

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)

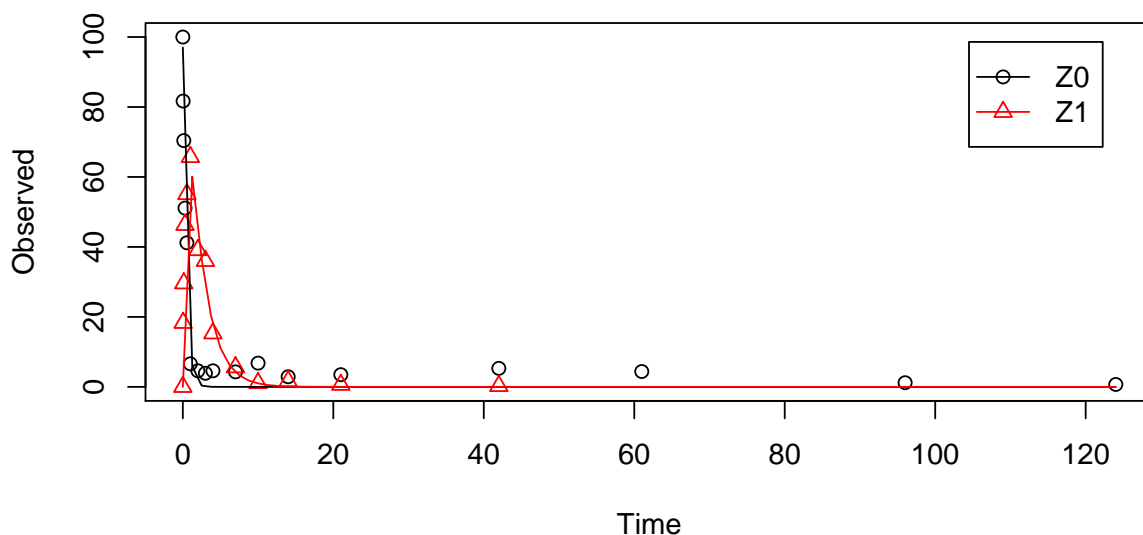
## Loading required package: mkin
## Loading required package: FME
## Loading required package: deSolve
## Loading required package: rootSolve
## Loading required package: minpack.lm
## Loading required package: MASS
## Loading required package: coda
## Loading required package: lattice

LOD = 0.5
FOCUS_2006_Z = data.frame(
  t = c(0, 0.04, 0.125, 0.29, 0.54, 1, 2, 3, 4, 7, 10, 14, 21,
        42, 61, 96, 124),
  Z0 = c(100, 81.7, 70.4, 51.1, 41.2, 6.6, 4.6, 3.9, 4.6, 4.3, 6.8,
        2.9, 3.5, 5.3, 4.4, 1.2, 0.7),
  Z1 = c(0, 18.3, 29.6, 46.3, 55.1, 65.7, 39.1, 36, 15.3, 5.6, 1.1,
        1.6, 0.6, 0.5 * LOD, NA, NA, NA),
  Z2 = c(0, NA, 0.5 * LOD, 2.6, 3.8, 15.3, 37.2, 31.7, 35.6, 14.5,
        0.8, 2.1, 1.9, 0.5 * LOD, NA, NA, NA),
  Z3 = c(0, NA, NA, NA, NA, 0.5 * LOD, 9.2, 13.1, 22.3, 28.4, 32.5,
        25.2, 17.2, 4.8, 4.5, 2.8, 4.4))
FOCUS_2006_Z_mkin <- mkin_wide_to_long(FOCUS_2006_Z)
```

2 Parent compound and one metabolite

The next step is to set up the models used for the kinetic analysis. As the simultaneous fit of parent and the first metabolite is usually straightforward, Step 1 (SFO for parent only) is skipped here. We start with the model 2a, with formation and decline of metabolite Z1 and the pathway from parent directly to sink included (default in mkin).

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



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

## mkin version:      0.9.25
## R version:         3.0.2
## Date of fit:       Sun Nov 17 15:23:40 2013
## Date of summary:   Sun Nov 17 15:23:40 2013
##
## Equations:
## [1] d_Z0 = - k_Z0_sink * Z0 - k_Z0_Z1 * Z0
## [2] d_Z1 = + k_Z0_Z1 * Z0 - k_Z1_sink * Z1
##
## Method used for solution of differential equation system:
## eigen
```

```

##
## Weighting: none
##
## Starting values for optimised parameters:
##           value    type transformed
## Z0_0       100.0000 state      100.000
## k_Z0_sink   0.1000 deparm      -2.303
## k_Z0_Z1     0.1001 deparm      -2.302
## k_Z1_sink   0.1002 deparm      -2.301
##
## Fixed parameter values:
##           value    type
## Z1_0         0 state
##
## Optimised, transformed parameters:
##           Estimate Std. Error Lower Upper
## Z0_0           97.000          NA    NA    NA
## k_Z0_sink     -36.400          NA    NA    NA
## k_Z0_Z1         0.805          NA    NA    NA
## k_Z1_sink     -0.730          NA    NA    NA
##
## Backtransformed parameters:
##           Estimate Lower Upper
## Z0_0       9.70e+01    NA    NA
## k_Z0_sink  1.62e-16    NA    NA
## k_Z0_Z1    2.24e+00    NA    NA
## k_Z1_sink  4.82e-01    NA    NA
##
## Residual standard error: 5.06 on 27 degrees of freedom
##
## Chi2 error levels in percent:
##           err.min n.optim df
## All data    17.9      4 26
## Z0          18.0      3 14
## Z1          15.1      1 12
##
## Estimated disappearance times:
##           DT50 DT90
## Z0 0.31 1.03
## Z1 1.44 4.78
##
## Estimated formation fractions:

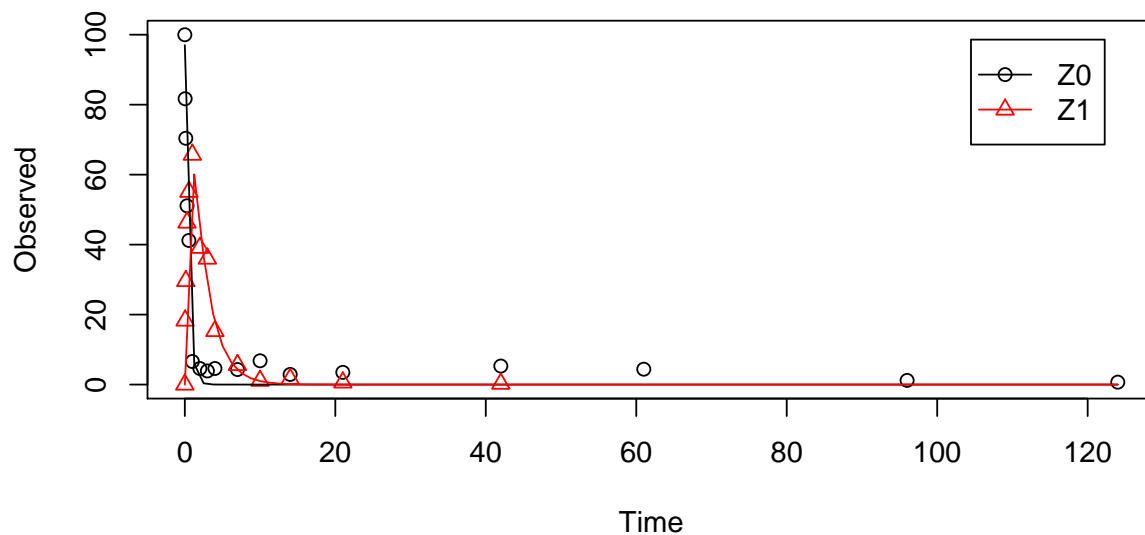
```

```
##          ff
## Z0_sink 7.23e-17
## Z0_Z1   1.00e+00
## Z1_sink 1.00e+00
##
## Parameter correlation:
## Could not estimate covariance matrix; singular system:
```

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

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

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



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

```

## mkin version:      0.9.25
## R version:        3.0.2
## Date of fit:       Sun Nov 17 15:23:41 2013
## Date of summary:  Sun Nov 17 15:23:41 2013
##
## Equations:
## [1] d_Z0 = - k_Z0 * Z0
## [2] d_Z1 = + f_Z0_to_Z1 * k_Z0 * Z0 - k_Z1 * Z1
##
## Method used for solution of differential equation system:
## eigen
##
## Weighting: none
##
## Starting values for optimised parameters:
##           value   type transformed
## Z0_0       100.0000 state    100.0000
## k_Z0         0.1000 deparm    -2.3026
## f_Z0_to_Z1   0.2000 deparm    -0.9803
## k_Z1         0.1001 deparm    -2.3016
##
## Fixed parameter values:
##           value   type
## Z1_0         0 state
##
## Optimised, transformed parameters:
##           Estimate Std. Error Lower Upper
## Z0_0           97.000         NA     NA     NA
## k_Z0            0.805         NA     NA     NA
## f_Z0_to_Z1     15.100         NA     NA     NA
## k_Z1           -0.730         NA     NA     NA
##
## Backtransformed parameters:
##           Estimate Lower Upper
## Z0_0           97.000     NA     NA
## k_Z0            2.240     NA     NA
## f_Z0_to_Z1      1.000     NA     NA
## k_Z1            0.482     NA     NA
##
## Residual standard error: 5.06 on 27 degrees of freedom
##
## Chi2 error levels in percent:

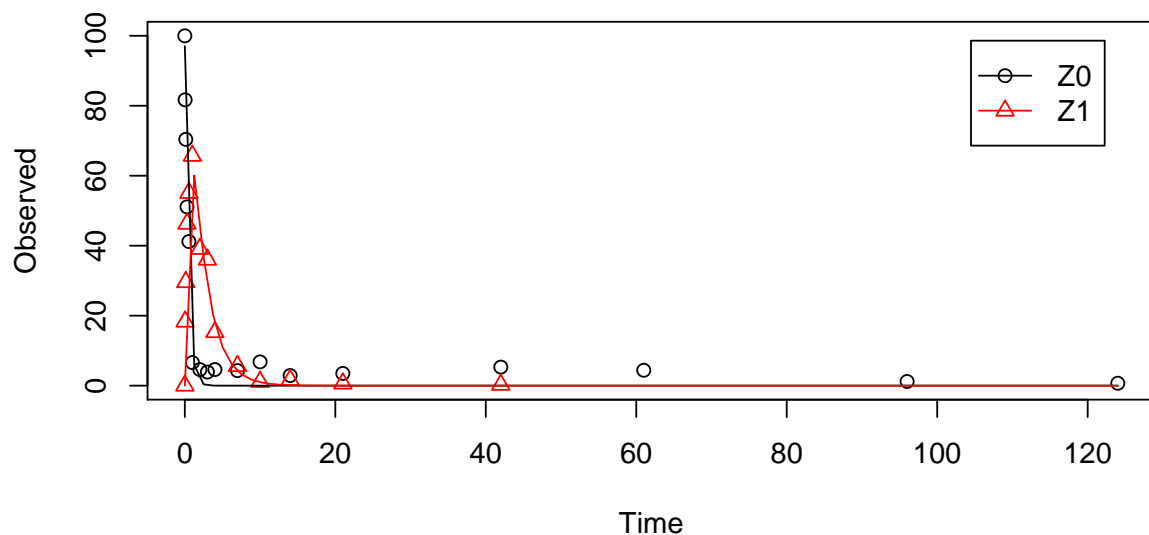
```

```
##           err.min n.optim df
## All data      17.9      4 26
## Z0           17.6      2 15
## Z1           15.6      2 11
##
## Estimated disappearance times:
##      DT50 DT90
## Z0 0.31 1.03
## Z1 1.44 4.78
##
## Parameter correlation:
## Could not estimate covariance matrix; singular system:
```

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

The simplified model is obtained by setting the list component `sink` to `FALSE`. This model definition is not supported when formation fractions are used.

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



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

## mkin version:      0.9.25
## R version:         3.0.2
## Date of fit:       Sun Nov 17 15:23:42 2013
## Date of summary:   Sun Nov 17 15:23:42 2013
##
## Equations:
## [1] d_Z0 = - 0 - k_Z0_Z1 * Z0
## [2] d_Z1 = + k_Z0_Z1 * Z0 - k_Z1_sink * Z1
##
## Method used for solution of differential equation system:
## eigen
##
## Weighting: none
##
## Starting values for optimised parameters:
##           value    type transformed
## Z0_0       100.0000 state      100.000
## k_Z0_Z1     0.1000 deparm      -2.303
## k_Z1_sink   0.1001 deparm      -2.302
##
## Fixed parameter values:
##           value    type
## Z1_0         0 state
##
## Optimised, transformed parameters:
##           Estimate Std. Error  Lower  Upper
## Z0_0           97.000      2.6800 91.500 103.000
## k_Z0_Z1         0.805      0.0657  0.670  0.939
## k_Z1_sink      -0.730      0.0885 -0.911 -0.548
##
## Backtransformed parameters:
##           Estimate  Lower  Upper
## Z0_0           97.000 91.500 103.000
## k_Z0_Z1         2.240  1.950  2.560
## k_Z1_sink        0.482  0.402  0.578
##
## Residual standard error: 4.97 on 28 degrees of freedom
##
## Chi2 error levels in percent:
##           err.min n.optim df
```

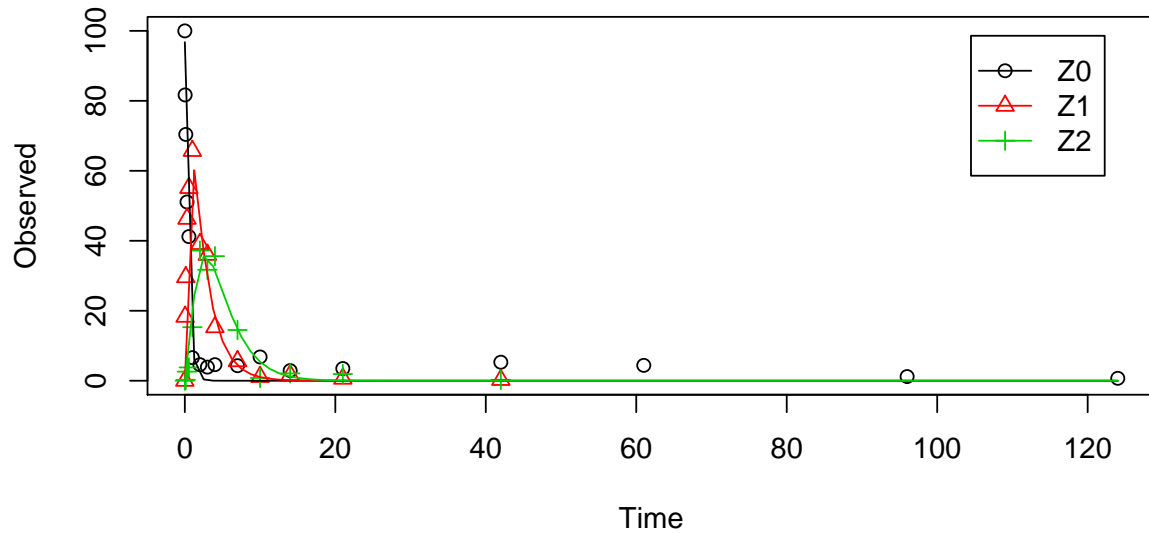


```
## All data      17.6      3 27
## Z0           17.6      2 15
## Z1           15.1      1 12
##
## Estimated disappearance times:
##      DT50 DT90
## Z0 0.31 1.03
## Z1 1.44 4.78
##
## Estimated formation fractions:
##      ff
## Z0_sink 1
## Z0_Z1   1
## Z1_sink 1
##
## Parameter correlation:
##      Z0_0 k_Z0_Z1 k_Z1_sink
## Z0_0      1.000 0.1063 0.4104
## k_Z0_Z1   0.106 1.0000 0.0434
## k_Z1_sink 0.410 0.0434 1.0000
```

3 Including metabolites Z2 and Z3

As suggested in the FOCUS report, the pathway to sink was removed for metabolite Z1 as well in the next step. While this step appears questionable on the basis of the above results, it is followed here for the purpose of comparison. Also, in the FOCUS report, it is assumed that there is additional empirical evidence that Z1 quickly and exclusively hydrolyses to Z2.

```
Z.5 <- mkinmod(Z0 = list(type = "SF0", to = "Z1", sink = FALSE),
              Z1 = list(type = "SF0", to = "Z2", sink = FALSE),
              Z2 = list(type = "SF0"))
m.Z.5 <- mkinfit(Z.5, FOCUS_2006_Z_mkin, quiet = TRUE)
plot(m.Z.5)
```



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

## mkin version:    0.9.25
## R version:      3.0.2
## Date of fit:     Sun Nov 17 15:23:43 2013
## Date of summary: Sun Nov 17 15:23:43 2013
##
## Equations:
## [1] d_Z0 = - 0 - k_Z0_Z1 * Z0
## [2] d_Z1 = + k_Z0_Z1 * Z0 - 0 - k_Z1_Z2 * Z1
## [3] d_Z2 = + k_Z1_Z2 * Z1 - k_Z2_sink * Z2
##
## Method used for solution of differential equation system:
## eigen
##
## Weighting: none
##
## Starting values for optimised parameters:
##           value  type transformed
## Z0_0       100.0000 state      100.000
## k_Z0_Z1     0.1000 deparm      -2.303
## k_Z1_Z2     0.1001 deparm      -2.302
## k_Z2_sink   0.1002 deparm      -2.301
##
```

```

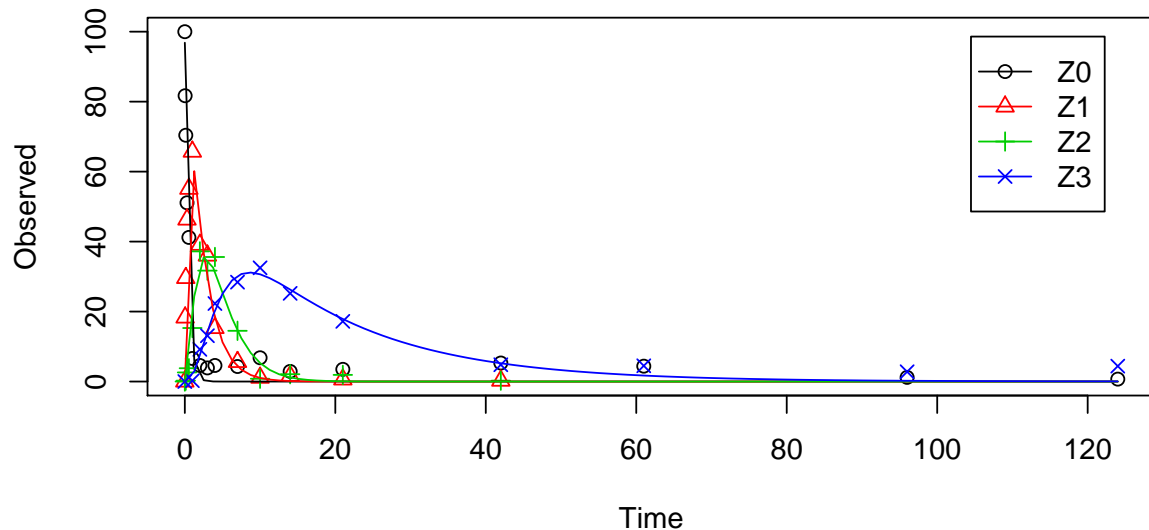
## Fixed parameter values:
##      value  type
## Z1_0      0 state
## Z2_0      0 state
##
## Optimised, transformed parameters:
##      Estimate Std. Error  Lower  Upper
## Z0_0      96.800    2.2700 92.200 101.000
## k_Z0_Z1     0.795    0.0584  0.677  0.913
## k_Z1_Z2    -0.741    0.0682 -0.879 -0.603
## k_Z2_sink  -0.803    0.1110 -1.030 -0.579
##
## Backtransformed parameters:
##      Estimate  Lower  Upper
## Z0_0      96.800 92.200 101.000
## k_Z0_Z1     2.210  1.970  2.490
## k_Z1_Z2     0.477  0.415  0.547
## k_Z2_sink    0.448  0.358  0.561
##
## Residual standard error: 4.49 on 40 degrees of freedom
##
## Chi2 error levels in percent:
##      err.min n.optim df
## All data   19.1      4 38
## Z0         17.4      2 15
## Z1         15.3      1 12
## Z2         19.6      1 11
##
## Estimated disappearance times:
##      DT50 DT90
## Z0 0.313 1.04
## Z1 1.454 4.83
## Z2 1.547 5.14
##
## Estimated formation fractions:
##      ff
## Z0_sink 1
## Z0_Z1    1
## Z1_sink  1
## Z1_Z2    1
## Z2_sink  1
##

```

```
## Parameter correlation:
##           Z0_0 k_Z0_Z1 k_Z1_Z2 k_Z2_sink
## Z0_0       1.0000  0.0578  0.2875   0.3179
## k_Z0_Z1    0.0578  1.0000 -0.0436   0.0121
## k_Z1_Z2    0.2875 -0.0436  1.0000   0.2402
## k_Z2_sink  0.3179  0.0121  0.2402   1.0000
```

Finally, metabolite Z3 is added to the model. The fit is 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,
                    parms.ini = m.Z.5$bparms.ode,
                    quiet = TRUE)
plot(m.Z.FOCUS)
```



```
summary(m.Z.FOCUS, data = FALSE)
## mkin version:    0.9.25
## R version:       3.0.2
## Date of fit:      Sun Nov 17 15:23:45 2013
## Date of summary:  Sun Nov 17 15:23:45 2013
```

```

##
## Equations:
## [1] d_Z0 = - 0 - k_Z0_Z1 * Z0
## [2] d_Z1 = + k_Z0_Z1 * Z0 - 0 - k_Z1_Z2 * Z1
## [3] d_Z2 = + k_Z1_Z2 * Z1 - k_Z2_sink * Z2 - k_Z2_Z3 * Z2
## [4] d_Z3 = + k_Z2_Z3 * Z2 - k_Z3_sink * Z3
##
## Method used for solution of differential equation system:
## eigen
##
## Weighting: none
##
## Starting values for optimised parameters:
##           value  type transformed
## Z0_0       100.0000  state    100.0000
## k_Z0_Z1     2.2140 deparm     0.7948
## k_Z1_Z2     0.4766 deparm    -0.7410
## k_Z2_sink   0.4481 deparm    -0.8027
## k_Z2_Z3     0.1000 deparm    -2.3026
## k_Z3_sink   0.1001 deparm    -2.3016
##
## Fixed parameter values:
##           value  type
## Z1_0         0  state
## Z2_0         0  state
## Z3_0         0  state
##
## Optimised, transformed parameters:
##           Estimate Std. Error  Lower  Upper
## Z0_0           96.800     2.0600  92.700 101.000
## k_Z0_Z1         0.795     0.0533   0.688   0.902
## k_Z1_Z2        -0.738     0.0612  -0.860  -0.615
## k_Z2_sink       -1.430     0.1720  -1.780  -1.090
## k_Z2_Z3        -1.550     0.1230  -1.790  -1.300
## k_Z3_sink       -2.840     0.2440  -3.320  -2.350
##
## Backtransformed parameters:
##           Estimate  Lower  Upper
## Z0_0           96.8000  92.700 101.0000
## k_Z0_Z1         2.2200   1.990   2.4700
## k_Z1_Z2         0.4780   0.423   0.5410
## k_Z2_sink        0.2390   0.169   0.3370

```

```

## k_Z2_Z3      0.2130  0.166   0.2720
## k_Z3_sink    0.0587  0.036   0.0957
##
## Residual standard error: 4.1 on 51 degrees of freedom
##
## Chi2 error levels in percent:
##           err.min n.optim df
## All data      19.2      6 48
## Z0            17.4      2 15
## Z1            15.2      1 12
## Z2            20.3      2 10
## Z3            11.9      1 11
##
## Estimated disappearance times:
##      DT50  DT90
## Z0  0.313  1.04
## Z1  1.449  4.81
## Z2  1.535  5.10
## Z3 11.810 39.23
##
## Estimated formation fractions:
##           ff
## Z0_sink 1.000
## Z0_Z1   1.000
## Z1_sink 1.000
## Z1_Z2   1.000
## Z2_sink 0.528
## Z2_Z3   0.472
## Z3_sink 1.000
##
## Parameter correlation:
##           Z0_0 k_Z0_Z1 k_Z1_Z2 k_Z2_sink k_Z2_Z3 k_Z3_sink
## Z0_0      1.0000  0.0539  0.2727   0.3701 -0.0730  -0.1135
## k_Z0_Z1   0.0539  1.0000 -0.0521   0.0244 -0.0358  -0.0252
## k_Z1_Z2   0.2727 -0.0521  1.0000   0.2938 -0.1213  -0.1915
## k_Z2_sink 0.3701  0.0244  0.2938   1.0000 -0.1889  -0.6430
## k_Z2_Z3  -0.0730 -0.0358 -0.1213  -0.1889  1.0000   0.5516
## k_Z3_sink -0.1135 -0.0252 -0.1915  -0.6430  0.5516   1.0000

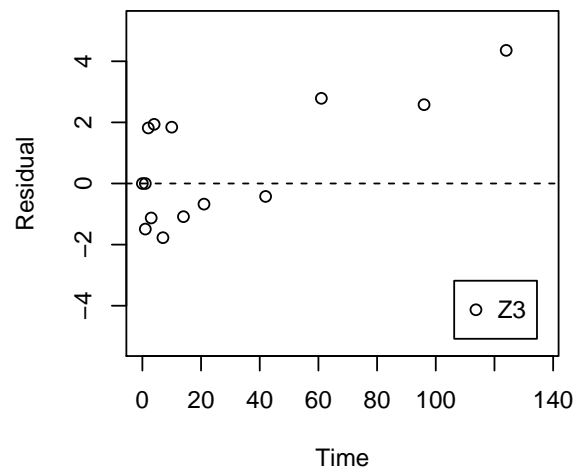
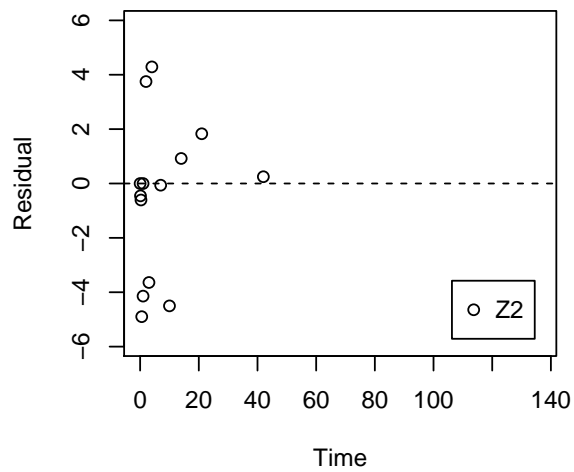
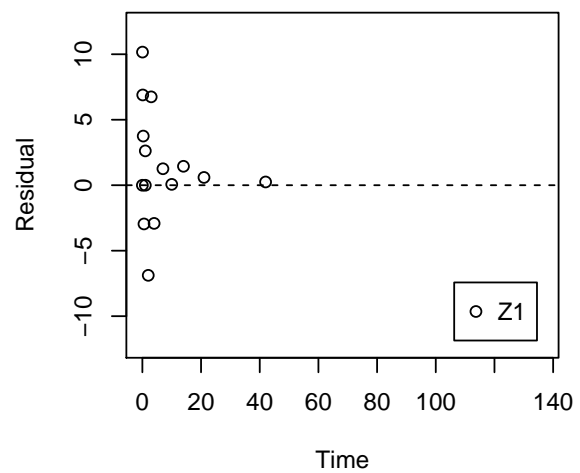
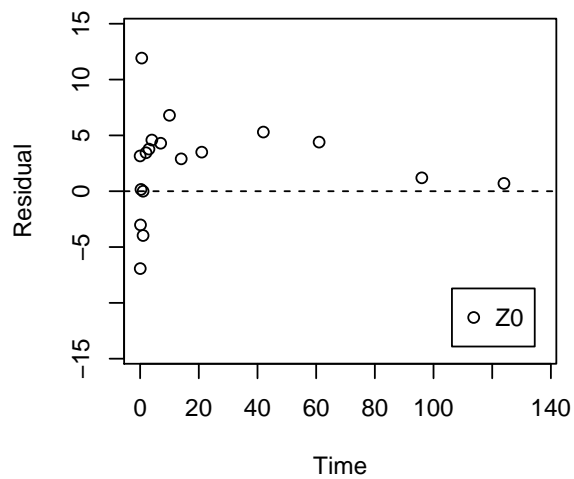
```

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

```

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

```

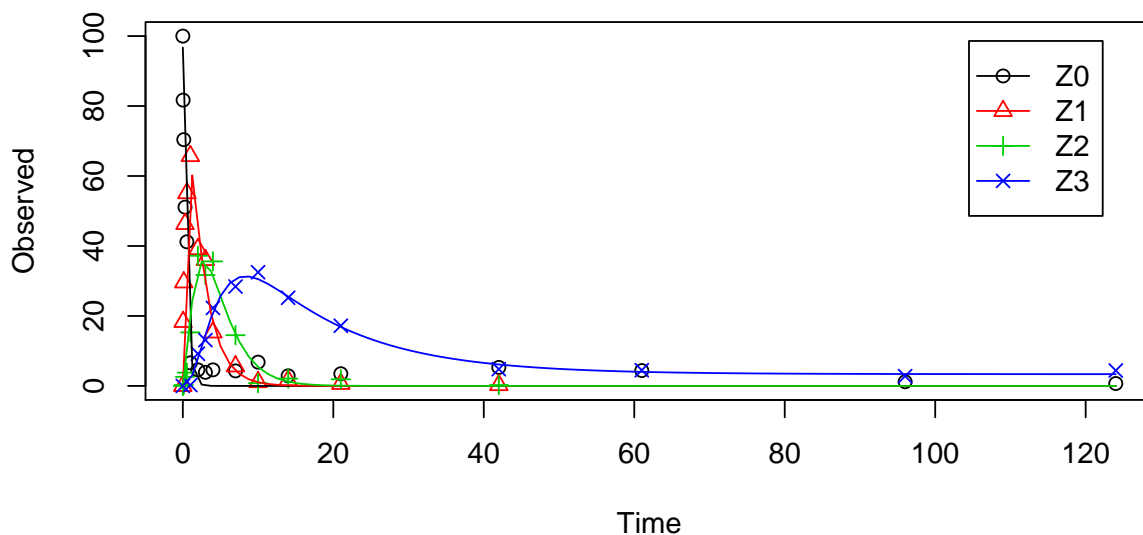


4 Using the SFORB model for parent and metabolites

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

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

```
Z.mkin.1 <- mkinmod(Z0 = list(type = "SFO", to = "Z1", sink = FALSE),
  Z1 = list(type = "SFO", to = "Z2", sink = FALSE),
  Z2 = list(type = "SFO", to = "Z3"),
  Z3 = list(type = "SFORB"))
m.Z.mkin.1 <- mkinfit(Z.mkin.1, FOCUS_2006_Z_mkin,
  parms.ini = c(k_Z0_Z1 = 0.5, k_Z1_Z2 = 0.3),
  quiet = TRUE)
plot(m.Z.mkin.1)
```



```
summary(m.Z.mkin.1, data = FALSE)
## mkin version:    0.9.25
## R version:       3.0.2
## Date of fit:      Sun Nov 17 15:23:54 2013
```



```

## Date of summary: Sun Nov 17 15:23:54 2013
##
## Equations:
## [1] d_Z0 = - 0 - k_Z0_Z1 * Z0
## [2] d_Z1 = + k_Z0_Z1 * Z0 - 0 - k_Z1_Z2 * Z1
## [3] d_Z2 = + k_Z1_Z2 * Z1 - k_Z2_sink * Z2 - k_Z2_Z3_free * Z2
## [4] d_Z3_free = + k_Z2_Z3_free * Z2 - k_Z3_free_sink * Z3_free - k_Z3_free_bound *
## [5] d_Z3_bound = + k_Z3_free_bound * Z3_free - k_Z3_bound_free * Z3_bound
##
## Method used for solution of differential equation system:
## eigen
##
## Weighting: none
##
## Starting values for optimised parameters:
##
##          value   type transformed
## Z0_0          100.0000 state    100.0000
## k_Z0_Z1         0.5000 deparm    -0.6931
## k_Z1_Z2         0.3000 deparm    -1.2040
## k_Z2_sink       0.1000 deparm    -2.3026
## k_Z2_Z3_free    0.1001 deparm    -2.3016
## k_Z3_free_sink  0.1002 deparm    -2.3006
## k_Z3_free_bound 0.1000 deparm    -2.3026
## k_Z3_bound_free 0.0200 deparm    -3.9120
##
## Fixed parameter values:
##          value   type
## Z1_0         0 state
## Z2_0         0 state
## Z3_free_0     0 state
## Z3_bound_0    0 state
##
## Optimised, transformed parameters:
##          Estimate Std. Error   Lower   Upper
## Z0_0          96.700   2.07e+00  9.26e+01  1.01e+02
## k_Z0_Z1         0.795   5.35e-02  6.87e-01  9.02e-01
## k_Z1_Z2        -0.743   6.19e-02 -8.67e-01 -6.18e-01
## k_Z2_sink       -1.490   2.02e-01 -1.90e+00 -1.09e+00
## k_Z2_Z3_free    -1.500   1.54e-01 -1.81e+00 -1.19e+00
## k_Z3_free_sink  -2.650   3.05e-01 -3.27e+00 -2.04e+00
## k_Z3_free_bound -5.240   8.80e-01 -7.01e+00 -3.48e+00
## k_Z3_bound_free -21.000   2.68e+06 -5.39e+06  5.39e+06

```

```

##
## Backtransformed parameters:
##           Estimate      Lower      Upper
## Z0_0          9.67e+01 9.26e+01 101.0000
## k_Z0_Z1        2.21e+00 1.99e+00  2.4600
## k_Z1_Z2        4.76e-01 4.20e-01  0.5390
## k_Z2_sink       2.24e-01 1.49e-01  0.3370
## k_Z2_Z3_free    2.22e-01 1.63e-01  0.3030
## k_Z3_free_sink  7.03e-02 3.81e-02  0.1300
## k_Z3_free_bound 5.28e-03 9.01e-04  0.0309
## k_Z3_bound_free 7.37e-10 0.00e+00      Inf
##
## Residual standard error: 4.11 on 49 degrees of freedom
##
## Chi2 error levels in percent:
##           err.min n.optim df
## All data    19.23      8 46
## Z0          17.43      2 15
## Z1          15.27      1 12
## Z2          20.28      2 10
## Z3           8.22      3  9
##
## Estimated disappearance times:
##           DT50  DT90
## Z0  0.313  1.04
## Z1  1.457  4.84
## Z2  1.552  5.16
## Z3 10.198 45.33
##
## Estimated formation fractions:
##           ff
## Z0_sink    1.000
## Z0_Z1      1.000
## Z1_sink    1.000
## Z1_Z2      1.000
## Z2_sink    0.502
## Z2_Z3_free 0.498
## Z3_free_sink 1.000
##
## Estimated Eigenvalues of SFORB model(s):
##           Z3_b1  Z3_b2
## 7.56e-02 6.86e-10

```

```
##
## Parameter correlation:
##
```

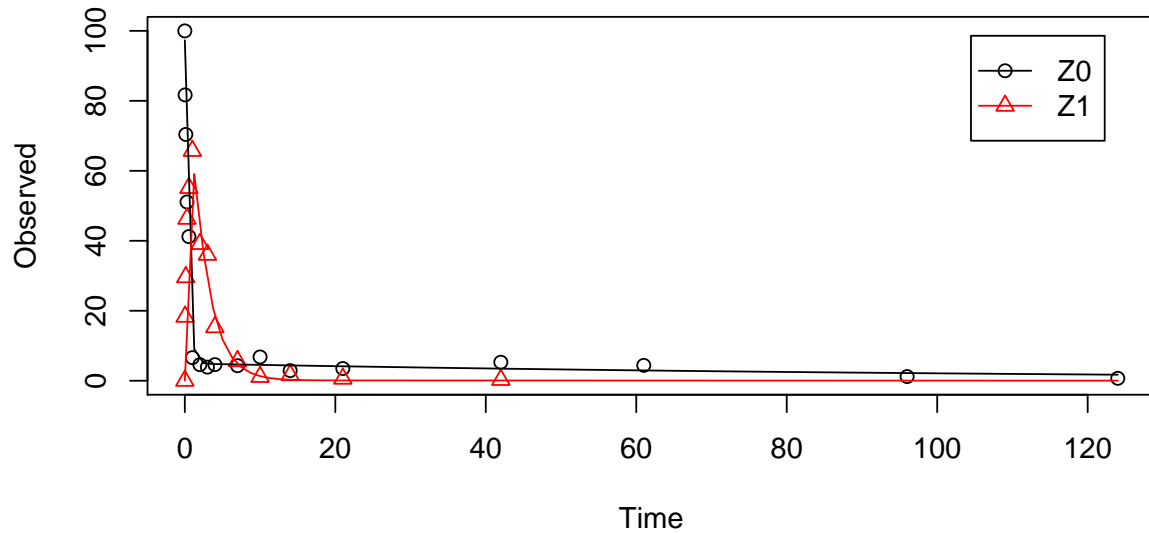
	Z0_0	k_Z0_Z1	k_Z1_Z2	k_Z2_sink	k_Z2_Z3_free
## Z0_0	1.0000	0.0549	0.2797	0.367	-0.1112
## k_Z0_Z1	0.0549	1.0000	-0.0491	0.032	-0.0437
## k_Z1_Z2	0.2797	-0.0491	1.0000	0.322	-0.1848
## k_Z2_sink	0.3671	0.0320	0.3222	1.000	-0.3689
## k_Z2_Z3_free	-0.1112	-0.0437	-0.1848	-0.369	1.0000
## k_Z3_free_sink	-0.1358	-0.0325	-0.2274	-0.723	0.6204
## k_Z3_free_bound	-0.0528	-0.0147	-0.0884	-0.301	0.3179
## k_Z3_bound_free	-0.0541	-0.0175	-0.0905	-0.127	0.5045

```
##
```

	k_Z3_free_sink	k_Z3_free_bound	k_Z3_bound_free
## Z0_0	-0.1358	-0.0528	-0.0541
## k_Z0_Z1	-0.0325	-0.0147	-0.0175
## k_Z1_Z2	-0.2274	-0.0884	-0.0905
## k_Z2_sink	-0.7235	-0.3006	-0.1273
## k_Z2_Z3_free	0.6204	0.3179	0.5045
## k_Z3_free_sink	1.0000	0.5689	0.1509
## k_Z3_free_bound	0.5689	1.0000	0.1594
## k_Z3_bound_free	0.1509	0.1594	1.0000

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:    0.9.25
## R version:      3.0.2
## Date of fit:     Sun Nov 17 15:23:55 2013
## Date of summary: Sun Nov 17 15:23:55 2013
##
## Equations:
## [1] d_Z0_free = - 0 - k_Z0_free_bound * Z0_free + k_Z0_bound_free * Z0_bound - k_Z
## [2] d_Z0_bound = + k_Z0_free_bound * Z0_free - k_Z0_bound_free * Z0_bound
## [3] d_Z1 = + k_Z0_free_Z1 * Z0_free - k_Z1_sink * Z1
##
## Method used for solution of differential equation system:
## eigen
##
## Weighting: none
##
## Starting values for optimised parameters:
##           value   type transformed
## Z0_free_0    100.0000 state     100.000
## k_Z0_free_bound  0.1000 deparm     -2.303
## k_Z0_bound_free  0.0200 deparm     -3.912
## k_Z0_free_Z1    0.1002 deparm     -2.301
## k_Z1_sink       0.1003 deparm     -2.300
```

```

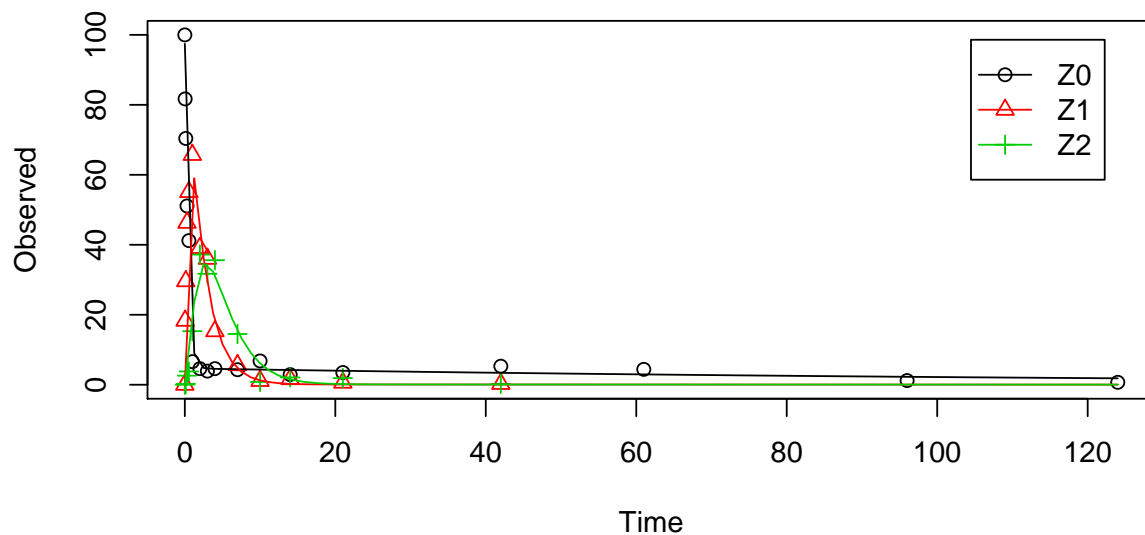
##
## Fixed parameter values:
##      value  type
## Z0_bound_0    0 state
## Z1_0          0 state
##
## Optimised, transformed parameters:
##      Estimate Std. Error  Lower  Upper
## Z0_free_0      97.300    2.4000 92.400 102.000
## k_Z0_free_bound -2.080    0.4320 -2.970 -1.190
## k_Z0_bound_free -4.720    1.6000 -8.020 -1.420
## k_Z0_free_Z1     0.855    0.0643  0.723  0.987
## k_Z1_sink       -0.793    0.0851 -0.968 -0.619
##
## Backtransformed parameters:
##      Estimate  Lower  Upper
## Z0_free_0      97.30000 9.24e+01 102.000
## k_Z0_free_bound  0.12500 5.13e-02  0.303
## k_Z0_bound_free  0.00891 3.29e-04  0.241
## k_Z0_free_Z1     2.35000 2.06e+00  2.680
## k_Z1_sink        0.45200 3.80e-01  0.539
##
## Residual standard error: 4.44 on 26 degrees of freedom
##
## Chi2 error levels in percent:
##      err.min n.optim df
## All data    15.6      5 25
## Z0          14.7      4 13
## Z1          14.3      1 12
##
## Estimated disappearance times:
##      DT50 DT90
## Z0 0.302 1.19
## Z1 1.532 5.09
##
## Estimated formation fractions:
##      ff
## Z0_free_sink 1
## Z0_free_Z1   1
## Z1_sink      1
##
## Estimated Eigenvalues of SFORB model(s):

```

```
## Z0_b1 Z0_b2
## 2.47631 0.00846
##
## Parameter correlation:
##
##      Z0_free_0 k_Z0_free_bound k_Z0_bound_free k_Z0_free_Z1
## Z0_free_0      1.00000      0.00649      0.0332      0.1118
## k_Z0_free_bound 0.00649      1.00000      0.5465      0.4139
## k_Z0_bound_free 0.03324      0.54647      1.0000      0.1584
## k_Z0_free_Z1    0.11182      0.41393      0.1584      1.0000
## k_Z1_sink       0.39155     -0.29191     -0.1260     -0.0419
##
##      k_Z1_sink
## Z0_free_0      0.3916
## k_Z0_free_bound -0.2919
## k_Z0_bound_free -0.1260
## k_Z0_free_Z1    -0.0419
## k_Z1_sink       1.0000
```

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

```
Z.mkin.3 <- mkinmod(Z0 = list(type = "SFORB", to = "Z1", sink = FALSE),
                  Z1 = list(type = "SFO", to = "Z2"),
                  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:    0.9.25
## R version:      3.0.2
## Date of fit:     Sun Nov 17 15:23:59 2013
## Date of summary: Sun Nov 17 15:23:59 2013
##
## Equations:
## [1] d_Z0_free = - 0 - k_Z0_free_bound * Z0_free + k_Z0_bound_free * Z0_bound - k_Z
## [2] d_Z0_bound = + k_Z0_free_bound * Z0_free - k_Z0_bound_free * Z0_bound
## [3] d_Z1 = + k_Z0_free_Z1 * Z0_free - k_Z1_sink * Z1 - k_Z1_Z2 * Z1
## [4] d_Z2 = + k_Z1_Z2 * Z1 - k_Z2_sink * Z2
##
## Method used for solution of differential equation system:
## eigen
##
## Weighting: none
##
## Starting values for optimised parameters:
##           value   type transformed
## Z0_free_0    100.0000 state    100.000
## k_Z0_free_bound  0.1000 deparm    -2.303
## k_Z0_bound_free  0.0200 deparm    -3.912
## k_Z0_free_Z1    0.1002 deparm    -2.301
```

```

## k_Z1_sink      0.1003 deparm      -2.300
## k_Z1_Z2        0.1004 deparm      -2.299
## k_Z2_sink      0.1005 deparm      -2.298
##
## 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
## Z0_free_0       97.400    2.13e+00  9.31e+01  1.02e+02
## k_Z0_free_bound  -2.150    4.10e-01 -2.98e+00 -1.32e+00
## k_Z0_bound_free  -4.840    1.63e+00 -8.15e+00 -1.53e+00
## k_Z0_free_Z1      0.846    5.91e-02  7.26e-01  9.65e-01
## k_Z1_sink       -21.600    2.83e+06 -5.74e+06  5.74e+06
## k_Z1_Z2         -0.781    8.75e-02 -9.59e-01 -6.04e-01
## k_Z2_sink       -0.861    1.22e-01 -1.11e+00 -6.13e-01
##
## Backtransformed parameters:
##           Estimate      Lower      Upper
## Z0_free_0       9.74e+01 9.31e+01 102.000
## k_Z0_free_bound  1.17e-01 5.08e-02  0.268
## k_Z0_bound_free  7.92e-03 2.89e-04  0.217
## k_Z0_free_Z1     2.33e+00 2.07e+00  2.630
## k_Z1_sink        4.09e-10 0.00e+00    Inf
## k_Z1_Z2          4.58e-01 3.83e-01  0.547
## k_Z2_sink        4.23e-01 3.30e-01  0.542
##
## Residual standard error: 4.14 on 37 degrees of freedom
##
## Chi2 error levels in percent:
##           err.min n.optim df
## All data      17.5      7 35
## Z0            14.7      4 13
## Z1            14.9      2 11
## Z2            20.3      1 11
##
## Estimated disappearance times:
##           DT50 DT90
## Z0 0.304 1.18

```



```

## Z1 1.514 5.03
## Z2 1.639 5.44
##
## Estimated formation fractions:
##           ff
## Z0_free_sink 1.00e+00
## Z0_free_Z1   1.00e+00
## Z1_sink      8.94e-10
## Z1_Z2        1.00e+00
## Z2_sink      1.00e+00
##
## Estimated Eigenvalues of SFORB model(s):
##   Z0_b1   Z0_b2
## 2.44664 0.00754
##
## Parameter correlation:
##           Z0_free_0 k_Z0_free_bound k_Z0_bound_free k_Z0_free_Z1
## Z0_free_0          1.0000          0.0643          0.0650          0.09060
## k_Z0_free_bound    0.0643          1.0000          0.5430          0.42352
## k_Z0_bound_free    0.0650          0.5430          1.0000          0.16290
## k_Z0_free_Z1       0.0906          0.4235          0.1629          1.00000
## k_Z1_sink          0.1731         -0.0545         -0.0270          0.00683
## k_Z1_Z2            0.0759         -0.1346         -0.0469         -0.08022
## k_Z2_sink          0.1656         -0.1578         -0.0564         -0.04623
##           k_Z1_sink k_Z1_Z2 k_Z2_sink
## Z0_free_0          0.17310 0.0759   0.1656
## k_Z0_free_bound   -0.05451 -0.1346  -0.1578
## k_Z0_bound_free   -0.02697 -0.0469  -0.0564
## k_Z0_free_Z1       0.00683 -0.0802  -0.0462
## k_Z1_sink          1.00000 -0.6605  -0.4824
## k_Z1_Z2            -0.66050 1.0000   0.4981
## k_Z2_sink          -0.48244 0.4981   1.0000

```

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

Finally, Z3 is added as well. This model appears overparameterised (no covariance matrix returned) if the sink for Z1 is left in the model.

```

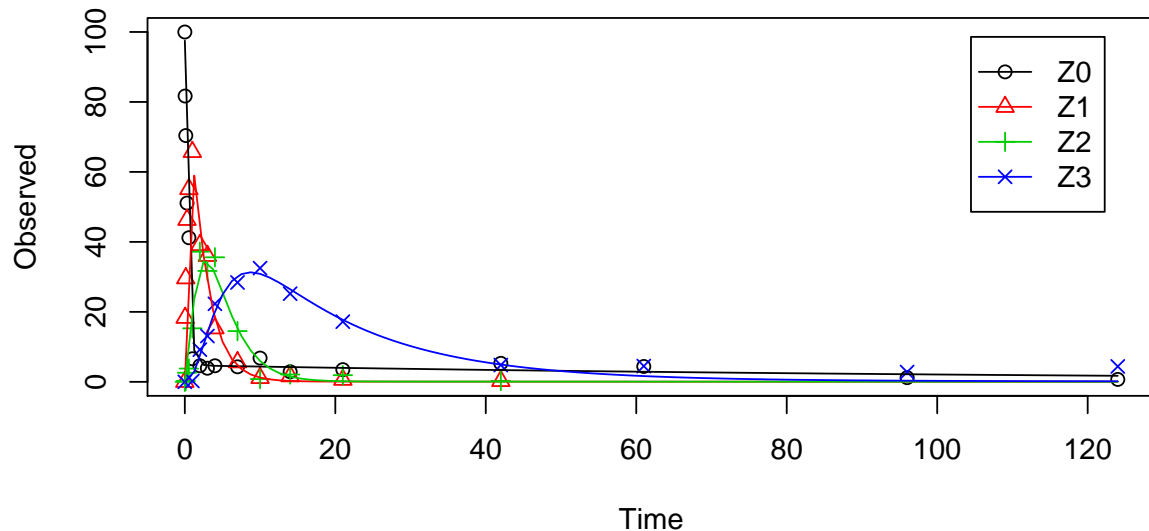
Z.mkin.4 <- mkinmod(Z0 = list(type = "SFORB", to = "Z1", sink = FALSE),
                  Z1 = list(type = "SFO", to = "Z2", sink = FALSE),
                  Z2 = list(type = "SFO", to = "Z3"),
                  Z3 = list(type = "SFO"))
m.Z.mkin.4 <- mkinfit(Z.mkin.4, FOCUS_2006_Z_mkin,

```

```

      parms.ini = c(k_Z1_Z2 = 0.05),
      quiet = TRUE)
plot(m.Z.mkin.4)

```



```

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

## mkin version:    0.9.25
## R version:      3.0.2
## Date of fit:     Sun Nov 17 15:24:07 2013
## Date of summary: Sun Nov 17 15:24:07 2013
##
## Equations:
## [1] d_Z0_free = - 0 - k_Z0_free_bound * Z0_free + k_Z0_bound_free * Z0_bound - k_Z
## [2] d_Z0_bound = + k_Z0_free_bound * Z0_free - k_Z0_bound_free * Z0_bound
## [3] d_Z1 = + k_Z0_free_Z1 * Z0_free - 0 - k_Z1_Z2 * Z1
## [4] d_Z2 = + k_Z1_Z2 * Z1 - k_Z2_sink * Z2 - k_Z2_Z3 * Z2
## [5] d_Z3 = + k_Z2_Z3 * Z2 - k_Z3_sink * Z3
##
## Method used for solution of differential equation system:
## eigen
##
## Weighting: none
##
## Starting values for optimised parameters:

```

```

##          value    type transformed
## Z0_free_0    100.0000 state      100.000
## k_Z1_Z2       0.0500 deparm     -2.996
## k_Z0_free_bound 0.1000 deparm     -2.303
## k_Z0_bound_free 0.0200 deparm     -3.912
## k_Z0_free_Z1   0.1002 deparm     -2.301
## k_Z2_sink      0.1003 deparm     -2.300
## k_Z2_Z3        0.1004 deparm     -2.299
## k_Z3_sink      0.1005 deparm     -2.298
##
## Fixed parameter values:
##          value    type
## Z0_bound_0      0 state
## Z1_0            0 state
## Z2_0            0 state
## Z3_0            0 state
##
## Optimised, transformed parameters:
##          Estimate Std. Error  Lower  Upper
## Z0_free_0      97.500      1.8900 93.700 101.000
## k_Z1_Z2       -0.777      0.0583 -0.894 -0.660
## k_Z0_free_bound -2.140      0.3680 -2.880 -1.400
## k_Z0_bound_free -4.770      1.4200 -7.610 -1.920
## k_Z0_free_Z1     0.847      0.0534  0.740  0.954
## k_Z2_sink      -1.560      0.1830 -1.930 -1.190
## k_Z2_Z3        -1.530      0.1140 -1.760 -1.300
## k_Z3_sink      -2.770      0.2250 -3.220 -2.320
##
## Backtransformed parameters:
##          Estimate  Lower  Upper
## Z0_free_0    97.50000 9.37e+01 101.0000
## k_Z1_Z2       0.46000 4.09e-01  0.5170
## k_Z0_free_bound 0.11800 5.64e-02  0.2470
## k_Z0_bound_free 0.00852 4.94e-04  0.1470
## k_Z0_free_Z1   2.33000 2.10e+00  2.6000
## k_Z2_sink      0.21000 1.45e-01  0.3030
## k_Z2_Z3        0.21700 1.73e-01  0.2730
## k_Z3_sink      0.06270 3.99e-02  0.0985
##
## Residual standard error: 3.74 on 49 degrees of freedom
##
## Chi2 error levels in percent:

```

```

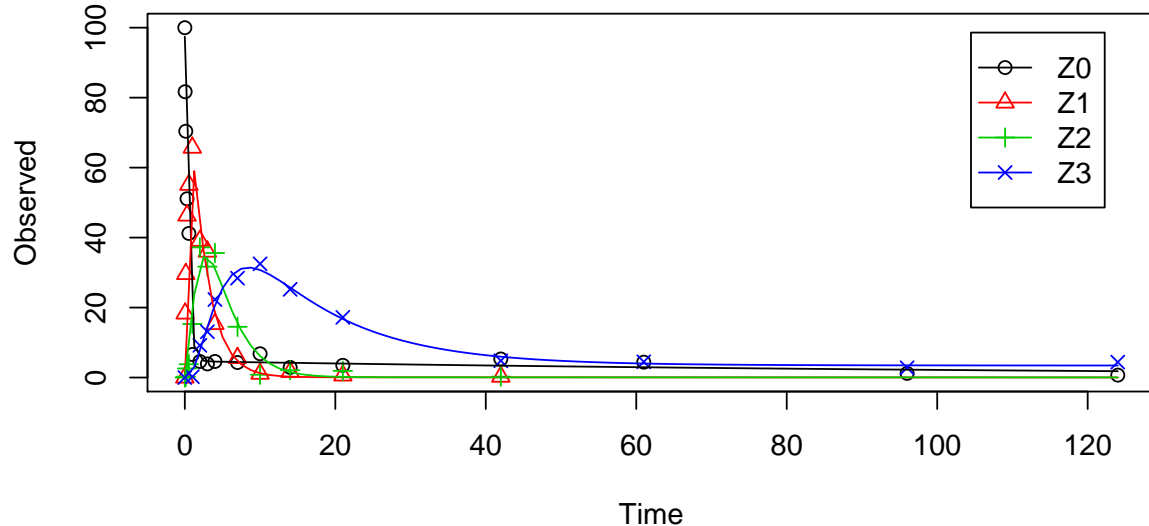
##          err.min n.optim df
## All data      17.5      8 46
## Z0            14.7      4 13
## Z1            14.4      1 12
## Z2            21.0      2 10
## Z3            11.8      1 11
##
## Estimated disappearance times:
##      DT50  DT90
## Z0  0.304  1.19
## Z1  1.507  5.01
## Z2  1.623  5.39
## Z3 11.051 36.71
##
## Estimated formation fractions:
##          ff
## Z0_free_sink 1.000
## Z0_free_Z1   1.000
## Z1_sink      1.000
## Z1_Z2        1.000
## Z2_sink      0.492
## Z2_Z3        0.508
## Z3_sink      1.000
##
## Estimated Eigenvalues of SFORB model(s):
##   Z0_b1  Z0_b2
## 2.45126 0.00811
##
## Parameter correlation:
##          Z0_free_0 k_Z1_Z2 k_Z0_free_bound k_Z0_bound_free
## Z0_free_0      1.0000  0.2424      0.0782      0.0692
## k_Z1_Z2        0.2424  1.0000     -0.2274     -0.0893
## k_Z0_free_bound 0.0782 -0.2274      1.0000      0.5398
## k_Z0_bound_free 0.0692 -0.0893      0.5398      1.0000
## k_Z0_free_Z1    0.0888 -0.1084      0.4277      0.1628
## k_Z2_sink       0.3299  0.3405     -0.2633     -0.1274
## k_Z2_Z3        -0.0749 -0.1489      0.0670      0.0608
## k_Z3_sink       -0.1046 -0.2249      0.1385      0.1253
##          k_Z0_free_Z1 k_Z2_sink k_Z2_Z3 k_Z3_sink
## Z0_free_0      0.0888  0.3299 -0.0749  -0.1046
## k_Z1_Z2        -0.1084  0.3405 -0.1489  -0.2249
## k_Z0_free_bound 0.4277  -0.2633  0.0670   0.1385

```

```
## k_Z0_bound_free      0.1628   -0.1274   0.0608   0.1253
## k_Z0_free_Z1         1.0000   -0.0531  -0.0128   0.0186
## k_Z2_sink            -0.0531    1.0000  -0.2547  -0.6832
## k_Z2_Z3              -0.0128   -0.2547   1.0000   0.5639
## k_Z3_sink            0.0186   -0.6832   0.5639   1.0000
```

The error level of the fit, but especially of metabolite Z3, can be improved if the SFORB model is chosen for this metabolite, as this model is capable of representing the tailing of the metabolite decline phase.

```
Z.mkin.5 <- mkinmod(Z0 = list(type = "SFORB", to = "Z1", sink = FALSE),
  Z1 = list(type = "SFO", to = "Z2", sink = FALSE),
  Z2 = list(type = "SFO", to = "Z3"),
  Z3 = list(type = "SFORB"))
m.Z.mkin.5 <- mkinfit(Z.mkin.5, FOCUS_2006_Z_mkin,
  parms.ini = m.Z.mkin.4$bparms.ode[1:5],
  quiet = TRUE)
plot(m.Z.mkin.5)
```



```
summary(m.Z.mkin.5, data = FALSE)
## mkin version:      0.9.25
## R version:         3.0.2
## Date of fit:       Sun Nov 17 15:24:11 2013
## Date of summary:   Sun Nov 17 15:24:11 2013
```

```

##
## Equations:
## [1] d_Z0_free = - 0 - k_Z0_free_bound * Z0_free + k_Z0_bound_free * Z0_bound - k_Z
## [2] d_Z0_bound = + k_Z0_free_bound * Z0_free - k_Z0_bound_free * Z0_bound
## [3] d_Z1 = + k_Z0_free_Z1 * Z0_free - 0 - k_Z1_Z2 * Z1
## [4] d_Z2 = + k_Z1_Z2 * Z1 - k_Z2_sink * Z2 - k_Z2_Z3_free * Z2
## [5] d_Z3_free = + k_Z2_Z3_free * Z2 - k_Z3_free_sink * Z3_free - k_Z3_free_bound *
## [6] d_Z3_bound = + k_Z3_free_bound * Z3_free - k_Z3_bound_free * Z3_bound
##
## Method used for solution of differential equation system:
## eigen
##
## Weighting: none
##
## Starting values for optimised parameters:
##
##          value   type transformed
## Z0_free_0      100.00000 state    100.0000
## k_Z0_free_bound  0.11810 deparm    -2.1362
## k_Z0_bound_free  0.00852 deparm    -4.7653
## k_Z0_free_Z1     2.33275 deparm     0.8470
## k_Z1_Z2          0.45981 deparm    -0.7769
## k_Z2_sink        0.20993 deparm    -1.5610
## k_Z2_Z3_free     0.10000 deparm    -2.3026
## k_Z3_free_sink   0.10010 deparm    -2.3016
## k_Z3_free_bound  0.10000 deparm    -2.3026
## k_Z3_bound_free  0.02000 deparm    -3.9120
##
## Fixed parameter values:
##          value   type
## Z0_bound_0      0 state
## 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
## Z0_free_0      97.400     1.8900   93.600  101.000
## k_Z0_free_bound -2.150     0.3680   -2.890   -1.400
## k_Z0_bound_free -4.820     1.4500   -7.750   -1.900
## k_Z0_free_Z1     0.846     0.0533    0.739    0.953
## k_Z1_Z2        -0.782     0.0584   -0.899   -0.664

```

```

## k_Z2_sink      -1.630      0.2480      -2.130      -1.130
## k_Z2_Z3_free   -1.490      0.1640      -1.810      -1.160
## k_Z3_free_sink -2.590      0.2880      -3.170      -2.010
## k_Z3_free_bound -5.260      0.8090      -6.890      -3.630
## k_Z3_bound_free -13.700     930.0000    -1880.000    1860.000
##
## Backtransformed parameters:
##           Estimate      Lower      Upper
## Z0_free_0      9.74e+01  93.60000  101.0000
## k_Z0_free_bound 1.17e-01  0.05580  0.2450
## k_Z0_bound_free 8.04e-03  0.00043  0.1500
## k_Z0_free_Z1   2.33e+00  2.09000  2.5900
## k_Z1_Z2        4.58e-01  0.40700  0.5150
## k_Z2_sink      1.96e-01  0.11900  0.3220
## k_Z2_Z3_free   2.26e-01  0.16300  0.3150
## k_Z3_free_sink  7.47e-02  0.04190  0.1330
## k_Z3_free_bound 5.21e-03  0.00102  0.0265
## k_Z3_bound_free 1.07e-06  0.00000      Inf
##
## Residual standard error: 3.73 on 47 degrees of freedom
##
## Chi2 error levels in percent:
##           err.min n.optim df
## All data      17.4      10 44
## Z0             14.7       4 13
## Z1             14.4       1 12
## Z2             21.0       2 10
## Z3             8.1        3 9
##
## Estimated disappearance times:
##           DT50  DT90
## Z0 0.304  1.19
## Z1 1.515  5.03
## Z2 1.642  5.45
## Z3 9.573 41.14
##
## Estimated formation fractions:
##           ff
## Z0_free_sink 1.000
## Z0_free_Z1   1.000
## Z1_sink      1.000
## Z1_Z2        1.000

```

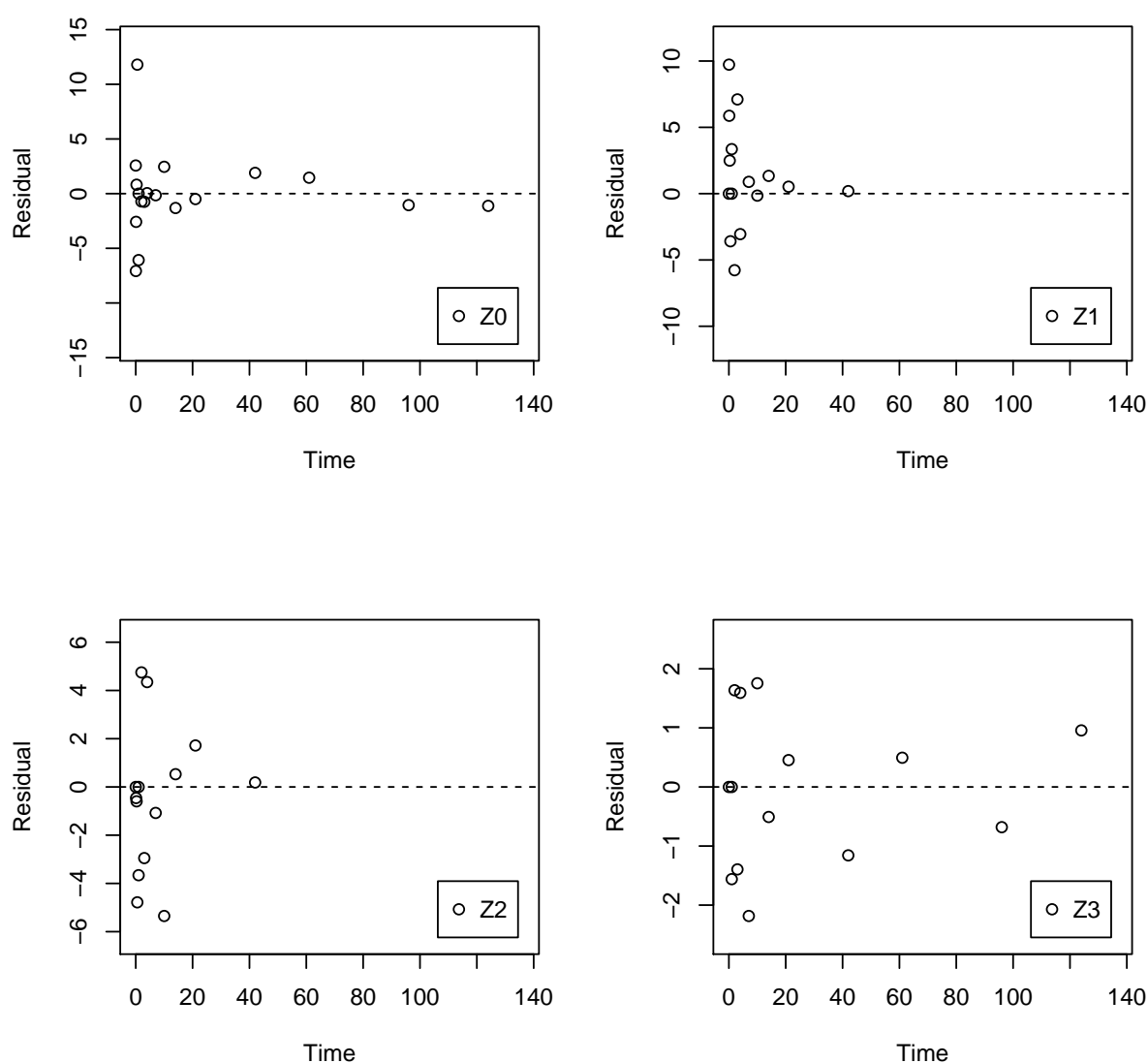
```

## Z2_sink      0.463
## Z2_Z3_free   0.537
## Z3_free_sink 1.000
##
## Estimated Eigenvalues of SFORB model(s):
##      Z0_b1      Z0_b2      Z3_b1      Z3_b2
## 2.447642 0.007653 0.079955 0.000001
##
## Parameter correlation:
##
##      Z0_free_0 k_Z0_free_bound k_Z0_bound_free k_Z0_free_Z1
## Z0_free_0      1.00000      0.0822      0.0784      0.09006
## k_Z0_free_bound 0.08217      1.0000      0.5403      0.42868
## k_Z0_bound_free 0.07836      0.5403      1.0000      0.16689
## k_Z0_free_Z1    0.09006      0.4287      0.1669      1.00000
## k_Z1_Z2         0.24672     -0.2193     -0.0743     -0.10541
## k_Z2_sink       0.27812     -0.2657     -0.1731     -0.06626
## k_Z2_Z3_free    -0.06659      0.0522      0.0765     -0.02823
## k_Z3_free_sink  -0.12891      0.0330     -0.0660     -0.00849
## k_Z3_free_bound -0.02259      0.1328      0.2445      0.03083
## k_Z3_bound_free -0.00314      0.0661      0.0588      0.04888
##
##      k_Z1_Z2 k_Z2_sink k_Z2_Z3_free k_Z3_free_sink
## Z0_free_0    0.2467    0.2781     -0.0666     -0.12891
## k_Z0_free_bound -0.2193   -0.2657      0.0522      0.03303
## k_Z0_bound_free -0.0743   -0.1731      0.0765     -0.06604
## k_Z0_free_Z1   -0.1054   -0.0663     -0.0282     -0.00849
## k_Z1_Z2        1.0000    0.3230     -0.1298     -0.22504
## k_Z2_sink      0.3230    1.0000      0.0446     -0.74724
## k_Z2_Z3_free   -0.1298    0.0446      1.0000      0.23544
## k_Z3_free_sink -0.2250   -0.7472      0.2354      1.00000
## k_Z3_free_bound -0.0898   -0.2631      0.2350      0.47267
## k_Z3_bound_free -0.0305   -0.4977     -0.6526      0.32280
##
##      k_Z3_free_bound k_Z3_bound_free
## Z0_free_0          -0.0226     -0.00314
## k_Z0_free_bound      0.1328      0.06614
## k_Z0_bound_free      0.2445      0.05880
## k_Z0_free_Z1         0.0308      0.04888
## k_Z1_Z2             -0.0898     -0.03050
## k_Z2_sink           -0.2631     -0.49767
## k_Z2_Z3_free         0.2350     -0.65255
## k_Z3_free_sink       0.4727      0.32280
## k_Z3_free_bound      1.0000     -0.01339
## k_Z3_bound_free     -0.0134      1.00000

```


Looking at the confidence intervals of the SFORB model parameters of Z3, it is clear that nothing can be said about the degradation rate of Z3 towards the end of the experiment. However, this appears to be a feature of the data.

```
par(mfrow = c(2, 2))
mkinresplot(m.Z.mkin.5, "Z0", lpos = "bottomright")
mkinresplot(m.Z.mkin.5, "Z1", lpos = "bottomright")
mkinresplot(m.Z.mkin.5, "Z2", lpos = "bottomright")
mkinresplot(m.Z.mkin.5, "Z3", lpos = "bottomright")
```



As expected, the residual plots are much more random than in the case of the all SFO

model for which they were shown above. In conclusion, the model Z.mkin.5 is proposed as the best-fit model for the dataset from Appendix 7 of the FOCUS report.

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>.