

# 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)

## 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, 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))

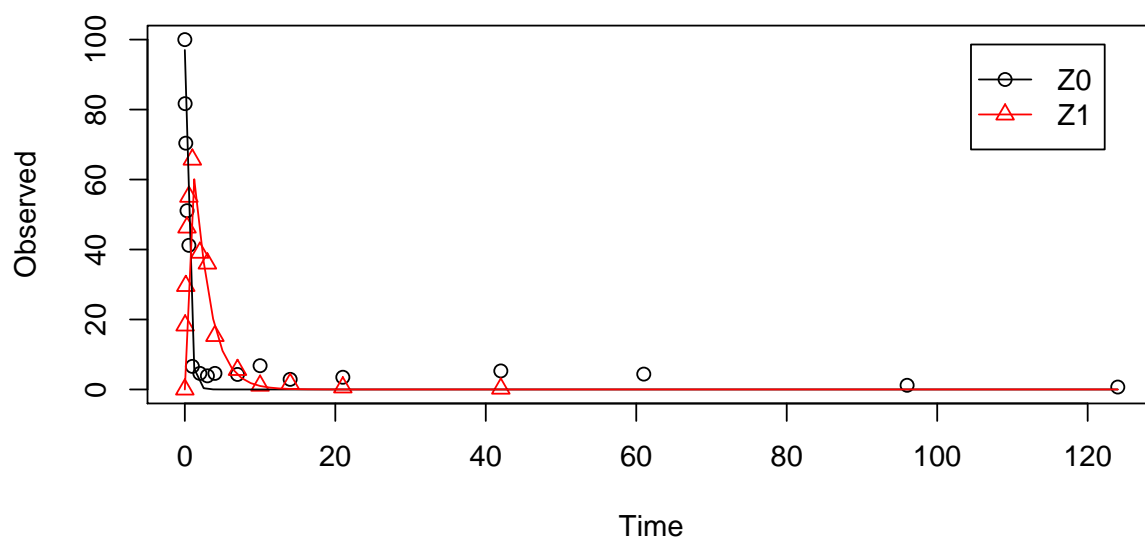
FOCUS_2006_Z_mkin <- mkin_wide_to_long(FOCUS_2006_Z)
```

## 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:         3.1.2
## Date of fit:       Wed Nov 12 18:10:29 2014
## Date of summary:  Wed Nov 12 18:10:29 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 4.059 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:
##           Estimate Std. Error Lower Upper t value Pr(>|t|) Pr(>t)
## Z0_0          97.0100         NA     NA     NA     NA     NA     NA
## log_k_Z0_sink -21.9100         NA     NA     NA     NA     NA     NA
## log_k_Z0_Z1    0.8047         NA     NA     NA     NA     NA     NA
## log_k_Z1_sink  -0.7296         NA     NA     NA     NA     NA     NA
##
## Parameter correlation:
## Could not estimate covariance matrix; singular system:
##
## Residual standard error: 5.064 on 27 degrees of freedom
##
## Backtransformed parameters:
##           Estimate Lower Upper
## Z0_0      9.701e+01     NA     NA
## k_Z0_sink 3.061e-10     NA     NA
## k_Z0_Z1   2.236e+00     NA     NA
## k_Z1_sink 4.821e-01     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 1.369e-10
## Z0_Z1    1.000e+00
## Z1_sink 1.000e+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")

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:         3.1.2
## Date of fit:        Wed Nov 12 18:10:33 2014
## Date of summary:    Wed Nov 12 18:10:33 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 3.906 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:
##           Estimate Std. Error Lower Upper t value Pr(>|t|) Pr(>t)
## Z0_0          97.0100         NA    NA    NA    NA      NA    NA
## log_k_Z0       0.8047         NA    NA    NA    NA      NA    NA
## log_k_Z1      -0.7296         NA    NA    NA    NA      NA    NA
## f_Z0_ilr_1    15.8900         NA    NA    NA    NA      NA    NA
##
## Parameter correlation:
## Could not estimate covariance matrix; singular system:
##
## Residual standard error: 5.064 on 27 degrees of freedom
##
## Backtransformed parameters:
##           Estimate Lower Upper
## Z0_0          97.0100    NA    NA
## k_Z0           2.2360    NA    NA
## k_Z1           0.4821    NA    NA
## f_Z0_to_Z1     1.0000    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   1.73e-10
##
## Estimated disappearance times:

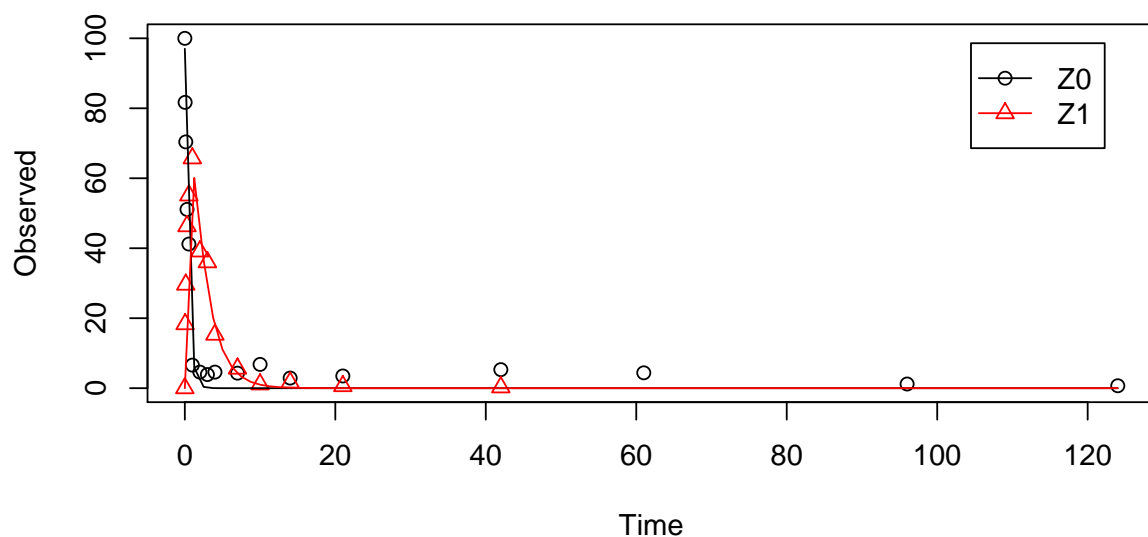
```

```
##      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 = "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:         3.1.2
## Date of fit:       Wed Nov 12 18:10:34 2014
## Date of summary:   Wed Nov 12 18:10:34 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 1.166 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:
##      Estimate Std. Error  Lower  Upper t value Pr(>|t|)
## Z0_0      97.0100    2.68200 91.5200 102.5000   36.18 4.727e-25
## log_k_Z0   0.8047    0.06568  0.6702   0.9392   12.25 9.117e-13
## log_k_Z1  -0.7296    0.08854 -0.9109  -0.5482   -8.24 5.738e-09
##      Pr(>t)
## Z0_0      2.364e-25
## log_k_Z0  4.558e-13
## log_k_Z1  2.869e-09
##
## Parameter correlation:
##      Z0_0 log_k_Z0 log_k_Z1
## Z0_0      1.0000  0.10629  0.41038
## log_k_Z0  0.1063  1.00000  0.04345
## log_k_Z1  0.4104  0.04345  1.00000
##
## Residual standard error: 4.973 on 28 degrees of freedom
##
## Backtransformed parameters:

```

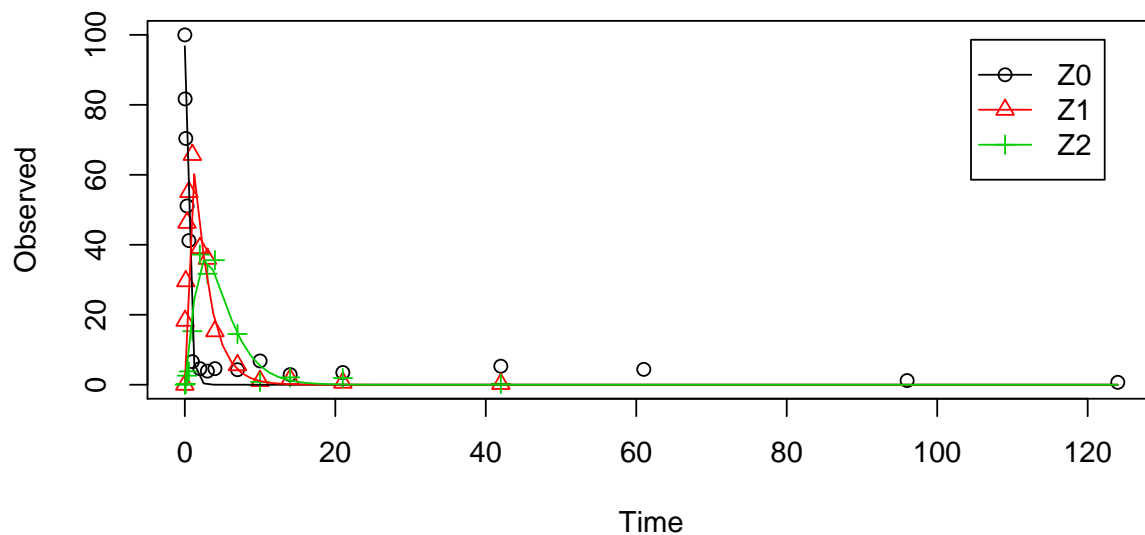
```
##      Estimate   Lower   Upper
## Z0_0  97.0100  91.5200 102.500
## k_Z0   2.2360   1.9550   2.558
## k_Z1   0.4821   0.4022   0.578
##
## Chi2 error levels in percent:
##           err.min n.optim df
## All data   17.61      3 27
## Z0         17.56      2 15
## Z1         15.08      1 12
##
## 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 = "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:      3.1.2
## Date of fit:     Wed Nov 12 18:10:38 2014
## Date of summary: Wed Nov 12 18:10:38 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 3.556 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
```

```

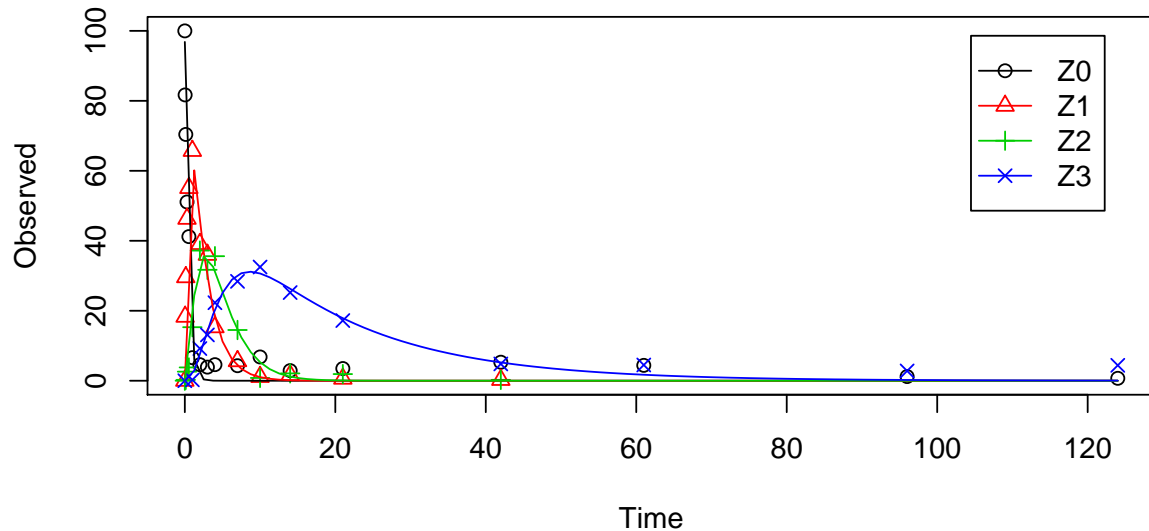
##
## Starting values for the transformed parameters actually optimised:
##           value lower upper
## Z0_0      100.000000 -Inf  Inf
## log_k_Z0_Z1 -2.302585 -Inf  Inf
## log_k_Z1_Z2 -2.301586 -Inf  Inf
## log_k_Z2_sink -2.300587 -Inf  Inf
##
## Fixed parameter values:
##           value type
## Z1_0      0 state
## Z2_0      0 state
##
## Optimised, transformed parameters:
##           Estimate Std. Error Lower Upper t value Pr(>|t|)
## Z0_0      96.7700  2.26600 92.1900 101.3000 42.710 5.434e-35
## log_k_Z0_Z1  0.7948  0.05843 0.6767  0.9129 13.600 1.361e-16
## log_k_Z1_Z2 -0.7410  0.06821 -0.8789 -0.6032 -10.860 1.682e-13
## log_k_Z2_sink -0.8027  0.11090 -1.0270 -0.5785 -7.237 8.786e-09
##           Pr(>t)
## Z0_0      2.717e-35
## log_k_Z0_Z1 6.804e-17
## log_k_Z1_Z2 8.409e-14
## log_k_Z2_sink 4.393e-09
##
## Parameter correlation:
##           Z0_0 log_k_Z0_Z1 log_k_Z1_Z2 log_k_Z2_sink
## Z0_0      1.00000  0.05781  0.28748  0.31786
## log_k_Z0_Z1 0.05781  1.00000 -0.04361  0.01213
## log_k_Z1_Z2 0.28748 -0.04361  1.00000  0.24019
## log_k_Z2_sink 0.31786  0.01213  0.24019  1.00000
##
## Residual standard error: 4.486 on 40 degrees of freedom
##
## Backtransformed parameters:
##           Estimate Lower Upper
## Z0_0      96.7700 92.1900 101.3000
## k_Z0_Z1    2.2140  1.9670  2.4920
## k_Z1_Z2    0.4766  0.4152  0.5471
## k_Z2_sink  0.4481  0.3581  0.5607
##
## 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"))
m.Z.FOCUS <- mkinfit(Z.FOCUS, FOCUS_2006_Z_mkin,
                    quiet = TRUE)
plot(m.Z.FOCUS)
```



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

## mkin version:      0.9.34
## R version:        3.1.2
## Date of fit:       Wed Nov 12 18:10:47 2014
## Date of summary:  Wed Nov 12 18:10:47 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 400 model solutions performed in 8.795 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:
##      Estimate Std. Error   Lower   Upper t value Pr(>|t|)
## Z0_0         96.8400    2.05900 92.7100 101.0000  47.040 1.116e-43
## log_k_Z0_Z1    0.7954    0.05332  0.6884   0.9025  14.920 3.078e-20
## log_k_Z1_Z2   -0.7375    0.06123 -0.8605  -0.6146 -12.040 1.569e-16
## log_k_Z2_sink -1.4330    0.17160 -1.7770  -1.0880  -8.348 4.167e-11
## log_k_Z2_Z3   -1.5470    0.12260 -1.7930  -1.3010 -12.620 2.598e-17
## log_k_Z3_sink -2.8350    0.24360 -3.3240  -2.3460 -11.640 5.637e-16
##      Pr(>t)
## Z0_0         5.581e-44
## log_k_Z0_Z1   1.539e-20
## log_k_Z1_Z2   7.843e-17
## log_k_Z2_sink 2.084e-11
## log_k_Z2_Z3   1.299e-17
## log_k_Z3_sink 2.819e-16
##
## Parameter correlation:
##      Z0_0 log_k_Z0_Z1 log_k_Z1_Z2 log_k_Z2_sink
## Z0_0      1.00000    0.05387    0.2727    0.37006
## log_k_Z0_Z1 0.05387    1.00000   -0.0521    0.02443
## log_k_Z1_Z2 0.27275   -0.05210    1.0000    0.29385
## log_k_Z2_sink 0.37006    0.02443    0.2939    1.00000
## log_k_Z2_Z3 -0.07297   -0.03582   -0.1213   -0.18897

```

```

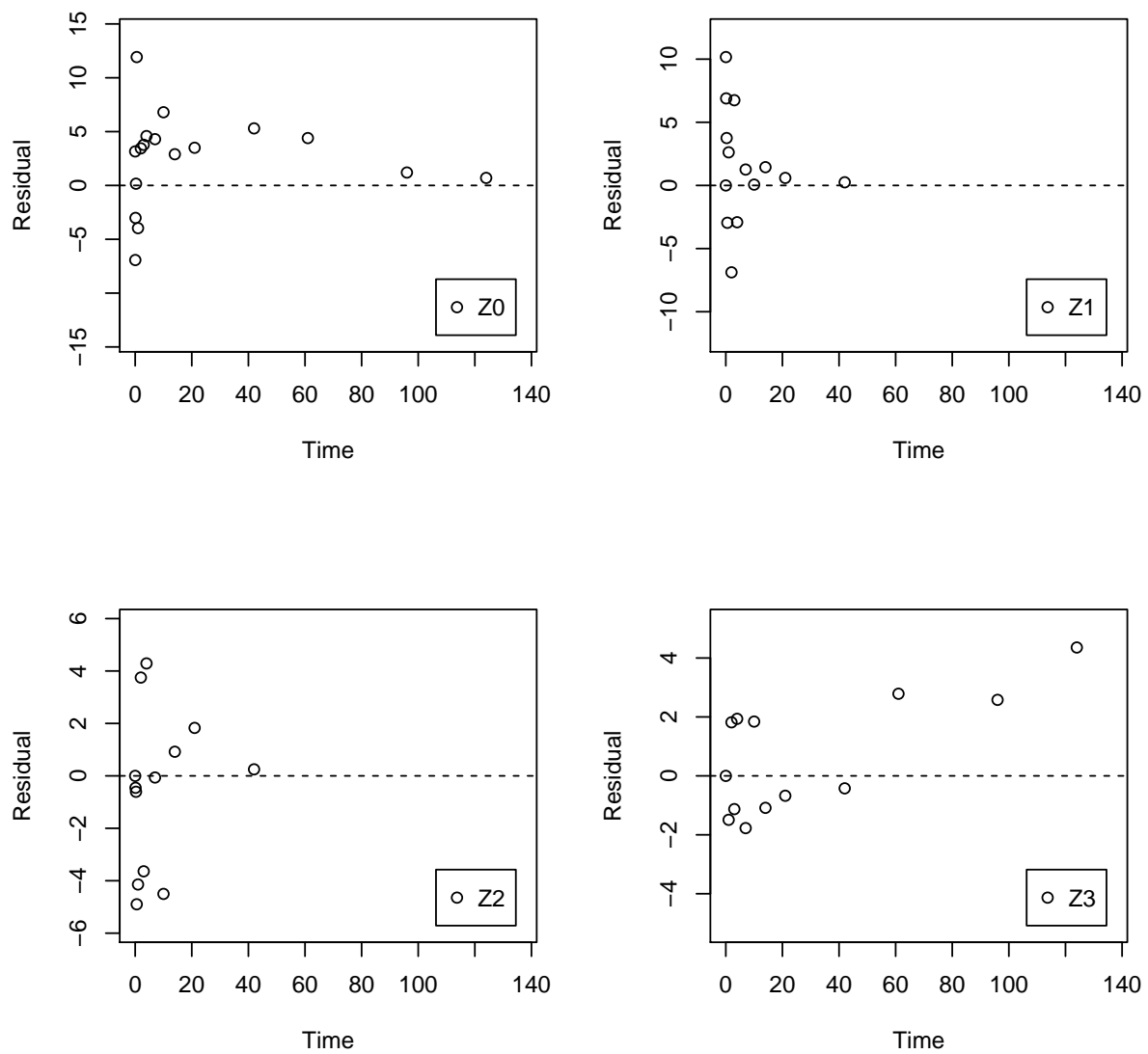
## log_k_Z3_sink -0.11349    -0.02522    -0.1915    -0.64299
##              log_k_Z2_Z3 log_k_Z3_sink
## Z0_0          -0.07297    -0.11349
## log_k_Z0_Z1   -0.03582    -0.02522
## log_k_Z1_Z2   -0.12135    -0.19146
## log_k_Z2_sink -0.18897    -0.64299
## log_k_Z2_Z3    1.00000     0.55158
## log_k_Z3_sink  0.55158     1.00000
##
## Residual standard error: 4.1 on 51 degrees of freedom
##
## Backtransformed parameters:
##           Estimate      Lower      Upper
## Z0_0          96.84000 92.71000 101.00000
## k_Z0_Z1        2.21500  1.99100  2.46600
## k_Z1_Z2        0.47830  0.42300  0.54090
## k_Z2_sink      0.23870  0.16910  0.33690
## k_Z2_Z3        0.21290  0.16650  0.27230
## k_Z3_sink      0.05869  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   11.8096 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")
```

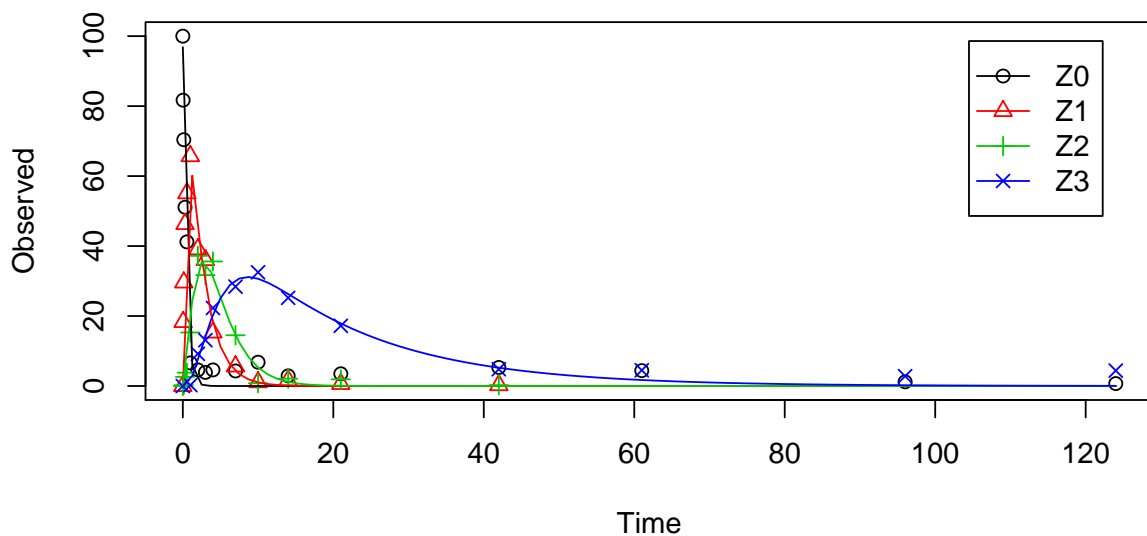


We can also investigate the confidence interval for the formation fraction from Z2 to Z3 by specifying the model using formation fractions.

```

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")
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:         3.1.2
## Date of fit:       Wed Nov 12 18:10:55 2014
## Date of summary:   Wed Nov 12 18:10:55 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 8.449 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:
##           Estimate Std. Error  Lower  Upper  t value  Pr(>|t|)
## Z0_0         96.84000    2.05900  92.7100 101.0000  47.0400 1.116e-43
## log_k_Z0      0.79540    0.05332   0.6884   0.9025  14.9200 3.078e-20
## log_k_Z1     -0.73750    0.06123  -0.8605  -0.6146 -12.0400 1.569e-16
## log_k_Z2     -0.79490    0.09790  -0.9915  -0.5984  -8.1200 9.442e-11
## log_k_Z3     -2.83500    0.24360  -3.3240  -2.3460 -11.6400 5.637e-16
## f_Z2_ilr_1   -0.08068    0.16190  -0.4057   0.2443  -0.4983 6.204e-01
##
##           Pr(>t)
## Z0_0         5.581e-44
## log_k_Z0      1.539e-20
## log_k_Z1      7.844e-17
## log_k_Z2      4.721e-11
## log_k_Z3      2.819e-16
## f_Z2_ilr_1    3.102e-01

```

```

##
## Parameter correlation:
##           Z0_0  log_k_Z0 log_k_Z1  log_k_Z2 log_k_Z3 f_Z2_ilr_1
## Z0_0         1.00000  0.053865  0.2727  0.299769 -0.11349  -0.31644
## log_k_Z0      0.05387  1.000000 -0.0521  0.001489 -0.02522  -0.03748
## log_k_Z1      0.27275 -0.052100  1.0000  0.200610 -0.19146  -0.28521
## log_k_Z2      0.29977  0.001489  0.2006  1.000000 -0.27012  -0.38859
## log_k_Z3     -0.11349 -0.025216 -0.1915 -0.270121  1.00000  0.77720
## f_Z2_ilr_1   -0.31644 -0.037480 -0.2852 -0.388595  0.77720  1.00000
##
## Residual standard error: 4.1 on 51 degrees of freedom
##
## Backtransformed parameters:
##           Estimate      Lower      Upper
## Z0_0          96.84000  92.71000 101.00000
## k_Z0           2.21500  1.99100  2.46600
## k_Z1           0.47830  0.42300  0.54090
## k_Z2           0.45160  0.37100  0.54970
## k_Z3           0.05869  0.03599  0.09571
## f_Z2_to_Z3    0.47150  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  5.099
## Z3   11.8097 39.231

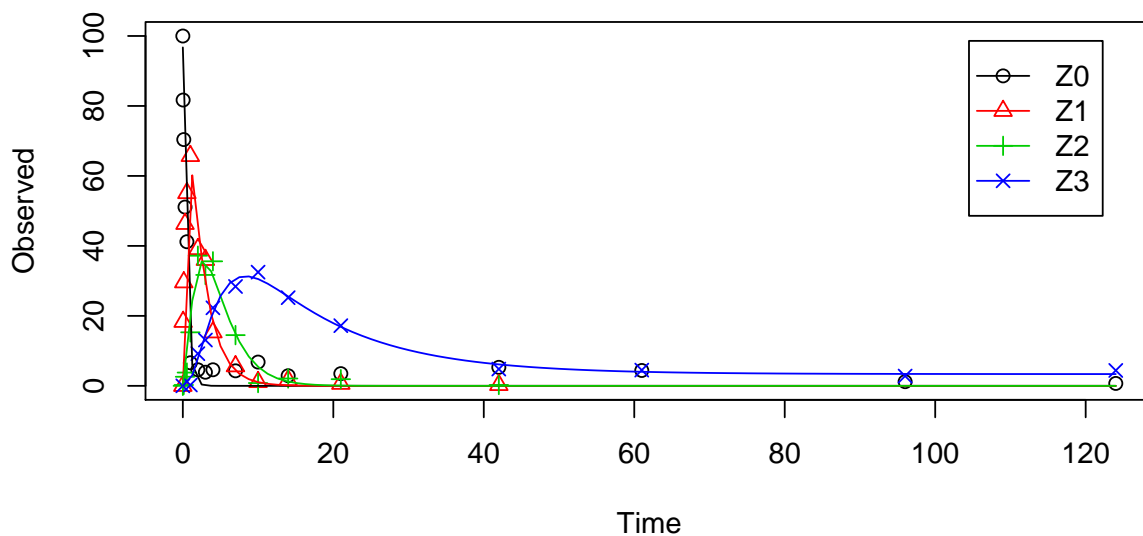
```

## 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"))
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:      0.9.34
## R version:         3.1.2
## Date of fit:       Wed Nov 12 18:11:15 2014
## Date of summary:   Wed Nov 12 18:11:15 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 - 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 804 model solutions performed in 19.534 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:
##               Estimate Std. Error Lower Upper t value Pr(>|t|)
## Z0_0           96.7400          NA      NA      NA      NA      NA
## log_k_Z0_Z1     0.7947          NA      NA      NA      NA      NA
## log_k_Z1_Z2    -0.7426          NA      NA      NA      NA      NA
## log_k_Z2_sink   -1.4950          NA      NA      NA      NA      NA
## log_k_Z2_Z3_free -1.5040          NA      NA      NA      NA      NA
## log_k_Z3_free_sink -2.6540          NA      NA      NA      NA      NA
## log_k_Z3_free_bound -5.2440          NA      NA      NA      NA      NA
## log_k_Z3_bound_free -22.1700          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: 4.107 on 49 degrees of freedom
##
## Backtransformed parameters:
##               Estimate Lower Upper
## Z0_0           9.674e+01      NA      NA
## k_Z0_Z1         2.214e+00      NA      NA
## k_Z1_Z2         4.759e-01      NA      NA
## k_Z2_sink       2.243e-01      NA      NA
## k_Z2_Z3_free    2.222e-01      NA      NA
## k_Z3_free_sink   7.034e-02      NA      NA
## k_Z3_free_bound  5.279e-03      NA      NA
## k_Z3_bound_free  2.358e-10      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 2.194e-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 10.1978 45.329      9.166    3.16e+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:       3.1.2
## Date of fit:     Wed Nov 12 18:11:17 2014
## Date of summary: Wed Nov 12 18:11:17 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 1.992 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
```

```

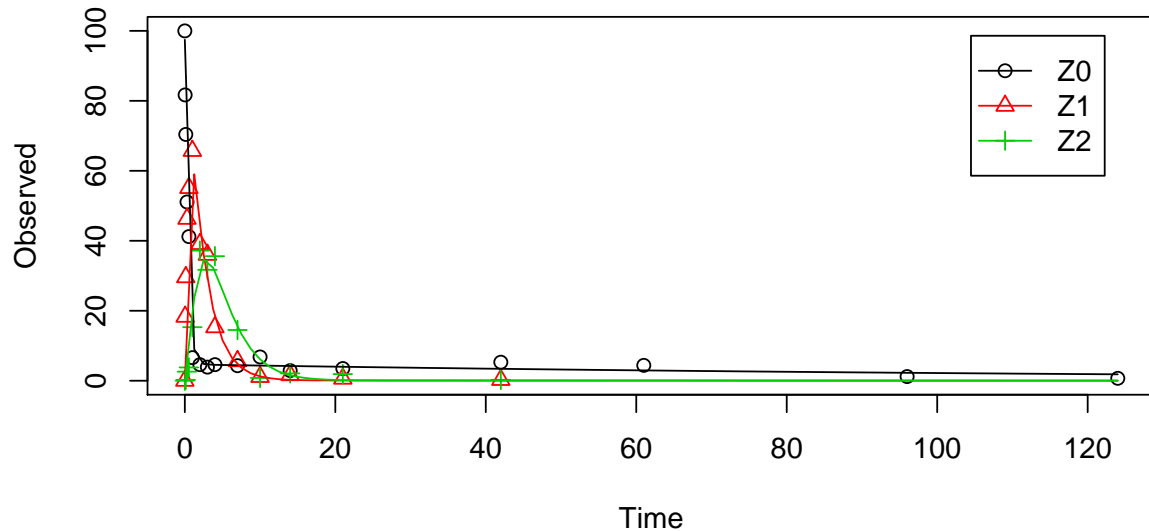
## k_Z0_bound_free    0.0200 deparm
## k_Z0_free_Z1       0.1002 deparm
## k_Z1_sink          0.1003 deparm
##
## Starting values for the transformed parameters actually optimised:
##               value lower upper
## Z0_free_0       100.000000 -Inf  Inf
## log_k_Z0_free_bound -2.302585 -Inf  Inf
## 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:
##           Estimate Std. Error   Lower   Upper t value
## Z0_free_0       97.2900    2.39500 92.3600 102.2000  40.610
## log_k_Z0_free_bound -2.0820    0.43220 -2.9710  -1.1940  -4.818
## log_k_Z0_bound_free -4.7200    1.60500 -8.0190  -1.4210  -2.941
## log_k_Z0_free_Z1    0.8549    0.06430  0.7227   0.9871  13.300
## log_k_Z1_sink      -0.7934    0.08506 -0.9682  -0.6185  -9.327
##           Pr(>|t|)   Pr(>t)
## Z0_free_0       4.728e-25 2.364e-25
## log_k_Z0_free_bound 5.440e-05 2.720e-05
## log_k_Z0_bound_free 6.785e-03 3.393e-03
## log_k_Z0_free_Z1   4.185e-13 2.092e-13
## log_k_Z1_sink      8.860e-10 4.430e-10
##
## Parameter correlation:
##           Z0_free_0 log_k_Z0_free_bound log_k_Z0_bound_free
## Z0_free_0       1.000000           0.006492           0.03324
## log_k_Z0_free_bound 0.006492           1.000000           0.54647
## log_k_Z0_bound_free 0.033236           0.546472           1.00000
## log_k_Z0_free_Z1   0.111818           0.413920           0.15837
## log_k_Z1_sink      0.391554          -0.291910          -0.12597
##           log_k_Z0_free_Z1 log_k_Z1_sink
## Z0_free_0           0.11182           0.39155
## log_k_Z0_free_bound 0.41392          -0.29191
## log_k_Z0_bound_free 0.15837          -0.12597

```

```
## 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:
##              Estimate      Lower      Upper
## Z0_free_0      97.290000  92.360000 102.2000
## k_Z0_free_bound 0.124700  0.051280  0.3031
## k_Z0_bound_free 0.008912  0.000329  0.2414
## k_Z0_free_Z1    2.351000  2.060000  2.6830
## k_Z1_sink       0.452300  0.379800  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
## 2.476315 0.008462
##
## 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"))
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:      3.1.2
## Date of fit:     Wed Nov 12 18:11:24 2014
## Date of summary: Wed Nov 12 18:11:24 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 6.003 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:
##               Estimate Std. Error   Lower   Upper t value
## Z0_free_0        97.4400    2.07100  93.2400 101.6000  47.050
## log_k_Z0_free_bound -2.1490    0.40400 -2.9670  -1.3310  -5.319
## log_k_Z0_bound_free -4.8380    1.61100 -8.1000  -1.5770  -3.003
## log_k_Z0_free_Z1     0.8457    0.05831  0.7277   0.9638  14.500
## log_k_Z1_Z2        -0.7812    0.06485 -0.9125  -0.6499 -12.050
## log_k_Z2_sink       -0.8606    0.10570 -1.0750  -0.6466  -8.140
##               Pr(>|t|)   Pr(>t)
## Z0_free_0        2.697e-35 1.348e-35
## log_k_Z0_free_bound 4.880e-06 2.440e-06
## log_k_Z0_bound_free 4.708e-03 2.354e-03
## log_k_Z0_free_Z1   4.501e-17 2.251e-17
## log_k_Z1_Z2        1.522e-14 7.611e-15
## log_k_Z2_sink       7.465e-10 3.732e-10
##
## Parameter correlation:
##               Z0_free_0 log_k_Z0_free_bound log_k_Z0_bound_free
## Z0_free_0        1.00000                0.07502                0.07080
## log_k_Z0_free_bound 0.07502                1.00000                0.54254
## log_k_Z0_bound_free 0.07080                0.54254                1.00000

```

```

## log_k_Z0_free_Z1      0.09079      0.42453      0.16315
## log_k_Z1_Z2           0.25718     -0.22757     -0.08626
## log_k_Z2_sink         0.28877     -0.21050     -0.07922
##           log_k_Z0_free_Z1 log_k_Z1_Z2 log_k_Z2_sink
## Z0_free_0            0.09079      0.25718      0.28877
## log_k_Z0_free_bound   0.42453     -0.22757     -0.21050
## log_k_Z0_bound_free   0.16315     -0.08626     -0.07922
## log_k_Z0_free_Z1      1.00000     -0.10084     -0.04901
## log_k_Z1_Z2           -0.10084      1.00000      0.27278
## log_k_Z2_sink         -0.04901      0.27278      1.00000
##
## Residual standard error: 4.081 on 38 degrees of freedom
##
## Backtransformed parameters:
##           Estimate      Lower      Upper
## Z0_free_0      97.44000 9.324e+01 101.6000
## k_Z0_free_bound 0.11660 5.148e-02  0.2642
## k_Z0_bound_free 0.00792 3.036e-04  0.2066
## k_Z0_free_Z1    2.33000 2.070e+00  2.6220
## k_Z1_Z2         0.45790 4.015e-01  0.5221
## k_Z2_sink       0.42290 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
## 2.446638 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

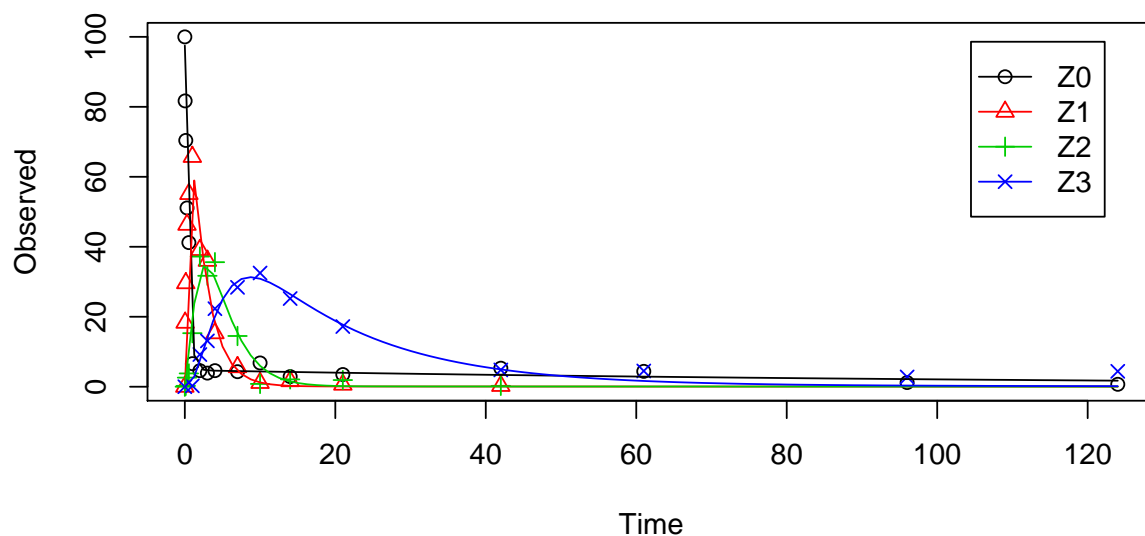
```

```
## 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"))
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:      0.9.34
## R version:         3.1.2
## Date of fit:        Wed Nov 12 18:11:36 2014
## Date of summary:    Wed Nov 12 18:11:36 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 531 model solutions performed in 12.625 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:
##
##      Estimate Std. Error   Lower   Upper t value
## Z0_free_0      97.5300    1.88700 93.7400 101.3000  51.700
## log_k_Z0_free_bound -2.1360    0.36810 -2.8760 -1.3960  -5.803
## log_k_Z0_bound_free -4.7650    1.41800 -7.6140 -1.9160  -3.362
## log_k_Z0_free_Z1      0.8470    0.05339  0.7398  0.9543  15.860
## log_k_Z1_Z2     -0.7769    0.05834 -0.8942 -0.6597 -13.320
## log_k_Z2_sink     -1.5610    0.18260 -1.9280 -1.1940  -8.550
## log_k_Z2_Z3     -1.5280    0.11350 -1.7560 -1.2990 -13.450
## log_k_Z3_sink     -2.7690    0.22460 -3.2200 -2.3180 -12.330
##
##      Pr(>|t|)   Pr(>t)
## Z0_free_0      2.066e-44 1.033e-44
## log_k_Z0_free_bound 4.709e-07 2.354e-07
## log_k_Z0_bound_free 1.510e-03 7.548e-04
## log_k_Z0_free_Z1  6.130e-21 3.065e-21
## log_k_Z1_Z2       6.658e-18 3.329e-18
## log_k_Z2_sink     2.793e-11 1.396e-11
## log_k_Z2_Z3       4.487e-18 2.244e-18
## log_k_Z3_sink     1.246e-16 6.232e-17
##
## 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            0.16280
## 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            0.12525
##
##      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 0.16280     -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     0.01857     -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

```

```

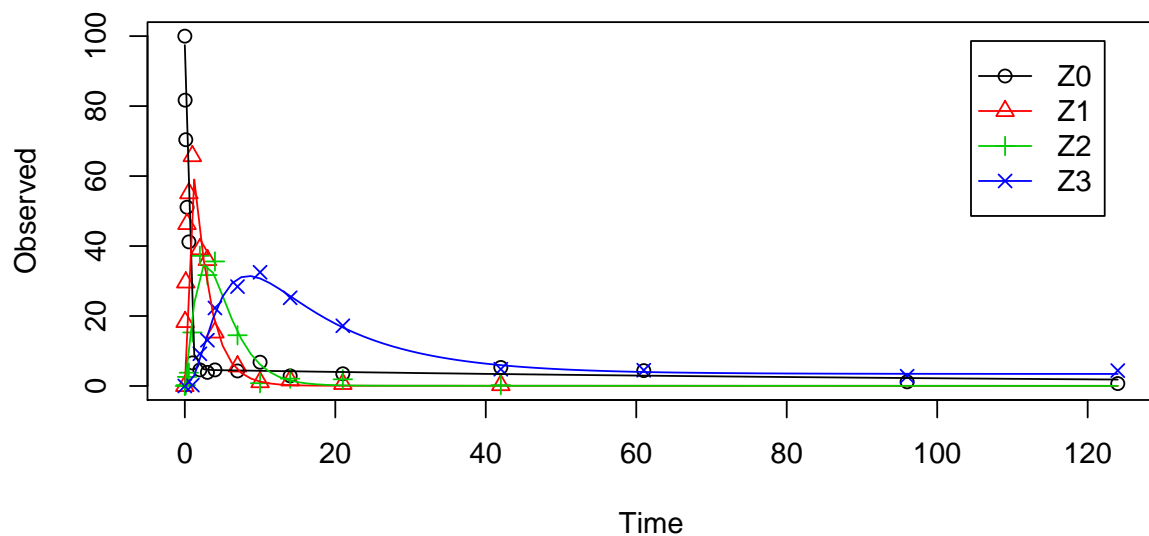
## log_k_Z0_bound_free      0.06082      0.12525
## log_k_Z0_free_Z1        -0.01281      0.01857
## log_k_Z1_Z2             -0.14885     -0.22486
## log_k_Z2_sink           -0.25473     -0.68320
## log_k_Z2_Z3              1.00000      0.56390
## log_k_Z3_sink            0.56390      1.00000
##
## Residual standard error: 3.737 on 49 degrees of freedom
##
## Backtransformed parameters:
##           Estimate      Lower      Upper
## Z0_free_0      97.530000 9.374e+01 101.3000
## k_Z0_free_bound 0.118100 5.636e-02 0.2475
## k_Z0_bound_free 0.008522 4.936e-04 0.1471
## k_Z0_free_Z1    2.333000 2.095e+00 2.5970
## k_Z1_Z2         0.459800 4.089e-01 0.5170
## k_Z2_sink       0.209900 1.455e-01 0.3030
## k_Z2_Z3         0.217000 1.728e-01 0.2727
## k_Z3_sink       0.062720 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"))
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 Lower Upper
## Z0_free_0      9.742495e+01    NA    NA
## k_Z0_free_bound 1.167578e-01    NA    NA
## k_Z0_bound_free 7.890227e-03    NA    NA
## k_Z0_free_Z1    2.330001e+00    NA    NA
## k_Z1_Z2         4.575901e-01    NA    NA
## k_Z2_sink       1.957094e-01    NA    NA
```

```
## k_Z2_Z3_free      2.265847e-01    NA    NA
## k_Z3_free_sink    7.478923e-02    NA    NA
## k_Z3_free_bound   5.217941e-03    NA    NA
## k_Z3_bound_free   2.124871e-10    NA    NA
```

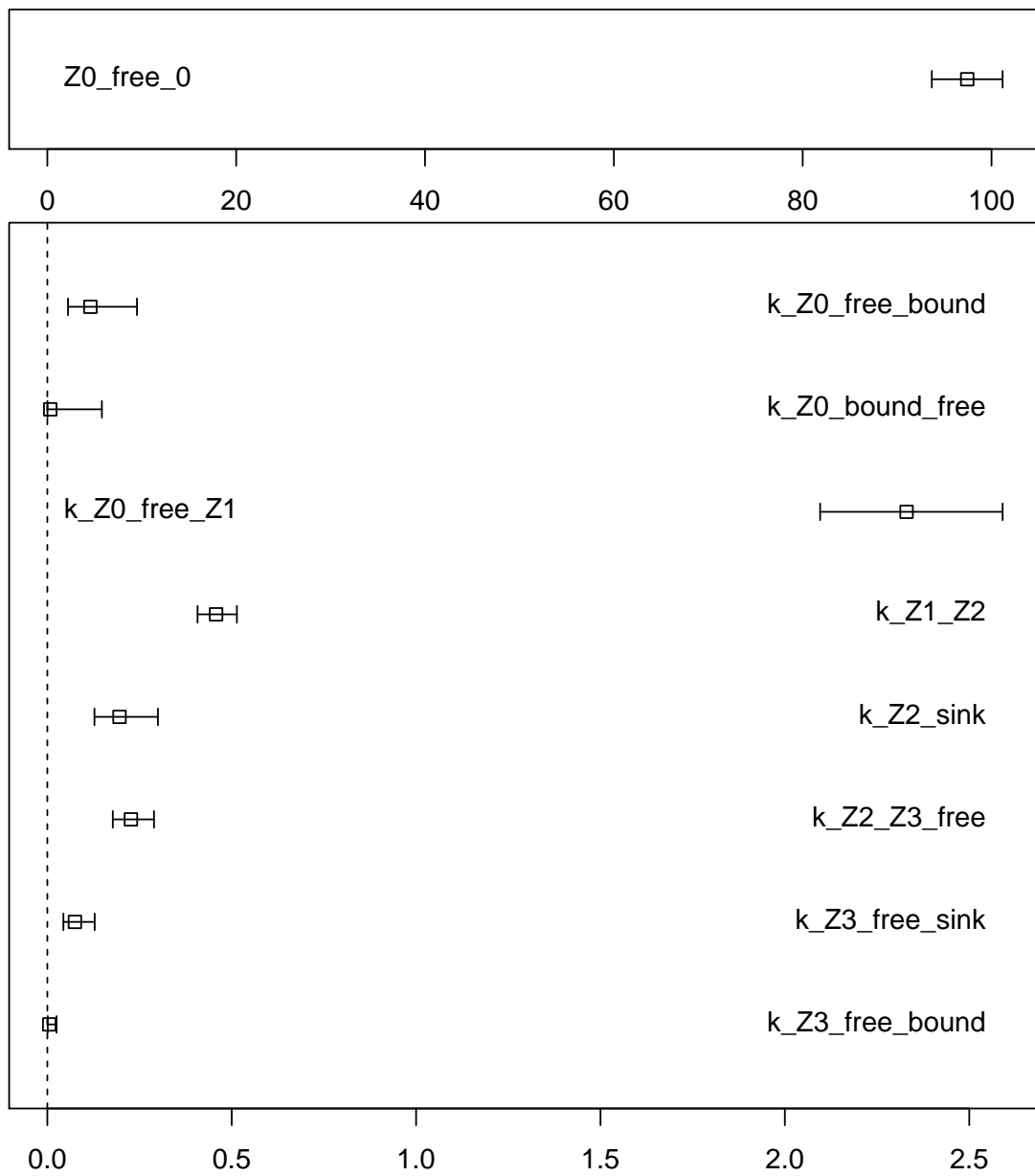
The summary view of the backtransformed parameters shows that we get no confidence intervals due to overparameterisation. As the optimized `k_Z3_bound_free` is excessively small, it seems reasonable to fix it to zero.

```
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     | Lower        | Upper        |
|--------------------|--------------|--------------|--------------|
| ## Z0_free_0       | 97.424951441 | 9.367615e+01 | 101.17374975 |
| ## k_Z0_free_bound | 0.116757743  | 5.608481e-02 | 0.24306708   |
| ## k_Z0_bound_free | 0.007890254  | 4.209026e-04 | 0.14791097   |
| ## k_Z0_free_Z1    | 2.330000647  | 2.095695e+00 | 2.59050229   |
| ## k_Z1_Z2         | 0.457590216  | 4.073552e-01 | 0.51402026   |
| ## k_Z2_sink       | 0.195709289  | 1.277412e-01 | 0.29984148   |
| ## k_Z2_Z3_free    | 0.226584870  | 1.775283e-01 | 0.28919721   |
| ## k_Z3_free_sink  | 0.074789319  | 4.353592e-02 | 0.12847879   |
| ## k_Z3_free_bound | 0.005217940  | 1.092645e-03 | 0.02491835   |

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
##   1.000000      1.000000      0.463443      0.536557      1.000000
##
## $SFORB
##   Z0_b1      Z0_b2      Z3_b1      Z3_b2
## 2.447136068 0.007512577 0.080007259 0.000000000
```

```
##
## $distimes
##          DT50          DT90 DT50_Z0_b1 DT50_Z0_b2 DT50_Z3_b1 DT50_Z3_b2
## Z0 0.3043058  1.184811  0.2832483    92.26491          NA          NA
## Z1 1.5147771  5.031981          NA          NA          NA          NA
## Z2 1.6413847  5.452562          NA          NA          NA          NA
## Z3 9.5674970 41.136541          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>.