Example evaluation of FOCUS dataset Z

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Contents

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1 The data

The following code defines the example dataset from Appendix 7 to the FOCUS kinetics report (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)
```

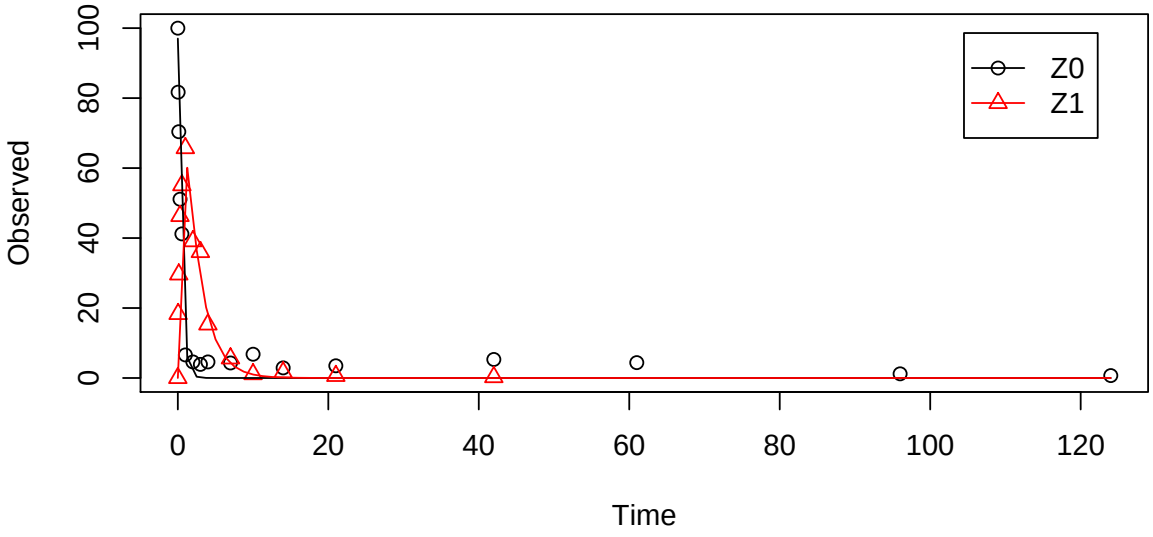
2 Parent compound and one metabolite

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).

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

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

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




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

```
## mkin version:    0.9.41
## R version:       3.2.2
## Date of fit:     Mon Nov  9 10:20:10 2015
## Date of summary: Mon Nov  9 10:20:10 2015
##
## 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 1.315 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  2.734e+00  9.141e+01  1.026e+02
## log_k_Z0_sink -25.0800  3.244e+05 -6.657e+05  6.656e+05
## log_k_Z0_Z1     0.8047  9.448e-02  6.108e-01  9.985e-01
## log_k_Z1_sink  -0.7296  9.030e-02 -9.148e-01 -5.443e-01
##
## Parameter correlation:
##                  Z0_0 log_k_Z0_sink log_k_Z0_Z1 log_k_Z1_sink
## Z0_0           1.0000      -0.04450    0.106603      0.406943
## log_k_Z0_sink -0.0445       1.00000   -0.706243      0.054352
## log_k_Z0_Z1    0.1066      -0.70624    1.000000     -0.007661
## log_k_Z1_sink  0.4069       0.05435   -0.007661      1.000000
##
## 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 91.4100 102.6000
## k_Z0_sink 1.281e-11 5.647e-11 5.000e-01  0.0000      Inf
```

```
## k_Z0_Z1   2.236e+00 1.355e+01 7.397e-14  1.8420   2.7140
## k_Z1_sink 4.821e-01 7.321e+00 3.552e-08  0.4006   0.5803
##
## 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 5.731e-12
## 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
```

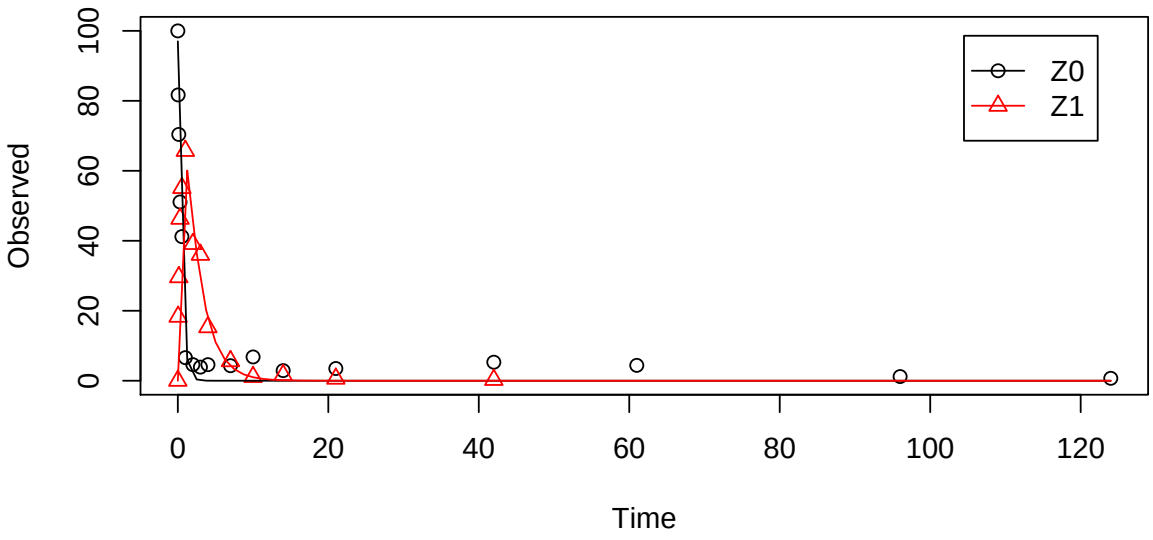
As obvious from the summary, the kinetic rate constant from parent compound Z to sink is negligible. Accordingly, the exact magnitude of the fitted parameter `log k_Z_sink` is ill-defined and the covariance matrix is not returned. This suggests, in agreement with the analysis in the FOCUS kinetics report,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

```
## 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:    0.9.41
## R version:       3.2.2
## Date of fit:     Mon Nov  9 10:20:12 2015
## Date of summary: Mon Nov  9 10:20:12 2015
##
## 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 1.288 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  2.771e+00  9.133e+01  1.027e+02
## log_k_Z0     0.8047  1.043e-01  5.907e-01  1.019e+00
## log_k_Z1    -0.7296  9.140e-02 -9.171e-01 -5.420e-01
## f_Z0_ilr_1  15.8900  5.756e+05 -1.181e+06  1.181e+06
##
## Parameter correlation:
##                Z0_0 log_k_Z0 log_k_Z1 f_Z0_ilr_1
## Z0_0        1.00000 -0.06309  0.42676    -0.1698
## log_k_Z0   -0.06309  1.00000 -0.09811     0.7672
## log_k_Z1    0.42676 -0.09811  1.00000    -0.1637
## f_Z0_ilr_1 -0.16981  0.76721 -0.16372     1.0000
##
## 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 91.3300 102.7000
## k_Z0         2.2360  10.310 3.662e-11  1.8050   2.7690
## k_Z1         0.4821   7.321 3.552e-08  0.3997   0.5816
## f_Z0_to_Z1   1.0000   9.855 9.707e-11  0.0000   1.0000
##
## 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   1.000e+00
## Z0_sink 1.741e-10
##
## Estimated disappearance times:
##     DT50  DT90
## Z0 0.310 1.030
## Z1 1.438 4.776
```

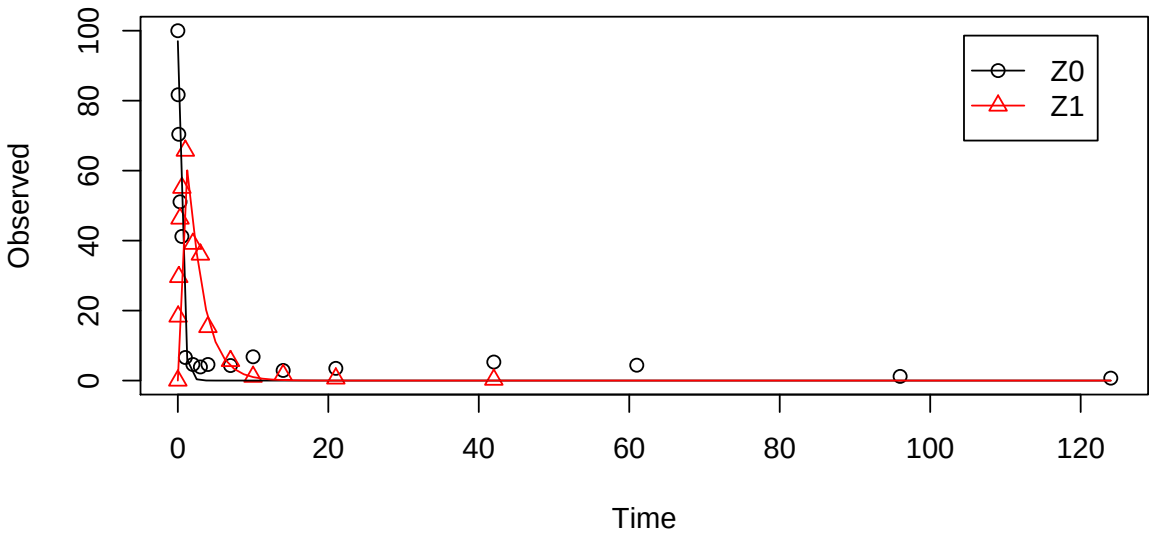
Here, the ilr transformed formation fraction fitted in the model takes a very large value, and the backtransformed formation fraction from parent Z to Z1 is practically unity. Again, the covariance matrix is not returned as the model is overparameterised.

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

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

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:    0.9.41
## R version:       3.2.2
## Date of fit:     Mon Nov  9 10:20:12 2015
## Date of summary: Mon Nov  9 10:20:12 2015
##
## 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 0.375 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  0.10629  0.41038
```

```
## log_k_Z0 0.1063  1.00000  0.04346
## log_k_Z1 0.4104  0.04346  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 2.247e-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.

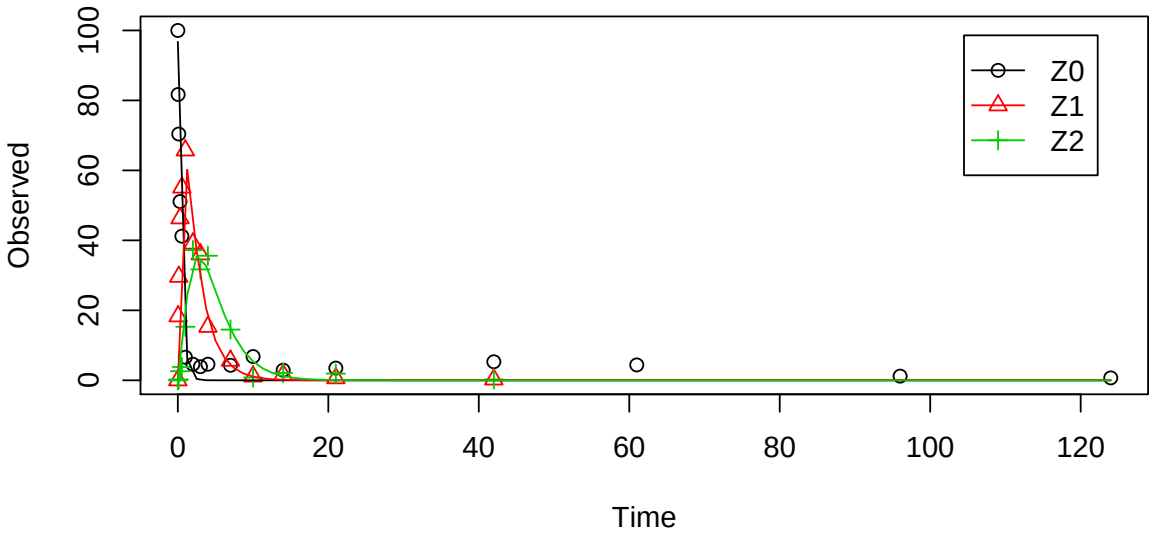
3 Including metabolites Z2 and Z3

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




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

```
## mkin version:    0.9.41
## R version:       3.2.2
## Date of fit:     Mon Nov  9 10:20:14 2015
## Date of summary: Mon Nov  9 10:20:14 2015
##
## 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 199 model solutions performed in 1.086 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     0.28748       0.31786
## log_k_Z0_Z1   0.05782     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:
## 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
##
```

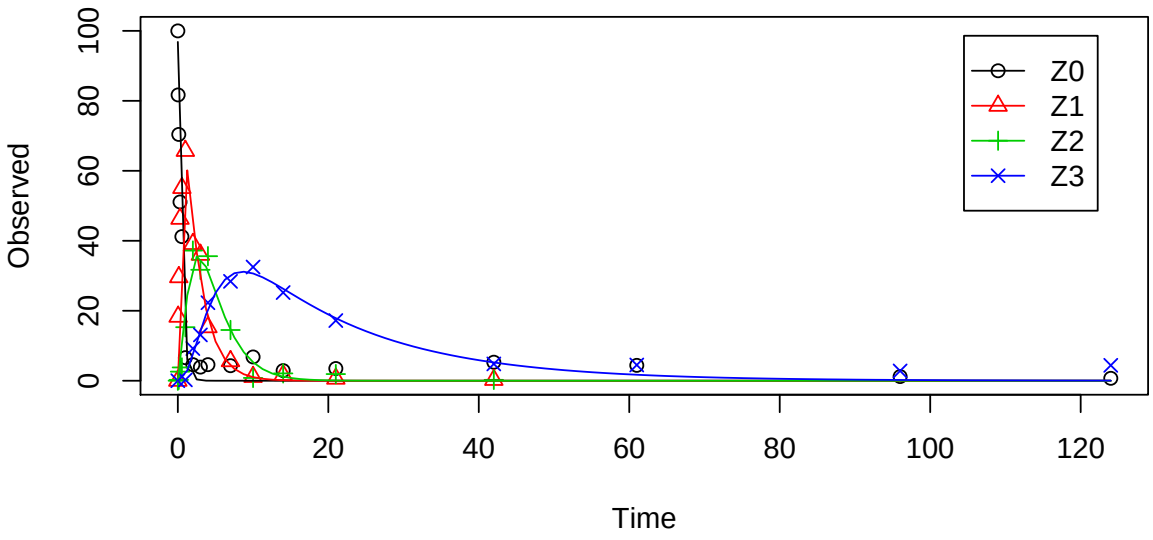
```
## Chi2 error levels in percent:
##          err.min n.optim df
## All data   19.10       4 38
## Z0         17.43       2 15
## Z1         15.27       1 12
## Z2         19.57       1 11
##
## Resulting formation fractions:
##         ff
## Z0_Z1    1
## Z1_Z2    1
## Z2_sink  1
##
## Estimated 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 accellerated by using the starting parameters from the previous fit.

```
Z.FOCUS <- mkinmod(Z0 = list(type = "SFO", to = "Z1", sink = FALSE),
                   Z1 = list(type = "SFO", to = "Z2", sink = FALSE),
                   Z2 = list(type = "SFO", to = "Z3"),
                   Z3 = list(type = "SFO"))
```

```
## 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)
```

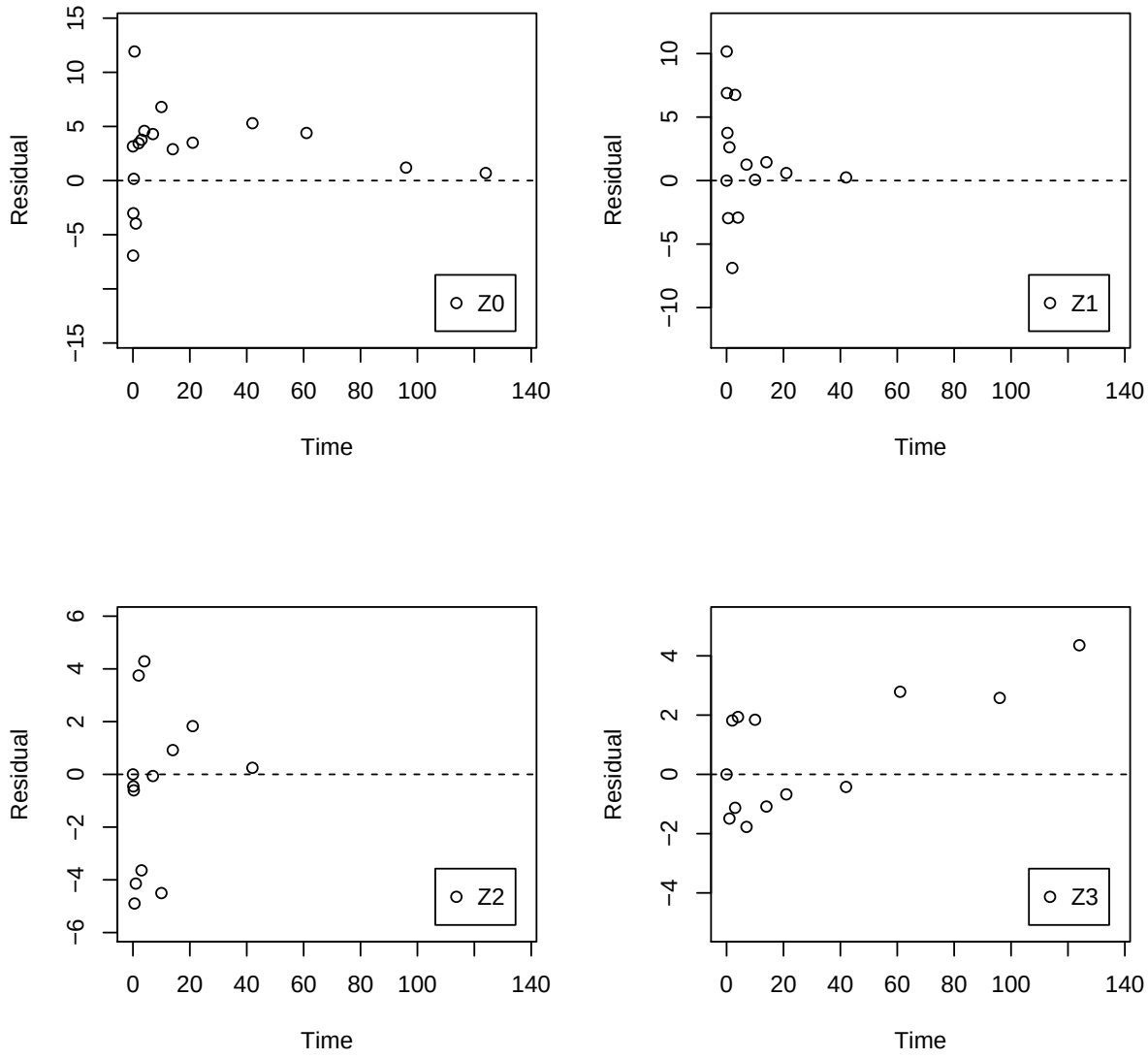
```
## mkin version:    0.9.41
## R version:       3.2.2
## Date of fit:     Mon Nov  9 10:20:17 2015
## Date of summary: Mon Nov  9 10:20:17 2015
##
## Equations:
## d_Z0 = - 0 - k_Z0_Z1 * Z0
## d_Z1 = + k_Z0_Z1 * Z0 - 0 - k_Z1_Z2 * Z1
## d_Z2 = + k_Z1_Z2 * Z1 - k_Z2_sink * Z2 - k_Z2_Z3 * Z2
## d_Z3 = + k_Z2_Z3 * Z2 - k_Z3_sink * Z3
##
## Model predictions using solution type deSolve
##
## Fitted with method Port using 465 model solutions performed in 3.343 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    0.06117 -0.8603  -0.6147
## log_k_Z2_sink  -1.4330    0.17150 -1.7770  -1.0880
## log_k_Z2_Z3    -1.5470    0.12250 -1.7930  -1.3010
## log_k_Z3_sink  -2.8350    0.24350 -3.3240  -2.3470
##
## Parameter correlation:
##                   Z0_0 log_k_Z0_Z1 log_k_Z1_Z2 log_k_Z2_sink
## Z0_0           1.00000     0.05396     0.27244       0.36977
## log_k_Z0_Z1    0.05396     1.00000    -0.05186       0.02481
## log_k_Z1_Z2    0.27244    -0.05186     1.00000       0.29261
## log_k_Z2_sink  0.36977     0.02481     0.29261       1.00000
## log_k_Z2_Z3   -0.07264    -0.03613    -0.12025      -0.18859
## log_k_Z3_sink -0.11291    -0.02580    -0.18947      -0.64263
##               log_k_Z2_Z3 log_k_Z3_sink
## Z0_0             -0.07264       -0.1129
## log_k_Z0_Z1      -0.03613       -0.0258
## log_k_Z1_Z2      -0.12025       -0.1895
## log_k_Z2_sink    -0.18859       -0.6426
## log_k_Z2_Z3       1.00000        0.5514
```

```
## log_k_Z3_sink     0.55142        1.0000
##
## 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 5.580e-44 92.7100 101.00000
## k_Z0_Z1    2.21500  18.750 7.735e-25  1.9910   2.46600
## k_Z1_Z2    0.47830  16.330 3.337e-22  0.4230   0.54080
## k_Z2_sink  0.23870   5.827 1.912e-07  0.1691   0.33680
## k_Z2_Z3    0.21290   8.160 4.089e-11  0.1665   0.27230
## k_Z3_sink  0.05869   4.106 7.288e-05  0.0360   0.09569
##
## 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")
```

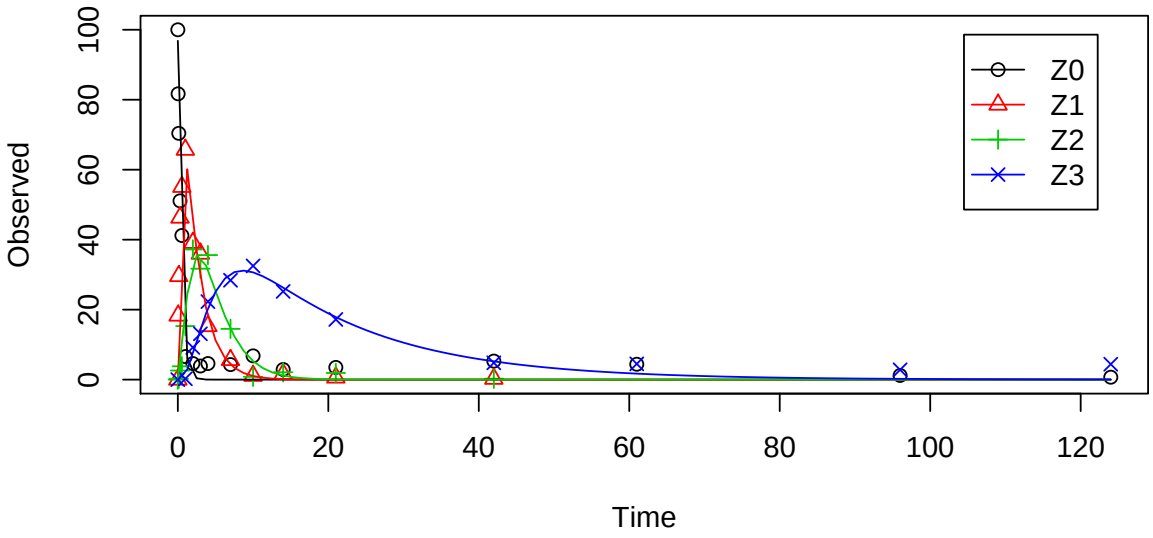


We can also investigate the confidence interval for the formation fraction from Z2 to Z3 by specifying the model using formation fractions.

```
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")
```

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

```
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:    0.9.41
## R version:       3.2.2
## Date of fit:     Mon Nov  9 10:20:21 2015
## Date of summary: Mon Nov  9 10:20:21 2015
##
## 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 423 model solutions performed in 3.054 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    0.05331  0.6884   0.9025
## log_k_Z1   -0.73750    0.06116 -0.8603  -0.6147
## log_k_Z2   -0.79490    0.09789 -0.9914  -0.5984
## log_k_Z3   -2.83500    0.24350 -3.3240  -2.3470
## f_Z2_ilr_1 -0.08067    0.16180 -0.4055   0.2442
##
## Parameter correlation:
##                Z0_0  log_k_Z0 log_k_Z1  log_k_Z2 log_k_Z3 f_Z2_ilr_1
## Z0_0        1.00000  0.054230  0.27243  0.299610 -0.11299   -0.31613
```

```
## log_k_Z0    0.05423  1.000000 -0.05099  0.001953 -0.02648   -0.03857
## log_k_Z1    0.27243 -0.050994  1.00000  0.199986 -0.18938   -0.28365
## log_k_Z2    0.29961  0.001953  0.19999  1.000000 -0.26974   -0.38832
## log_k_Z3   -0.11299 -0.026480 -0.18938 -0.269738  1.00000    0.77699
## f_Z2_ilr_1 -0.31613 -0.038565 -0.28365 -0.388324  0.77699    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 5.582e-44 92.7100 101.00000
## k_Z0        2.21500  18.760 7.693e-25  1.9910   2.46600
## k_Z1        0.47830  16.330 3.330e-22  0.4230   0.54080
## k_Z2        0.45160  10.210 3.110e-14  0.3710   0.54970
## k_Z3        0.05869   4.106 7.285e-05  0.0360   0.09569
## f_Z2_to_Z3  0.47150   8.265 2.809e-11  0.3604   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 11.8096 39.231
```

4 Using the SFORB model for parent and metabolites

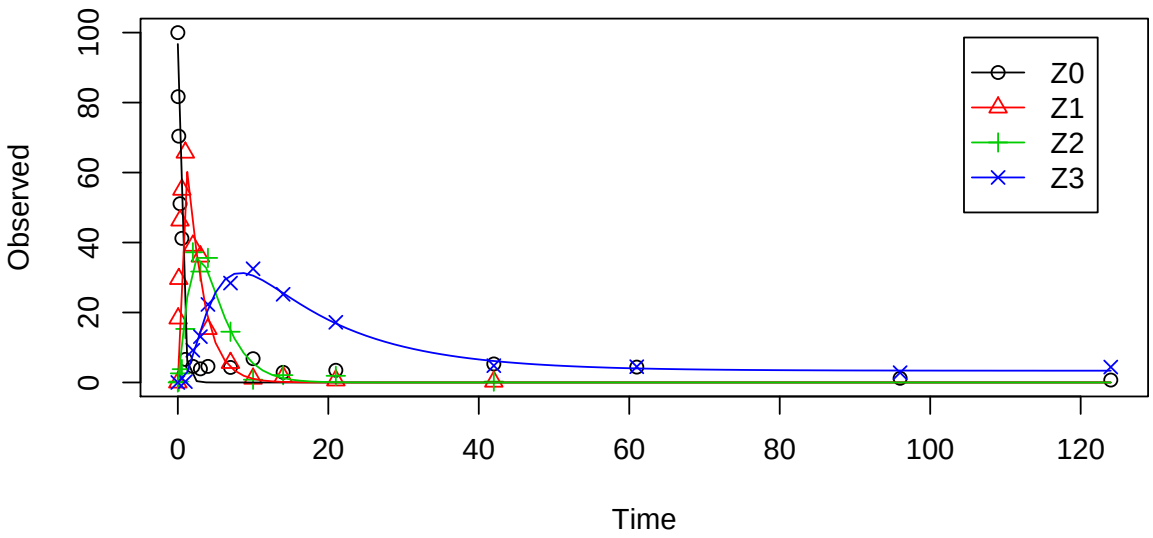
As the FOCUS report states, there is a certain tailing of the time course of metabolite Z3. Also, the time course of the parent compound is not fitted very well using the SFO model, as residues at a certain low level remain.

Therefore, an additional model is offered here, using the single first-order reversible binding (SFORB) model for metabolite Z3. As expected, the χ^2 error level is lower for metabolite Z3 using this model and the graphical fit for Z3 is improved. However, the covariance matrix is not returned.

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

```
## 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:    0.9.41
## R version:       3.2.2
```

```
## Date of fit:     Mon Nov  9 10:20:27 2015
## Date of summary: Mon Nov  9 10:20:27 2015
##
## 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 829 model solutions performed in 6.239 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 -22.0900         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 2.944e-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 4.243e-03    NA    NA
## k_Z3_free_bound 5.279e-03 4.513e-01 3.269e-01    NA    NA
## k_Z3_bound_free 2.562e-10 8.607e-09 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
```

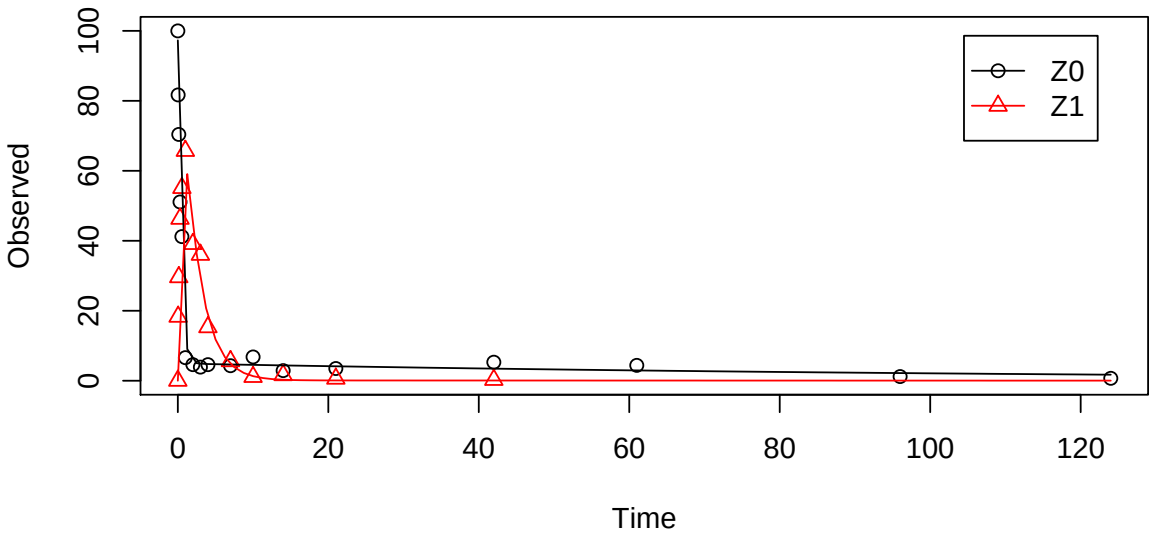
```
## Z3         8.562       3 10
##
## Estimated Eigenvalues of SFORB model(s):
##     Z3_b1     Z3_b2
## 7.562e-02 2.383e-10
##
## Resulting formation fractions:
##                  ff
## Z0_Z1        1.0000
## Z1_Z2        1.0000
## Z2_sink      0.5024
## Z2_Z3_free   0.4976
## Z3_free_sink 1.0000
##
## 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  2.909e+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:    0.9.41
## R version:       3.2.2
## Date of fit:     Mon Nov  9 10:20:28 2015
## Date of summary: Mon Nov  9 10:20:28 2015
##
## 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 176 model solutions performed in 0.694 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    0.43260 -2.9710  -1.1930
## log_k_Z0_bound_free  -4.7200    1.60000 -8.0090  -1.4310
## log_k_Z0_free_Z1      0.8549    0.06431  0.7227   0.9871
## log_k_Z1_sink        -0.7934    0.08507 -0.9682  -0.6185
##
## Parameter correlation:
##                      Z0_free_0 log_k_Z0_free_bound log_k_Z0_bound_free
## Z0_free_0              1.00000              0.0066             0.03334
## log_k_Z0_free_bound    0.00660              1.0000             0.54771
## log_k_Z0_bound_free    0.03334              0.5477             1.00000
## log_k_Z0_free_Z1       0.11184              0.4141             0.15887
## log_k_Z1_sink          0.39149             -0.2922            -0.12655
##                      log_k_Z0_free_Z1 log_k_Z1_sink
## Z0_free_0                    0.11184       0.39149
## log_k_Z0_free_bound          0.41406      -0.29216
## log_k_Z0_bound_free          0.15887      -0.12655
## log_k_Z0_free_Z1             1.00000      -0.04204
## log_k_Z1_sink               -0.04204       1.00000
##
## Residual standard error: 4.438 on 26 degrees of freedom
##
## Backtransformed parameters:
```

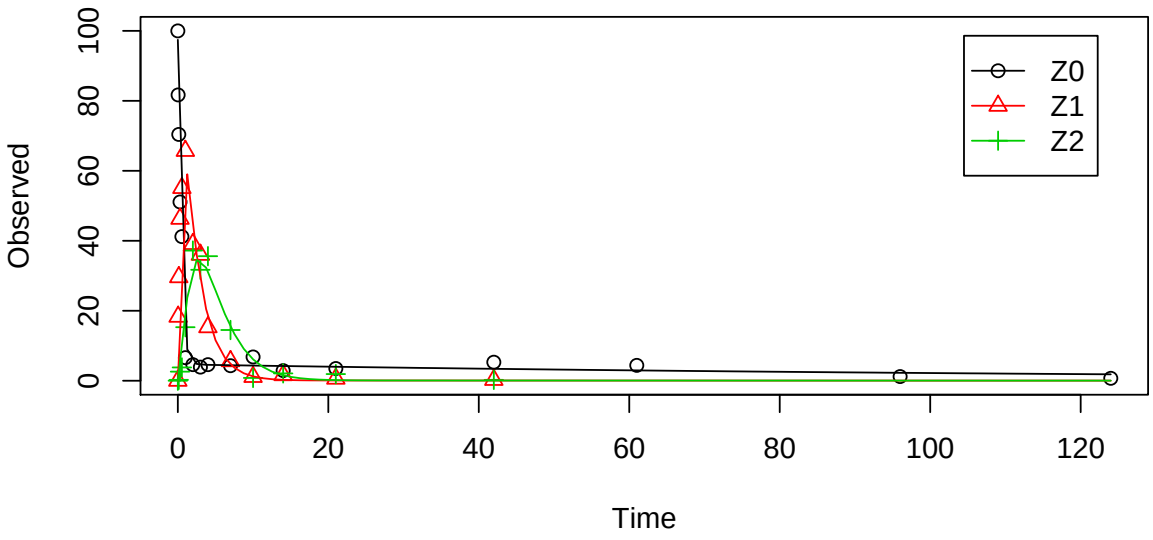
```
## Confidence intervals for internally transformed parameters are asymmetric.
## t-test (unrealistically) based on the assumption of normal distribution
## for estimators of untransformed parameters.
##                   Estimate t value    Pr(>t)     Lower    Upper
## Z0_free_0       97.290000 40.6100 2.364e-25 9.236e+01 102.2000
## k_Z0_free_bound  0.124700  2.3140 1.443e-02 5.123e-02   0.3034
## k_Z0_bound_free  0.008912  0.6231 2.693e-01 3.324e-04   0.2390
## k_Z0_free_Z1     2.351000 15.5500 5.521e-15 2.060e+00   2.6830
## k_Z1_sink        0.452300 11.7600 3.299e-12 3.797e-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
## 2.476315 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"))
```

```
## 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:    0.9.41
## R version:       3.2.2
## Date of fit:     Mon Nov  9 10:20:31 2015
## Date of summary: Mon Nov  9 10:20:31 2015
##
## 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 347 model solutions performed in 1.926 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    0.40310 -2.9650  -1.3330
## log_k_Z0_bound_free  -4.8380    1.57800 -8.0320  -1.6450
## log_k_Z0_free_Z1      0.8457    0.05831  0.7277   0.9638
## log_k_Z1_Z2          -0.7812    0.06484 -0.9124  -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             0.07538             0.07232
## log_k_Z0_free_bound   0.07538             1.00000             0.54571
## log_k_Z0_bound_free   0.07232             0.54571             1.00000
## log_k_Z0_free_Z1      0.09094             0.42470             0.16631
## log_k_Z1_Z2           0.25720            -0.22703            -0.08737
## log_k_Z2_sink         0.28877            -0.21014            -0.08015
##                     log_k_Z0_free_Z1 log_k_Z1_Z2 log_k_Z2_sink
## Z0_free_0                   0.09094     0.25720       0.28877
## log_k_Z0_free_bound         0.42470    -0.22703      -0.21014
## log_k_Z0_bound_free         0.16631    -0.08737      -0.08015
```

```
## log_k_Z0_free_Z1             1.00000    -0.10056      -0.04878
## log_k_Z1_Z2                 -0.10056     1.00000       0.27259
## log_k_Z2_sink               -0.04878     0.27259       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 47.0500 1.353e-35 9.324e+01 101.6000
## k_Z0_free_bound  0.11660  2.4730 8.993e-03 5.157e-02   0.2638
## k_Z0_bound_free  0.00792  0.6198 2.695e-01 3.248e-04   0.1931
## k_Z0_free_Z1     2.33000 17.1400 8.899e-20 2.070e+00   2.6220
## k_Z1_Z2          0.45790 15.4200 3.057e-18 4.015e-01   0.5221
## k_Z2_sink        0.42290  9.4580 7.842e-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
## 2.446636 0.007541
##
## 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
```

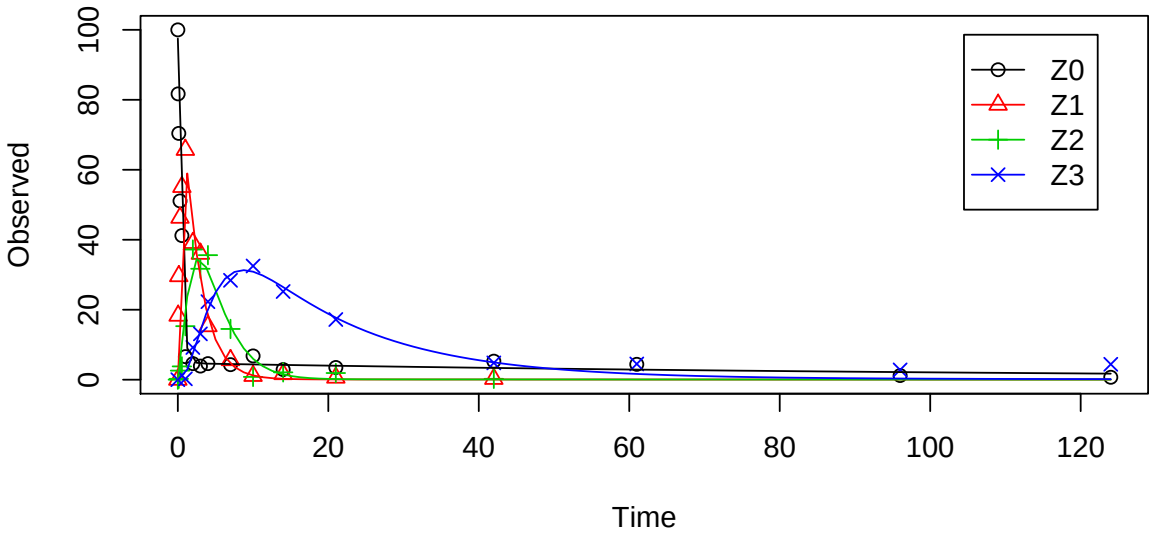
This results in a much better representation of the behaviour of the parent compound Z0.

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:    0.9.41
## R version:       3.2.2
## Date of fit:     Mon Nov  9 10:20:36 2015
## Date of summary: Mon Nov  9 10:20:36 2015
##
## 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 607 model solutions performed in 4.466 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             0.42766             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             0.12525
##                     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         0.42766    -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       0.12525
## 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.
```

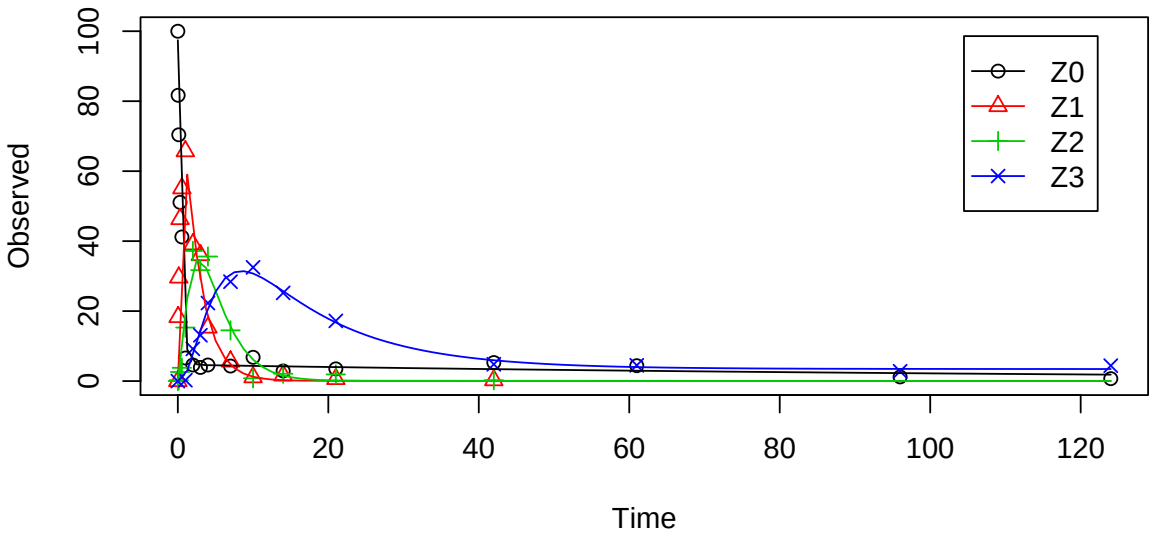
```
## t-test (unrealistically) based on the assumption of normal distribution
## for estimators of untransformed parameters.
##                  Estimate t value    Pr(>t)     Lower    Upper
## Z0_free_0       97.530000 51.7000 1.033e-44 9.374e+01 101.3000
## k_Z0_free_bound  0.118100  2.7160 4.548e-03 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 7.394e-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
## 2.45126 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       9.742496e+01 1.88615829 5.165259e+01 2.327739e-43
## k_Z0_free_bound 1.167582e-01 0.04302217 2.713909e+00 4.633395e-03
## k_Z0_bound_free 7.890260e-03 0.01162028 6.790077e-01 2.502316e-01
## k_Z0_free_Z1    2.330002e+00 0.12411426 1.877304e+01 8.893667e-24
## k_Z1_Z2         4.575901e-01 0.02681895 1.706219e+01 4.516643e-22
## k_Z2_sink       1.957097e-01 0.04395612 4.452388e+00 2.605224e-05
## k_Z2_Z3_free    2.265848e-01 0.02922696 7.752595e+00 2.987495e-10
## k_Z3_free_sink  7.478914e-02 0.02433843 3.072882e+00 1.761201e-03
## k_Z3_free_bound 5.217939e-03 0.01034277 5.045013e-01 3.081332e-01
## k_Z3_bound_free 4.406164e-10 0.02663898 1.654029e-08 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
```

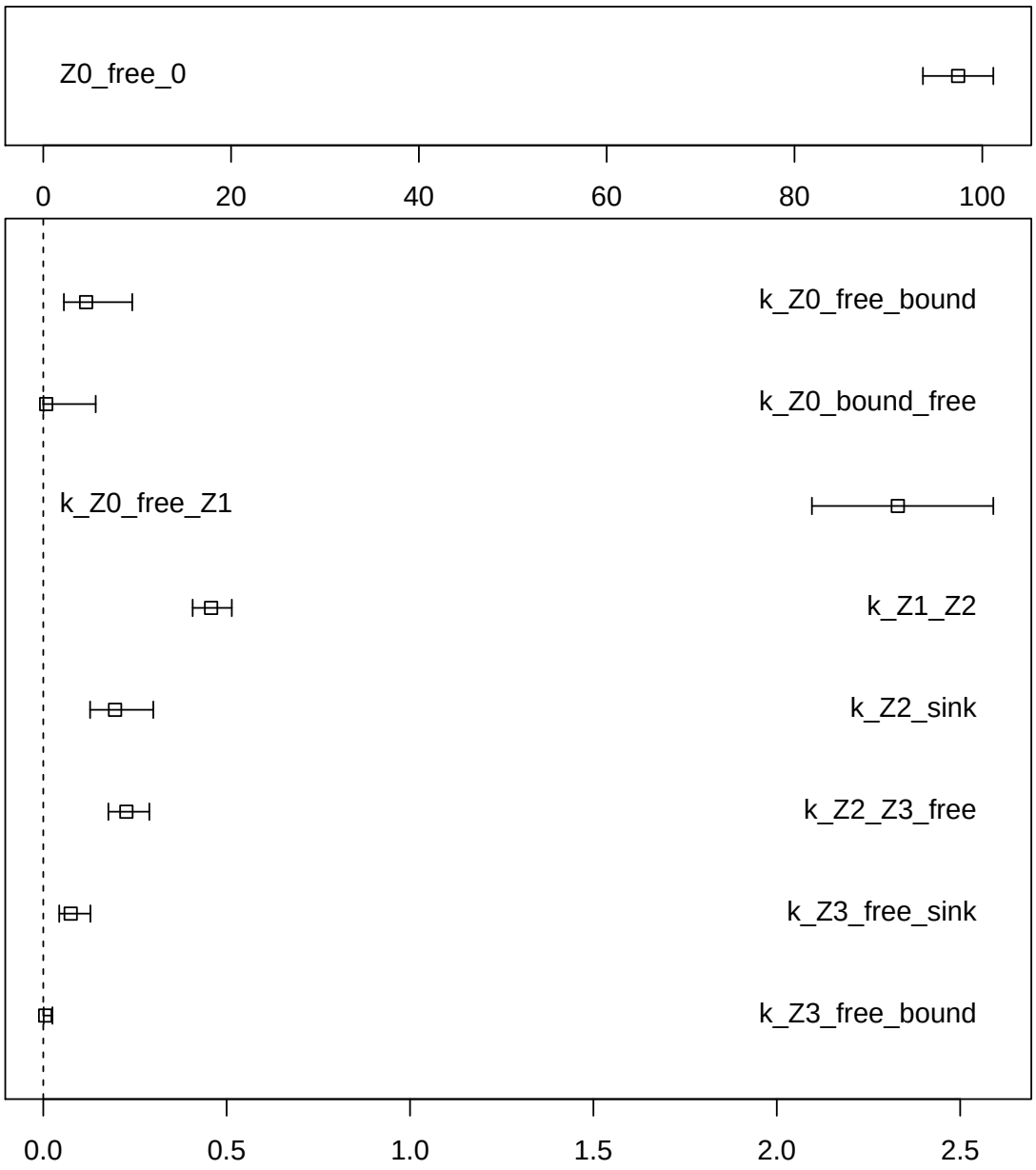
The summary view of the backtransformed parameters shows that we get no confidence intervals due to overparameterisation. As the optimized `k_Z3_bound_free` is excessively small, it seems reasonable to fix it to zero.

```
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       97.424978857 1.864396799 52.255496 2.880619e-44
## k_Z0_free_bound  0.116757939 0.042567928  2.742862 4.268192e-03
## k_Z0_bound_free  0.007890184 0.011498777  0.686176 2.479517e-01
## k_Z0_free_Z1     2.329999746 0.122793940 18.974876 2.997958e-24
## k_Z1_Z2          0.457590280 0.026468268 17.288260 1.494011e-22
## k_Z2_sink        0.195709803 0.041590057  4.705687 1.089697e-05
## k_Z2_Z3_free     0.226584724 0.027560331  8.221408 5.106652e-11
## k_Z3_free_sink   0.074789042 0.020202690  3.701935 2.757338e-04
## k_Z3_free_bound  0.005217923 0.004074066  1.280765 1.032150e-01
##                         Lower       Upper
## Z0_free_0       9.367767e+01 101.1722896
## k_Z0_free_bound 5.619674e-02   0.2425838
## k_Z0_bound_free 4.354112e-04   0.1429798
## k_Z0_free_Z1    2.095795e+00   2.5903768
## k_Z1_Z2         4.073348e-01   0.5140461
## k_Z2_sink       1.277075e-01   0.2999224
## k_Z2_Z3_free    1.775320e-01   0.2891908
## k_Z3_free_sink  4.353436e-02   0.1284825
## k_Z3_free_bound 1.098317e-03   0.0247895
```

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

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



The endpoints obtained with this model are

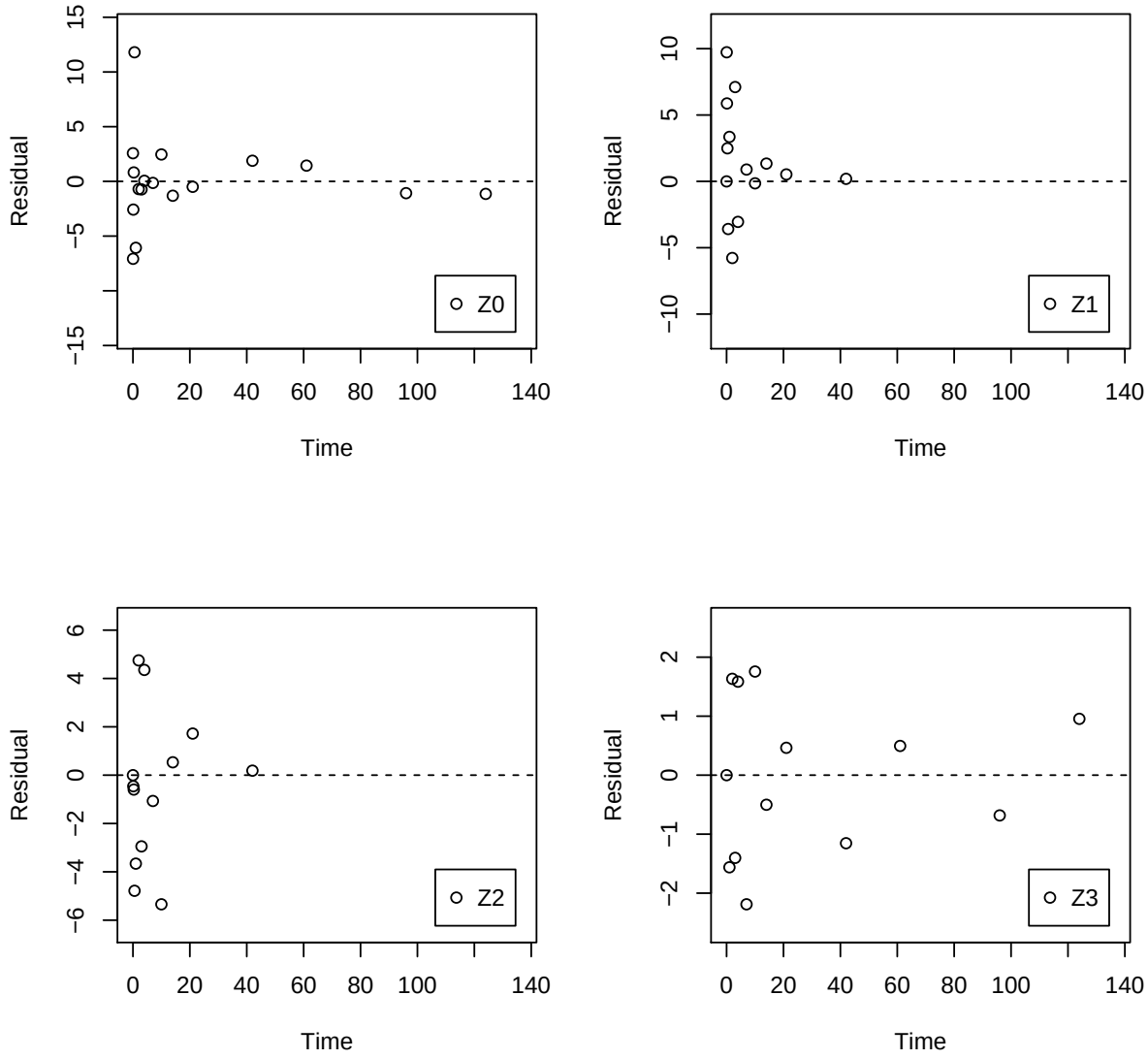
```
endpoints(m.Z.mkin.5a)
```

```
## $ff
##   Z0_free_Z1        Z1_Z2      Z2_sink   Z2_Z3_free Z3_free_sink
##    1.0000000    1.0000000    0.4634439    0.5365561    1.0000000
##
## $SFORB
```

```
##       Z0_b1       Z0_b2       Z3_b1       Z3_b2
## 2.447135360 0.007512509 0.080006965 0.000000000
##
## $distimes
##         DT50     DT90 DT50_Z0_b1 DT50_Z0_b2 DT50_Z3_b1 DT50_Z3_b2
## Z0 0.3042976 1.184811  0.2832484   92.26573         NA         NA
## Z1 1.5147769 5.031980         NA         NA         NA         NA
## Z2 1.6413833 5.452557         NA         NA         NA         NA
## Z3        NA       NA         NA         NA   8.663585        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.