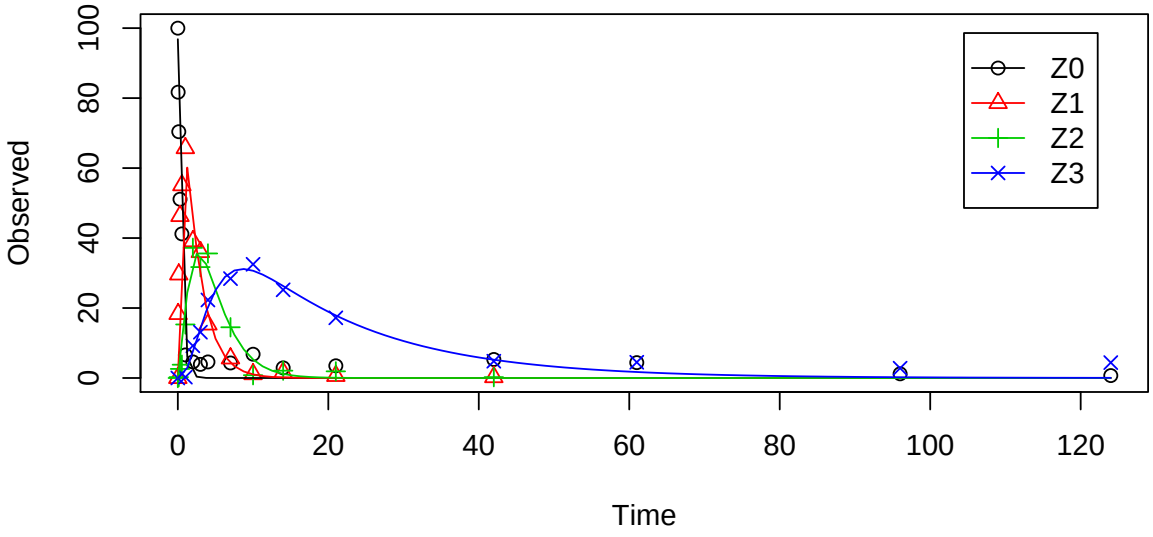
This is the fit corresponding to the final result chosen in Appendix 7 of the FOCUS report. The residual plots can be obtained by

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



We can also investigate the confidence interval for the formation fraction from Z2 to Z3 by specifying the model using formation fractions.

```
Z.FOCUS.ff <- 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"),
                     use_of_ff = "max")
m.Z.FOCUS.ff <- mkinfit(Z.FOCUS.ff, FOCUS_2006_Z_mkin, quiet = TRUE)
plot(m.Z.FOCUS.ff)
```




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

```
## mkin version:    0.9.34
## R version:       3.1.1
## Date of fit:     Wed Oct 15 00:59:31 2014
## Date of summary: Wed Oct 15 00:59:31 2014
##
## Equations:
## d_Z0 = - k_Z0 * Z0
## d_Z1 = + k_Z0 * Z0 - k_Z1 * Z1
## d_Z2 = + k_Z1 * Z1 - k_Z2 * Z2
## d_Z3 = + f_Z2_to_Z3 * k_Z2 * Z2 - k_Z3 * Z3
##
## Model predictions using solution type eigen
##
## Fitted with method Port using 355 model solutions performed in 7.778 s
```

```
##
## Weighting: none
##
## Starting values for parameters to be optimised:
##               value   type
## Z0_0       100.0000  state
## k_Z0         0.1000 deparm
## k_Z1         0.1001 deparm
## k_Z2         0.1002 deparm
## k_Z3         0.1003 deparm
## f_Z2_to_Z3   0.5000 deparm
##
## Starting values for the transformed parameters actually optimised:
##               value lower upper
## Z0_0       100.000  -Inf   Inf
## log_k_Z0    -2.303  -Inf   Inf
## log_k_Z1    -2.302  -Inf   Inf
## log_k_Z2    -2.301  -Inf   Inf
## log_k_Z3    -2.300  -Inf   Inf
## f_Z2_ilr_1   0.000  -Inf   Inf
##
## Fixed parameter values:
##      value  type
## Z1_0     0 state
## Z2_0     0 state
## Z3_0     0 state
##
## Optimised, transformed parameters:
##            Estimate Std. Error  Lower   Upper t value Pr(>|t|)
## Z0_0        96.8000     2.0600 92.700 101.000  47.000 1.12e-43
## log_k_Z0     0.7950     0.0533  0.688   0.902  14.900 3.08e-20
## log_k_Z1    -0.7380     0.0612 -0.860  -0.615 -12.000 1.57e-16
## log_k_Z2    -0.7950     0.0979 -0.991  -0.598  -8.120 9.44e-11
## log_k_Z3    -2.8400     0.2440 -3.320  -2.350 -11.600 5.64e-16
## f_Z2_ilr_1  -0.0807     0.1620 -0.406   0.244  -0.498 6.20e-01
##              Pr(>t)
## Z0_0       5.58e-44
## log_k_Z0   1.54e-20
## log_k_Z1   7.84e-17
## log_k_Z2   4.72e-11
## log_k_Z3   2.82e-16
## f_Z2_ilr_1 3.10e-01
```

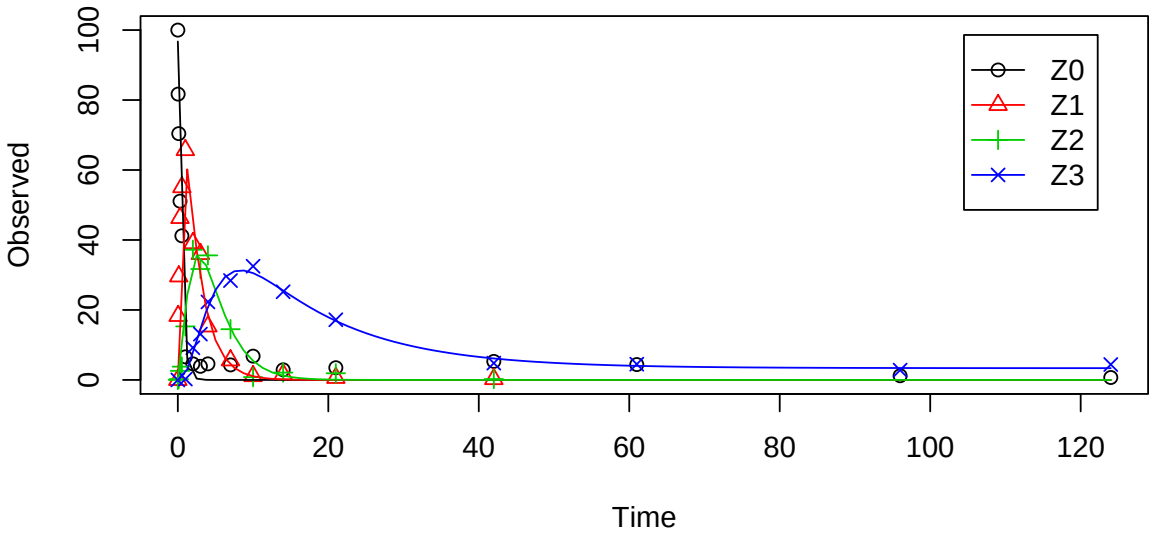
```
##
## Parameter correlation:
##               Z0_0 log_k_Z0 log_k_Z1 log_k_Z2 log_k_Z3 f_Z2_ilr_1
## Z0_0        1.0000  0.05387   0.2727  0.29977  -0.1135    -0.3164
## log_k_Z0    0.0539  1.00000  -0.0521  0.00149  -0.0252    -0.0375
## log_k_Z1    0.2727 -0.05210   1.0000  0.20061  -0.1915    -0.2852
## log_k_Z2    0.2998  0.00149   0.2006  1.00000  -0.2701    -0.3886
## log_k_Z3   -0.1135 -0.02522  -0.1915 -0.27012   1.0000     0.7772
## f_Z2_ilr_1 -0.3164 -0.03748  -0.2852 -0.38859   0.7772     1.0000
##
## Residual standard error: 4.1 on 51 degrees of freedom
##
## Backtransformed parameters:
##            Estimate  Lower    Upper
## Z0_0        96.8000 92.700 101.0000
## k_Z0         2.2200  1.990   2.4700
## k_Z1         0.4780  0.423   0.5410
## k_Z2         0.4520  0.371   0.5500
## k_Z3         0.0587  0.036   0.0957
## f_Z2_to_Z3   0.4720  0.360   0.5860
##
## Chi2 error levels in percent:
##          err.min n.optim df
## All data    19.2       6 48
## Z0          17.4       2 15
## Z1          15.2       1 12
## Z2          19.6       1 11
## Z3          12.3       2 10
##
## Resulting formation fractions:
##            ff
## Z2_Z3   0.472
## Z2_sink 0.528
##
## Estimated disappearance times:
##      DT50  DT90
## Z0  0.313  1.04
## Z1  1.449  4.81
## Z2  1.535  5.10
## Z3 11.810 39.23
```

4 Using the SFORB model for parent and metabolites

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.

```
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"))
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)
plot(m.Z.mkin.1)
```




```
summary(m.Z.mkin.1, data = FALSE)

## mkin version:    0.9.34
## R version:       3.1.1
## Date of fit:     Wed Oct 15 00:59:51 2014
```

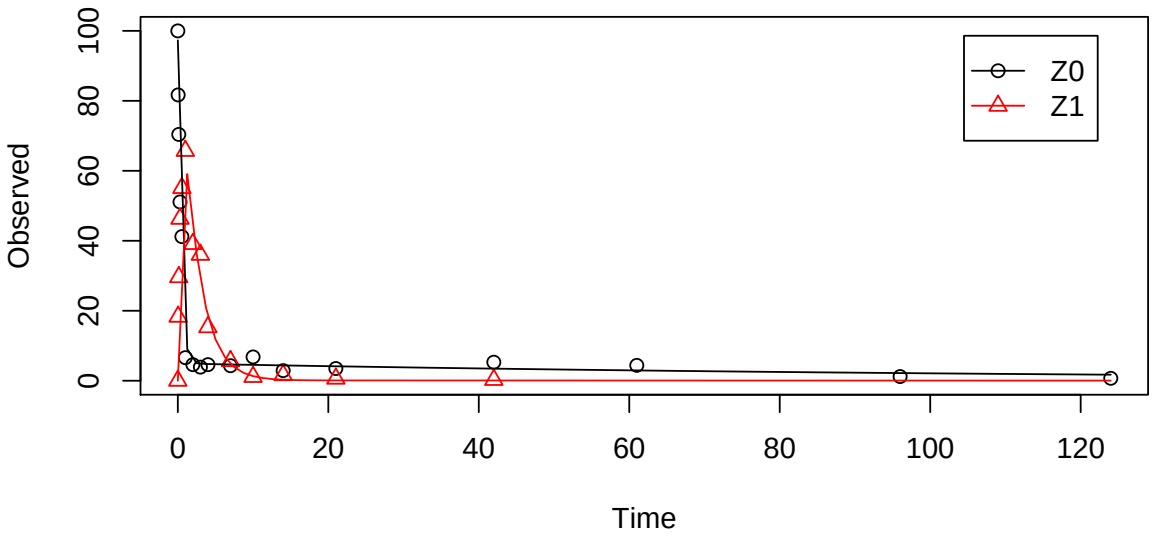
```
## Date of summary: Wed Oct 15 00:59:51 2014
##
## Equations:
## d_Z0 = - 0 - k_Z0_Z1 * Z0
## d_Z1 = + k_Z0_Z1 * Z0 - 0 - k_Z1_Z2 * Z1
## d_Z2 = + k_Z1_Z2 * Z1 - k_Z2_sink * Z2 - k_Z2_Z3_free * Z2
## d_Z3_free = + k_Z2_Z3_free * Z2 - k_Z3_free_sink * Z3_free -
##             k_Z3_free_bound * Z3_free + k_Z3_bound_free *
##             Z3_bound
## d_Z3_bound = + k_Z3_free_bound * Z3_free - k_Z3_bound_free *
##             Z3_bound
##
## Model predictions using solution type eigen
##
## Fitted with method Port using 785 model solutions performed in 19.51 s
##
## Weighting: none
##
## Starting values for parameters to be optimised:
##                     value   type
## Z0_0            100.0000  state
## k_Z0_Z1           0.5000 deparm
## k_Z1_Z2           0.3000 deparm
## k_Z2_sink         0.1000 deparm
## k_Z2_Z3_free      0.1001 deparm
## k_Z3_free_sink    0.1002 deparm
## k_Z3_free_bound   0.1000 deparm
## k_Z3_bound_free   0.0200 deparm
##
## Starting values for the transformed parameters actually optimised:
##                         value lower upper
## Z0_0                  100.0000  -Inf   Inf
## log_k_Z0_Z1            -0.6931  -Inf   Inf
## log_k_Z1_Z2            -1.2040  -Inf   Inf
## log_k_Z2_sink          -2.3026  -Inf   Inf
## log_k_Z2_Z3_free       -2.3016  -Inf   Inf
## log_k_Z3_free_sink     -2.3006  -Inf   Inf
## log_k_Z3_free_bound    -2.3026  -Inf   Inf
## log_k_Z3_bound_free    -3.9120  -Inf   Inf
##
## Fixed parameter values:
##             value  type
```

```
## Z1_0           0 state
## Z2_0           0 state
## Z3_free_0      0 state
## Z3_bound_0     0 state
##
## Optimised, transformed parameters:
##                      Estimate Std. Error Lower Upper t value Pr(>|t|)
## Z0_0                   96.700         NA    NA    NA      NA       NA
## log_k_Z0_Z1             0.795         NA    NA    NA      NA       NA
## log_k_Z1_Z2            -0.743         NA    NA    NA      NA       NA
## log_k_Z2_sink          -1.490         NA    NA    NA      NA       NA
## log_k_Z2_Z3_free       -1.500         NA    NA    NA      NA       NA
## log_k_Z3_free_sink     -2.650         NA    NA    NA      NA       NA
## log_k_Z3_free_bound    -5.240         NA    NA    NA      NA       NA
## log_k_Z3_bound_free   -22.300         NA    NA    NA      NA       NA
##                      Pr(>t)
## Z0_0                     NA
## log_k_Z0_Z1              NA
## log_k_Z1_Z2              NA
## log_k_Z2_sink            NA
## log_k_Z2_Z3_free         NA
## log_k_Z3_free_sink       NA
## log_k_Z3_free_bound      NA
## log_k_Z3_bound_free      NA
##
## Parameter correlation:
## Could not estimate covariance matrix; singular system:
##
## Residual standard error: 4.11 on 49 degrees of freedom
##
## Backtransformed parameters:
##                  Estimate Lower Upper
## Z0_0             9.67e+01    NA    NA
## k_Z0_Z1          2.21e+00    NA    NA
## k_Z1_Z2          4.76e-01    NA    NA
## k_Z2_sink        2.24e-01    NA    NA
## k_Z2_Z3_free     2.22e-01    NA    NA
## k_Z3_free_sink   7.03e-02    NA    NA
## k_Z3_free_bound  5.28e-03    NA    NA
## k_Z3_bound_free  2.10e-10    NA    NA
##
## Chi2 error levels in percent:
```

```
##          err.min n.optim df
## All data   19.41       8 47
## Z0         17.43       2 15
## Z1         15.27       1 12
## Z2         20.28       2 10
## Z3          8.56       3 10
##
## Estimated Eigenvalues of SFORB model(s):
##    Z3_b1    Z3_b2
## 7.56e-02 1.96e-10
##
## Resulting formation fractions:
##                 ff
## Z0_Z1        1.000
## Z1_Z2        1.000
## Z2_sink      0.502
## Z2_Z3_free   0.498
## Z3_free_sink 1.000
##
## Estimated disappearance times:
##      DT50  DT90 DT50_Z3_b1 DT50_Z3_b2
## Z0  0.313  1.04         NA         NA
## Z1  1.457  4.84         NA         NA
## Z2  1.552  5.16         NA         NA
## Z3 10.198 45.33       9.17   3.54e+09
```

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.

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




```
summary(m.Z.mkin.2, data = FALSE)
```

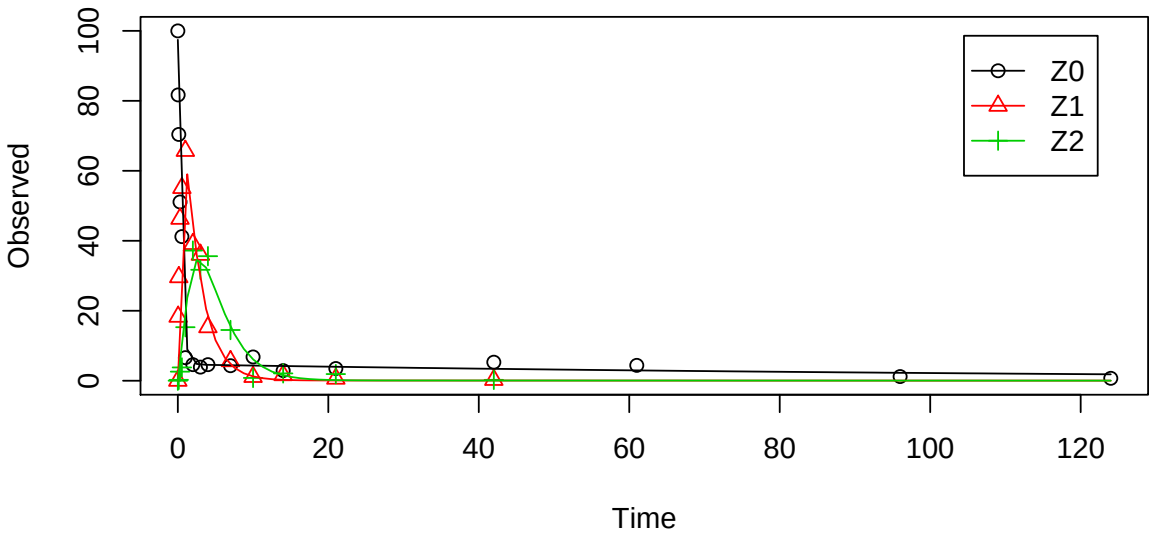
```
## mkin version:    0.9.34
## R version:       3.1.1
## Date of fit:     Wed Oct 15 00:59:53 2014
## Date of summary: Wed Oct 15 00:59:53 2014
##
## Equations:
## d_Z0_free = - 0 - k_Z0_free_bound * Z0_free + k_Z0_bound_free
##             * Z0_bound - k_Z0_free_Z1 * Z0_free
## d_Z0_bound = + k_Z0_free_bound * Z0_free - k_Z0_bound_free *
##             Z0_bound
## d_Z1 = + k_Z0_free_Z1 * Z0_free - k_Z1_sink * Z1
##
## Model predictions using solution type eigen
##
## Fitted with method Port using 149 model solutions performed in 1.954 s
##
## Weighting: none
##
## Starting values for parameters to be optimised:
##                     value   type
## Z0_free_0       100.0000  state
## k_Z0_free_bound   0.1000 deparm
```

```
## k_Z0_bound_free   0.0200 deparm
## k_Z0_free_Z1      0.1002 deparm
## k_Z1_sink         0.1003 deparm
##
## Starting values for the transformed parameters actually optimised:
##                       value lower upper
## Z0_free_0           100.000  -Inf   Inf
## log_k_Z0_free_bound  -2.303  -Inf   Inf
## log_k_Z0_bound_free  -3.912  -Inf   Inf
## log_k_Z0_free_Z1     -2.301  -Inf   Inf
## log_k_Z1_sink        -2.300  -Inf   Inf
##
## Fixed parameter values:
##            value  type
## Z0_bound_0     0 state
## Z1_0           0 state
##
## Optimised, transformed parameters:
##                      Estimate Std. Error  Lower   Upper t value
## Z0_free_0             97.300     2.4000 92.400 102.000   40.60
## log_k_Z0_free_bound   -2.080     0.4320 -2.970  -1.190   -4.82
## log_k_Z0_bound_free   -4.720     1.6000 -8.020  -1.420   -2.94
## log_k_Z0_free_Z1       0.855     0.0643  0.723   0.987   13.30
## log_k_Z1_sink         -0.793     0.0851 -0.968  -0.619   -9.33
##                      Pr(>|t|)   Pr(>t)
## Z0_free_0           4.73e-25 2.36e-25
## log_k_Z0_free_bound 5.44e-05 2.72e-05
## log_k_Z0_bound_free 6.79e-03 3.39e-03
## log_k_Z0_free_Z1    4.18e-13 2.09e-13
## log_k_Z1_sink       8.86e-10 4.43e-10
##
## Parameter correlation:
##                      Z0_free_0 log_k_Z0_free_bound log_k_Z0_bound_free
## Z0_free_0             1.00000             0.00649              0.0332
## log_k_Z0_free_bound   0.00649             1.00000              0.5465
## log_k_Z0_bound_free   0.03324             0.54647              1.0000
## log_k_Z0_free_Z1      0.11182             0.41392              0.1584
## log_k_Z1_sink         0.39155            -0.29191             -0.1260
##                      log_k_Z0_free_Z1 log_k_Z1_sink
## Z0_free_0                     0.1118        0.3916
## log_k_Z0_free_bound           0.4139       -0.2919
## log_k_Z0_bound_free           0.1584       -0.1260
```

```
## log_k_Z0_free_Z1               1.0000       -0.0419
## log_k_Z1_sink                 -0.0419        1.0000
##
## Residual standard error: 4.44 on 26 degrees of freedom
##
## Backtransformed parameters:
##                 Estimate    Lower   Upper
## Z0_free_0       97.30000 9.24e+01 102.000
## k_Z0_free_bound  0.12500 5.13e-02   0.303
## k_Z0_bound_free  0.00891 3.29e-04   0.241
## k_Z0_free_Z1     2.35000 2.06e+00   2.680
## k_Z1_sink        0.45200 3.80e-01   0.539
##
## Chi2 error levels in percent:
##          err.min n.optim df
## All data    15.6       5 25
## Z0          14.7       4 13
## Z1          14.3       1 12
##
## Estimated Eigenvalues of SFORB model(s):
##   Z0_b1   Z0_b2
## 2.47631 0.00846
##
## Resulting formation fractions:
##            ff
## Z0_free_Z1  1
## Z1_sink     1
##
## Estimated disappearance times:
##     DT50 DT90 DT50_Z0_b1 DT50_Z0_b2
## Z0 0.302 1.19       0.28       81.9
## Z1 1.532 5.09         NA         NA
```

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

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




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

```
## mkin version:    0.9.34
## R version:       3.1.1
## Date of fit:     Wed Oct 15 01:00:00 2014
## Date of summary: Wed Oct 15 01:00:00 2014
##
## Equations:
## d_Z0_free = - 0 - k_Z0_free_bound * Z0_free + k_Z0_bound_free
##             * Z0_bound - k_Z0_free_Z1 * Z0_free
## d_Z0_bound = + k_Z0_free_bound * Z0_free - k_Z0_bound_free *
##             Z0_bound
## d_Z1 = + k_Z0_free_Z1 * Z0_free - 0 - k_Z1_Z2 * Z1
## d_Z2 = + k_Z1_Z2 * Z1 - k_Z2_sink * Z2
##
## Model predictions using solution type eigen
##
## Fitted with method Port using 337 model solutions performed in 6.677 s
##
## Weighting: none
##
## Starting values for parameters to be optimised:
##                    value   type
## Z0_free_0       100.0000  state
```

```
## k_Z0_free_bound   0.1000 deparm
## k_Z0_bound_free   0.0200 deparm
## k_Z0_free_Z1      0.1002 deparm
## k_Z1_Z2           0.1003 deparm
## k_Z2_sink         0.1004 deparm
##
## Starting values for the transformed parameters actually optimised:
##                       value lower upper
## Z0_free_0           100.000  -Inf   Inf
## log_k_Z0_free_bound  -2.303  -Inf   Inf
## log_k_Z0_bound_free  -3.912  -Inf   Inf
## log_k_Z0_free_Z1     -2.301  -Inf   Inf
## log_k_Z1_Z2          -2.300  -Inf   Inf
## log_k_Z2_sink        -2.299  -Inf   Inf
##
## Fixed parameter values:
##            value  type
## Z0_bound_0     0 state
## Z1_0           0 state
## Z2_0           0 state
##
## Optimised, transformed parameters:
##                      Estimate Std. Error  Lower   Upper t value
## Z0_free_0              97.400     2.0700 93.200 102.000   47.10
## log_k_Z0_free_bound   -2.150     0.4040 -2.970  -1.330   -5.32
## log_k_Z0_bound_free   -4.840     1.6100 -8.100  -1.580   -3.00
## log_k_Z0_free_Z1       0.846     0.0583  0.728   0.964   14.50
## log_k_Z1_Z2           -0.781     0.0649 -0.912  -0.650  -12.00
## log_k_Z2_sink         -0.861     0.1060 -1.070  -0.647   -8.14
##                      Pr(>|t|)   Pr(>t)
## Z0_free_0            2.70e-35 1.35e-35
## log_k_Z0_free_bound  4.88e-06 2.44e-06
## log_k_Z0_bound_free  4.71e-03 2.35e-03
## log_k_Z0_free_Z1     4.50e-17 2.25e-17
## log_k_Z1_Z2          1.52e-14 7.61e-15
## log_k_Z2_sink        7.46e-10 3.73e-10
##
## Parameter correlation:
##                      Z0_free_0 log_k_Z0_free_bound log_k_Z0_bound_free
## Z0_free_0               1.0000               0.075              0.0708
## log_k_Z0_free_bound     0.0750               1.000              0.5425
## log_k_Z0_bound_free     0.0708               0.543              1.0000
```

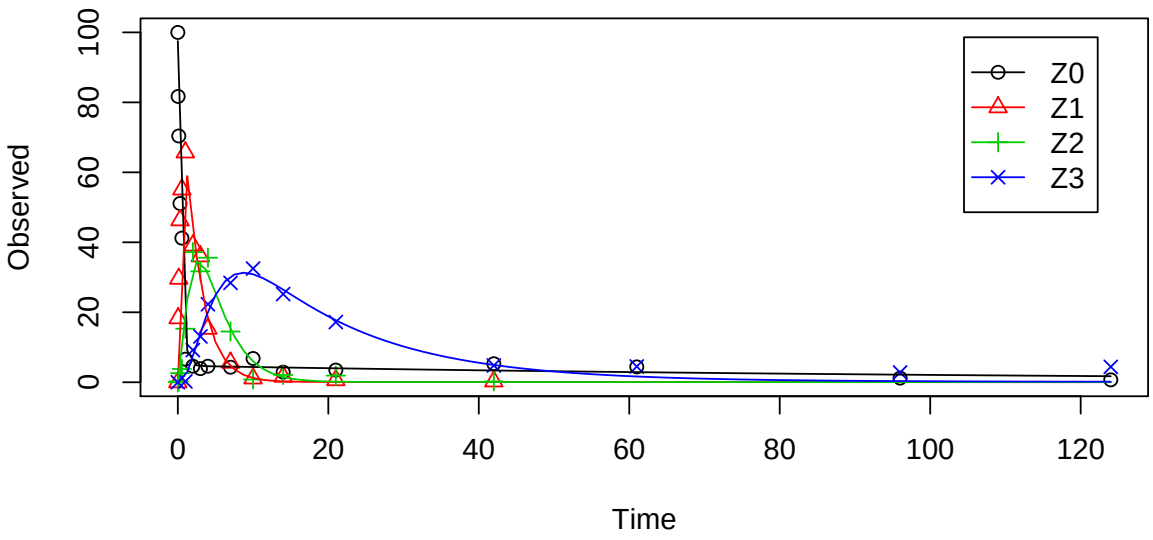
```
## log_k_Z0_free_Z1       0.0908               0.425              0.1632
## log_k_Z1_Z2            0.2572              -0.228             -0.0863
## log_k_Z2_sink          0.2888              -0.211             -0.0792
##                     log_k_Z0_free_Z1 log_k_Z1_Z2 log_k_Z2_sink
## Z0_free_0                    0.0908      0.2572        0.2888
## log_k_Z0_free_bound          0.4245     -0.2276       -0.2105
## log_k_Z0_bound_free          0.1632     -0.0863       -0.0792
## log_k_Z0_free_Z1             1.0000     -0.1008       -0.0490
## log_k_Z1_Z2                 -0.1008      1.0000        0.2728
## log_k_Z2_sink               -0.0490      0.2728        1.0000
##
## Residual standard error: 4.08 on 38 degrees of freedom
##
## Backtransformed parameters:
##                 Estimate    Lower   Upper
## Z0_free_0       97.40000 9.32e+01 102.000
## k_Z0_free_bound  0.11700 5.15e-02   0.264
## k_Z0_bound_free  0.00792 3.04e-04   0.207
## k_Z0_free_Z1     2.33000 2.07e+00   2.620
## k_Z1_Z2          0.45800 4.02e-01   0.522
## k_Z2_sink        0.42300 3.41e-01   0.524
##
## Chi2 error levels in percent:
##          err.min n.optim df
## All data    17.3       6 36
## Z0          14.7       4 13
## Z1          14.4       1 12
## Z2          20.3       1 11
##
## Estimated Eigenvalues of SFORB model(s):
##   Z0_b1   Z0_b2
## 2.44664 0.00754
##
## Resulting formation fractions:
##            ff
## Z0_free_Z1  1
## Z1_Z2       1
## Z2_sink     1
##
## Estimated disappearance times:
##     DT50 DT90 DT50_Z0_b1 DT50_Z0_b2
## Z0 0.304 1.18      0.283       91.9
```

```
## Z1 1.514 5.03          NA          NA
## Z2 1.639 5.44          NA          NA
```

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

Finally, Z3 is added as well. These models appear overparameterised (no covariance matrix returned) if the sink for Z1 is left in the models.

```
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"))
m.Z.mkin.4 <- mkinfit(Z.mkin.4, FOCUS_2006_Z_mkin,
                      parms.ini = c(k_Z1_Z2 = 0.05),
                      quiet = TRUE)
plot(m.Z.mkin.4)
```




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

```
## mkin version:    0.9.34
## R version:       3.1.1
## Date of fit:     Wed Oct 15 01:00:13 2014
## Date of summary: Wed Oct 15 01:00:13 2014
##
## Equations:
```

```
## d_Z0_free = - 0 - k_Z0_free_bound * Z0_free + k_Z0_bound_free
##             * Z0_bound - k_Z0_free_Z1 * Z0_free
## d_Z0_bound = + k_Z0_free_bound * Z0_free - k_Z0_bound_free *
##             Z0_bound
## d_Z1 = + k_Z0_free_Z1 * Z0_free - 0 - k_Z1_Z2 * Z1
## d_Z2 = + k_Z1_Z2 * Z1 - k_Z2_sink * Z2 - k_Z2_Z3 * Z2
## d_Z3 = + k_Z2_Z3 * Z2 - k_Z3_sink * Z3
##
## Model predictions using solution type eigen
##
## Fitted with method Port using 541 model solutions performed in 12.63 s
##
## Weighting: none
##
## Starting values for parameters to be optimised:
##                     value   type
## Z0_free_0       100.0000  state
## k_Z1_Z2           0.0500 deparm
## k_Z0_free_bound   0.1000 deparm
## k_Z0_bound_free   0.0200 deparm
## k_Z0_free_Z1      0.1002 deparm
## k_Z2_sink         0.1003 deparm
## k_Z2_Z3           0.1004 deparm
## k_Z3_sink         0.1005 deparm
##
## Starting values for the transformed parameters actually optimised:
##                       value lower upper
## Z0_free_0           100.000  -Inf   Inf
## log_k_Z1_Z2          -2.996  -Inf   Inf
## log_k_Z0_free_bound  -2.303  -Inf   Inf
## log_k_Z0_bound_free  -3.912  -Inf   Inf
## log_k_Z0_free_Z1     -2.301  -Inf   Inf
## log_k_Z2_sink        -2.300  -Inf   Inf
## log_k_Z2_Z3          -2.299  -Inf   Inf
## log_k_Z3_sink        -2.298  -Inf   Inf
##
## Fixed parameter values:
##            value  type
## Z0_bound_0     0 state
## Z1_0           0 state
## Z2_0           0 state
## Z3_0           0 state
```

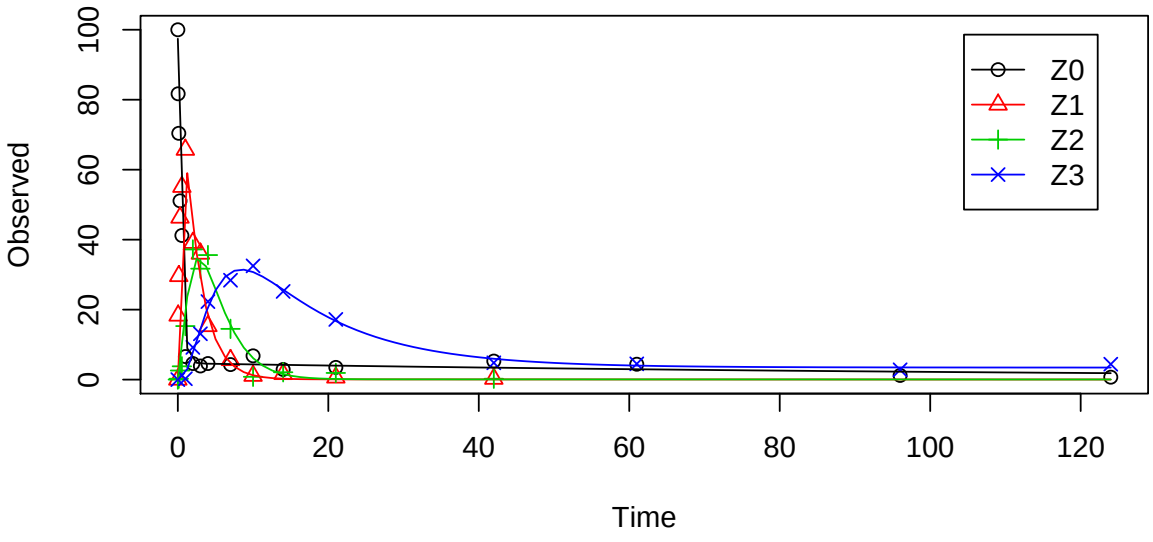
```
##
## Optimised, transformed parameters:
##                     Estimate Std. Error  Lower   Upper t value
## Z0_free_0             97.500     1.8900 93.700 101.000   51.70
## log_k_Z1_Z2           -0.777     0.0583 -0.894  -0.660  -13.30
## log_k_Z0_free_bound   -2.140     0.3680 -2.880  -1.400   -5.80
## log_k_Z0_bound_free   -4.770     1.4200 -7.610  -1.920   -3.36
## log_k_Z0_free_Z1       0.847     0.0534  0.740   0.954   15.90
## log_k_Z2_sink         -1.560     0.1830 -1.930  -1.190   -8.55
## log_k_Z2_Z3           -1.530     0.1140 -1.760  -1.300  -13.50
## log_k_Z3_sink         -2.770     0.2250 -3.220  -2.320  -12.30
##                     Pr(>|t|)   Pr(>t)
## Z0_free_0           2.07e-44 1.03e-44
## log_k_Z1_Z2         6.66e-18 3.33e-18
## log_k_Z0_free_bound 4.71e-07 2.35e-07
## log_k_Z0_bound_free 1.51e-03 7.55e-04
## log_k_Z0_free_Z1    6.13e-21 3.06e-21
## log_k_Z2_sink       2.79e-11 1.40e-11
## log_k_Z2_Z3         4.49e-18 2.24e-18
## log_k_Z3_sink       1.25e-16 6.23e-17
##
## Parameter correlation:
##                     Z0_free_0 log_k_Z1_Z2 log_k_Z0_free_bound
## Z0_free_0              1.0000      0.2424              0.0782
## log_k_Z1_Z2            0.2424      1.0000             -0.2274
## log_k_Z0_free_bound    0.0782     -0.2274              1.0000
## log_k_Z0_bound_free    0.0692     -0.0894              0.5398
## log_k_Z0_free_Z1       0.0888     -0.1084              0.4277
## log_k_Z2_sink          0.3299      0.3405             -0.2633
## log_k_Z2_Z3           -0.0749     -0.1489              0.0670
## log_k_Z3_sink         -0.1046     -0.2249              0.1384
##                     log_k_Z0_bound_free log_k_Z0_free_Z1
## Z0_free_0                        0.0692           0.0888
## log_k_Z1_Z2                     -0.0894          -0.1084
## log_k_Z0_free_bound              0.5398           0.4277
## log_k_Z0_bound_free              1.0000           0.1628
## log_k_Z0_free_Z1                 0.1628           1.0000
## log_k_Z2_sink                   -0.1274          -0.0531
## log_k_Z2_Z3                      0.0608          -0.0128
## log_k_Z3_sink                    0.1253           0.0186
##                     log_k_Z2_sink log_k_Z2_Z3 log_k_Z3_sink
## Z0_free_0                  0.3299     -0.0749       -0.1046
```

```
## log_k_Z1_Z2                0.3405     -0.1489       -0.2249
## log_k_Z0_free_bound       -0.2633      0.0670        0.1384
## log_k_Z0_bound_free       -0.1274      0.0608        0.1253
## log_k_Z0_free_Z1          -0.0531     -0.0128        0.0186
## log_k_Z2_sink              1.0000     -0.2547       -0.6832
## log_k_Z2_Z3               -0.2547      1.0000        0.5639
## log_k_Z3_sink             -0.6832      0.5639        1.0000
##
## Residual standard error: 3.74 on 49 degrees of freedom
##
## Backtransformed parameters:
##                 Estimate    Lower    Upper
## Z0_free_0       97.50000 9.37e+01 101.0000
## k_Z1_Z2          0.46000 4.09e-01   0.5170
## k_Z0_free_bound  0.11800 5.64e-02   0.2470
## k_Z0_bound_free  0.00852 4.94e-04   0.1470
## k_Z0_free_Z1     2.33000 2.10e+00   2.6000
## k_Z2_sink        0.21000 1.45e-01   0.3030
## k_Z2_Z3          0.21700 1.73e-01   0.2730
## k_Z3_sink        0.06270 3.99e-02   0.0985
##
## Chi2 error levels in percent:
##          err.min n.optim df
## All data    17.5       8 46
## Z0          14.7       4 13
## Z1          14.4       1 12
## Z2          21.0       2 10
## Z3          11.8       1 11
##
## Estimated Eigenvalues of SFORB model(s):
##   Z0_b1   Z0_b2
## 2.45126 0.00811
##
## Resulting formation fractions:
##               ff
## Z0_free_Z1 1.000
## Z1_Z2      1.000
## Z2_sink    0.492
## Z2_Z3      0.508
## Z3_sink    1.000
##
## Estimated disappearance times:
```

```
##       DT50  DT90 DT50_Z0_b1 DT50_Z0_b2
## Z0   0.304  1.19      0.283       85.5
## Z1   1.507  5.01         NA         NA
## Z2   1.623  5.39         NA         NA
## Z3  11.051 36.71         NA         NA
```

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.

```
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"))
m.Z.mkin.5 <- mkinfit(Z.mkin.5, FOCUS_2006_Z_mkin,
                      parms.ini = m.Z.mkin.4$bparms.ode[1:5],
                      quiet = TRUE)
plot(m.Z.mkin.5)
```




```
summary(m.Z.mkin.5, data = FALSE)$bpar
```

```
##                   Estimate Lower Upper
## Z0_free_0        9.742e+01    NA    NA
## k_Z0_free_bound  1.168e-01    NA    NA
## k_Z0_bound_free  7.890e-03    NA    NA
```

```
## k_Z0_free_Z1    2.330e+00    NA    NA
## k_Z1_Z2         4.576e-01    NA    NA
## k_Z2_sink       1.957e-01    NA    NA
## k_Z2_Z3_free    2.266e-01    NA    NA
## k_Z3_free_sink  7.479e-02    NA    NA
## k_Z3_free_bound 5.218e-03    NA    NA
## k_Z3_bound_free 1.841e-10    NA    NA
```

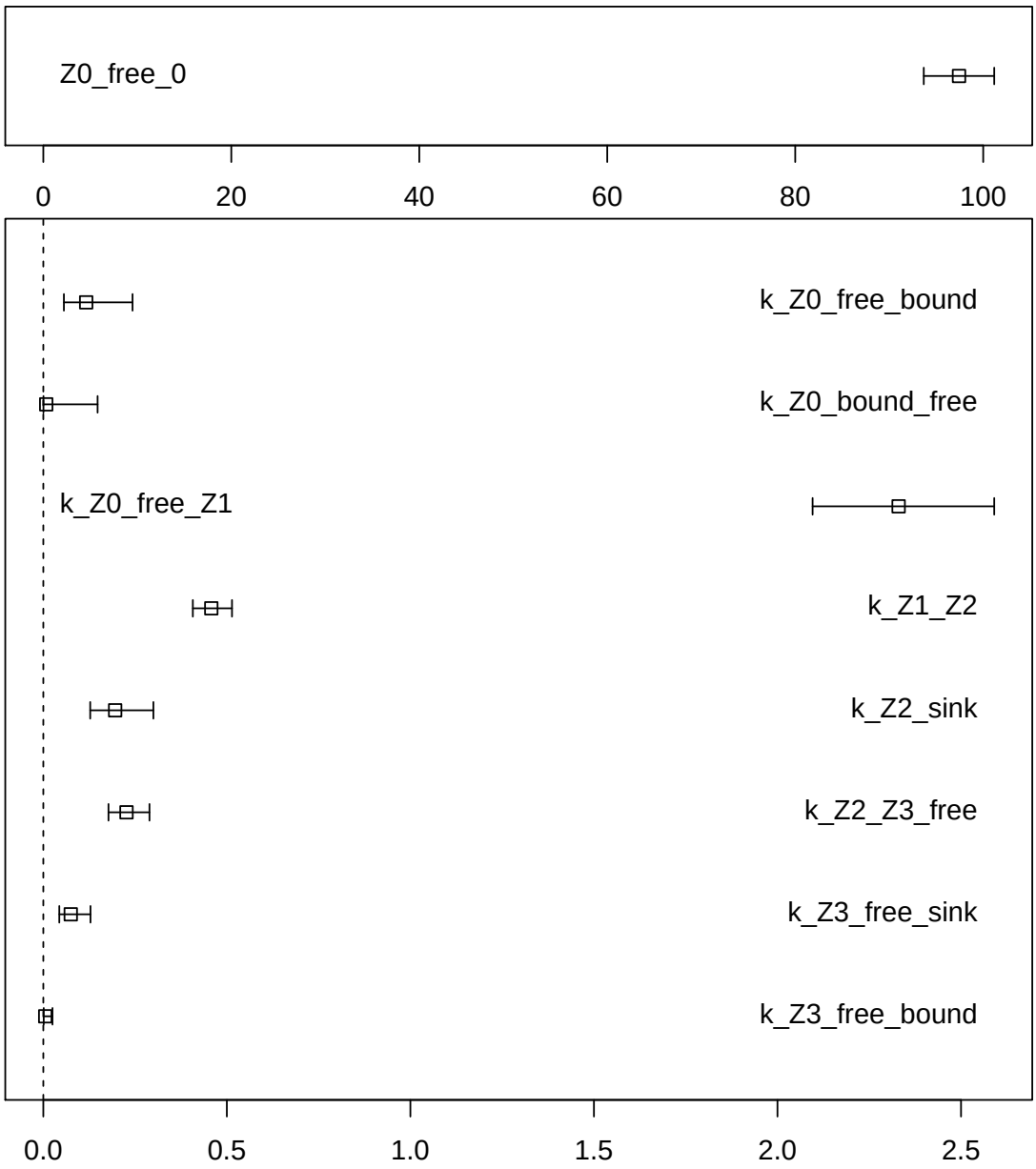
The summary view of the backtransformed parameters shows that we get no confidence intervals due to overparameterisation. As the optimized `k_Z3_bound_free` is excessively small, it is reasonable to fix it to zero.

```
m.Z.mkin.5a <- mkinfit(Z.mkin.5, FOCUS_2006_Z_mkin,
                       parms.ini = c(m.Z.mkin.4$bparms.ode[1:5],
                                     k_Z3_bound_free = 0),
                       fixed_parms = "k_Z3_bound_free",
                       quiet = TRUE)
summary(m.Z.mkin.5a, data = FALSE)$bpar
```

```
##                  Estimate     Lower     Upper
## Z0_free_0       97.424951 9.368e+01 101.17375
## k_Z0_free_bound  0.116758 5.608e-02   0.24307
## k_Z0_bound_free  0.007890 4.209e-04   0.14791
## k_Z0_free_Z1     2.330001 2.096e+00   2.59050
## k_Z1_Z2          0.457590 4.074e-01   0.51402
## k_Z2_sink        0.195709 1.277e-01   0.29984
## k_Z2_Z3_free     0.226585 1.775e-01   0.28920
## k_Z3_free_sink   0.074789 4.354e-02   0.12848
## k_Z3_free_bound  0.005218 1.093e-03   0.02492
```

A graphical representation of the confidence intervals can finally be obtained.

```
mkinparplot(m.Z.mkin.5a)
```



The endpoints obtained with this model are

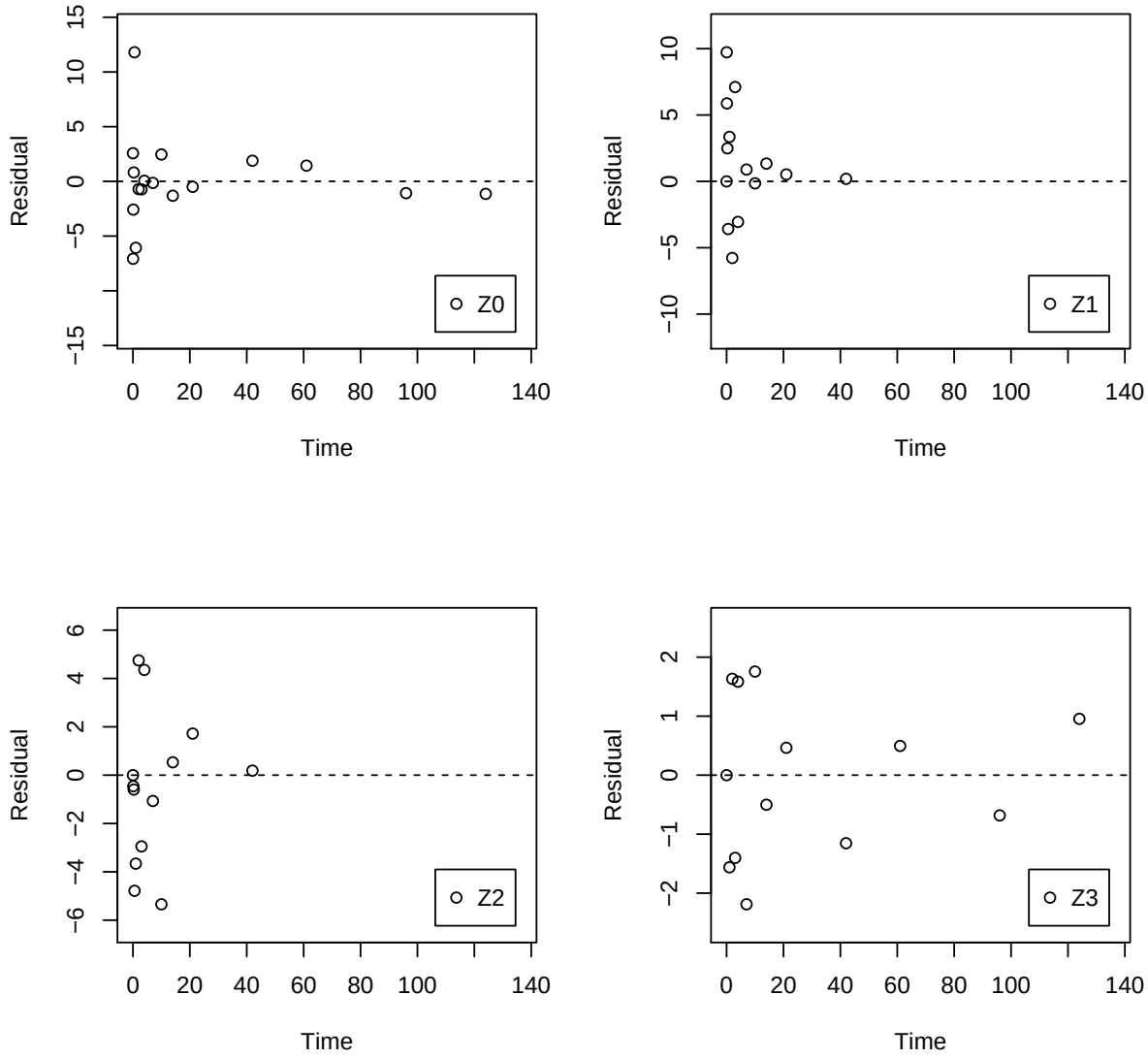
```
endpoints(m.Z.mkin.5a)
```

```
## $ff
##   Z0_free_Z1        Z1_Z2      Z2_sink   Z2_Z3_free Z3_free_sink
##       1.0000       1.0000       0.4634       0.5366       1.0000
##
## $SFORB
##    Z0_b1    Z0_b2    Z3_b1    Z3_b2
## 2.447136 0.007513 0.080007 0.000000
```

```
##
## $distimes
##      DT50   DT90 DT50_Z0_b1 DT50_Z0_b2 DT50_Z3_b1 DT50_Z3_b2
## Z0 0.3043  1.185     0.2832      92.27         NA         NA
## Z1 1.5148  5.032         NA         NA         NA         NA
## Z2 1.6414  5.453         NA         NA         NA         NA
## Z3 9.5675 41.137         NA         NA      8.664        Inf
```

It is clear the degradation rate of Z3 towards the end of the experiment is very low as DT50_Z3_b2 is reported to be infinity. However, this appears to be a feature of the data.

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