Progress in regulatory degradation kinetics

SETAC Europe 24th Annual Meeting

PD Dr. Johannes Ranke
Scientific consultant

Basel, 13 May 2014

How do you define progress?

Outline

Outline

Outline

Outline

The tasks of regulatory degradation kinetics

- Derive endpoints for fate modelling

The tasks of regulatory degradation kinetics

- Derive endpoints for fate modelling
- Provide endpoints for comparison with trigger values

The tasks of regulatory degradation kinetics

- Derive endpoints for fate modelling
- Provide endpoints for comparison with trigger values
  - Triggers for further data requirements
    (EU pesticides: FOCUS kinetics “persistence endpoints”)

The tasks of regulatory degradation kinetics

- Derive endpoints for fate modelling
- Provide endpoints for comparison with trigger values
  - Triggers for further data requirements
    (EU pesticides: FOCUS kinetics "persistence endpoints")
  - Triggers for persistence, P and vP
    (Regulation 1107/2009, REACH)

The tasks of regulatory degradation kinetics

- Derive endpoints for fate modelling
- Provide endpoints for comparison with trigger values
  - Triggers for further data requirements
    (EU pesticides: FOCUS kinetics "persistence endpoints")
  - Triggers for persistence, P and vP
    (Regulation 1107/2009, REACH)
- Reflect endpoint uncertainty

Motivation to improve regulatory degradation kinetics

Provide the best possible foundation

- Transparency

Motivation to improve regulatory degradation kinetics

Provide the best possible foundation

- Transparency
- Scientific quality

Motivation to improve regulatory degradation kinetics

Provide the best possible foundation

- Transparency
- Scientific quality
- Technical quality

Motivation to improve regulatory degradation kinetics

Provide the best possible foundation

- Transparency
- Scientific quality
- Technical quality
- Collaboration

Motivation to improve regulatory degradation kinetics

Provide the best possible foundation

- Transparency ✓
- Scientific quality
- Technical quality
- Collaboration

www.r-project.org

Motivation to improve regulatory degradation kinetics

Provide the best possible foundation

- Transparency ✓
- Scientific quality ?
- Technical quality
- Collaboration

Version: 1.0
Date: 23 November 2011

Generic guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration

www.r-project.org - focus.jrc.ec.europa.eu/dk

Motivation to improve regulatory degradation kinetics

Provide the best possible foundation

- Transparency ✓
- Scientific quality ?
- Technical quality
- Collaboration

Version: 1.0
Date: 23 November 2011

```
Model cost at call  161 :  242.2306
Model cost at call  165 :  242.2306
Model cost at call  171 :  242.2306
Model cost at call  175 :  242.2306
 done successfully.
```

Generic guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration

```
Executing test function test.SFO_solution_types  ...  done successfully.

------------------- UNIT TEST SUMMARY ---------------------

RUNIT TEST PROTOCOL -- Fri May  9 16:30:24 2014
***********************************************
Number of test functions: 8
Number of errors: 0
Number of failures: 0


1 Test Suite :
mkin  Unit Tests - 8 test functions, 0 errors, 0 failures
```

www.r-project.org - focus.jrc.ec.europa.eu/dk
`R CMD check mkin_0.9-27.tar.gz`

Motivation to improve regulatory degradation kinetics

Provide the best possible foundation

- Transparency ✓
- Scientific quality ?
- Technical quality
- Collaboration !

Version: 1.0
Date: 23 November 2011

```
Model cost at call  161 :  242.2306
Model cost at call  165 :  242.2306
Model cost at call  171 :  242.2306
Model cost at call  175 :  242.2306
 done successfully.
```

Generic guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration

```
Executing test function test.SFO_solution_types  ...  done successfully.

------------------- UNIT TEST SUMMARY ---------------------

RUNIT TEST PROTOCOL -- Fri May  9 16:30:24 2014
***********************************************
Number of test functions: 8
Number of errors: 0
Number of failures: 0


1 Test Suite :
mkin  Unit Tests - 8 test functions, 0 errors, 0 failures
```

www.r-project.org - focus.jrc.ec.europa.eu/dk
R CMD check mkin_0.9-27.tar.gz - **github.com/jranke/mkin**

Critical areas

- t-test for parameter significance (assumes normal distribution for estimator)
- Confidence intervals for fitted parameters
- Modelling biphasic behaviour of metabolites

Elements of success

mkin was first published in May 2010, including biphasic models for metabolites since May 18. It was then used to develop

- KinGUII (Bayer Crop Science)
- CAKE (Syngenta)

by adding a graphical user interface (GUI), iteratively reweighted least squares (IRLS) and Markov Chain Monte Carlo (MCMC)

Elements of success

mkin was first published in May 2010, including biphasic models for metabolites since May 18. It was then used to develop

- KinGUII (Bayer Crop Science)
- CAKE (Syngenta)

by adding a graphical user interface (GUI), iteratively reweighted least squares (IRLS) and Markov Chain Monte Carlo (MCMC)

Isometric logratio transformation (ILR) for fitting formation fractions together with René Lehmann (UBA) in 2012

Elements of success

mkin was first published in May 2010, including biphasic models for metabolites since May 18. It was then used to develop

- KinGUII (Bayer Crop Science)
- CAKE (Syngenta)

by adding a graphical user interface (GUI), iteratively reweighted least squares (IRLS) and Markov Chain Monte Carlo (MCMC)

Isometric logratio transformation (ILR) for fitting formation fractions together with René Lehmann (UBA) in 2012

Parameter confidence intervals based on transformed parameters (2013)

Elements of success

mkin was first published in May 2010, including biphasic models for metabolites since May 18. It was then used to develop

- KinGUII (Bayer Crop Science)
- CAKE (Syngenta)

by adding a graphical user interface (GUI), iteratively reweighted least squares (IRLS) and Markov Chain Monte Carlo (MCMC)

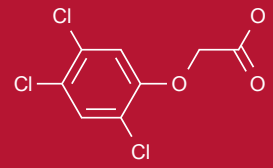
Isometric logratio transformation (ILR) for fitting formation fractions together with René Lehmann (UBA) in 2012

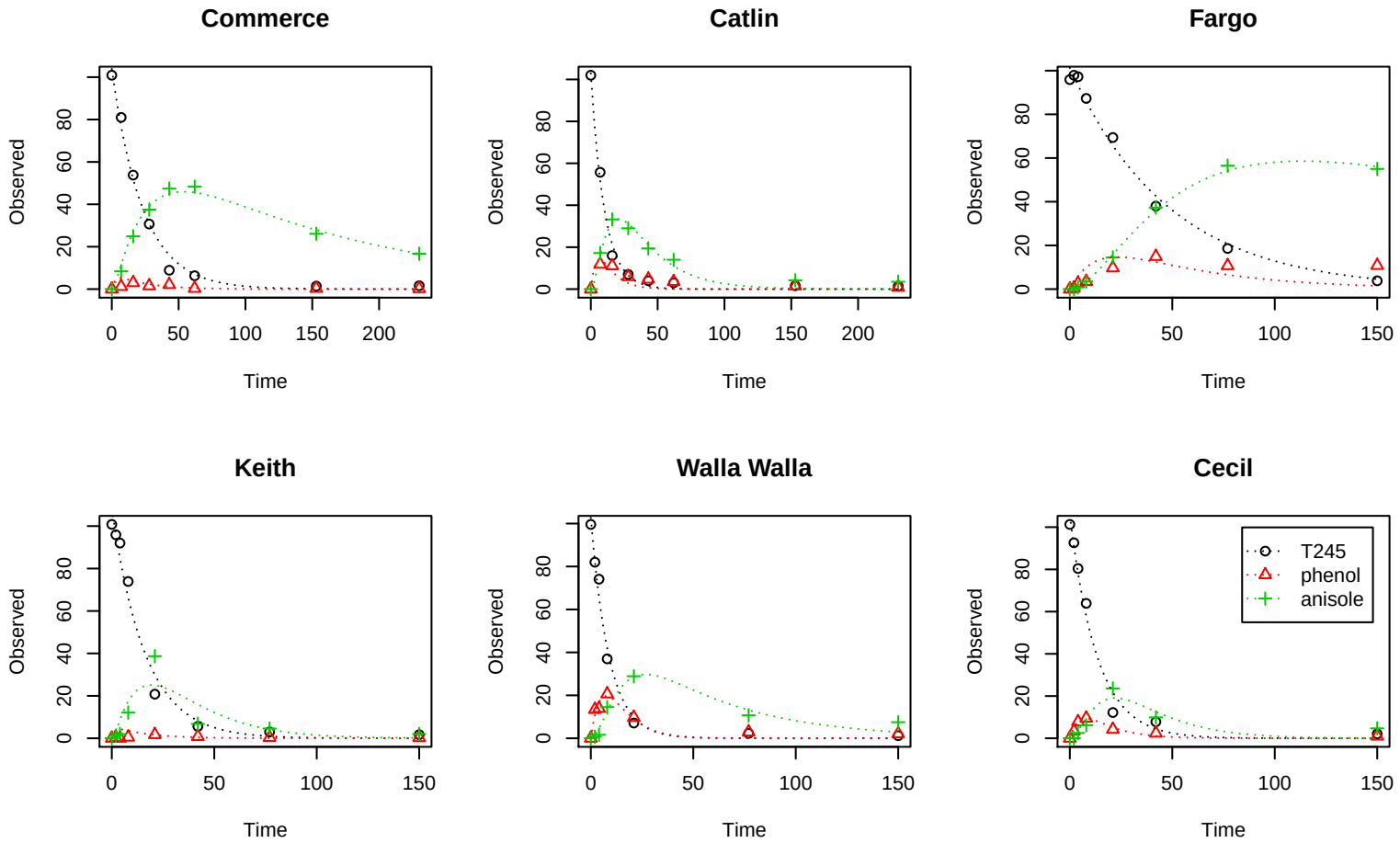
Parameter confidence intervals based on transformed parameters (2013)

mkin ($\geq$ 0.9-27) allows for fitting models with or without formation fractions, with or without parameter transformations.

Ranke and Lehmann, SETAC World 20-24 May 2012, Berlin
mkin 0.9-27 published on CRAN 10 May 2014

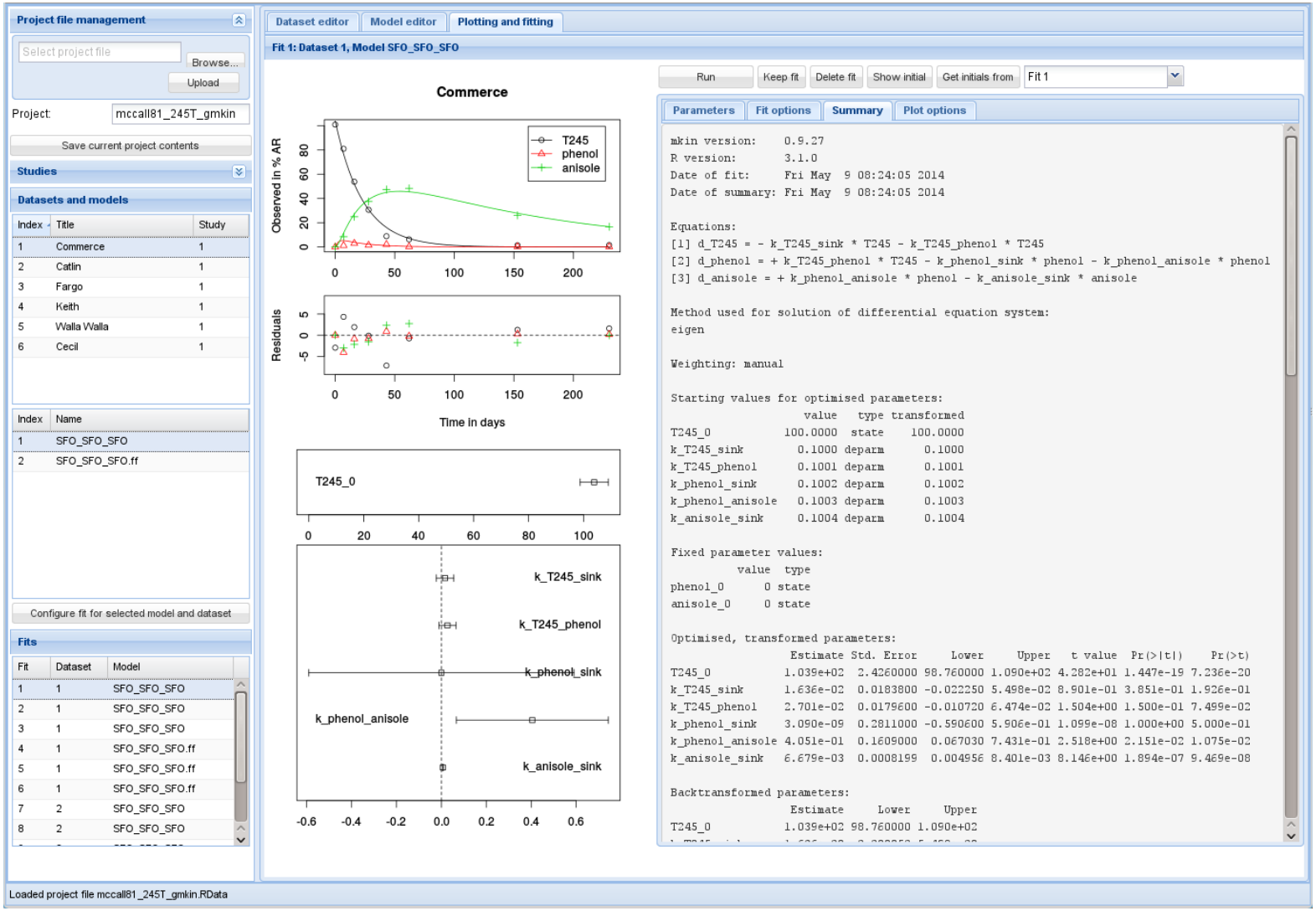
Soil metabolism of 2,4,5-T in the lab





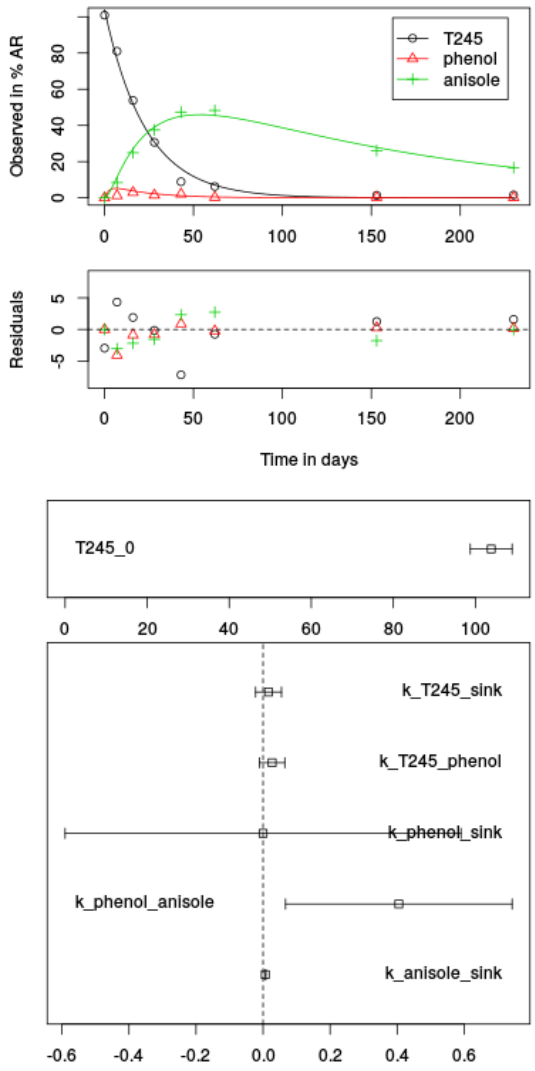
McCall *et al.* (1981) *J Agric Food Chem* **29** 100-107

2,4,5-T in Commerce soil in gmkin



gmkin user interface

Pathway from 2,4,5-T-phenol to sink?

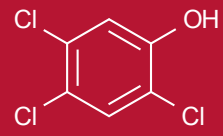


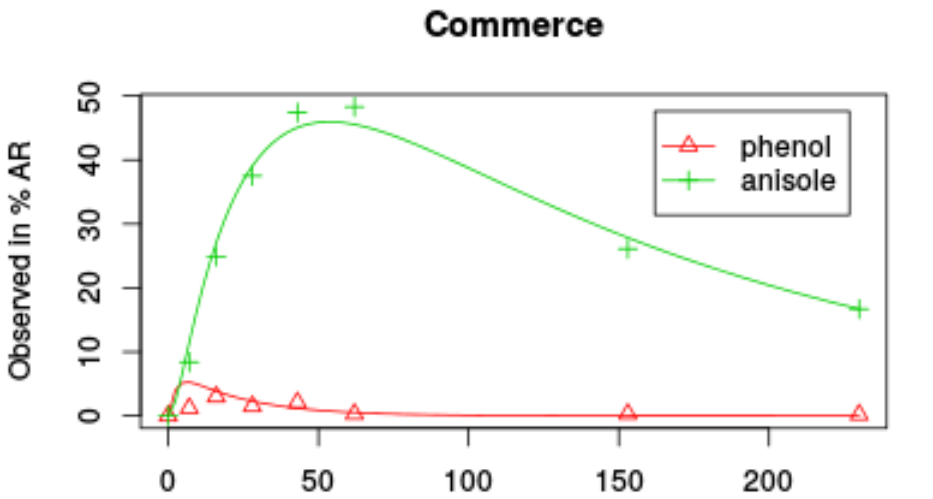
Parameters | Fit options | Summary | Plot options

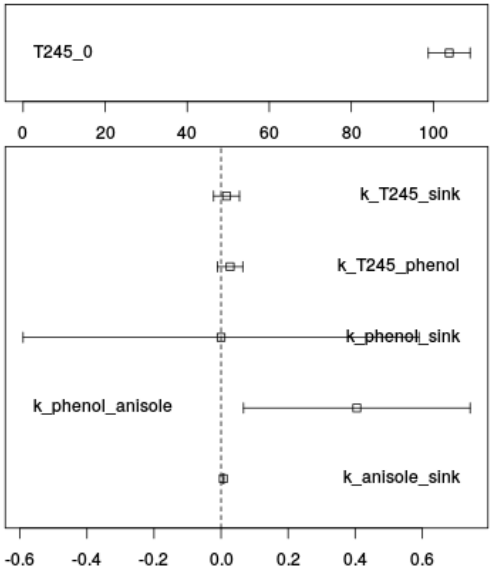
```
mkin version:    0.9.27
R version:       3.1.0
Date of fit:     Fri May  9 08:24:05 2014
Date of summary: Fri May  9 08:24:05 2014

Equations:
[1] d_T245 = - k_T245_sink * T245 - k_T245_phenol * T245
[2] d_phenol = + k_T245_phenol * T245 - k_phenol_sink * phenol - k_phenol_anisole * phenol
[3] d_anisole = + k_phenol_anisole * phenol - k_anisole_sink * anisole

Method used for solution of differential equation system:
eigen

Weighting: manual

Starting values for optimised parameters:
                 value   type transformed
T245_0           100.0  state     100.0000
k_T245_sink        0.1 deparm       0.1000
k_T245_phenol      0.1 deparm       0.1001
k_phenol_sink      0.1 deparm       0.1002
k_phenol_anisole   0.1 deparm       0.1003
k_anisole_sink     0.1 deparm       0.1004

Fixed parameter values:
          value  type
phenol_0      0 state
anisole_0     0 state

Optimised, transformed parameters:
                 Estimate Std. Error     Lower     Upper  t value  Pr(>|t|)    Pr(>t)
T245_0           1.039e+02  2.4260000 98.760000 1.090e+02 4.282e+01 1.447e-19 7.236e-20
k_T245_sink      1.636e-02  0.0183800 -0.022250 5.498e-02 8.901e-01 3.851e-01 1.926e-01
k_T245_phenol    2.701e-02  0.0179600 -0.010720 6.474e-02 1.504e+00 1.500e-01 7.499e-02
k_phenol_sink    3.090e-09  0.2811000 -0.590600 5.906e-01 1.099e-08 1.000e+00 5.000e-01
k_phenol_anisole 4.051e-01  0.1609000  0.067030 7.431e-01 2.518e+00 2.151e-02 1.075e-02
k_anisole_sink   6.679e-03  0.0008199  0.004956 8.401e-03 8.146e+00 1.894e-07 9.469e-08

Backtransformed parameters:
                 Estimate     Lower     Upper
T245_0           1.039e+02 98.760000 1.090e+02
```

`k_phenol_sink` negligible - fix to zero

Pathway from 2,4,5-T-phenol to sink?





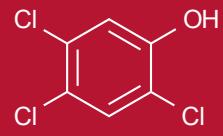


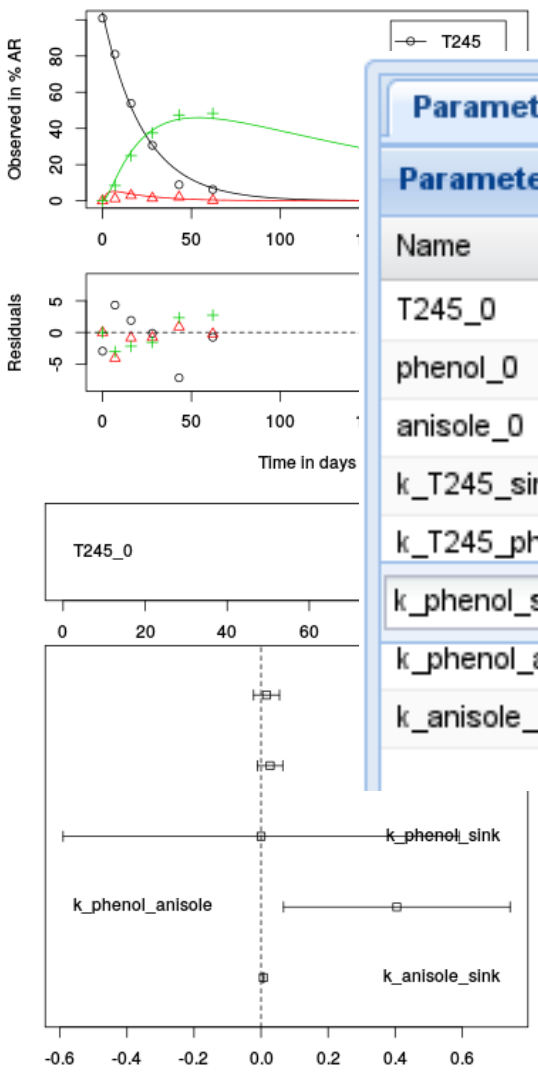
Plot options

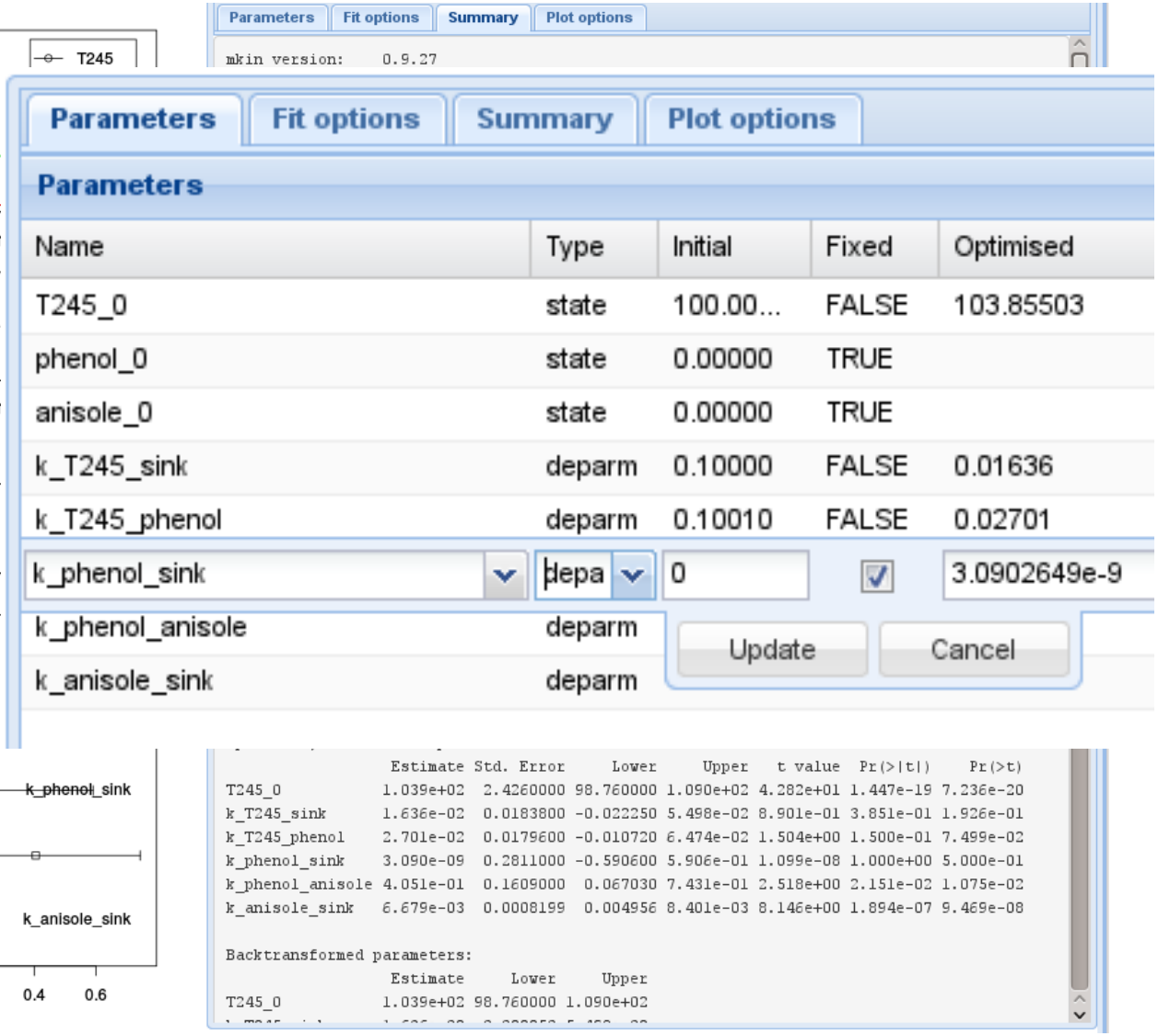
```
:24:05 2014
:24:05 2014

245 - k_T245_phenol * T245
 * T245 - k_phenol_sink * phenol - k_phenol_anisole * phenol
sole * phenol - k_anisole_sink * anisole

fferential equation system:

arameters:
e transformed
                100.0000
k_T245_sink       0.1000 deparm      0.1000
k_T245_phenol     0.1001 deparm      0.1001
k_phenol_sink     0.1002 deparm      0.1002
k_phenol_anisole  0.1003 deparm      0.1003
k_anisole_sink    0.1004 deparm      0.1004

Fixed parameter values:
          value  type
phenol_0      0 state
anisole_0     0 state

Optimised, transformed parameters:
                 Estimate Std. Error     Lower     Upper   t value  Pr(>|t|)     Pr(>t)
T245_0           1.039e+02  2.4260000 98.760000 1.090e+02 4.282e+01 1.447e-19 7.236e-20
k_T245_sink      1.636e-02  0.0183800 -0.022250 5.498e-02 8.901e-01 3.851e-01 1.926e-01
k_T245_phenol    2.701e-02  0.0179600 -0.010720 6.474e-02 1.504e+00 1.500e-01 7.499e-02
k_phenol_sink    3.090e-09  0.2811000 -0.590600 5.906e-01 1.099e-08 1.000e+00 5.000e-01
k_phenol_anisole 4.051e-01  0.1609000  0.067030 7.431e-01 2.518e+00 2.151e-02 1.075e-02
k_anisole_sink   6.679e-03  0.0008199  0.004956 8.401e-03 8.146e+00 1.894e-07 9.469e-08

Backtransformed parameters:
                 Estimate     Lower     Upper
T245_0           1.039e+02 98.760000 1.090e+02
```

`k_phenol_sink` negligible - fix to zero

Pathway from 2,4,5-T-phenol to sink?

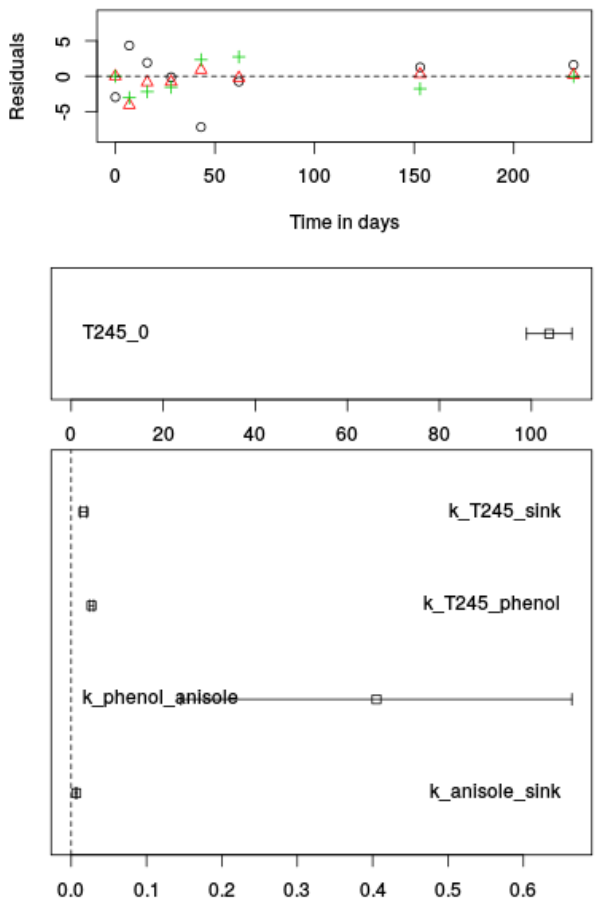






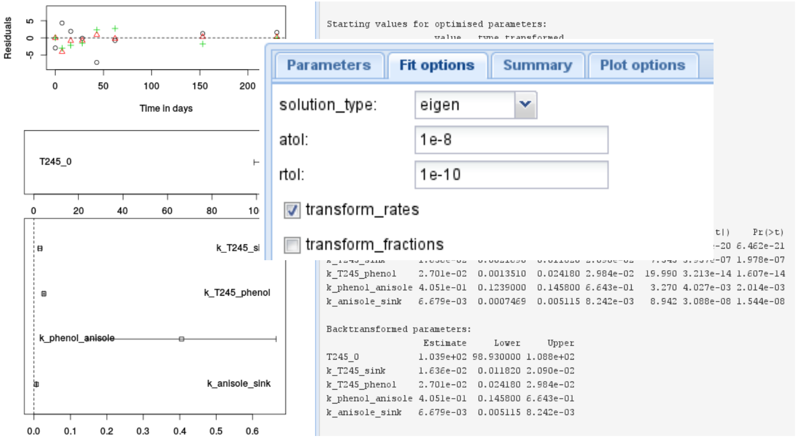
`k_phenol_sink` negligible - fix to zero

2,4,5-T in Commerce soil, no path to sink

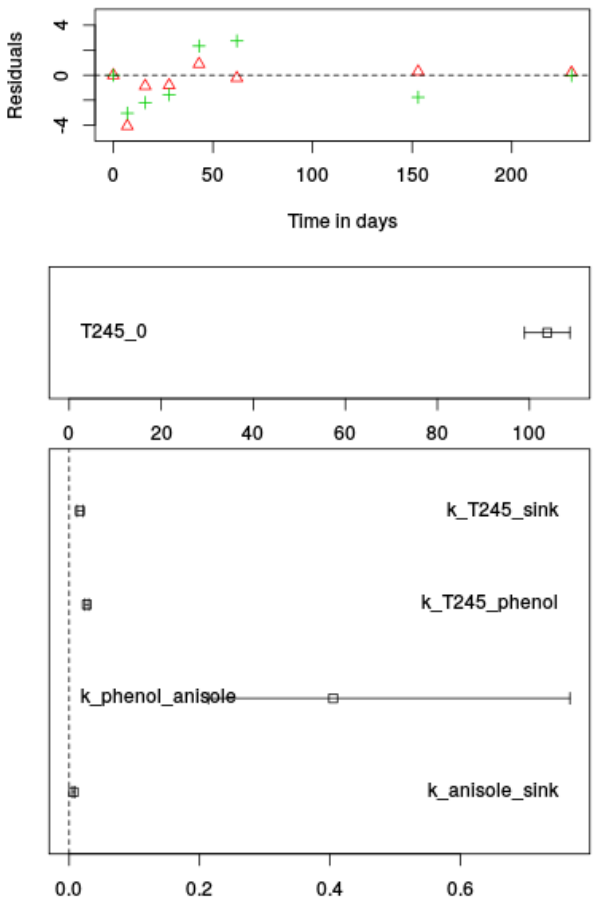



```
Starting values for optimised parameters:
                  value   type transformed
T245_0           100.0000  state    100.0000
k_T245_sink        0.1000 deparm      0.1000
k_T245_phenol      0.1001 deparm      0.1001
k_phenol_anisole   0.1003 deparm      0.1003
k_anisole_sink     0.1004 deparm      0.1004

Fixed parameter values:
            value   type
phenol_0        0  state
anisole_0       0  state
k_phenol_sink   0 deparm

Optimised, transformed parameters:
                 Estimate Std. Error     Lower     Upper t value  Pr(>|t|)     Pr(>t)
T245_0           1.039e+02  2.3520000 98.930000 1.088e+02  44.160 1.292e-20 6.462e-21
k_T245_sink      1.636e-02  0.0021690  0.011820 2.090e-02   7.545 3.957e-07 1.978e-07
k_T245_phenol    2.701e-02  0.0013510  0.024180 2.984e-02  19.990 3.213e-14 1.607e-14
k_phenol_anisole 4.051e-01  0.1239000  0.145800 6.643e-01   3.270 4.027e-03 2.014e-03
k_anisole_sink   6.679e-03  0.0007469  0.005115 8.242e-03   8.942 3.088e-08 1.544e-08

Backtransformed parameters:
                 Estimate     Lower     Upper
T245_0           1.039e+02 98.930000 1.088e+02
k_T245_sink      1.636e-02  0.011820 2.090e-02
k_T245_phenol    2.701e-02  0.024180 2.984e-02
k_phenol_anisole 4.051e-01  0.145800 6.643e-01
k_anisole_sink   6.679e-03  0.005115 8.242e-03
```

2,4,5-T in Commerce soil, no path to sink



Rate parameters log transformed during fit




```
Starting values for optimised parameters:
                   value   type transformed
T245_0           100.0000  state  100.000000
k_T245_sink        0.1000 deparm   -2.302585
k_T245_phenol      0.1001 deparm   -2.301586
k_phenol_anisole   0.1003 deparm   -2.299590
k_anisole_sink     0.1004 deparm   -2.298593

Fixed parameter values:
             value   type
phenol_0         0  state
anisole_0        0  state
k_phenol_sink    0 deparm

Optimised, transformed parameters:
                Estimate Std. Error  Lower    Upper t value  Pr(>|t|)    Pr(>t)
T245_0           103.9000    2.35200 98.930 108.8000   44.160 1.292e-20 6.462e-21
k_T245_sink       -4.1130    0.13250 -4.390  -3.8350 -31.030 9.646e-18 4.823e-18
k_T245_phenol     -3.6120    0.05002 -3.716  -3.5070 -72.200 1.199e-24 5.997e-25
k_phenol_anisole  -0.9037    0.30580 -1.544  -0.2637  -2.955 8.127e-03 4.063e-03
k_anisole_sink    -5.0090    0.11180 -5.243  -4.7750 -44.790 9.901e-21 4.950e-21

Backtransformed parameters:
                 Estimate     Lower     Upper
T245_0           1.039e+02 98.930000 108.80000
k_T245_sink      1.636e-02  0.012400   0.02159
k_T245_phenol    2.701e-02  0.024320   0.02999
k_phenol_anisole 4.051e-01  0.213600   0.76820
k_anisole_sink   6.679e-03  0.005285   0.00844
```

Proposal regarding t-test

Instead of testing rate constants for significant difference from zero:

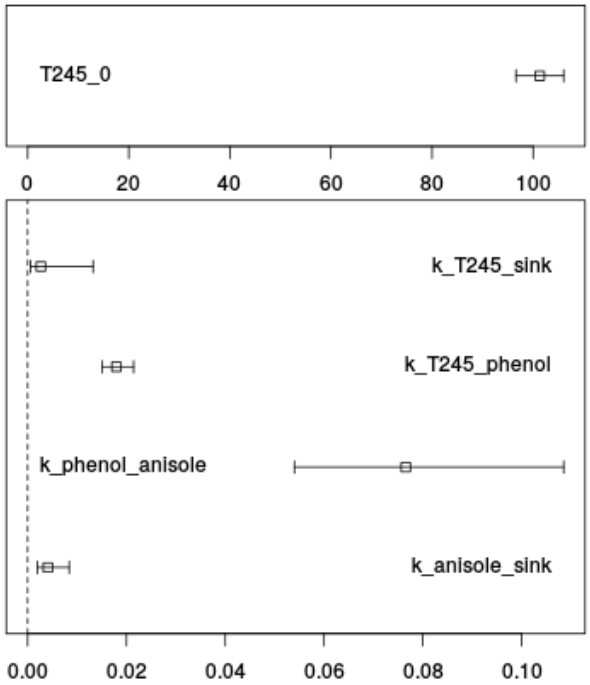
- Use best available estimate

Proposal regarding t-test

Instead of testing rate constants for significant difference from zero:

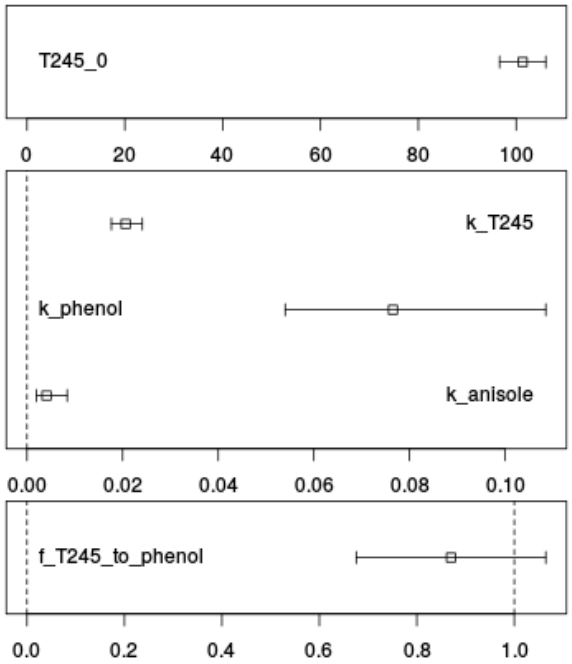
- Use best available estimate
- Consider if it is neglibly small

2,4,5-T in Fargo soil




```
Fixed parameter values:
              value   type
phenol_0          0  state
anisole_0         0  state
k_phenol_sink     0 deparm

Optimised, transformed parameters:
                Estimate Std. Error  Lower   Upper t value  Pr(>|t|)    Pr(>t)
T245_0           101.300    2.25600 96.610 106.100  44.910 9.396e-21 4.698e-21
k_T245_sink       -5.922    0.76330 -7.519  -4.324  -7.758 2.638e-07 1.319e-07
k_T245_phenol     -4.020    0.08497 -4.198  -3.842 -47.310 3.530e-21 1.765e-21
k_phenol_anisole  -2.570    0.16670 -2.919  -2.221 -15.420 3.394e-12 1.697e-12
k_anisole_sink    -5.499    0.34400 -6.219  -4.779 -15.980 1.790e-12 8.948e-13

Backtransformed parameters:
                 Estimate     Lower     Upper
T245_0           1.013e+02 9.661e+01 1.061e+02
k_T245_sink      2.681e-03 5.425e-04 1.325e-02
k_T245_phenol    1.796e-02 1.503e-02 2.145e-02
k_phenol_anisole 7.654e-02 5.399e-02 1.085e-01
k_anisole_sink   4.090e-03 1.991e-03 8.404e-03
```

2,4,5-T in Fargo soil
Model with formation fractions



Fixed parameter values:

	value	type
phenol_0	0	state
anisole_0	0	state
f_phenol_to_anisole	1	deparm

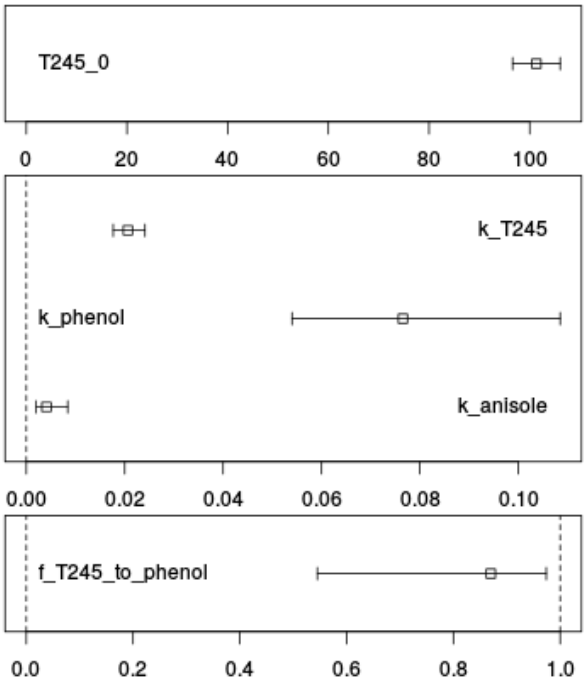
Optimised, transformed parameters:

	Estimate	Std. Error	Lower	Upper	t value	Pr(>|t|)	Pr(>t)
T245_0	101.3000	2.25600	96.6100	106.100	44.910	9.396e-21	4.698e-21
k_T245	-3.8810	0.07455	-4.0370	-3.725	-52.050	5.828e-22	2.914e-22
f_T245_to_phenol	0.8701	0.09293	0.6756	1.065	9.363	1.502e-08	7.511e-09
k_phenol	-2.5700	0.16670	-2.9190	-2.221	-15.420	3.394e-12	1.697e-12
k_anisole	-5.4990	0.34400	-6.2190	-4.779	-15.980	1.789e-12	8.947e-13

Backtransformed parameters:

	Estimate	Lower	Upper
T245_0	101.30000	96.610000	1.061e+02
k_T245	0.02064	0.017660	2.412e-02
f_T245_to_phenol	0.87010	0.675600	1.065e+00
k_phenol	0.07654	0.053990	1.085e-01
k_anisole	0.00409	0.001991	8.404e-03

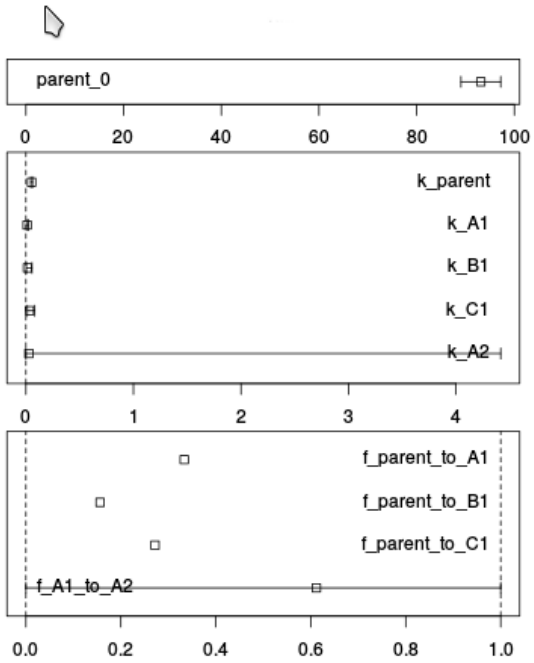
2,4,5-T in Fargo soil
Model with transformed formation fractions




```
Fixed parameter values:
                    value    type
phenol_0                0   state
anisole_0               0   state
f_phenol_to_anisole     1 deparm

Optimised, transformed parameters:
                Estimate Std. Error  Lower   Upper t value  Pr(>|t|)     Pr(>t)
T245_0           101.300    2.25600 96.610 106.100  44.910 9.396e-21 4.698e-21
k_T245            -3.881    0.07455 -4.037  -3.725 -52.050 5.828e-22 2.914e-22
k_phenol          -2.570    0.16670 -2.919  -2.221 -15.420 3.395e-12 1.697e-12
k_anisole         -5.499    0.34400 -6.219  -4.779 -15.980 1.790e-12 8.949e-13
f_T245_to_phenol   1.345    0.58140  0.128   2.562   2.313 3.207e-02 1.604e-02

Backtransformed parameters:
                Estimate     Lower     Upper
T245_0          101.30000 96.610000 1.061e+02
k_T245            0.02064  0.017660 2.412e-02
k_phenol          0.07654  0.053990 1.085e-01
k_anisole         0.00409  0.001991 8.404e-03
f_T245_to_phenol  0.87010  0.545100 9.740e-01
```

We get a plausible confidence interval without doing an MCMC simulation

Parallel formation of metabolites



Optimised, transformed parameters:

	Estimate	Std. Error	Lower	Upper	t value	Pr(>|t|)	Pr(>t)
parent_0	93.14000	1.9660	89.0700	97.2000	47.3800	1.945e-24	9.727e-25
k_parent	-2.94600	0.0671	-3.0850	-2.8070	-43.9000	1.101e-23	5.505e-24
f_parent_to_A1	0.53640	0.2495	0.0202	1.0530	2.1500	4.234e-02	2.117e-02
f_parent_to_B1	-0.14300	0.2581	-0.6768	0.3909	-0.5540	5.850e-01	2.925e-01
f_parent_to_C1	0.01254	0.5360	-1.0960	1.1210	0.0234	9.815e-01	4.908e-01
k_A1	-4.48100	0.3412	-5.1870	-3.7750	-13.1300	3.579e-12	1.790e-12
f_A1_to_A2	0.32170	3.1700	-6.2370	6.8800	0.1015	9.201e-01	4.600e-01
k_B1	-4.27600	0.6800	-5.6830	-2.8690	-6.2880	2.043e-06	1.021e-06
k_C1	-3.40200	0.4373	-4.3060	-2.4970	-7.7780	6.929e-08	3.464e-08
k_A2	-3.58800	2.4520	-8.6610	1.4850	-1.4630	1.570e-01	7.849e-02

Backtransformed parameters:

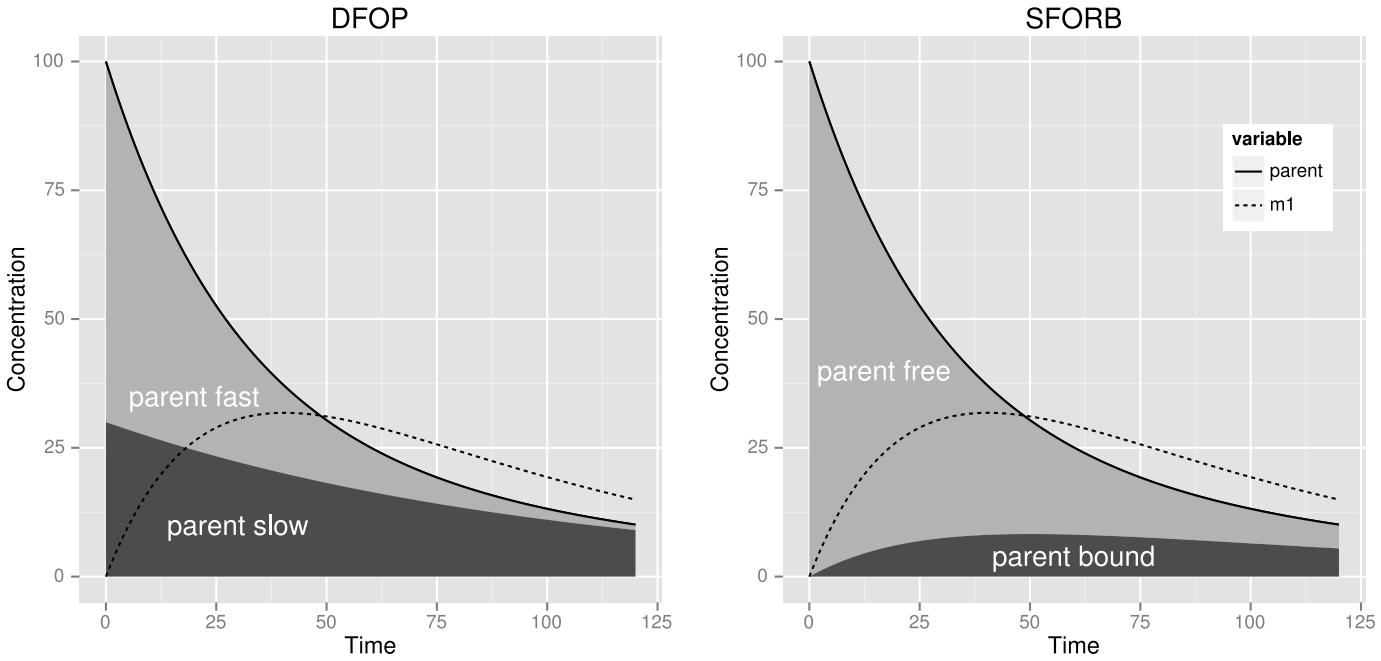
	Estimate	Lower	Upper
parent_0	93.14000	8.907e+01	97.20000
k_parent	0.05256	4.575e-02	0.06038
f_parent_to_A1	0.33350	NA	NA
f_parent_to_B1	0.15620	NA	NA
f_parent_to_C1	0.27190	NA	NA
k_A1	0.01132	5.591e-03	0.02294
f_A1_to_A2	0.61180	1.478e-04	0.99990
k_B1	0.01389	3.403e-03	0.05673
k_C1	0.03332	1.348e-02	0.08234
k_A2	0.02765	1.732e-04	4.41500

Confidence intervals only for single formation fractions
(slide corrected after the meeting)

Data from Schäfer *et al.* (2007) Proc. XIII Symposium Pesticide Chemistry, Piacenza, 2007, p. 916-923

Conceptual comparison of DFOP and SFORB

Both possible for metabolites, but with two extra parameters.



Alternative with one extra parameter

We could also use an Indeterminate Order Rate Equation (IORE) for metabolites

$$\frac{dm}{dt} = \ldots - k_m m^n \ldots$$

This is used in North America for parent compounds as an equivalent alternative to the FOMC model, with the possibility to test if n is different from unity.

NAFTA Technical Working Group on Pesticides, Guidance for Evaluating and Calculating Degradation Kinetics in Environmental Media (7 p)

Challenges for the future

- Better collaboration

Challenges for the future

- Better collaboration
- Improve error model

Challenges for the future

- Better collaboration
- Improve error model
- Evaluate related datasets in one step (mixed effect models)

Challenges for the future

- Better collaboration
- Improve error model
- Evaluate related datasets in one step (mixed effect models)
- Improve model comparisons (ANOVA, AIC)

Conclusions

- With gmkin + mkin, we now have a completely open sourced software toolset

Conclusions

- With gmkin + mkin, we now have a completely open sourced software toolset
- Biphasic models for metabolites can conveniently be fitted

Conclusions

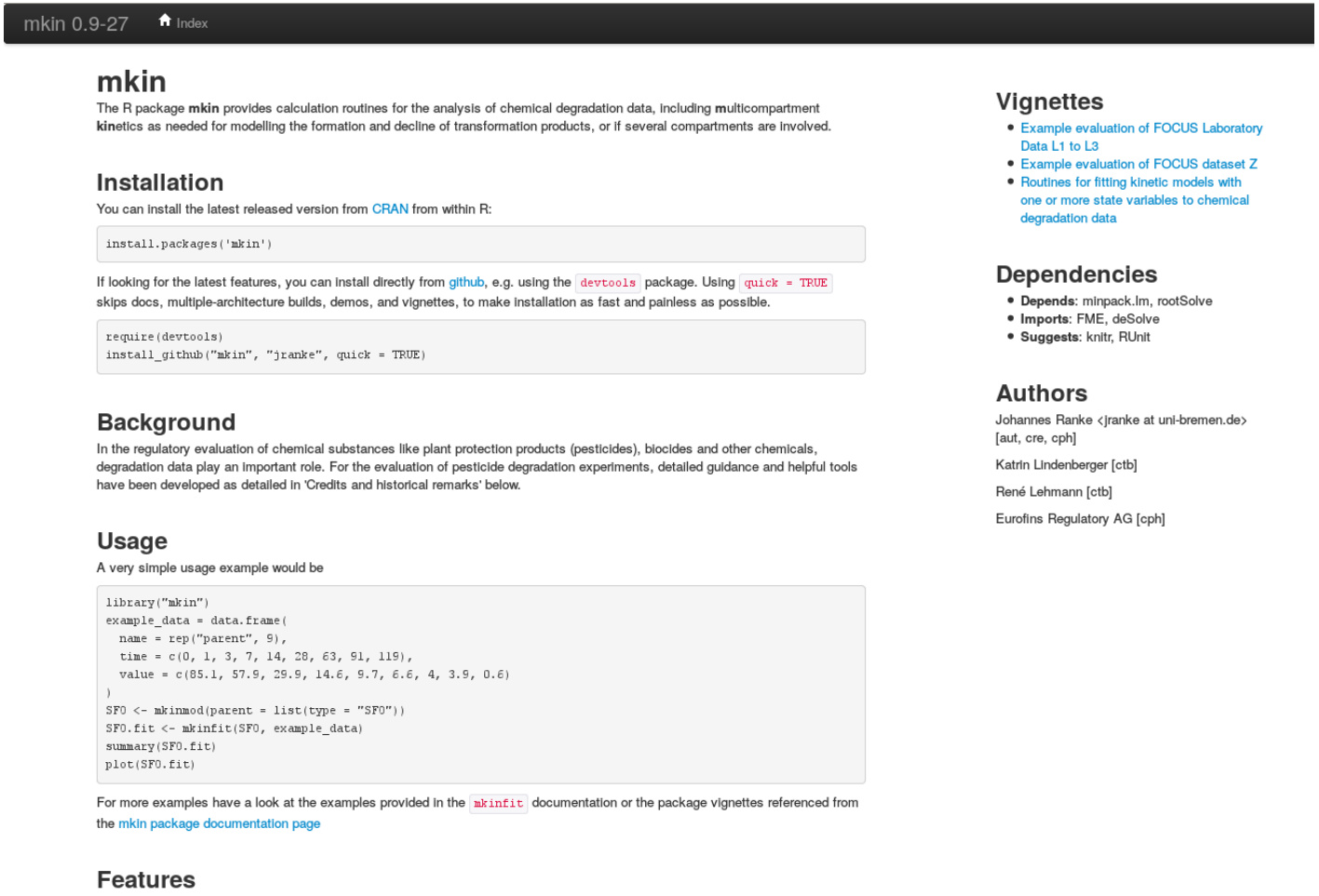
- With gmkin + mkin, we now have a completely open sourced software toolset
- Biphasic models for metabolites can conveniently be fitted
- The use of t-tests for parameter significance is questioned

Conclusions

- With gmkin + mkin, we now have a completely open sourced software toolset
- Biphasic models for metabolites can conveniently be fitted
- The use of t-tests for parameter significance is questioned
- Plausible confidence intervals for rate constants and single formation fractions are easily available

(slide corrected after the meeting)

Documentation



mkin 0.9-27 Index

mkin

The R package **mkin** provides calculation routines for the analysis of chemical degradation data, including **m**ulticompartment **kin**etics as needed for modelling the formation and decline of transformation products, or if several compartments are involved.

Installation

You can install the latest released version from CRAN from within R:

```
install.packages('mkin')
```

If looking for the latest features, you can install directly from github, e.g. using the `devtools` package. Using `quick = TRUE` skips docs, multiple-architecture builds, demos, and vignettes, to make installation as fast and painless as possible.

```
require(devtools)
install_github("mkin", "jranke", quick = TRUE)
```

Background

In the regulatory evaluation of chemical substances like plant protection products (pesticides), biocides and other chemicals, degradation data play an important role. For the evaluation of pesticide degradation experiments, detailed guidance and helpful tools have been developed as detailed in 'Credits and historical remarks' below.

Usage

A very simple usage example would be

```
library("mkin")
example_data = data.frame(
  name = rep("parent", 9),
  time = c(0, 1, 3, 7, 14, 28, 63, 91, 119),
  value = c(85.1, 57.9, 29.9, 14.6, 9.7, 6.6, 4, 3.9, 0.6)
)
SFO <- mkinmod(parent = list(type = "SFO"))
SFO.fit <- mkinfit(SFO, example_data)
summary(SFO.fit)
plot(SFO.fit)
```

For more examples have a look at the examples provided in the `mkinfit` documentation or the package vignettes referenced from the mkin package documentation page

Features

Vignettes

- Example evaluation of FOCUS Laboratory Data L1 to L3
- Example evaluation of FOCUS dataset Z
- Routines for fitting kinetic models with one or more state variables to chemical degradation data

Dependencies

- **Depends**: minpack.lm, rootSolve
- **Imports**: FME, deSolve
- **Suggests**: knitr, RUnit

Authors

Johannes Ranke <jranke at uni-bremen.de> [aut, cre, cph]

Katrin Lindenberger [ctb]

René Lehmann [ctb]

Eurofins Regulatory AG [cph]

kinfit.r-forge.r-project.org/mkin_static