Example evaluation of FOCUS dataset Z

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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)
```

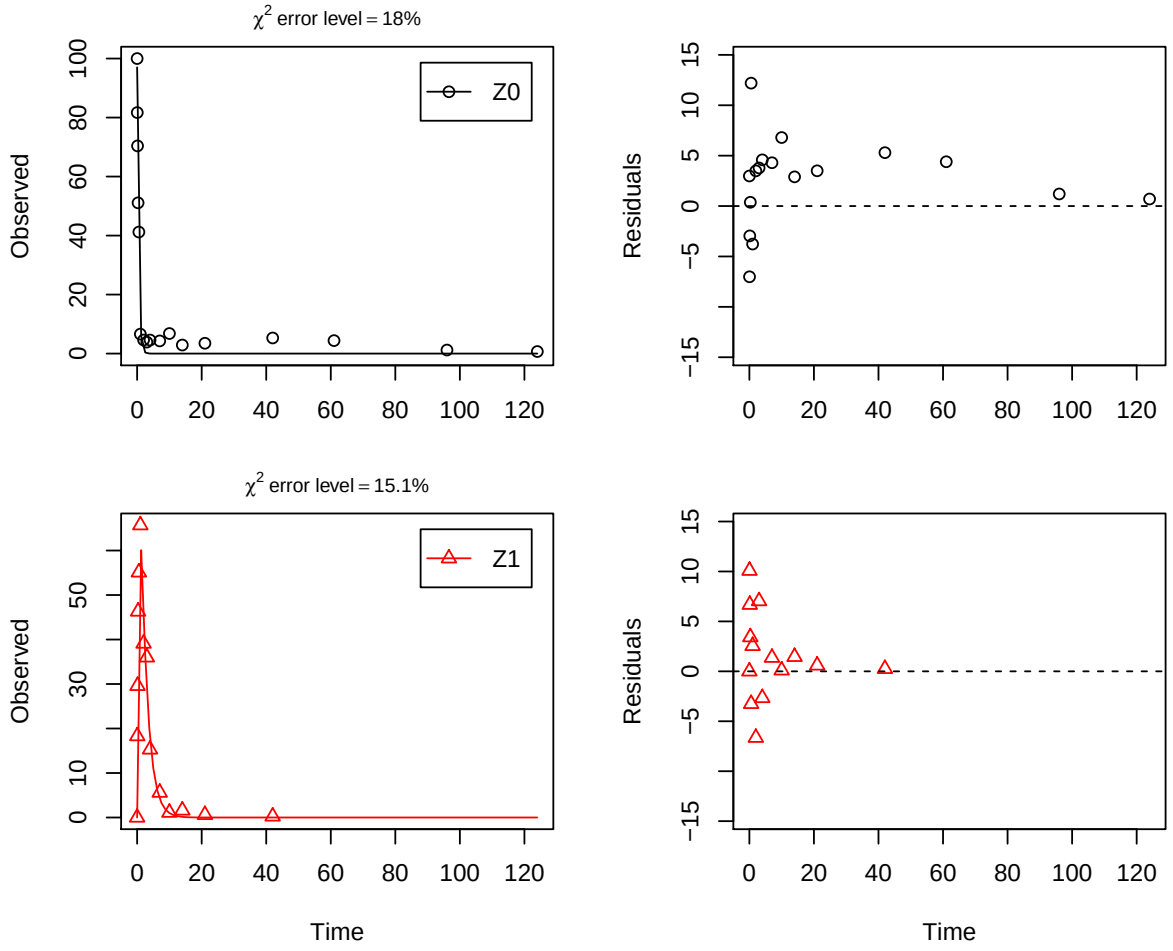
2 Parent compound and one metabolite

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).

```
Z.2a <- mkinmod(Z0 = mkinsub("SFO", "Z1"),
                Z1 = mkinsub("SFO"))
```

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

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




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

```
##               Estimate se_notrans      t value       Pr(>t)
## Z0_0      9.701488e+01 3.55313531 2.730402e+01 1.679194e-21
## k_Z0_sink 6.213452e-10 0.22689429 2.738479e-09 5.000000e-01
## k_Z0_Z1   2.236006e+00 0.16507349 1.354552e+01 7.393893e-14
## k_Z1_sink 4.821248e-01 0.06585366 7.321154e+00 3.551981e-08
```

```
##                Lower       Upper
## Z0_0      91.4013833 102.6283792
## k_Z0_sink  0.0000000         Inf
## k_Z0_Z1    1.8374087   2.7210739
## k_Z1_sink  0.4005976   0.5802439
```

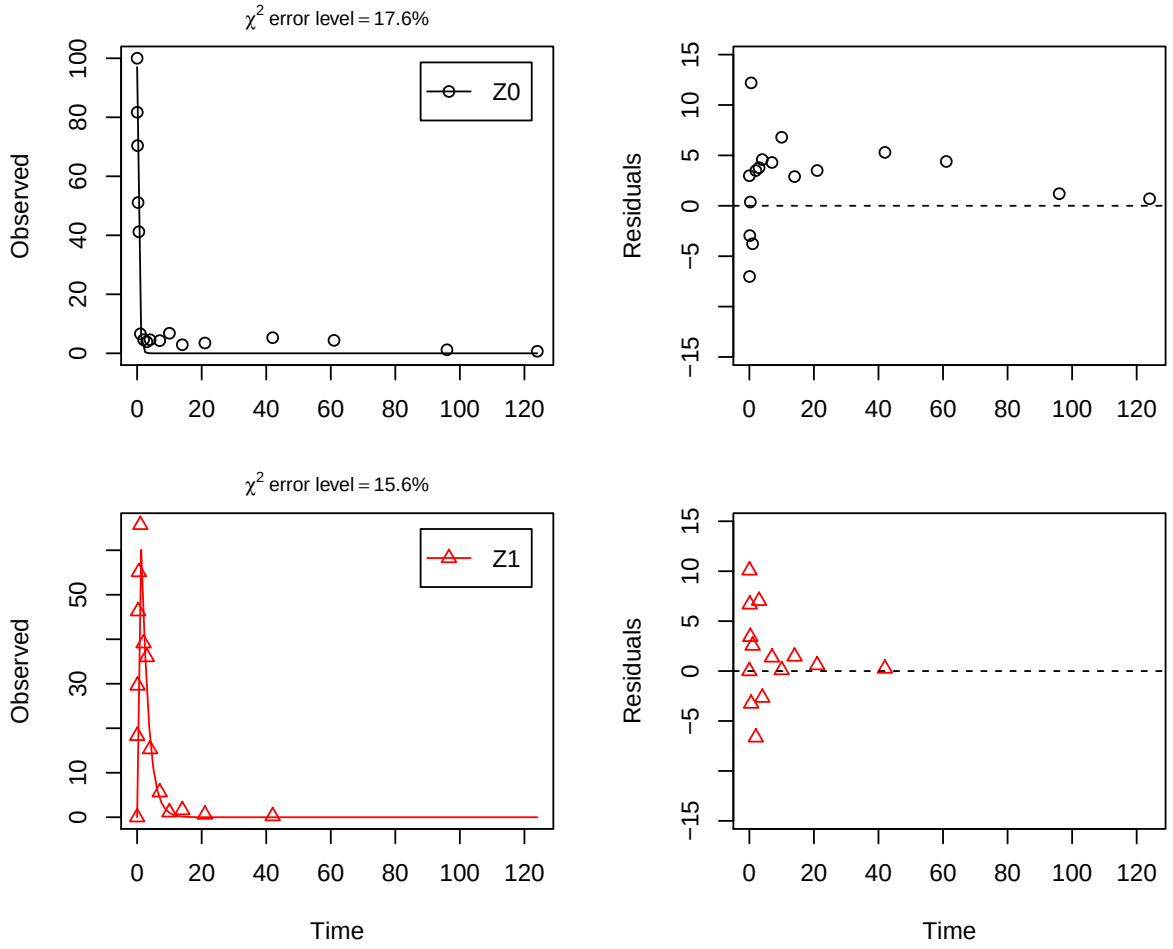
As obvious from the parameter summary (the `bpar` component of the summary), the kinetic rate constant from parent compound Z to sink is negligible. Accordingly, the exact magnitude of the fitted parameter `log k_Z0_sink` is ill-defined and the covariance matrix is not returned (not shown, would be visible in the complete summary). 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 = mkinsub("SFO", "Z1"),
                   Z1 = mkinsub("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_sep(m.Z.2a.ff)
```




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

```
##              Estimate se_notrans   t value       Pr(>t) Lower Upper
## Z0_0       97.0148813 3.55314638 27.303936 1.679331e-21    NA    NA
## k_Z0        2.2360064 0.21684717 10.311439 3.661734e-11    NA    NA
## k_Z1        0.4821248 0.06585372  7.321147 3.552046e-08    NA    NA
## f_Z0_to_Z1  1.0000000 0.10147344  9.854795 9.707117e-11    NA    NA
```

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`.[1]

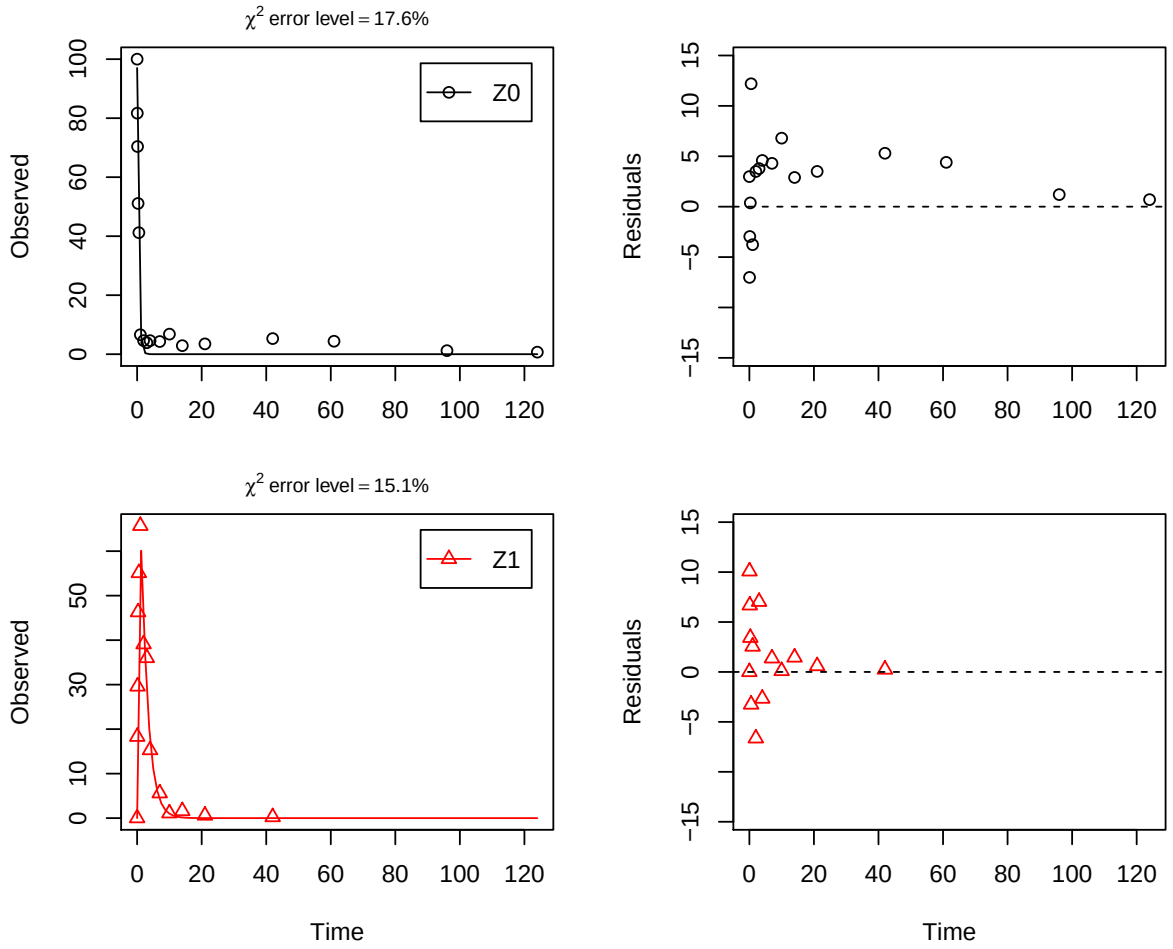
[1] If the model formulation without formation fractions is used, the same effect can be obtained by fixing the parameter `k_Z_sink` to a value of zero.

In the following, we use the parameterisation with formation fractions in order to be able to compare with the results in the FOCUS guidance, and as it makes it easier to use parameters obtained in a previous fit when adding a further metabolite.

```
Z.3 <- mkinmod(Z0 = mkinsub("SFO", "Z1", sink = FALSE),
               Z1 = mkinsub("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_sep(m.Z.3)
```




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

```
##        Estimate se_notrans  t value       Pr(>t)      Lower
## Z0_0 97.0148816 2.68177104 36.17568 2.363590e-25 91.5215232
```

```
## k_Z0  2.2360064 0.14686238 15.22518 2.247007e-15  1.9545318
## k_Z1  0.4821248 0.04268711 11.29439 3.068559e-12  0.4021552
##            Upper
## Z0_0 102.5082401
## k_Z0   2.5580166
## k_Z1   0.5779966
```

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

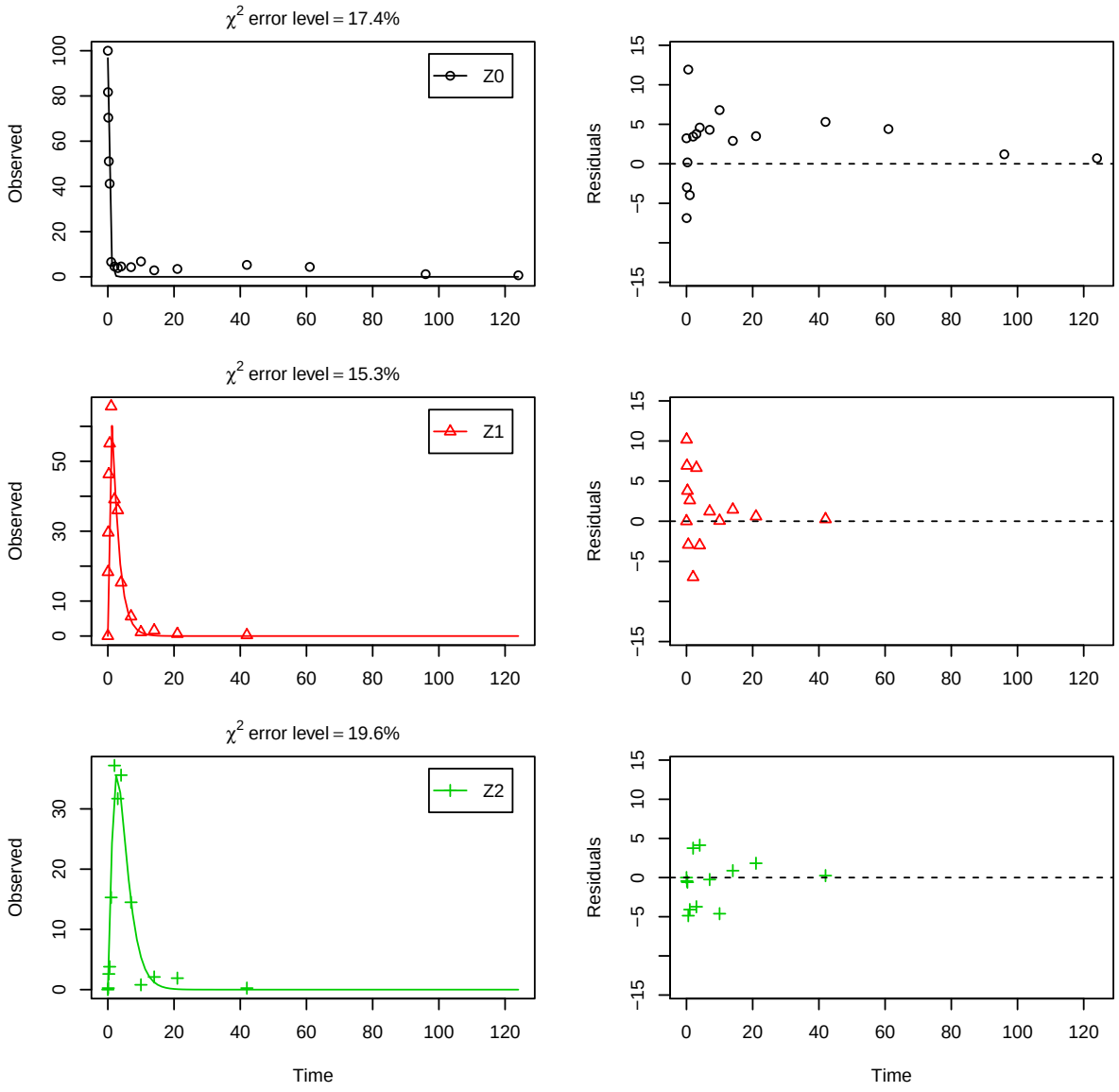
3 Including metabolites Z2 and Z3

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 = mkinsub("SFO", "Z1", sink = FALSE),
               Z1 = mkinsub("SFO", "Z2", sink = FALSE),
               Z2 = mkinsub("SFO"), use_of_ff = "max")
```

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

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



Finally, metabolite Z3 is added to the model. We use the optimised differential equation parameter values from the previous fit in order to accelerate the optimization.

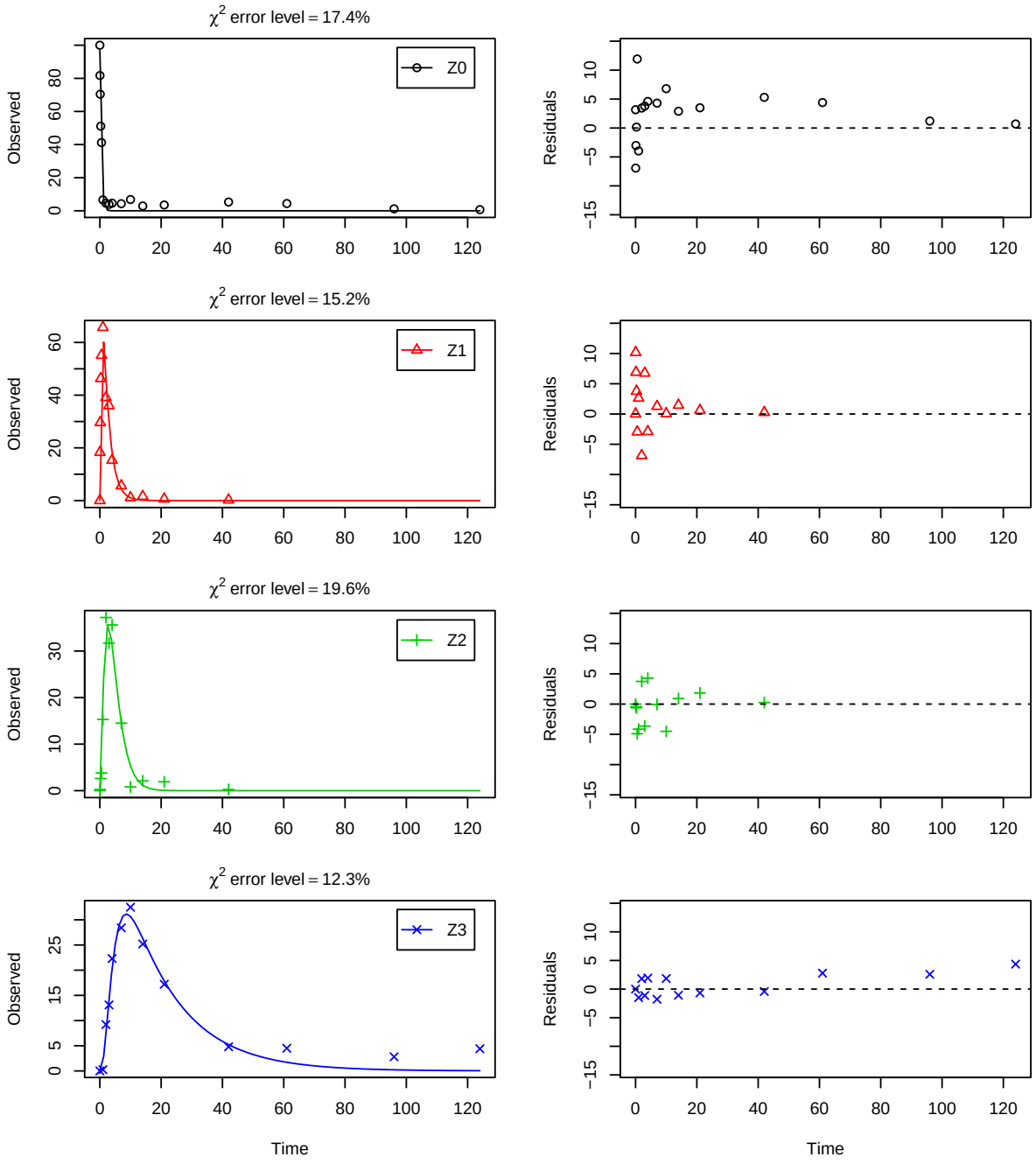
```
Z.FOCUS <- mkinmod(Z0 = mkinsub("SFO", "Z1", sink = FALSE),
                   Z1 = mkinsub("SFO", "Z2", sink = FALSE),
                   Z2 = mkinsub("SFO", "Z3"),
                   Z3 = mkinsub("SFO"),
                   use_of_ff = "max")

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

```
m.Z.FOCUS <- mkinfit(Z.FOCUS, FOCUS_2006_Z_mkin,
                     parms.ini = m.Z.5$bparms.ode,
                     quiet = TRUE)
```

```
## Warning in mkinfit(Z.FOCUS, FOCUS_2006_Z_mkin, parms.ini = m.Z.5$bparms.ode,
:  Optimisation by method Port did not converge.
## Convergence code is 1
```

```
plot_sep(m.Z.FOCUS)
```




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

```
##              Estimate se_notrans   t value       Pr(>t)       Lower
## Z0_0      96.84024413 2.05881366 47.036916 5.572278e-44 92.70685169
## k_Z0       2.21540096 0.11812759 18.754306 7.736886e-25  1.99050408
## k_Z1       0.47835870 0.02929353 16.329843 3.344317e-22  0.42303496
```

```
## k_Z2        0.45166296 0.04418624 10.221801 3.036447e-14  0.37106537
## k_Z3        0.05868971 0.01428961  4.107158 7.256030e-05  0.03598292
## f_Z2_to_Z3  0.47147387 0.05702672  8.267596 2.779011e-11  0.36029541
##                   Upper
## Z0_0       100.97363656
## k_Z0         2.46570780
## k_Z1         0.54091758
## k_Z2         0.54976682
## k_Z3         0.09572548
## f_Z2_to_Z3   0.58555627
```

```
endpoints(m.Z.FOCUS)
```

```
## $ff
##     Z2_Z3   Z2_sink
## 0.4714739 0.5285261
##
## $SFORB
## logical(0)
##
## $distimes
##          DT50      DT90
## Z0  0.3128766  1.039354
## Z1  1.4490113  4.813512
## Z2  1.5346558  5.098016
## Z3 11.8103701 39.233200
```

This fit corresponds to the final result chosen in Appendix 7 of the FOCUS report. Confidence intervals returned by mkin are based on internally transformed parameters, however.

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.

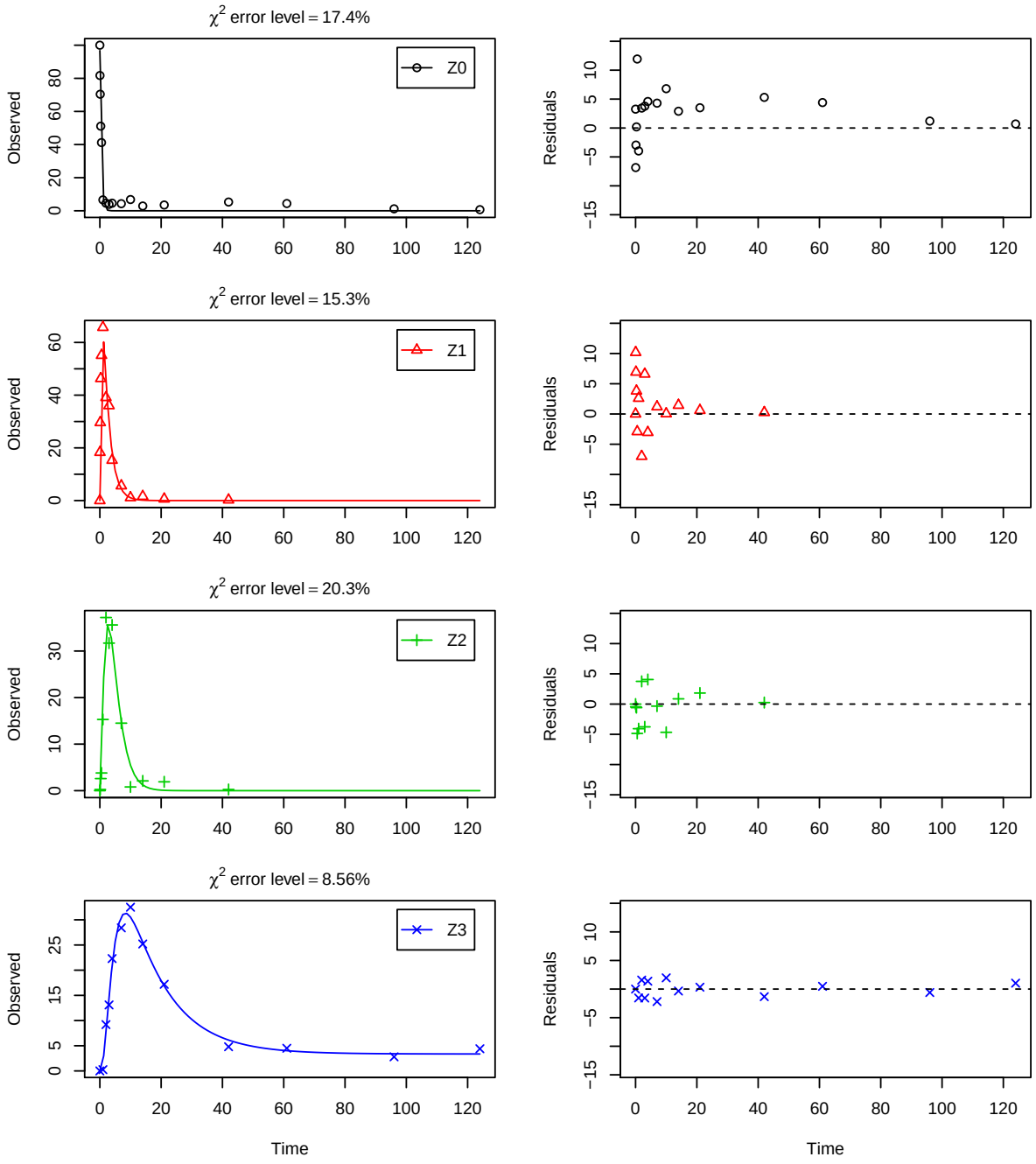
Therefore, an additional model is offered here, using the single first-order reversible binding (SFORB) model for metabolite Z3. As expected, the χ^2 error level is lower for metabolite Z3 using this model and the graphical fit for Z3 is improved. However, the covariance matrix is not returned.

```
Z.mkin.1 <- mkinmod(Z0 = mkinsub("SFO", "Z1", sink = FALSE),
                    Z1 = mkinsub("SFO", "Z2", sink = FALSE),
```

```
                    Z2 = mkinsub("SFO", "Z3"),
                    Z3 = mkinsub("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_sep(m.Z.mkin.1)
```




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

## NULL
```

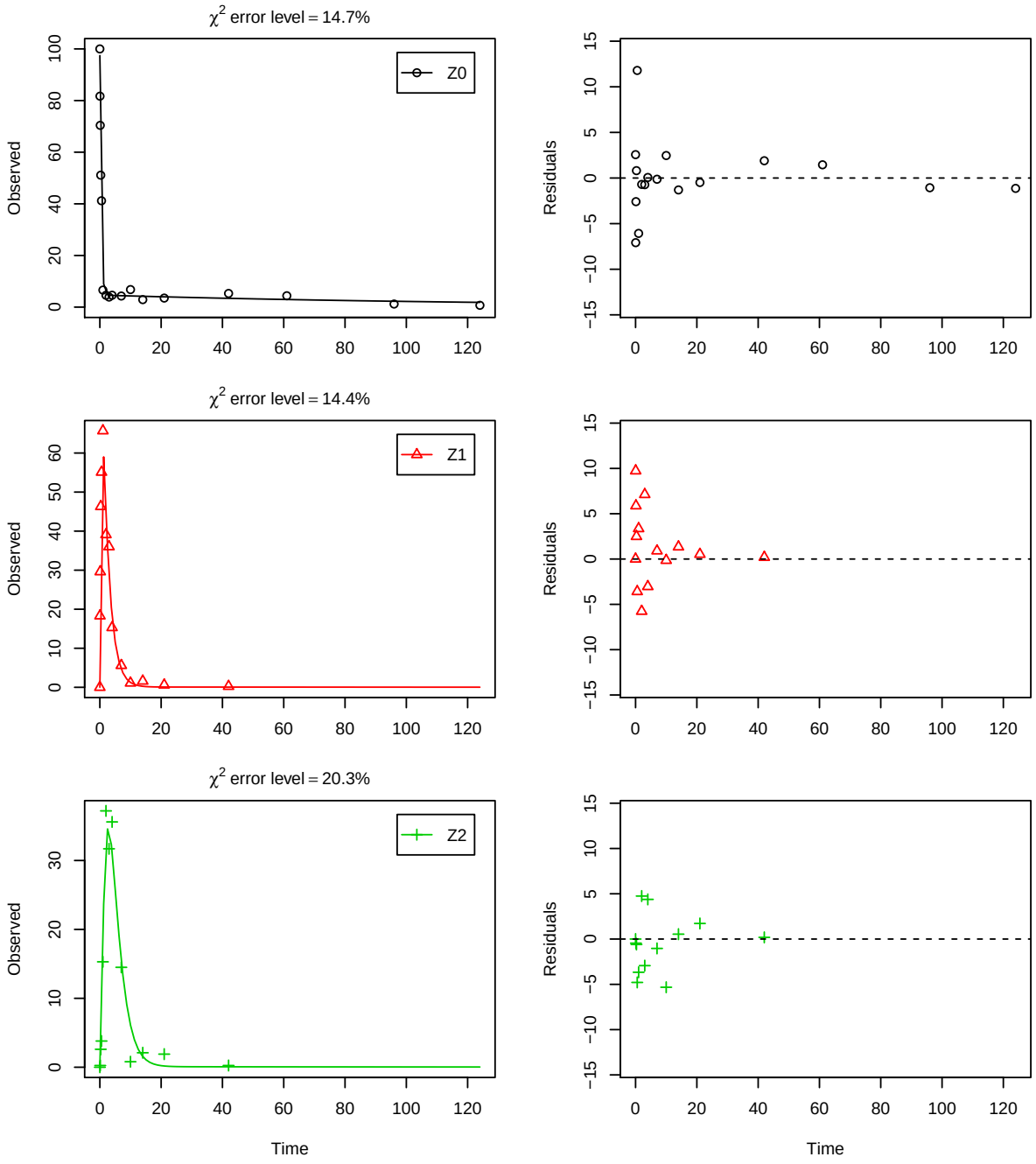
Therefore, a further stepwise model building is performed starting from the stage of parent and two metabolites, starting from the assumption that the model fit for the parent

compound can be improved by using the SFORB model.

```
Z.mkin.3 <- mkinmod(Z0 = mkinsub("SFORB", "Z1", sink = FALSE),
                    Z1 = mkinsub("SFO", "Z2", sink = FALSE),
                    Z2 = mkinsub("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_sep(m.Z.mkin.3)
```



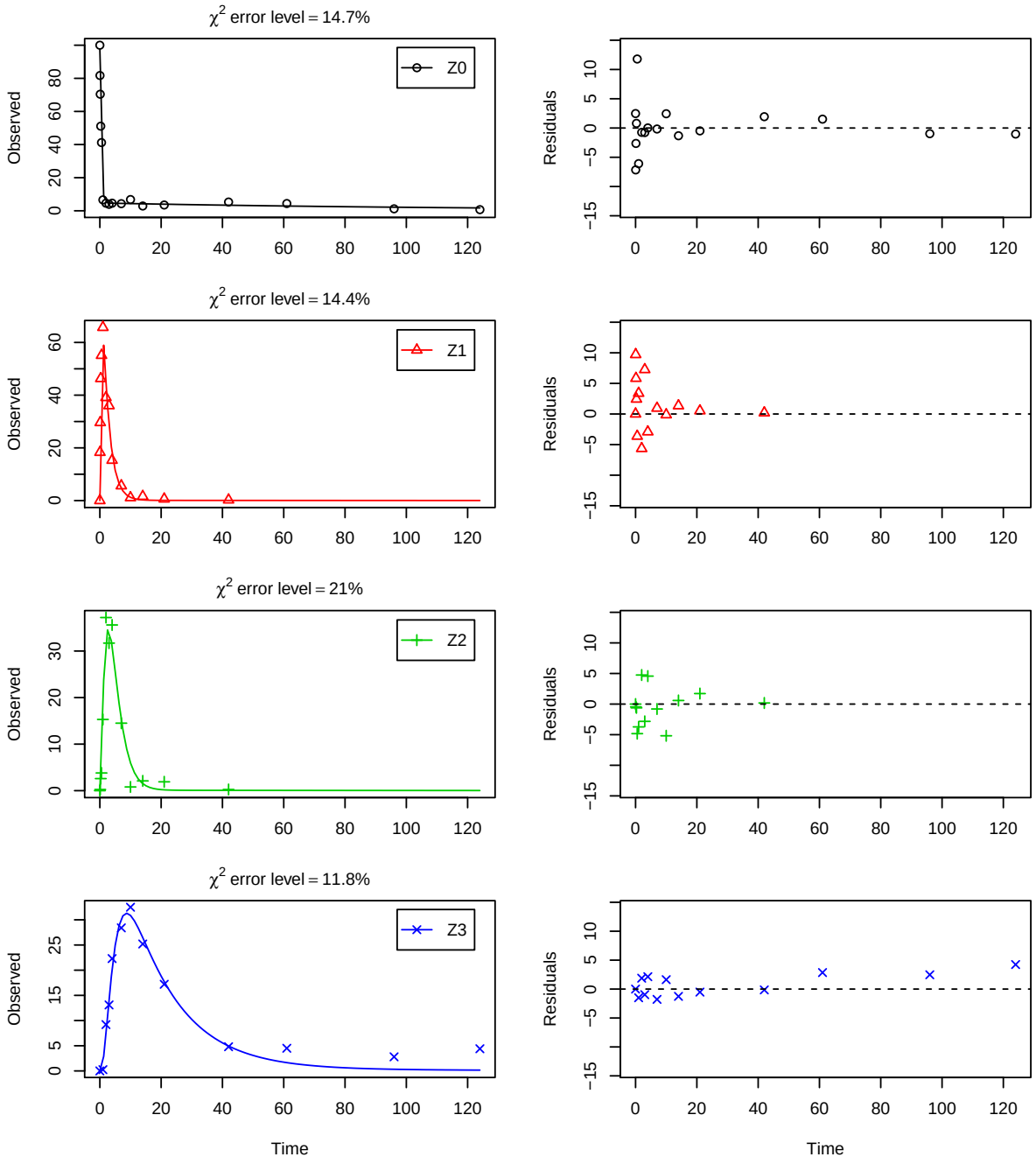
This results in a much better representation of the behaviour of the parent compound Z0.

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 = mkinsub("SFORB", "Z1", sink = FALSE),
                    Z1 = mkinsub("SFO", "Z2", sink = FALSE),
                    Z2 = mkinsub("SFO", "Z3"),
                    Z3 = mkinsub("SFO"))
```

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

```
m.Z.mkin.4 <- mkinfit(Z.mkin.4, FOCUS_2006_Z_mkin,
                      parms.ini = m.Z.mkin.3$bparms.ode,
                      quiet = TRUE)
plot_sep(m.Z.mkin.4)
```



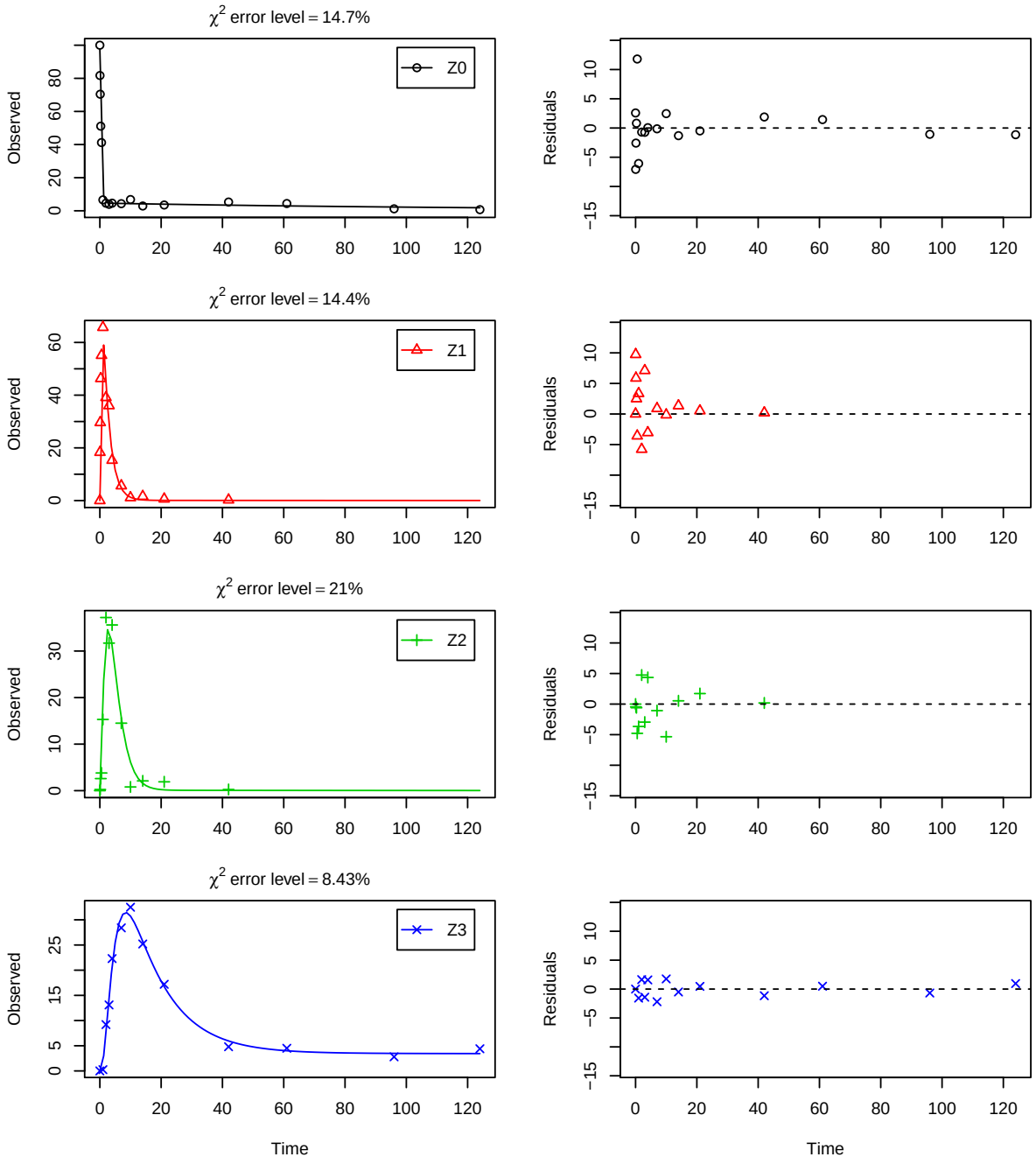
The error level of the fit, but especially of metabolite Z3, can be improved if the SFORB model is chosen for this metabolite, as this model is capable of representing the tailing of the metabolite decline phase.

```
Z.mkin.5 <- mkinmod(Z0 = mkinsub("SFORB", "Z1", sink = FALSE),
                    Z1 = mkinsub("SFO", "Z2", sink = FALSE),
```

```
                    Z2 = mkinsub("SFO", "Z3"),
                    Z3 = mkinsub("SFORB"))
```

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

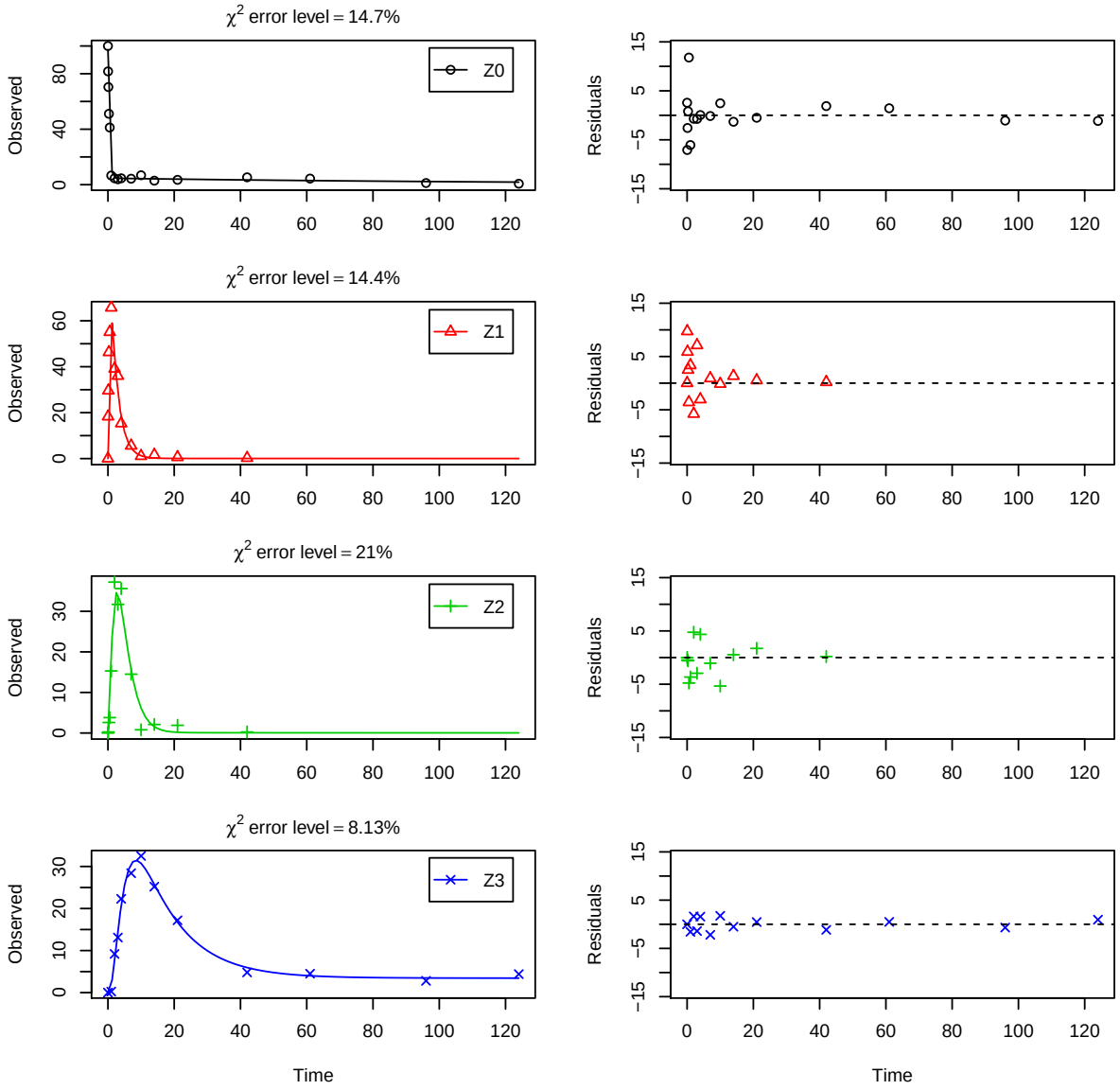
```
m.Z.mkin.5 <- mkinfit(Z.mkin.5, FOCUS_2006_Z_mkin,
                      parms.ini = m.Z.mkin.4$bparms.ode[1:4],
                      quiet = TRUE)
plot_sep(m.Z.mkin.5)
```



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(m.Z.mkin.5$bparms.ode[1:7],
```

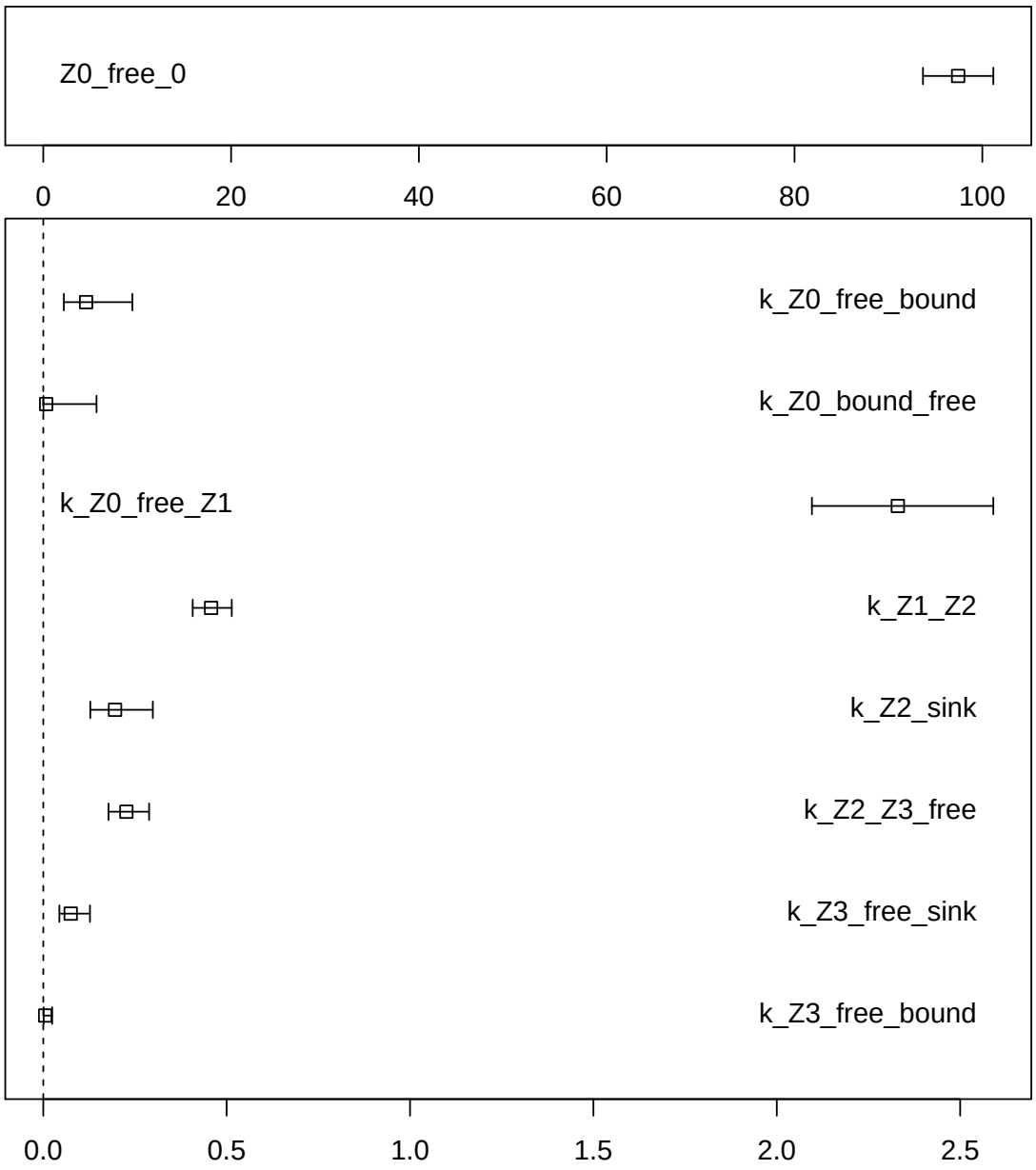
```
                                    k_Z3_bound_free = 0),
                        fixed_parms = "k_Z3_bound_free",
                        quiet = TRUE)
plot_sep(m.Z.mkin.5a)
```



As expected, the residual plots for Z0 and Z3 are more random than in the case of the all SFO model for which they were shown above. In conclusion, the model `Z.mkin.5a` is proposed as the best-fit model for the dataset from Appendix 7 of the FOCUS report.

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    0.4634423    0.5365577    1.0000000
##
## $SFORB
```

```
##       Z0_b1       Z0_b2       Z3_b1       Z3_b2
## 2.447137325 0.007512576 0.080007563 0.000000000
##
## $distimes
##         DT50     DT90 DT50_Z0_b1 DT50_Z0_b2 DT50_Z3_b1 DT50_Z3_b2
## Z0 0.3042974 1.184810  0.2832482   92.26492         NA         NA
## Z1 1.5147780 5.031984         NA         NA         NA         NA
## Z2 1.6413852 5.452564         NA         NA         NA         NA
## Z3        NA       NA         NA         NA   8.663521        Inf
```

It is clear the degradation rate of Z3 towards the end of the experiment is very low as DT50_Z3_b2 (the second Eigenvalue of the system of two differential equations representing the SFORB system for Z3, corresponding to the slower rate constant of the DFOP model) is reported to be infinity. However, this appears to be a feature of the data.

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://esdac.jrc.ec.europa.eu/projects/degradation-kinetics.