

# Example evaluation of FOCUS dataset Z

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**Key words:** Kinetics, FOCUS, nonlinear optimisation

## 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)
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).

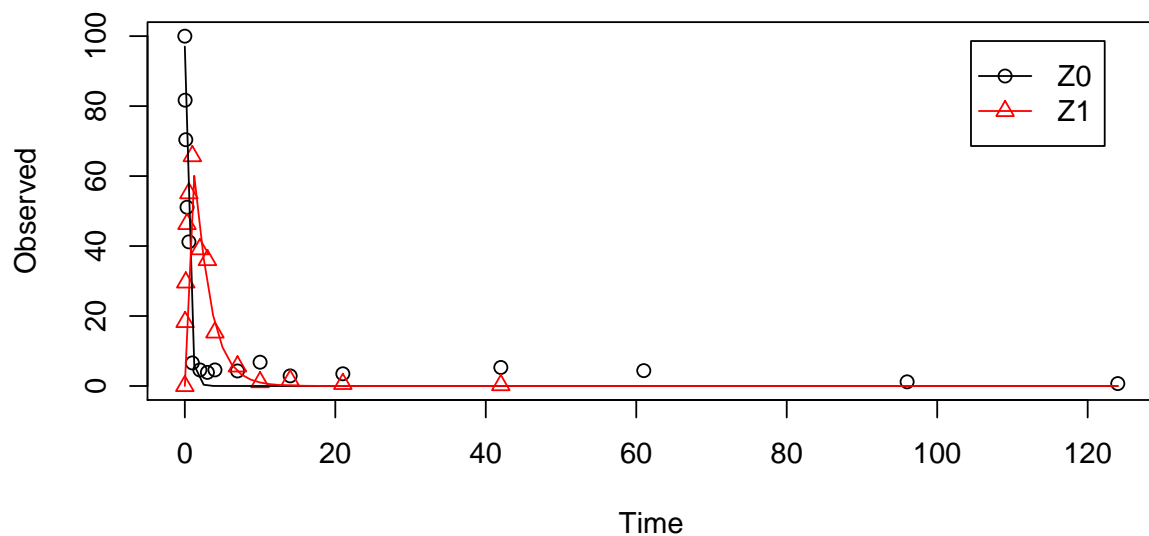
```

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

## Successfully compiled differential equation model from auto-generated C
code.

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

```



```

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

## mkin version:      0.9.42
## R version:         3.2.4
## Date of fit:        Tue Mar 29 16:12:47 2016
## Date of summary:    Tue Mar 29 16:12:47 2016
##
## 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 deSolve
##
## Fitted with method Port using 340 model solutions performed in 2.677 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      100.000000 -Inf  Inf
## log_k_Z0_sink -2.302585 -Inf  Inf
## log_k_Z0_Z1  -2.301586 -Inf  Inf
## log_k_Z1_sink -2.300587 -Inf  Inf
##
## Fixed parameter values:
##      value  type
## Z1_0      0  state
##
## Optimised, transformed parameters with symmetric confidence intervals:
##      Estimate Std. Error Lower Upper
## Z0_0      97.0100          NA    NA    NA
## log_k_Z0_sink -25.0800          NA    NA    NA
## log_k_Z0_Z1   0.8047          NA    NA    NA
## log_k_Z1_sink -0.7296          NA    NA    NA
##
## Parameter correlation:
## Could not estimate covariance matrix; singular system:
##
## Residual standard error: 5.064 on 27 degrees of freedom
##
## Backtransformed parameters:
## Confidence intervals for internally transformed parameters are asymmetric.
## t-test (unrealistically) based on the assumption of normal distribution
## for estimators of untransformed parameters.
##      Estimate  t value  Pr(>t) Lower Upper
## Z0_0      9.701e+01 2.730e+01 1.679e-21    NA    NA
## k_Z0_sink 1.279e-11 5.637e-11 5.000e-01    NA    NA
## k_Z0_Z1   2.236e+00 1.355e+01 7.394e-14    NA    NA
## k_Z1_sink 4.821e-01 7.321e+00 3.552e-08    NA    NA
##
## Chi2 error levels in percent:

```

```
##          err.min n.optim df
## All data    17.89      4 26
## Z0         18.04      3 14
## Z1         15.08      1 12
##
## Resulting formation fractions:
##          ff
## Z0_sink 5.72e-12
## Z0_Z1   1.00e+00
## Z1_sink 1.00e+00
##
## Estimated disappearance times:
##      DT50  DT90
## Z0 0.310 1.030
## Z1 1.438 4.776
```

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")

## Successfully compiled differential equation model from auto-generated C code.

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:      0.9.42
## R version:        3.2.4
## Date of fit:       Tue Mar 29 16:12:50 2016
## Date of summary:   Tue Mar 29 16:12:50 2016
##
## Equations:
## d_Z0 = - k_Z0 * Z0
## d_Z1 = + f_Z0_to_Z1 * k_Z0 * Z0 - k_Z1 * Z1
##
## Model predictions using solution type deSolve
##
## Fitted with method Port using 329 model solutions performed in 2.59 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      100.000000 -Inf  Inf
## log_k_Z0   -2.302585 -Inf  Inf
## log_k_Z1   -2.301586 -Inf  Inf
## f_Z0_ilr_1  0.000000 -Inf  Inf
##
## Fixed parameter values:
##      value  type
## Z1_0      0 state
##
## Optimised, transformed parameters with symmetric confidence intervals:
##           Estimate Std. Error Lower Upper
## Z0_0          97.0100         NA    NA    NA
## log_k_Z0        0.8047         NA    NA    NA
## log_k_Z1       -0.7296         NA    NA    NA
## f_Z0_ilr_1    15.8900         NA    NA    NA
##
## Parameter correlation:
## Could not estimate covariance matrix; singular system:
##
## Residual standard error: 5.064 on 27 degrees of freedom
##
## Backtransformed parameters:
## Confidence intervals for internally transformed parameters are asymmetric.
## t-test (unrealistically) based on the assumption of normal distribution
## for estimators of untransformed parameters.
##           Estimate t value    Pr(>t) Lower Upper
## Z0_0          97.0100  27.300 1.679e-21    NA    NA
## k_Z0           2.2360  10.310 3.662e-11    NA    NA
## k_Z1           0.4821   7.321 3.552e-08    NA    NA
## f_Z0_to_Z1     1.0000   9.855 9.707e-11    NA    NA
##
## Chi2 error levels in percent:
##           err.min n.optim df
## All data    17.89      4 26
## Z0          17.56      2 15
## Z1          15.59      2 11
##
## Resulting formation fractions:
##           ff
## Z0_Z1     1.00e+00

```

```
## Z0_sink 1.75e-10
##
## Estimated disappearance times:
##      DT50  DT90
## Z0  0.310  1.030
## Z1  1.438  4.776
```

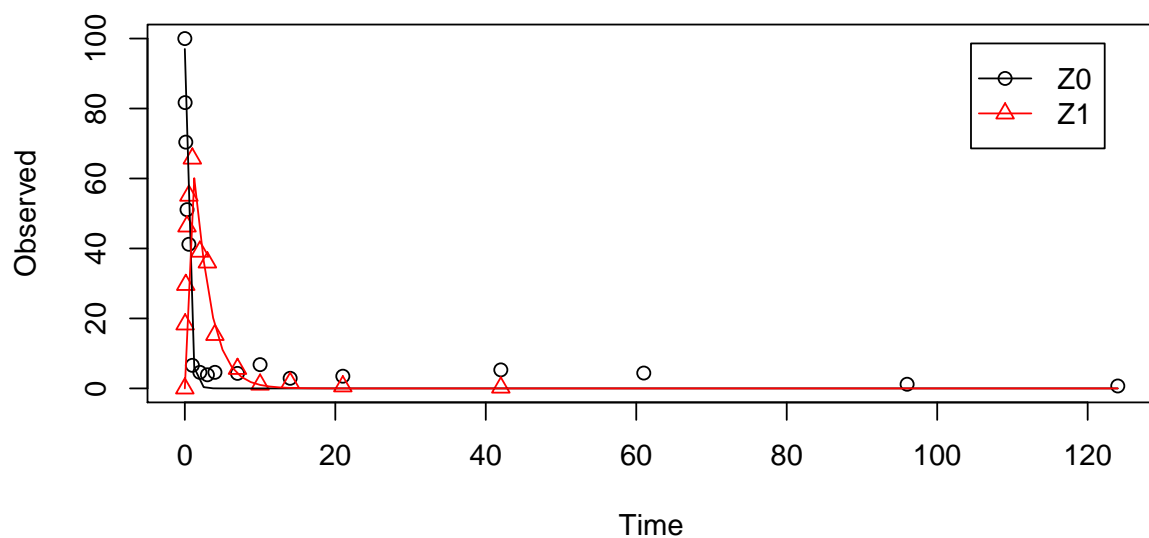
Here, the  $\text{ilr}$  transformed formation fraction fitted in the model takes a very large value, and the backtransformed formation fraction from parent Z to Z1 is practically unity. Again, the covariance matrix is not returned as the model is overparameterised.

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

```
Z.3 <- mkinmod(Z0 = list(type = "SF0", to = "Z1", sink = FALSE),
               Z1 = list(type = "SF0"), use_of_ff = "max")

## Successfully compiled differential equation model from auto-generated C code.

m.Z.3 <- mkinfit(Z.3, FOCUS_2006_Z_mkin, quiet = TRUE)
plot(m.Z.3)
```



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

## mkin version:    0.9.42
## R version:      3.2.4
```



```

## Date of fit:      Tue Mar 29 16:12:52 2016
## Date of summary: Tue Mar 29 16:12:52 2016
##
## Equations:
## d_Z0 = - k_Z0 * Z0
## d_Z1 = + k_Z0 * Z0 - k_Z1 * Z1
##
## Model predictions using solution type deSolve
##
## Fitted with method Port using 100 model solutions performed in 0.774 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    100.000000 -Inf  Inf
## log_k_Z0 -2.302585 -Inf  Inf
## log_k_Z1 -2.301586 -Inf  Inf
##
## Fixed parameter values:
##      value type
## Z1_0      0 state
##
## Optimised, transformed parameters with symmetric confidence intervals:
##      Estimate Std. Error  Lower  Upper
## Z0_0      97.0100    2.68200 91.5200 102.5000
## log_k_Z0   0.8047    0.06568  0.6702  0.9392
## log_k_Z1  -0.7296    0.08854 -0.9109 -0.5482
##
## Parameter correlation:
##      Z0_0 log_k_Z0 log_k_Z1
## Z0_0    1.0000  0.10628  0.41038
## log_k_Z0 0.1063  1.00000  0.04344
## log_k_Z1 0.4104  0.04344  1.00000
##
## Residual standard error: 4.973 on 28 degrees of freedom

```

```
##
## Backtransformed parameters:
## Confidence intervals for internally transformed parameters are asymmetric.
## t-test (unrealistically) based on the assumption of normal distribution
## for estimators of untransformed parameters.
##      Estimate t value      Pr(>t)      Lower      Upper
## Z0_0  97.0100    36.18 2.364e-25  91.5200 102.500
## k_Z0   2.2360    15.23 2.246e-15   1.9550   2.558
## k_Z1   0.4821    11.29 3.069e-12   0.4022   0.578
##
## Chi2 error levels in percent:
##      err.min n.optim df
## All data   17.61      3 27
## Z0          17.56      2 15
## Z1          15.08      1 12
##
## Estimated disappearance times:
##      DT50  DT90
## Z0 0.310 1.030
## Z1 1.438 4.776
```

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

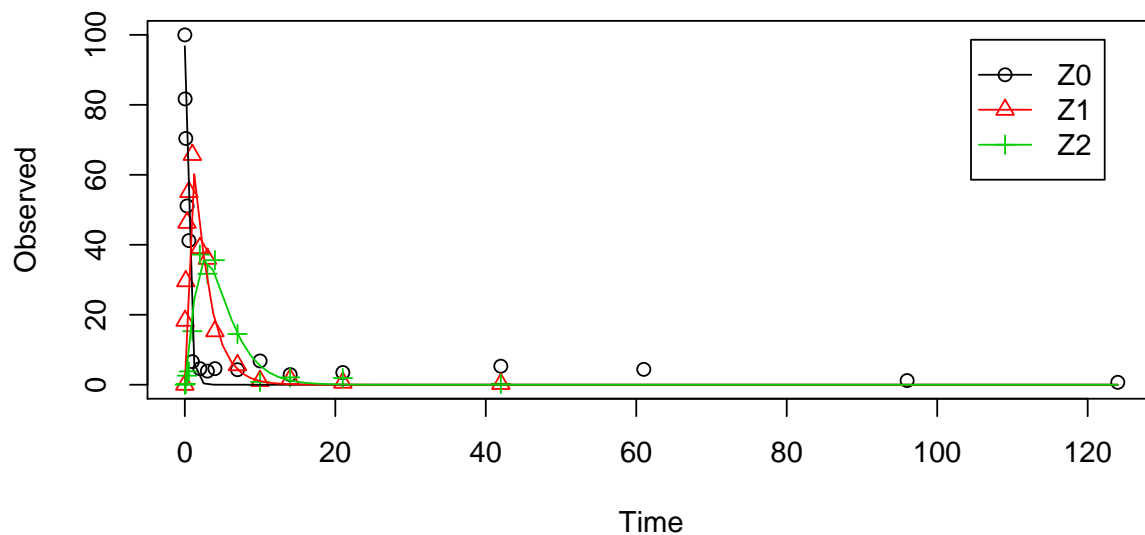
### 3 Including metabolites Z2 and Z3

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 = "SF0", to = "Z1", sink = FALSE),
              Z1 = list(type = "SF0", to = "Z2", sink = FALSE),
              Z2 = list(type = "SF0"))

## Successfully compiled differential equation model from auto-generated C
code.

m.Z.5 <- mkinfit(Z.5, FOCUS_2006_Z_mkin, quiet = TRUE)
plot(m.Z.5)
```



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

## mkin version:    0.9.42
## R version:      3.2.4
## Date of fit:     Tue Mar 29 16:12:54 2016
## Date of summary: Tue Mar 29 16:12:54 2016
##
## 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 deSolve
##
## Fitted with method Port using 184 model solutions performed in 2.079 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          100.000000 -Inf  Inf
## log_k_Z0_Z1    -2.302585 -Inf  Inf
## log_k_Z1_Z2    -2.301586 -Inf  Inf
## log_k_Z2_sink  -2.300587 -Inf  Inf
##
## Fixed parameter values:
##           value type
## Z1_0           0 state
## Z2_0           0 state
##
## Optimised, transformed parameters with symmetric confidence intervals:
##           Estimate Std. Error   Lower   Upper
## Z0_0          96.7700    2.26600 92.1900 101.3000
## log_k_Z0_Z1     0.7948    0.05843  0.6767  0.9129
## log_k_Z1_Z2    -0.7410    0.06821 -0.8789 -0.6032
## log_k_Z2_sink  -0.8027    0.11090 -1.0270 -0.5785
##
## Parameter correlation:
##           Z0_0 log_k_Z0_Z1 log_k_Z1_Z2 log_k_Z2_sink
## Z0_0          1.00000    0.05782    0.2875    0.31786
## log_k_Z0_Z1   0.05782    1.00000   -0.0436    0.01213
## log_k_Z1_Z2   0.28748   -0.04360    1.0000    0.24019
## log_k_Z2_sink 0.31786    0.01213    0.2402    1.00000
##
## Residual standard error: 4.486 on 40 degrees of freedom
##
## Backtransformed parameters:
## Confidence intervals for internally transformed parameters are asymmetric.
## t-test (unrealistically) based on the assumption of normal distribution
## for estimators of untransformed parameters.
##           Estimate t value   Pr(>t)   Lower   Upper
## Z0_0          96.7700  42.710 2.717e-35 92.1900 101.3000
## k_Z0_Z1        2.2140  17.120 2.615e-20  1.9670  2.4920
## k_Z1_Z2         0.4766  14.660 5.572e-18  0.4152  0.5471
## k_Z2_sink       0.4481   9.016 1.754e-11  0.3581  0.5607
##
## Chi2 error levels in percent:
##           err.min n.optim df
## All data      19.10      4 38

```

```
## Z0          17.43          2 15
## Z1          15.27          1 12
## Z2          19.57          1 11
##
## Resulting formation fractions:
##          ff
## Z0_Z1     1
## Z1_Z2     1
## Z2_sink   1
##
## Estimated disappearance times:
##          DT50  DT90
## Z0 0.3131 1.040
## Z1 1.4543 4.831
## Z2 1.5468 5.138
```

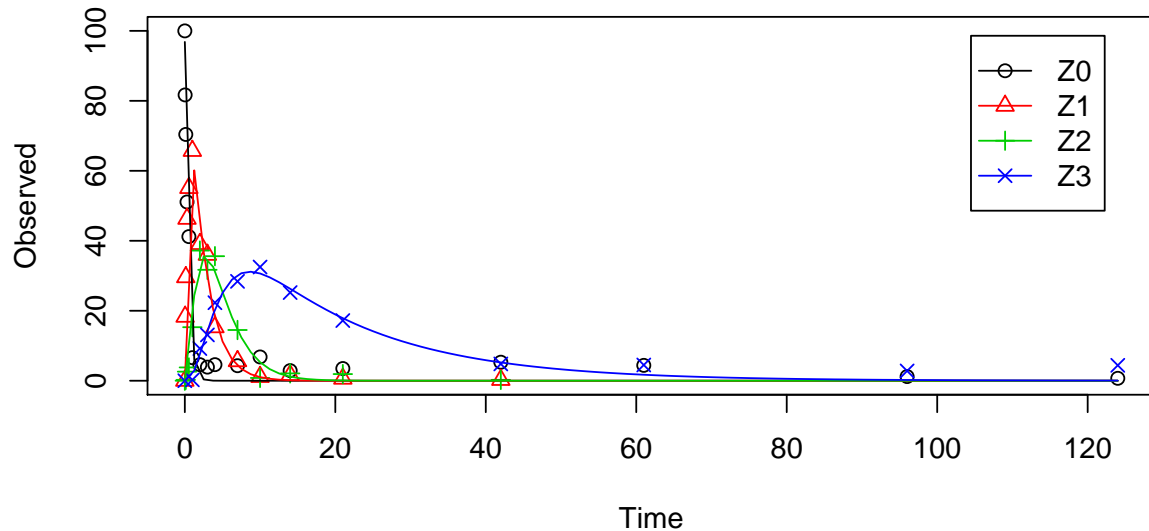
Finally, metabolite Z3 is added to the model. The fit is accelerated by using the starting parameters from the previous fit.

```
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"))

## Successfully compiled differential equation model from auto-generated C code.

m.Z.FOCUS <- mkinfit(Z.FOCUS, FOCUS_2006_Z_mkin,
                    quiet = TRUE)

plot(m.Z.FOCUS)
```



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

## mkin version:      0.9.42
## R version:        3.2.4
## Date of fit:       Tue Mar 29 16:13:03 2016
## Date of summary:  Tue Mar 29 16:13:03 2016
##
## 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 deSolve
##
## Fitted with method Port using 505 model solutions performed in 7.453 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
## k_Z2_Z3      0.1003 deparm
## k_Z3_sink    0.1004 deparm
##
## Starting values for the transformed parameters actually optimised:
##               value lower upper
## Z0_0          100.000000 -Inf  Inf
## log_k_Z0_Z1   -2.302585 -Inf  Inf
## log_k_Z1_Z2   -2.301586 -Inf  Inf
## log_k_Z2_sink -2.300587 -Inf  Inf
## log_k_Z2_Z3   -2.299590 -Inf  Inf
## log_k_Z3_sink -2.298593 -Inf  Inf
##
## Fixed parameter values:
##      value  type
## Z1_0      0 state
## Z2_0      0 state
## Z3_0      0 state
##
## Optimised, transformed parameters with symmetric confidence intervals:
##      Estimate Std. Error  Lower  Upper
## Z0_0         96.8400    2.05900 92.7100 101.0000
## log_k_Z0_Z1    0.7954    0.05332  0.6884  0.9025
## log_k_Z1_Z2   -0.7375    0.06123 -0.8605 -0.6146
## log_k_Z2_sink -1.4330    0.17160 -1.7770 -1.0880
## log_k_Z2_Z3   -1.5470    0.12250 -1.7930 -1.3010
## log_k_Z3_sink -2.8350    0.24360 -3.3240 -2.3460
##
## Parameter correlation:
##      Z0_0 log_k_Z0_Z1 log_k_Z1_Z2 log_k_Z2_sink
## Z0_0      1.00000    0.05384    0.27273    0.36998
## log_k_Z0_Z1 0.05384    1.00000   -0.05212    0.02437
## log_k_Z1_Z2 0.27273   -0.05212    1.00000    0.29389
## log_k_Z2_sink 0.36998    0.02437    0.29389    1.00000
## log_k_Z2_Z3 -0.07289   -0.03577   -0.12139   -0.18895
## log_k_Z3_sink -0.11335   -0.02513   -0.19154   -0.64296
##      log_k_Z2_Z3 log_k_Z3_sink
## Z0_0      -0.07289   -0.11335
## log_k_Z0_Z1 -0.03577   -0.02513
## log_k_Z1_Z2 -0.12139   -0.19154
## log_k_Z2_sink -0.18895   -0.64296
## log_k_Z2_Z3    1.00000    0.55157

```

```

## log_k_Z3_sink      0.55157      1.00000
##
## Residual standard error: 4.1 on 51 degrees of freedom
##
## Backtransformed parameters:
## Confidence intervals for internally transformed parameters are asymmetric.
## t-test (unrealistically) based on the assumption of normal distribution
## for estimators of untransformed parameters.
##           Estimate t value    Pr(>t)    Lower    Upper
## Z0_0      96.84000  47.040 5.575e-44  92.71000 101.00000
## k_Z0_Z1    2.21500  18.750 7.743e-25   1.99000   2.46600
## k_Z1_Z2    0.47830  16.330 3.339e-22   0.42300   0.54090
## k_Z2_sink  0.23870   5.827 1.911e-07   0.16910   0.33680
## k_Z2_Z3    0.21290   8.160 4.088e-11   0.16650   0.27230
## k_Z3_sink  0.05869   4.106 7.287e-05   0.03599   0.09571
##
## Chi2 error levels in percent:
##           err.min n.optim df
## All data   19.23      6 48
## Z0         17.45      2 15
## Z1         15.24      1 12
## Z2         20.32      2 10
## Z3         11.89      1 11
##
## Resulting formation fractions:
##           ff
## Z0_Z1     1.0000
## Z1_Z2     1.0000
## Z2_sink   0.5285
## Z2_Z3     0.4715
## Z3_sink   1.0000
##
## Estimated disappearance times:
##           DT50   DT90
## Z0   0.3129   1.039
## Z1   1.4492   4.814
## Z2   1.5348   5.099
## Z3  11.8097  39.231

```

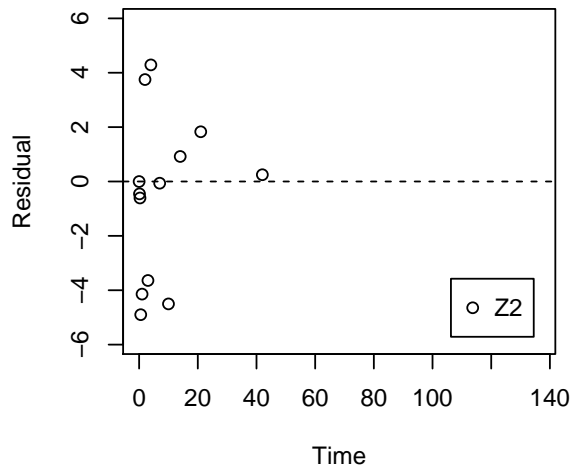
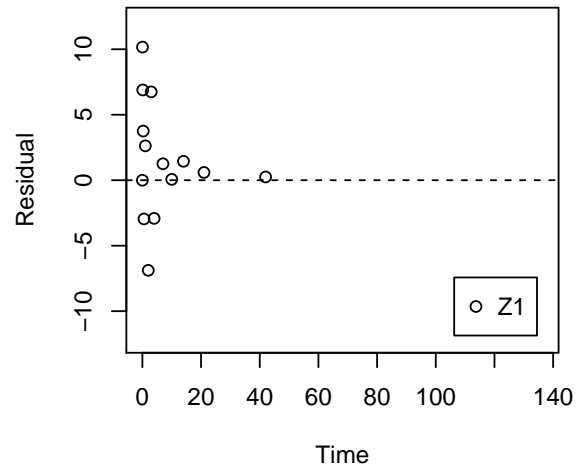
This is the fit corresponding to the final result chosen in Appendix 7 of the FOCUS report. The residual plots can be obtained by



```

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")

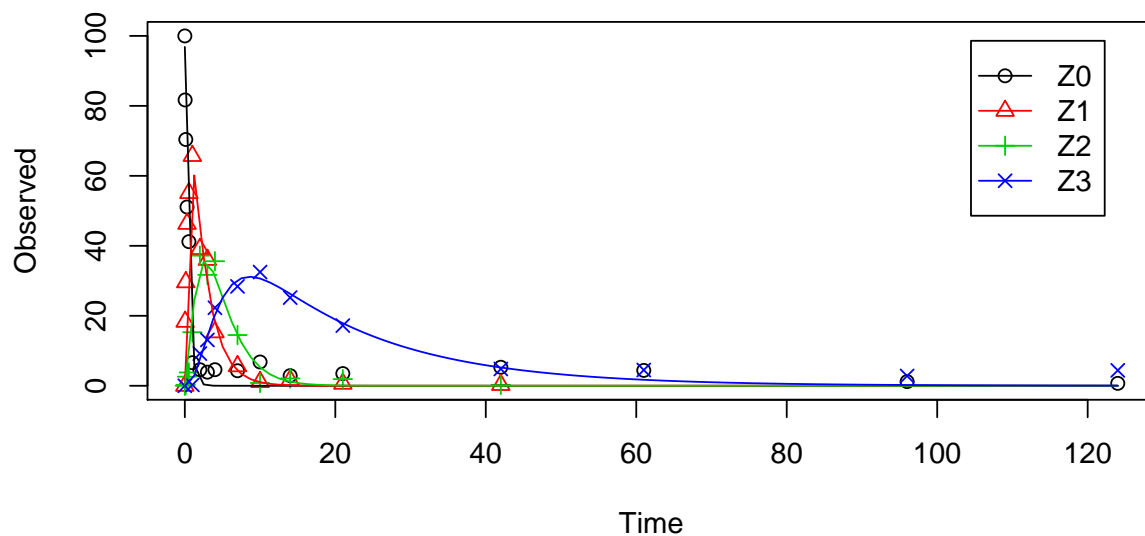
## Successfully compiled differential equation model from auto-generated C
code.

m.Z.FOCUS.ff <- mkinfit(Z.FOCUS.ff, FOCUS_2006_Z_mkin, quiet = TRUE)

## Warning in mkinfit(Z.FOCUS.ff, FOCUS_2006_Z_mkin, quiet = TRUE): Optimisation
by method Port did not converge.
## Convergence code is 1

plot(m.Z.FOCUS.ff)

```



```

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

## mkin version:    0.9.42
## R version:       3.2.4
## Date of fit:      Tue Mar 29 16:13:13 2016
## Date of summary: Tue Mar 29 16:13:13 2016
##
##
## Warning: Optimisation by method Port did not converge.

```

```

## Convergence code is 1
##
##
## 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 deSolve
##
## Fitted with method Port using 614 model solutions performed in 9.181 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       100.000000 -Inf  Inf
## log_k_Z0    -2.302585 -Inf  Inf
## log_k_Z1    -2.301586 -Inf  Inf
## log_k_Z2    -2.300587 -Inf  Inf
## log_k_Z3    -2.299590 -Inf  Inf
## f_Z2_ilr_1   0.000000 -Inf  Inf
##
## Fixed parameter values:
##           value  type
## Z1_0         0 state
## Z2_0         0 state
## Z3_0         0 state
##
## Optimised, transformed parameters with symmetric confidence intervals:
##           Estimate Std. Error  Lower  Upper
## Z0_0       96.84000    2.05900  92.7100 101.0000

```

```

## log_k_Z0      0.79540      0.05332  0.6884   0.9025
## log_k_Z1     -0.73750      0.06124 -0.8605  -0.6146
## log_k_Z2     -0.79490      0.09790 -0.9915  -0.5984
## log_k_Z3     -2.83500      0.24360 -3.3240  -2.3460
## f_Z2_ilr_1   -0.08069      0.16190 -0.4057   0.2443
##
## Parameter correlation:
##           Z0_0  log_k_Z0 log_k_Z1  log_k_Z2 log_k_Z3 f_Z2_ilr_1
## Z0_0         1.00000  0.053861  0.2728  0.299775 -0.1135  -0.31643
## log_k_Z0     0.05386  1.000000 -0.0521  0.001486 -0.0252  -0.03747
## log_k_Z1     0.27276 -0.052105  1.0000  0.200682 -0.1917  -0.28540
## log_k_Z2     0.29977  0.001486  0.2007  1.000000 -0.2702  -0.38864
## log_k_Z3    -0.11348 -0.025199 -0.1917 -0.270190  1.0000  0.77723
## f_Z2_ilr_1  -0.31643 -0.037466 -0.2854 -0.388640  0.7772  1.00000
##
## Residual standard error: 4.1 on 51 degrees of freedom
##
## Backtransformed parameters:
## Confidence intervals for internally transformed parameters are asymmetric.
## t-test (unrealistically) based on the assumption of normal distribution
## for estimators of untransformed parameters.
##           Estimate t value      Pr(>t)      Lower      Upper
## Z0_0         96.84000  47.040 5.573e-44  92.71000 101.00000
## k_Z0          2.21500  18.750 7.744e-25  1.99100  2.46600
## k_Z1          0.47830  16.330 3.329e-22  0.42300  0.54090
## k_Z2          0.45160  10.210 3.108e-14  0.37100  0.54970
## k_Z3          0.05869   4.106 7.282e-05  0.03599  0.09571
## f_Z2_to_Z3   0.47150   8.265 2.804e-11  0.36040  0.58550
##
## Chi2 error levels in percent:
##           err.min n.optim df
## All data    19.23      6 48
## Z0          17.45      2 15
## Z1          15.24      1 12
## Z2          19.61      1 11
## Z3          12.32      2 10
##
## Resulting formation fractions:
##           ff
## Z2_Z3      0.4715
## Z2_sink    0.5285
##

```

```
## Estimated disappearance times:
##      DT50   DT90
## Z0  0.3129  1.039
## Z1  1.4492  4.814
## Z2  1.5348  5.098
## Z3 11.8097 39.231
```

## 4 Using the SFORB model for parent and metabolites

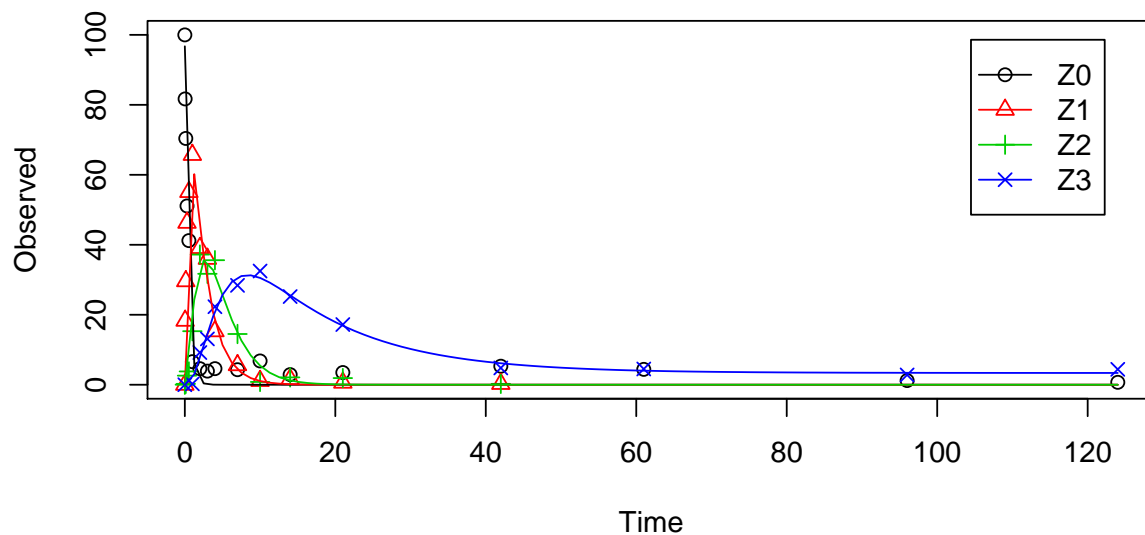
As the FOCUS report states, there is a certain tailing of the time course of metabolite Z3. Also, the time course of the parent compound is not fitted very well using the SFO model, as residues at a certain low level remain.

Therefore, an additional model is offered here, using the single first-order reversible binding (SFORB) model for metabolite Z3. As expected, the  $\chi^2$  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"))

## Successfully compiled differential equation model from auto-generated C code.

m.Z.mkin.1 <- mkinfit(Z.mkin.1, FOCUS_2006_Z_mkin, quiet = TRUE)
plot(m.Z.mkin.1)
```



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

## mkin version:    0.9.42
## R version:      3.2.4
## Date of fit:     Tue Mar 29 16:13:27 2016
## Date of summary: Tue Mar 29 16:13:27 2016
##
## 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 deSolve
##
## Fitted with method Port using 890 model solutions performed in 13.47 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
## k_Z2_Z3_free    0.1003 deparm
## k_Z3_free_sink  0.1004 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          100.000000 -Inf  Inf
## log_k_Z0_Z1    -2.302585 -Inf  Inf
## log_k_Z1_Z2    -2.301586 -Inf  Inf
## log_k_Z2_sink  -2.300587 -Inf  Inf
## log_k_Z2_Z3_free -2.299590 -Inf  Inf
## log_k_Z3_free_sink -2.298593 -Inf  Inf
## log_k_Z3_free_bound -2.302585 -Inf  Inf
## log_k_Z3_bound_free -3.912023 -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 with symmetric confidence intervals:
##              Estimate Std. Error Lower Upper
## Z0_0           96.7400          NA    NA    NA
## log_k_Z0_Z1      0.7947          NA    NA    NA
## log_k_Z1_Z2     -0.7426          NA    NA    NA
## log_k_Z2_sink    -1.4950          NA    NA    NA
## log_k_Z2_Z3_free -1.5040          NA    NA    NA
## log_k_Z3_free_sink -2.6540          NA    NA    NA
## log_k_Z3_free_bound -5.2440          NA    NA    NA
## log_k_Z3_bound_free -21.8100          NA    NA    NA
##
## Parameter correlation:
## Could not estimate covariance matrix; singular system:
##

```

```

## Residual standard error: 4.107 on 49 degrees of freedom
##
## Backtransformed parameters:
## Confidence intervals for internally transformed parameters are asymmetric.
## t-test (unrealistically) based on the assumption of normal distribution
## for estimators of untransformed parameters.
##
##           Estimate   t value   Pr(>t) Lower Upper
## Z0_0          9.674e+01 4.683e+01 1.197e-42    NA    NA
## k_Z0_Z1        2.214e+00 1.871e+01 2.943e-24    NA    NA
## k_Z1_Z2        4.759e-01 1.619e+01 1.328e-21    NA    NA
## k_Z2_sink      2.243e-01 4.747e+00 9.147e-06    NA    NA
## k_Z2_Z3_free   2.222e-01 7.134e+00 2.059e-09    NA    NA
## k_Z3_free_sink 7.034e-02 2.743e+00 4.242e-03    NA    NA
## k_Z3_free_bound 5.279e-03 4.513e-01 3.269e-01    NA    NA
## k_Z3_bound_free 3.357e-10 1.128e-08 5.000e-01    NA    NA
##
## Chi2 error levels in percent:
##           err.min n.optim df
## All data  19.406      8 47
## Z0         17.429      2 15
## Z1         15.275      1 12
## Z2         20.279      2 10
## Z3          8.562      3 10
##
## Estimated Eigenvalues of SFORB model(s):
##           Z3_b1      Z3_b2
## 7.562e-02 3.123e-10
##
## Resulting 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 disappearance times:
##           DT50   DT90 DT50_Z3_b1 DT50_Z3_b2
## Z0  0.3131  1.040      NA      NA
## Z1  1.4566  4.839      NA      NA
## Z2  1.5523  5.157      NA      NA
## Z3 10.1979 45.329     9.166  2.22e+09

```

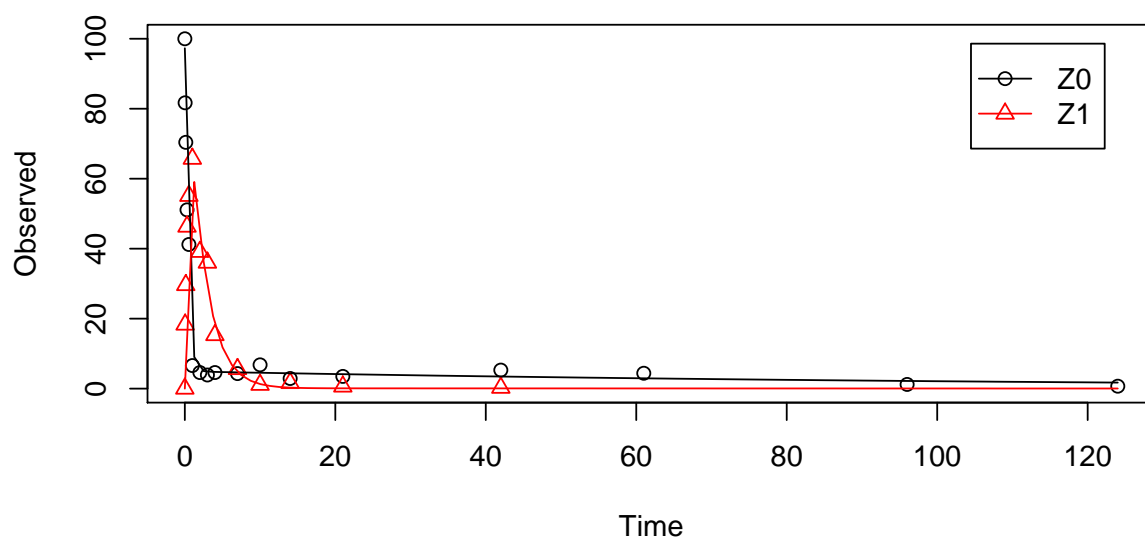


Therefore, a further stepwise model building is performed starting from the stage of parent and one metabolite, starting from the assumption that the model fit for the parent compound can be improved by using the SFORB model.

```
Z.mkin.2 <- mkinmod(Z0 = list(type = "SFORB", to = "Z1", sink = FALSE),
                    Z1 = list(type = "SFO"))

## Successfully compiled differential equation model from auto-generated C
## code.

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:      0.9.42
## R version:         3.2.4
## Date of fit:        Tue Mar 29 16:13:29 2016
## Date of summary:    Tue Mar 29 16:13:29 2016
##
## 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 deSolve
##
## Fitted with method Port using 139 model solutions performed in 1.129 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      100.000000  -Inf  Inf
## log_k_Z0_free_bound -2.302585  -Inf  Inf
## log_k_Z0_bound_free -3.912023  -Inf  Inf
## log_k_Z0_free_Z1    -2.300587  -Inf  Inf
## log_k_Z1_sink       -2.299590  -Inf  Inf
##
## Fixed parameter values:
##           value  type
## Z0_bound_0      0  state
## Z1_0            0  state
##
## Optimised, transformed parameters with symmetric confidence intervals:
##
##           Estimate Std. Error  Lower  Upper
## Z0_free_0      97.2900    2.39500  92.3600 102.2000
## log_k_Z0_free_bound -2.0820    0.43220 -2.9700  -1.1940
## log_k_Z0_bound_free -4.7200    1.60400 -8.0180  -1.4220
## log_k_Z0_free_Z1     0.8549    0.06430  0.7227   0.9871
## log_k_Z1_sink       -0.7934    0.08506 -0.9682  -0.6185
##
## Parameter correlation:
##
##           Z0_free_0 log_k_Z0_free_bound log_k_Z0_bound_free
## Z0_free_0      1.000000          0.006481          0.03321
## log_k_Z0_free_bound 0.006481          1.000000          0.54640
## log_k_Z0_bound_free 0.033215          0.546405          1.00000
## log_k_Z0_free_Z1    0.111812          0.413909          0.15831

```

```

## log_k_Z1_sink      0.391560      -0.291886      -0.12595
##                  log_k_Z0_free_Z1 log_k_Z1_sink
## Z0_free_0          0.11181      0.39156
## log_k_Z0_free_bound 0.41391      -0.29189
## log_k_Z0_bound_free 0.15831      -0.12595
## log_k_Z0_free_Z1    1.00000      -0.04187
## log_k_Z1_sink       -0.04187      1.00000
##
## Residual standard error: 4.438 on 26 degrees of freedom
##
## Backtransformed parameters:
## Confidence intervals for internally transformed parameters are asymmetric.
## t-test (unrealistically) based on the assumption of normal distribution
## for estimators of untransformed parameters.
##           Estimate t value    Pr(>t)      Lower    Upper
## Z0_free_0    97.290000 40.6100 2.364e-25 9.236e+01 102.2000
## k_Z0_free_bound 0.124700 2.3140 1.442e-02 5.128e-02 0.3031
## k_Z0_bound_free 0.008912 0.6231 2.693e-01 3.294e-04 0.2411
## k_Z0_free_Z1   2.351000 15.5500 5.520e-15 2.060e+00 2.6830
## k_Z1_sink      0.452300 11.7600 3.299e-12 3.798e-01 0.5387
##
## Chi2 error levels in percent:
##           err.min n.optim df
## All data    15.63      5 25
## Z0          14.74      4 13
## Z1          14.31      1 12
##
## Estimated Eigenvalues of SFORB model(s):
##      Z0_b1      Z0_b2
## 2.476314 0.008461
##
## 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 1.190      0.2799      81.92
## Z1 1.532 5.091          NA          NA

```

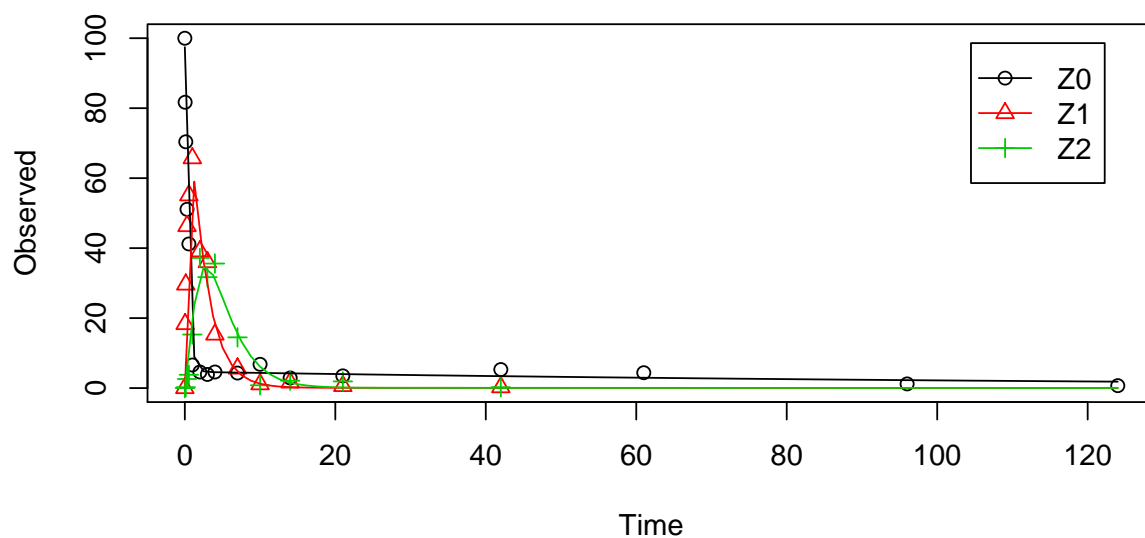
When metabolite Z2 is added, the additional sink for Z1 is turned off again, for the same

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"))

## Successfully compiled differential equation model from auto-generated C
## code.

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:    0.9.42
## R version:       3.2.4
## Date of fit:      Tue Mar 29 16:13:34 2016
## Date of summary: Tue Mar 29 16:13:34 2016
##
## 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 deSolve
##
## Fitted with method Port using 355 model solutions performed in 4.129 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      100.000000  -Inf  Inf
## log_k_Z0_free_bound -2.302585  -Inf  Inf
## log_k_Z0_bound_free -3.912023  -Inf  Inf
## log_k_Z0_free_Z1    -2.300587  -Inf  Inf
## log_k_Z1_Z2         -2.299590  -Inf  Inf
## log_k_Z2_sink       -2.298593  -Inf  Inf
##
## Fixed parameter values:
##           value  type
## Z0_bound_0      0  state
## Z1_0            0  state
## Z2_0            0  state
##
## Optimised, transformed parameters with symmetric confidence intervals:
##
##           Estimate Std. Error  Lower  Upper
## Z0_free_0      97.4400    2.07100  93.2400 101.6000
## log_k_Z0_free_bound -2.1490    0.40510 -2.9690 -1.3290
## log_k_Z0_bound_free -4.8380    1.62600 -8.1310 -1.5460
## log_k_Z0_free_Z1     0.8457    0.05832  0.7276  0.9638
## log_k_Z1_Z2        -0.7812    0.06486 -0.9125 -0.6499
## log_k_Z2_sink       -0.8606    0.10570 -1.0750 -0.6466
##
## Parameter correlation:
##
##           Z0_free_0 log_k_Z0_free_bound log_k_Z0_bound_free

```

```

## Z0_free_0      1.00000      0.0761      0.07283
## log_k_Z0_free_bound 0.07610      1.0000      0.54561
## log_k_Z0_bound_free 0.07283      0.5456      1.00000
## log_k_Z0_free_Z1 0.09111      0.4249      0.16182
## log_k_Z1_Z2      0.25675      -0.2290      -0.09190
## log_k_Z2_sink    0.28871      -0.2097      -0.07849
##               log_k_Z0_free_Z1 log_k_Z1_Z2 log_k_Z2_sink
## Z0_free_0      0.09111      0.2567      0.28871
## log_k_Z0_free_bound 0.42492      -0.2290      -0.20973
## log_k_Z0_bound_free 0.16182      -0.0919      -0.07849
## log_k_Z0_free_Z1 1.00000      -0.1015      -0.04913
## log_k_Z1_Z2      -0.10153      1.0000      0.27278
## log_k_Z2_sink    -0.04913      0.2728      1.00000
##
## Residual standard error: 4.081 on 38 degrees of freedom
##
## Backtransformed parameters:
## Confidence intervals for internally transformed parameters are asymmetric.
## t-test (unrealistically) based on the assumption of normal distribution
## for estimators of untransformed parameters.
##               Estimate t value      Pr(>t)      Lower      Upper
## Z0_free_0      97.44000 47.0400 1.357e-35 9.324e+01 101.6000
## k_Z0_free_bound 0.11660  2.4700 9.068e-03 5.137e-02  0.2648
## k_Z0_bound_free 0.00792  0.6191 2.698e-01 2.944e-04  0.2131
## k_Z0_free_Z1    2.33000 17.1200 9.291e-20 2.070e+00  2.6220
## k_Z1_Z2          0.45790 15.4200 3.058e-18 4.015e-01  0.5221
## k_Z2_sink        0.42290  9.4590 7.825e-12 3.414e-01  0.5238
##
## Chi2 error levels in percent:
##               err.min n.optim df
## All data      17.33      6 36
## Z0             14.67      4 13
## Z1             14.41      1 12
## Z2             20.29      1 11
##
## Estimated Eigenvalues of SFORB model(s):
##      Z0_b1      Z0_b2
## 2.446637 0.007542
##
## 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 0.3043 1.185      0.2833      91.91
## Z1 1.5138 5.029      NA          NA
## Z2 1.6391 5.445      NA          NA
```

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

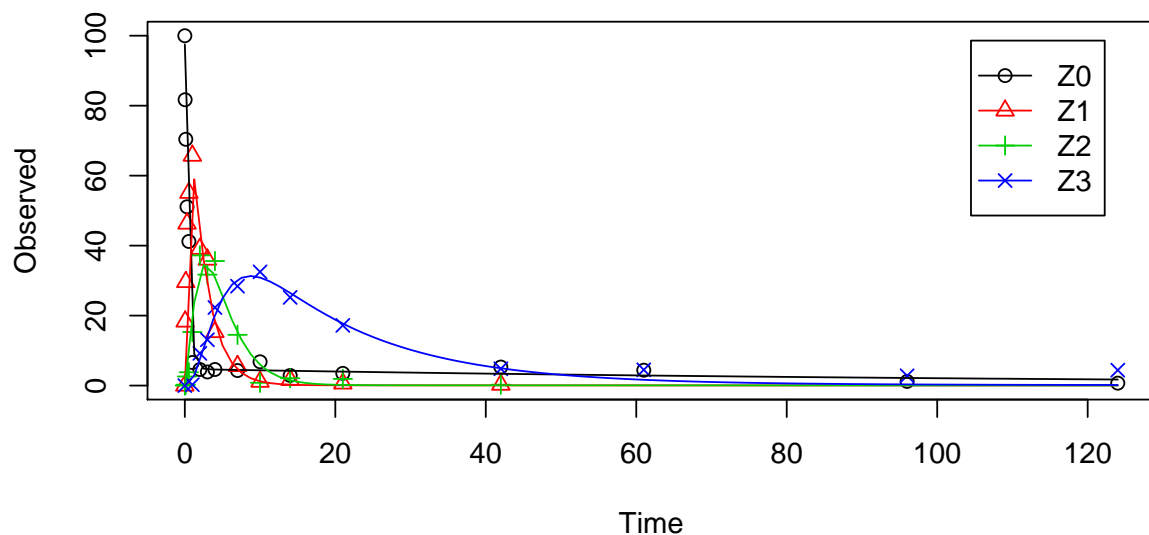
Finally, Z3 is added as well. These models appear overparameterised (no covariance matrix returned) if the sink for Z1 is left in the models.

```
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"))

## Successfully compiled differential equation model from auto-generated C
## code.

m.Z.mkin.4 <- mkinfit(Z.mkin.4, FOCUS_2006_Z_mkin,
                    quiet = TRUE)

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



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

## mkin version:      0.9.42
## R version:         3.2.4
## Date of fit:        Tue Mar 29 16:13:42 2016
## Date of summary:    Tue Mar 29 16:13:42 2016
##
## 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 deSolve
##
## Fitted with method Port using 521 model solutions performed in 7.803 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
## k_Z2_Z3          0.1005 deparm
## k_Z3_sink        0.1006 deparm
##
## Starting values for the transformed parameters actually optimised:
##              value lower upper
## Z0_free_0      100.000000 -Inf  Inf
## log_k_Z0_free_bound -2.302585 -Inf  Inf
## log_k_Z0_bound_free -3.912023 -Inf  Inf
## log_k_Z0_free_Z1    -2.300587 -Inf  Inf
## log_k_Z1_Z2        -2.299590 -Inf  Inf
## log_k_Z2_sink       -2.298593 -Inf  Inf
## log_k_Z2_Z3        -2.297598 -Inf  Inf
```



```

## log_k_Z3_sink      -2.296603  -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 with symmetric confidence intervals:
##           Estimate Std. Error   Lower   Upper
## Z0_free_0       97.5300    1.88700  93.7400 101.3000
## log_k_Z0_free_bound -2.1360    0.36820 -2.8760 -1.3960
## log_k_Z0_bound_free -4.7650    1.41800 -7.6140 -1.9160
## log_k_Z0_free_Z1    0.8470    0.05339  0.7398  0.9543
## log_k_Z1_Z2     -0.7769    0.05834 -0.8942 -0.6597
## log_k_Z2_sink    -1.5610    0.18260 -1.9280 -1.1940
## log_k_Z2_Z3     -1.5280    0.11350 -1.7560 -1.2990
## log_k_Z3_sink    -2.7690    0.22460 -3.2200 -2.3180
##
## Parameter correlation:
##           Z0_free_0 log_k_Z0_free_bound log_k_Z0_bound_free
## Z0_free_0      1.00000           0.07822           0.06921
## log_k_Z0_free_bound 0.07822           1.00000           0.53978
## log_k_Z0_bound_free 0.06921           0.53978           1.00000
## log_k_Z0_free_Z1    0.08882           0.42765           0.16282
## log_k_Z1_Z2         0.24238          -0.22742          -0.08936
## log_k_Z2_sink       0.32993          -0.26331          -0.12744
## log_k_Z2_Z3        -0.07494           0.06698           0.06082
## log_k_Z3_sink       -0.10456           0.13844           0.12526
##           log_k_Z0_free_Z1 log_k_Z1_Z2 log_k_Z2_sink
## Z0_free_0           0.08882    0.24238    0.32993
## log_k_Z0_free_bound 0.42765   -0.22742   -0.26331
## log_k_Z0_bound_free 0.16282   -0.08936   -0.12744
## log_k_Z0_free_Z1    1.00000   -0.10841   -0.05309
## log_k_Z1_Z2        -0.10841    1.00000    0.34052
## log_k_Z2_sink      -0.05309    0.34052    1.00000
## log_k_Z2_Z3        -0.01281   -0.14885   -0.25473
## log_k_Z3_sink       0.01858   -0.22486   -0.68320
##           log_k_Z2_Z3 log_k_Z3_sink
## Z0_free_0       -0.07494   -0.10456
## log_k_Z0_free_bound 0.06698    0.13844

```

```

## log_k_Z0_bound_free      0.06082      0.12526
## log_k_Z0_free_Z1        -0.01281      0.01858
## log_k_Z1_Z2             -0.14885     -0.22486
## log_k_Z2_sink           -0.25473     -0.68320
## log_k_Z2_Z3             1.00000      0.56390
## log_k_Z3_sink           0.56390      1.00000
##
## Residual standard error: 3.737 on 49 degrees of freedom
##
## Backtransformed parameters:
## Confidence intervals for internally transformed parameters are asymmetric.
## t-test (unrealistically) based on the assumption of normal distribution
## for estimators of untransformed parameters.
##
##           Estimate t value    Pr(>t)      Lower      Upper
## Z0_free_0      97.530000 51.7000 1.033e-44 9.374e+01 101.3000
## k_Z0_free_bound 0.118100 2.7160 4.548e-03 5.636e-02 0.2475
## k_Z0_bound_free 0.008522 0.7054 2.419e-01 4.936e-04 0.1471
## k_Z0_free_Z1   2.333000 18.7300 2.790e-24 2.095e+00 2.5970
## k_Z1_Z2        0.459800 17.1400 1.223e-22 4.089e-01 0.5170
## k_Z2_sink      0.209900 5.4770 7.393e-07 1.455e-01 0.3030
## k_Z2_Z3        0.217000 8.8070 5.733e-12 1.728e-01 0.2727
## k_Z3_sink      0.062720 4.4520 2.459e-05 3.994e-02 0.0985
##
## Chi2 error levels in percent:
##           err.min n.optim df
## All data    17.50      8 46
## Z0          14.69      4 13
## Z1          14.39      1 12
## Z2          21.05      2 10
## Z3          11.76      1 11
##
## Estimated Eigenvalues of SFORB model(s):
##   Z0_b1  Z0_b2
## 2.45127 0.00811
##
## Resulting formation fractions:
##           ff
## Z0_free_Z1 1.0000
## Z1_Z2      1.0000
## Z2_sink    0.4917
## Z2_Z3      0.5083
## Z3_sink    1.0000

```

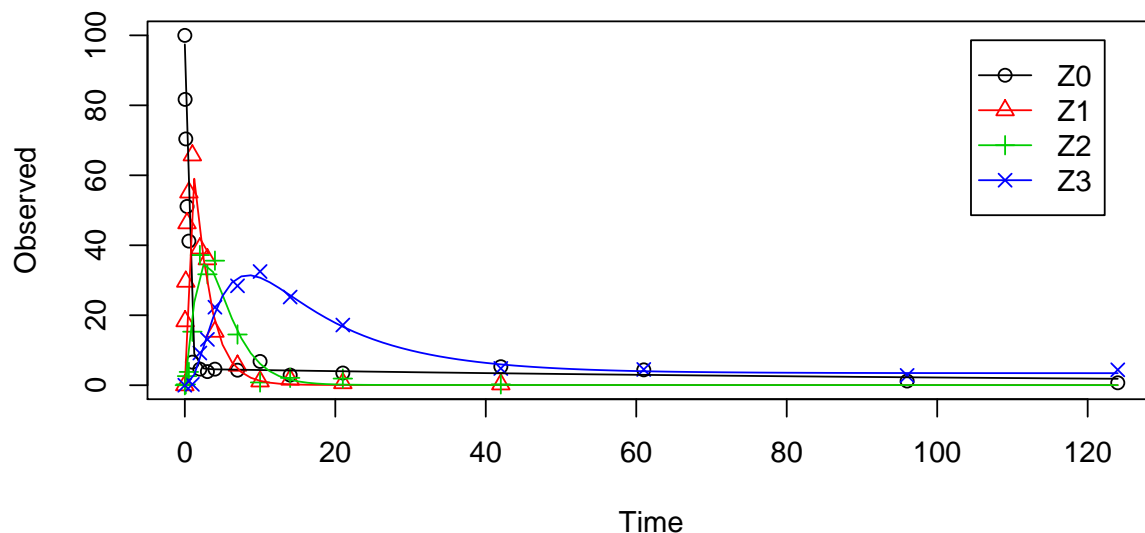
```
##
## Estimated disappearance times:
##      DT50   DT90 DT50_Z0_b1 DT50_Z0_b2
## Z0  0.304  1.186      0.2828      85.47
## Z1  1.507  5.008          NA          NA
## Z2  1.623  5.393          NA          NA
## Z3 11.051 36.712          NA          NA
```

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"))

## Successfully compiled differential equation model from auto-generated C
## code.

m.Z.mkin.5 <- mkinfit(Z.mkin.5, FOCUS_2006_Z_mkin, quiet = TRUE)
plot(m.Z.mkin.5)
```



```
summary(m.Z.mkin.5, data = FALSE)$bpar
##      Estimate se_notrans      t value      Pr(>t)
```

```
## Z0_free_0      9.742492e+01 1.88598230 5.165739e+01 2.317770e-43
## k_Z0_free_bound 1.167577e-01 0.04302935 2.713443e+00 4.639007e-03
## k_Z0_bound_free 7.890217e-03 0.01162538 6.787061e-01 2.503263e-01
## k_Z0_free_Z1   2.330000e+00 0.12411169 1.877341e+01 8.886332e-24
## k_Z1_Z2        4.575902e-01 0.02681632 1.706387e+01 4.498639e-22
## k_Z2_sink      1.957089e-01 0.04390924 4.457123e+00 2.565165e-05
## k_Z2_Z3_free   2.265847e-01 0.02917124 7.767400e+00 2.838550e-10
## k_Z3_free_sink  7.478934e-02 0.02428902 3.079142e+00 1.730764e-03
## k_Z3_free_bound 5.217955e-03 0.01033260 5.049994e-01 3.079595e-01
## k_Z3_bound_free 1.681758e-10 0.02671860 6.294335e-09 5.000000e-01
##               Lower Upper
## Z0_free_0      NA      NA
## k_Z0_free_bound NA      NA
## k_Z0_bound_free NA      NA
## k_Z0_free_Z1   NA      NA
## k_Z1_Z2        NA      NA
## k_Z2_sink      NA      NA
## k_Z2_Z3_free   NA      NA
## k_Z3_free_sink  NA      NA
## k_Z3_free_bound NA      NA
## k_Z3_bound_free 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 seems reasonable to fix it to zero.

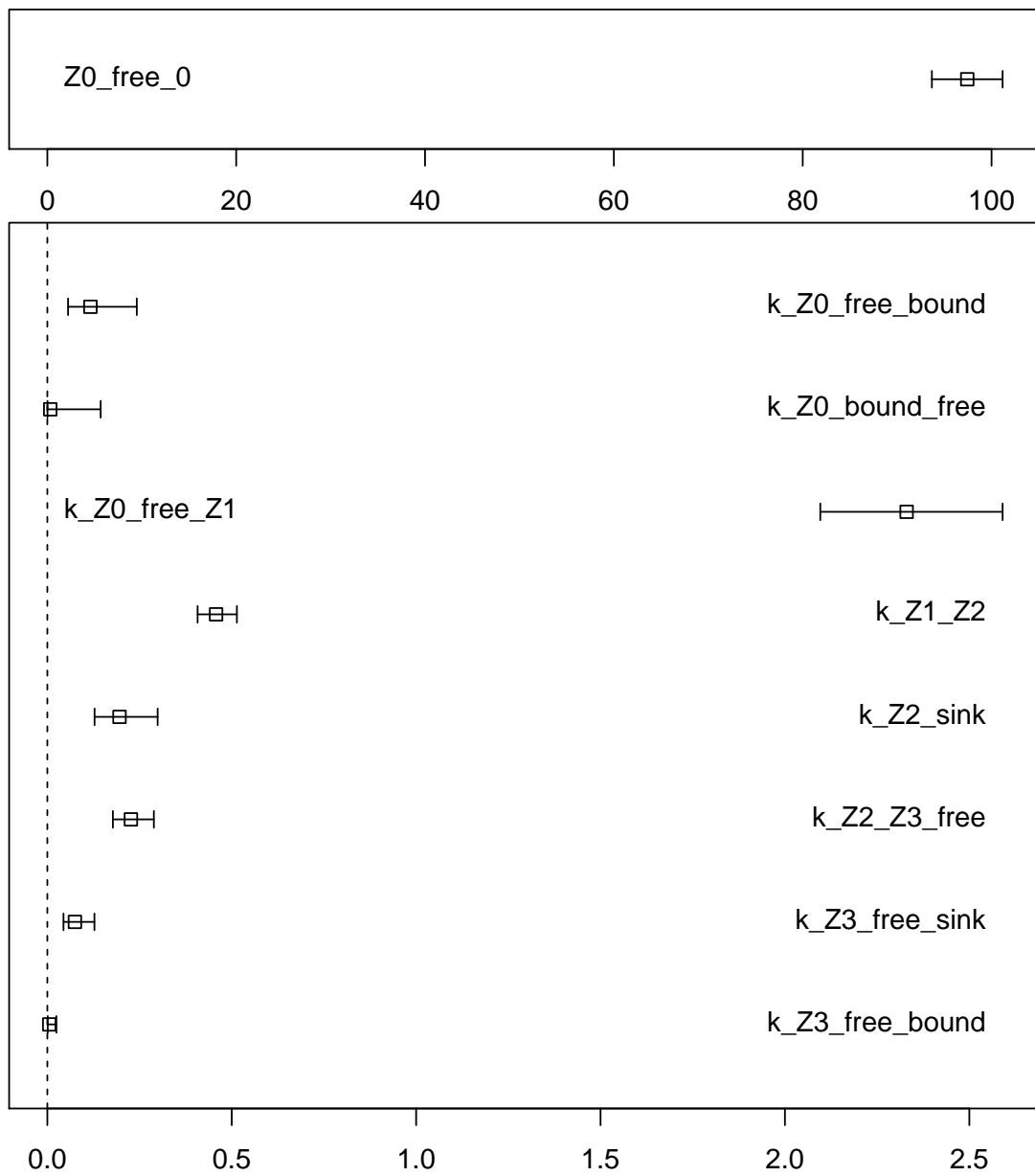
```
m.Z.mkin.5a <- mkinfit(Z.mkin.5, FOCUS_2006_Z_mkin,
  parms.ini = c(k_Z3_bound_free = 0),
  fixed_parms = "k_Z3_bound_free",
  quiet = TRUE)
summary(m.Z.mkin.5a, data = FALSE)$bpar

##               Estimate se_notrans      t value      Pr(>t)
## Z0_free_0      97.424949627 1.864206573 52.2608122 2.866823e-44
## k_Z0_free_bound 0.116758072 0.042594555 2.7411502 4.287410e-03
## k_Z0_bound_free 0.007890362 0.011516391 0.6851419 2.482749e-01
## k_Z0_free_Z1   2.330001269 0.122812327 18.9720472 3.016997e-24
## k_Z1_Z2        0.457590149 0.026446751 17.3023202 1.444452e-22
## k_Z2_sink      0.195709301 0.041414039 4.7256753 1.019075e-05
## k_Z2_Z3_free   0.226584924 0.027406955 8.2674243 4.355148e-11
## k_Z3_free_sink 0.074789313 0.020015159 3.7366335 2.478957e-04
## k_Z3_free_bound 0.005217946 0.004032924 1.2938370 1.009565e-01
##               Lower      Upper
```

```
## Z0_free_0      9.367838e+01 101.17151960
## k_Z0_free_bound 5.620707e-02  0.24253975
## k_Z0_bound_free 4.306506e-04  0.14456686
## k_Z0_free_Z1   2.095869e+00  2.59028835
## k_Z1_Z2        4.074130e-01  0.51394711
## k_Z2_sink      1.280681e-01  0.29907634
## k_Z2_Z3_free   1.777345e-01  0.28886198
## k_Z3_free_sink  4.379588e-02  0.12771615
## k_Z3_free_bound 1.113594e-03  0.02444962
```

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

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



The endpoints obtained with this model are

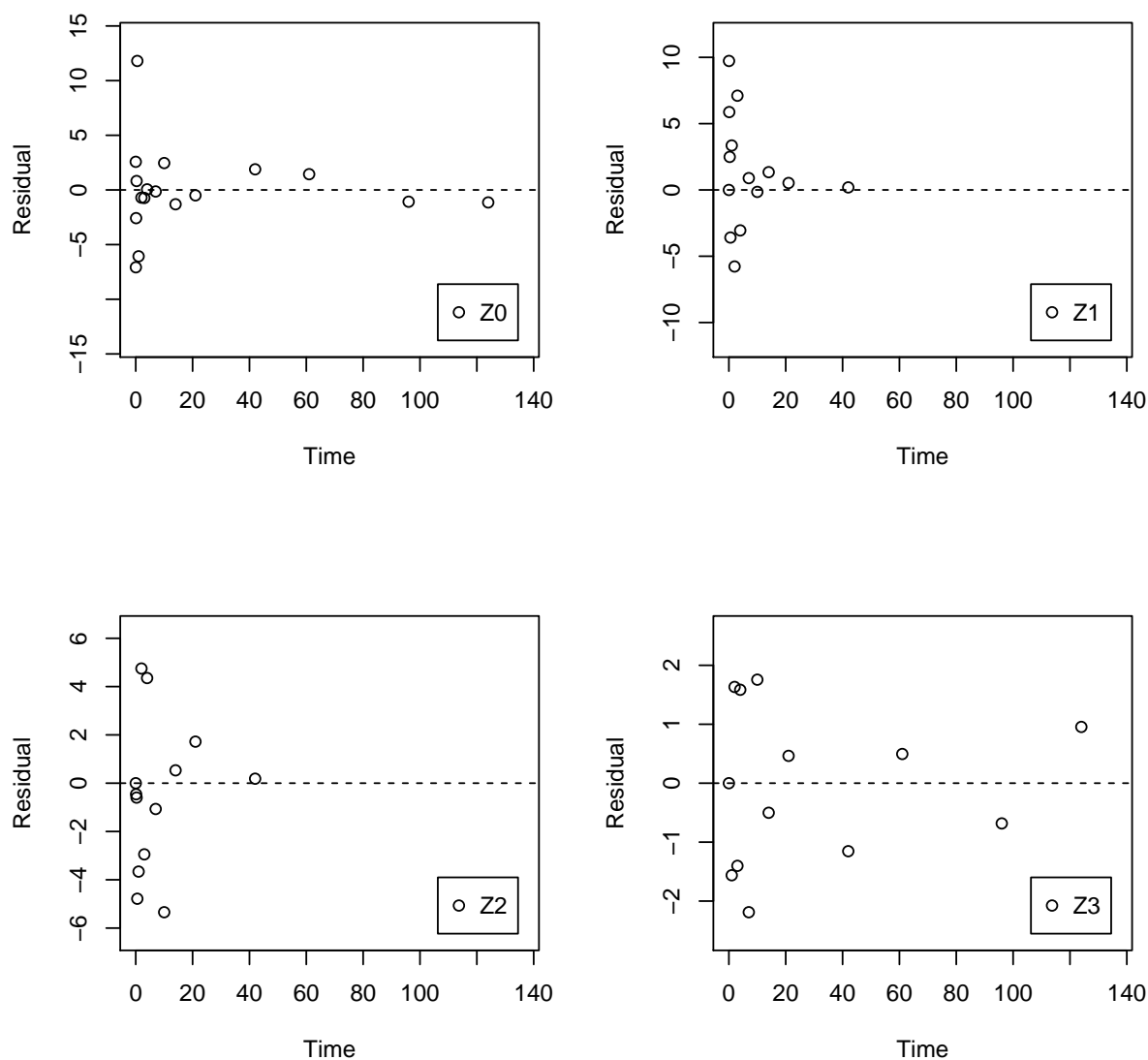
```
endpoints(m.Z.mkin.5a)

## $ff
##   Z0_free_Z1      Z1_Z2      Z2_sink      Z2_Z3_free      Z3_free_sink
##   1.000000      1.000000      0.463443      0.536557      1.000000
##
## $SFORB
##      Z0_b1      Z0_b2      Z3_b1      Z3_b2
## 2.447137024 0.007512678 0.080007259 0.000000000
```

```
##
## $distimes
##          DT50          DT90 DT50_Z0_b1 DT50_Z0_b2 DT50_Z3_b1 DT50_Z3_b2
## Z0 0.3042974 1.184810 0.2832482 92.26366          NA          NA
## Z1 1.5147773 5.031981          NA          NA          NA          NA
## Z2 1.6413845 5.452561          NA          NA          NA          NA
## Z3          NA          NA          NA          NA 8.663554          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>.