Example evaluation of FOCUS dataset Z

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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 (**?**), p.350.

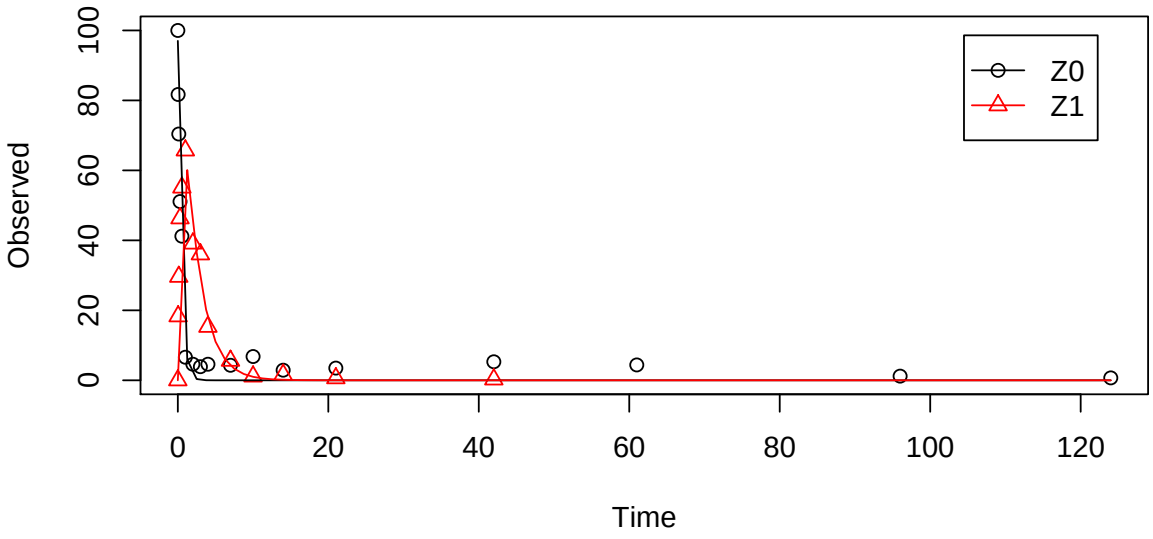
```
require(mkin)
LOD = 0.5
FOCUS_2006_Z = data.frame(
  t = c(0, 0.04, 0.125, 0.29, 0.54, 1, 2, 3, 4, 7, 10, 14, 21,
        42, 61, 96, 124),
  Z0 = c(100, 81.7, 70.4, 51.1, 41.2, 6.6, 4.6, 3.9, 4.6, 4.3, 6.8,
         2.9, 3.5, 5.3, 4.4, 1.2, 0.7),
  Z1 = c(0, 18.3, 29.6, 46.3, 55.1, 65.7, 39.1, 36,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

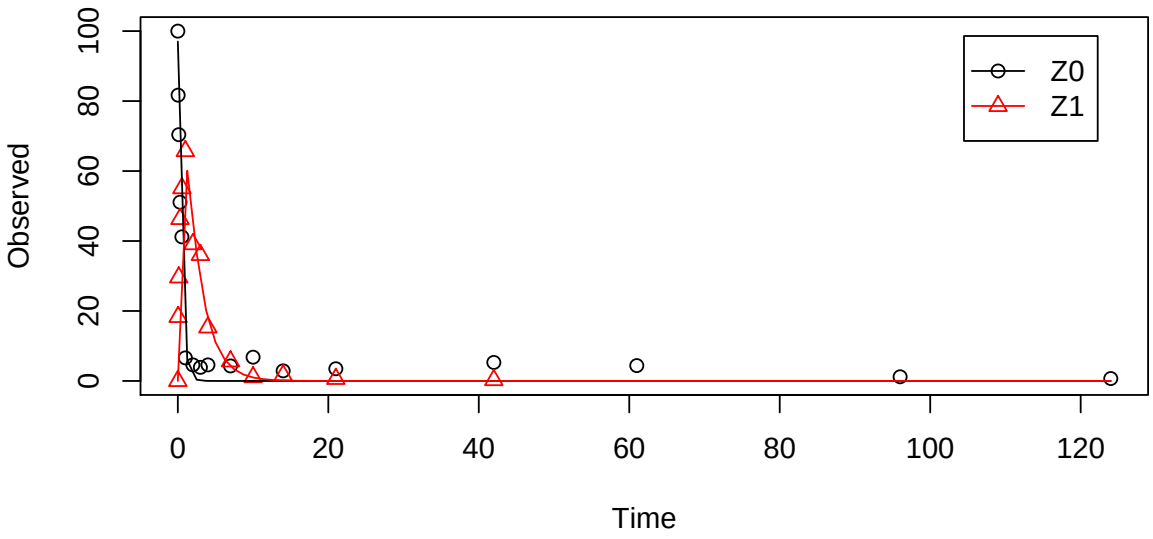
```
## mkin version:    0.9.26
## R version:       3.1.0
## Date of fit:     Fri May  2 18:26:47 2014
## Date of summary: Fri May  2 18:26:47 2014
##
## Equations:
## [1] d_Z0 = - k_Z0_sink * Z0 - k_Z0_Z1 * Z0
## [2] d_Z1 = + k_Z0_Z1 * Z0 - k_Z1_sink * Z1
##
## Method used for solution of differential equation system:
## eigen
##
## Weighting: none
##
## Starting values for optimised parameters:
##              value   type transformed
## Z0_0      100.0000  state     100.000
## k_Z0_sink   0.1000 deparm      -2.303
## k_Z0_Z1     0.1001 deparm      -2.302
```

```
## k_Z1_sink   0.1002 deparm      -2.301
##
## 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
## k_Z0_sink  -36.400         NA    NA    NA      NA       NA     NA
## k_Z0_Z1      0.805         NA    NA    NA      NA       NA     NA
## k_Z1_sink   -0.730         NA    NA    NA      NA       NA     NA
##
## Backtransformed parameters:
##           Estimate Lower Upper
## Z0_0      9.70e+01    NA    NA
## k_Z0_sink 1.62e-16    NA    NA
## k_Z0_Z1   2.24e+00    NA    NA
## k_Z1_sink 4.82e-01    NA    NA
##
## Residual standard error: 5.06 on 27 degrees of freedom
##
## 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
##
## Estimated disappearance times:
##    DT50 DT90
## Z0 0.31 1.03
## Z1 1.44 4.78
##
## Estimated formation fractions:
##               ff
## Z0_sink 7.23e-17
## Z0_Z1   1.00e+00
## Z1_sink 1.00e+00
##
## Parameter correlation:
## Could not estimate covariance matrix; singular system:
```

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

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

```
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:    0.9.26
## R version:       3.1.0
## Date of fit:     Fri May  2 18:26:48 2014
## Date of summary: Fri May  2 18:26:48 2014
##
## Equations:
## [1] d_Z0 = - k_Z0 * Z0
## [2] d_Z1 = + f_Z0_to_Z1 * k_Z0 * Z0 - k_Z1 * Z1
```

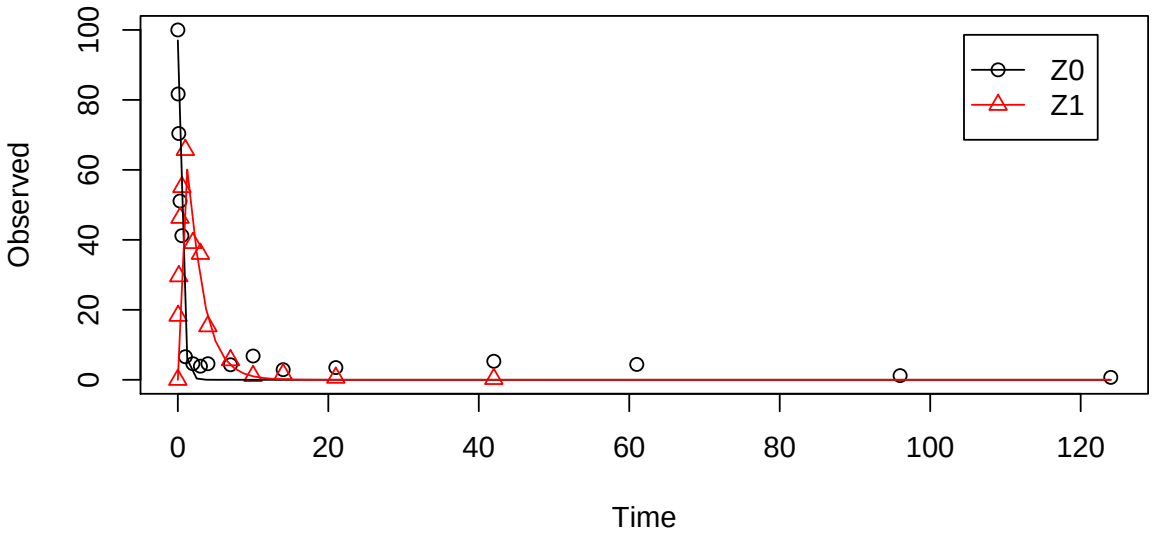
```
##
## Method used for solution of differential equation system:
## eigen
##
## Weighting: none
##
## Starting values for optimised parameters:
##               value   type transformed
## Z0_0       100.0000  state    100.0000
## k_Z0         0.1000 deparm     -2.3026
## f_Z0_to_Z1   0.2000 deparm     -0.9803
## k_Z1         0.1001 deparm     -2.3016
##
## 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
## k_Z0          0.805         NA    NA    NA      NA       NA     NA
## f_Z0_to_Z1   24.100         NA    NA    NA      NA       NA     NA
## k_Z1         -0.730         NA    NA    NA      NA       NA     NA
##
## Backtransformed parameters:
##            Estimate Lower Upper
## Z0_0         97.000    NA    NA
## k_Z0          2.240    NA    NA
## f_Z0_to_Z1    1.000    NA    NA
## k_Z1          0.482    NA    NA
##
## Residual standard error: 5.06 on 27 degrees of freedom
##
## 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
##
## Estimated disappearance times:
##    DT50 DT90
## Z0 0.31 1.03
```

```
## Z1 1.44 4.78
##
## Parameter correlation:
## Could not estimate covariance matrix; singular system:
```

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

The simplified model is obtained by setting the list component `sink` to `FALSE`.

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




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

```
## mkin version:    0.9.26
## R version:       3.1.0
## Date of fit:     Fri May  2 18:26:48 2014
## Date of summary: Fri May  2 18:26:48 2014
##
## Equations:
## [1] d_Z0 = - 0 - k_Z0_Z1 * Z0
```

```
## [2] d_Z1 = + k_Z0_Z1 * Z0 - k_Z1_sink * Z1
##
## Method used for solution of differential equation system:
## eigen
##
## Weighting: none
##
## Starting values for optimised parameters:
##              value   type transformed
## Z0_0      100.0000  state     100.000
## k_Z0_Z1     0.1000 deparm      -2.303
## k_Z1_sink   0.1001 deparm      -2.302
##
## 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
## k_Z0_Z1      0.805     0.0657  0.670   0.939   12.30 9.12e-13 4.56e-13
## k_Z1_sink   -0.730     0.0885 -0.911  -0.548   -8.24 5.74e-09 2.87e-09
##
## Backtransformed parameters:
##           Estimate  Lower   Upper
## Z0_0        97.000 91.500 103.000
## k_Z0_Z1      2.240  1.950   2.560
## k_Z1_sink    0.482  0.402   0.578
##
## Residual standard error: 4.97 on 28 degrees of freedom
##
## 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
##
```

```
## Estimated formation fractions:
##         ff
## Z0_sink  1
## Z0_Z1    1
## Z1_sink  1
##
## Parameter correlation:
##            Z0_0 k_Z0_Z1 k_Z1_sink
## Z0_0      1.000  0.1063    0.4104
## k_Z0_Z1   0.106  1.0000    0.0434
## k_Z1_sink 0.410  0.0434    1.0000
```

This model definition is not supported when formation fractions are used, but the formation fraction can be fixed to unity.

```
Z.3.ff <- mkinmod(Z0 = list(type = "SFO", to = "Z1"),
                  Z1 = list(type = "SFO"), use_of_ff = "max")
m.Z.3.ff <- mkinfit(Z.3.ff, FOCUS_2006_Z_mkin,
                    parms.ini = c(f_Z0_to_Z1 = 1),
                    fixed_parms = "f_Z0_to_Z1",
                    quiet = TRUE)
summary(m.Z.3.ff, data = FALSE)
```

```
## mkin version:    0.9.26
## R version:       3.1.0
## Date of fit:     Fri May  2 18:26:49 2014
## Date of summary: Fri May  2 18:26:49 2014
##
## Equations:
## [1] d_Z0 = - k_Z0 * Z0
## [2] d_Z1 = + f_Z0_to_Z1 * k_Z0 * Z0 - k_Z1 * Z1
##
## Method used for solution of differential equation system:
## eigen
##
## Weighting: none
##
## Starting values for optimised parameters:
##         value   type transformed
## Z0_0 100.0000  state     100.000
## k_Z0   0.1000 deparm      -2.303
## k_Z1   0.1001 deparm      -2.302
##
```

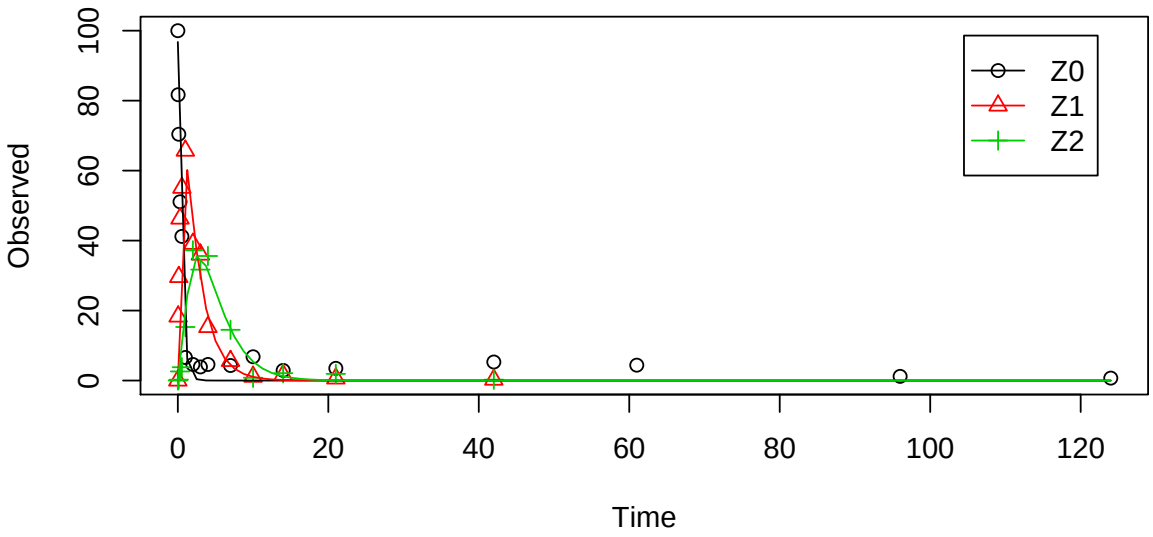
```
## Fixed parameter values:
##            value   type
## Z1_0           0  state
## f_Z0_to_Z1     1 deparm
##
## Optimised, transformed parameters:
##      Estimate Std. Error  Lower   Upper t value Pr(>|t|)   Pr(>t)
## Z0_0   97.000     2.6800 91.500 103.000   36.20 4.73e-25 2.36e-25
## k_Z0    0.805     0.0657  0.670   0.939   12.30 9.12e-13 4.56e-13
## k_Z1   -0.730     0.0885 -0.911  -0.548   -8.24 5.74e-09 2.87e-09
##
## 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
##
## Residual standard error: 4.97 on 28 degrees of freedom
##
## 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
##
## Parameter correlation:
##       Z0_0   k_Z0   k_Z1
## Z0_0 1.000 0.1063 0.4104
## k_Z0 0.106 1.0000 0.0434
## k_Z1 0.410 0.0434 1.0000
```

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)
```

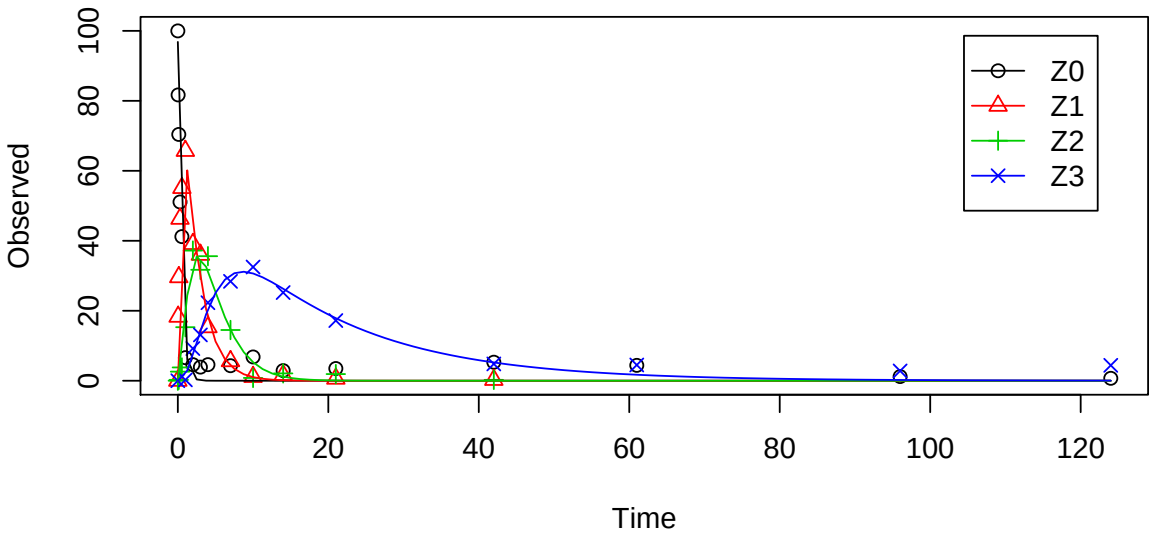
```
## mkin version:    0.9.26
## R version:       3.1.0
## Date of fit:     Fri May  2 18:26:50 2014
## Date of summary: Fri May  2 18:26:50 2014
##
## Equations:
## [1] d_Z0 = - 0 - k_Z0_Z1 * Z0
## [2] d_Z1 = + k_Z0_Z1 * Z0 - 0 - k_Z1_Z2 * Z1
## [3] d_Z2 = + k_Z1_Z2 * Z1 - k_Z2_sink * Z2
##
## Method used for solution of differential equation system:
## eigen
##
```

```
## Weighting: none
##
## Starting values for optimised parameters:
##              value   type transformed
## Z0_0      100.0000  state     100.000
## k_Z0_Z1     0.1000 deparm      -2.303
## k_Z1_Z2     0.1001 deparm      -2.302
## k_Z2_sink   0.1002 deparm      -2.301
##
## 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|)   Pr(>t)
## Z0_0        96.800     2.2700 92.200 101.000   42.70 5.43e-35 2.72e-35
## k_Z0_Z1      0.795     0.0584  0.677   0.913   13.60 1.36e-16 6.80e-17
## k_Z1_Z2     -0.741     0.0682 -0.879  -0.603  -10.90 1.68e-13 8.41e-14
## k_Z2_sink   -0.803     0.1110 -1.030  -0.579   -7.24 8.79e-09 4.39e-09
##
## 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
##
## Residual standard error: 4.49 on 40 degrees of freedom
##
## 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
##
## Estimated disappearance times:
##     DT50 DT90
## Z0 0.313 1.04
## Z1 1.454 4.83
## Z2 1.547 5.14
```

```
##
## Estimated formation fractions:
##         ff
## Z0_sink  1
## Z0_Z1    1
## Z1_sink  1
## Z1_Z2    1
## Z2_sink  1
##
## Parameter correlation:
##             Z0_0 k_Z0_Z1 k_Z1_Z2 k_Z2_sink
## Z0_0      1.0000  0.0578  0.2875    0.3179
## k_Z0_Z1   0.0578  1.0000 -0.0436    0.0121
## k_Z1_Z2   0.2875 -0.0436  1.0000    0.2402
## k_Z2_sink 0.3179  0.0121  0.2402    1.0000
```

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)
```

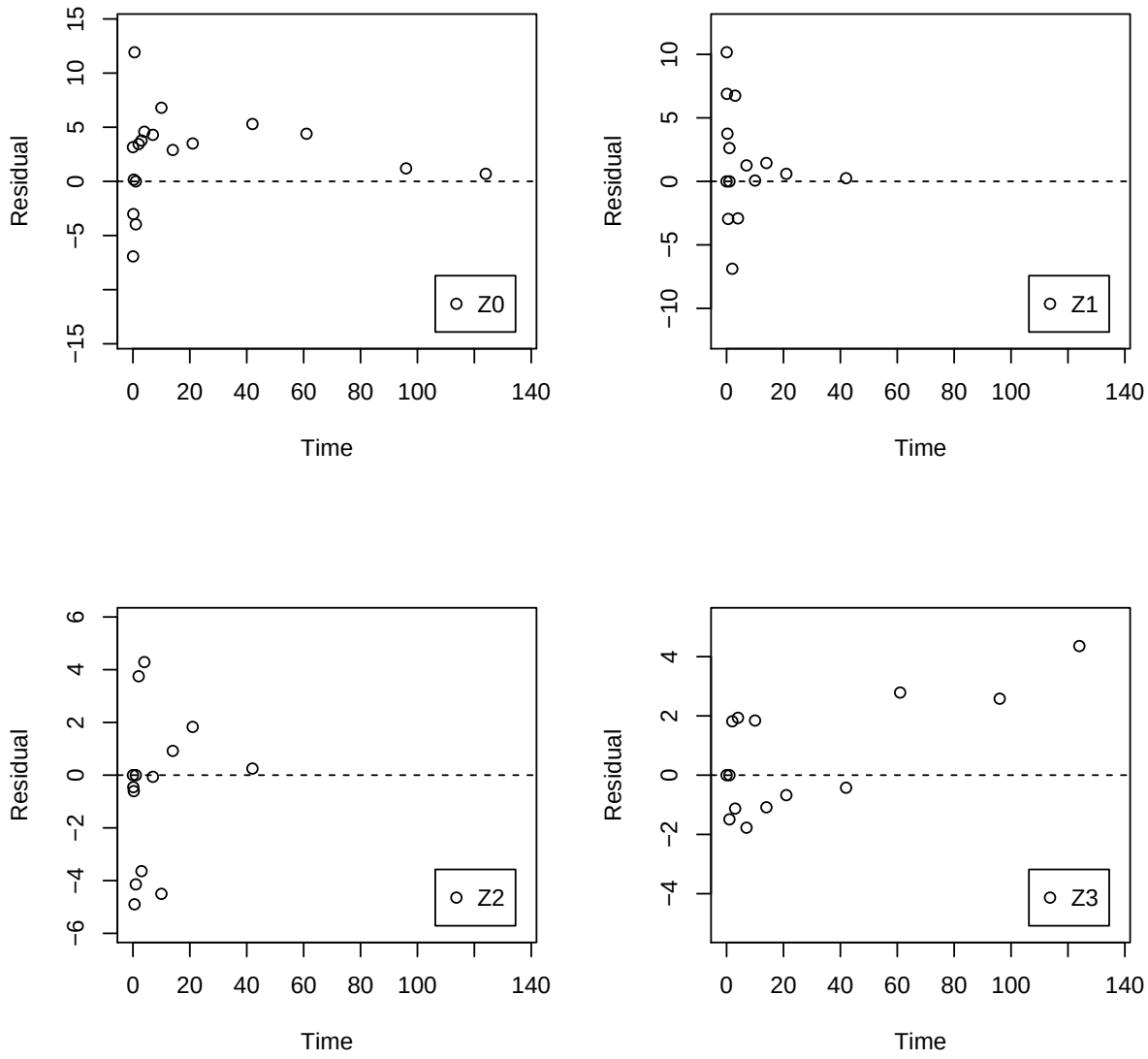
```
## mkin version:    0.9.26
## R version:       3.1.0
## Date of fit:     Fri May  2 18:26:51 2014
## Date of summary: Fri May  2 18:26:51 2014
##
## Equations:
## [1] d_Z0 = - 0 - k_Z0_Z1 * Z0
## [2] d_Z1 = + k_Z0_Z1 * Z0 - 0 - k_Z1_Z2 * Z1
## [3] d_Z2 = + k_Z1_Z2 * Z1 - k_Z2_sink * Z2 - k_Z2_Z3 * Z2
## [4] d_Z3 = + k_Z2_Z3 * Z2 - k_Z3_sink * Z3
##
## Method used for solution of differential equation system:
## eigen
##
## Weighting: none
##
## Starting values for optimised parameters:
##               value   type transformed
## Z0_0      100.0000  state    100.0000
## k_Z0_Z1     2.2140 deparm      0.7948
## k_Z1_Z2     0.4766 deparm     -0.7410
## k_Z2_sink   0.4481 deparm     -0.8027
```

```
## k_Z2_Z3     0.1000 deparm     -2.3026
## k_Z3_sink   0.1001 deparm     -2.3016
##
## 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|)   Pr(>t)
## Z0_0        96.800     2.0600 92.700 101.000   47.00 1.12e-43 5.58e-44
## k_Z0_Z1      0.795     0.0533  0.688   0.902   14.90 3.08e-20 1.54e-20
## k_Z1_Z2     -0.738     0.0612 -0.860  -0.615  -12.00 1.57e-16 7.84e-17
## k_Z2_sink   -1.430     0.1720 -1.780  -1.090   -8.35 4.16e-11 2.08e-11
## k_Z2_Z3     -1.550     0.1230 -1.790  -1.300  -12.60 2.60e-17 1.30e-17
## k_Z3_sink   -2.840     0.2440 -3.320  -2.350  -11.60 5.64e-16 2.82e-16
##
## 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
##
## Residual standard error: 4.1 on 51 degrees of freedom
##
## 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
##
## 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
##
## Estimated formation fractions:
##            ff
## Z0_sink 1.000
## Z0_Z1   1.000
## Z1_sink 1.000
## Z1_Z2   1.000
## Z2_sink 0.528
## Z2_Z3   0.472
## Z3_sink 1.000
##
## Parameter correlation:
##              Z0_0 k_Z0_Z1 k_Z1_Z2 k_Z2_sink k_Z2_Z3 k_Z3_sink
## Z0_0       1.0000  0.0539  0.2727    0.3701 -0.0730   -0.1135
## k_Z0_Z1    0.0539  1.0000 -0.0521    0.0244 -0.0358   -0.0252
## k_Z1_Z2    0.2727 -0.0521  1.0000    0.2938 -0.1213   -0.1915
## k_Z2_sink  0.3701  0.0244  0.2938    1.0000 -0.1889   -0.6430
## k_Z2_Z3   -0.0730 -0.0358 -0.1213   -0.1889  1.0000    0.5516
## k_Z3_sink -0.1135 -0.0252 -0.1915   -0.6430  0.5516    1.0000
```

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")
```



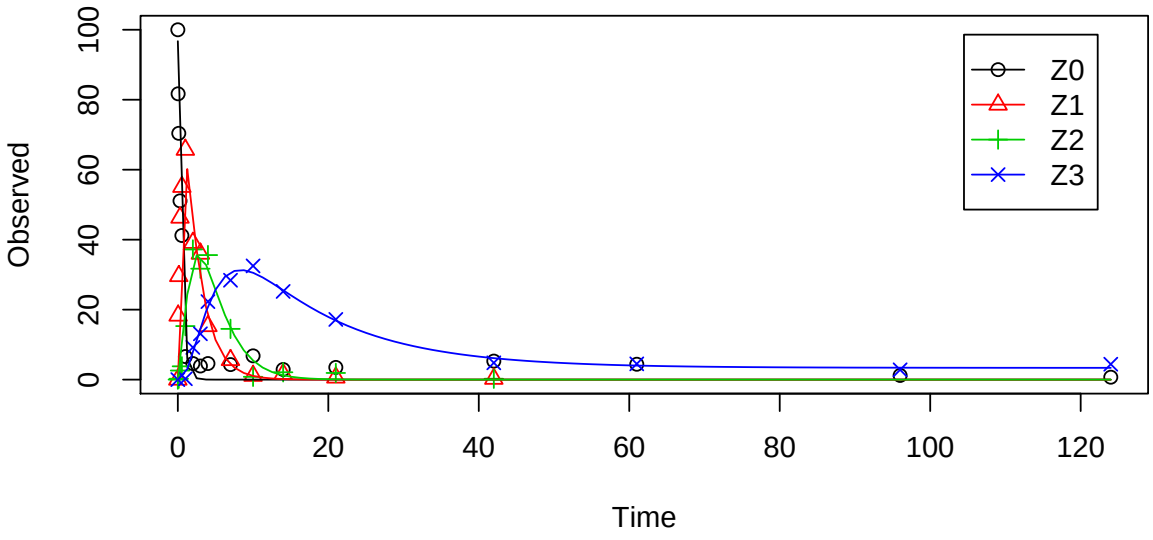
4 Using the SFORB model for parent and metabolites

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)
```

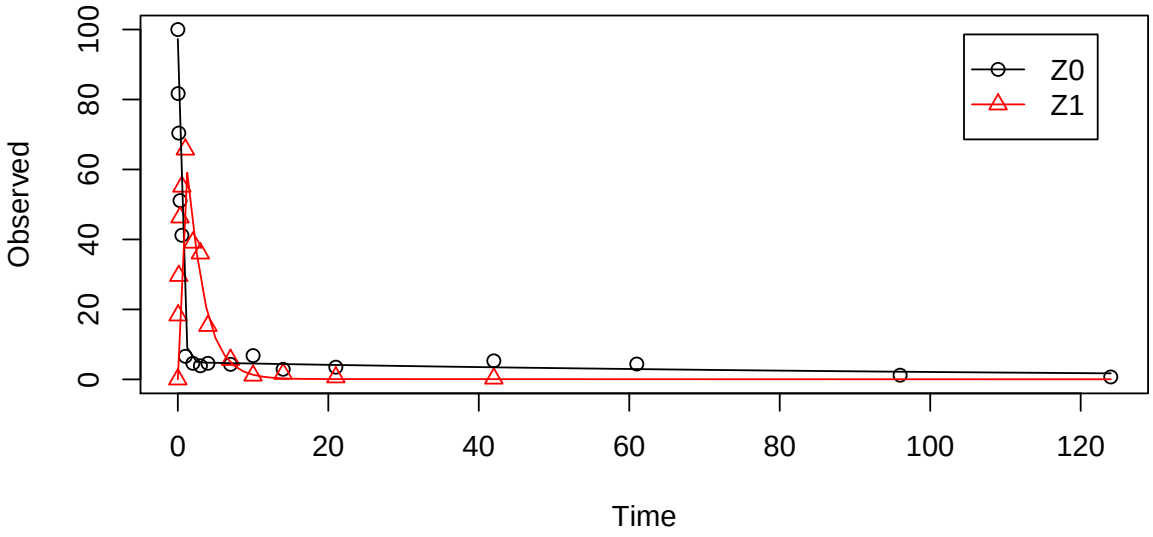
```
## mkin version:    0.9.26
## R version:       3.1.0
## Date of fit:     Fri May  2 18:26:56 2014
## Date of summary: Fri May  2 18:26:56 2014
##
## Equations:
## [1] d_Z0 = - 0 - k_Z0_Z1 * Z0
## [2] d_Z1 = + k_Z0_Z1 * Z0 - 0 - k_Z1_Z2 * Z1
## [3] d_Z2 = + k_Z1_Z2 * Z1 - k_Z2_sink * Z2 - k_Z2_Z3_free * Z2
## [4] d_Z3_free = + k_Z2_Z3_free * Z2 - k_Z3_free_sink * Z3_free - k_Z3_free_bound *
## [5] d_Z3_bound = + k_Z3_free_bound * Z3_free - k_Z3_bound_free * Z3_bound
```

```
##
## Method used for solution of differential equation system:
## eigen
##
## Weighting: none
##
## Starting values for optimised parameters:
##                   value   type transformed
## Z0_0            100.0000  state    100.0000
## k_Z0_Z1           0.5000 deparm     -0.6931
## k_Z1_Z2           0.3000 deparm     -1.2040
## k_Z2_sink         0.1000 deparm     -2.3026
## k_Z2_Z3_free      0.1001 deparm     -2.3016
## k_Z3_free_sink    0.1002 deparm     -2.3006
## k_Z3_free_bound   0.1000 deparm     -2.3026
## k_Z3_bound_free   0.0200 deparm     -3.9120
##
## 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|) Pr(>t)
## Z0_0              96.700         NA    NA    NA      NA       NA     NA
## k_Z0_Z1            0.795         NA    NA    NA      NA       NA     NA
## k_Z1_Z2           -0.743         NA    NA    NA      NA       NA     NA
## k_Z2_sink         -1.490         NA    NA    NA      NA       NA     NA
## k_Z2_Z3_free      -1.500         NA    NA    NA      NA       NA     NA
## k_Z3_free_sink    -2.650         NA    NA    NA      NA       NA     NA
## k_Z3_free_bound   -5.240         NA    NA    NA      NA       NA     NA
## k_Z3_bound_free  -21.400         NA    NA    NA      NA       NA     NA
##
## 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 5.06e-10    NA    NA
##
## Residual standard error: 4.11 on 49 degrees of freedom
##
## Chi2 error levels in percent:
##          err.min n.optim df
## All data   19.23       8 46
## Z0         17.43       2 15
## Z1         15.27       1 12
## Z2         20.28       2 10
## Z3          8.22       3  9
##
## Estimated disappearance times:
##      DT50  DT90
## Z0  0.313  1.04
## Z1  1.457  4.84
## Z2  1.552  5.16
## Z3 10.198 45.33
##
## Estimated formation fractions:
##                 ff
## Z0_sink      1.000
## Z0_Z1        1.000
## Z1_sink      1.000
## Z1_Z2        1.000
## Z2_sink      0.502
## Z2_Z3_free   0.498
## Z3_free_sink 1.000
##
## Estimated Eigenvalues of SFORB model(s):
##    Z3_b1    Z3_b2
## 7.56e-02 4.71e-10
##
## Parameter correlation:
## Could not estimate covariance matrix; singular system:
```

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

```
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)
```

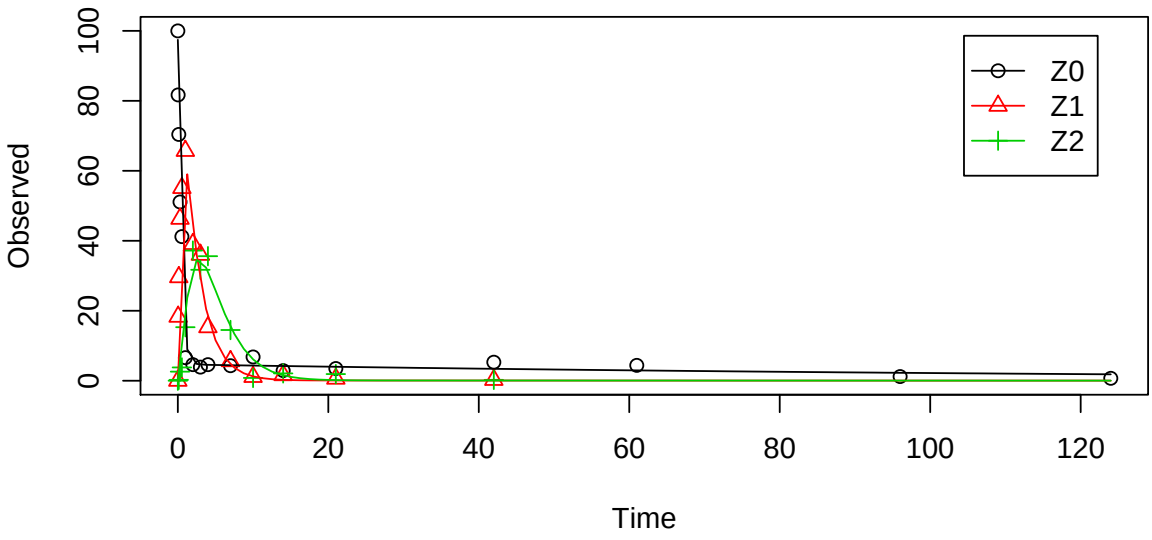
```
## mkin version:    0.9.26
## R version:       3.1.0
## Date of fit:     Fri May  2 18:26:57 2014
## Date of summary: Fri May  2 18:26:57 2014
##
## Equations:
## [1] d_Z0_free = - 0 - k_Z0_free_bound * Z0_free + k_Z0_bound_free * Z0_bound - k_Z
## [2] d_Z0_bound = + k_Z0_free_bound * Z0_free - k_Z0_bound_free * Z0_bound
## [3] d_Z1 = + k_Z0_free_Z1 * Z0_free - k_Z1_sink * Z1
##
## Method used for solution of differential equation system:
## eigen
##
## Weighting: none
##
## Starting values for optimised parameters:
##                    value   type transformed
```

```
## Z0_free_0       100.0000  state     100.000
## k_Z0_free_bound   0.1000 deparm      -2.303
## k_Z0_bound_free   0.0200 deparm      -3.912
## k_Z0_free_Z1      0.1002 deparm      -2.301
## k_Z1_sink         0.1003 deparm      -2.300
##
## Fixed parameter values:
##            value  type
## Z0_bound_0     0 state
## Z1_0           0 state
##
## Optimised, transformed parameters:
##                 Estimate Std. Error  Lower   Upper t value Pr(>|t|)
## Z0_free_0         97.300     2.4000 92.400 102.000   40.60 4.73e-25
## k_Z0_free_bound   -2.080     0.4320 -2.970  -1.190   -4.82 5.44e-05
## k_Z0_bound_free   -4.720     1.6000 -8.020  -1.420   -2.94 6.78e-03
## k_Z0_free_Z1       0.855     0.0643  0.723   0.987   13.30 4.18e-13
## k_Z1_sink         -0.793     0.0851 -0.968  -0.619   -9.33 8.86e-10
##                   Pr(>t)
## Z0_free_0       2.36e-25
## k_Z0_free_bound 2.72e-05
## k_Z0_bound_free 3.39e-03
## k_Z0_free_Z1    2.09e-13
## k_Z1_sink       4.43e-10
##
## 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
##
## Residual standard error: 4.44 on 26 degrees of freedom
##
## 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 disappearance times:
```

```
##     DT50 DT90
## Z0 0.302 1.19
## Z1 1.532 5.09
##
## Estimated formation fractions:
##              ff
## Z0_free_sink  1
## Z0_free_Z1    1
## Z1_sink       1
##
## Estimated Eigenvalues of SFORB model(s):
##   Z0_b1   Z0_b2
## 2.47631 0.00846
##
## Parameter correlation:
##                 Z0_free_0 k_Z0_free_bound k_Z0_bound_free k_Z0_free_Z1
## Z0_free_0         1.00000         0.00649          0.0332       0.1118
## k_Z0_free_bound   0.00649         1.00000          0.5465       0.4139
## k_Z0_bound_free   0.03324         0.54647          1.0000       0.1584
## k_Z0_free_Z1      0.11182         0.41393          0.1584       1.0000
## k_Z1_sink         0.39155        -0.29191         -0.1260      -0.0419
##                 k_Z1_sink
## Z0_free_0          0.3916
## k_Z0_free_bound   -0.2919
## k_Z0_bound_free   -0.1260
## k_Z0_free_Z1      -0.0419
## k_Z1_sink          1.0000
```

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:    0.9.26
## R version:       3.1.0
## Date of fit:     Fri May  2 18:26:58 2014
## Date of summary: Fri May  2 18:26:58 2014
##
## Equations:
## [1] d_Z0_free = - 0 - k_Z0_free_bound * Z0_free + k_Z0_bound_free * Z0_bound - k_Z
## [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
##
## Method used for solution of differential equation system:
## eigen
##
## Weighting: none
##
## Starting values for optimised parameters:
##                     value   type transformed
## Z0_free_0       100.0000  state     100.000
## k_Z0_free_bound   0.1000 deparm      -2.303
## k_Z0_bound_free   0.0200 deparm      -3.912
## k_Z0_free_Z1      0.1002 deparm      -2.301
```

```
## k_Z1_Z2           0.1003 deparm      -2.300
## k_Z2_sink         0.1004 deparm      -2.299
##
## 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 Pr(>|t|)
## Z0_free_0         97.400     2.0700 93.200 102.000   47.00 2.70e-35
## k_Z0_free_bound   -2.150     0.4040 -2.970  -1.330   -5.32 4.88e-06
## k_Z0_bound_free   -4.840     1.6100 -8.100  -1.580   -3.00 4.71e-03
## k_Z0_free_Z1       0.846     0.0583  0.728   0.964   14.50 4.50e-17
## k_Z1_Z2           -0.781     0.0649 -0.912  -0.650  -12.00 1.52e-14
## k_Z2_sink         -0.861     0.1060 -1.070  -0.647   -8.14 7.47e-10
##                   Pr(>t)
## Z0_free_0       1.35e-35
## k_Z0_free_bound 2.44e-06
## k_Z0_bound_free 2.35e-03
## k_Z0_free_Z1    2.25e-17
## k_Z1_Z2         7.61e-15
## k_Z2_sink       3.73e-10
##
## 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
##
## Residual standard error: 4.08 on 38 degrees of freedom
##
## 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
```

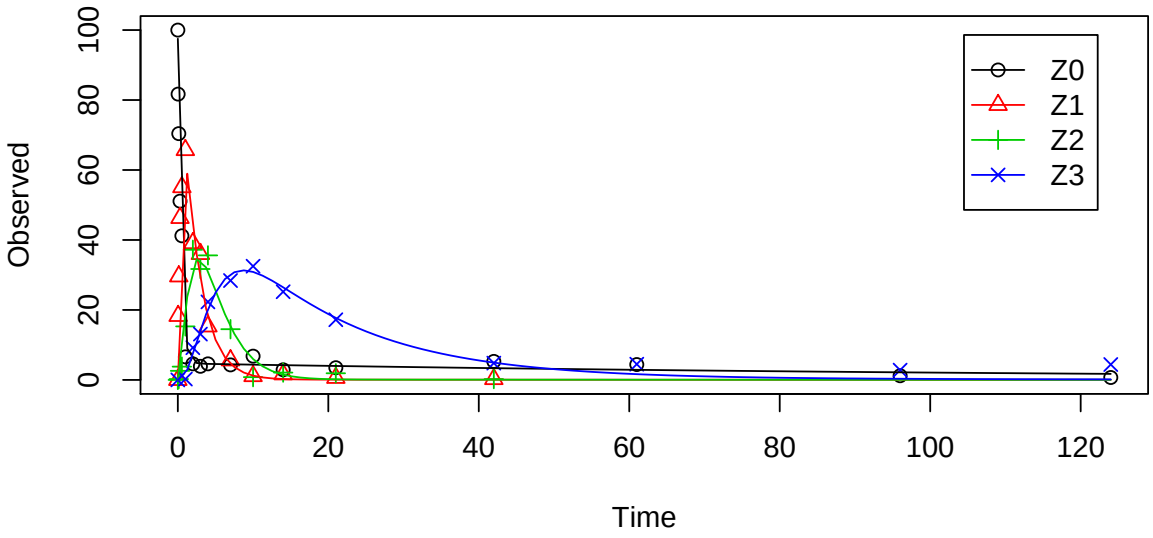
```
##
## Estimated disappearance times:
##     DT50 DT90
## Z0 0.304 1.18
## Z1 1.514 5.03
## Z2 1.639 5.44
##
## Estimated formation fractions:
##              ff
## Z0_free_sink  1
## Z0_free_Z1    1
## Z1_sink       1
## Z1_Z2         1
## Z2_sink       1
##
## Estimated Eigenvalues of SFORB model(s):
##   Z0_b1   Z0_b2
## 2.44664 0.00754
##
## Parameter correlation:
##                 Z0_free_0 k_Z0_free_bound k_Z0_bound_free k_Z0_free_Z1
## Z0_free_0          1.0000           0.075          0.0708       0.0908
## k_Z0_free_bound    0.0750           1.000          0.5425       0.4245
## k_Z0_bound_free    0.0708           0.543          1.0000       0.1632
## k_Z0_free_Z1       0.0908           0.425          0.1632       1.0000
## k_Z1_Z2            0.2572          -0.228         -0.0863      -0.1008
## k_Z2_sink          0.2888          -0.211         -0.0792      -0.0490
##                 k_Z1_Z2 k_Z2_sink
## Z0_free_0        0.2572    0.2888
## k_Z0_free_bound -0.2276   -0.2105
## k_Z0_bound_free -0.0863   -0.0792
## k_Z0_free_Z1    -0.1008   -0.0490
## k_Z1_Z2          1.0000    0.2728
## k_Z2_sink        0.2728    1.0000
```

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:    0.9.26
## R version:       3.1.0
## Date of fit:     Fri May  2 18:27:03 2014
## Date of summary: Fri May  2 18:27:03 2014
##
## Equations:
## [1] d_Z0_free = - 0 - k_Z0_free_bound * Z0_free + k_Z0_bound_free * Z0_bound - k_Z
## [2] d_Z0_bound = + k_Z0_free_bound * Z0_free - k_Z0_bound_free * Z0_bound
## [3] d_Z1 = + k_Z0_free_Z1 * Z0_free - 0 - k_Z1_Z2 * Z1
## [4] d_Z2 = + k_Z1_Z2 * Z1 - k_Z2_sink * Z2 - k_Z2_Z3 * Z2
## [5] d_Z3 = + k_Z2_Z3 * Z2 - k_Z3_sink * Z3
##
## Method used for solution of differential equation system:
## eigen
##
## Weighting: none
```

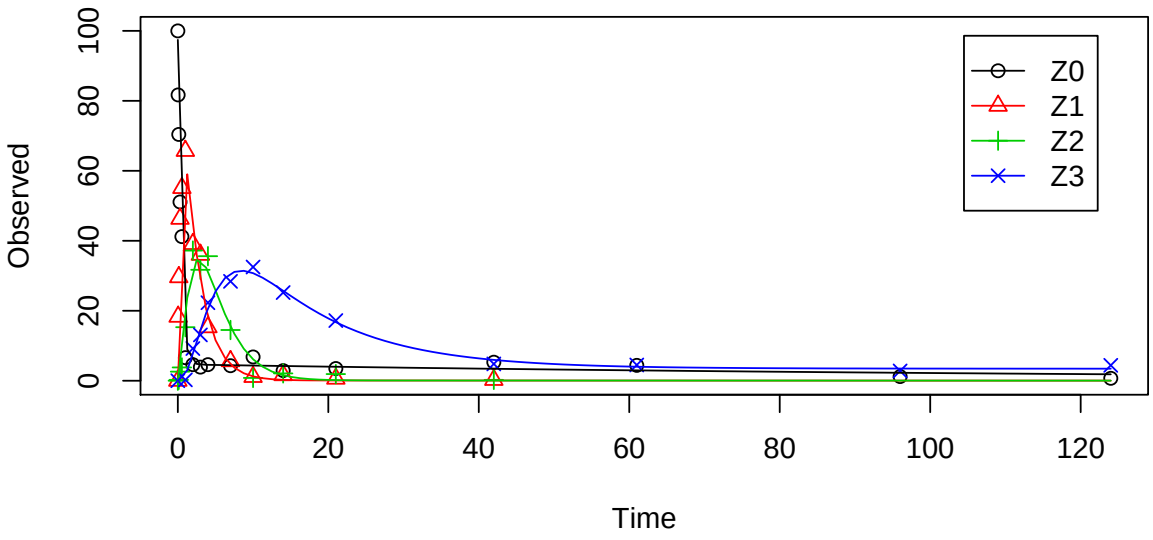
```
##
## Starting values for optimised parameters:
##                    value   type transformed
## Z0_free_0       100.0000  state     100.000
## k_Z1_Z2           0.0500 deparm      -2.996
## k_Z0_free_bound   0.1000 deparm      -2.303
## k_Z0_bound_free   0.0200 deparm      -3.912
## k_Z0_free_Z1      0.1002 deparm      -2.301
## k_Z2_sink         0.1003 deparm      -2.300
## k_Z2_Z3           0.1004 deparm      -2.299
## k_Z3_sink         0.1005 deparm      -2.298
##
## 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 Pr(>|t|)
## Z0_free_0         97.500     1.8900 93.700 101.000   51.70 2.07e-44
## k_Z1_Z2           -0.777     0.0583 -0.894  -0.660  -13.30 6.66e-18
## k_Z0_free_bound   -2.140     0.3680 -2.880  -1.400   -5.80 4.71e-07
## k_Z0_bound_free   -4.760     1.4200 -7.610  -1.920   -3.36 1.52e-03
## k_Z0_free_Z1       0.847     0.0534  0.740   0.954   15.90 6.13e-21
## k_Z2_sink         -1.560     0.1830 -1.930  -1.190   -8.55 2.79e-11
## k_Z2_Z3           -1.530     0.1140 -1.760  -1.300  -13.50 4.49e-18
## k_Z3_sink         -2.770     0.2250 -3.220  -2.320  -12.30 1.25e-16
##                   Pr(>t)
## Z0_free_0       1.03e-44
## k_Z1_Z2         3.33e-18
## k_Z0_free_bound 2.36e-07
## k_Z0_bound_free 7.58e-04
## k_Z0_free_Z1    3.06e-21
## k_Z2_sink       1.40e-11
## k_Z2_Z3         2.24e-18
## k_Z3_sink       6.23e-17
##
## 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   0.2480
## k_Z0_bound_free  0.00852 4.93e-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
##
## Residual standard error: 3.74 on 49 degrees of freedom
##
## 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 disappearance times:
##      DT50  DT90
## Z0  0.304  1.19
## Z1  1.507  5.01
## Z2  1.623  5.39
## Z3 11.051 36.71
##
## Estimated formation fractions:
##                 ff
## Z0_free_sink 1.000
## Z0_free_Z1   1.000
## Z1_sink      1.000
## Z1_Z2        1.000
## Z2_sink      0.492
## Z2_Z3        0.508
## Z3_sink      1.000
##
## Estimated Eigenvalues of SFORB model(s):
##   Z0_b1   Z0_b2
## 2.45127 0.00811
##
## Parameter correlation:
##                 Z0_free_0 k_Z1_Z2 k_Z0_free_bound k_Z0_bound_free
## Z0_free_0          1.0000  0.2424          0.0782          0.0692
```

```
## k_Z1_Z2             0.2424  1.0000          -0.2274          -0.0894
## k_Z0_free_bound     0.0782 -0.2274           1.0000           0.5398
## k_Z0_bound_free     0.0692 -0.0894           0.5398           1.0000
## k_Z0_free_Z1        0.0888 -0.1084           0.4276           0.1628
## k_Z2_sink           0.3299  0.3405          -0.2633          -0.1275
## k_Z2_Z3            -0.0749 -0.1489           0.0670           0.0608
## k_Z3_sink          -0.1046 -0.2249           0.1384           0.1252
##                  k_Z0_free_Z1 k_Z2_sink k_Z2_Z3 k_Z3_sink
## Z0_free_0              0.0888    0.3299 -0.0749   -0.1046
## k_Z1_Z2               -0.1084    0.3405 -0.1489   -0.2249
## k_Z0_free_bound        0.4276   -0.2633  0.0670    0.1384
## k_Z0_bound_free        0.1628   -0.1275  0.0608    0.1252
## k_Z0_free_Z1           1.0000   -0.0531 -0.0128    0.0186
## k_Z2_sink             -0.0531    1.0000 -0.2547   -0.6832
## k_Z2_Z3               -0.0128   -0.2547  1.0000    0.5639
## k_Z3_sink              0.0186   -0.6832  0.5639    1.0000
```

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  5.013e-22    NA    NA
```

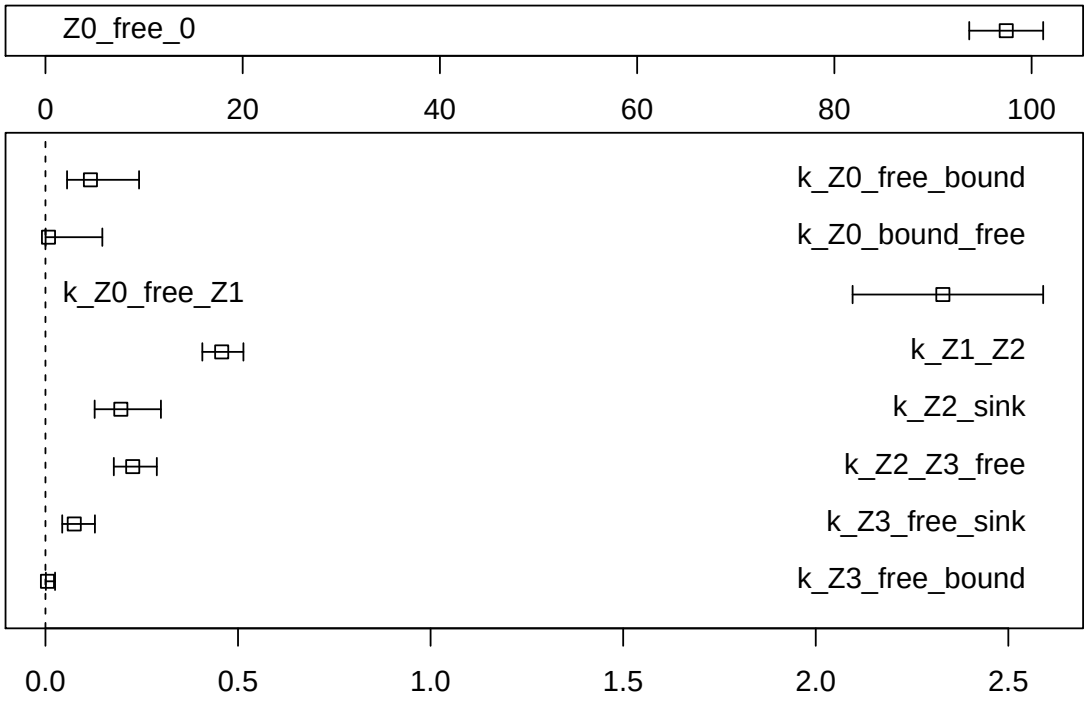
The summary view of the backtransformed parameters shows that we get no confidence intervals due to overparameterisation. As the optimized `k_Z3_bound_free` is excessively small, it is reasonable to fix it to zero.

```
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       97.424939 9.368e+01 101.17374
## k_Z0_free_bound  0.116755 5.608e-02   0.24306
## k_Z0_bound_free  0.007889 4.213e-04   0.14772
## k_Z0_free_Z1     2.329999 2.096e+00   2.59050
## k_Z1_Z2          0.457590 4.074e-01   0.51402
## k_Z2_sink        0.195710 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
```

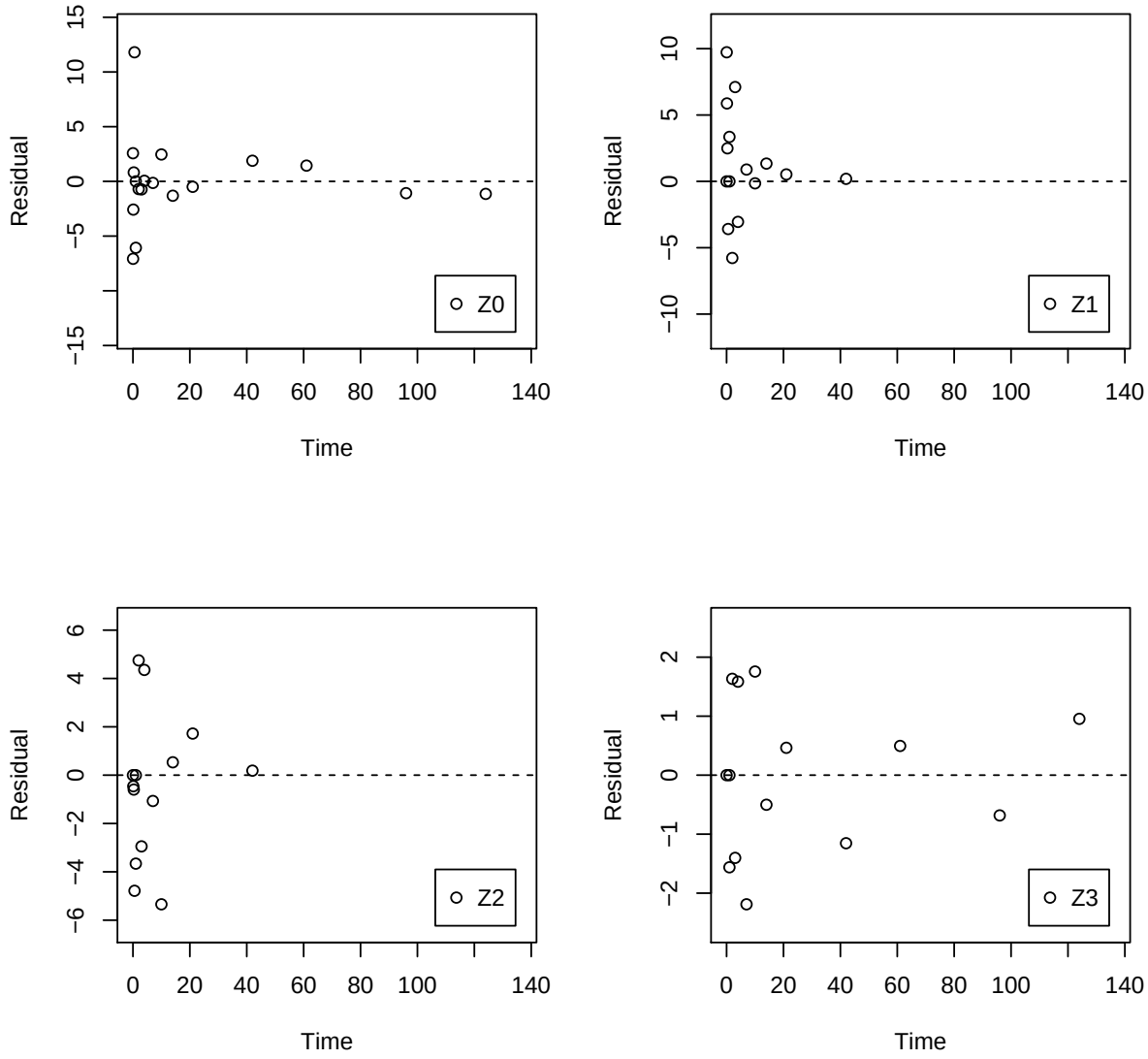
A graphical representation of the confidence intervals can finally be obtained.

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



It is clear that nothing can be said about the degradation rate of Z3 towards the end of the experiment. However, this appears to be a feature of the data.

```
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.