

Examples for kinetic evaluations using mkin

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Key words: Kinetics, FOCUS, nonlinear optimisation

1 Kinetic evaluations for parent compounds

These examples are also evaluated in a parallel vignette of the **kinfit** package ([Ranke, 2012](#)). The datasets are from Appendix 3, of the FOCUS kinetics report ([FOCUS Work Group on Degradation Kinetics, 2006, 2011](#)).

1.1 Laboratory Data L1

The following code defines example dataset L1 from the FOCUS kinetics report, p. 284

```
R> library("mkin")
R> FOCUS_2006_L1 = data.frame(
+   t = rep(c(0, 1, 2, 3, 5, 7, 14, 21, 30), each = 2),
+   parent = c(88.3, 91.4, 85.6, 84.5, 78.9, 77.6,
+              72.0, 71.9, 50.3, 59.4, 47.0, 45.1,
+              27.7, 27.3, 10.0, 10.4, 2.9, 4.0))
R> FOCUS_2006_L1_mkin <- mkin_wide_to_long(FOCUS_2006_L1)
```

The next step is to set up the models used for the kinetic analysis. Note that the model definitions contain the names of the observed variables in the data. In this case, there is only one variable called **parent**.

```
R> SFO <- mkinmod(parent = list(type = "SFO"))
R> FOMC <- mkinmod(parent = list(type = "FOMC"))
R> DFOP <- mkinmod(parent = list(type = "DFOP"))
```

The three models cover the first assumption of simple first order (SFO), the case of declining rate constant over time (FOMC) and the case of two different phases of the kinetics

(DFOP). For a more detailed discussion of the models, please see the FOCUS kinetics report.

The following two lines fit the model and produce the summary report of the model fit. This covers the numerical analysis given in the FOCUS report.

```
R> m.L1.SF0 <- mkinfit(SF0, FOCUS_2006_L1_mkin, quiet=TRUE)
R> summary(m.L1.SF0)
```

```
mkim version:      0.9.14
R version:         2.15.2
Date of fit:       Thu Feb 21 22:53:11 2013
Date of summary:   Thu Feb 21 22:53:11 2013
```

```
Equations:
[1] d_parent = - k_parent_sink * parent
```

```
Starting values for optimised parameters:
              initial   type transformed
parent_0      100.0   state  100.000000
k_parent_sink    0.1 deparm  -2.302585
```

```
Fixed parameter values:
None
```

```
Optimised, transformed parameters:
              Estimate Std. Error
parent_0      92.471      1.368
k_parent_sink -2.347      0.041
```

```
Backtransformed parameters:
              Estimate
parent_0      92.471
k_parent_sink  0.096
```

```
Residual standard error: 2.948 on 16 degrees of freedom
```

```
Chi2 error levels in percent:
              err.min n.optim df
All data    3.424      2    7
parent      3.424      2    7
```

```
Estimated disappearance times:
              DT50 DT90
parent 7.249 24.08
```

```
Estimated formation fractions:
              ff
parent_sink  1
```

Parameter correlation:

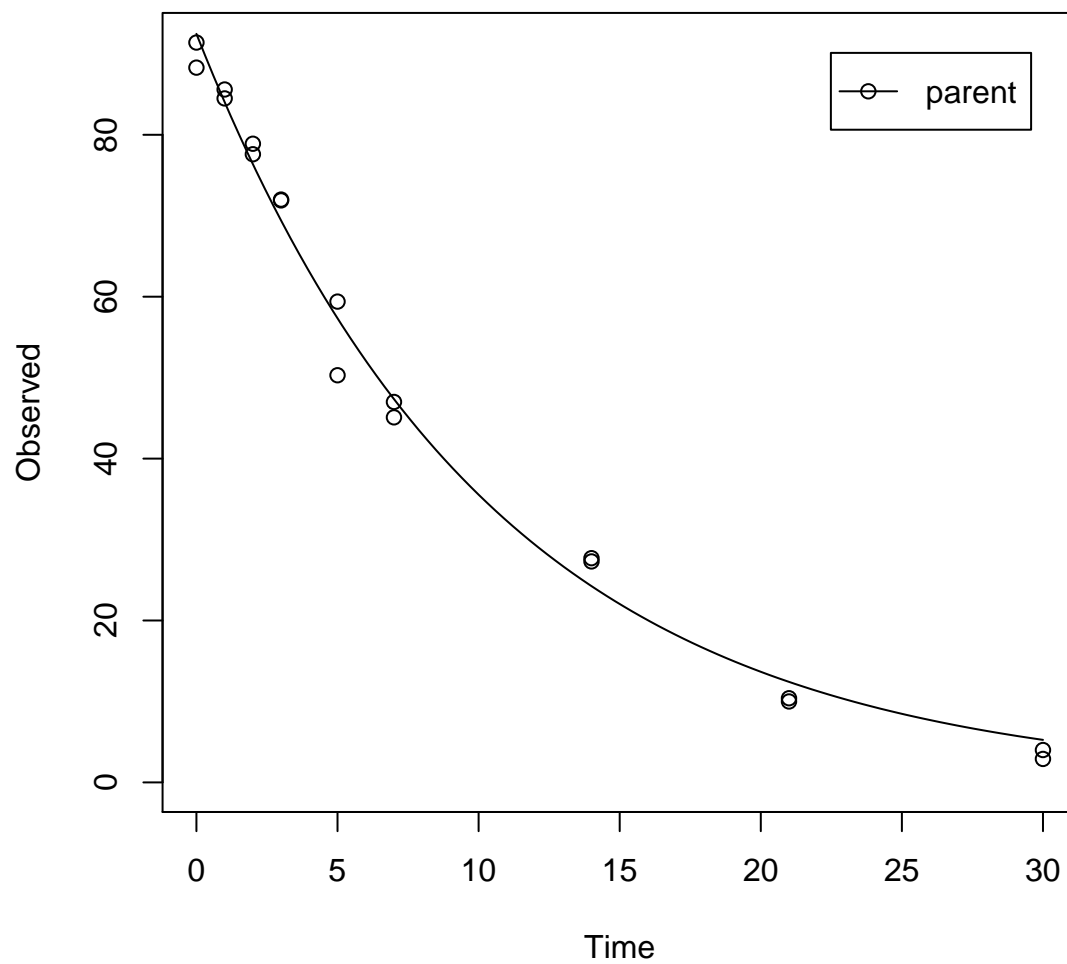
	parent_0	k_parent_sink
parent_0	1.0000	0.6248
k_parent_sink	0.6248	1.0000

Data:

time	variable	observed	predicted	residual
0	parent	88.3	92.471	-4.1710
0	parent	91.4	92.471	-1.0710
1	parent	85.6	84.039	1.5610
1	parent	84.5	84.039	0.4610
2	parent	78.9	76.376	2.5241
2	parent	77.6	76.376	1.2241
3	parent	72.0	69.412	2.5884
3	parent	71.9	69.412	2.4884
5	parent	50.3	57.330	-7.0301
5	parent	59.4	57.330	2.0699
7	parent	47.0	47.352	-0.3515
7	parent	45.1	47.352	-2.2515
14	parent	27.7	24.247	3.4527
14	parent	27.3	24.247	3.0527
21	parent	10.0	12.416	-2.4163
21	parent	10.4	12.416	-2.0163
30	parent	2.9	5.251	-2.3513
30	parent	4.0	5.251	-1.2513

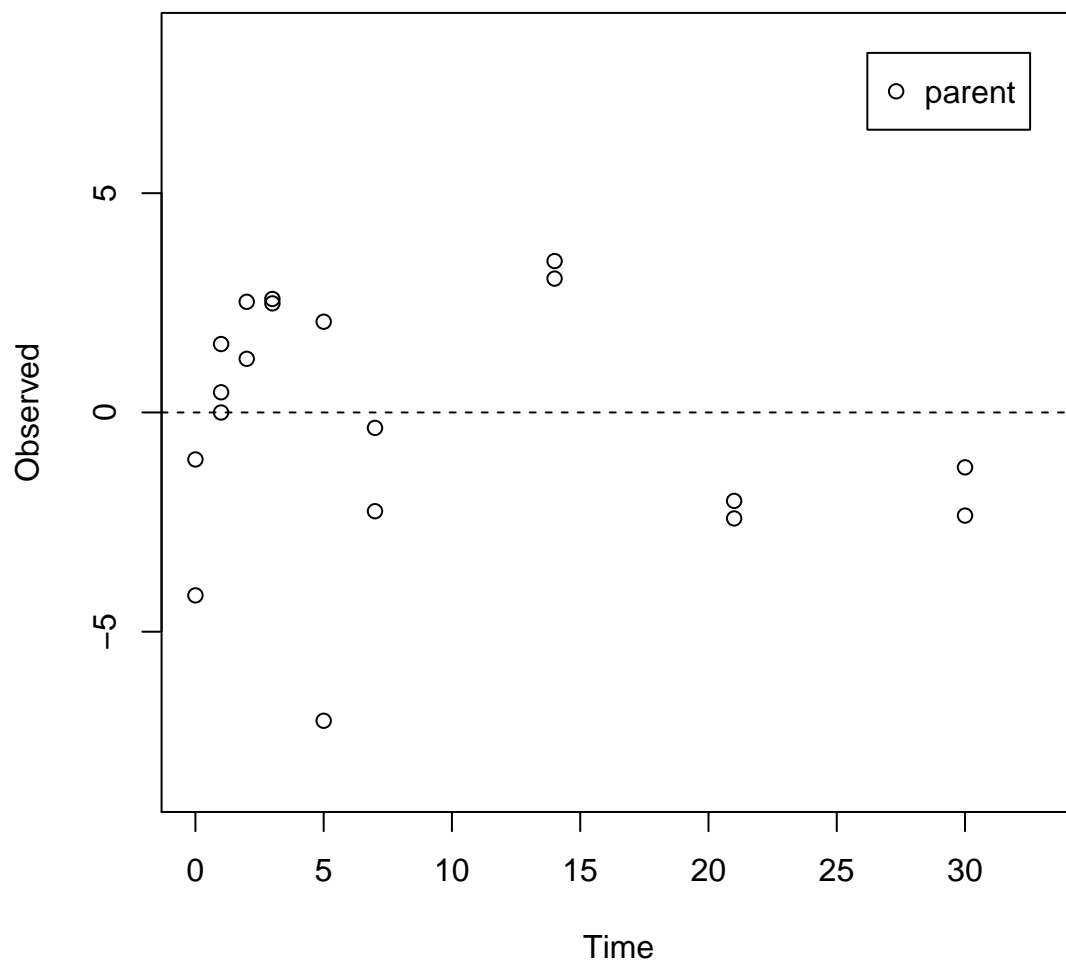
A plot of the fit is obtained with the plot function for mkinfit objects.

```
R> plot(m.L1.SF0)
```



The residual plot can be easily obtained by

```
R> mkinresplot(m.L1.SF0, ylab = "Observed", xlab = "Time")
```



For comparison, the FOMC model is fitted as well, and the χ^2 error level is checked.

```
R> m.L1.FOMC <- mkinfit(FOMC, FOCUS_2006_L1_mkin, quiet=TRUE)
```

```
R> summary(m.L1.FOMC)
```

```
mkin version:    0.9.14
R version:       2.15.2
Date of fit:     Thu Feb 21 22:53:12 2013
Date of summary: Thu Feb 21 22:53:12 2013
```

Equations:

```
[1] d_parent = - (alpha/beta) * ((time/beta) + 1)^-1 * parent
```

Starting values for optimised parameters:

	initial	type	transformed
parent_0	100	state	100.000000
alpha	1	deparm	0.000000
beta	10	deparm	2.302585

Fixed parameter values:
None

Optimised, transformed parameters:

	Estimate	Std. Error
parent_0	92.47	NA
alpha	25.63	NA
beta	27.98	NA

Backtransformed parameters:

	Estimate
parent_0	9.247e+01
alpha	1.350e+11
beta	1.412e+12

Residual standard error: 3.045 on 15 degrees of freedom

Chi2 error levels in percent:

	err.min	n.optim	df
All data	3.619	3	6
parent	3.619	3	6

Estimated disappearance times:

	DT50	DT90
parent	7.249	24.08

Data:

time	variable	observed	predicted	residual
0	parent	88.3	92.471	-4.1711
0	parent	91.4	92.471	-1.0711
1	parent	85.6	84.038	1.5618
1	parent	84.5	84.038	0.4618
2	parent	78.9	76.377	2.5233
2	parent	77.6	76.377	1.2233
3	parent	72.0	69.412	2.5884
3	parent	71.9	69.412	2.4884
5	parent	50.3	57.331	-7.0306
5	parent	59.4	57.331	2.0694
7	parent	47.0	47.351	-0.3510
7	parent	45.1	47.351	-2.2510
14	parent	27.7	24.247	3.4526
14	parent	27.3	24.247	3.0526
21	parent	10.0	12.416	-2.4162
21	parent	10.4	12.416	-2.0162

30	parent	2.9	5.251	-2.3513
30	parent	4.0	5.251	-1.2513

Due to the higher number of parameters, and the lower number of degrees of freedom of the fit, the χ^2 error level is actually higher for the FOMC model (3.6%) than for the SFO model (3.4%).

The χ^2 error levels reported in Appendix 3 and Appendix 7 to the FOCUS kinetics report are rounded to integer percentages and partly deviate by one percentage point from the results calculated by **mk**in. The reason for this is not known. However, **mk**in gives the same χ^2 error levels as the **kinfit** package. Furthermore, the calculation routines of the kinfit package have been extensively compared to the results obtained by the KinGUI software, as documented in the kinfit package vignette. KinGUI is a widely used standard package in this field. Therefore, the reason for the difference was not investigated further.

1.2 Laboratory Data L2

The following code defines example dataset L2 from the FOCUS kinetics report, p. 287

```
R> FOCUS_2006_L2 = data.frame(
+   t = rep(c(0, 1, 3, 7, 14, 28), each = 2),
+   parent = c(96.1, 91.8, 41.4, 38.7,
+             19.3, 22.3, 4.6, 4.6,
+             2.6, 1.2, 0.3, 0.6))
R> FOCUS_2006_L2_mkin <- mkin_wide_to_long(FOCUS_2006_L2)
```

Again, the SFO model is fitted and a summary is obtained.

```
R> m.L2.SFO <- mkinfit(SFO, FOCUS_2006_L2_mkin, quiet=TRUE)
R> summary(m.L2.SFO)
```

```
mk version:      0.9.14
R version:       2.15.2
Date of fit:     Thu Feb 21 22:53:12 2013
Date of summary: Thu Feb 21 22:53:12 2013
```

```
Equations:
[1] d_parent = - k_parent_sink * parent
```

```
Starting values for optimised parameters:
              initial   type transformed
parent_0      100.0   state  100.000000
k_parent_sink    0.1 deparm  -2.302585
```

```
Fixed parameter values:
None
```


Optimised, transformed parameters:

	Estimate	Std. Error
parent_0	91.4656	3.807
k_parent_sink	-0.4112	0.107

Backtransformed parameters:

	Estimate
parent_0	91.466
k_parent_sink	0.663

Residual standard error: 5.51 on 10 degrees of freedom

Chi2 error levels in percent:

	err.min	n.optim	df
All data	14.38	2	4
parent	14.38	2	4

Estimated disappearance times:

	DT50	DT90
parent	1.046	3.474

Estimated formation fractions:

	ff
parent_sink	1

Parameter correlation:

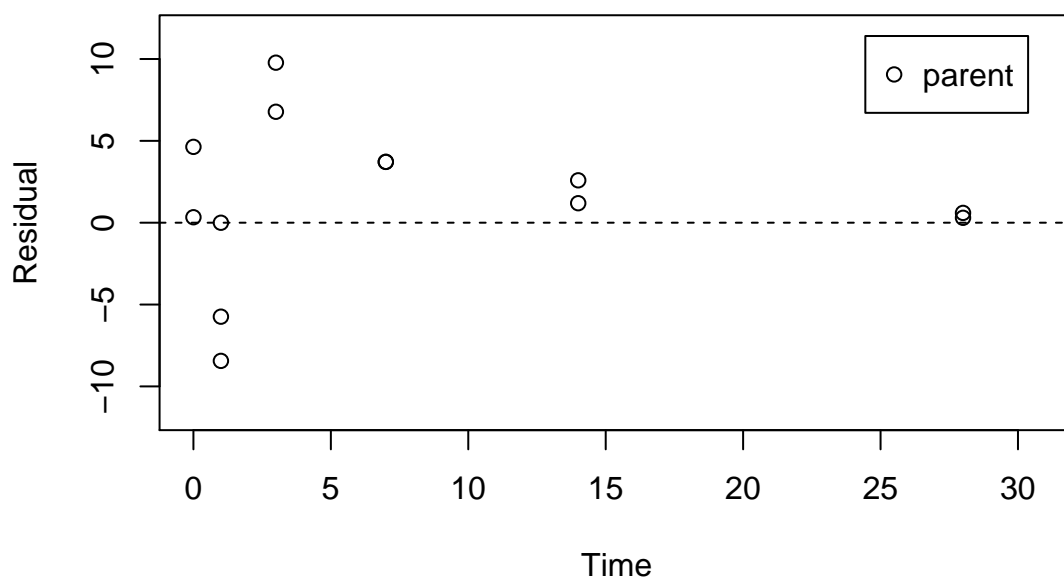
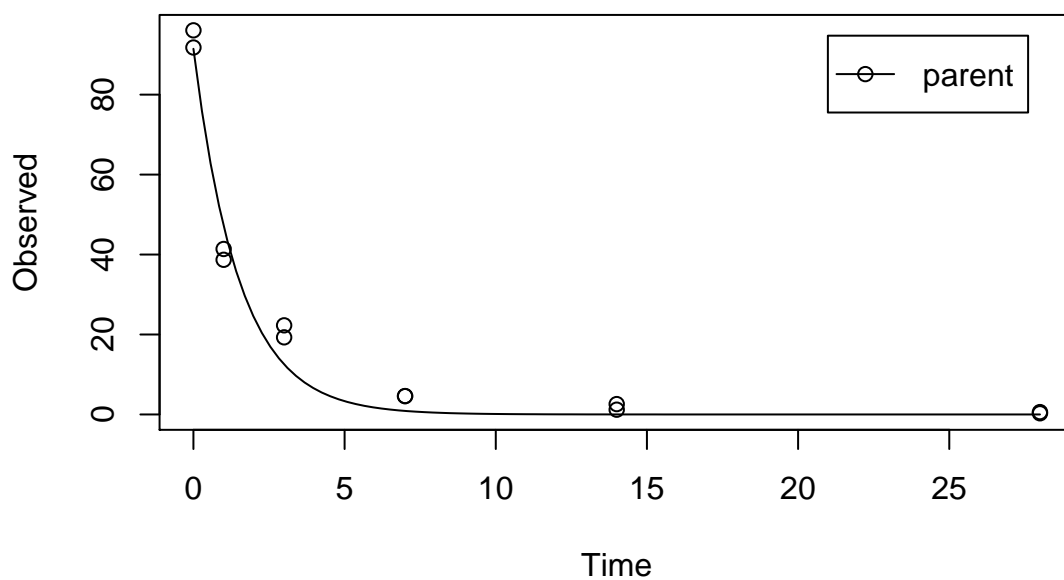
	parent_0	k_parent_sink
parent_0	1.0000	0.4295
k_parent_sink	0.4295	1.0000

Data:

time	variable	observed	predicted	residual
0	parent	96.1	91.4656079103	4.6344
0	parent	91.8	91.4656079103	0.3344
1	parent	41.4	47.1395280371	-5.7395
1	parent	38.7	47.1395280371	-8.4395
3	parent	19.3	12.5210295280	6.7790
3	parent	22.3	12.5210295280	9.7790
7	parent	4.6	0.8833842647	3.7166
7	parent	4.6	0.8833842647	3.7166
14	parent	2.6	0.0085318162	2.5915
14	parent	1.2	0.0085318162	1.1915
28	parent	0.3	0.0000007958	0.3000
28	parent	0.6	0.0000007958	0.6000

The χ^2 error level of 14% suggests that the model does not fit very well. This is also obvious from the plots of the fit and the residuals.

```
R> par(mfrow = c(2, 1))  
R> plot(m.L2.SF0)  
R> mkinresplot(m.L2.SF0)
```



In the FOCUS kinetics report, it is stated that there is no apparent systematic error observed from the residual plot up to the measured DT90 (approximately at day 5), and

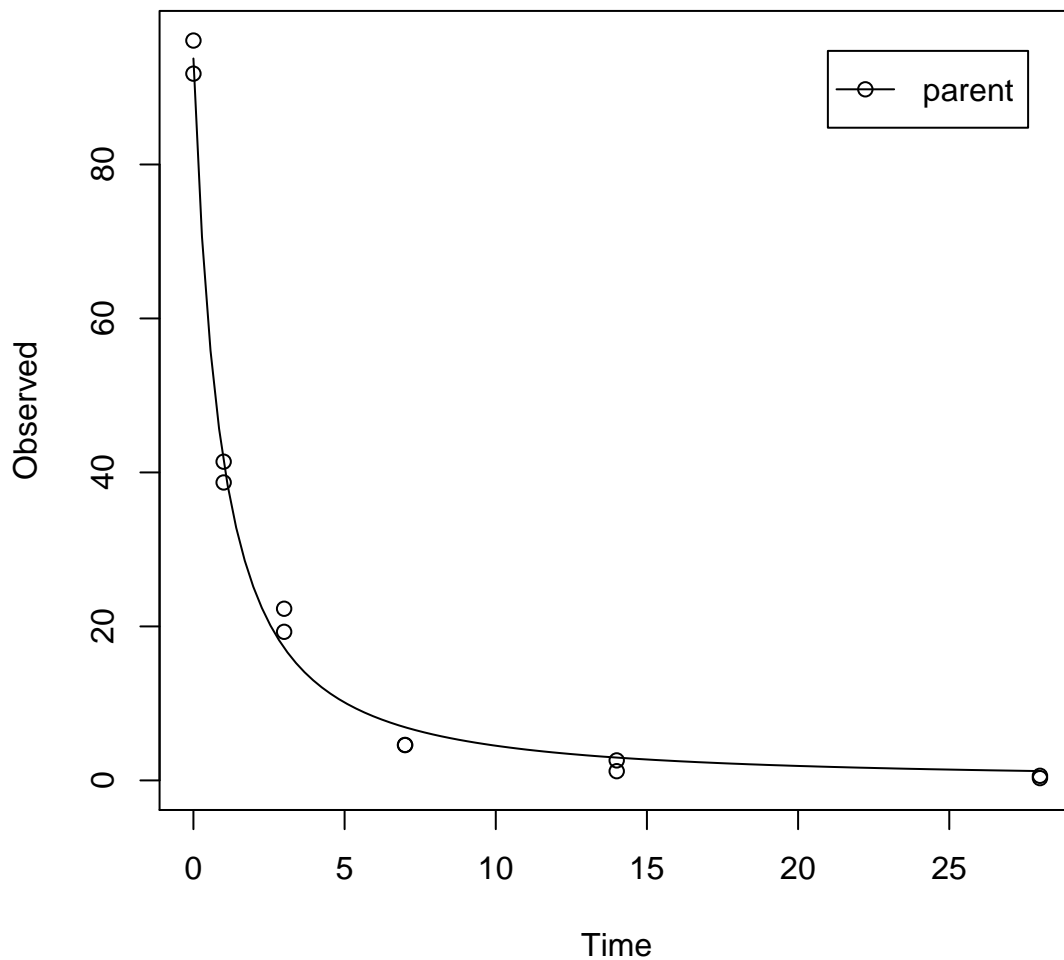
there is an underestimation beyond that point.

We may add that it is difficult to judge the random nature of the residuals just from the three samplings at days 0, 1 and 3. Also, it is not clear *a priori* why a consistent underestimation after the approximate DT90 should be irrelevant. However, this can be rationalised by the fact that the FOCUS fate models generally only implement SFO kinetics.

For comparison, the FOMC model is fitted as well, and the χ^2 error level is checked.

```
R> m.L2.FOMC <- mkinfit(FOMC, FOCUS_2006_L2_mkin, quiet=TRUE)
R> plot(m.L2.FOMC)
R> s.m.L2.FOMC <- summary(m.L2.FOMC)
R> s.m.L2.FOMC$errmin
```

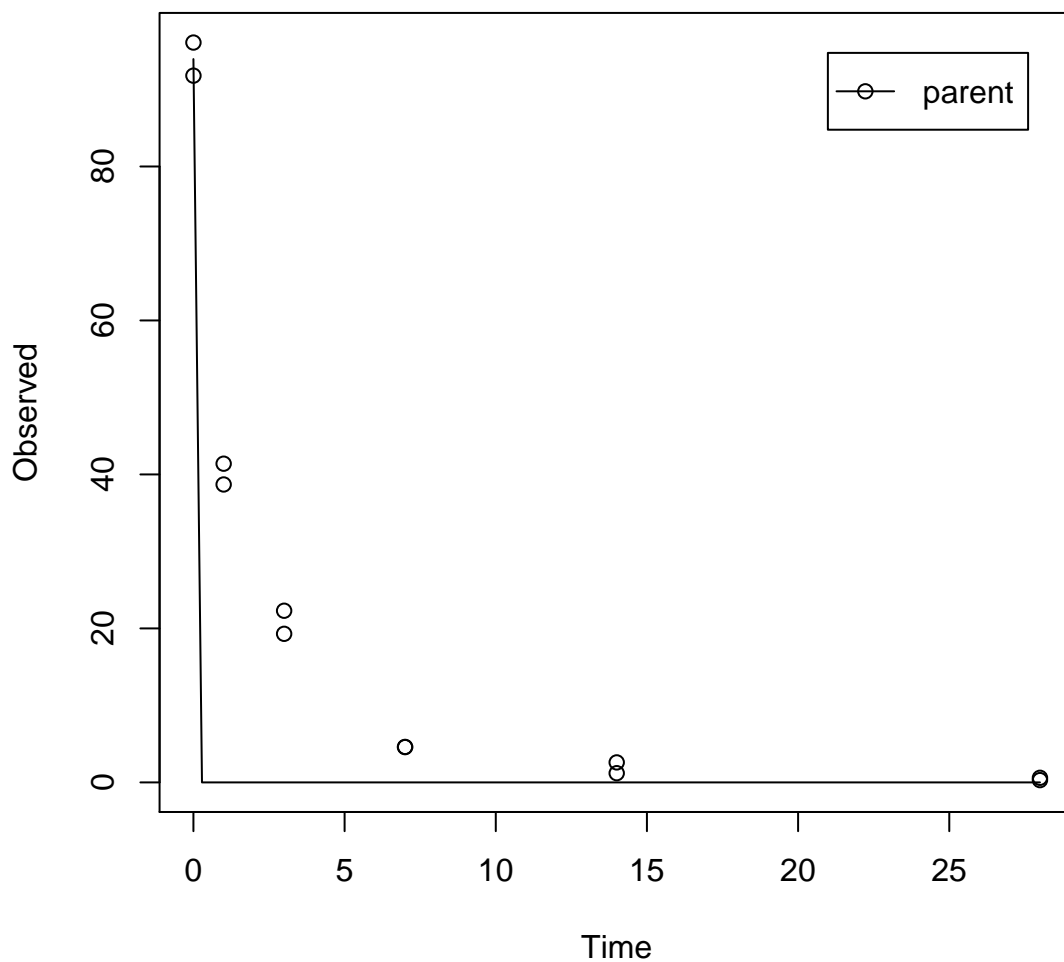
	<i>err.min</i>	<i>n.optim</i>	<i>df</i>
<i>All data</i>	0.06204245	3	3
<i>parent</i>	0.06204245	3	3



The error level at which the χ^2 test passes is much lower in this case. Therefore, the FOMC model provides a better description of the data, as less experimental error has to be assumed in order to explain the data.

Fitting the four parameter DFOP model does not further reduce the χ^2 error level.

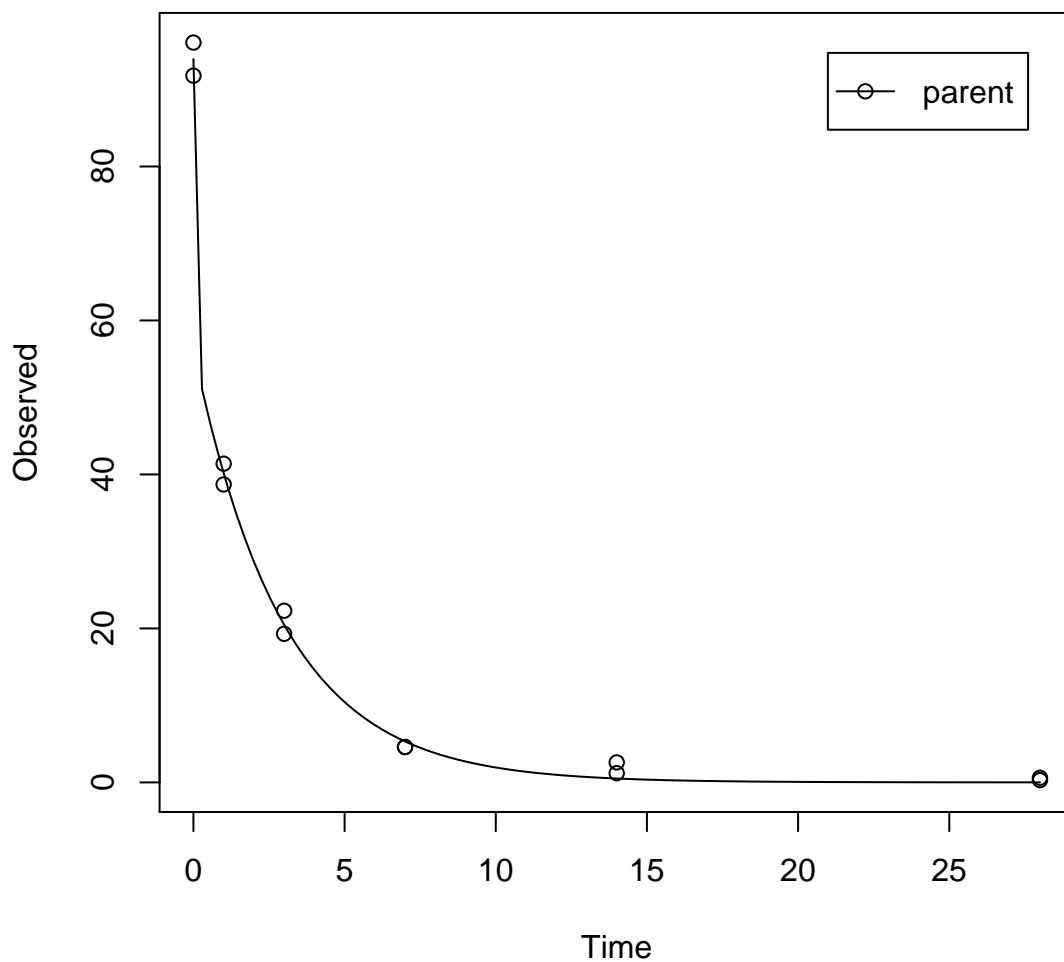
```
R> m.L2.DFOP <- mkinfit(DFOP, FOCUS_2006_L2_mkin, quiet=TRUE)
R> plot(m.L2.DFOP)
```



Here, the default starting parameters for the DFOP model obviously do not lead to a reasonable solution. Therefore the fit is repeated with different starting parameters.

```
R> m.L2.DFOP <- mkinfit(DFOP, FOCUS_2006_L2_mkin,
+   parms.ini = c(k1 = 1, k2 = 0.01, g = 0.8),
+   quiet=TRUE)
R> plot(m.L2.DFOP)
R> s.m.L2.DFOP <- summary(m.L2.DFOP)
R> s.m.L2.DFOP$errmin
```

```
      err.min n.optim df
All data 0.02528763    4  2
parent   0.02528763    4  2
```



Therefore, the FOMC model is clearly the best-fit model for dataset L1 based on the χ^2 error level criterion.

1.3 Laboratory Data L3

The following code defines example dataset L3 from the FOCUS kinetics report, p. 290.

```
R> FOCUS_2006_L3 = data.frame(
+   t = c(0, 3, 7, 14, 30, 60, 91, 120),
+   parent = c(97.8, 60, 51, 43, 35, 22, 15, 12))
R> FOCUS_2006_L3_mkin <- mkin_wide_to_long(FOCUS_2006_L3)
```

SFO model, summary and plot:

```
R> m.L3.SF0 <- mkinfit(SF0, FOCUS_2006_L3_mkin, quiet = TRUE)
```

```
R> summary(m.L3.SF0)
```

```
mkim version:      0.9.14
R version:         2.15.2
Date of fit:       Thu Feb 21 22:53:13 2013
Date of summary:   Thu Feb 21 22:53:13 2013
```

Equations:

```
[1] d_parent = - k_parent_sink * parent
```

Starting values for optimised parameters:

	initial	type	transformed
parent_0	100.0	state	100.000000
k_parent_sink	0.1	deparm	-2.302585

Fixed parameter values:

None

Optimised, transformed parameters:

	Estimate	Std. Error
parent_0	74.873	8.458
k_parent_sink	-3.678	0.326

Backtransformed parameters:

	Estimate
parent_0	74.873
k_parent_sink	0.025

Residual standard error: 12.91 on 6 degrees of freedom

Chi2 error levels in percent:

	err.min	n.optim	df
All data	21.24	2	6
parent	21.24	2	6

Estimated disappearance times:

	DT50	DT90
parent	27.43	91.12

Estimated formation fractions:

	ff
parent_sink	1

Parameter correlation:

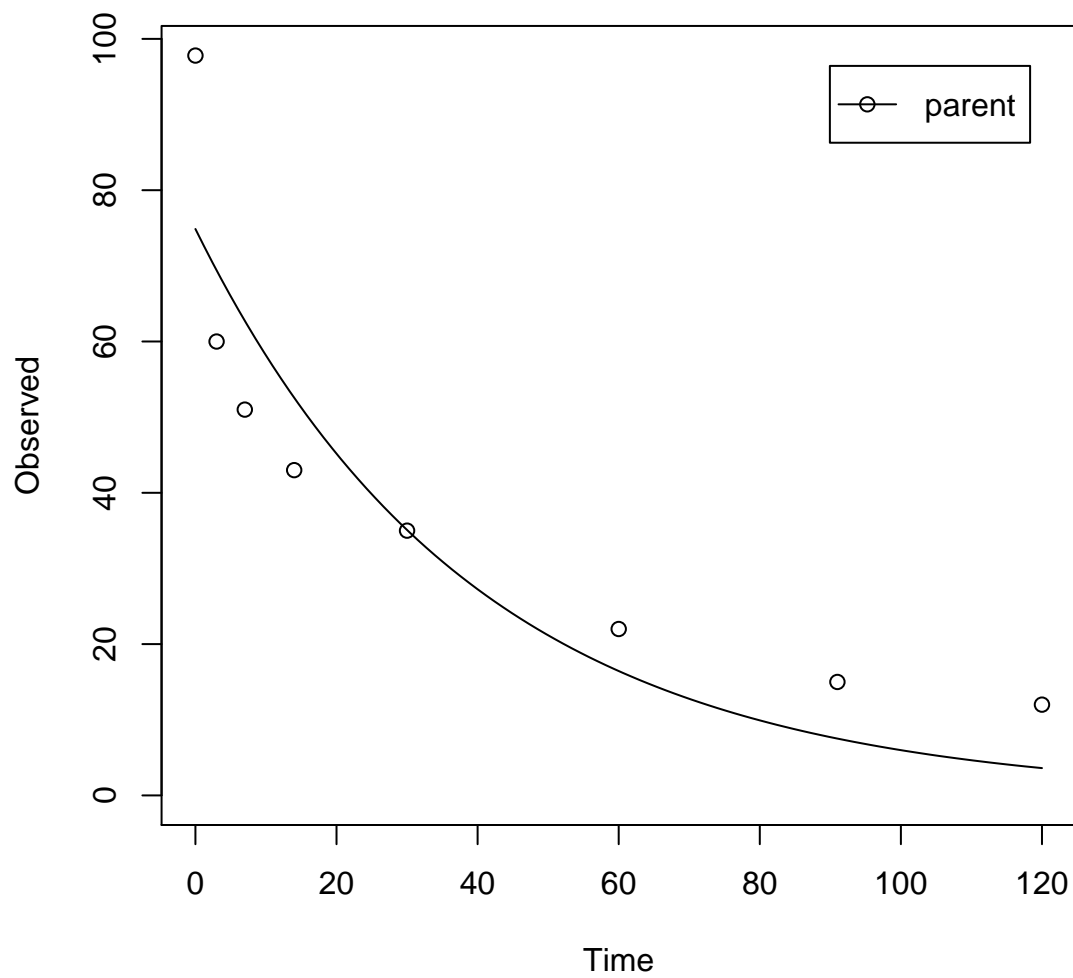
	parent_0	k_parent_sink
parent_0	1.0000	0.5484

k_parent_sink 0.5484 1.0000

Data:

<i>time</i>	<i>variable</i>	<i>observed</i>	<i>predicted</i>	<i>residual</i>
0	parent	97.8	74.873	22.92734
3	parent	60.0	69.407	-9.40654
7	parent	51.0	62.734	-11.73403
14	parent	43.0	52.563	-9.56336
30	parent	35.0	35.083	-0.08281
60	parent	22.0	16.439	5.56137
91	parent	15.0	7.510	7.48961
120	parent	12.0	3.609	8.39083

R> plot(m.L3.SF0)



The χ^2 error level of 22% as well as the plot suggest that the model does not fit very well.

The FOMC model performs better:

```
R> m.L3.FOMC <- mkinfit(FOMC, FOCUS_2006_L3_mkin, quiet = TRUE)
R> plot(m.L3.FOMC)
R> s.m.L3.FOMC <- summary(m.L3.FOMC)
R> s.m.L3.FOMC$errmin
```

	<i>err.min</i>	<i>n.optim</i>	<i>df</i>
<i>All data</i>	0.07321867	3	5
<i>parent</i>	0.07321867	3	5

```
R> endpoints(m.L3.FOMC)
```

\$distimes

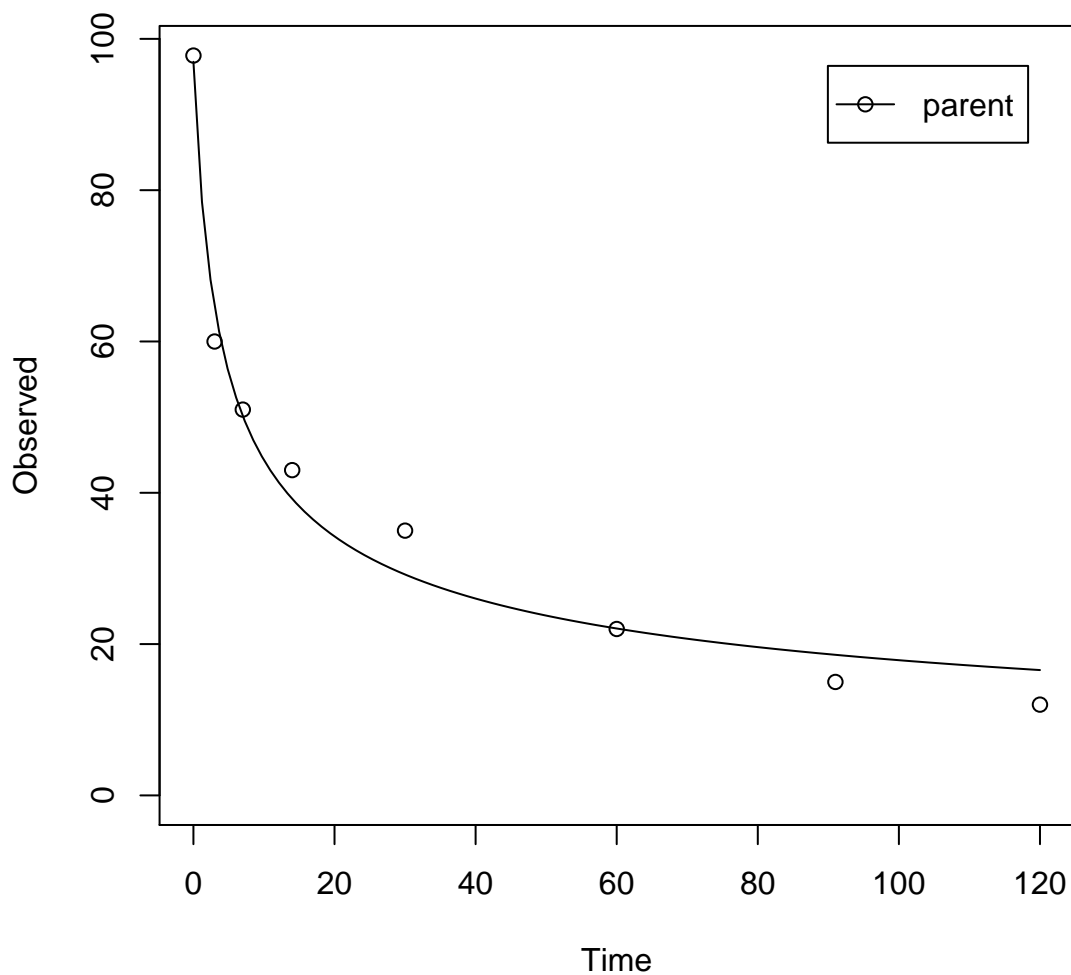
	<i>DT50</i>	<i>DT90</i>
<i>parent</i>	7.729478	431.2428

\$ff

logical(0)

\$SFORB

logical(0)

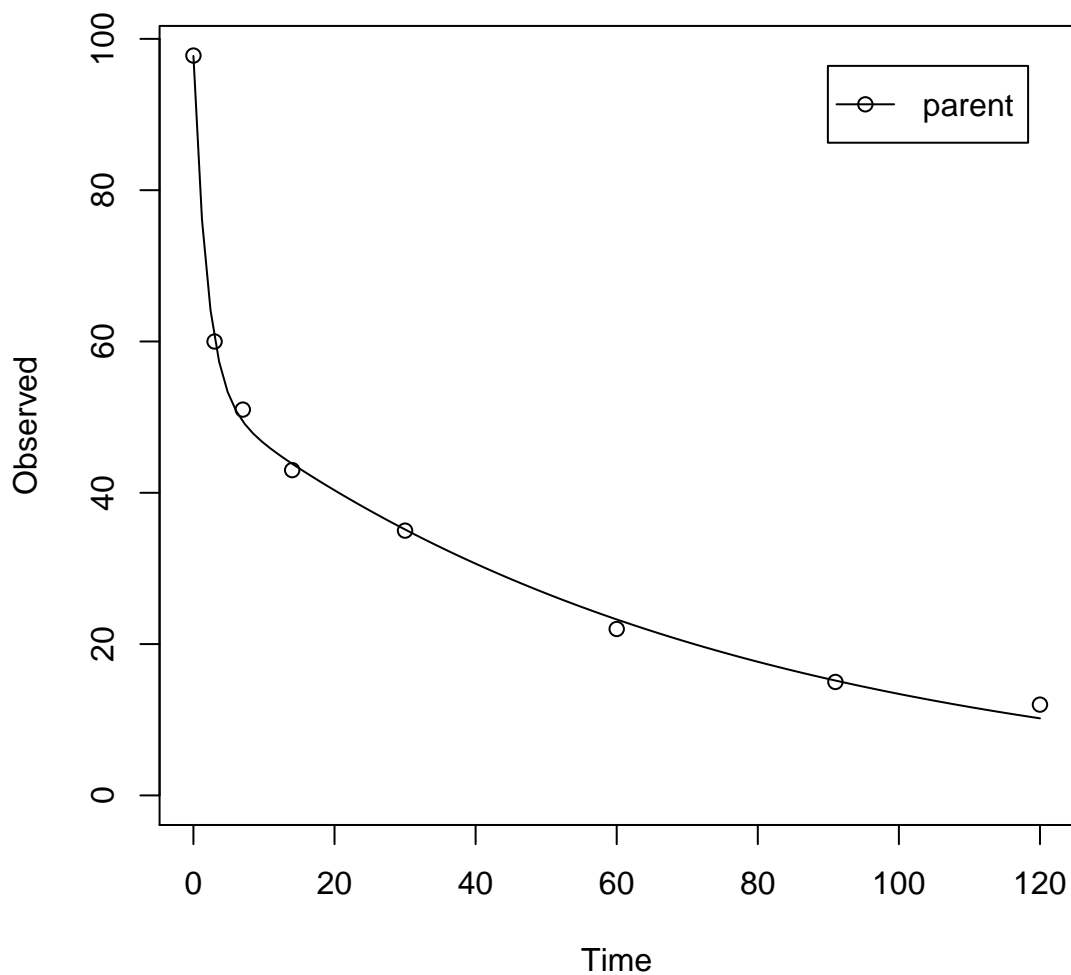


The error level at which the χ^2 test passes is 7% in this case.

Fitting the four parameter DFOP model further reduces the χ^2 error level considerably:

```
R> m.L3.DFOP <- mkinfit(DFOP, FOCUS_2006_L3_mkin, quiet = TRUE)
R> plot(m.L3.DFOP)
R> s.m.L3.DFOP <- summary(m.L3.DFOP)
R> s.m.L3.DFOP$errmin
```

```
      err.min n.optim df
All data 0.02223992    4  4
parent   0.02223992    4  4
```



Therefore, the DFOP model is the best-fit model based on the χ^2 error level criterion for laboratory data L3.

1.4 Laboratory Data L4

The following code defines example dataset L4 from the FOCUS kinetics report, p. 293

```
R> FOCUS_2006_L4 = data.frame(
+   t = c(0, 3, 7, 14, 30, 60, 91, 120),
+   parent = c(96.6, 96.3, 94.3, 88.8, 74.9, 59.9, 53.5, 49.0))
R> FOCUS_2006_L4_mkin <- mkin_wide_to_long(FOCUS_2006_L4)
```

SFO model, summary and plot:

```
R> m.L4.SF0 <- mkinfit(SF0, FOCUS_2006_L4_mkin, quiet = TRUE)
```

```
R> summary(m.L4.SF0)
```

```
mkim version:      0.9.14
R version:         2.15.2
Date of fit:       Thu Feb 21 22:53:14 2013
Date of summary:   Thu Feb 21 22:53:14 2013
```

Equations:

```
[1] d_parent = - k_parent_sink * parent
```

Starting values for optimised parameters:

	initial	type	transformed
parent_0	100.0	state	100.000000
k_parent_sink	0.1	deparm	-2.302585

Fixed parameter values:

None

Optimised, transformed parameters:

	Estimate	Std. Error
parent_0	96.44	1.949
k_parent_sink	-5.03	0.080

Backtransformed parameters:

	Estimate
parent_0	96.442
k_parent_sink	0.007

Residual standard error: 3.651 on 6 degrees of freedom

Chi2 error levels in percent:

	err.min	n.optim	df
All data	3.288	2	6
parent	3.288	2	6

Estimated disappearance times:

	DT50	DT90
parent	106	352

Estimated formation fractions:

	ff
parent_sink	1

Parameter correlation:

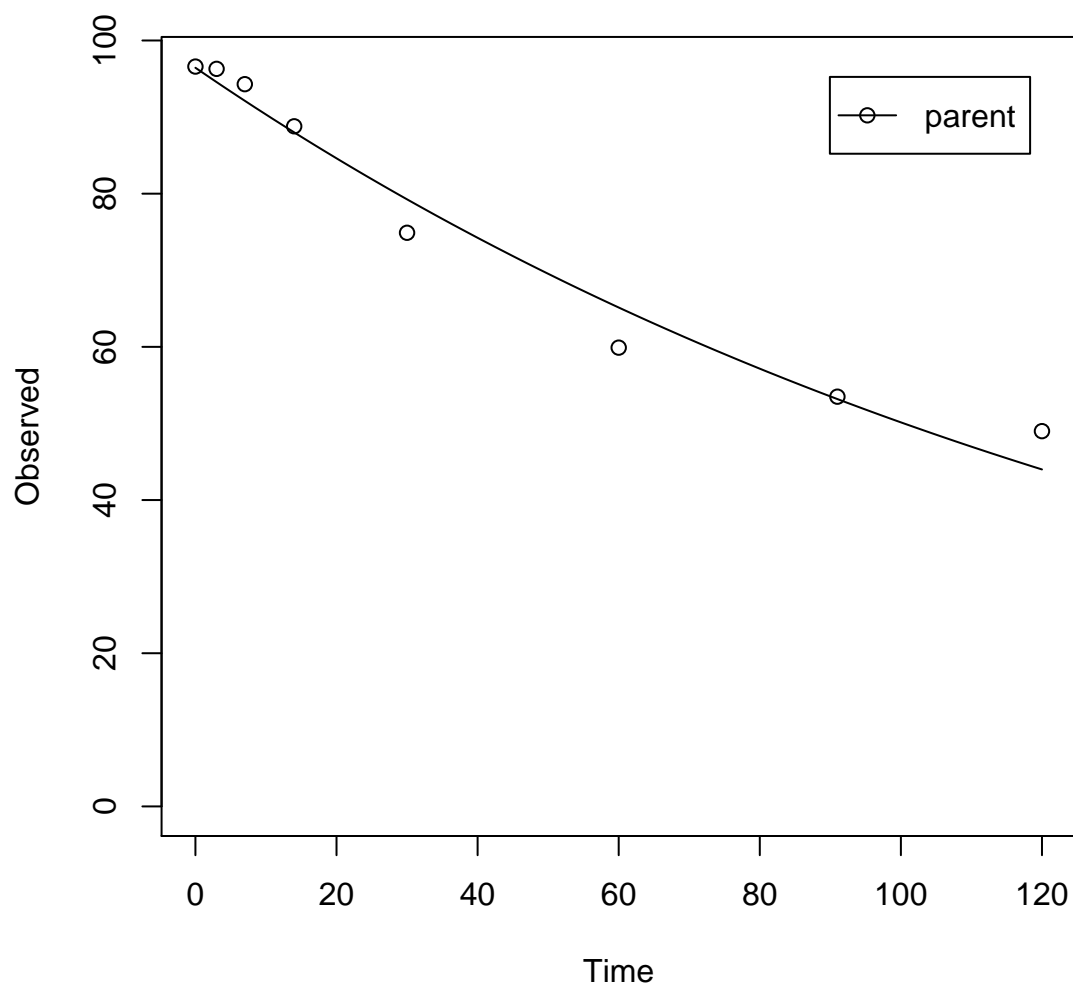
	parent_0	k_parent_sink
parent_0	1.0000	0.5865

```
k_parent_sink    0.5865    1.0000
```

Data:

time	variable	observed	predicted	residual
0	parent	96.6	96.44	0.1585
3	parent	96.3	94.57	1.7324
7	parent	94.3	92.13	2.1744
14	parent	88.8	88.00	0.7972
30	parent	74.9	79.26	-4.3589
60	parent	59.9	65.14	-5.2376
91	parent	53.5	53.18	0.3167
120	parent	49.0	43.99	5.0054

```
R> plot(m.L4.SF0)
```

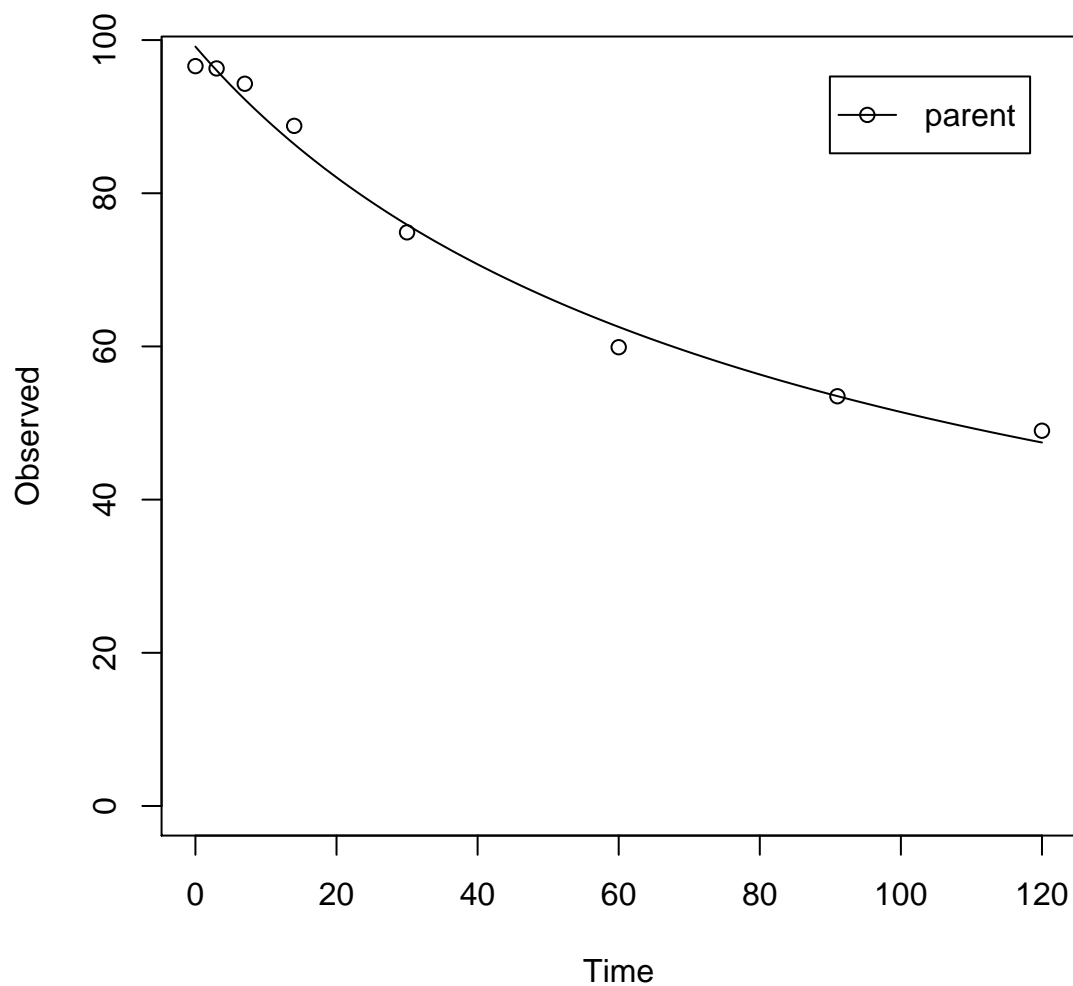


The χ^2 error level of 3.3% as well as the plot suggest that the model fits very well.

The FOMC model for comparison

```
R> m.L4.FOMC <- mkinfit(FOMC, FOCUS_2006_L4_mkin, quiet = TRUE)
R> plot(m.L4.FOMC)
R> s.m.L4.FOMC <- summary(m.L4.FOMC)
R> s.m.L4.FOMC$errmin
```

	<i>err.min</i>	<i>n.optim</i>	<i>df</i>
<i>All data</i>	0.02027643	3	5
<i>parent</i>	0.02027643	3	5



The error level at which the χ^2 test passes is slightly lower for the FOMC model. However, the difference appears negligible.

2 Kinetic evaluations for parent and metabolites

2.1 Laboratory Data for example compound Z

The following code defines the example dataset from Appendix 7 to the FOCUS kinetics report, p.350

```
R> LOD = 0.5
R> 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))
R> FOCUS_2006_Z_mkin <- mkin_wide_to_long(FOCUS_2006_Z)
```

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"))
R> m.Z.2a <- mkinfit(Z.2a, FOCUS_2006_Z_mkin, quiet = TRUE)
R> summary(m.Z.2a, data = FALSE)
```

```
mkin version:      0.9.14
R version:         2.15.2
Date of fit:       Thu Feb 21 22:53:15 2013
Date of summary:   Thu Feb 21 22:53:15 2013
```

```
Equations:
[1] d_Z0 = - k_Z0_sink * Z0 - k_Z0_Z1 * Z0
[2] d_Z1 = + k_Z0_Z1 * Z0 - k_Z1_sink * Z1
```


Starting values for optimised parameters:

	initial	type	transformed
Z0_0	100.0	state	100.000000
k_Z0_sink	0.1	deparm	-2.302585
k_Z0_Z1	0.1	deparm	-2.302585
k_Z1_sink	0.1	deparm	-2.302585

Fixed parameter values:

	value	type
Z1	0	state

Optimised, transformed parameters:

	Estimate	Std. Error
Z0_0	97.0149	NA
k_Z0_sink	-36.2915	NA
k_Z0_Z1	0.8047	NA
k_Z1_sink	-0.7296	NA

Backtransformed parameters:

	Estimate
Z0_0	97.015
k_Z0_sink	0.000
k_Z0_Z1	2.236
k_Z1_sink	0.482

Residual standard error: 5.064 on 27 degrees of freedom

Chi2 error levels in percent:

	err.min	n.optim	df
All data	18.20	4	27
Z0	18.04	3	14
Z1	15.75	1	13

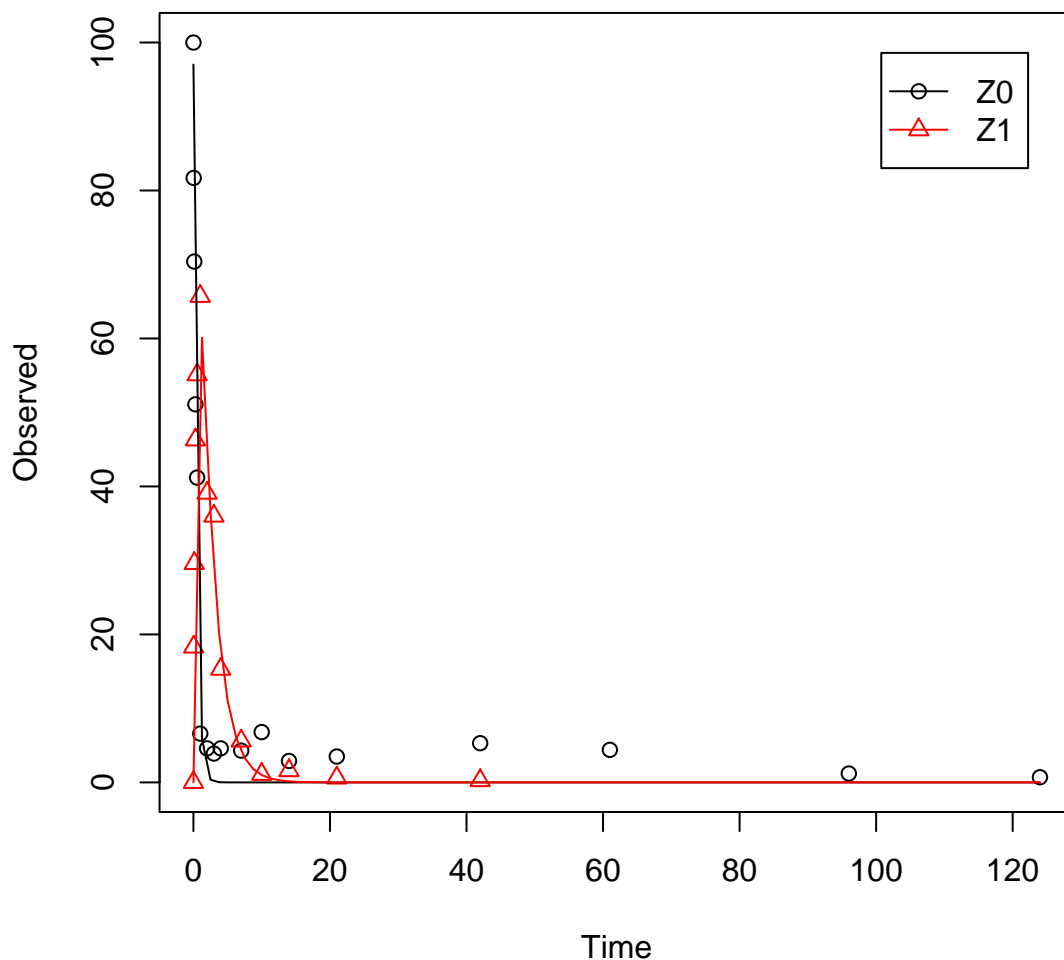
Estimated disappearance times:

	DT50	DT90
Z0	0.310	1.030
Z1	1.438	4.776

Estimated formation fractions:

	ff
Z0_sink	7.75e-17
Z0_Z1	1.00e+00
Z1_sink	1.00e+00

R> plot(m.Z.2a)



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 to 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:

```
R> Z.2a.ff <- mkinmod(Z0 = list(type = "SF0", to = "Z1"),
+                    Z1 = list(type = "SF0"), use_of_ff = "max")
R> m.Z.2a.ff <- mkinfit(Z.2a.ff, FOCUS_2006_Z_mkin, quiet = TRUE)
R> summary(m.Z.2a.ff, data = FALSE)
```

```

mkin version:    0.9.14
R version:       2.15.2
Date of fit:     Thu Feb 21 22:53:15 2013
Date of summary: Thu Feb 21 22:53:15 2013

```

Equations:

```

[1] d_Z0 = - k_Z0 * Z0
[2] d_Z1 = + f_Z0_to_Z1 * k_Z0 * Z0 - k_Z1 * Z1

```

Starting values for optimised parameters:

	initial	type	transformed
Z0_0	100.0	state	100.0000000
k_Z0	0.1	deparm	-2.3025851
f_Z0_to_Z1	0.2	deparm	-0.9802581
k_Z1	0.1	deparm	-2.3025851

Fixed parameter values:

	value	type
Z1	0	state

Optimised, transformed parameters:

	Estimate	Std. Error
Z0_0	9.701e+01	NA
k_Z0	8.047e-01	NA
f_Z0_to_Z1	8.862e+06	NA
k_Z1	-7.296e-01	NA

Backtransformed parameters:

	Estimate
Z0_0	97.015
k_Z0	2.236
f_Z0_to_Z1	1.000
k_Z1	0.482

Residual standard error: 5.064 on 27 degrees of freedom

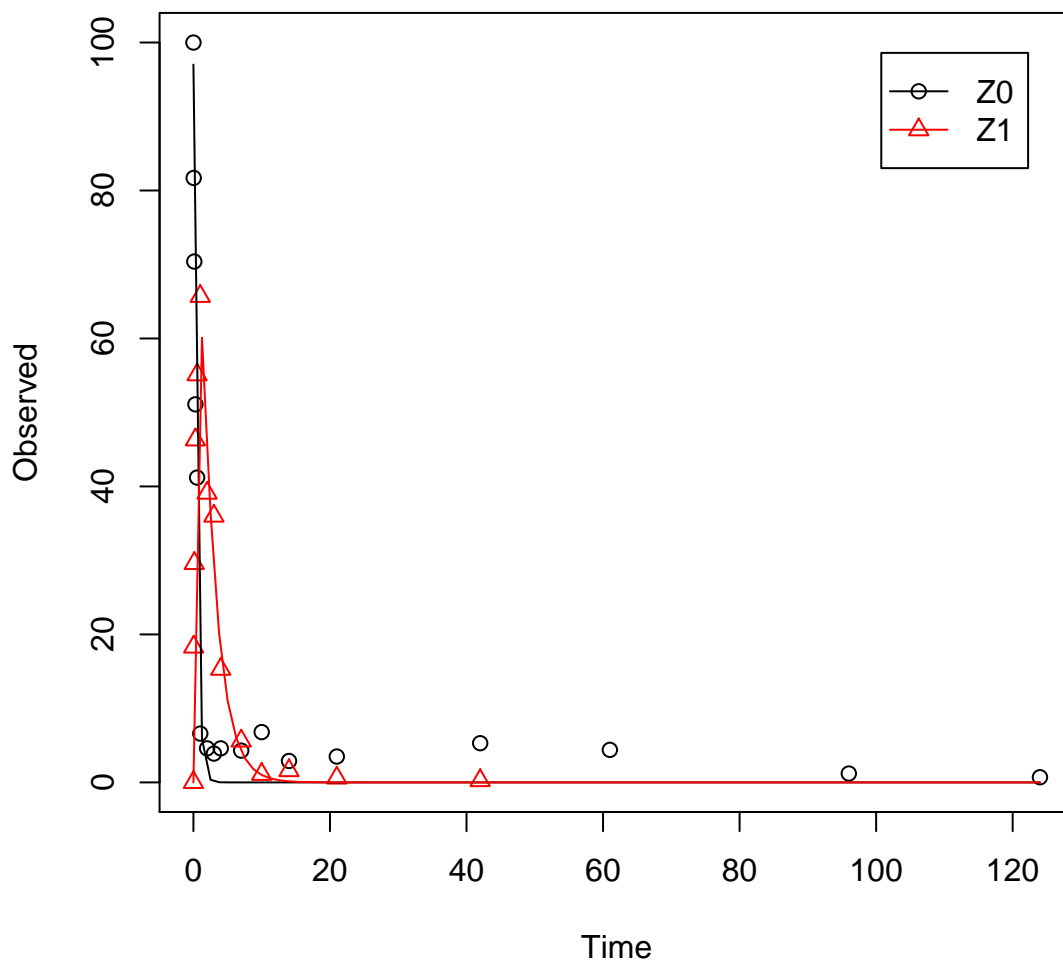
Chi2 error levels in percent:

	err.min	n.optim	df
All data	18.20	4	27
Z0	17.56	2	15
Z1	16.25	2	12

Estimated disappearance times:

	DT50	DT90
Z0	0.310	1.030
Z1	1.438	4.776

```
R> plot(m.Z.2a.ff)
```



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.

```
R> Z.3 <- mkinmod(Z0 = list(type = "SF0", to = "Z1", sink = FALSE),
+               Z1 = list(type = "SF0"))
R> m.Z.3 <- mkinfit(Z.3, FOCUS_2006_Z_mkin, parms.ini = c(k_Z0_Z1 = 0.5),
+               quiet = TRUE)
R> m.Z.3 <- mkinfit(Z.3, FOCUS_2006_Z_mkin, solution_type = "deSolve",
+               quiet = TRUE)
```

```

R> summary(m.Z.3, data = FALSE)

mkin version:      0.9.14
R version:         2.15.2
Date of fit:       Thu Feb 21 22:53:19 2013
Date of summary:   Thu Feb 21 22:53:19 2013

Equations:
[1] d_Z0 = - 0 - k_Z0_Z1 * Z0
[2] d_Z1 = + k_Z0_Z1 * Z0 - k_Z1_sink * Z1

Starting values for optimised parameters:
      initial   type transformed
Z0_0      100.0  state  100.000000
k_Z0_Z1     0.1 deparm  -2.302585
k_Z1_sink    0.1 deparm  -2.302585

Fixed parameter values:
      value   type
Z1         0 state

Optimised, transformed parameters:
      Estimate Std. Error
Z0_0       97.0149      2.682
k_Z0_Z1     0.8047      0.066
k_Z1_sink  -0.7296      0.089

Backtransformed parameters:
      Estimate
Z0_0       97.015
k_Z0_Z1     2.236
k_Z1_sink    0.482

Residual standard error: 4.973 on 28 degrees of freedom

Chi2 error levels in percent:
      err.min n.optim df
All data   17.93      3 28
Z0         17.56      2 15
Z1         15.75      1 13

Estimated disappearance times:
      DT50 DT90
Z0 0.310 1.030
Z1 1.438 4.776

Estimated formation fractions:
      ff
Z0_Z1  1

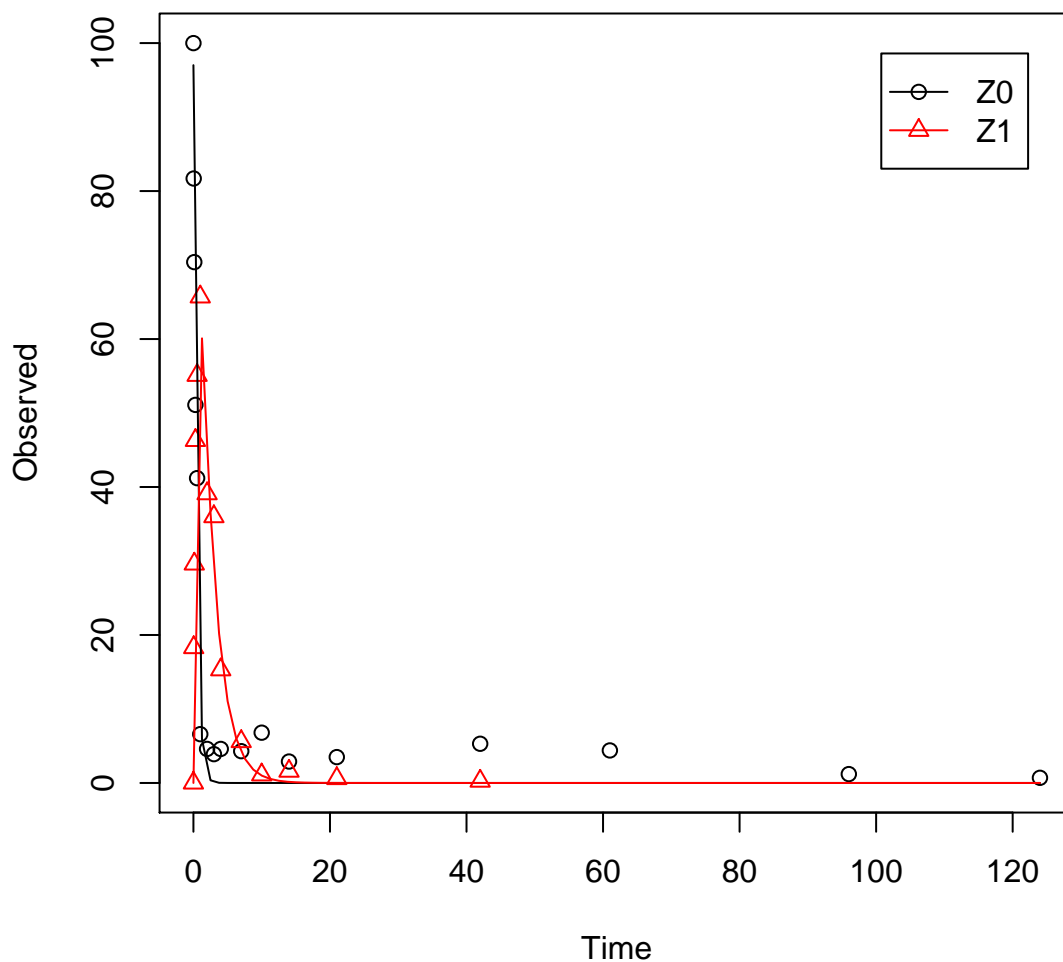
```

```
Z1_sink 1
```

```
Parameter correlation:
```

	Z0_0	k_Z0_Z1	k_Z1_sink
Z0_0	1.0000	0.10629	0.41038
k_Z0_Z1	0.1063	1.00000	0.04346
k_Z1_sink	0.4104	0.04346	1.00000

```
R> plot(m.Z.3)
```



The first attempt to fit the model fails, as the default solution type chosen by `mkinf` is based on eigenvalues, and the system defined by the starting parameters is identified as being singular to the solver. This is caused by the fact that the rate constants for both state variables are the same using the default starting parameters. Setting a different starting

value for one of the parameters overcomes this. Alternatively, the **deSolve** based model solution can be chosen, at the cost of a bit more computing time.

```
R> Z.4a <- mkinmod(Z0 = list(type = "SF0", to = "Z1", sink = FALSE),
+                 Z1 = list(type = "SF0", to = "Z2"),
+                 Z2 = list(type = "SF0"))
R> m.Z.4a <- mkinfit(Z.4a, FOCUS_2006_Z_mkin, parms.ini = c(k_Z0_Z1 = 0.5),
+                  quiet = TRUE)
R> summary(m.Z.4a, data = FALSE)
```

```

mkin version:      0.9.14
R version:         2.15.2
Date of fit:       Thu Feb 21 22:53:21 2013
Date of summary:   Thu Feb 21 22:53:21 2013

```

Equations:

```

[1] d_Z0 = - 0 - k_Z0_Z1 * Z0
[2] d_Z1 = + k_Z0_Z1 * Z0 - k_Z1_sink * Z1 - k_Z1_Z2 * Z1
[3] d_Z2 = + k_Z1_Z2 * Z1 - k_Z2_sink * Z2

```

Starting values for optimised parameters:

	initial	type	transformed
Z0_0	100.0	state	100.0000000
k_Z0_Z1	0.5	deparm	-0.6931472
k_Z1_sink	0.1	deparm	-2.3025851
k_Z1_Z2	0.1	deparm	-2.3025851
k_Z2_sink	0.1	deparm	-2.3025851

Fixed parameter values:

	value	type
Z1	0	state
Z2	0	state

Optimised, transformed parameters:

	Estimate	Std. Error
Z0_0	96.9587	2.447
k_Z0_Z1	0.7970	0.060
k_Z1_sink	-4.1044	4.847
k_Z1_Z2	-0.7667	0.146
k_Z2_sink	-0.8410	0.229

Backtransformed parameters:

	Estimate
Z0_0	96.959
k_Z0_Z1	2.219
k_Z1_sink	0.017
k_Z1_Z2	0.465
k_Z2_sink	0.431

Residual standard error: 4.54 on 39 degrees of freedom

Chi2 error levels in percent:

	err.min	n.optim	df
All data	19.78	5	39
Z0	17.47	2	15
Z1	16.37	2	12
Z2	20.47	1	12

Estimated disappearance times:

	DT50	DT90
Z0	0.3124	1.038
Z1	1.4410	4.787
Z2	1.6072	5.339

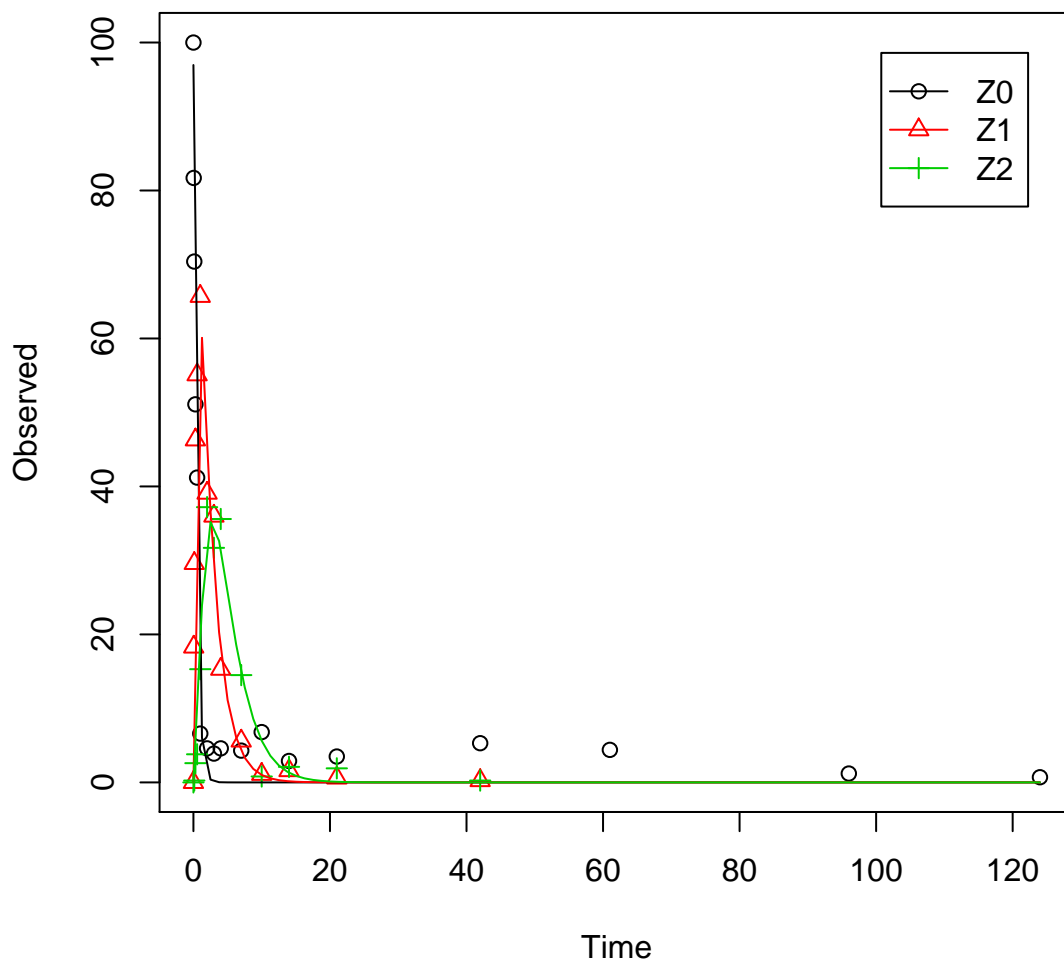
Estimated formation fractions:

	ff
Z0_Z1	1.0000
Z1_sink	0.0343
Z1_Z2	0.9657
Z2_sink	1.0000

Parameter correlation:

	Z0_0	k_Z0_Z1	k_Z1_sink	k_Z1_Z2	k_Z2_sink
Z0_0	1.0000	0.1064	0.3492	-0.1769	-0.1534
k_Z0_Z1	0.1064	1.0000	0.1479	-0.1501	-0.1226
k_Z1_sink	0.3492	0.1479	1.0000	-0.8745	-0.8656
k_Z1_Z2	-0.1769	-0.1501	-0.8745	1.0000	0.8190
k_Z2_sink	-0.1534	-0.1226	-0.8656	0.8190	1.0000

R> plot(m.Z.4a)



As suggested in the FOCUS report, the pathway to sink was removed for metabolite Z1 as well. 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. Again, in order to avoid a singular system when using default starting parameters, the starting parameter for the pathway without sink term has to be adapted.

```
R> Z.5 <- mkinmod(Z0 = list(type = "SF0", to = "Z1", sink = FALSE),
+               Z1 = list(type = "SF0", to = "Z2", sink = FALSE),
+               Z2 = list(type = "SF0"))
R> m.Z.5 <- mkinfit(Z.5, FOCUS_2006_Z_mkin,
+               parms.ini = c(k_Z0_Z1 = 0.5, k_Z1_Z2 = 0.2), quiet = TRUE)
```

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

```
mkim version:      0.9.14
R version:         2.15.2
Date of fit:       Thu Feb 21 22:53:22 2013
Date of summary:   Thu Feb 21 22:53:22 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
```

Starting values for optimised parameters:

	initial	type	transformed
Z0_0	100.0	state	100.0000000
k_Z0_Z1	0.5	deparm	-0.6931472
k_Z1_Z2	0.2	deparm	-1.6094379
k_Z2_sink	0.1	deparm	-2.3025851

Fixed parameter values:

	value	type
Z1	0	state
Z2	0	state

Optimised, transformed parameters:

	Estimate	Std. Error
Z0_0	96.7690	2.266
k_Z0_Z1	0.7948	0.058
k_Z1_Z2	-0.7410	0.068
k_Z2_sink	-0.8027	0.111

Backtransformed parameters:

	Estimate
Z0_0	96.769
k_Z0_Z1	2.214
k_Z1_Z2	0.477
k_Z2_sink	0.448

Residual standard error: 4.486 on 40 degrees of freedom

Chi2 error levels in percent:

	err.min	n.optim	df
All data	19.58	4	40
Z0	17.43	2	15
Z1	15.94	1	13
Z2	20.51	1	12

Estimated disappearance times:

DT50 DT90

```
Z0 0.3131 1.040
Z1 1.4543 4.831
Z2 1.5468 5.138
```

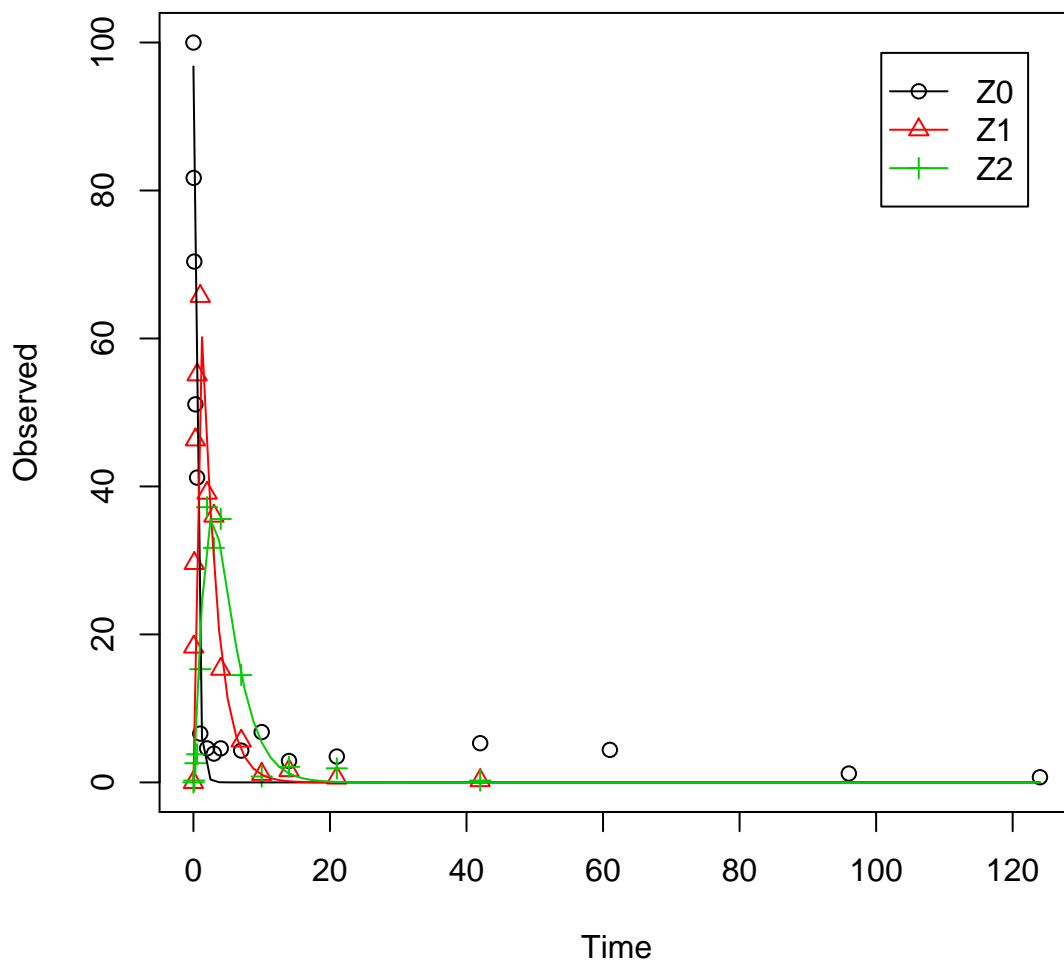
Estimated formation fractions:

```
      ff
Z0_Z1  1
Z1_Z2  1
Z2_sink 1
```

Parameter correlation:

	Z0_0	k_Z0_Z1	k_Z1_Z2	k_Z2_sink
Z0_0	1.00000	0.05781	0.28747	0.31786
k_Z0_Z1	0.05781	1.00000	-0.04361	0.01212
k_Z1_Z2	0.28747	-0.04361	1.00000	0.24018
k_Z2_sink	0.31786	0.01212	0.24018	1.00000

```
R> plot(m.Z.5)
```



Finally, metabolite Z3 is added to the model.

```
R> Z.FOCUS <- mkinmod(Z0 = list(type = "SF0", to = "Z1", sink = FALSE),
+                     Z1 = list(type = "SF0", to = "Z2", sink = FALSE),
+                     Z2 = list(type = "SF0", to = "Z3"),
+                     Z3 = list(type = "SF0"))
R> m.Z.FOCUS <- mkinfit(Z.FOCUS, FOCUS_2006_Z_mkin,
+                       parms.ini = c(k_Z0_Z1 = 0.5, k_Z1_Z2 = 0.2, k_Z2_Z3 = 0.3),
+                       quiet = TRUE)
R> summary(m.Z.FOCUS, data = FALSE)

mkin version:      0.9.14
R version:         2.15.2
```

Date of fit: Thu Feb 21 22:53:23 2013

Date of summary: Thu Feb 21 22:53:23 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
```

Starting values for optimised parameters:

	initial	type	transformed
Z0_0	100.0	state	100.0000000
k_Z0_Z1	0.5	deparm	-0.6931472
k_Z1_Z2	0.2	deparm	-1.6094379
k_Z2_Z3	0.3	deparm	-1.2039728
k_Z2_sink	0.1	deparm	-2.3025851
k_Z3_sink	0.1	deparm	-2.3025851

Fixed parameter values:

	value	type
Z1	0	state
Z2	0	state
Z3	0	state

Optimised, transformed parameters:

	Estimate	Std. Error
Z0_0	96.8386	2.059
k_Z0_Z1	0.7954	0.053
k_Z1_Z2	-0.7375	0.061
k_Z2_Z3	-1.5467	0.123
k_Z2_sink	-1.4326	0.172
k_Z3_sink	-2.8355	0.244

Backtransformed parameters:

	Estimate
Z0_0	96.839
k_Z0_Z1	2.215
k_Z1_Z2	0.478
k_Z2_Z3	0.213
k_Z2_sink	0.239
k_Z3_sink	0.059

Residual standard error: 4.1 on 51 degrees of freedom

Chi2 error levels in percent:

	err.min	n.optim	df
All data	19.78	6	51
Z0	17.45	2	15
Z1	15.92	1	13

Z2	21.24	2 11
Z3	12.46	1 12

Estimated disappearance times:

	DT50	DT90
Z0	0.3129	1.039
Z1	1.4492	4.814
Z2	1.5348	5.099
Z3	11.8100	39.232

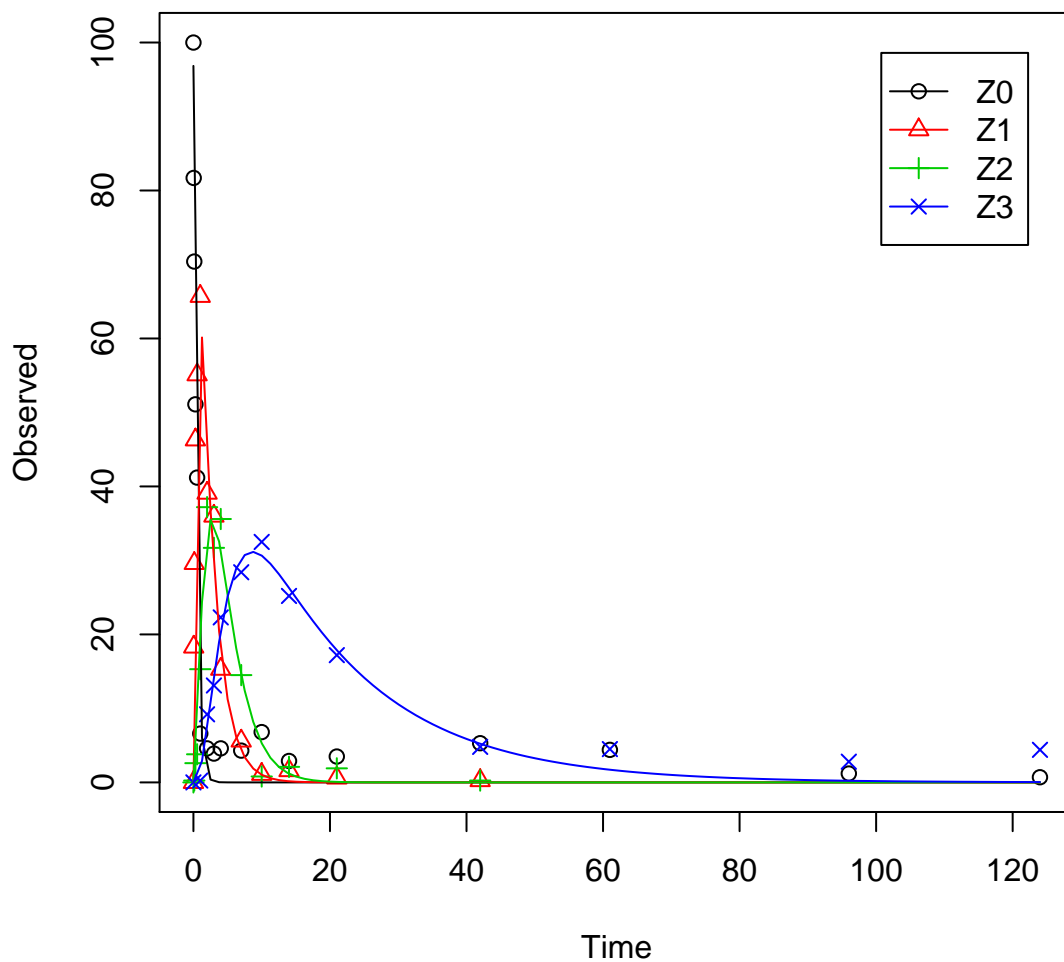
Estimated formation fractions:

	ff
Z0_Z1	1.0000
Z1_Z2	1.0000
Z2_sink	0.5285
Z2_Z3	0.4715
Z3_sink	1.0000

Parameter correlation:

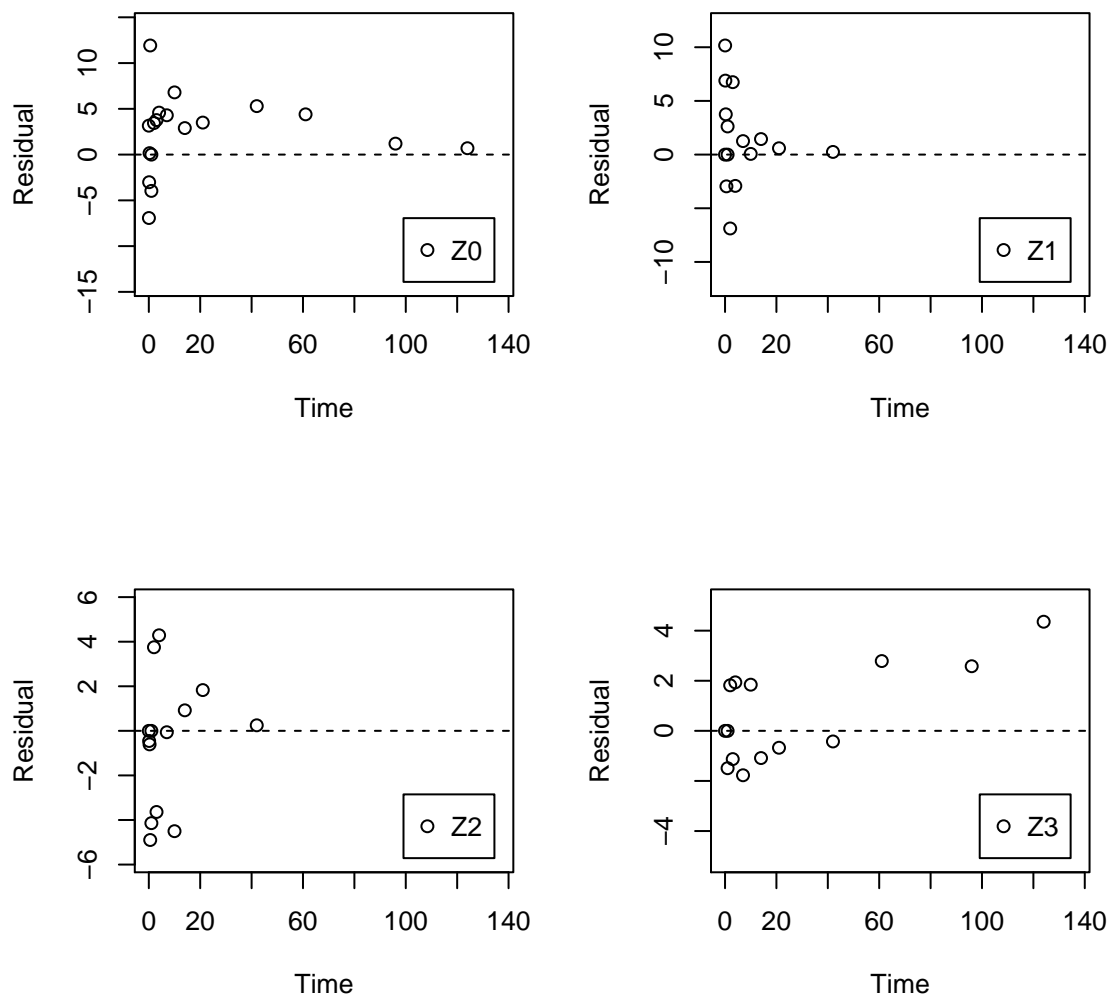
	Z0_0	k_Z0_Z1	k_Z1_Z2	k_Z2_Z3	k_Z2_sink	k_Z3_sink
Z0_0	1.00000	0.05387	0.2727	-0.07295	0.37006	-0.11348
k_Z0_Z1	0.05387	1.00000	-0.0521	-0.03581	0.02442	-0.02521
k_Z1_Z2	0.27275	-0.05210	1.0000	-0.12132	0.29384	-0.19144
k_Z2_Z3	-0.07295	-0.03581	-0.1213	1.00000	-0.18887	0.55154
k_Z2_sink	0.37006	0.02442	0.2938	-0.18887	1.00000	-0.64293
k_Z3_sink	-0.11348	-0.02521	-0.1914	0.55154	-0.64293	1.00000

R> plot(m.Z.FOCUS)



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

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



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.

```
R> 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"))
```



```

R> 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, k_Z2_Z3 = 0.2),
+                          quiet = TRUE)
R> summary(m.Z.mkin.1, data = FALSE)

mkin version:      0.9.14
R version:         2.15.2
Date of fit:       Thu Feb 21 22:53:28 2013
Date of summary:   Thu Feb 21 22:53:28 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 * Z3_free
[5] d_Z3_bound = + k_Z3_free_bound * Z3_free - k_Z3_bound_free * Z3_bound

Starting values for optimised parameters:
              initial   type transformed
Z0_0          1e+02  state 100.0000000
k_Z0_Z1        5e-01 deparm -0.6931472
k_Z1_Z2        3e-01 deparm -1.2039728
k_Z2_Z3        2e-01 deparm -1.6094379
k_Z2_sink      1e-01 deparm -2.3025851
k_Z2_Z3_free   1e-01 deparm -2.3025851
k_Z3_free_sink 1e-01 deparm -2.3025851
k_Z3_free_bound 1e-01 deparm -2.3025851
k_Z3_bound_free 2e-02 deparm -3.9120230

Fixed parameter values:
      value type
Z1         0 state
Z2         0 state
Z3_free    0 state
Z3_bound   0 state

Optimised, transformed parameters:
              Estimate Std. Error
Z0_0          96.7375         NA
k_Z0_Z1        0.7947         NA
k_Z1_Z2       -0.7426         NA
k_Z2_Z3       -1.6094         NA
k_Z2_sink     -1.4946         NA
k_Z2_Z3_free  -1.5042         NA
k_Z3_free_sink -2.6544         NA
k_Z3_free_bound -5.2440         NA
k_Z3_bound_free -19.8427         NA

Backtransformed parameters:

```

	Estimate
Z0_0	96.738
k_Z0_Z1	2.214
k_Z1_Z2	0.476
k_Z2_Z3	0.200
k_Z2_sink	0.224
k_Z2_Z3_free	0.222
k_Z3_free_sink	0.070
k_Z3_free_bound	0.005
k_Z3_bound_free	0.000

Residual standard error: 4.149 on 48 degrees of freedom

Chi2 error levels in percent:

	err.min	n.optim	df
All data	19.931	9	48
Z0	17.429	2	15
Z1	15.949	1	13
Z2	21.967	3	10
Z3	8.561	3	10

Estimated disappearance times:

	DT50	DT90
Z0	0.3131	1.040
Z1	1.4566	4.839
Z2	1.5523	5.157
Z3	10.1977	45.329

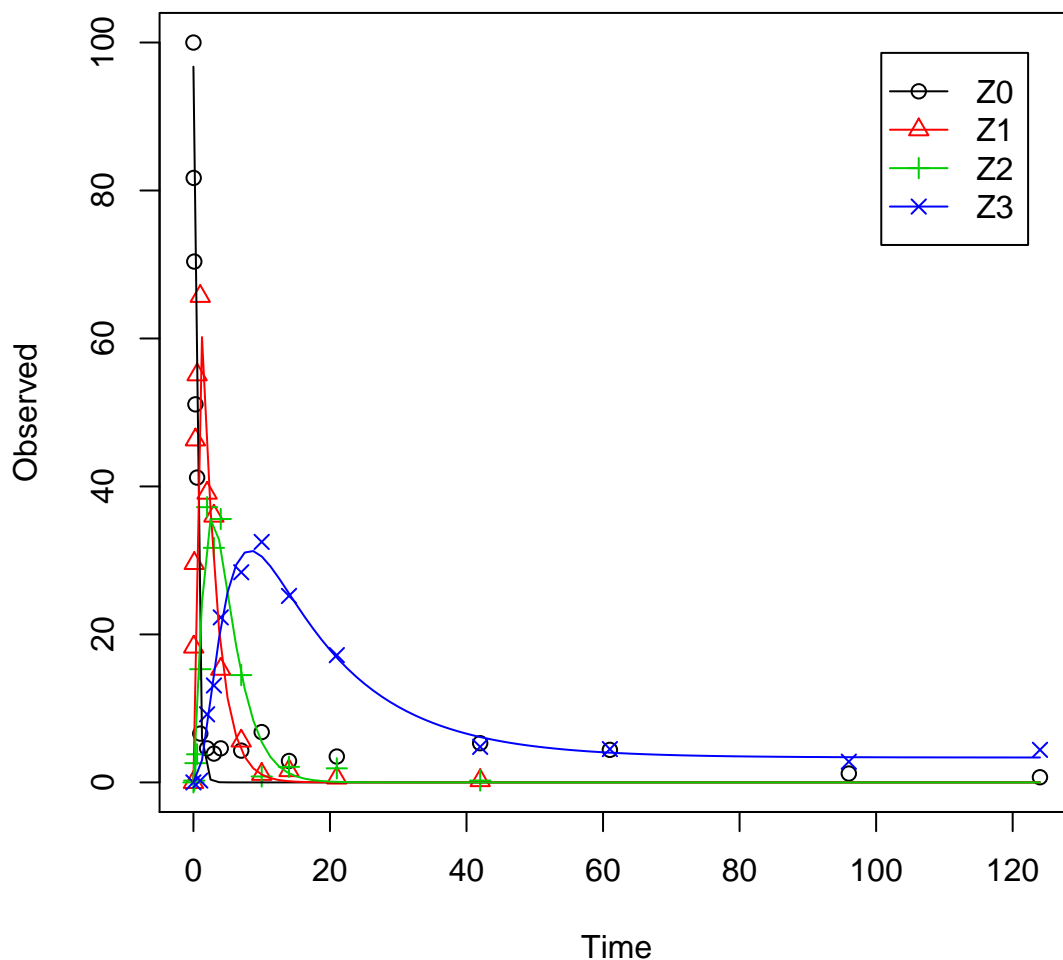
Estimated formation fractions:

	ff
Z0_Z1	1.0000
Z1_Z2	1.0000
Z2_sink	0.5024
Z2_Z3_free	0.4976
Z3_free_sink	1.0000

Estimated Eigenvalues of SFORB model(s):

Z3_b1	Z3_b2
7.562e-02	2.244e-09

R> plot(m.Z.mkin.1)



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.

```
R> Z.mkin.2 <- mkinmod(Z0 = list(type = "SFORB", to = "Z1", sink = FALSE),
+                      Z1 = list(type = "SF0"))
R> m.Z.mkin.2 <- mkinfit(Z.mkin.2, FOCUS_2006_Z_mkin, quiet = TRUE)
R> summary(m.Z.mkin.2, data = FALSE)
```

```
mkim version:    0.9.14
R version:       2.15.2
Date of fit:     Thu Feb 21 22:53:28 2013
Date of summary: Thu Feb 21 22:53:28 2013
```

Equations:

```
[1] d_Z0_free = - 0 - k_Z0_free_bound * Z0_free + k_Z0_bound_free * Z0_bound - k_Z0_free
[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
```

Starting values for optimised parameters:

	initial	type	transformed
Z0_free_0	1e+02	state	100.000000
k_Z0_free_bound	1e-01	deparm	-2.302585
k_Z0_bound_free	2e-02	deparm	-3.912023
k_Z0_free_Z1	1e-01	deparm	-2.302585
k_Z1_sink	1e-01	deparm	-2.302585

Fixed parameter values:

	value	type
Z0_bound	0	state
Z1	0	state

Optimised, transformed parameters:

	Estimate	Std. Error
Z0_free_0	97.2851	2.395
k_Z0_free_bound	-2.0821	0.432
k_Z0_bound_free	-4.7204	1.605
k_Z0_free_Z1	0.8549	0.064
k_Z1_sink	-0.7934	0.085

Backtransformed parameters:

	Estimate
Z0_free_0	97.285
k_Z0_free_bound	0.125
k_Z0_bound_free	0.009
k_Z0_free_Z1	2.351
k_Z1_sink	0.452

Residual standard error: 4.438 on 26 degrees of freedom

Chi2 error levels in percent:

	err.min	n.optim	df
All data	15.89	5	26
Z0	14.74	4	13
Z1	14.94	1	13

Estimated disappearance times:

	DT50	DT90
Z0	0.302	1.190
Z1	1.532	5.091

Estimated formation fractions:

```

ff
Z0_free_Z1 1
Z1_sink 1

```

Estimated Eigenvalues of SFORB model(s):

```

Z0_b1 Z0_b2
2.476313 0.008461

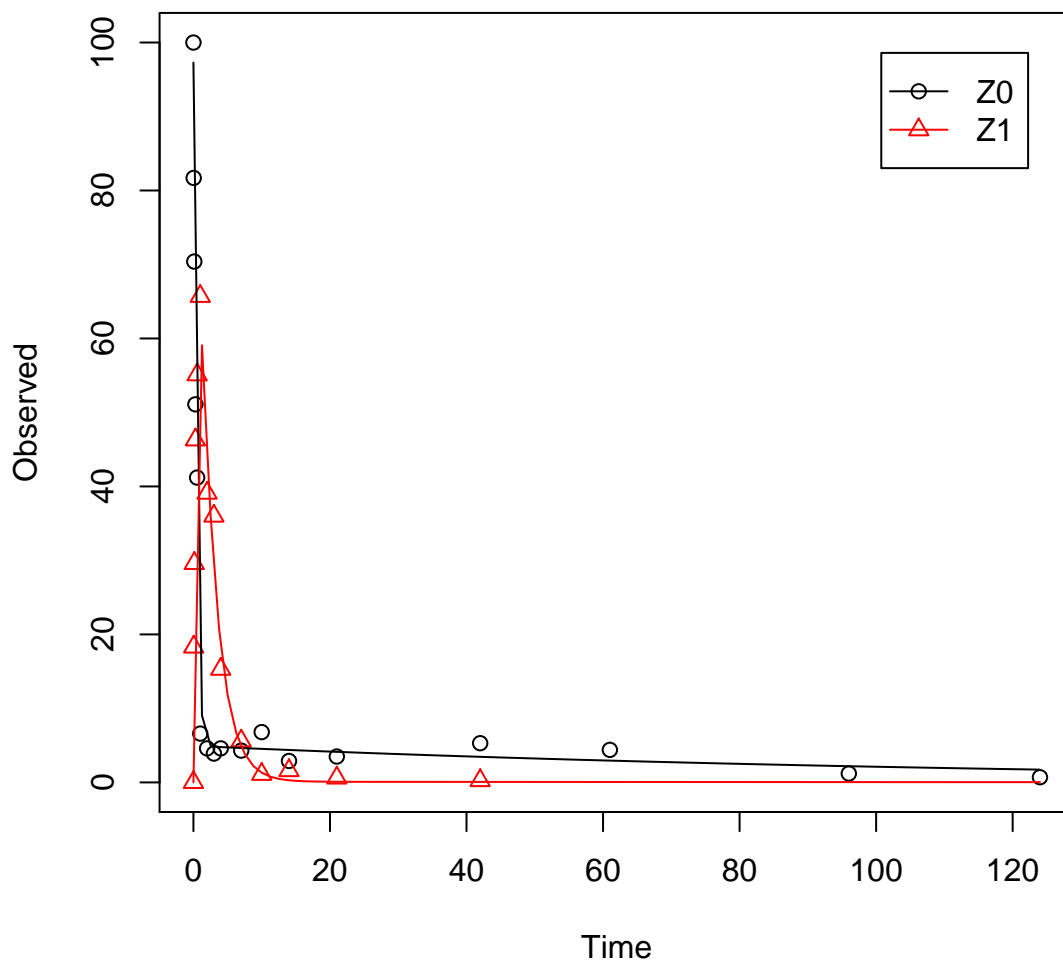
```

Parameter correlation:

	Z0_free_0	k_Z0_free_bound	k_Z0_bound_free	k_Z0_free_Z1
Z0_free_0	1.000000	0.006494	0.03324	0.11182
k_Z0_free_bound	0.006494	1.000000	0.54646	0.41393
k_Z0_bound_free	0.033238	0.546465	1.00000	0.15837
k_Z0_free_Z1	0.111819	0.413926	0.15837	1.00000
k_Z1_sink	0.391553	-0.291912	-0.12597	-0.04188

	k_Z1_sink
Z0_free_0	0.39155
k_Z0_free_bound	-0.29191
k_Z0_bound_free	-0.12597
k_Z0_free_Z1	-0.04188
k_Z1_sink	1.00000

```
R> plot(m.Z.mkin.2)
```



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

```
R> Z.mkin.3 <- mkinmod(Z0 = list(type = "SFORB", to = "Z1", sink = FALSE),
+                     Z1 = list(type = "SFO", to = "Z2"),
+                     Z2 = list(type = "SFO"))
R> m.Z.mkin.3 <- mkinfit(Z.mkin.3, FOCUS_2006_Z_mkin, quiet = TRUE)
R> summary(m.Z.mkin.3, data = FALSE)
```

```
mkin version:    0.9.14
R version:       2.15.2
Date of fit:     Thu Feb 21 22:53:32 2013
Date of summary: Thu Feb 21 22:53:32 2013
```

Equations:

```
[1] d_Z0_free = - 0 - k_Z0_free_bound * Z0_free + k_Z0_bound_free * Z0_bound - k_Z0_free_bound * Z0_bound
[2] d_Z0_bound = + k_Z0_free_bound * Z0_free - k_Z0_bound_free * Z0_bound + k_Z0_free_bound * 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
```

Starting values for optimised parameters:

	initial	type	transformed
Z0_free_0	1e+02	state	100.000000
k_Z0_free_bound	1e-01	deparm	-2.302585
k_Z0_bound_free	2e-02	deparm	-3.912023
k_Z0_free_Z1	1e-01	deparm	-2.302585
k_Z1_sink	1e-01	deparm	-2.302585
k_Z1_Z2	1e-01	deparm	-2.302585
k_Z2_sink	1e-01	deparm	-2.302585

Fixed parameter values:

	value	type
Z0_bound	0	state
Z1	0	state
Z2	0	state

Optimised, transformed parameters:

	Estimate	Std. Error
Z0_free_0	97.4356	2.124e+00
k_Z0_free_bound	-2.1488	4.120e-01
k_Z0_bound_free	-4.8383	1.636e+00
k_Z0_free_Z1	0.8457	5.900e-02
k_Z1_sink	-19.3794	1.751e+06
k_Z1_Z2	-0.7812	8.800e-02
k_Z2_sink	-0.8606	1.440e-01

Backtransformed parameters:

	Estimate
Z0_free_0	97.436
k_Z0_free_bound	0.117
k_Z0_bound_free	0.008
k_Z0_free_Z1	2.330
k_Z1_sink	0.000
k_Z1_Z2	0.458
k_Z2_sink	0.423

Residual standard error: 4.136 on 37 degrees of freedom

Chi2 error levels in percent:

	err.min	n.optim	df
All data	17.95	7	37
Z0	14.67	4	13

Z1	15.52	2 12
Z2	21.27	1 12

Estimated disappearance times:

	DT50	DT90
Z0	0.3043	1.185
Z1	1.5138	5.029
Z2	1.6391	5.445

Estimated formation fractions:

	ff
Z0_free_Z1	1.000e+00
Z1_sink	8.373e-09
Z1_Z2	1.000e+00
Z2_sink	1.000e+00

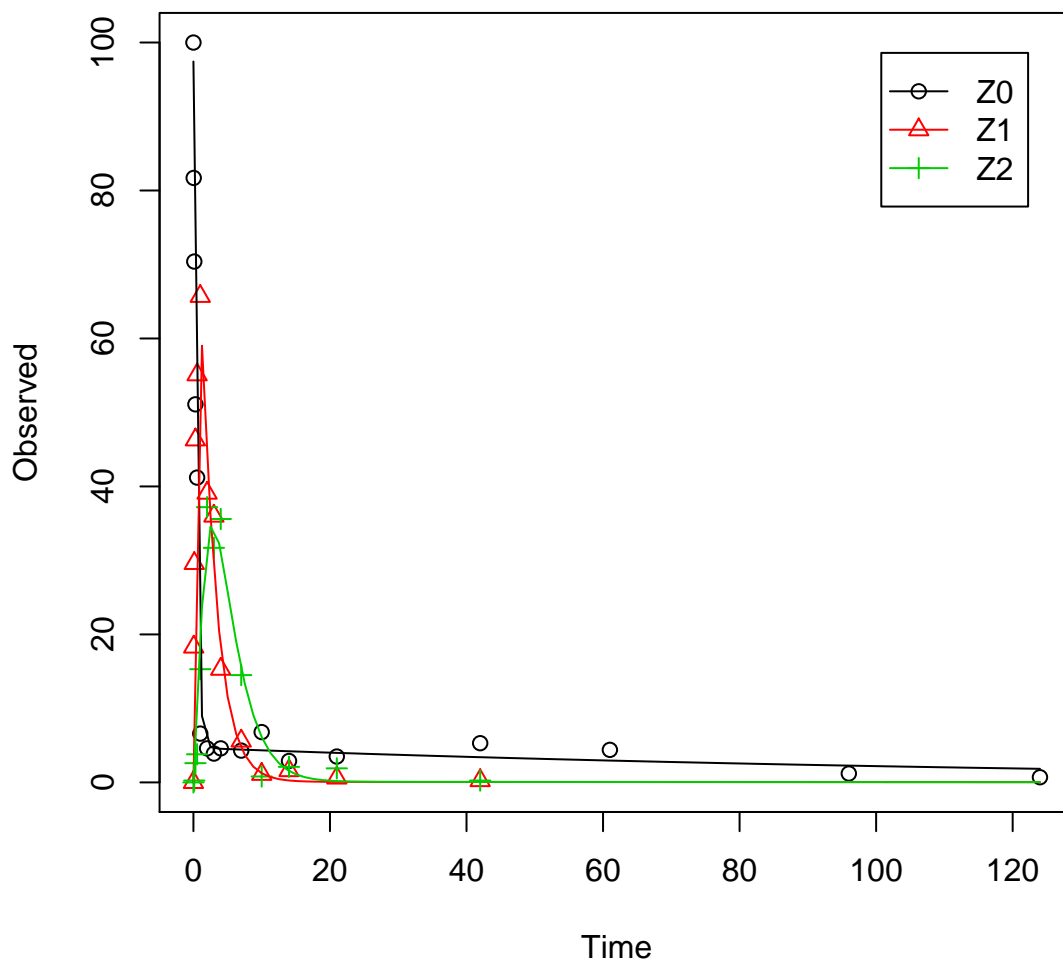
Estimated Eigenvalues of SFORB model(s):

	Z0_b1	Z0_b2
	2.446638	0.007542

Parameter correlation:

	Z0_free_0	k_Z0_free_bound	k_Z0_bound_free	k_Z0_free_Z1
Z0_free_0	1.00000	0.05615	0.06063	0.09324
k_Z0_free_bound	0.05615	1.00000	0.54490	0.41896
k_Z0_bound_free	0.06063	0.54490	1.00000	0.16141
k_Z0_free_Z1	0.09324	0.41896	0.16141	1.00000
k_Z1_sink	0.15262	-0.11467	-0.06038	0.02314
k_Z1_Z2	0.08997	-0.09395	-0.02475	-0.09097
k_Z2_sink	0.11068	-0.07920	-0.01859	-0.05193
	k_Z1_sink	k_Z1_Z2	k_Z2_sink	
Z0_free_0	0.15262	0.08997	0.11068	
k_Z0_free_bound	-0.11467	-0.09395	-0.07920	
k_Z0_bound_free	-0.06038	-0.02475	-0.01859	
k_Z0_free_Z1	0.02314	-0.09097	-0.05193	
k_Z1_sink	1.00000	-0.66065	-0.66738	
k_Z1_Z2	-0.66065	1.00000	0.59341	
k_Z2_sink	-0.66738	0.59341	1.00000	

R> plot(m.Z.mkin.3)



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

```
R> Z.mkin.4 <- mkinmod(Z0 = list(type = "SFORB", to = "Z1", sink = FALSE),
+                     Z1 = list(type = "SF0", to = "Z2", sink = FALSE),
+                     Z2 = list(type = "SF0", to = "Z3"),
+                     Z3 = list(type = "SF0"))
R> m.Z.mkin.4 <- mkinfit(Z.mkin.4, FOCUS_2006_Z_mkin,
+   parms.ini = c(k_Z1_Z2 = 0.05), quiet = TRUE)
R> summary(m.Z.mkin.4, data = FALSE)
```

```
mkin version:    0.9.14
R version:       2.15.2
```

Date of fit: Thu Feb 21 22:53:36 2013

Date of summary: Thu Feb 21 22:53:36 2013

Equations:

```
[1] d_Z0_free = - 0 - k_Z0_free_bound * Z0_free + k_Z0_bound_free * Z0_bound - k_Z0_free
[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
```

Starting values for optimised parameters:

	initial	type	transformed
Z0_free_0	1e+02	state	100.000000
k_Z1_Z2	5e-02	deparm	-2.995732
k_Z0_free_bound	1e-01	deparm	-2.302585
k_Z0_bound_free	2e-02	deparm	-3.912023
k_Z0_free_Z1	1e-01	deparm	-2.302585
k_Z2_sink	1e-01	deparm	-2.302585
k_Z2_Z3	1e-01	deparm	-2.302585
k_Z3_sink	1e-01	deparm	-2.302585

Fixed parameter values:

	value	type
Z0_bound	0	state
Z1	0	state
Z2	0	state
Z3	0	state

Optimised, transformed parameters:

	Estimate	Std. Error
Z0_free_0	97.5296	1.887
k_Z1_Z2	-0.7769	0.058
k_Z0_free_bound	-2.1362	0.368
k_Z0_bound_free	-4.7653	1.417
k_Z0_free_Z1	0.8470	0.053
k_Z2_sink	-1.5610	0.183
k_Z2_Z3	-1.5276	0.114
k_Z3_sink	-2.7691	0.225

Backtransformed parameters:

	Estimate
Z0_free_0	97.530
k_Z1_Z2	0.460
k_Z0_free_bound	0.118
k_Z0_bound_free	0.009
k_Z0_free_Z1	2.333
k_Z2_sink	0.210
k_Z2_Z3	0.217
k_Z3_sink	0.063

Residual standard error: 3.737 on 49 degrees of freedom

Chi2 error levels in percent:

	err.min	n.optim	df
All data	17.98	8	49
Z0	14.69	4	13
Z1	15.03	1	13
Z2	21.99	2	11
Z3	12.32	1	12

Estimated disappearance times:

	DT50	DT90
Z0	0.304	1.186
Z1	1.507	5.008
Z2	1.623	5.393
Z3	11.051	36.712

Estimated 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 Eigenvalues of SFORB model(s):

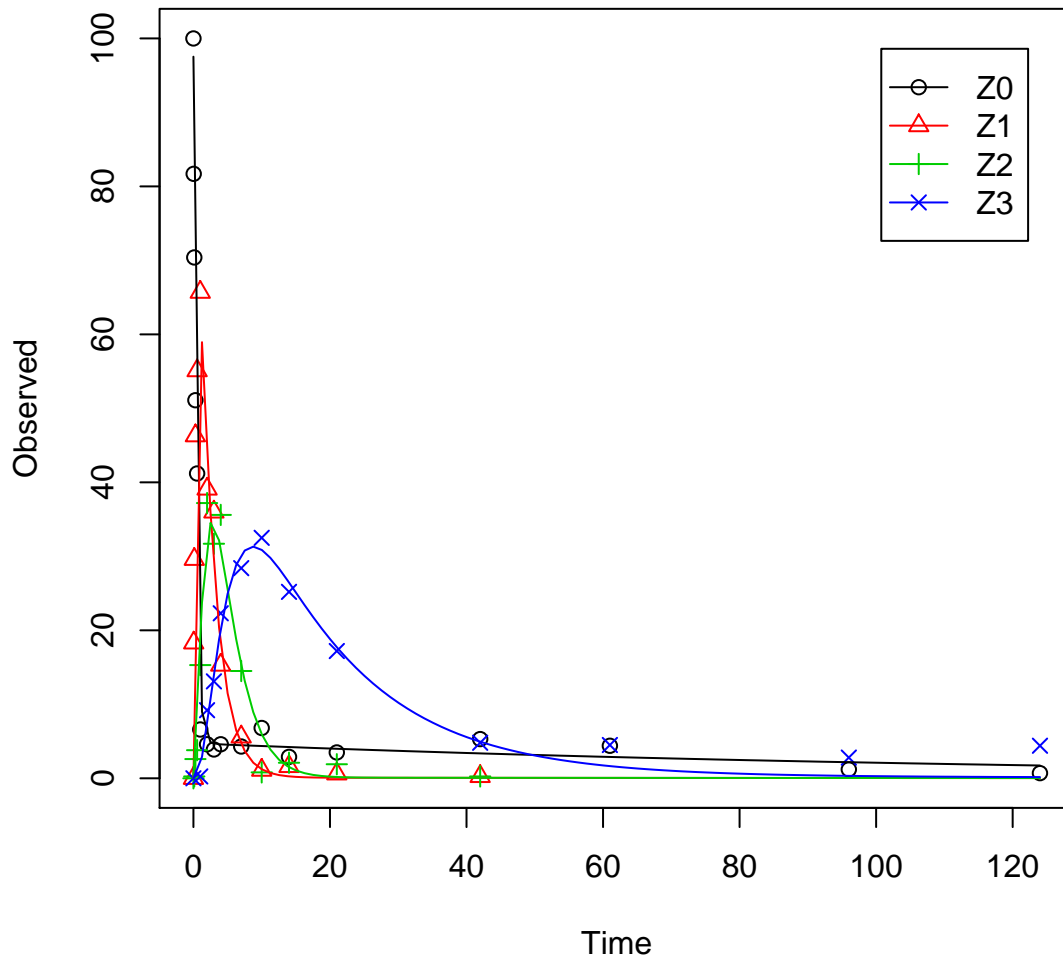
	Z0_b1	Z0_b2
	2.451259	0.008108

Parameter correlation:

	Z0_free_0	k_Z1_Z2	k_Z0_free_bound	k_Z0_bound_free	k_Z0_free_Z1
Z0_free_0	1.00000	0.24238	0.07823	0.06921	0.08883
k_Z1_Z2	0.24238	1.00000	-0.22742	-0.08934	-0.10841
k_Z0_free_bound	0.07823	-0.22742	1.00000	0.53976	0.42766
k_Z0_bound_free	0.06921	-0.08934	0.53976	1.00000	0.16281
k_Z0_free_Z1	0.08883	-0.10841	0.42766	0.16281	1.00000
k_Z2_sink	0.32993	0.34052	-0.26331	-0.12743	-0.05310
k_Z2_Z3	-0.07494	-0.14885	0.06698	0.06082	-0.01281
k_Z3_sink	-0.10456	-0.22486	0.13845	0.12526	0.01858

	k_Z2_sink	k_Z2_Z3	k_Z3_sink
Z0_free_0	0.3299	-0.07494	-0.10456
k_Z1_Z2	0.3405	-0.14885	-0.22486
k_Z0_free_bound	-0.2633	0.06698	0.13845
k_Z0_bound_free	-0.1274	0.06082	0.12526
k_Z0_free_Z1	-0.0531	-0.01281	0.01858
k_Z2_sink	1.0000	-0.25473	-0.68320
k_Z2_Z3	-0.2547	1.00000	0.56390
k_Z3_sink	-0.6832	0.56390	1.00000

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



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.

Using the SFORB additionally for Z1 or Z2 did not further improve the result. Therefore, the model Z.mkin.5 is proposed as the best-fit model for the dataset from Appendix 7 of the FOCUS report.

```
R> Z.mkin.5 <- mkinmod(Z0 = list(type = "SFORB", to = "Z1", sink = FALSE),
+                      Z1 = list(type = "SF0", to = "Z2", sink = FALSE),
+                      Z2 = list(type = "SF0", to = "Z3"),
```

```
+          Z3 = list(type = "SFORB"))
R> m.Z.mkin.5 <- mkinfit(Z.mkin.5, FOCUS_2006_Z_mkin,
+   parms.ini = c(k_Z1_Z2 = 0.2), quiet = TRUE)
R> summary(m.Z.mkin.5, data = FALSE)
```

```
mkkin version:    0.9.14
R version:        2.15.2
Date of fit:      Thu Feb 21 22:53:48 2013
Date of summary:  Thu Feb 21 22:53:48 2013
```

Equations:

```
[1] d_Z0_free = - 0 - k_Z0_free_bound * Z0_free + k_Z0_bound_free * Z0_bound - k_Z0_free
[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 * Z3_free
[6] d_Z3_bound = + k_Z3_free_bound * Z3_free - k_Z3_bound_free * Z3_bound
```

Starting values for optimised parameters:

	initial	type	transformed
Z0_free_0	1e+02	state	100.000000
k_Z1_Z2	2e-01	deparm	-1.609438
k_Z0_free_bound	1e-01	deparm	-2.302585
k_Z0_bound_free	2e-02	deparm	-3.912023
k_Z0_free_Z1	1e-01	deparm	-2.302585
k_Z2_sink	1e-01	deparm	-2.302585
k_Z2_Z3_free	1e-01	deparm	-2.302585
k_Z3_free_sink	1e-01	deparm	-2.302585
k_Z3_free_bound	1e-01	deparm	-2.302585
k_Z3_bound_free	2e-02	deparm	-3.912023

Fixed parameter values:

	value	type
Z0_bound	0	state
Z1	0	state
Z2	0	state
Z3_free	0	state
Z3_bound	0	state

Optimised, transformed parameters:

	Estimate	Std. Error
Z0_free_0	97.4307	1.887
k_Z1_Z2	-0.7813	0.059
k_Z0_free_bound	-2.1467	0.369
k_Z0_bound_free	-4.8228	1.463
k_Z0_free_Z1	0.8459	0.053
k_Z2_sink	-1.6296	0.221
k_Z2_Z3_free	-1.4854	0.196
k_Z3_free_sink	-2.5954	0.385

k_Z3_free_bound	-5.2565	1.372
k_Z3_bound_free	-13.4386	282.033

Backtransformed parameters:

	Estimate
Z0_free_0	97.431
k_Z1_Z2	0.458
k_Z0_free_bound	0.117
k_Z0_bound_free	0.008
k_Z0_free_Z1	2.330
k_Z2_sink	0.196
k_Z2_Z3_free	0.226
k_Z3_free_sink	0.075
k_Z3_free_bound	0.005
k_Z3_bound_free	0.000

Residual standard error: 3.73 on 47 degrees of freedom

Chi2 error levels in percent:

	err.min	n.optim	df
All data	17.890	10	47
Z0	14.666	4	13
Z1	15.049	1	13
Z2	21.991	2	11
Z3	8.433	3	10

Estimated disappearance times:

	DT50	DT90
Z0	0.3043	1.185
Z1	1.5141	5.030
Z2	1.6409	5.451
Z3	9.5901	41.258

Estimated formation fractions:

	ff
Z0_free_Z1	1.000
Z1_Z2	1.000
Z2_sink	0.464
Z2_Z3_free	0.536
Z3_free_sink	1.000

Estimated Eigenvalues of SFORB model(s):

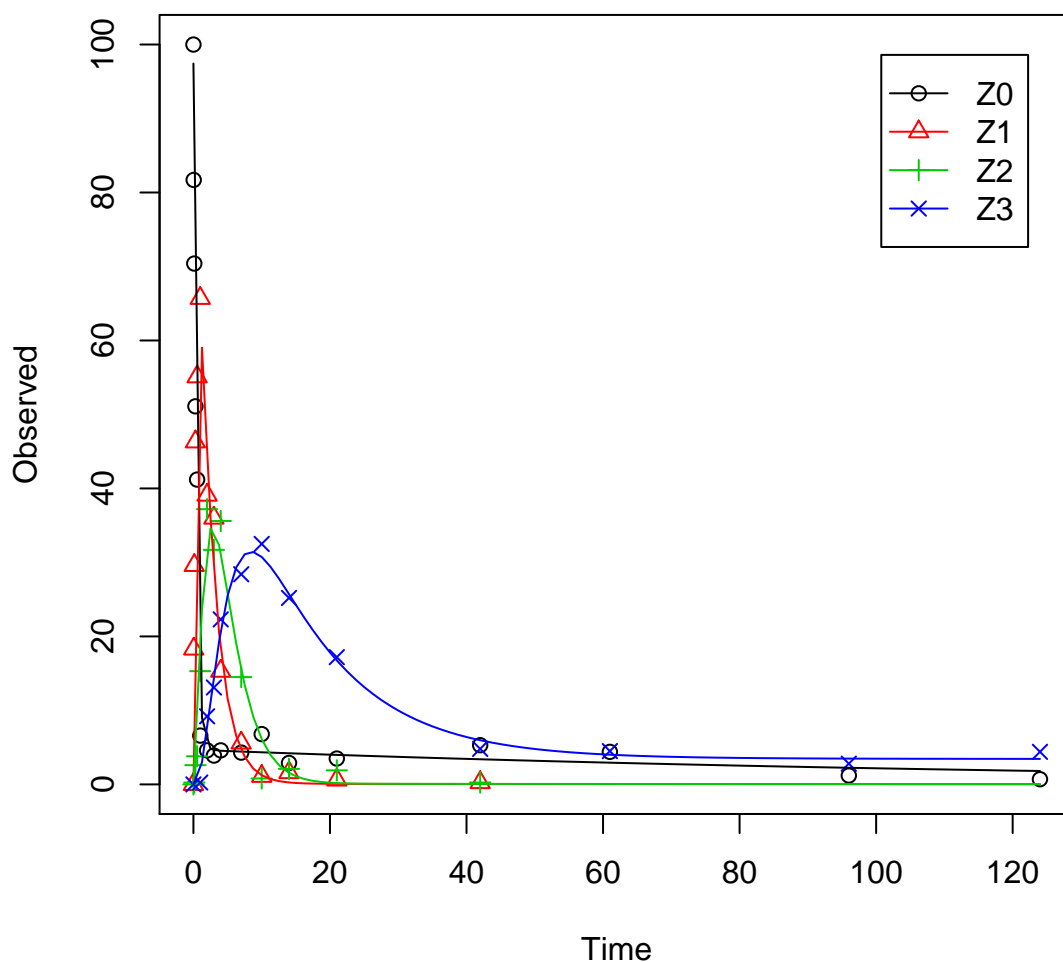
Z0_b1	Z0_b2	Z3_b1	Z3_b2
2.447e+00	7.658e-03	7.983e-02	1.363e-06

Parameter correlation:

	Z0_free_0	k_Z1_Z2	k_Z0_free_bound	k_Z0_bound_free	k_Z0_free_Z1
Z0_free_0	1.00000	0.24874	0.079292	0.073662	0.09072
k_Z1_Z2	0.24874	1.00000	-0.223885	-0.082769	-0.10368

k_Z0_free_bound	0.07929	-0.22389	1.000000	0.542876	0.42696
k_Z0_bound_free	0.07366	-0.08277	0.542876	1.000000	0.16540
k_Z0_free_Z1	0.09072	-0.10368	0.426958	0.165399	1.00000
k_Z2_sink	0.31619	0.36741	-0.287122	-0.168222	-0.08266
k_Z2_Z3_free	-0.03185	-0.05441	0.008287	0.013478	-0.01908
k_Z3_free_sink	-0.12037	-0.22367	0.070584	0.024242	-0.01842
k_Z3_free_bound	0.01629	0.01906	0.009670	0.031837	0.03607
k_Z3_bound_free	0.03781	0.08868	-0.055136	-0.008975	-0.06574
	k_Z2_sink	k_Z2_Z3_free	k_Z3_free_sink	k_Z3_free_bound	
Z0_free_0	0.31619	-0.031847	-0.12037	0.01629	
k_Z1_Z2	0.36741	-0.054405	-0.22367	0.01906	
k_Z0_free_bound	-0.28712	0.008287	0.07058	0.00967	
k_Z0_bound_free	-0.16822	0.013478	0.02424	0.03184	
k_Z0_free_Z1	-0.08266	-0.019083	-0.01842	0.03607	
k_Z2_sink	1.00000	-0.069361	-0.66596	0.02603	
k_Z2_Z3_free	-0.06936	1.000000	-0.26493	0.73477	
k_Z3_free_sink	-0.66596	-0.264933	1.00000	-0.36064	
k_Z3_free_bound	0.02603	0.734774	-0.36064	1.00000	
k_Z3_bound_free	0.23931	0.774434	-0.71123	0.81143	
	k_Z3_bound_free				
Z0_free_0	0.037814				
k_Z1_Z2	0.088677				
k_Z0_free_bound	-0.055136				
k_Z0_bound_free	-0.008975				
k_Z0_free_Z1	-0.065741				
k_Z2_sink	0.239308				
k_Z2_Z3_free	0.774434				
k_Z3_free_sink	-0.711226				
k_Z3_free_bound	0.811433				
k_Z3_bound_free	1.000000				

R> plot(m.Z.mkin.5)



References

- FOCUS Work Group on Degradation Kinetics. *Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. Report of the FOCUS Work Group on Degradation Kinetics*, 2006. URL <http://focus.jrc.ec.europa.eu/dk>. EC Document Reference Sanco/10058/2005 version 2.0.
- 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>.

Johannes Ranke. *kinfit: Routines for fitting simple kinetic models to chemical degradation data*, 2012. URL <http://CRAN.R-project.org>.